IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Xu et al.	Application Number: 10/987,732
Filed: Novemebr 12, 2004	Group Art Unit: 1744
Docket No. 20191101-21	Examiner: Redding, David A.
Title: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS	Confirmation No. 1601
	Customer No.: 22878

FILED ELECTRONICALLY VIA EFS-WEB
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Request to Change Entity Status

Commissioner:

Applicant hereby requests a change in entity status from Small Entity to Undiscounted.

Respectfully submitted,

WAGENKNECHT IP LAW GROUP PC
By: /Raymond Wagenknecht/
Raymond Wagenknecht
Registration No. 59538

Dated: February 8, 2022

12396 World Trade Drive, Suite 110
San Diego, CA 92128
Telephone: 858-592-4141

ATTORNEY FOR APPLICANTS

Ex. 2008
Axion v. Agilent
IPR2024-01467

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Xu et al.	Application Number: 10/987,732
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P.O. Box 1450
Alexandria, VA 22313-1450

Request to Enter Name of Assignee and Update Docket Number

Commissioner:

The previous owner of the above-identified application has merged with/been acquired by Agilent Technologies, Inc. An assignment transferring all rights to Agilent Technologies, Inc. was recorded at reel/frame 055409/0874 on February 25, 2021. Accordingly, we request the following changes for this case.

Please update the name and address of the Assignee as follows:

FROM	TO
ACEA Biosciences, Inc.	Agilent Technologies, Inc.
6779 Mesa Ridge Rd. #100	5301 Stevens Creek Blvd., 1A-PB
San Diego, CA 92121	Santa Clara, CA 95051

Please update the docket number to **20191101-21**.

To assist with the above, we provide herewith a marked-up ADS showing the change in Assignee.

Ex. 2008
Axion v. Agilent
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Respectfully submitted,

WAGENKNECHT IP LAW GROUP PC
By: /Raymond Wagenknecht/
Raymond Wagenknecht
Registration No. 59538

Dated: February 8, 2022

12396 World Trade Drive, Suite 110
San Diego, CA 92128
Telephone: 858-592-4141

ATTORNEY FOR APPLICANTS

Ex. 2008
Axion v. Agilent
IPR2024-01467

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	10/987,732
Title of Invention		

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City		State/Province		Country of Residence	

Mailing Address of Inventor:

Address 1	
Address 2	

City		State/Province	
Postal Code		**Country**	

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number			
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention			
Attorney Docket Number		**Small Entity Status Claimed**	☐
Application Type			
Subject Matter			
Total Number of Drawing Sheets (if any)		**Suggested Figure for Publication (if any)**	

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	
Title of Invention		

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	22878		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	
Title of Invention		

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	
Title of Invention		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	
Title of Invention		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◯ Assignee ◯ Legal Representative under 35 U.S.C. 117 ◯ Joint Inventor

◯ Person to whom the inventor is obligated to assign. ◯ Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Applicant:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	
Title of Invention		

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

Organization Name	~~ACEA Biosciences, Inc.~~ Agilent Technologies, Inc.

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	~~6779 Mira Mesa Rd. #100~~ 5301 Stevens Creek Blvd., 1A-PB		
Address 2			
City	~~San Diego~~ Santa Clara	**State/Province**	CA
Country i	US	Postal Code	~~92121~~ 95051
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Raymond Wagenknecht/			Date (YYYY-MM-DD)	2022-02-08
First Name	Raymond	Last Name	Wagenknecht	Registration Number	50948

Additional Signature may be generated within this form by selecting the Add button.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	
	Application Number	
Title of Invention		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	44954007
Application Number:	10987732
International Application Number:	
Confirmation Number:	1601
Title of Invention:	REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS
First Named Inventor/Applicant Name:	Xiao Xu
Customer Number:	22878
Filer:	Raymond C. Wagenknecht/Katie Vinson
Filer Authorized By:	Raymond C. Wagenknecht
Attorney Docket Number:	20191101-21
Receipt Date:	09-FEB-2022
Filing Date:	12-NOV-2004
Time Stamp:	13:30:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	20191101-21_Loss_of_Small_Entity.pdf	89794 d68e0fd3fbb938361f6646eb5c1348abbd3438a3	no	1

Warnings:

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Axion v. Agilent
IPR2024-01467

Information:					
2	Transmittal Letter	20191101-21_Change_of_Name.pdf	95424 9ea19657349ac4c4cd9dad8dcd8aa76f1d52d1ed	no	2
Warnings:					
Information:					
3	Application Data Sheet	20191101-21_ADS_Change_NonApplicant_Assignee.pdf	174602 423565e07a5735ad94815bc4bcc5f86341e90f98	no	8
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
			Total Files Size (in bytes):	359820	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Ex. 2008
Axion v. Agilent
IPR2024-01467

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY.DOCKET NO./TITLE	REQUEST ID
10/987,732	11/12/2004	Xiao Xu	20191101-21	157524

Acknowledgement of Loss of Entitlement to Entity Status Discount

The entity status change request below filed through Private PAIR on 01/28/2022 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

X Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be notification of loss of entitlement to small or micro entity status, as applicable.

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Robert Kramer/
Name:	Robert Kramer
Registration Number:	51242

Ex. 2008
Axion v. Agilent
IPR2024-01467

PTO/AIA/123 (08-12)
Approved for use through 03/31/2021. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

CHANGE OF CORRESPONDENCE ADDRESS
Patent

Address to:
Mail Stop Post Issue
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Patent Number	7,192,752
Issue Date	March 20, 2007
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Attorney Docket Number	ACEA102P1US

Please change the Correspondence Address for the above-identified patent to:

☑ The address associated with Customer Number: 22878

OR

☐ **Firm *or* Individual Name**

Address

City | **State** | **ZIP**

Country

Telephone | **Email**

This form cannot be used to change the data associated with a Customer Number. To change the data associated with an existing Customer Number use "Request for Customer Number Data Change" (PTO/SB/124).

This form will not affect any "fee address" provided for the above-identified patent. To change a "fee address" use the "Fee Address Indication Form" (PTO/SB/47).

I am the:

☐ Patentee.

☐ If the Patentee was not the applicant for patent (37 CFR 1.42), then a Statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is enclosed or was filed on _____. See 37 CFR 3.71.

☑ Attorney or agent of record. Registration Number 50,948.

☐ Patent practitioner acting in a representative capacity whose correspondence address is the correspondence address of record. Notice has been given to the patentee or owner. Registration Number 50,948.

Signature /Raymond Wagenknecht/

Typed or Printed Name Raymond Wagenknecht

Date 2/26/2021 | Telephone 858-592-4141

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☑ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Post Issue, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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Axion v. Agilent
IPR2024-01467

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Electronic Acknowledgement Receipt

EFS ID:	42043424
Application Number:	10987732
International Application Number:	
Confirmation Number:	1601
Title of Invention:	REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS
First Named Inventor/Applicant Name:	Xiao Xu
Customer Number:	59538
Filer:	Raymond C. Wagenknecht/Antonia Gordon
Filer Authorized By:	Raymond C. Wagenknecht
Attorney Docket Number:	ACEA102P1US
Receipt Date:	26-FEB-2021
Filing Date:	12-NOV-2004
Time Stamp:	20:11:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Change of Address	10101-100022_Change_of_Correspondence_Address.pdf	251734 2c4426dfb16fcd92a30da7076820d69aef9687f1	no	2

Warnings:

Ex. 2008
Axion v. Agilent
IPR2024-01467

Information:		
	Total Files Size (in bytes):	251734

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Patent
Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
Xu et al.	)	Examiner: David A. Redding
	)	
Patent Number: 7,192,752	)	
	)	
Application Number: 10/987,732	)	
	)	Group Art Unit: 1744
Filed: November 12, 2004	)	
	)	
For: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL BASED ASSAYS	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Change of Entity Status to Large Entity
& Supplemental Payment

Sir:

Applicants respectfully request change of entity status to large entity for the above-identified issued patent. Accordingly, please change the entity status to "large entity." The entity status is changed without any current or previous deceptive intent. The fourth year maintenance fee for small entity was unintentionally paid for the present issued patent on June 8, 2010 in the amount of $490.

The fourth year maintenance fee for a large entity is $980. Thus the remainder due for the fourth year maintenance fee is $490.

Accordingly, Applicants provide herewith the supplemental payment in the amount of $490 as required for the remaining portion of fourth year maintenance fee for a large entity.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Respectfully submitted,

6/9/2010

Date

Raymond Wagenknecht
Reg. No. 50,948

Biotech Beach Law Group, PC
5677 Oberlin Drive, Ste. 204
San Diego, CA 92121
(858) 587-2510 x 201

Ex. 2008
Axion v. Agilent
IPR2024-01467

Electronic Patent Application Fee Transmittal

Application Number:	10987732
Filing Date:	12-Nov-2004
Title of Invention:	REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS
First Named Inventor/Applicant Name:	Xiao Xu
Filer:	Raymond C. Wagenknecht/Case Kamshad
Attorney Docket Number:	ACE-00102.P.1-US

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 2 months with $0 paid	1252	1	490	490

Ex. 2008
Axion v. Agilent
IPR2024-01467

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**490**

Ex. 2008
Axion v. Agilent
IPR2024-01467

Electronic Acknowledgement Receipt

EFS ID:	7782440
Application Number:	10987732
International Application Number:	
Confirmation Number:	1601
Title of Invention:	REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS
First Named Inventor/Applicant Name:	Xiao Xu
Customer Number:	59538
Filer:	Raymond C. Wagenknecht/Case Kamshad
Filer Authorized By:	Raymond C. Wagenknecht
Attorney Docket Number:	ACE-00102.P.1-US
Receipt Date:	09-JUN-2010
Filing Date:	12-NOV-2004
Time Stamp:	18:50:23
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$490
RAM confirmation Number	7269
Deposit Account	504352
Authorized User	WAGENKNECHT,RAYMOND

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Ex. 2008
Axion v. Agilent
IPR2024-01467

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Filing_papers.pdf	232352 5cd43fc98467c3832872848cc97b21f1356ee0a2	yes	3

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Transmittal Letter	1	1
Miscellaneous Incoming Letter	2	3

Warnings:

Information:

2	Fee Worksheet (PTO-875)	fee-info.pdf	30972 4d502e1f75aa55e4305004d68471693dbeb4ef054	no	2

Warnings:

Information:

Total Files Size (in bytes):	263324

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Doc Code: TRAN.LET
Document Description: Transmittal Letter

PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission

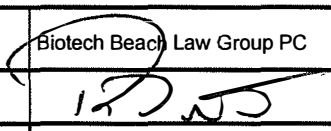
Application Number	10/987,732
Filing Date	December 1, 2008
First Named Inventor	Xiao Xu
Art Unit	1744
Examiner Name	David A. Redding
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES *(Check all that apply)*

- [✓] Fee Transmittal Form
  - [✓] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [✓] Other Enclosure(s) (please Identify below):
  1. Change of entity status

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Biotech Beach Law Group PC		
Signature			
Printed name	Raymond Wagenknecht		
Date	06/09/2010	Reg. No.	50948

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name		Date	

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Ex. 2008
Axion v. Agilent
IPR2024-01467

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US

CONFIRMATION NO. 1601

59538
BIOTECH BEACH LAW GROUP , PC
625 BROADWAY
Suite 1210
SAN DIEGO, CA 92101

POA ACCEPTANCE LETTER

OC00000033177894

Date Mailed: 11/21/2008

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/12/2008.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/tle/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US

CONFIRMATION NO. 1601

POWER OF ATTORNEY NOTICE

24232
DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

OC000000033177887

Date Mailed: 11/21/2008

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/12/2008.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

/tle/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Patent

Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
XU et al	)	Examiner: David Redding
	)	
Application Number:	)	
10/987,732	)	
	)	Group Art Unit: 1744
Filed: November 12, 2004	)	
	)	
For: REAL TIME ELECTRONIC	)	
CELL SENSING SYSTEMS	)	
AND APPLICATIONS FOR	)	
CELL BASED ASSAYS	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REVOCATION OF POWER OF ATTORNEY WITH APPOINTMENT OF NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

1. I, the undersigned, am authorized to act on behalf of ACEA BIOSCIENCES, INC., and my title is provided below.

2. ACEA BIOSCIENCES, INC. is the assignee of record of the entire right, title and interest in the above-identified application; a STATEMENT UNDER 37 CFR 3.73(b) provided herewith.

Ex. 2008
Axion v. Agilent
IPR2024-01467

3. ACEA BIOSCIENCES, INC. hereby revokes all previous powers of attorney given in the above-identified application.

4. ACEA BIOSCIENCES, INC. hereby appoints the practitioners associated with CUSTOMER NUMBER 59538 in the name of Biotech Beach Law Group PC as attorneys of record to prosecute the above-identified application and to conduct all business before the United States Patent and Trademark Office in connection therewith.

5. Please change the correspondence address for the above-identified application to the address associated with CUSTOMER NUMBER 59538 in the name of Biotech Beach Law Group PC.

Respectfully submitted,



Xiaobo Wang
Vice President

NOV 12, 2008
Date

ACEA BIOSCIENCES, INC.
6779 Mesa Ridge Rd.
Suite 100
San Diego, CA 92121

1(858)724-0928

Ex. 2008
Axion v. Agilent
IPR2024-01467

Electronic Acknowledgement Receipt

EFS ID:	4280139
Application Number:	10987732
International Application Number:	
Confirmation Number:	1601
Title of Invention:	REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS
First Named Inventor/Applicant Name:	Xiao Xu
Customer Number:	24232
Filer:	Raymond C. Wagenknecht
Filer Authorized By:	
Attorney Docket Number:	ACE-00102.P.1-US
Receipt Date:	12-NOV-2008
Filing Date:	12-NOV-2004
Time Stamp:	20:42:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		PowerOfAttorney.PDF	121453 a603d40fe0b47936b8aadbff89a899be1a21e6f7	yes	4

Ex. 2008
Axion v. Agilent
IPR2024-01467

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Assignee showing of ownership per 37 CFR 3.73(b).	1	2
	Power of Attorney	3	4

Warnings:	
Information:	
Total Files Size (in bytes):	121453

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Patent

Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
XU et al	)	Examiner: David Redding
	)	
Application Number:	)	
10/987,732	)	
	)	Group Art Unit: 1744
Filed: November 12, 2004	)	
	)	
For: REAL TIME ELECTRONIC	)	
CELL SENSING SYSTEMS	)	
AND APPLICATIONS FOR	)	
CELL BASED ASSAYS	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT UNDER 37 CFR 3.73(b)

1. I, the undersigned, am authorized to act on behalf of ACEA BIOSCIENCES, INC., and my title is provided below.

2. ACEA BIOSCIENCES, INC. states that it is the assignee of the entire right, title and interest in the patent application identified above by virtue of a chain of title from the inventors of the patent application, to the current assignee as follows:

a. From: Xiao Xu To: ACEA BIOSCIENCES; The assignment recorded with the United States Patent and Trademark Office at Reel 016389, Frame 0559 on 3/24/2005;

Ex. 2008
Axion v. Agilent
IPR2024-01467

b. From: Yama A. Abassi To: ACEA BIOSCIENCES; The assignment recorded with the United States Patent and Trademark Office at Reel 016389, Frame 0559 on 3/24/2005;

c. From: Xiaobo Wang To: ACEA BIOSCIENCES; The assignment recorded with the United States Patent and Trademark Office at Reel 016389, Frame 0559 on 3/24/2005; and

d. From: Jiangbo Gan To: ACEA BIOSCIENCES; The assignment recorded with the United States Patent and Trademark Office at Reel 016389, Frame 0559 on 3/24/2005

3. As required by 37 CFR 3.73(b)(1), the documentary evidence of the chain of title from the original owner to the assignee was submitted for recordation pursuant to 37 CFR 3.11.

Respectfully submitted,

X _____ May 12, 2008

Xiaobo Wang
Vice President

Date

ACEA BIOSCIENCES, INC.
6779 Mesa Ridge Rd.
Suite 100
San Diego, CA 92121
1(858)724-0928

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	03/20/2007	7192752	ACE-00102.P.1-US	1601

24232 7590 02/28/2007

DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Xiao Xu, San Diego, CA;
Yama Abassi, San Diego, CA;
Xiaobo Wang, San Diego, CA;
Jiangbo Gan, San Diego, CA;

Ex. 2008
Axion v. Agilent
IPR2024-01467

PART B - FEE(S) TRANSMITTAL

PTO/SB/31 (07-06)
JAN 0 8 2007
PATENT & TRADEMARK OFFICE

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

24232 7590 11/01/2006

DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

01/09/2007 HDERESS2 00000093 501321 10987732

01 FC:2501 700.00 OP
02 FC:1504 300.00 OP
03 FC:8001 30.00 DA

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

David R Preston (Depositor's name)
(Signature)
Jan 4, 2007 (Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

TITLE OF INVENTION: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$300	$0	$1000	02/01/2007

EXAMINER	ART UNIT	CLASS-SUBCLASS
REDDING, DAVID A	1744	435-173100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 David R Preston
a + Associates
2 a David Preston

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: ACEA BioSciences

(B) RESIDENCE: (CITY and STATE OR COUNTRY): San Diego, CA

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☑ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☑ Issue Fee
☑ Publication Fee (No small entity discount permitted)
☑ Advance Order - # of Copies 10

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☑ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number 501321 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date Jan 4, 2007

Typed or printed name David R Preston Registration No. 38,710

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Ex. 2008
Axion v. Agilent
IPR2024-01467

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

24232 7590 12/21/2006

DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

EXAMINER
REDDING, DAVID A

ART UNIT	PAPER NUMBER
1744	

MAIL DATE	DELIVERY MODE
12/21/2006	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Supplemental Notice of Allowability	Application No. 10/987,732	Applicant(s) XU ET AL.	
	Examiner David A. Redding	Art Unit 1744	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☐ This communication is responsive to _____.

2. ☒ The allowed claim(s) is/are *51-60,72-81,101-103,160-163, renumbered 1-27, respectively.*

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 12/19/05
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

David A Redding
Primary Examiner
Art Unit: 1744

Ex. 2008
Axion v. Agilent
IPR2024-01467

OIPE IAP59
DEC 19 2005
PATENT & TRADEMARK OFFICE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Docket Number: ACE-00102.P.1-US	Application Number: 10/987,732
	Applicant: XU et al.	
	Filing Date: November 12, 2004	Group Art Unit: 1744

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
DR	P1	2003/0032000	02/2003	Liu et al			
	P2	2005/0014130	01/2005	Liu et al			
	P3	USRE38,323	11/2003	Sugihara et al			
	P4	6,368,795	04/2002	Hefti			
	P5	6,376,233	04/2002	Wolf et al			
	P6	6,461,808	10/2002	Bodner et al			
	P7	6,485,905	11/2002	Hefti			
	P8	6,566,079	05/2003	Hefti			
	P9	6,573,063	03/2003	Hochman			
	P10	6,626,902	09/2003	Kucharczyk et al			
	P11	6,627,461	09/2003	Chapman et al			
	P12	6,686,193	02/2004	Maher et al			
DR	P13	6,716,620	04/2004	Bashir et al			

Examiner Signature	/David Redding/ (12/19/2006)	Date Considered	

Ex. 2008
Axion v. Agilent
IPR2024-01467

FOREIGN PATENT DOCUMENTS								
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	Translation	
							YES	NO
DR	F1	EP 1195432B1	09/2004	EPO				
	F2	01/25769	04/2001	PCT				
	F3	02/04943	01/2002	PCT				
	F4	02/42766	05/2002	PCT				
	F5	03/016887	02/2003	PCT				
DR	F6	05/005979	01/2005	PCT				

OTHER DOCUMENTS (Including Author, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
DR	D1	Bieberich and Guiseppi-Elie, Biosensors and Bioelectronics, 19:923-931 (2004)
DR	D2	Burnett et al., J. Biomo. Screening, 8(6):660-667 (2003)
DR	D3	Ciambrone et al., J. Biomo. Screening, 9(6):467-480 (2004)
DR	D4	Ehret et al., Med. Biol. Eng. Comput. 36:365-370 (1998)
DR	D5	Ehret et al.,Biosensors and Bioelectronics, 12(1):29-41 (1996)
DR	D6	Gutmann et al., Pharmaceutical Research, 16(3):402-407 (1999)
DR	D7	Hug, Assay and Drug Dev. Tech., 1(3):479-488 (2003)

Examiner Signature	/David Redding/ (12/19/2006)	Date Considered	

Ex. 2008
Axion v. Agilent
IPR2024-01467

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
DR	D8	Lin and Huang, J. Micromech. Microeng., 11:542-547 (2001)
DR	D9	Lin et al., Min. For Chem., Bio., & Bioeng., 4:104-108 (2004)
DR	D10	Wegener et al., Eur. J. Physiol., 437:925-934 (1999)
DR	D11	Wolf et al., Biosensors and Bioelectronics, 13:501-509 (1998)
DR	D12	Xiao and Luong, Biotechnol. Prog., 19:1000-1005 (2003)
DR	D13	Xiao et al., Anal. Chem., 74:5748-5753 (2002)
DR	D14	Yamauchi et al., Nuc. Acids Res., 32(22):1-8 (2004)
DR	D15	Loffert et al., QIAGENNews, 4:15-18 (1997)

Examiner Signature	/David Redding/ (12/19/2006)	Date Considered	

Ex. 2008
Axion v. Agilent
IPR2024-01467

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

24232 7590 11/01/2006

DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

EXAMINER	
REDDING, DAVID A	
ART UNIT	PAPER NUMBER
1744	

DATE MAILED: 11/01/2006

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

TITLE OF INVENTION: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$300	$0	$1000	02/01/2007

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

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Axion v. Agilent
IPR2024-01467

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

24232 7590 11/01/2006

DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

TITLE OF INVENTION: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$300	$0	$1000	02/01/2007

EXAMINER	ART UNIT	CLASS-SUBCLASS
REDDING, DAVID A	1744	435-173100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☐ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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Ex. 2008
Axion v. Agilent
IPR2024-01467

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

24232 7590 11/01/2006

DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

EXAMINER	
REDDING, DAVID A	
ART UNIT	PAPER NUMBER
1744	

DATE MAILED: 11/01/2006

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No. 10/987,732	Applicant(s) XU ET AL.	
	Examiner David A. Redding	Art Unit 1744	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *amendment filed 7/27/06.*

2. ☒ The allowed claim(s) is/are *51-60,72-81,101-103,160-163, renumbered 1-27, respectively.*

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
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4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
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   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

David A Redding
Primary Examiner
Art Unit: 1744

Ex. 2008
Axion v. Agilent
IPR2024-01467

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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Bib Data Sheet

CONFIRMATION NO. 1601

SERIAL NUMBER	FILING OR 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
10/987,732	11/12/2004 RULE	205	1744	ACE-00102.P.1-US

APPLICANTS

Xiao Xu, San Diego, CA;
Yama Abassi, San Diego, CA;
Xiaobo Wang, San Diego, CA;
Jiangbo Gan, San Diego, CA;

**** CONTINUING DATA *******************************

This application is a CIP of 10/705,447 11/10/2003 and is a CIP of 10/705,615 11/10/2003
which claims benefit of 60/435,400 12/20/2002
and claims benefit of 60/469,572 05/09/2003
This application 10/987,732
claims benefit of 60/519,567 11/12/2003
and claims benefit of 60/542,927 02/09/2004
and claims benefit of 60/548,713 02/27/2004

**** FOREIGN APPLICATIONS *******************************

UNITED STATES OF AMERICA PCT/US03/22537 07/18/2003
UNITED STATES OF AMERICA PCT/US03/22557 07/18/2003

IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** SMALL ENTITY **
** 01/14/2005

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☑ no 35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance Verified and Acknowledged Examiner's Signature ( ) Initials	CA	26	~~23~~ 27	1

ADDRESS
24232

TITLE

Real time electronic cell sensing systems and applications for cell-based assays

FILING FEE RECEIVED		
587	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other ☐ Credit

Ex. 2008
Axion v. Agilent
IPR2024-01467

Issue Classification	Application/Control No.	Applicant(s)/Patent under Reexamination	
	10/987,732	XU ET AL.	
	Examiner	Art Unit	
	David A. Redding	1744	

ISSUE CLASSIFICATION

ORIGINAL		CROSS REFERENCE(S)							
CLASS	SUBCLASS	CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)						
435	173.1	435	173.4	288.4	288.5				
INTERNATIONAL CLASSIFICATION		204	403.03						
C 1 2 N	13/00								
	/								
	/								
	/								
	/								

(Assistant Examiner) (Date)

(Legal Instruments Examiner) (Date) 10/17/06

David Redding 10/16/06

(Primary Examiner) (Date)

Total Claims Allowed: 27

O.G. Print Claim(s)	O.G. Print Fig.
1	4

☒ **Claims renumbered in the same order as presented by applicant** ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		31		61		91		121		151		181
	2		32		62		92		122		152		182
	3		33		63		93		123		153		183
	4		34		64		94		124		154		184
	5		35		65		95		125		155		185
	6		36		66		96		126		156		186
	7		37		67		97		127		157		187
	8		38		68		98		128		158		188
	9		39		69		99		129		159		189
	10		40		70		100		130		160		190
	11		41		71		101		131		161		191
	12		42		72		102		132		162		192
	13		43		73		103		133		163		193
	14		44		74		104		134		164		194
	15		45		75		105		135		165		195
	16		46		76		106		136		166		196
	17		47		77		107		137		167		197
	18		48		78		108		138		168		198
	19		49		79		109		139		169		199
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	21		51		81		111		141		171		201
	22		52		82		112		142		172		202
	23		53		83		113		143		173		203
	24		54		84		114		144		174		204
	25		55		85		115		145		175		205
	26		56		86		116		146		176		206
	27		57		87		117		147		177		207
	28		58		88		118		148		178		208
	29		59		89		119		149		179		209
	30		60		90		120		150		180		210

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Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	10/987,732	XU ET AL.	
	Examiner	Art Unit	
	David A. Redding	1744	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner
204	**403.03**	10/12/2006	**DAR**
435	288.4		
	288.5 305.2		
	305.3 173.1 173.4		

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
updated	10/16/2006	DAR

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	46	("2656508"|"20020032531"|"20020076690"|"20020086280"|"20020110847"|"20020150886"|"20030032000"|"20050014130"|"3259842"|"3743581"|"3890201"|"4072578"|"4225410"|"4686190"|"4920047"|"5134070"|"5187096"|"5218312"|"5278048"|"5284753"|"5563067"|"5626734"|"5643742"|"5801055"|"5810725"|"5851422"|"6132683"|"6169394"|"6232062"|"6235520"|"6280586"|"6288527"|"6368795"|"6368851"|"6376233"|"6448030"|"6448794"|"6461808"|"6472144"|"6485905"|"6566079"|"6573063"|"6626902"|"6627461"|"6686193"|"6716620").PN.	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:29
L2	3	1 and (individually with addressed)	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:30
L3	3	1 and (individually with addressed)	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:30
L4	18	1 and (addressed or addressable or sam)	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:30
L5	3587228	3 or "18"	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:30
L6	18	3 or 4	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:30
L7	9	6 and (uniform)	US-PGPUB; USPAT	OR	OFF	2006/10/16 12:43
L8	5	7 and (impedance)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2006/10/16 12:44

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IFW

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 8

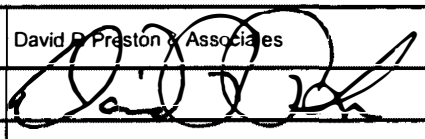
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Art Unit	1744
Examiner Name	David Redding
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
  - [x] Fee Attached
- [x] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):
  1. Postcard

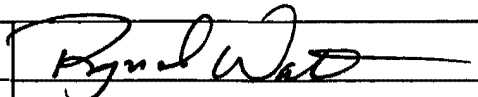
Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R Preston & Associates		
Signature			
Printed name	David R Preston		
Date	7.24.06	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name	Raymond Wagenknecht	Date	7/24/2006

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2006

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 75.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Examiner Name	David Redding
Art Unit	1744
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R Preston

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION (All the fees below are due upon filing or may be subject to a surcharge.)

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fees Paid ($)**
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 20 or HP =	3	x 25	= 75

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____	x _____	= 0

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**
_____	0

3. APPLICATION SIZE FEE

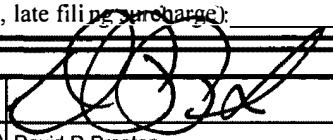
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____	/ 50 = _____ (round up to a whole number)	x _____	= _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): _____	

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R Preston		Date 7-24-06

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Axion v. Agilent
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Patent
Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
XU et al	)	Examiner: David Redding
	)	
Application Number: 10/987,732	)	
	)	Group Art Unit: 1744
Filed: November 12, 2004	)	
	)	
For: A REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATIONS FOR CELL BASED ASSAYS	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO OFFICE ACTION

Sir:

Applicants are responding to the Office Action mailed July 3, 2006. Applicants are responding within one (1) month from the mailing date of the Office Action. Thus no extension of time is deemed necessary.

Amendments to the claims begin on page 2 of this paper.

Remarks / Arguments begin on page 7 of this paper.

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Amendments to the Claims

Applicants amend claim 51 and add new claims 161-163. Thus, Claims 51-60, 72-81, 101-103 and 160-163 remain pending upon entry of this Reply to Office Action.

Listing of Claims

Claims 1-50 (cancelled)

Claim 51 (currently amended) A system for monitoring cell-substrate impedance, comprising:

a) one or more multiple well cell-substrate impedance monitoring devices, wherein at least two of the multiple wells of said device comprise an electrode array at the bottom of the well, wherein said electrode array is individually addressed, further wherein said device can be used to measure differences in impedance values that relate to cell behavior;

b) an impedance analyzer;

c) a device station comprising electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer; and

d) a software program that can ~~said~~ control said device station and record and analyze data obtained from said impedance analyzer;

wherein said electrode array has an approximately uniform electrode resistance distribution across said electrode array so that the electrode resistances between two locations on said array do not differ by more than 30%.

Claim 52 (original) The system of claim 51, wherein said device station comprises electronic switches that can switch on and off connections of each of said two or more electrode arrays to said impedance analyzer.

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Claim 53 (previously presented) The system of claim 51, wherein said software program can direct said device station to measure impedance values at regular or irregular time intervals from one or more of said two or more electrode arrays.

Claim 54 (original) The system of claim 51, wherein said impedance analyzer can measure impedance at one or more frequencies.

Claim 55 (previously presented) The system of claim 51, wherein said software program can store said data.

Claim 56 (previously presented) The system of claim 51 wherein said system can display said data.

Claim 57 (previously presented) The system of claim 51, wherein said software can calculate a cell index for one or more time points for one or more wells of said multi-well plate based on impedance values measured from said one or more electrode arrays localized to said one or more wells.

Claim 58 (previously presented) The system of claim 56 wherein said system can display said cell index for said one or more time points data for said one or more wells.

Claim 59 (original) The system of claim 51, wherein said software is programmable to direct impedance monitoring of any of said two or more electrode arrays at chosen time intervals.

Claim 60 (original) The system of claim 55, wherein said software allows entry and display of experimental parameters.

Claims 61-71 (cancelled)

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Claim 72 (original) A method of performing an assay that monitors cell-substrate impedance, comprising:

providing the system of claim 51;

introducing cells into at least one well of said system;

monitoring cell-substrate impedance of said at least one well.

Claim 73 (original) The method of claim 72, wherein said monitoring comprises analyzing impedance changes at three or more time points.

Claim 74 (original) The method of claim 73, wherein at least three of said three or more time points are spaced at regular intervals.

Claim 75 (original) A method of performing an assay that monitors cell-substrate impedance in response to one or more test compounds, comprising:

providing the system of claim 51;

introducing cells into one or more wells of said system that comprise electrode arrays;

adding at least one test compound to said one or more wells; and

monitoring cell-substrate impedance of said one or more wells before and after adding said at least one test compound.

Claim 76 (original) The method of claim 75, wherein impedance is monitored at three or more time points.

Claim 77 (original) The method of claim 75, further comprising analyzing impedance values before and after adding said at least one test compound.

Claim 78 (original) The method of claim 75, further comprising introducing cells to at least one well to which a test compound is not added to create a control well.

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Claim 79 (original) The method of claim 77, wherein said analyzing comprises calculating a cell index.

Claim 80 (original) The method of claim 77, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a compound, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

Claim 81 (original) The method of claim 79, wherein said cell index is used as an indicator of cytotoxicity.

Claims 82-100 (cancelled)

Claim 101 (original) A method for monitoring changes in cell-substrate impedance in response to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system, wherein each of said one or more wells comprises an electrode array;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance of at least one of said one or more wells of said system.

Claim 102 (original) The method of claim 101, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

Claim 103 (original) The method of claim 102, wherein said cells are mast cells.

Claims 104-159 (canceled)

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Claim 160 (previously presented) The system of claim 51, wherein said electrode array is electrically connected to two connection pads.

Claim 161 (new) The system of claim 51, wherein said electrode resistances between said two locations on said array do not differ by more than 15%.

Claim 162 (new) The system of claim 51, wherein said electrode resistances between said two locations on said array do not differ by more than 5%.

Claim 163 (new) The system of claim 51, wherein said electrode resistances between said two locations on said array do not differ by more than 3%.

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REMARKS / ARGUMENTS

Applicants amend claim 51 and add new claims 161-163. Thus, Claims 51-60, 72-81,101-103 and 160-163 remain pending upon entry of this Reply to Office Action.

Support for Amendments

Applicants amend claim 51 to recite, "the electrode resistances between two locations on said array do not differ by more than 30%." Support for the amendment may be found throughout the specification and drawings as originally filed. For example, support for this amendment may be found on page 27, lines 9-11, "Preferably, the electrode resistance at a first location on an array of the device and the electrode resistance at a second location on the same array does not differ by more than 30%."

Applicants add new claim 161, which depends from claim 51, to recite, "The system of claim 51, wherein said electrode resistances between said two locations on said array do not differ by more than 15%." Support for this dependent claim may be found on page 27, lines 11-13, "More preferably, the electrode resistance at a first location on an array of the device and the electrode resistance at a second location on the same array does not differ by more than 15%."

Applicants add new claim 162, which depends from claim 51, to recite, "The system of claim 51, wherein said electrode resistances between said two locations on said array do not differ by more than 5%." Support for this dependent claim may be found on page 27, lines 13-15, "Even more preferably, the electrode resistance at a first location on an array of the device and a second location on the same array does not differ by more than 5%."

Applicants add new claim 163, which depends from claim 51, to recite, "The system of claim 51, wherein said electrode resistances between said two locations on said array do not differ by more than 2%." Support may be found on page 27, lines 15-16, "More preferably yet, the electrode resistance at a first location on an array of the device and a second location on the same array does not differ by more than 2%."

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Response to Claim Rejections – 35 U.S.C §112

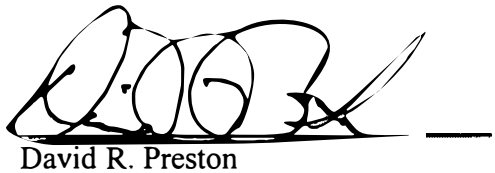
The Examiner has rejected claims 51-60, 72-81, 100-103 and 160 under 35 U.S.C. §112 as allegedly being indefinite because the Examiner argues the term "approximately uniform electrode resistance distribution across the electrode array" is a relative term. The Examiner has also indicated claims 51-60, 72-81, 100-103 and 160 would be allowable if rewritten or amended to overcome the rejection under 35 U.S.C. §112.

To expedite prosecution of the present application to allowance and for clarity Applicants have amended independent claim 51. Claims 52-60, 72-81, 100-103 and 160 as well as new claims 161-163 depend from claim 51. To expedite prosecution of the present application to allowance and for clarity, Applicants have amended claim 51 to recite, "said electrode array has an approximately uniform electrode resistance distribution across said electrode array so that the electrode resistances between two locations on said array do not differ by more than 30%." Thus, Applicants respectfully request the term be deemed definite and the rejection be withdrawn.

Applicants respectfully submit that the claims are in condition for allowance.

Respectfully submitted,

Date: July 24, 2006



David R. Preston
Reg. No. 38,710

David R. Preston & Associates A.P.C.
5850 Oberlin Drive
Suite 300
San Diego, CA 92130

Telephone: 858.724.0375
Facsimile: 858.724.0384

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PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2004

Application or Docket Number: 10987732

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	23	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	23 minus 20=	* 3
INDEPENDENT CLAIMS	1 minus 3 =	* 0
MULTIPLE DEPENDENT CLAIM PRESENT	☐	

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	395.00	OR	BASIC FEE	790.00
X$ 9=	27	OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL	422	OR	TOTAL	

7.27.06

CLAIMS AS AMENDED - PART II

AMENDMENT A	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 27	Minus	** 23	= 4
Independent	* 1	Minus	*** 3	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM	☐			

after paid 25.00

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=	75.00	OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM	☐			

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM	☐			

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

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DAVID R PRESTON & ASSOCIATES APC
5850 OBERLIN DRIVE
SUITE 300
SAN DIEGO, CA 92121

EXAMINER	
REDDING, DAVID A	

ART UNIT	PAPER NUMBER
1744	

DATE MAILED: 07/03/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/987,732	XU ET AL.	
	Examiner	Art Unit	
	David A. Redding	1744	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 April 2006.*

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *51-60,72-81,100-103 and 160* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *51-60,72-81,100-103,160* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

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DETAILED ACTION

This Office Action is in response to the reply filed 4/13/06.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The term "approximately uniform electrode resistance distribution across the electrode array" in claim 51-60,72-81,100-103,160 is a relative term which renders the claim indefinite. The term "approximately uniform electrode resistance distribution across said electrode array" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The asserted difference between the claims and the prior art is that the prior art devices do not provide “approximately uniform electrode resistance distribution across the electrode array”. However, the specification as to what range of electrode resistance distribution across the electrode array constitutes “approximately uniform”. Without this feature being clearly defined it’s not possible to determine how the present claims distinguish over the prior art.

Response to Arguments

Applicants arguments as to the prior art having electrode arrays with variable lengths between electrical contacts and the measurement electrodes results in variable electrode resistances is persuasive and the prior art rejections withdrawn.

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Allowable Subject Matter

Claims 51-60,72-81,100-103,160 would be allowable if rewritten or amended to overcome the rejection(s) under 35 U.S.C. 112, 2nd paragraph, set forth in this Office action.

As allowable subject matter has been indicated, applicant's reply must either comply with all formal requirements or specifically traverse each requirement not complied with. See 37 CFR 1.111(b) and MPEP § 707.07(a).

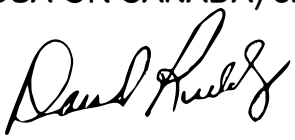
Any inquiry concerning this communication or earlier communications from the examiner should be directed to David A. Redding whose telephone number is 571-272-1276. The examiner can normally be reached on Mon.-Fri. 6:00 - 3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gladys Corcoran-Piazza can be reached on 571-272-1224. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

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For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.



David A Redding
Primary Examiner
Art Unit 1744

DAR

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Search Notes

Application/Control No.	Applicant(s)/Patent under Reexamination
10/987,732	XU ET AL.
Examiner	**Art Unit**
David A. Redding	1744

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
UPDATED	6/26/2006	DAR

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APR 13 2006
PATENT & TRADEMARK OFFICE

Patent
Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
XU et al	Examiner: David Redding
Application Number: 10/987,732	Group Art Unit: 1744
Filed: November 12, 2004	
For: A REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATIONS FOR CELL BASED ASSAYS	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO OFFICE ACTION

Sir:

Applicants are responding to the Office Action mailed December 2, 2005. Applicants are responding within five (5) months from the mailing date of the Office Action. Applicants respectfully request a two (2) month extension of time to file a reply and enclose the fee of $ 225.00 as indicated under 37 CFR §1.17(a)(2).

Amendments to the claims begin on page 2 of this paper.

Remarks / Arguments begin on page 7 of this paper.

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Amendments to the Claims

Applicant adds new claim 160. Thus, Claims 51-60, 72-81, 101-103 and 160 remain pending upon entry of this Response to Office Action.

Listing of Claims

Claims 1-50 (cancelled)

Claim 51 (previously amended) A system for monitoring cell-substrate impedance, comprising:

a) one or more multiple well cell-substrate impedance monitoring devices, wherein at least two of the multiple wells of said device comprise an electrode array at the bottom of the well, wherein said electrode array is individually addressed, further wherein said device can be used to measure differences in impedance values that relate to cell behavior;

b) an impedance analyzer;

c) a device station comprising electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer; and

d) a software program that can said control device station and record and analyze data obtained from said impedance analyzer;

wherein said electrode array has an approximately uniform electrode resistance distribution across said electrode array.

Claim 52 (original) The system of claim 51, wherein said device station comprises electronic switches that can switch on and off connections of each of said two or more electrode arrays to said impedance analyzer.

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Claim 53 (previously amended) The system of claim 51, wherein said software program can direct said device station to measure impedance values at regular or irregular time intervals from one or more of said two or more electrode arrays.

Claim 54 (original) The system of claim 51, wherein said impedance analyzer can measure impedance at one or more frequencies.

Claim 55 (previously amended) The system of claim 51, wherein said software program can store said data.

Claim 56 (previously amended) The system of claim 51 wherein said system can display said data.

Claim 57 (previously amended) The system of claim 51, wherein said software can calculate a cell index for one or more time points for one or more wells of said multi-well plate based on impedance values measured from said one or more electrode arrays localized to said one or more wells.

Claim 58 (previously amended) The system of claim 56 wherein said system can display said cell index for said one or more time points data for said one or more wells.

Claim 59 (original) The system of claim 51, wherein said software is programmable to direct impedance monitoring of any of said two or more electrode arrays at chosen time intervals.

Claim 60 (original) The system of claim 55, wherein said software allows entry and display of experimental parameters.

Claims 61-71 (cancelled)

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Claim 72 (original) A method of performing an assay that monitors cell-substrate impedance, comprising:

providing the system of claim 51;

introducing cells into at least one well of said system;

monitoring cell-substrate impedance of said at least one well.

Claim 73 (original) The method of claim 72, wherein said monitoring comprises analyzing impedance changes at three or more time points.

Claim 74 (original) The method of claim 73, wherein at least three of said three or more time points are spaced at regular intervals.

Claim 75 (original) A method of performing an assay that monitors cell-substrate impedance in response to one or more test compounds, comprising:

providing the system of claim 51;

introducing cells into one or more wells of said system that comprise electrode arrays;

adding at least one test compound to said one or more wells; and

monitoring cell-substrate impedance of said one or more wells before and after adding said at least one test compound.

Claim 76 (original) The method of claim 75, wherein impedance is monitored at three or more time points.

Claim 77 (original) The method of claim 75, further comprising analyzing impedance values before and after adding said at least one test compound.

Claim 78 (original) The method of claim 75, further comprising introducing cells to at least one well to which a test compound is not added to create a control well.

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Claim 79 (original) The method of claim 77, wherein said analyzing comprises calculating a cell index.

Claim 80 (original) The method of claim 77, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a compound, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

Claim 81 (original) The method of claim 79, wherein said cell index is used as an indicator of cytotoxicity.

Claims 82-100 (cancelled)

Claim 101 (original) A method for monitoring changes in cell-substrate impedance in response to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system, wherein each of said one or more wells comprises an electrode array;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance of at least one of said one or more wells of said system.

Claim 102 (original) The method of claim 101, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

Claim 103 (original) The method of claim 102, wherein said cells are mast cells.

Claims 104-159 (canceled)

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Claim 160 (new) The system of claim 51, wherein said electrode array is electrically connected to two connection pads.

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REMARKS / ARGUMENTS

Applicant adds new Claim 160. Thus, Claims 51-60, 72-81,101-103 and 160 remain pending upon entry of this Response to Office Action.

Support for Amendments

Applicants add new dependent Claim 160. Support for the new dependent claim may be found throughout the specification and drawings as originally filed. New Claim 160 adds that the electrode array can be in contact with two connection pads. Support for this amendment may be found on page 26, lines 25, "Each of two electrode structures of an electrode array is connected to a separate connection pad that is located at the edge of the substrate."

Response to Provisional Double Patenting Rejection

The Examiner has provisionally rejected Claims 51-60, 72-81 and 101-103 as being unpatentable over Claims 118-131, 166, 179-183, 200-202, 277, 278, 281-286 of copending U.S. Patent Application No. 10/705,447 (the '447 application) in view of Pancrazio et al (Sensors and Actuators B53 (1998) 179-185).

The Examiner states that while the '447 application is silent as to the electrodes being addressable and uniform electrode resistance, Pancrazio et al disclose a microelectronic device for monitoring cell impedance using an array of microelectrodes, which are individually addressable and have substantially uniform electrical resistance. Thus, the Examiner alleges it would have been obvious to one skilled in the art to provide the system of the '447 application with individually addressable electrodes with substantially uniform electrical resistance in view of the known practice.

Pancrazio et al. does not disclose an array with approximate uniform electrode resistance. Uniform electrode resistance and its significance are discussed throughout the

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specification of the present application as originally filed. For example, referring to page 27, lines 4-8 of the present application,

> "Each electrode array of the device has an approximately uniform electrode resistance distribution across the entire array. "[Uniform electrode resistance means] that when a measurement voltage is applied across the electrode structures of the array, the electrode resistance at any given location of the array is approximately equal to the electrode resistance at any other location on the array."

Providing an array with approximate uniform resistance is one important aspect of the present application as filed. Approximate uniform electrode resistance would ensure the electrical field applied to an individual cell is consistent across the array and therefore the positioning of the cell is not crucial. For example, referring to page 27, lines 19-27,

> "[Cells,] no matter where they land and attach to the electrode surfaces, to contribute similarly to the total impedance change measured for the electrode array. Thus, it is desirable to have similar electric field strengths at any two locations within any given array of the device when a measurement voltage is applied to the electrode array. At any given location of the array, the field strength is related to the potential difference between the nearest point on a first electrode structure of the array and the nearest point on a second electrode structure of the array. It is therefore desirable to have similar electric potential drops across the electrode elements and across the electrode buses of a given array. Based on this requirement, it is preferred to have an approximately uniform electrode resistance distribution across the whole array….. "

The electrode resistance at a location in an electrode array is described in page 27, line 27 to page 28, line 3,

> "[The] electrode resistance at a location of interest is equal to the sum of the electrode resistance between the nearest point on a first electrode structure (that is the point on the first electrode structure nearest the location of interest) and a first connection pad connected to the first electrode structure and the electrode resistance between the nearest point on a second electrode structure and a second connection pad[.]"

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A discussion regarding the construction of an appropriate array is provided on page 28, lines 5-9 and 13-17 of the present application,

> "[Uniform electrode resistance distribution] can be achieved, for example by having electrode structures and electrode buses of particular spacing and dimensions (lengths, widths, thicknesses and geometrical shapes) such that the resistance at any single location of the array is approximately equal to the resistance at any single other location on the array . . . Thus, in these preferred embodiments, an array is designed such that the lengths and geometrical shapes of electrode elements or structures, the lengths and geometrical shapes of electrode traces, and the lengths and geometrical shapes of buses allow for approximately uniform electrode resistance distribution across the array."

Furthermore, specific examples of electrode arrays that provide approximate uniform electrode resistance are also provided in the instance patent application. For example, page 29, line 29 to page 30, line 2 provides,

> "In Figure 1C, the electrodes or electrode elements are circle-on-a-line configuration with diameter of the circle being about 90 microns, the line width about 30 microns and the gaps between the lines about 80 microns. The inner diameter of the electrode bus is about 5.75 mm and the outer diameter for the electrode bus is about 6.070 mm."

Referring to the above provided discussion regarding uniform electrode resistance, Pancrazio et al. do not disclose an array with approximate uniform electrode resistance. More specifically, Pancrazio et al. demonstrate an electrode array including electrodes and interconnecting traces having a variety of lengths and spaces. The lengths of the electrodes, the interconnecting traces and the spaces between them contribute to the electrode resistance across the array. Therefore an array with a variety of lengths and spaces would yield a variety of resistances across the array and not be approximately uniform. Yet there is no discussion of how the configuration has been modified to address the variety of electrode resistances that would occur across the array resulting from the variety of displayed lengths and spaces. The lack of discussion may be due to

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the observation that the Pancrazio et al. device is designed to detect electrical pulses from a cell such as a cardiac cell and not intended to monitor impedance. Detection of an electrical pulse using specially-designed circuitry in Pancarzio et al. does not require approximate uniform resistance across the array.

Thus the '447 application and Pancrazio et al either alone or in combination do not teach each of the elements of the provisionally rejected claims. Therefore Applicants respectfully request the rejections be withdrawn.

In addition, Applicants add new Claim 160 to include the array is in contact with two connection pads. Pancrazio et al does not disclose an array in contact with two connection pads. Thus Applicants respectfully request Claim 160 be deemed allowed.

Response to Claim Rejections – 35 U.S.C. §102

The Examiner has rejected Claims 51-56, 59, 60, 72-81, 101-103 under 35 U.S.C. §102(b) as allegedly being anticipated by Pancrazio et al. (Sensors and Actuators B53 (1998) 179-185).

The Examiner alleges Figure 2 of Pancrazio et al illustrates a portable biosensor system comprising a plurality of arrays of microelectrodes (FIG.1), which are contained at the bottom of a culture dish. The Examiner alleges the microelectrodes are individually addressable and have a uniform electrode resistance.

The Pancrazio et al. does not disclose one or more multiple well cell-substrate impedance monitoring devices or one or more electrode arrays having approximate uniform resistance. Instead, Pancrazio et al. disclose a portable device for extracellular recording of excitable cells. More specifically, Pancrazio et al. records electrical signals produced by excited cells and does not monitor impedance across an array having approximate uniform electrode resistance.

In Pancrazio et al. the excitable cells produce an electrical signal pulse that is detected by the device. For example, page 184, lines 2-3 state, "After 3 - 4 days in culture, chick cardiac myocytes exhibited a spontaneously pulsating monolayer." The Pancrazio et al. device then detects the signals produced by the excitable cells. For example, page 184, lines 13-15 state, "[Delays] between spikes at neighboring

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microelectronic sites were extremely consistent beat-to-beat." Thus the Pancrazio et al device records signals initiated by the excitable cells.

In contrast, Claims 51-56, 59, 60, 72-81, 101-103 of the present invention do not require an excitable cell to produce a signal but provide a system including one or more multiple well cell-substrate impedance monitoring devices and an impedance analyzer. In the present invention, the impedance analyzer provides the voltage and does not rely on the pulsing of excited cells. For example, referring to page 21, lines 16 - 26 of the present application,

> "The method for the measurement of such impedance is achieved [by] applying a voltage between or among said electrodes at a given frequency (or multiple frequencies, or having specific voltage waveform) and monitoring the electrical current through said electrodes at the frequency (or multiple frequencies, or having specific waveform) . . .[or] applying an electric current of a single frequency component (or multiple frequencies or having specific current wave form) through said electrodes and at the frequency (or multiple frequencies, or having specific waveform) . . .[or other methods].

Therefore the present invention monitors impedance and unlike Poncrazio et al, the present invention does not require excitable cells.

Secondly, Pancrazio et al. does not disclose an electrode array having approximate uniform electrode resistance across the electrode array. The arguments set forth above in the Response to the Provisional Double Patenting Rejection are herein incorporated by reference. As a brief review uniform electrode resistance is referred to on page 27, lines 5-8 of the present application,

> "By 'uniform resistance distribution across the array' is meant that when a measurement voltage is applied across the electrode structures of the array, the electrode resistance at any given location of the array is approximately equal to the electrode resistance at any other location on the array."

The electrode resistance at a location in an electrode array is referred in page 27, line 27 to page 28, line 3

> "[The] electrode resistance at a location of interest is equal to the sum of the electrode resistance between the nearest

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> point on a first electrode structure (that is the point on the first electrode structure nearest the location of interest) and a first connection pad connected to the first electrode structure and the electrode resistance between the nearest point on a second electrode structure and a second connection pad[.]"

The Examiner alleges the Pancrazio et al device in Fig. 1 includes approximate electrode resistance across the array. However, Fig. 1 of Pancrazio et al demonstrates an electrode array having electrodes and interconnecting traces having a variety of lengths and spaces. Lengths of the electrodes and interconnecting traces and spaces between them affect the electrode resistance across the array. Therefore an array with a variety of lengths and spaces would not yield an approximate uniform electrode resistance without further modification. Yet there is no discussion of how the configuration has been modified to address the variety of electrode resistances that would occur across the array resulting from the variety of displayed lengths and spaces.

The present invention addresses the problem of variations in electrode resistance occurring across an array for cell-substrate impedance measurement and has provided a solution to create an approximate uniform resistance across the array. Referring to page 28, lines 5-9 of the present application,

> "[A uniform electrode resistance] can be achieved, for example by having electrode structures and electrode buses of particular spacing and dimensions (lengths, widths, thicknesses and geometrical shapes) such that the resistance at any single location of the array is approximately equal to the resistance at any single other location on the array."

Specific examples of electrode arrays that provide approximate uniform electrode resistance are also provided in the instance patent application. For example, page 29, line 29 to page 30, line 2 provides,

> "In Figure 1C, the electrodes or electrode elements are circle-on-a-line configuration with diameter of the circle being about 90 microns, the line width about 30 microns and the gaps between the lines about 80 microns. The inner diameter of the electrode bus is about 5.75 mm and the outer diameter for the electrode bus is about 6.070 mm."

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Thus, the Pancrazio et al. does not disclose one or more multiple well cell-substrate impedance monitoring devices or one or more electrode arrays having approximate uniform resistance. Therefore the Claims of the present invention are not anticipated by Pancrazio et al. under 35 USC §102(b) and Applicants respectfully request the Examiner's rejections be withdrawn.

Applicants add new Claim 160 to include the electrode array is in contact with two connection pads. Pancrazio does not include an array in contact with two connection pads.

Response to Claim Rejections – 35 USC §103

The Examiner has rejected Claim 51 as allegedly being obvious in view of U.S Patent 6,132,683 (the '683 patent).

The Examiner alleges Figure 1 shows the embodiment which substantially reads on the claimed invention. The Examiner alleges the '683 patent differs from the instant invention in that it includes only one well instead of a plurality claimed.

The device disclosed in the '683 patent does not render the claimed invention obvious. Claim 51 includes an electrode array having an approximately uniform electrode resistance distribution across the electrode array. In contrast, the device in the '683 patent discloses a cell potential measuring electrode assembly suitable for measuring electrical potential in a neural sample including a plurality of measurement electrodes and a plurality of reference electrodes, wherein each of the reference electrodes has an impedance smaller than each of the plurality of measurement electrodes when measured in an electrolyte. More specifically the device includes reference electrodes **10** are connected to a conductive pattern **12** and electrical contacts **7**. Referring to FIG. 3 the device also includes measurement electrodes **11** connected to the conductive pattern **12** and electrical contacts **7**.

Referring to FIGS 1 and 3, the conductive pattern **12** is shown having variable lengths between the electrical contacts **7** and the measurement electrodes **11**. Since resistance increases when the length of the conductive pattern increases, a variety of

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lengths of the conductive pattern **12** results in a variety of resistances across the array. Thus the resulting array does not have approximate uniform resistance.

As previously described in this Response to Office Action, the present invention addresses the problem of variations in electrode resistance occurring across an array for cell-substrate impedance measurement and provides a solution to create an approximate uniform resistance across the array. The arguments previously provided in the present response are herein incorporated by reference. In review, referring to page 28, lines 5-9 of the present application,

> "[A uniform electrode resistance] can be achieved, for example by having electrode structures and electrode buses of particular spacing and dimensions (lengths, widths, thicknesses and geometrical shapes) such that the resistance at any single location of the array is approximately equal to the resistance at any single other location on the array."

Specific examples of electrode arrays that provide approximate uniform electrode resistance are also provided in the instance patent application. For example, page 29, line 29 to page 30, line 2 provides,

> "In Figure 1C, the electrodes or electrode elements are circle-on-a-line configuration with diameter of the circle being about 90 microns, the line width about 30 microns and the gaps between the lines about 80 microns. The inner diameter of the electrode bus is about 5.75 mm and the outer diameter for the electrode bus is about 6.070 mm."

In summary, the '683 patent fails to render the claimed invention obvious. The '683 patent does not report an electrode array having an approximately uniform electrode resistance distribution across the electrode array. Thus the cited reference does not suggest or teach each element of the claims as filed or amended. Applicants respectfully request the rejection be withdrawn.

Applicants adds new Claim 160 to include the electrode array is in contact with two connection pads. The '683 patent does not teach or suggest this element. Thus, Applicants respectfully request Claim 160 be deemed allowable.

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Conclusion

Applicants respectfully request all rejections be withdrawn by the Examiner and respectfully submit that the claims are in condition for allowance.

Respectfully submitted,

Date: April 10, 2006



David R. Preston
Reg. No. 38,710

David R. Preston & Associates A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, CA 92130

Telephone: 858.724.0375
Facsimile: 858.724.0384

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 15

Application Number	10/987,732
Filing Date	11/12/2004
First Named Inventor	Xiao Xu
Art Unit	1744
Examiner Name	REDDING, David
Attorney Docket Number	ACE-00102.P.1-US

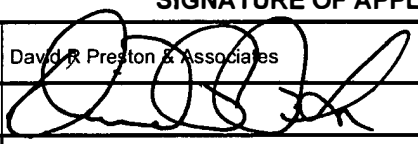
ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
- [x] Fee Attached
- [x] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

1. Postcard

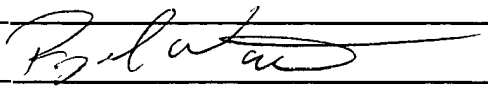
Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R Preston & Associates		
Signature			
Printed name	David R Preston		
Date	April 10, 2006	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name	Raymond Wagenknecht	Date	4-10-06

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Ex. 2008
Axion v. Agilent
IPR2024-01467

OIPE IAP22 APR 19 2006 PATENT & TRADEMARK OFFICE

PTO/SB/17 (01-06)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number

Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2006

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 225.00

Complete if Known	
Application Number	10/987,732
Filing Date	11/12/2004
First Named Inventor	Xiao Xu
Examiner Name	REDDING, David
Art Unit	1744
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R Preston

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☐ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION (All the fees below are due upon filing or may be subject to a surcharge.)

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims		Extra Claims		Fee ($)		Fee Paid ($)
_____	- 20 or HP =	_____	x	_____	=	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		Extra Claims		Fee ($)		Fee Paid ($)
_____	- 3 or HP =	_____	x	_____	=	_____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

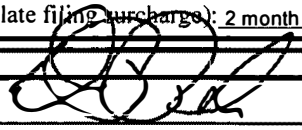
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof		Fee ($)		Fee Paid ($)
_____	- 100 =	_____	/ 50 =	_____	(round up to a whole number) x	_____	=	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 2 month extension of time 37 CFR 1.17(a)(2)	225.00

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R Preston		Date 4-10-2006

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 15

Application Number	10/987,732
Filing Date	11/12/2004
First Named Inventor	Xiao Xu
Art Unit	1744
Examiner Name	REDDING, David
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
- [x] Fee Attached
- [x] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
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SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R Preston & Associates		
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Typed or printed name	Raymond Wagenknecht	Date	4-10-06

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For FY 2006

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Filing Date	11/12/2004
First Named Inventor	Xiao Xu
Examiner Name	REDDING, David
Art Unit	1744
Attorney Docket No.	ACE-00102.P.1-US

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1. BASIC FILING, SEARCH, AND EXAMINATION FEES

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		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

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Fee Description	Fee ($)	Small Entity Fee ($)
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Total Claims		Extra Claims		Fee ($)		Fee Paid ($)
_____	- 20 or HP =	_____	x	_____	=	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		Extra Claims		Fee ($)		Fee Paid ($)
_____	- 3 or HP =	_____	x	_____	=	_____

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Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

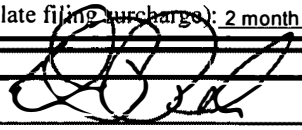
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	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
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SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R Preston		Date 4-10-2006

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Ex. 2008
Axion v. Agilent
IPR2024-01467

10/987732

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2004

Application or Docket Number: 10987732

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	23	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	23 minus 20=	* 3
INDEPENDENT CLAIMS	1 minus 3 =	* 0
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐			OR	OTHER THAN SMALL ENTITY	
RATE	FEE			RATE	FEE
BASIC FEE	395.00		OR	BASIC FEE	790.00
X$ 9=	27		OR	X$18=	
X44=			OR	X88=	
+150=			OR	+300=	
TOTAL	422		OR	TOTAL	

11.12.04

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 23	Minus	** 23	= —
Independent	* 1	Minus	*** 3	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY			OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE			RATE	ADDITIONAL FEE
X$ 9=			OR	X$18=	
X44=			OR	X88=	
+150=			OR	+300=	
TOTAL ADDIT. FEE			OR	TOTAL ADDIT. FEE	

1.19.05

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 23	Minus	** 23	= —
Independent	* 1	Minus	*** 3	= —
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		OR	RATE	ADDITIONAL FEE
X$ 9=			OR	X$18=	
X44=			OR	X88=	
+150=			OR	+300=	
TOTAL ADDIT. FEE			OR	TOTAL ADDIT. FEE	

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 24	Minus	** 23	= 1
Independent	* 1	Minus	*** 3	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		OR	RATE	ADDITIONAL FEE
X$ 9=	25		OR	X$18=	
X44=			OR	X88=	
+150=			OR	+300=	
TOTAL ADDIT. FEE	25		OR	TOTAL ADDIT. FEE	

If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

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Axion v. Agilent
IPR2024-01467

United States Patent and Trademark Office
- Sales Receipt -

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01 FC:2202 25.00 DA

Ex. 2008
Axion v. Agilent
IPR2024-01467

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
10/987,732	XU ET AL.
Examiner	**Art Unit**
David A. Redding	1744

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
=	Allowed	+	Restricted	I	Interference	O	Objected

Claim		Date								
Final	Original									
	1									
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Claim		Date								
Final	Original	11/28/05								
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Claim		Date								
Final	Original	11/28/05								
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Axion v. Agilent
IPR2024-01467

OIPE IAP59
DEC 19 2005
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 6

Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	XU
Art Unit	1744
Examiner Name	REDDING, David A.
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES *(Check all that apply)*

- [✓] Fee Transmittal Form
  - [ ] Fee Attached
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- [ ] Status Letter
- [✓] Other Enclosure(s) (please Identify below):

Remarks

1. Listing of References on Form 1449;
2. Postcard
3. Check for $180.00

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R Preston & Associates		
Signature			
Printed name	David R Preston		
Date	December 14, 2005	Reg. No.	38,710

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Signature	Hal Gibson		
Typed or printed name	Hal Gibson	Date	December 14, 2005

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OIPE IAP59 DEC 19 2005 PATENT & TRADEMARK OFFICE

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Effective on 12/8/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 180.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	XU
Examiner Name	REDDING, David A.
Art Unit	1744
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R. Preston

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims _____ - 20 or HP = _____ x _____ = _____ Extra Claims / Fee ($) / Fee Paid ($)

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims _____ - 3 or HP = _____ x _____ = _____ Extra Claims / Fee ($) / Fee Paid ($)

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims: Fee ($) _____ Fee Paid ($) _____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets _____ - 100 = _____ Extra Sheets / 50 = _____ Number of each additional 50 or fraction thereof (round up to a whole number) x _____ Fee ($) = _____ Fee Paid ($)

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Information Disclosure Statement	180.00

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R Preston		Date December 14, 2005

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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DEC 19 2005
PATENT & TRADEMARK OFFICE

Patent
Docket Number: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
XU et al.	Examiner: REDDING, David A.
Application No.: 10/987,732	Art Unit: 1744
Filed: November 12, 2004	
For: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL BASED ASSAYS	

Commissioner for Patents
Alexandria, VA 22313

INFORMATION DISCLOSURE STATEMENT

Sir:

This Information Disclosure Statement is being filed after the mailing of a First Office Action on the merits under 37 C.F.R. § 1.97(a)(3). Therefore, a fee of $180.00 is enclosed herein.

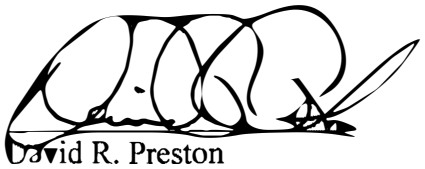
Applicants respectfully submit herewith a listing of references on the attached Form 1449. Applicant has provided a copy of each reference cited on the attached Form 1449 with Patent Application Serial Number 10/705,447.

12/20/2005 MAHMED1 00000025 10987732
01 FC:1806 180.00 OP

Please apply any charges not covered, or any credits, to Deposit Account number 501321 in the name of David R. Preston & Associates, having Customer Number 24232.

Respectfully submitted,

Date: Dec 14, 2005



David R. Preston
Reg. No. 38,710

David R. Preston & Associates, APC
12625 High Bluff Drive
Suite 205
San Diego, CA 92130
phone: 858.724.0375
facsimile: 858.724.0384

OIPE IAP59
DEC 19 2005
PATENT & TRADEMARK OFFICE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use several sheets if necessary)

Docket Number: ACE-00102.P.1-US	Application Number: 10/987,732
Applicant: XU et al.	
Filing Date: November 12, 2004	Group Art Unit: 1744

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	P1	2003/0032000	02/2003	Liu et al			
	P2	2005/0014130	01/2005	Liu et al			
	P3	USRE38,323	11/2003	Sugihara et al			
	P4	6,368,795	04/2002	Hefti			
	P5	6,376,233	04/2002	Wolf et al			
	P6	6,461,808	10/2002	Bodner et al			
	P7	6,485,905	11/2002	Hefti			
	P8	6,566,079	05/2003	Hefti			
	P9	6,573,063	03/2003	Hochman			
	P10	6,626,902	09/2003	Kucharczyk et al			
	P11	6,627,461	09/2003	Chapman et al			
	P12	6,686,193	02/2004	Maher et al			
	P13	6,716,620	04/2004	Bashir et al			

Examiner Signature		Date Considered	

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FOREIGN PATENT DOCUMENTS								
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	Translation	
							YES	NO
	F1	EP 1195432B1	09/2004	EPO				
	F2	01/25769	04/2001	PCT				
	F3	02/04943	01/2002	PCT				
	F4	02/42766	05/2002	PCT				
	F5	03/016887	02/2003	PCT				
	F6	05/005979	01/2005	PCT				

OTHER DOCUMENTS (Including Author, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D1	Bieberich and Guiseppi-Elie, Biosensors and Bioelectronics, 19:923-931 (2004)
	D2	Burnett et al., J. Biomo. Screening, 8(6):660-667 (2003)
	D3	Ciambrone et al., J. Biomo. Screening, 9(6):467-480 (2004)
	D4	Ehret et al., Med. Biol. Eng. Comput. 36:365-370 (1998)
	D5	Ehret et al.,Biosensors and Bioelectronics, 12(1):29-41 (1996)
	D6	Gutmann et al., Pharmaceutical Research, 16(3):402-407 (1999)
	D7	Hug, Assay and Drug Dev. Tech., 1(3):479-488 (2003)

Examiner Signature		Date Considered	

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OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D8	Lin and Huang, J. Micromech. Microeng., 11:542-547 (2001)
	D9	Lin et al., Min. For Chem., Bio., & Bioeng., 4:104-108 (2004)
	D10	Wegener et al., Eur. J. Physiol., 437:925-934 (1999)
	D11	Wolf et al., Biosensors and Bioelectronics, 13:501-509 (1998)
	D12	Xiao and Luong, Biotechnol. Prog., 19:1000-1005 (2003)
	D13	Xiao et al., Anal. Chem., 74:5748-5753 (2002)
	D14	Yamauchi et al., Nuc. Acids Res., 32(22):1-8 (2004)
	D15	Loffert et al., QIAGENNews, 4:15-18 (1997)

Examiner Signature		Date Considered	

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

24232 7590 12/02/2005

DAVID R PRESTON & ASSOCIATES APC
12625 HIGH BLUFF DRIVE
SUITE 205
SAN DIEGO, CA 92130

EXAMINER	
REDDING, DAVID A	
ART UNIT	**PAPER NUMBER**
1744	

DATE MAILED: 12/02/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/987,732	XU ET AL.	
	Examiner	Art Unit	
	David A. Redding	1744	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 September 2005.*

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *51-60,72-81 and 101-103* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *51-60,72-81 and 101-103* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *7/11/05.*
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

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DETAILED ACTION

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 51-60,72-81,101-103 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 118-131,166,179-183,200-202,277,278,281-286 of copending Application No. 10/705,447 in view of and Pancrazio et al. (Sensors and Actuators B53 (1998) 179-185).

The 10/705,447 application is silent as to the electrodes being addressable and uniform electrode resistance. The Pancrazio et al. article discloses a microelectronic device for monitoring cell impedance using an array of microelectrodes which are individually addressable and substantially uniform electrical resistance. Accordingly, it would have been obvious to one skilled in the art to provide the system of the '447 application with individually addressable electrodes with substantially uniform electrical resistance in view of the known practice.

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This is a provisional obviousness-type double patenting rejection.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 51-56,59,60,72-81,101-103, are rejected under 35 U.S.C. 102(b) as being anticipated by Pancrazio et al. (Sensors and Actuators B 53 (1998) 179-185).

Figure 2 illustrates a portable biosensor system comprising a plurality of arrays of microelectrodes (figure 1) which are contained at the bottom of a culture dish. The microelectrodes are individually addressed and have a uniform electrical resistance (see entire article).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

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2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claim 51 rejected under 35 U.S.C. 103 as being obvious in view of USP 6,132,683 ('683).

Figure 1 shows the embodiment which substantially reads on the claimed invention. The patent differs from the claimed invention in that it includes only one well (6) instead of a plurality claimed.

In re Harza, 274 F.2d 669, 124 USPQ 378 (CCPA 1960) the court held that mere duplication of parts has no patentable significance unless a new and unexpected result is produced.). Accordingly, the plurality of wells is considered to be an obvious duplication of the wells in the '683 patent.

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to David A. Redding whose telephone number is 571-272-1276. The examiner can normally be reached on Mon.-Fri. 6:00 - 3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John Kim can be reached on 571-272-9178. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



David A Redding
Primary Examiner
Art Unit 1744

DAR

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JUL 11 2005
PATENT & TRADEMARK OFFICE

10/705,447

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use several sheets if necessary)

Docket Number: ACE-00107.P.1-US	Application Number: ~~11/055,639~~ 10/989,732
Applicant: Xiao Xu	
Filing Date: February 9, 2005	Group Art Unit: 2818

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
DAR	P1	2002/0032531	03/2002	Mansky et al			
	P2	2002/0076690	06/2002	Miles et al			
	P3	2002/0086280	07/2002	Lynes et al			
	P4	2002/0110847	08/2002	Baumann et al			
	P5	2002/0150886	10/2002	Miles et al			
	P6	2,656,508	10/1953	Coulter			
	P7	3,259,842	07/1966	Coulter et al			
	P8	3,743,581	07/1973	Cady et al			
	P9	3,890,201	06/1975	Cady			
	P10	4,072,578	02/1978	Cady et al			
	P11	4,225,410	09/1980	Pace			
	P12	4,686,190	08/1987	Cramer et al			
	P13	4,920,047	04/1990	Giaever et al			
	P14	5,134,070	07/1992	Casnig			
	P15	5,187,096	02/1993	Giaever et al			
	P16	5,218,312	06/1993	Moro			
	P17	5,278,048	01/1994	Parce et al			
DAR	P18	5,284,753	02/1994	Goodwin			

Examiner Signature	Daniel Ruddy	Date Considered	11/28/05

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	P19	5,563,067	10/1996	Sugihara et al			
	P20	5,626,734	05/1997	Docoslis et al			
	P21	5,643,742	07/1997	Mālin et al			
	P22	5,801,055	09/1998	Henderson			
	P23	5,810,725	10/1998	Sugihara et al			
	P24	5,851,489	12/1998	Wolf et al			
	P25	5,981,268	11/1999	Kovacs et al			
	P26	6,051,422	04/2000	Kovacs et al			
	P27	6,132,683	10/2000	Sugihara et al			
	P28	6,169,394	01/2001	Frazier et al			
	P29	6,232,062	05/2001	Kayyem et al			
	P30	6,235,520	05/2001	Malin et al			
	P31	6,280,586	08/2001	Wolf et al			
	P32	6,288,527	09/2001	Sugihara et al			
	P33	6,368,851	04/2002	Baumann et al			
	P34	6,376,233	04/2002	Wolf et al			
	P35	6,448,030	09/2002	Rust et al			
	P36	6,448,794	09/2002	Cheng et al			
	P37	6,472,144	10/2002	Malin et al			
	P38						

Examiner Signature	Daniel Ruddy	Date Considered	11/28/05

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FOREIGN PATENT DOCUMENTS								
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	Translation	
							YES	NO
	F1	96/01836	01/1996	PCT				
	F2	99/66329	12/1999	PCT				
	F3	00/71669	11/2000	PCT				
	F4	01/038873	05/2001	PCT				
	F5	02/42766	05/2002	PCT				
	F6							

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D1	Aravanis et al. A genetically engineered cell-based biosensor for functional classification of agents. Biosensors & Bioelectronics 16:571-577 (2001)
	D2	Baumann et al. Microelectronic sensor system for microphysiological application on living cells. Sensors & Accuators B55:77-89 (1999)
	D3	Becker et al, Separation of human breast cancer cells from blood by differential dielectric affinity. Cell Biology. 92:960-964 (1995)
	D4	Berens et al, The role of extracellular matrix in human astrocytoma migration and proliferation studied in a microliter scale assay. Clin. Exp. Metastasis 12:405-415 (1994)
	D5	Bergveld, A critical evaluation of direct electrical protein detection methods, Biosensors& Bioelectronics. 6:55-72 (1991)
	D6	Burns et al, Neutrophil Transendothelial Migration Is Independent of Tight Junctions and Occurs Preferentially at Tricellular Corners. Journal of Immunology 2893-2903 (1997)

Examiner Signature	Daniel Rudy	Date Considered	11/28/05

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Axion v. Agilent
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OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D7	Duan et al, Separation-Free Sandwich Enzyme Immnoassays Using Microporous Gold Electrodes and Self-Assembled Monolayer/Immobilized Capture Antibodies, Anal. Chem. 66:1369-1377 (1994)
	D8	Connolly et al., An extracellular microelectrode array for monitoring electrogenic cells in culture Biosensors & Bioelectronics 5: 223-234 (1990)
	D9	Ehret et al, Monitoring of cellular behaviour by impedance measurements on interdigitated electrode structures. Biosensors and Bioelectronics 12(1):29-41 (1997)
	D10	Ehret et al, On-line control of cellular adhesion with impedance measurements using interdigitated electrode structures, Meidcal & Biolgical Engineering and Computing 36:365-370
	D11	Falk et al, A 48-well Micro Chemotaxis Assembly for Rapid and Accurate Measruement of Leukocyte Migration. J Immunol. Meth. 33:239-247 (1980)
	D12	Fuhr et al, Positioning and Manipulatin of Cells and Microparticles Using Miniaturized Electric Field Traps and Travelling Waves. Sensors and Materials 7(2):131-146 (1995)
	D13	Gaiever et al, Monitoring fibroblast behavior in tissue culture with an applied electric field. Proc. Natl. Acad. Sci 81:3761-3764 (1984)
	D14	Giaever et al, Micromotion of mammalian cells measured electrically. Proc. Natl. Acad. USA 88: 7896-7900 (1991)
	D15	Hadjout et al., Automated Real-Time Measurement of Chemotactic Cell Motility BioTechniques 31: 1130-1138 (2001)
	D16	Henning et al, Approach to a multiparametric sensor-chip-based tumor chemosensitivity assay, Anti-Cancer Drugs 12:21-32 (2001)
	D17	Hidalgo et al, Characterization of the Human Colon Carcinoma Cell Line (Caco-2) as a Model System for Intestinal Epithelial Permeability. Gastroenterology 96:736-749 (1989)
	D18	Huang et al., Dielectrophoretic Cell Separation and Gene Expression Profiling on Microelectronic Chip Arrays. Anal. Chem. 74:3362-3371 (2002)

Examiner Signature	Daniel Ruddy	Date Considered	11/28/05

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D19	Keese et al, Real-time impedance assay to follow the invasive activities of metastatic cells in culture. Biotechniques 33:842-850 (2002)
	D20	Kleinmann et al, Basement Membrane Complexes with Biological Activity. Biochemistry. 26:312-318 (1986)
	D21	Kowolenko et al., Measurement of macrophage adherence and spreading with weak electric fields. Journal of Immunological Methods 127: 71-77 (1990)
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Examiner Signature		Date Considered	11/28/05

Notice of References Cited	Application/Control No. 10/987,732	Applicant(s)/Patent Under Reexamination XU ET AL.	
	Examiner David A. Redding	Art Unit 1744	Page 1 of 1

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,132,683	10-2000	Sugihara et al.	422/82.01
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
10/987,732	XU ET AL.
Examiner	**Art Unit**
David A. Redding	1744

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
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Claim		Date								
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Search Notes

Application/Control No.	Applicant(s)/Patent under Reexamination	
10/987,732	XU ET AL.	
Examiner	**Art Unit**	
David A. Redding	1744	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
UPDATED	11/28/2005	DAR

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	614	Stenger.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:24
L2	30	1 and (electrode)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 14:24
L3	9	2 and (array or arrays or microelectrode)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 14:26
L4	17	borkholder.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:41
L5	12	4 and (address or addressed or addressable)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:37
L6	10	5 and (uniform)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:36
L7	10	5 and plurality	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:38
L8	15	4 and (array or arrayed or arrays)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:45
L9	567	ehret.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:45
L10	25	9 and (electrodes or microelectrodes)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:50
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L12	6	10 and (array or arrayed or arrays)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 14:55

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L19	7	16 or 18	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 15:01
L20	6	("6132683").URPN.	USPAT	OR	OFF	2005/11/28 15:09
L21	6	("6132683").URPN.	USPAT	OR	OFF	2005/11/28 15:17
L22	1	("6376233").PN.	US-PGPUB; USPAT	OR	OFF	2005/11/28 15:17
L23	1	22 and (impedence or impedance)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 15:18
L24	1	23 and (array or arrays or arrayed)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 15:18
L25	0	24 and (uniform)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 15:18
L26	0	24 and (board)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 15:18

L27	0	24 and (connector)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 15:19
L28	1	24 and (circuit)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/11/28 15:29
L29	82002	(impedance) and (electrode or electrodes or microelectrodes)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 15:30
L30	264	29 and (uniform near3 resistance)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 15:30
L31	4	30 and ("435"/$.ccls.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 15:31
L32	19	30 and ("204"/$.ccls.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 15:31
L33	23	31 or 32	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/11/28 15:31

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Patent
Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
XU et al	)	Examiner: Davis Redding
	)	
Aplication Number: 10/987,732	)	
	)	Group Art Unit: 1744
Filed: November 12, 2004	)	
	)	
For: A REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATIONS FOR CELL BASED ASSAYS	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO OFFICE ACTION

Sir:

Applicants are responding to the Office Action mailed June 15, 2005. Applicants are responding within four (4) months from the mailing date of the Office Action. Applicants respectfully request a one (1) month extension of time to file a reply and enclose the fee of 60.00 as indicated under 37 CFR §1.17(a)(1).

Amendments to the claims begin on page 2 of this paper.

Remarks / Arguments begin on page 6 of this paper.

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Amendments to the Claims

Applicants amend claims 51, 53, and 55-58 and cancel claims 82-100 and 104. Thus claims 51-60, 72-81 and 101-103 are pending upon entry of this amendment.

Listing of Claims

Claims 1-50 (cancelled)

Claim 51 (currently amended) A system for monitoring cell-substrate impedance, comprising:

a) one or more multiple well cell-substrate impedance monitoring devices, wherein at least two of the multiple wells of said device comprise an electrode array at the bottom of the well, wherein said electrode array is individually addressed, further wherein said device can be used to measure differences in impedance values that relate to cell behavior;

b) an impedance analyzer;

c) a device station comprising electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer; and

d) a software program that can said control device station and record and analyze data obtained from said impedance analyzer[.];

wherein said electrode array has an approximately uniform electrode resistance distribution across said electrode array.

Claim 52 (original) The system of claim 51, wherein said device station comprises electronic switches that can switch on and off connections of each of said two or more electrode arrays to said impedance analyzer.

Claim 53 (currently amended) The ~~device station~~ system of claim 51, wherein said software program can direct said device station to measure impedance values at

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regular or irregular time intervals from one or more of said two or more electrode arrays.

Claim 54 (original) The system of claim 51, wherein said impedance analyzer can measure impedance at one or more frequencies.

Claim 55 (currently amended) The ~~device station~~ system of claim 51, wherein said software program can store said data.

Claim 56 (currently amended) The ~~device station~~ system of claim 51 wherein said system can display said data.

Claim 57 (currently amended) The ~~device station~~ system of claim 51, wherein said software can calculate a cell index for one or more time points for one or more wells of said multi-well plate based on impedance values measured from said one or more electrode arrays localized to said one or more wells.

Claim 58 (currently amended) The ~~device station~~ system of claim 56 wherein said system can display said cell index for said one or more time points data for said one or more wells.

Claim 59 (original) The system of claim 51, wherein said software is programmable to direct impedance monitoring of any of said two or more electrode arrays at chosen time intervals.

Claim 60 (original) The system of claim 55, wherein said software allows entry and display of experimental parameters.

Claims 61-71 (cancelled)

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Claim 72 (original) A method of performing an assay that monitors cell-substrate impedance, comprising:

providing the system of claim 51;

introducing cells into at least one well of said system;

monitoring cell-substrate impedance of said at least one well.

Claim 73 (original) The method of claim 72, wherein said monitoring comprises analyzing impedance changes at three or more time points.

Claim 74 (original) The method of claim 73, wherein at least three of said three or more time points are spaced at regular intervals.

Claim 75 (original) A method of performing an assay that monitors cell-substrate impedance in response to one or more test compounds, comprising:

providing the system of claim 51;

introducing cells into one or more wells of said system that comprise electrode arrays;

adding at least one test compound to said one or more wells; and

monitoring cell-substrate impedance of said one or more wells before and after adding said at least one test compound.

Claim 76 (original) The method of claim 75, wherein impedance is monitored at three or more time points.

Claim 77 (original) The method of claim 75, further comprising analyzing impedance values before and after adding said at least one test compound.

Claim 78 (original) The method of claim 75, further comprising introducing cells to at least one well to which a test compound is not added to create a control well.

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Claim 79 (original) The method of claim 77, wherein said analyzing comprises calculating a cell index.

Claim 80 (original) The method of claim 77, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a comound, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

Claim 81 (original) The method of claim 79, wherein said cell index is used as an indicator of cytotoxicity.

Claims 82-100 (cancelled)

Claim 101 (original) A method for monitoring changes in cell-substrate impedance in response to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system, wherein each of said one or more wells comprises an electrode array;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance of at least one of said one or more wells of said system.

Claim 102 (original) The method of claim 101, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

Claim 103 (original) The method of claim 102, wherein said cells are mast cells.

Claims 104-159 (canceled)

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REMARKS / ARGUMENTS

Applicant amends claim 51, 53, and 55-58 and cancels claims 82-100 and 104. Thus, claims 51-60, 72-81, and 101-103 are pending upon entry of this amendment.

Support for Claim Amendments

Independent claim 51 is amended to recite, "wherein said electrode array has an approximately uniform electrode resistance distribution across said electrode array." Support for the present amendment may be found throughout the application as originally filed. As a non-limiting example, Page 27, lines 4-5 of the specification states, "Each electrode array of the device has an approximately uniform electrode resistance distribution across the entire array."

Dependant claims 53 and 55-58 were amended to correct a typographical error.

Response to Examiner's Arguments

The Examiner notes the listing of references in the specification is not a proper information disclosure statement (IDS).

Applicants provided a proper IDS on July 8, 2005. Thus Applicant requests consideration of the submitted IDS and requests this objection be withdrawn.

The Examiner has rejected Claims 51-60, 72-81 and 101-103 under the judicially created doctrine of obvious type double patenting as allegedly being unpatentable over claims 118-131, 166, 179-183, 200-202, 277, 278, 281-286 of copending U.S. Application No. 10/705,447 (the '447 Application) in view of what is known in the art.

Claims 118-131, 166, 179-183, 200-202, 277, 278, 281-286 of the '447 Application are not pending. However, to expedite allowance of the present application, Applicant has amended independent claim 51 to newly recite, "wherein said electrode array has an approximately uniform electrode resistance distribution across said electrode

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array." The claims of the '447 Application do not recite the newly provided recitation. Thus, Applicants respectfully request this rejection with withdrawn.

Applicants respectfully submit that the claims are in condition for allowance.

Respectfully submitted,

Date: Sep 16, 2005

David R. Preston
Reg. No. 38,710

David R. Preston & Associates A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, CA 92130

Telephone: 858.724.0375
Facsimile: 858.724.0384

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 7

Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Art Unit	1744
Examiner Name	Davis Redding
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES *(Check all that apply)*

- [✓] Fee Transmittal Form
  - [✓] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [✓] Other Enclosure(s) (please Identify below):
  1. Postcard
  2. Check

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R Preston & Associates		
Signature			
Printed name	David R Preson		
Date	9-16-05	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name	Raymond Wagenknecht	Date	9/16/05

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 60.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12 2004
First Named Inventor	Xiao Xu
Examiner Name	Davis Redding
Art Unit	1744
Attorney Docket No.	ART-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: _____

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims		Extra Claims		Fee ($)		Fee Paid ($)
_____	- 20 or HP =	_____	x	_____	=	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		Extra Claims		Fee ($)		Fee Paid ($)
_____	- 3 or HP =	_____	x	_____	=	_____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof		Fee ($)		Fee Paid ($)
_____	- 100 =	_____	/ 50 =	_____	(round **up** to a whole number) x	_____	=	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): small entity 1 month extension of time	60.00

SUBMITTED BY

Signature	[signature]	Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R Preston		Date 9-16-05

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

09/20/2005 RFEKADU1 00000021 10987732 60.00 OP

Ex. 2008
Axion v. Agilent
IPR2024-01467

10/987732

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2004

Application or Docket Number: 10987732

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	23	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	23 minus 20=	* 3
INDEPENDENT CLAIMS	1 minus 3 =	* 0
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	395.00	OR	BASIC FEE	790.00
X$ 9=	27	OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL	422	OR	TOTAL	

11.1.04

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 23	Minus	** 23	= —
Independent	* 1	Minus	*** 3	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

9.19.05

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 23	Minus	** 23	= —
Independent	* 1	Minus	*** 3	= —
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
10/984,132	
Examiner	Art Unit

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

Claim		Date								
Final	Original									
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IPR2024-01467

OIPE JUL 11 2005 PATENT & TRADEMARK OFFICE

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	9

Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Art Unit	1745
Examiner Name	David A. Redding
Attorney Docket Number	ACE-00102.P.1-US

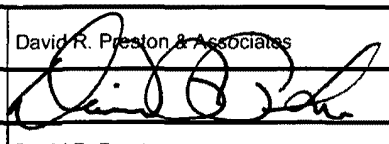
ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
  - [x] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [x] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Remarks

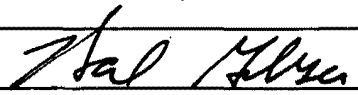
1) Listing of references on Form 1449;
2) Postcard

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R. Preston & Associates		
Signature			
Printed name	David R. Preston		
Date	July 8, 2005	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name	Hal Gibson	Date	July 8 '05

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Axion v. Agilent
IPR2024-01467

OIPE JUL 11 2005 PATENT & TRADEMARK OFFICE

PTO/SB/17 (12-04v2)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 180.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Examiner Name	David A. REdding
Art Unit	1745
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R. Preston

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims _____ - 20 or HP = _____ x Fee ($) _____ = Fee Paid ($) _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims _____ - 3 or HP = _____ x Fee ($) _____ = Fee Paid ($) _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims Fee ($) _____ Fee Paid ($) _____

3. APPLICATION SIZE FEE

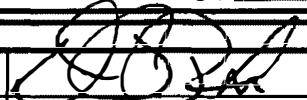
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets _____ - 100 = Extra Sheets _____ / 50 = Number of each additional 50 or fraction thereof _____ (round **up** to a whole number) x Fee ($) _____ = Fee Paid ($) _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Information Disclosure Statement	180.00

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R. Preston		Date Jul 8, 2005

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Axion v. Agilent
IPR2024-01467



Patent
Docket Number: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Xu et al.	Examiner: David A. Redding
Application No.: 10/987,732	Art Unit: 1745
Filed: November 12, 2004	
For: REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS	

Commissioner for Patents
Alexandria, VA 22313

INFORMATION DISCLOSURE STATEMENT

Sir:

Applicant respectfully submits this information disclosure statement for the above referenced U.S. Patent Application. This statement is filed after the First Office Action on the merits, and a fee of $180.00 is enclosed herein.

Applicants respectfully submit herewith a listing of references on the attached Form 1449. Applicant has provided a copy of each reference cited on the attached Form 1449 with Patent Application Serial Number 10/705,447.

07/13/2005 MAHMED1 00000007 10987732
01 FC:1806 180.00 OP

Ex. 2008
Axion v. Agilent
IPR2024-01467

Please apply any charges not covered, or any credits, to Deposit Account number 501321 in the name of David R. Preston & Associates, having Customer Number 24232.

Respectfully submitted,

Date: July 8, 2005

David R. Preston
Reg. No. 38,710

David R. Preston & Associates, APC
12625 High Bluff Drive
Suite 205
San Diego, CA 92130
phone: 858.724.0375
facsimile: 858.724.0384

OIPE
JUL 11 2005
PATENT & TRADEMARK OFFICE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Docket Number: ACE-00107.P.1-US	Application Number: 11/055,639
	Applicant: Xiao Xu	
	Filing Date: February 9, 2005	Group Art Unit: 2818

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	P1	2002/0032531	03/2002	Mansky et al			
	P2	2002/0076690	06/2002	Miles et al			
	P3	2002/0086280	07/2002	Lynes et al			
	P4	2002/0110847	08/2002	Baumann et al			
	P5	2002/0150886	10/2002	Miles et al			
	P6	2,656,508	10/1953	Coulter			
	P7	3,259,842	07/1966	Coulter et al			
	P8	3,743,581	07/1973	Cady et al			
	P9	3,890,201	06/1975	Cady			
	P10	4,072,578	02/1978	Cady et al			
	P11	4,225,410	09/1980	Pace			
	P12	4,686,190	08/1987	Cramer et al			
	P13	4,920,047	04/1990	Giaever et al			
	P14	5,134,070	07/1992	Casnig			
	P15	5,187,096	02/1993	Giaever et al			
	P16	5,218,312	06/1993	Moro			
	P17	5,278,048	01/1994	Parce et al			
	P18	5,284,753	02/1994	Goodwin			

Examiner Signature		Date Considered	

Ex. 2008
Axion v. Agilent
IPR2024-01467

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	P19	5,563,067	10/1996	Sugihara et al			
	P20	5,626,734	05/1997	Docoslis et al			
	P21	5,643,742	07/1997	Malin et al			
	P22	5,801,055	09/1998	Henderson			
	P23	5,810,725	10/1998	Sugihara et al			
	P24	5,851,489	12/1998	Wolf et al			
	P25	5,981,268	11/1999	Kovacs et al			
	P26	6,051,422	04/2000	Kovacs et al			
	P27	6,132,683	10/2000	Sugihara et al			
	P28	6,169,394	01/2001	Frazier et al			
	P29	6,232,062	05/2001	Kayyem et al			
	P30	6,235,520	05/2001	Malin et al			
	P31	6,280,586	08/2001	Wolf et al			
	P32	6,288,527	09/2001	Sugihara et al			
	P33	6,368,851	04/2002	Baumann et al			
	P34	6,376,233	04/2002	Wolf et al			
	P35	6,448,030	09/2002	Rust et al			
	P36	6,448,794	09/2002	Cheng et al			
	P37	6,472,144	10/2002	Malin et al			
	P38						

Examiner Signature		Date Considered	

Ex. 2008
Axion v. Agilent
IPR2024-01467

FOREIGN PATENT DOCUMENTS								
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	Translation	
							YES	NO
	F1	96/01836	01/1996	PCT				
	F2	99/66329	12/1999	PCT				
	F3	00/71669	11/2000	PCT				
	F4	01/038873	05/2001	PCT				
	F5	02/42766	05/2002	PCT				
	F6							

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D1	Aravanis et al. A genetically engineered cell-based biosensor for functional classification of agents. Biosensors & Bioelectronics 16:571-577 (2001)
	D2	Baumann et al. Microelectronic sensor system for microphysiological application on living cells. Sensors & Accuators B55:77-89 (1999)
	D3	Becker et al, Separation of human breast cancer cells from blood by differential dielectric affinity. Cell Biology. 92:960-964 (1995)
	D4	Berens et al, The role of extracellular matrix in human astrocytoma migration and proliferation studied in a microliter scale assay. Clin. Exp. Metastasis 12:405-415 (1994)
	D5	Bergveld, A critical evaluation of direct electrical protein detection methods, Biosensors& Bioelectronics. 6:55-72 (1991)
	D6	Burns et al, Neutrophil Transendothelial Migration Is Independent of Tight Junctions and Occurs Preferentially at Tricellular Corners. Journal of Immunology 2893-2903 (1997)

Examiner Signature		Date Considered	

Ex. 2008
Axion v. Agilent
IPR2024-01467

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D7	Duan et al, Separation-Free Sandwich Enzyme Immnoassays Using Microporous Gold Electrodes and Self-Assembled Monolayer/Immobilized Capture Antibodies, Anal. Chem. 66:1369-1377 (1994)
	D8	Connolly et al., An extracellular microelectrode array for monitoring electrogenic cells in culture Biosensors & Bioelectronics 5: 223-234 (1990)
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	D11	Falk et al, A 48-well Micro Chemotaxis Assembly for Rapid and Accurate Measruement of Leukocyte Migration. J Immunol. Meth. 33:239-247 (1980)
	D12	Fuhr et al, Positioning and Manipulatin of Cells and Microparticles Using Miniaturized Electric Field Traps and Travelling Waves. Sensors and Materials 7(2):131-146 (1995)
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	D15	Hadjout et al., Automated Real-Time Measurement of Chemotactic Cell Motility BioTechniques 31: 1130-1138 (2001)
	D16	Henning et al, Approach to a multiparametric sensor-chip-based tumor chemosensitivity assay, Anti-Cancer Drugs 12:21-32 (2001)
	D17	Hidalgo et al, Characterization of the Human Colon Carcinoma Cell Line (Caco-2) as a Model System for Intestinal Epithelial Permeability. Gastroenterology 96:736-749 (1989)
	D18	Huang et al., Dielectrophoretic Cell Separation and Gene Expression Profiling on Microelectronic Chip Arrays. Anal. Chem. 74:3362-3371 (2002)

Examiner Signature		Date Considered	

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Axion v. Agilent
IPR2024-01467

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
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	D19	Keese et al, Real-time impedance assay to follow the invasive activities of metastatic cells in culture. Biotechniques 33:842-850 (2002)
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	D43	Wang et al, Selective dielectrophoretic confinement of bioparticles in potential energy wells. Appl. Phys. 26:1278-1285 (1993)
	D44	Wang et al, Cell Separation by Dielectrophoretic Field-flow-fractionation. Anal. Chem. 72:832-839 (2000)

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	D46	Wang et al, Separation of Polystyrene Microbeads Using Dielectrophoretic/Graviational Field-Flow-Fractionation. Biophysical Journal 74:2689-2701 (1998)
	D47	Wang et al., Electronic Manipulation of Cells on Microchip-Based Devices. In Biochip Technology (eds.) Harwood Academic Publishers, PA U.S.A. 135-159
	D48	Warburg, Ueber die Polarisationscapacitat des Platins. Ann. Phy. 6:125-135 (1901)
	D49	Wegener et al, Electric cell-substrate impedance sensing system (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces, Experimental Cell Research, 259:158-166 (2000)
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	D56	New Products page. Science 298:2409 (2002)
	D57	Abstract: Real-Time Impedance Assay to Follow the Invasive Activities of Metastatic Cells in Culture. Biotechniques 33: 842 (2002)
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 9

Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Art Unit	1745
Examiner Name	David A. Redding
Attorney Docket Number	ACE-00102.P.1-US

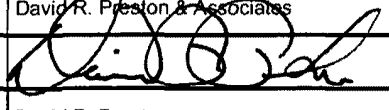
ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
- [x] Fee Attached
- [ ] Amendment/Reply
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  - [ ] Affidavits/declaration(s)
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- [ ] Express Abandonment Request
- [x] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
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- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
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- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
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- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Remarks

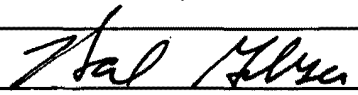
1) Listing of references on Form 1449;
2) Postcard

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R. Preston & Associates		
Signature			
Printed name	David R. Preston		
Date	July 8, 2005	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name	Hal Gibson	Date	July 8 '05

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PTO/SB/17 (12-04v2)
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Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 180.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Examiner Name	David A. REdding
Art Unit	1745
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R. Preston

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims _____ - 20 or HP = _____ x Fee ($) _____ = Fee Paid ($) _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims _____ - 3 or HP = _____ x Fee ($) _____ = Fee Paid ($) _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims Fee ($) _____ Fee Paid ($) _____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets _____ - 100 = Extra Sheets _____ / 50 = Number of each additional 50 or fraction thereof _____ (round **up** to a whole number) x Fee ($) _____ = Fee Paid ($) _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Information Disclosure Statement	180.00

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R. Preston		Date July 8, 2005

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Patent
Docket Number: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Xu et al.	Examiner: David A. Redding
Application No.: 10/987,732	Art Unit: 1745
Filed: November 12, 2004	
For: REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS	

Commissioner for Patents
Alexandria, VA 22313

INFORMATION DISCLOSURE STATEMENT

Sir:

Applicant respectfully submits this information disclosure statement for the above referenced U.S. Patent Application. This statement is filed after the First Office Action on the merits, and a fee of $180.00 is enclosed herein.

Applicants respectfully submit herewith a listing of references on the attached Form 1449. Applicant has provided a copy of each reference cited on the attached Form 1449 with Patent Application Serial Number 10/705,447.

07/13/2005 MAHMED1 00000007 10987732
01 FC:1806 180.00 OP

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Axion v. Agilent
IPR2024-01467

Please apply any charges not covered, or any credits, to Deposit Account number 501321 in the name of David R. Preston & Associates, having Customer Number 24232.

Respectfully submitted,

Date: July 8, 2005

David R. Preston
Reg. No. 38,710

David R. Preston & Associates, APC
12625 High Bluff Drive
Suite 205
San Diego, CA 92130
phone: 858.724.0375
facsimile: 858.724.0384

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JUL 11 2005
PATENT & TRADEMARK OFFICE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Docket Number: ACE-00107.P.1-US	Application Number: 11/055,639
	Applicant: Xiao Xu	
	Filing Date: February 9, 2005	Group Art Unit: 2818

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	P1	2002/0032531	03/2002	Mansky et al			
	P2	2002/0076690	06/2002	Miles et al			
	P3	2002/0086280	07/2002	Lynes et al			
	P4	2002/0110847	08/2002	Baumann et al			
	P5	2002/0150886	10/2002	Miles et al			
	P6	2,656,508	10/1953	Coulter			
	P7	3,259,842	07/1966	Coulter et al			
	P8	3,743,581	07/1973	Cady et al			
	P9	3,890,201	06/1975	Cady			
	P10	4,072,578	02/1978	Cady et al			
	P11	4,225,410	09/1980	Pace			
	P12	4,686,190	08/1987	Cramer et al			
	P13	4,920,047	04/1990	Giaever et al			
	P14	5,134,070	07/1992	Casnig			
	P15	5,187,096	02/1993	Giaever et al			
	P16	5,218,312	06/1993	Moro			
	P17	5,278,048	01/1994	Parce et al			
	P18	5,284,753	02/1994	Goodwin			

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U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	P19	5,563,067	10/1996	Sugihara et al			
	P20	5,626,734	05/1997	Docoslis et al			
	P21	5,643,742	07/1997	Malin et al			
	P22	5,801,055	09/1998	Henderson			
	P23	5,810,725	10/1998	Sugihara et al			
	P24	5,851,489	12/1998	Wolf et al			
	P25	5,981,268	11/1999	Kovacs et al			
	P26	6,051,422	04/2000	Kovacs et al			
	P27	6,132,683	10/2000	Sugihara et al			
	P28	6,169,394	01/2001	Frazier et al			
	P29	6,232,062	05/2001	Kayyem et al			
	P30	6,235,520	05/2001	Malin et al			
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	P32	6,288,527	09/2001	Sugihara et al			
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	P34	6,376,233	04/2002	Wolf et al			
	P35	6,448,030	09/2002	Rust et al			
	P36	6,448,794	09/2002	Cheng et al			
	P37	6,472,144	10/2002	Malin et al			
	P38						

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FOREIGN PATENT DOCUMENTS								
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	Translation	
							YES	NO
	F1	96/01836	01/1996	PCT				
	F2	99/66329	12/1999	PCT				
	F3	00/71669	11/2000	PCT				
	F4	01/038873	05/2001	PCT				
	F5	02/42766	05/2002	PCT				
	F6							

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
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	D2	Baumann et al. Microelectronic sensor system for microphysiological application on living cells. Sensors & Accuators B55:77-89 (1999)
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	D4	Berens et al, The role of extracellular matrix in human astrocytoma migration and proliferation studied in a microliter scale assay. Clin. Exp. Metastasis 12:405-415 (1994)
	D5	Bergveld, A critical evaluation of direct electrical protein detection methods, Biosensors& Bioelectronics. 6:55-72 (1991)
	D6	Burns et al, Neutrophil Transendothelial Migration Is Independent of Tight Junctions and Occurs Preferentially at Tricellular Corners. Journal of Immunology 2893-2903 (1997)

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	D21	Kowolenko et al., Measurement of macrophage adherence and spreading with weak electric fields. Journal of Immunological Methods 127: 71-77 (1990)
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	D23	Lo et al, Monitoring motion of confluent cells in tissue culture, Experimental Cell Research 204:102-109 (1993)
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EXAMINER INITIALS		CITATION
	D32	Pancrazio et al, Portable cell-based biosensor system for toxin detection. Sensors and Actuators B 53:179-185 (1998)
	D33	Patolsky et al, Detection of single-base DNA mutations by enzyme-amplified electronic transduction. Nature Biotechnology 19:253-257 (2001)
	D34	Pethig et al, Positive and negative dielectrophoretic collection of colloidal particles using interdigitated castellated microelectrodes. Appl. Phys. 24:881-888 (1992)
	D35	Richards et al, A Modified Microchamber Method For Chemotaxis and Chemokinesis. Immunological Communications 13(1):49-62 (1984)
	D36	Rishpon et al, An amperometric enzyme-channeling immunosensor, Biosensors & Bioelectronics, 12(3):195-204 (1997)
	D37	Simpson et al., Whole-cell biocomputing Trends in Biotechnology 19: 317-323 (2001)
	D38	Sohn et al, Capacitance cytometry: Measuring biological cells one by one. Proc. Nat. Acad. Sci. 97(20)10687-10690 (2000)
	D39	Stenger et al., Detection of physiologically active compounds using cell-based biosensors. Trends in Biotechnology 19: 304-309 (2001)
	D40	Svetlicic et al., Charge displacement by adhesion and spreading of a cell Bioelectrochemistry 53: 79-86 (2000)
	D41	Tiruppathi et al, Electrical method for detection of endothelial cell shape change in time: assessment of endothelial barrier function. Proc Natl Acad Sci USA 89:7919-7923 (1992)
	D42	Wang et al, A theoretical method of electrical field analysis for dielectrophoretic electrode arrays using Green's theorem. Appl. Phys. 1649-1660 (1996)
	D43	Wang et al, Selective dielectrophoretic confinement of bioparticles in potential energy wells. Appl. Phys. 26:1278-1285 (1993)
	D44	Wang et al, Cell Separation by Dielectrophoretic Field-flow-fractionation. Anal. Chem. 72:832-839 (2000)

Examiner Signature		Date Considered	

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Axion v. Agilent
IPR2024-01467

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)		
EXAMINER INITIALS		CITATION
	D45	Wang et al, Dielectrophoretic Manipulation of Cells with Spiral Electrodes. Biophysical Journal 72:1887-1899 (1997)
	D46	Wang et al, Separation of Polystyrene Microbeads Using Dielectrophoretic/Graviational Field-Flow-Fractionation. Biophysical Journal 74:2689-2701 (1998)
	D47	Wang et al., Electronic Manipulation of Cells on Microchip-Based Devices. In Biochip Technology (eds.) Harwood Academic Publishers, PA U.S.A. 135-159
	D48	Warburg, Ueber die Polarisationscapacitat des Platins. Ann. Phy. 6:125-135 (1901)
	D49	Wegener et al, Electric cell-substrate impedance sensing system (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces, Experimental Cell Research, 259:158-166 (2000)
	D50	Wolf et al, Monitoring of cellular signalling and metabolism with modular sensor-technique: The PhysioControl0Microsystem (PCM). Biosensors & Bioelectronics 13:501-509 (1998)
	D51	Xiao et al, An in-depth Analysis of Electric Cell-Substrate Impedance Sensing To Study the Attachment and Spreading of Mammalian Cells, Anal. Chem 74:1333-1339 (2002)
	D52	Yang et al, Cell Separation on Microfabricated Electrodes Using Dielectrophoretic/Gravitational Field-Flow Fractionation. Anal. Chem. 71:911-918 (1999)
	D53	http://www.neuroprobe.com/protocol/pt_96a.html
	D54	http://www.bdbiosciences.com/discovery_labware/Products/inserts/BD_Falcon_HTS_fluoroblok_inserts/individual_fluoroblok_inserts/index.html
	D55	http://www.tecan.com/migration_intro1.pdf
	D56	New Products page. Science 298:2409 (2002)
	D57	Abstract: Real-Time Impedance Assay to Follow the Invasive Activities of Metastatic Cells in Culture. Biotechniques 33: 842 (2002)
	D58	http://www.biophysics.com/pages/front.html

Examiner Signature		Date Considered	



Patent
Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Xiao Xu Yama Abassi Xiabo Wang Jiangbo Gan Application Number: 10/987,732 Filed: November 12, 2004 For: REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS	Examiner: To Be Determined Group Art Unit: 1745

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CORRECTION OF FILING RECEIPT

Sir;

Please correct the updated filing receipt for the referenced patent application as follows and as is marked on the enclosed copy of the corrected filing receipt mailed April 4, 2005.

In the "Foreign Applications" field, above please add "UNITED STATES OF AMERICA PCT/US03/22557 07/18/2003." This information was provided in the original Patent Application as well as the signed Declaration.

The correction is not due to any error on the part of Applicants, thus no fee under 37 C.F.R. 1.19(h) is deemed necessary.

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Axion v. Agilent
IPR2024-01467

Please charge any necessary additional fees or apply any credits to **Deposit Account number 501321** in the name of David R. Preston & Associates having **Customer Number 24232**.

Respectfully submitted,

Date: June 29, 2005

David R. Preston
Reg. No. 38,710

David R. Preston & Associates
12625 High Bluff Drive
Suite 205
San Diego, CA 92130

Telephone: 858.724.0375
Facsimile: 858.725.0384

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Axion v. Agilent
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UNITED STATES PATENT AND TRADEMARK OFFICE

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
10/987,732	11/12/2004	1745	487	ACE-00102.P.1-US	26	23	1

CONFIRMATION NO. 1601

UPDATED FILING RECEIPT

OC000000015648062

24232
DAVID R PRESTON & ASSOCIATES
12625 HIGH BLUFF DRIVE
SUITE 205
SAN DIEGO, CA 92130

Date Mailed: 04/04/2005

Receipt is acknowledged of this regular Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please mail to the Commissioner for Patents P.O. Box 1450 Alexandria Va 22313-1450. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Xiao Xu, San Diego, CA;
Yama Abassi, San Diego, CA;
Xiaobo Wang, San Diego, CA;
Jiangbo Gan, San Diego, CA;

Power of Attorney: The patent practitioners associated with Customer Number **24232**.

Domestic Priority data as claimed by applicant

This application is a CIP of 10/705,447 11/10/2003
and is a CIP of 10/705,615 11/10/2003
which claims benefit of 60/435,400 12/20/2002
and claims benefit of 60/469,572 05/09/2003
This application 10/987,732
claims benefit of 60/519,567 11/12/2003
and claims benefit of 60/542,927 02/09/2004
and claims benefit of 60/548,713 02/27/2004

Foreign Applications UNITED STATES OF AMERICA PCT/US03/22537 07/18/2003

UNITED STATES OF AMERICA PCT/US03/22537 07/18/2003

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BY:

If Required, Foreign Filing License Granted: 01/14/2005

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US10/987,732

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PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 4

Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	XU, Xiao
Art Unit	1745
Examiner Name	TBD
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES (Check all that apply)

- [x] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):
  1. Request for Corrected Filing Receipt
  2. Copy of Corrected Filing Receipt
  3. Postcard

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R. Preston & Associates, APC		
Signature			
Printed name	David R. Preston		
Date	June 29, 2005	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature	Hal Gibson		
Typed or printed name	Hal Gibson	Date	June 29 '05

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PTO/SB/17 (12-04v2)
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Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 0.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	XU
Examiner Name	TBD
Art Unit	1745
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☐ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R. Preston & Assoc.

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 20 or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims Fee ($)	Multiple Dependent Claims Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____ / 50 =	_____ (round **up** to a whole number)	x _____	= _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): _____	

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 38,710	Telephone 858-724-0375
Name (Print/Type)	David R. Preston		Date 6-29-05

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P.1-US	1601

24232 7590 06/15/2005

DAVID R PRESTON & ASSOCIATES APC
12625 HIGH BLUFF DRIVE
SUITE 205
SAN DIEGO, CA 92130

EXAMINER	
REDDING, DAVID A	

ART UNIT	PAPER NUMBER
1744	

DATE MAILED: 06/15/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/987,732	XU ET AL.	
	Examiner	Art Unit	
	David A. Redding	1744	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 November 2004.*

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *51-60,72-81 and 101-103* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *51-60,72-81 and 101-103* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

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DETAILED ACTION

Information Disclosure Statement

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 51-60,72-81,101-103 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 118-131,166,179-183,200-202,277,278,281-286 of copending Application No. 10/705,447 in view of that which is known in the art.

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The 10/705,447 application is silent as to the electrodes being addressible. However, such technology is known and would have been obvious to provide selectivity to the 10/705,447 device.

This is a provisional obviousness-type double patenting rejection.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The US patents cited are considered to be generally related to the claimed invention.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David A. Redding whose telephone number is 571-272-1276. The examiner can normally be reached on Mon.-Fri. 6:00 - 3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John Kim can be reached on 571-272-9178. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

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For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David A Redding
Primary Examiner
Art Unit 1744

DAR

Ex. 2008
Axion v. Agilent
IPR2024-01467

Notice of References Cited	Application/Control No. 10/987,732	Applicant(s)/Patent Under Reexamination XU ET AL.	
	Examiner David A. Redding	Art Unit 1744	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-6,132,683	10-2000	Sugihara et al.	422/82.01
	B	US-6,376,233	04-2002	Wolf et al.	435/288.4
	C	US-6,716,620	04-2004	Bashir et al.	435/287.2
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	10/987,732	XU ET AL.	
	Examiner	Art Unit	
	David A. Redding	1744	

SEARCHED			
Class	Subclass	Date	Examiner
435	285.2	6/13/2005	DAR
	287.2		
	288.4		
	305.2		
	305.3		
204	403.01		
	403.02		

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
INVENTOR NAME SEARCH EAST	6/13/2005	DAR

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5	(("6492122") or ("6379897") or ("6132683") or ("6376233") or ("6716620")).PN.	US-PGPUB; USPAT	OR	OFF	2005/06/13 13:59
L2	4	1 and (addressed or addressable)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:00
L3	4	2 and (multple or plurality)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:07
L4	3	1 and (impedence or impedance)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:08
L5	2	4 and (addressed or addressable)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:09
L6	2	4 and (individual or individually)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:11
L7	228	435/288.4,288.5,305.2,305.3.ccls. and (electrode or electrodes)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:12
L8	50	7 and (impedence or impedance)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:12
L9	31	8 and (wells)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:15
L10	25	9 and (addressed or addressable)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/13 14:16

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P. 1-US

CONFIRMATION NO. 1601

24232
DAVID R PRESTON & ASSOCIATES
12625 HIGH BLUFF DRIVE
SUITE 205
SAN DIEGO, CA 92130

OIPE JC19
MAR 21 2005
PATENT & TRADEMARK OFFICE

FORMALITIES LETTER

OC000000014967855

Date Mailed: 01/18/2005

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is unsigned.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(e) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$65** for a Small Entity

- **$65** Late oath or declaration Surcharge.

Replies should be mailed to: Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

03/22/2005 LWONDIH1 00000025 10987732
01 FC:2051 65.00 DP

A copy of this notice MUST be returned with the reply.

Ex. 2008
Axion v. Agilent
IPR2024-01467

OIPE JC19
MAR 21 2005
PATENT & TRADEMARK OFFICE

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 8

Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Art Unit	1745
Examiner Name	to be determined
Attorney Docket Number	ACE-00102.P.1-US

ENCLOSURES *(Check all that apply)*

- [✓] Fee Transmittal Form
  - [✓] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [✓] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [✓] Other Enclosure(s) (please Identify below):

Remarks

1. Copy of Notice to File Missing Parts;
2. Declaration; and
3. Postcard

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	David R. Preston & Associates		
Signature			
Printed name	David R Preston		
Date	March 18, 2005	Reg. No.	38,710

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Signature			
Typed or printed name	Raymond Wagenknecht	Date	3/18/2005

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Ex. 2008
Axion v. Agilent
IPR2024-01467

OIPE JC75
MAR 21 2005
PATENT & TRADEMARK OFFICE

PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 65.00

Complete if Known	
Application Number	10/987,732
Filing Date	November 12, 2004
First Named Inventor	Xiao Xu
Examiner Name	to be determined
Art Unit	1745
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 501321 Deposit Account Name: David R. Preston

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 20 or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____	/ 50 = _____ (round up to a whole number)	x _____	= _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Surcharge late filing of declaration	65.00

SUBMITTED BY

Signature	[signature]	Registration No. (Attorney/Agent) 38,710	Telephone 858/724-0378
Name (Print/Type)	David R Preston		Date 3/18/05

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Ex. 2008
Axion v. Agilent
IPR2024-01467

OIPE JC19
MAR 21 2005
PATENT & TRADEMARK OFFICE

Patent
Docket Number: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
XU et al	)	Examiner: To be determined
	)	
Application No.: 10/987,732	)	Art Unit: 1745
	)	
Filed: November 12, 2004	)	
	)	
For: REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS	)	

Commissioner for Patents
Alexandria, VA 22313

RESPONSE TO NOTICE TO FILE MISSING PARTS

Sir:

Applicants are responding to the Notice to File Missing Parts of Nonprovisional Application mailed January 18, 2005, notifying Applicants that the Oath or Declaration filed with the present application was unsigned. Applicants are responding within two months from the mailing of the Notice to File Missing Parts of Nonprovisional Application. Thus no additional fee is deemed necessary.

Enclosed herewith are the signed declaration and our fee for $65.00 for the late oath or declaration surcharge.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Please apply any charges not covered, or any credits, to **Deposit Account 501321 in the name of David R. Preston & Associates having Customer No.: 24232.**

Respectfully submitted,

Date: March 18, 2005



David R. Preston
Reg. No. 38,710

David R. Preston & Associates, A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, CA 92130

Telephone: 858.724.0375
Facsimile: 858.724.0384

Ex. 2008
Axion v. Agilent
IPR2024-01467

PE JC79
MAR 21 2005
PATENT & TRADEMARK OFFICE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number:	ACE-00102.P.1-US
	First Named Inventor:	Xiao Xu
	COMPLETE IF KNOWN	
	Application Number:	10/987,732
	Filing Date:	November 12, 2004
[ ] Declaration Submitted with Initial Filing, OR	Group Art Unit:	1745
[x] Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e) required)	Examiner:	To be determined

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS

the specification and preliminary amendment of which
[ ] is attached hereto
OR
[x] was filed on November 12, 2004 as United States Application Number or PCT International Application Number 10/987,732 and was amended _____ (if applicable).
I hereby state that I have reviewed and understand the contents of the above- identified specification, including the claims, as amended by any amendment specifically referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date	Priority Not Claimed	Certified Copy Attached? YES	NO

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below:

Application Number(s)	Filing Date	[ ] Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto
60/397,749	July 20, 2002	
60/435,400	December 20, 2002	
60/469,572	May 9, 2003	
60/519,567	November 12, 2003	
60/542,927	February 9, 2004	
60/548,713	February 27, 2004	

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR. 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

U.S. Parent Application or PCT Parent Number	Parent Fling Date	Parent Patent Number (if applicable)
10/705,447	November 10, 2003	
10/705,615	November 10, 2003	
PCT/US03/22557	July 18, 2003	
PCT/US03/22537	July 18, 2003	

[ ] Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the United States Patent and Trademark Office connected herewith:

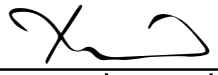
[x] Customer Number **24232** [Customer Number Bar Code Label]
OR
[ ] Registered practitioner(s) name/registration number listed below:

Name	Registration Number	Name	Registration Number
David R. Preston	38,710	Elizabeth Orr	45,937

[ ] Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: [X] Customer Number 24232 or Bar Code Label [Attached Bar Code Label] OR [ ] Correspondence address below:					
Name	David R. Preston & Associates, A.P.C.				
Address	David R. Preston				
Address	12625 High Bluff Drive, Suite 205				
City	San Diego	State	California	Zip Code	92130
Country	USA	Telephone	858.724.0375	Facsimile	858.724.0384

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR							[ ] A petition has been filed for this unsigned inventor
Given Name (first and middle, if any)						Family Name or Surname	
Xiao						Xu	
Inventor's Signature						Date	03/16/05
Residence: City	San Diego	State	California	Country	USA	Citizenship	USA
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						

[ ] Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

DECLARATION

ADDITIONAL INVENTOR(S)
SUPPLEMENTAL SHEET
PAGE _1___ OF __2___

NAME OF ADDITIONAL INVENTOR				[ ] A petition has been filed for this unsigned inventor			
Given Name (first and middle, if any)				Family Name or Surname			
Yama				Abassi			
Inventor's Signature						Date 03-11-05	
Residence: City	San Diego	State	CA	Country	USA	Citizenship	USA
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						
City	San Diego	State	CA	Zip Code	92121	Country	USA

Given Name (first and middle, if any)				Family Name or Surname			
Xiaobo				Wang			
Inventor's Signature						Date 3-11-05	
Residence: City	San Diego	State	CA	Country	USA	Citizenship	People's Republic of China
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						
City	San Diego	State	CA	Zip Code	92121	Country	USA

DECLARATION

ADDITIONAL INVENTOR(S)
SUPPLEMENTAL SHEET
PAGE 2 OF 2

NAME OF ADDITIONAL INVENTOR				[ ] A petition has been filed for this unsigned inventor			
Given Name (first and middle, if any)				Family Name or Surname			
Jiangbo				Gan			
Inventor's Signature						Date 03/01/05	
Residence: City	San Diego	State	CA	Country	USA	Citizenship	Peoples Republic of China
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						
City	San Diego	State	CA	Zip Code	92121	Country	USA

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/987,732	11/12/2004	Xiao Xu	ACE-00102.P. 1-US

CONFIRMATION NO. 1601

24232
DAVID R PRESTON & ASSOCIATES
12625 HIGH BLUFF DRIVE
SUITE 205
SAN DIEGO, CA 92130

FORMALITIES LETTER

OC000000014967855

Date Mailed: 01/18/2005

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is unsigned.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(e) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$65** for a Small Entity

- **$65** Late oath or declaration Surcharge.

Replies should be mailed to: Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

A copy of this notice MUST be returned with the reply.

Ex. 2008
Axion v. Agilent
IPR2024-01467

Syed Asaduddin

Customer Service Center
Initial Patent Examination Division (703) 308-1202

PART 3 - OFFICE COPY

Ex. 2008
Axion v. Agilent
IPR2024-01467

22713 U.S. PTO
111204

PTO/SB/05 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office. U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	ACE-00102.P.1-US
First Inventor	Xiao Xu
Title	REAL TIME ELECTRONIC CELL
Express Mail Label No.	EV 367469845 US

17510 U.S. PTO
10/987732
111204

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450**

1. ☑ Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. ☑ Applicant claims small entity status. See 37 CFR 1.27.
3. ☑ Specification [*Total Pages* 135]
   (preferred arrangement set forth below)
   - Descriptive title of the invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. ☑ Drawing(s) (*35 U.S.C. 113*) [*Total Sheets* 26]
5. Oath or Declaration [*Total Sheets* 5]
   a. ☑ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 18 completed)*
      i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☐ Application Data Sheet. See 37 CFR 1.76
7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)* ☑ Power of Attorney
11. ☐ English Translation Document *(if applicable)*
12. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
13. ☑ Preliminary Amendment
14. ☑ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. ☐ Nonpublication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☑ Other: Cover letter; Check for filing fee

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☐ Continuation ☐ Divisional ☑ Continuation-in-part (CIP) of prior application No.: 10/705,447

Prior application information: *Examiner* _____ *Art Unit:* 1632

For CONTINUATION OF DIVISIONAL APPS only; The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS

☑ Customer Number: 24232 *OR* ☐ Correspondence address below

Name	David R Preston				
Address	David Preston & Associates				
	12625 High Bluff Drive, Suite 205				
City	San Diego	*State*	CA	*Zip Code*	92130
Country	United States of America	*Telephone*	858-724-0375	*Fax*	858-724-0384

Name (Print/Type)	David R Preston	*Registration No. (Attorney/Agent)*	38,710
Signature		*Date*	Nov 12, 2004

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Ex. 2008
Axion v. Agilent
IPR2024-01467

22713 U.S. PTO 111204

PTO/SB/17 (01-03)
Approved for use through 04/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 412.00

Complete if Known	
Application Number	To be deterrnined
Filing Date	Herewith
First Named Inventor	Xiao Xu
Examiner Name	To be determined
Art Unit	To be determined
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 501321

Deposit Account Name: David R. Preston

The Commissioner is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) during the pendency of this application

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	385.00
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 385.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	Fee Paid
Total Claims	23	-20** =	3	x 9	= 27
Independent Claims	1	- 3** =	0	x 0	= 0
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 27.00

***or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($) 0.00

SUBMITTED BY				(Complete *(if applicable)*
Name (Print/Type)	David R. Preston	*Registration No. (Attorney/Agent)*	38,710	*Telephone* 858-724-0375
Signature				*Date* Nov 12, 2004

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David R. Preston & Associates, A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, California 92130

David R. Preston
Owen Smigelski‡
Mo Savari‡
Raymond Wagenknecht‡

‡ Of Counsel

"Express Mail" Mailing Label Number: EV367469845 US
Date of Deposit: November 12, 2004

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313

Re: Utility Patent Application

Entitled:	REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS
Appl. No.:	To be determined
Filed:	Herewith
Inventor:	Xiao Xu, Yama Abassi, Xiaobo Wang and Jiangbo Gan
Our Ref.:	ACE-00102.P.1-US

Sir:

The following documents are forwarded herewith for appropriate action by the United States Patent and Trademark Office:

1. Utility Patent Application Transmittal (in duplicate);

2. Fee Transmittal (in duplicate);

3. Declaration for Utility or Design Patent Application;

4. Complete U. S. Patent Application entitled:

REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS

and naming as inventor:

Xiao Xu, Yama Abassi, Xiaobo Wang and Jiangbo Gan

the utility application comprising:

1. Total pages of application: [161]
2. Pages of Disclosure: [110];
3. Sheets of Drawings: [26];
4. Pages of Title Page: [1];
5. Pages of Abstract: [1]; and
6. Pages of Claims: [24];

5. Preliminary Amendment;

6. One Return Post Card; and

7. Our Check number 3536 for $ 412.00 to cover the Application fee (small entity status).

It is respectfully requested that the attached postcard be stamped with the filing date and unofficial application number and returned as soon as possible.

Please apply any charges not covered, or any credits, to Deposit Account number 501321 in the name of David R. Preston & Associates, APC

The following attorneys are attorneys of record for prosecuting this application and transacting all business in the USPTO connected therewith:

David R. Preston, Esquire	Elizabeth Orr
Registration No. 38,710	Registration No. 45,937

Additional Attorneys or Agents of Record are those listed on Customer No. 24232

David R. Preston & Associates, A.P.C.
DRPNA®
www.drpna.com
phone: 858.724.0375
facsimile: 858.724.0384

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Please send all correspondence and direct all telephone calls to:

David R. Preston
David R. Preston & Associates, APC
12625 High Bluff Drive
Suite 205
San Diego, California 92130
858.724.0375

Respectfully Submitted,
DAVID R. PRESTON & ASSOCIATES, A.P.C.



David R. Preston
Attorney for Applicant
Registration No. 38,710

PATENT APPLICATION

on

REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS

by

Xiao Xu, Yama Abassi, Xiaobo Wang, and Jiangbo Gan

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David R. Preston & Associates, A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, CA 92130
ACE-00102.P.1-US

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Real Time Electronic Cell Sensing Systems and Applications for Cell-based Assays

This application is a continuation-in-part of U.S. Patent Application Number 10/705,447 filed November 10, 2003, entitled "Impedance Based Devices and Methods for Use in Assays" (Attorney docket number ACE-101.P.1.1-US) which claims priority to U. S. Provisional Application 60/397,749, filed July 20, 2002; U. S. Provisional Application 60/435,400, filed December 20, 2002; U. S. Provisional Application 60/469,572, filed May 9, 2003; and PCT application PCT/US03/22557, filed July 18, 2003. All of the applications referred to in this paragraph are incorporated by reference herein.

This application is also a continuation-in-part of United States patent application No.10/705,615, entitled "Impedance Based Apparatuses and Methods for Analyzing Cells and Particles" (Attorney Docket No. ACE-00101.P.1.2-US), filed on November 10, 2003, which claims priority to U. S. Provisional Application 60/397,749 filed July 20, 2002; U. S. Provisional Application 60/435,400, filed December 20, 2002; U. S. Provisional Application 60/469,572, filed May 9, 2003; and PCT application PCT/US03/22537, filed July 18, 2003. All of the applications referred to in this paragraph are incorporated by reference herein.

This application also claims priority to U. S. Provisional Application 60/519,567 filed November 12, 2003; U. S. Provisional Application 60/542,927 filed February 9, 2004; and U. S. Provisional Application 60/548,713, filed February 27, 2004. All of the applications referred to in this paragraph are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Technical Field

This invention relates to the field of cell-based assays. In particular, the invention provides impedance-based devices, apparatuses and systems for analyzing cells and for conducting cell-based assays.

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Background Art

A. Electronic Analysis of Cells

Bioelectronics is a progressing interdisciplinary research field that involves the integration of biomatereials with electronic devices. Bioelectronic methods have been used for analyzing cells and assaying biological molecules and cells. In one type of application, cells are cultured on microelectrodes and cell-electrode impedance is measured and determined to monitor cellular changes.

In PCT Application No. PCT/US03/22557,entitled "IMPEDANCE BASED DEVICES AND METHODS FOR USE IN ASSAYS", filed on July 18, 2003, a device for detecting cells and/or molecules on an electrode surface is disclosed. The device detects cells and/or molecules through measurement of impedance changes resulting from the attachment or binding of cells and/or molecules to the electrode surfaces. A number of embodiments of the device is disclosed, together with the apparatuses, system for using such devices to perform certain cell based assays.

B. Allergic diseases and IgE-mediated cell activation

Allergic diseases, also commonly known as immediate hypersensitivity disorder are the most common dysfunction of the immune system afflicting 20 % of all individuals in the United States. The immediate hypersensitivity response can range anywhere from a simple rash or itchy and watery eyes to the most extreme case of anaphylaxis, where the airways are restricted to the point of asphyxiation and death. Due to the severity of the hypersensitivity responses, the lack of adequate treatment and the high percentage of the population suffering from various forms of this condition, the pharmaceutical industry has taken a keen interest in developing novel drugs to effectively treat and combat the symptoms of this disabling and potentially life threatening disorder.

The primary cells of the immune system that are involved in the allergic response are mast cells, basophils and eisonophils. Basophils and eisonophils differentiate in the bone marrow, circulate in the blood and are recruited to the sites of the inflamed tissue in the late-phase of the reactions. In contrast, mast cells are normally distributed throughout the connective tissue and are the involved in the immediate phase of immunoglobulin E

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(IgE)-mediated allergic reactions (Sharma et al. Clin Rev Allergy Immunol. 2002 Apr;22(2):119-48). The initial encounter of an individual with an allergen leads to the production of IgE, which binds to the high affinity IgE receptor (Fc(epsilon)RI) on the surface of mast cells causing sensitization of the mast cells. Subsequent encounter with the allergen leads to cross-linking of the Fc(epsilon)RI-IgE complex on the surface of mast cells and stimulation of the mast cells to release mediators of immediate hypersensitivity. The cross-linking of receptor-bound IgE on the mast cell surface triggers a sequence of intracellular events, collectively referred to as mast cell activation that culminate in the extracellular release of potent inflammatory mediators, many of which are stored in the secretory granules, including histamine. Mast cell activation can be divided into an interdependent early and late phase. The early phase of mast cell activation include phosphorylation and activation of protein tyrosine kinases and their substrates, generation of the second messengers inositol-tris phosphate and diacylglycerol, elevation of intracellular calcium levels and fusion of secretory granules with the membrane (Stassen et al. Crit Rev Immunol. 2002;22(2):115-40). The late phase of mast cell activation includes dramatic morphological changes due to remodeling of the actin cytoskeleton, gene expression leading to the synthesis and secretion of potent inflammatory cytokines and synthesis of lipid mediators that have variety of effects on blood vessels, bronchial smooth muscle and leukocytes.

Based on the various steps involved in the initiation and execution of the immediate hypersensitivity response, there are multiple potential targets for pharmaceutical intervention. The bulk of the current therapies for immediate hypersensitivity disorders such as asthma seek to alleviate the symptoms of the condition rather than directly target the underlying cause. However, current promising efforts are underway to neutralize the IgE antibody by administration of humanized monoclonal anti-IgE antibody and to achieve long term alleviation of clinical symptoms (D'Amato et al. Monaldi Arch Chest Dis. 2003 Jan-Mar;59(1):25-9). Also, the elucidation of the intrinsic signaling pathways underlying IgE-mediated mast cell activation together with the advent of combinatorial chemistry provide ample opportunity to employ small molecular inhibitors to target key proteins and enzymes involved in mast cell activation. These compounds could potentially provide novel immunomodulators for the treatment

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of immediate hypersensitivity disorder. Small molecular inhibitors of several kinases, including PKC and the tyrosine kinase Syk have provided encouraging preclinical results in rodent studies in blocking some immediate hypersensitivity responses (Seow et al. Eur J Pharmacol. 2002 May 17; 443(1-3):189-96).

An increasing number of companies are utilizing large chemical libraries to screen for potential inhibitors of signaling pathways that maybe involved in various disease states. Hence, there is an urgent need for high-throughput molecular and cellular assays to screen for potential modulators of these signaling pathways. With regards to IgE-mediated signaling, the current assays measure mediators that are released into the media after degranulation. This is accomplished by either measuring the enzymatic activity of these mediators (Rac and phosphatidylinositol 3-kinase regulate the protein kinase B in Fc epsilon RI signaling in RBL 2H3 mast cells. J Immunol. 2001 Feb 1;166(3):1627-34), using radioactive precursors (Guillermot et al. J Cell Sci. 1997 Sep;110 (Pt 18):2215-25), or by ELISA, quantifying the amount of mediators that are released (Berger et al. Allergy. 2002 Jul;57(7):592-9). These assays are single point assays or endpoint assays which measure the cumulative release of these mediators and also involve utilization of reagents and manipulation of the cells, such as fixation or lysis. The fact that these are single point assays, which utilize expensive reagents such as antibodies and cellular manipulation, does not warrant adaptability for high-throughput analysis that is required to screen large chemical libraries.

C. Anticancer drug screening and discovery

In anticancer drug development, the study of the time dependence of cytotoxic and cell proliferation inhibitory effect of a drug is an important element for gaining information to use in the development of clinical dosing strategies. In particular, time dependent IC50's are derived and different time dependent patterns for IC50's are observed (e.g., see Hassan SB, Jonsson E, Larsson R and Karlsson MO in *J. Pharmacology and Experimental Therapeutics*, 2001, Vol. 299, No. 3, pp 1140-1147; Levasseur LM, Slocum HK, Rustum YM and Greco WR, in *Cancer Research*, 1998, vol. 58, pp 5749-5761.). Typically, these studies used end-point single-measurement assays. Each time point for a dose concentration of drug or compound applied to the cultured cells required

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a separate experiment. This limits the time resolution and the number of time points of such time-dependent cytotoxicity studies. Thus, new technologies or methods that can provide higher time resolution and permit measurements on many time points are needed.

The present invention further expands the inventions disclosed in PCT Application No. PCT/US03/22557, entitled "IMPEDANCE BASED DEVICES AND METHODS FOR USE IN ASSAYS", filed on July 18, 2003 and disclosed in United States patent application No.10/705,447, entitled "IMPEDANCE BASED DEVICES AND METHODS FOR USE IN ASSAYS," Attorney Docket No. ACE-00101.P.1.1-US, filed on November 10, 2003. The invention provides a real time cell electronic sensing system for conducting cell-based assays based on measurement of cell-substrate impedance and provides the method for using such a system to perform cell-based assays. Furthermore, the present invention is aimed at addressing the limitations in current methods and technologies for assaying *IgE*-mediated signaling and cell activation.

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SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to a device for monitoring cell-substrate impedance, which device comprises: a) a nonconducting substrate; b) two or more electrode arrays fabricated on the substrate, where each of the two or more electrode arrays comprises two electrode structures; and c) at least two connection pads, each of which is located on an edge of the substrate. For each of the two or more electrode arrays of the device, the first of the two electrode structures is connected to one of the two or more connection pads, and the second of the two electrode structures is connected to another of the two or more connection pads. Each of the two electrode structures of the two or more electrode arrays comprises multiple electrode elements, and each electrode array of the device has an approximately uniform electrode resistance distribution across the entire array. The substrate of the device has a surface suitable for cell attachment or growth; where cell attachment or growth on said substrate can result in a detectable change in impedance between or among the electrode structures within each electrode array. In preferred embodiments, each electrode array on the substrate of a device of the present invention is associated with a fluid-impermeable container.

In another aspect, the present invention is directed to a cell-substrate impedance measurement system comprising: a) at least one multiple-well device monitoring cell-substrate impedance, in which at least two of the multiple wells each comprise an electrode array at the bottom of the well; b) an impedance analyzer; c) a device station capable of engaging the one or more multiple-well devices and capable of selecting and electrically connecting electrode arrays within any of the multiple wells in to the impedance analyzer; and d) a software program to control the device station and perform data acquisition and data analysis on impedance values measured by the impedance analyzer. In preferred embodiments of this aspect of the present invention, each electrode array of the multiple-well device is individually addressed.

In yet another aspect, the present invention provides a method for monitoring cell-substrate impedance using a device of the present invention. The method includes: providing a multiple array device of the present invention; connecting said multiple array device to an impedance analyzer; depositing cells on at least one of the two or more

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arrays of the device; and monitoring cell-substrate impedance on one or more arrays of the device.

In yet another aspect, the present invention provides methods for calculating a Cell Index for quantifying and comparing cell-substrate impedance.

In yet another aspect, the present invention provides methods for calculating resistance of electrical traces connecting an array of a cell-substrate monitoring device with a connection pad. Such calculations of electrical trace resistance can be used for calculating Cell Index.

In yet another aspect, the present invention provides a method for monitoring cell-substrate impedance using a cell-substrate impedance measurement system of the present invention. The method includes: providing a cell-substrate impedance measurement system of the present invention, adding cells to at least one well of the multiple-well device that comprises an electrode array, and monitoring cell-substrate impedance from one or more of the wells that comprise cells. Impedance can be monitored at regular or irregular time intervals. In preferred embodiments, cell-substrate impedance is monitored in at least two wells of a multiple-well device.

In yet another aspect, the present invention provides a method for performing real-time cell-based assays investigating the effects of one or more compound on cells, comprising: providing an above described cell-substrate impedance measurement system; introducing cells into at least one well of the system that comprises an electrode array; adding one or more compounds to one or more of the wells containing cells; and monitoring cell-substrate impedance over the electrode array of the one or more wells before and after adding the one or more compounds. Preferably, cell-substrate impedance is monitored at regular or irregular time intervals after adding one or more compounds to the one or more of the wells containing cells. The time dependent impedance change can provide information about time dependent cell status before addition of the compound and about time dependent cell status under the interaction of the compound. This information can be used to determine the effect of a compound on the cells.

In yet another aspect, the present invention provides a method for performing real-time cytotoxicity assays of at least one compound, comprising: providing an above described cell-substrate impedance measurement system; introducing cells into one or

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more wells of the system that comprise an electrode array; adding one or more compounds to the one or more wells containing cells; and monitoring cell-substrate impedance of the one or more wells before and after adding the one or more compounds, wherein the time dependent impedance change provides information about time dependent cytotoxicity of the compound or compounds. Preferably, cell-substrate impedance is monitored at regular or irregular time intervals after adding one or more compounds to the one or more of the wells containing cells. The time dependent impedance change can provide information about any potential cytotoxic effects of the compound.

In one embodiment of the above method, multiple wells with same cell types are used, wherein different concentrations of a compound are added to different wells that comprise cells. The method can provide time-dependent and concentration-dependent cytotoxic responses.

In yet another aspect, the present invention provides a method for analyzing and comparing time-dependent cytotoxic effects of a first compound and a second compound on a cell type, comprising : a) performing a real-time cytotoxicity assay on a cell type with the first compound using the method described above; b) performing a real-time cytotoxicity assay on said cell type with the second compound using the method described above; and c) comparing the time-dependent cytotoxic responses of the first compound and the second compound.

In one embodiment of this method, time-dependent cytotoxic responses are determined for a first compound at multiple dose concentrations. In another embodiment, time-dependent cytotoxic responses are determined for a second compound at multiple dose concentrations. In yet another embodiment, time-dependent cytotoxic responses are determined for both a first compound and a second compound at multiple dose concentrations.

In yet another aspect, the present invention provides methods for cytotoxicity profiling for a compound on multiple cell types, comprising: a) performing real-time cytotoxicity assays on different cell types with the compound using the method described above, and b) analyzing real-time cytotoxic responses of different cell types to the compound to provide a cytotoxicity profile of the compound.

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In yet another embodiment, the above methods are applied to perform cytotoxicity profiling of multiple compounds on multiple cell types.

In yet another aspect, the present invention is directed to method to use electronic impedance technology to assess and quantify the morphological changes that occur in cells as a result of *IgE* stimulation and *IgE* receptor cross-linking with an antigen. The method includes : providing a cell-substrate impedance measurement system of the present invention; introducing cells into one or more wells of the system, adding IgE to the one or more wells comprising cells; adding an antigen to the one or more wells comprising cells and IgE; and monitoring cell-substrate impedance from the one or more wells of the system. Impedance can be monitored before and after adding IgE, and is preferably measured before adding IgE, after adding IgE and before adding antigen, and after adding antigen. The cell-substrate impedance can be used as an indicator of a cell's response to IgE stimulation through cell morphological changes.

In yet another aspect, the present invention is directed at a method to screen for inhibitors of signaling pathways that are initiated by engagement of the IgE-Fc(epsilon)RI complex by an antigen by utilizing electronic measurement and sensing of cells. The method includes : providing a cell-substrate impedance measurement system of the present invention, introducing cells into one or more wells of the system, adding at least one test compound to at least one of the one or more wells containing cells; providing at least one control well comprising cells in the absence of test compound; adding IgE to the one or more wells comprising cells and test compound and to the one or more control wells; adding an antigen to the one or more wells comprising cells and test compound and to the one or more control wells; and monitoring cell-substrate impedance from the one or more wells of the system. Impedance can be monitored before and after adding IgE, and is preferably measured before adding IgE, after adding IgE and before adding antigen, and after adding antigen. The cell-substrate impedance can be used as an indicator of a cell's response to IgE stimulation through cell morphological changes. Comparsion of the cell-substrate impedance in one or more wells comprising test compound with one or more control wells provides an assessment of the effect of a test compound on the cells' response to IgE stimulation. In particular, inhibitors of the IgE

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response can be identified by their property of reducing the cell-impedance response of cells treated with test compound when compared with the responses of control cells.

In one embodiment of this aspect, the present invention is directed to method to use electronic impedance technology to screen for potential inhibitors of IgE binding to the high affinity Fc(epsilon)RI receptor on the surfaces of mast cells or basophils.

In another embodiment of this aspect, the present invention is directed to method of using electronic impedance technology for screening of small molecular inhibitors of key enzymes and proteins involved in the signaling pathway that ensues from engagement of the high-affinity Fc(epsilon)RI receptor of cells in the presence or absence of IgE cross-linking by the antigen.

In yet another aspect, the present invention is directed to method to use electronic impedance technology for target validation purposes of key enzymes and proteins involved in the signaling pathway leading from engagement of the high-affinity FcεRI receptor of cells in the presence or absence of *IgE* cross-linking by the antigen. The method includes : providing a cell-substrate impedance measurement system of the present invention; introducing genetically modified cells into one or more wells of the system; providing at least one control well comprising cells that are not genetically modified; adding IgE to the wells comprising genetically modified cells and to the one or more control wells, adding an antigen to the wells comprising genetically modified cells and to the one or more control wells; adding an antigen to the wells comprising genetically modified cells and to the one or more control wells; monitoring cell-substrate impedance form the wells comprising genetically modified cells and to the one or more control wells; and analyzing the impedance data to determine the effect of the genetic alteration on the response of cells to IgE stimulation.

In yet another aspect, the present invention is directed to method to use electronic impedance technology for comparing IgE-mediated responses of cells of different genetic backgrounds. The method can be used for screening and validating genetic markers such as gene expression profiles, gene splicing variants, protein expression profiles, key single nucleotide polymorphisms (SNPs) or mutations and other genetic variants that determine or influence the IgE stimulation, IgE receptor interactions,

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antigen-mediated IgE receptor cross-linking, intracellular signal transduction pathways, and degranulation.

In yet another aspect, the present invention is directed to method to use electronic impedance technology for screening, discovering, and validating chemical structures of antigens (allergens) and half antigens, which lead to IgE cross-linking specifically or nonspecifically.

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BRIEF DESCIPTION OF THE DRAWINGS

Figure 1 shows schematic drawings of one design of a cell-substrate impedance measurement device of the present invention. A) depicts the substrate having 16 electrode arrays (or 16 electrode structure units) that are arranged in a 2-row by 8-column configuration on a substrate. B) depicts a single electrode array of a device. C) shows a schematic drawing of an electrode array, illustrating the requirement of approximately uniform distribution of electrode reistance across the array.

Figure 2 shows an image of a 16x device of the present invention.

Figure 3 shows an image of a 96 x device of the present invention.

Figure 4 shows one design of a 16x device station with 6 16x devices connected to the station

Figure 5 shows one design of a 96x device station with a 96-well plate placed on the station.

Figure 6 shows different pages from real-time cell electronic sensing software. A) An experimental note page allows the recording of key information about the experiment by the experimenter, such as the goals and procedures of the experiment. (B) An experimental layout page allows the recording of cells, cell number, compound and compound concentration added into each well. (C) Test time setting page allows for the recording and control of time intervals used for performing cell-substrate impedance measurement and multiple experimental steps each having different time interval values and different length times can be setup. (D) Cell index page is a result page where the software automatically update the measured and derived cell index values for all wells that are under test after the completion of each measurement at predetermined time interval as setup by the Test time setting page. (E) Experimental data plot page allows for flexible plotting and organization of experimental data.

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Figure 7 shows real-time monitoring of proliferation of H460 cells seeded at different initial cell seeding numbers on a cell substrate monitoring system of the present invention. Cell proliferation was continuously recorded every 15 minutes for over 125 hours. The cell growth curves in the log scale show exponential cell growth or cells in the stationary phase.

Figure 8 shows real time monitoring of cell attachment and spreading of NIH3T3 cells using a cell-substrate impedance monitoring system of the present invention. The cells were seeded onto devices coated with either poly- L-lysine or fibronectin. The cell attachment and cell spreading processes on the different coating surfaces were monitored every 3 minutes for over 3 hours in real time.

Figure 9 shows real-time monitoring of morphological changes in Cos-7 cells using a cell-substrate impedance monitoring system of the present invention. The cells were serum starved for 8 hours and stimulated with or 50 ng/mL EGF. Changes in cell morphology were monitored at 3 min intervals for 2 hours and then 1 hour interval for 14 hours. The initial jump in the signal in EGF-treated cells is due to membrane ruffling and actin dynamics in response to EGF. The arrow indicates the point of EGF stimulation.

Figure 10 shows a plots of time-dependent cell index for H460 cells treated by anticancer drug paclitaxel. Different wells of cultured H460 cells in their exponential growth phase were treated with different concentrations of Paclitaxel. The dynamic response of the cells to different doses of paclitaxel was monitored in real time every 15 minutes for 50 hours after treatment using a cell-substrate impedance monitoring system of the present invention.

Figure 11 shows plots of time-dependent cell index for H460 cells treated by anticancer drug AC101103. Different wells of cultured H460 cells were treated at their exponential growth phase with different concentrations of AC101103. The dynamic response of the cells to different doses of AC101103 was monitored in real time every 30 minutes for

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about 20 hours after treatment on a cell-substrate impedance monitoring system of the present invention.

Figure 12 shows dynamic drug response curves of A549 cells treated with doxorubicin. 10,000 A549 cells were seeded in each well of a 16X cell-substrate impedance monitoring device. Cell attachment and cell growth were monitored using a cell-substrate impedance monitoring system before treatment. When the cells were in exponential growth phase, doxorubicin at different concentration was added to the cells. The time and drug dose dependent cell response to doxorubicin was recorded in real time on the as shown in this figure.

Figure 13 is a plot of cell index recording indicating mast cell responses to IgE in the presence or absence of antigen. 20,000 RBL-2H3 mast cells were seeded in each well of a 16X device. Cells were allowed to adhere and grow for 22 hours while being recorded. At 22 hours the cells were treated with either 1 microgram/mL non-specific mouse IgG or 1 microgram/mL anti-DNP mouse IgE. The electronic impedance response of the cells were continued to be recorded for an additional 20 hours after which the media in the wells were aspirated and replaced with fresh serum free media. The cells were allowed to recover for 30 minutes and then treated with the 1 microgram/mL of the antigen, DNP-BSA. The electronic impedance response of the cells was measured for an additional 3 hours.

Figure 14 shows the results of another experiment monitoring cell-electrode impedance responses of RBL-2H3 mast cells sensitized with anti-DNP *Ig*E and activated by the application of DNP-BSA. RBL-2H3 mast cells were seeded at a density of 20,000 cells/ well onto the surface of a 16X device and the impedance was continuously measured and Cell Index recorded every 30 minutes using a cell-substrate impedance monitoring system of the present invention. Approximately 14 hours after seeding the cells were incubated with 100 ng/mL anti-DNP *Ig*E followed by application of 100 ng/mL DNP-BSA 24 hours later. Impedance measurements (indicated as Cell Index) were performed at 5 minute intervals post DNP-BSA application.

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Figure 15 shows correlation between cell-electrode impedance response of RBL-2H3 mast cells and cell morphological dynamics and mediator release. (A) RBL-2H3 mast cells were sensitized with 100 ng/mL IgE or treated with a control IgG antibody and subsequently activated with DNP-BSA. The cells were fixed with paraformaldehyde at the indicated time points, permeablized and stained with rhodamine-phalloidin. The cells were visualized and photographed with a Nikon E-400 immunoflourescence microscope equipped with a CCD camera. (B) RBL-2H3 cells were sensitized with IgE, activated by the addition of DNP-BSA.

Figure 16 shows IgE alone-mediated increase in mast cell-electrode impedance response and its effect on antigen-mediated activation step. (A) RBL-2H3 cells seeded on microelectronic sensor arrays were left untreated or treated with indicated concentration of anti-DNP IgE. The impedance value indicated as Cell Index was continuously monitored and recorded using a cell-substrate impedance monitoring system. (B) RBL-2H3 cells that had been sensitized with 15 ng/mL or 1 microgram/mL anti-DNP-IgE were activated by the application of 100 ng/mL DNP-BSA. The impedance value indicated as Cell Index was continuously monitored using the system.

Figure 17 shows monitoring of inhibitory effects of Protein kinase C inhbitor, Bisindolymaleimide on IgE-mediated RBL-2H3 mast cell activation by cell-electrode impedance measurement. RBL-2H3 mast cells were sensitized with 100 ng/mL anti-DNP-IgE and then incubated with the indicated concentrations of the Bisindolylmaleimide 1 hour prior to addition of 100 ng/mL DNP-BSA. The impedance value indicated as Cell Index was monitored using a cell-substrate impedance monitoring system of the present invention.

Figure 18 shows the beta-hexosaminidase activity of IgE-mediated RBL-2H3 mast cells measured in the presence of pharmacological inhibitors. RBL-2H3 mast cells were sensitized, incubated with DMSO, 16.6 micromolar SU 6656, 5 micromolar U73122, 16.6 micromolar Bisindolylmaleimide, 16.6 micromolar Piceatannol and 16.6 micromolar PD 98059 for 1 hour. The cells were activated by the addition of 100 ng/mL DNP-BSA.

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DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections that follow.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications referred to herein are incorporated by reference in their entirety. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

As used herein, "a" or "an" means "at least one" or "one or more."

As used herein, "membrane" is a sheet of material.

As used herein, "biocompatible membrane" means a membrane that does not have deleterious effects on cells, including the viability, attachment, spreading, motility, growth, or cell division.

When a suspension of viable, unimpaired, epithelial or endothelial cells is added to a vessel, a surface of the vessel "is suitable for cell attachment" when a significant percentage of the cells are adhering to the surface of the vessel within twelve hours. Preferably, at least 50% of the cells are adhering to the surface of the vessel within twelve hours. More preferably, a surface that is suitable for cell attachment has surface properties so that at least 70% of the cells are adhering to the surface within twelve hours of plating (i.e., adding cells to the vessel). Even more preferably, the surface properties of a surface that is suitable for cell attachment results in at least 90% of the cells adhering to the surface within twelve hours of plating. Most preferably, the surface properties of a surface that is suitable for cell attachment results in at least 90% of the cells adhering to the surface within eight, six, four, two hours of plating. To have desired surface properties for cell attachment, the surface may need to chemically-treated (e.g. treatment with an acid and/or with a base), and/or physically treated (e.g. treatment with plasma),

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and/or biochemically treated (e.g. coated with one or more molecules or biomolecules that promotes cell attachment). In the present invention, a biocompatible surface (such as a membrane) preferably is suitable for the attachment of cells of the type that are to be used in an assay that uses the biocompatible surface (e.g., membrane), and most preferably, allows the attachment of at least 90% of the cells that contact the biocompatible surface during the assay.

A "biomolecular coating" is a coating on a surface that comprises a molecule that is a naturally occurring biomolecule or biochemical, or a biochemical derived from or based on one or more naturally occurring biomolecules or biochemicals. For example, a biomolecular coating can comprise an extracellular matrix component (e.g., fibronectin, collagens), or a derivative thereof, or can comprise a biochemical such as polylysine or polyornithine, which are polymeric molecules based on the naturally occurring biochemicals lysine and ornithine. Polymeric molecules based on naturally occurring biochemicals such as amino acids can use isomers or enantiomers of the naturally-occuring biochemicals.

An "extracellular matrix component" is a molecule that occurs in the extracellular matrix of an animal. It can be a component of an extracellular matrix from any species and from any tissue type. Nonlimiting examples of extracellular matrix components include laminins, collagens fibronectins, other glycoproteins, peptides, glycosaminoglycans, proteoglycans, etc. Extracellular matrix components can also include growth factors.

An "electrode" is a structure having a high electrical conductivity, that is, an electrical conductivity much higher than the electrical conductivity of the surrounding materials.

As used herein, an "electrode structure" refers to a single electrode, particularly one with a complex structure (as, for example, a spiral electrode structure), or a collection of at least two electrode elements that are electrically connected together. All the electrode elements within an "electrode structure" are electrically connected.

As used herein, "electrode element" refers to a single structural feature of an electrode structure, such as, for example, a fingerlike projection of an interdigitated electrode structure.

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As used herein, an "electrode array" or "electrode structure unit" is two or more electrode structures that are constructed to have dimensions and spacing such that they can, when connected to a signal source, operate as a unit to generate an electrical field in the region of spaces around the electrode structures. Preferred electrode structure units of the present invention can measure impedance changes due to cell attachment to an electrode surface. Non-limiting examples of electrode structure units are interdigitated electrode structure units and concentric electrode structure units.

An "electrode bus" is a portion of an electrode that connects individual electrode elements or substructures. An electrode bus provides a common conduction path from individual electrode elements or individual electrode substructures to another electrical connection. In the devices of the present invention, an electrode bus can contact each electrode element of an electrode structure and provide an electrical connection path to electrical traces that lead to a connection pad.

"Electrode traces" or "electrically conductive traces" or "electrical traces", are electrically conductive paths that extend from electrodes or electrode elements or electrode structures toward one end or boundary of a device or apparatus for connecting the electrodes or electrode elements or electrode structures to an impedance analyzer. The end or boundary of a device may correspond to the connection pads on the device or apparatus.

A "connection pad" is an area on an apparatus or a device of the present invention which is electrically connected to at least one electrode or all electrode elements within at least one electrode structure on an apparatus or a device and which can be operatively connected to external electrical circuits (e.g., an impedance measurement circuit or a signal source). The electrical connection between a connection pad and an impedance measurement circuit or a signal source can be direct or indirect, through any appropriate electrical conduction means such as leads or wires. Such electrical conduction means may also go through electrode or electrical conduction paths located on other regions of the apparatus or device.

"Interdigitated" means having projections coming one direction that interlace with projections coming from a different direction in the manner of the fingers of folded hands

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(with the caveat that interdigitated electrode elements preferably do not contact one another).

As used herein, a "high probability of contacting an electrode element" means that, if a cell is randomly positioned within the sensor area of a device or apparatus of the present invention, the probability of a cell (or particle) contacting on an electrode element, calculated from the average diameter of a cell used on or in a device or apparatus of the present invention, the sizes of the electrode elements, and the size of the gaps between electrode elements, is greater than about 50%, more preferably greater than about 60%, yet more preferably greater than about 70%, and even more preferably greater than about 80%, greater than about 90%, or greater than about 95%.

As used herein, "at least two electrodes fabricated on said substrate" means that the at least two electrodes are fabricated or made or produced on the substrate. The at least two electrodes can be on the same side of the substrate or on the different side of the substrate. The substrate may have multiple layers, the at least two electrodes can be either on the same or on the different layers of the substrate.

As used herein, "at least two electrodes fabricated to a same side of said substrate" means that the at least two electrodes are fabricated on the same side of the substrate.

As used herein, "at least two electrodes fabricated to a same plane of said substrate" means that, if the nonconducting substrate has multiple layers, the at least two electrodes are fabricated to the same layer of the substrate.

As used herein, "said . . . electrodes [or electrode structures] have substantially the same surface area" means that the surface areas of the electrodes referred to are not substantially different from each other, so that the impedance change due to cell attachment or growth on any one of the electrodes (or electrode structures) referred to will contribute to the overall detectable change in impedance to a same or similar degree as the impedance change due to cell attachment or growth on any other of the electrodes (or electrode structures) referred to. In other words, where electrodes (or electrode structures) have substantially the same surface area, any one of the electrodes can contribute to overall change in impedance upon cell attachment or growth on the electrode. In most cases, the ratio of surface area between the largest electrode and the

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smallest electrode that have "substantially the same surface area" is less than 10. Preferably, the ratio of surface area between the largest electrode and the smallest electrode of an electrode array is less than 5, 4, 3, 2, 1.5, 1.2 or 1.1. More preferably, the at least two electrodes of an electrode structure have nearly identical or identical surface area.

As used herein, "said device has a surface suitable for cell attachment or growth" means that the electrode and/or non-electrode area of the apparatus has appropriate physical, chemical or biological properties such that cells of interest can viably attach on the surface and new cells can continue to attach, while the cell culture grows, on the surface of the apparatus. However, it is not necessary that the device, or the surface thereof, contain substances necessary for cell viability or growth. These necessary substances, e.g., nutrients or growth factors, can be supplied in a medium. Preferably, when a suspension of viable, unimpaired, epithelial or endothelial cells is added to the "surface suitable for cell attachment" when at least 50% of the cells are adhering to the surface within twelve hours. More preferably, a surface that is suitable for cell attachment has surface properties so that at least 70% of the cells are adhering to the surface within twelve hours of plating (i.e., adding cells to the chamber or well that comprises the said device). Even more preferably, the surface properties of a surface that is suitable for cell attachment results in at least 90% of the cells adhering to the surface within twelve hours of plating. Most preferably, the surface properties of a surface that is suitable for cell attachment results in at least 90% of the cells adhering to the surface within eight, six, four, two hours of plating.

As used herein, "detectable change in impedance between or among said electrodes" (or "detectable change in impedance between or among said electrode structures") means that the impedance between or among said electrodes (or electrode structures) would have a significant change that can be detected by an impedance analyzer or impedance measurement circuit when molecule binding reaction occurs on the electrode surfaces. The impedance change refers to the difference in impedance values when molecule binding reaction occurs on the electrode surface of the apparatus and when no molecular reaction occurs on the electrode surface. Alternatively, the impedance change refers to the difference in impedance values when cells are attached to

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the electrode surface and when cells are not attached to the electrode surface, or when the number, type, activity, adhesiveness, or morphology of cells attached to the electrode-comprising surface of the apparatus changes. In most cases, the change in impedance is larger than 0.1% to be detectable. Preferably, the detectable change in impedance is larger than 1%, 2%, 5%, or 8%. More preferably, the detectable change in impedance is larger than 10%. Impedance between or among electrodes is typically a function of the frequency of the applied electric field for measurement. "Detectable change in impedance between or among said electrodes" does not require the impedance change at all frequencies being detectable. "Detectable change in impedance between or among said electrodes" only requires a detectable change in impedance at any single frequency (or multiple frequencies). In addition, impedance has two components, resistance and reactance (reactance can be divided into two categories, capacitive reactance and inductive reactance). "Detectable change in impedance between or among said electrodes" requires only that either one of resistance and reactance has a detectable change at any single frequency or multiple frequencies. In the present application, impedance is the electrical or electronic impedance. The method for the measurement of such impedance is achieved by, (1) applying a voltage between or among said electrodes at a given frequency (or multiple frequencies, or having specific voltage waveform) and monitoring the electrical current through said electrodes at the frequency (or multiple frequencies, or having specific waveform), dividing the voltage amplitude value by the current amplitude value to derive the impedance value; (2) applying an electric current of a single frequency component (or multiple frequencies or having specific current wave form) through said electrodes and monitoring the voltage resulted between or among said electrodes at the frequency (or multiple frequencies, or having specific waveform), dividing the voltage amplitude value by the current amplitude value to derive the impedance value; (3) other methods that can measure or determine electric impedance. Note that in the description above of "dividing the voltage amplitude value by the current amplitude value to derive the impedance value", the "division" is done for the values of current amplitude and voltage amplitude at same frequencies. Measurement of such electric impedance is an electronic or electrical process that does not involve the use of any reagents.

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As used herein, "said at least two electrodes have substantially different surface area" means that the surface areas of any electrodes are not similar to each other so that the impedance change due to cell attachment or growth on the larger electrode will not contribute to the overall detectable impedance to a same or similar degree as the impedance change due to cell attachment or growth on the smaller electrodes. Preferably, any impedance change due to cell attachment or growth on the larger electrode is significantly smaller than the impedance change due to cell attachment or growth on the smaller electrode. Ordinarily, the ratio of surface area between the largest electrode and the smallest electrode is more than 10. Preferably, the ratio of surface area between the largest electrode and the smallest electrode is more than 20, 30, 40, 50 or 100.

As used herein, "multiple pairs of electrodes or electrode structures spatially arranged according to wells of a multi-well microplate" means that the multiple pairs of electrodes or electrode structures of a device or apparatus are spatially arranged to match the spatial configuration of wells of a multi-well microplate so that, when desirable, the device can be inserted into, joined with, or attached to a multiwell plate (for example, a bottomless multiwell plate) such that multiple wells of the multi-well microplate will comprise electrodes or electrode structures.

As used herein, "arranged in a row-column configuration" means that, in terms of electric connection, the position of an electrode, an electrode array or a switching circuit is identified by both a row position number and a column position number.

As used herein, "each well contains substantially same number . . . of cells" means that the lowest number of cells in a well is at least 50% of the highest number of cells in a well. Preferably, the lowest number of cells in a well is at least 60%, 70%, 80%, 90%, 95% or 99% of the highest number of cells in a well. More preferably, each well contains an identical number of cells.

As used herein, "each well contains . . .same type of cells" means that, for the intended purpose, each well contains same type of cells; it is not necessary that each well contains exactly identical type of cells. For example, if the intended purpose is that each well contains mammalian cells, it is permissible if each well contains same type of

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mammalian cells, *e.g.*, human cells, or different mammalian cells, *e.g.*, human cells as well as other non-human mammalian cells such as mice, goat or monkey cells, etc.

As used herein, "each well contains . . . serially different concentration of a test compound" means that each well contains a test compound with a serially diluted concentrations, *e.g.*, an one-tenth serially diluted concentrations of 1 M, 0. 1M, 0.01 M, etc.

As used herein, "dose-response curve" means the dependent relationship of response of cells on the dose concentration of a test compound. The response of cells can be measured by many different parameters. For example, a test compound is suspected to have cytotoxicity and cause cell death. Then the response of cells can be measured by percentage of non-viable (or viable) cells after the cells are treated by the test compound.

As used herein, "the electrodes have, along the length of the microchannel, a length that is substantially less than the largest single-dimension of a particle to be analyzed" means that the electrodes have, along the length of the microchannel, a length that is at least less than 90% of the largest single-dimension of a particle to be analyzed. Preferably, the electrodes have, along the length of the microchannel, a length that is at least less than 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5% of the largest single-dimension of a particle to be analyzed.

As used herein, "the microelectrodes span the entire height of the microchannel" means that the microelectrodes span at least 70% of the entire height of the microchannel. Preferably, microelectrodes span at least 80%, 90%, 95% of the entire height of the microchannel. More preferably, microelectrodes span at least 100% of the entire height of the microchannel.

As used herein, "an aperture having a pore size that equals to or is slightly larger than size of said particle" means that aperture has a pore size that at least equals to the particle size but less than 300% of the particle size. Here both pore size and particle size are measured in terms of single dimension value.

As used herein, "microelectrode strip or electrode strip" means that a non-conducting substrate strip on which electrodes or electrode structure units are fabricated or incorporated. The non-limiting examples of the non-conducting substrate strips include polymer membrane, glass, plastic sheets, ceramics, insulator-on-semiconductor,

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fiber glass (like those for manufacturing printed-circuits-board). Electrode structure units having different geometries can be fabricated or made on the substrate strip by any suitable microfabrication, micromachining, or other methods. Non-limiting examples of electrode geometries include interdigitated electrodes, circle-on-line electrodes, diamond-on-line electrodes, castellated electrodes, or sinusoidal electrodes. Characteristic dimensions of these electrode geometries may vary from as small as less than 5 micron, or less than 10 micron, to as large as over 200 micron, over 500 micron, over 1 mm. The characteristic dimensions of the electrode geometries refer to the smallest width of the electrode elements, or smallest gaps between the adjacent electrode elements, or size of a repeating feature on the electrode geometries. The microelectrode strip can be of any geometry for the present invention. One exemplary geometry for the microelectrode strips is rectangular shape – having the width of the strip between less than 50 micron to over 10 mm, and having the length of the strip between less than 60 micron to over 15 mm. An exemplary geometry of the microelectrode strips may have a geometry having a width of 200 micron and a length of 20 mm. A single microelectrode strip may have two electrodes serving as a measurement unit, or multiple such two-electrodes serving as multiple measurement units, or a single electrode structure unit as a measurement unit, or multiple electrode structure units serving as multiple electrode structure units. In one exemplary embodiment, when multiple electrode structure units are fabricated on a single microelectrode strip, these electrode structure units are positioned along the length direction of the strip. The electrode structure units may be of squared-shape, or rectangular-shape, or circle shapes. Each of electrode structure units may occupy size from less than 50 micron by 50 micron, to larger than 2 mm x 2mm.

As used herein, "sample" refers to anything which may contain a moiety to be isolated, manipulated, measured, quantified, detected or analyzed using apparatuses, microplates or methods in the present application. The sample may be a biological sample, such as a biological fluid or a biological tissue. Examples of biological fluids include suspension of cells in a medium such as cell culture medium, urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, amniotic fluid or the like. Biological tissues are aggregates of cells, usually of a particular kind together with their intercellular substance that form one of the structural materials of a

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human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues. Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s). The biological samples may further include cell suspensions, solutions containing biological molecules (e.g. proteins, enzymes, nucleic acids, carbonhydrates, chemical molecules binding to biological molecules) .

As used herein, a "liquid (fluid) sample" refers to a sample that naturally exists as a liquid or fluid, *e.g.*, a biological fluid. A "liquid sample" also refers to a sample that naturally exists in a non-liquid status, *e.g.*, solid or gas, but is prepared as a liquid, fluid, solution or suspension containing the solid or gas sample material. For example, a liquid sample can encompass a liquid, fluid, solution or suspension containing a biological tissue.

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B. Devices and systems for monitoring cell-substrate impedance

Devices for Measuring Cell-Substrate Impedance

The present invention includes devices for measuring cell-substrate impedance that comprise a nonconducting substrate; two or more electrode arrays fabricated on the substrate, where each of the two or more electrode arrays comprises two electrode structures; and at least two connection pads, each of which is located on an edge of the substrate. Each electrode array of the device has approximately uniform electrode resistance across the entire array. The substrate of the device has a surface suitable for cell attachment or growth; where cell attachment or growth on said substrate can result in a detectable change in impedance between or among the electrode structures within each electrode array.

An electrode array is two or more electrode structures that are constructed to have dimensions and spacing such that they can, when connected to a signal source, operate as a unit to generate an electrical field in the region of spaces around the electrode structures. An electrode structure refers to a single electrode, particularly one with a complex structure. (For example, an electrode structure can comprise two or more electrode elements that are electrically connected together.) In devices of the present invention, an electrode array comprises two electrode structures, each of which comprises multiple electrode elements, or substructures. In preferred embodiments of the present invention, the electrode structures of each of the two or more electrode arrays of a device have substantially the same surface area. In preferred embodiments of a device of the present invention, each of the two or more electrode arrays of a device comprise two electrode structures, and each electrode structure comprises multiple electrode elements. Each of the two electrode structures of an electrode array is connected to a separate connection pad that is located at the edge of the substrate.

Thus, in devices of the present invention, for each of the two or more electrode arrays of the device, the first of the two electrode structures is connected to one of the two or more connection pads, and the second of the two electrode structures is connected to another of the two or more connection pads. Preferably, each array of a device is individually addressed, meaning that the electrical traces and connection pads of the

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arrays are configured such that an array can be connected to an impedance analyzer in such a way that a measuring voltage can be applied across a single array at a given time by using switches (such as electronic switches).

Each electrode array of the device has an approximately uniform electrode resistance distribution across the entire array. By "uniform resistance distribution across the array" is meant that when a measurement voltage is applied across the electrode structures of the array, the electrode resistance at any given location of the array is approximately equal to the electrode resistance at any other location on the array. Preferably, the electrode resistance at a first location on an array of the device and the electrode resistance at a second location on the same array does not differ by more than 30%. More preferably, the electrode resistance at a first location on an array of the device and the electrode resistance at a second location on the same array does not differ by more than 15%. Even more preferably, the electrode resistance at a first location on an array of the device and a second location on the same array does not differ by more than 5%. More preferably yet, the electrode resistance at a first location on an array of the device and a second location on the same array does not differ by more than 2%.

For a device of the present invention, preferred arrangements for the electrode elements, gaps between the electrodes and electrode buses in a given electrode array are used to allow all cells, no matter where they land and attach to the electrode surfaces, to contribute similarly to the total impedance change measured for the electrode array. Thus, it is desirable to have similar electric field strengths at any two locations within any given array of the device when a measurement voltage is applied to the electrode array. At any given location of the array, the field strength is related to the potential difference between the nearest point on a first electrode structure of the array and the nearest point on a second electrode structure of the array. It is therefore desirable to have similar electric potential drops across the electrode elements and across the electrode buses of a given array. Based on this requirement, it is preferred to have an approximately uniform electrode resistance distribution across the whole array where the electrode resistance at a location of interest is equal to the sum of the electrode resistance between the nearest point on a first electrode structure (that is the point on the first electrode structure nearest the location of interest) and a first connection pad connected to the first electrode

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structure and the electrode resistance between the nearest point on a second electrode structure (that is the point on the first electrode structure nearest the location of interest) and a second connection pad connected to the second electrode structure.

Devices of the present invention are designed such that the arrays of the device have an approximately uniform distribution across the whole array. This can be achieved, for example, by having electrode structures and electrode buses of particular spacing and dimensions (lengths, widths, thicknesses and geometrical shapes) such that the resistance at any single location on the array is approximately equal to the resistance at any single other location on the array. In most embodiments, the electrode elements (or electrode structures) of a given array will have even spacing and be of similar thicknesses and widths, the electrode buses of a given array will be of similar thicknesses and widths, and the electrode traces leading from a given array to a connection pad will be of closely similar thicknesses and widths. Thus, in these preferred embodiments, an array is designed such that the lengths and geometrical shapes of electrode elements or structures, the lengths and geometrical shapes of electrode traces, and the lengths and geometrical shapes of buses allow for approximately uniform electrode resistance distribution across the array.

In some preferred embodiments of cell-substrate impedance measurement devices, electrode structures comprise multiple electrode elements, and each electrode element connects directly to an electrode bus. Electrode elements of a first electrode structure connect to a first electrode bus, and electrode elements of a second electrode structure connect to a second electrode bus. In these embodiments, each of the two electrode buses connects to a separate connection pad via an electrical trace. Although the resistances of the traces contribute to the resistance at a location on the array, for any two locations on the array the trace connections from the first bus to a first connection pad and from the second bus to a second connection pad are identical. Thus, in these preferred embodiments trace resistances do not need to be taken into account in designing the geometry of the array to provide for uniform resistances across the array.

Taking the electrode array in **Figure 1C** as an example, it is desirable that cells attached at location B and cells attached at location A contribute similarly to the total impedance change. Thus, it is desirable to have similar electric field strengths at location

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A and location B when a measurement voltage is applied to the electrode array. It is thus desirable to have similar electric potential drops across the electrode elements and across the electrode buses. Based on this requirement, it is preferred to have an approximately uniform electrode resistance distribution across the whole array where the electrode resistance at a location of interest is equal to the sum of the electrode resistances between the electrode points at the location and the two connection pads connected to the electrode array. The electrode resistance at location A and location B in **Figure 1C** is given by

$$R_{location\_A} = R_{trace1} + R_{1\text{-}to\text{-}A1} + R_{A1\text{-}to\text{-}A} + R_{A\text{-}to\text{-}A2} + R_{A2\text{-}to\text{-}2} + R_{trace2} \quad (1)$$

$$R_{location\_B} = R_{trace1} + R_{1\text{-}to\text{-}B1} + R_{B1\text{-}to\text{-}B} + R_{B\text{-}to\text{-}B2} + R_{B2\text{-}to\text{-}2} + R_{trace2} \quad (2)$$

where R_{trace1} is the impedance (i.e., resistance, since the reaction component is very very small) of the electrical connection trace from a connection pad to location 1 on the top electrode bus; R_{trace2} is the impedance (i.e., resistance, since the reaction component is very very small) of the electrical connection trace from a connection pad to location 2 on the bottom electrode bus; $R_{1\text{-}to\text{-}A1}$ is the impedance (i.e., resistance, the reaction component is very very small) between location 1 and location A1 along the top electrode bus; $R_{2\text{-}to\text{-}A2}$ is the impedance (i.e., resistance, the reaction component is very very small) between location 2 and location A2 along the bottom electrode bus; $R_{A1\text{-}to\text{-}A}$ is the impedance (i.e., resistance, the reaction component is very very small) between location A1 on the top electrode bus and location A along a connected electrode element; $R_{A2\text{-}to\text{-}A}$ is the impedance (i.e., resistance, the reaction component is very very small) between location A2 on the bottom electrode bus and location A along another connected electrode element. The requirement for an approximately uniform electrode resistance is that $R_{location\_A}$ is similar to $R_{location\_B}$.

Electrode structures shown in **Figure 1C** are designed to satisfy the requirement of approximately uniform electrode resistance. In **Figure 1C**, the electrodes or electrode elements are circle-on-a-line configuration with diameter of the circle being about 90 microns, the line width about 30 microns and the gaps between lines about 80 microns.

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The inner diameter of the electrode bus is about 5.75 mm and the outer diameter for the electrode bus is about 6.070 mm.

In preferred embodiments of the present invention, for the measurement of cell-substrate impedance, an impedance analyzer is connected indirectly through electronic switches to the connection pads on the substrate. The measured impedance Z_{total} at the impedance analyzer is given by

$$Z_{total} = Z_{switch} + Z_{trace} + Z_{electrode\text{-}array} \quad (3)$$

where Z_{switch} is the impedance of electronic switch at its on stage, Z_{trace} is the impedance of the electrical connection traces on the substrate between the connection pads and the electrode buses, $Z_{electrode\text{-}array}$ is the impedance of the electrode array with or without cell-being present. Major components of both Z_{switch} and Z_{trace} are electric resistance. The contribution of Z_{switch} and Z_{trace} to total measured impedance Z_{total} can be removed from the measured impedance if these values can be determined. By choosing electronic switches of good quality, the Z_{switch} values can be nearly-constant for different switches. Thus, the contribution of Z_{switch} to total measured impedance Z_{total} can be removed from the measured impedance. Z_{trace} is somewhat difficult to remove because different electrode arrays may have different impedance values and also because there is also a thickness variation in the conductive thin film used for making the electrode structures between different devices. For accurate measurement of cell-substrate impedance, it is preferred to have small electrical connection trace (or electrical traces) impedance Z_{trace}. For this reason, it is desirable to have electrical traces of large width, which will lead to a reduced size for the electrode array area. For this reason, in one embodiment of the device of the presentation, in order to have reasonably wide electrical traces, the diameter of the electrode array area, as exemplified in **Figure 1B** as the inner diameter of arc-shaped electrode buses, was made smaller than the bottom well diameter of a standard microtiter plate. To ensure the electrode buses are not exposed to solution during the assay, a plate having wells whose with a bottom diameter is small enough to exclude the electrode buses from the interior of the wells can be attached to the substrate.

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In preferred embodiments of the present invention, a device for monitoring cell-substrate impedance has two or more electrode arrays that share a connection pad. Preferably one of the electrode structures of at least one of the electrode arrays of the device is connected to a connection pad that also connects to an electrode structure of at least one other of the electrode arrays of the device. Preferably for at least two arrays of the device, each of the two or more arrays has a first electrode structure connected to a connection pad that connects with an electrode structure of at least one other electrode array, and each of the two or more arrays has a second electrode structure that connects to a connection pad that does not connect with any other electrode structures or arrays of the device. Thus, in preferred designs of a device there are at least two electrode arrays each of which has a first electrode structure that is connected to a common connection pad and a second electrode structure that is connected to an independent connection pad.

In some preferred embodiments of the present invention, each of the electrode structures of an array is connected to an electrode bus that is connected to one of the two or more connection pads of the device via an electrically conductive trace. In preferred embodiments, each of the two electrode structures is connected to a single bus, such that each array connects to two buses, one for each electrode structures. In this arrangement, each of the two buses connects to a separate connection pad of the substrate.

The electrically conductive traces that connect a bus with a connection can be fabricated of any electrically conductive material. The traces can be localized to the surface of the substrate, and can be optionally covered with an insulating layer. Alternatively the traces can be disposed in a second plane of the substrate. Description of arrangements and design of electrically conductive traces on impedance measurement devices can be found in parent U.S. Patent Application 10/705,447, herein incorporated by reference for disclosure on fabrication and design of electrically conductive trace on substrates.

Figure 1A shows an example of the device of the present invention. This device comprises a glass substrate (**101**) shown with 16 electrode arrays fabricated on the substrate. Each electrode array (**102**) comprises two electrode structures (shown in detail in **Figure 1B**). Each electrode array connects to two electrical traces (**103**), with each of the two traces connected one of the two electrode structures. These electrical connection

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traces (**103**) from the electrode array (**102**) are connected to the connection pads (**104**) at the edges of the substrate (**101**). As shown in **Figure 1A**, each the four electrode arrays in each of four quarters on the substrate (**101**) have one of their electrical connection traces (**103**) connected to a common connection pad (**104**). Thus, for the entire device there are four common connection pads (**104**), one for each quarter of the device. In addition, each electrode array has a separate electrical connection trace (**103**), connecting to an independent connection pad (**104**). Thus, there are total 20 connection pads (**104**) at the edges of the substrate (**101**).

An example of an electrode array that can be used on a device of the present invention (such as that of **Figure 1A**) is depicted in **Figure 1B**. Here, a single electrode array is shown. The electrode array has two electrode structures, where each electrode structure comprises multiple electrode elements (**105**) shown here having a circle-on-line geometry. In this electrode array structure, electrode elements (105) of one electrode structure of the array alternate with electrode elements (**105**) of the other electrode structure of the array. Each of the electrode structures is independently connected to its electrode bus (**106**), in this case, by means of direct connection of the electrode elements (**105**) to the electrode bus (**106**). Each electrode bus (**106**) forms an arc around the perimeter of the array, where the two buses of the array do not abut or overlap. The electrically conductive connection traces (**103** in **Figure 1A**) connect each bus with a connection pad (**104** in **Figure 1A**) on the edge of the substrate (**101** in **Figure 1A**)

Appropriate electronic connection means such as metal clips engaged onto the connection pads on the substrate and connected printed-circuit-boards can be used for leading the electronic connections from the connection pads on the devices to external electronic circuitry (e.g. an impedance analyzer). Description of the design of cell-substrate impedance devices and their manufacture can be found in U.S. Patent Application No. 10/705,447, herein incorporated by reference for description and disclosure of the design, features, and manufacture of impedance device comprising electrode arrays.

Preferably the nonconducting substrate is planar, and is flat or approximately flat. Exemplary substrates can comprise many materials, including, but not limited to, silicon dioxide on silicon, silicon-on-insulator (SOI) wafer, glass (e.g., quartz glass, lead glass or

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borosilicate glass), sapphire, ceramics, polymer, fiber glass, plastics, *e.g.*, polyimide (e.g. Kapton, polyimide film supplied by DuPont), polystyrene, polycarbonate, polyvinyl chloride, polyester, polypropylene and urea resin. Preferably, the substrate and the surface of the substrate are not going to interfere with molecular binding reactions that will occur at the substrate surface. For cell-substrate impedance monitoring, any surface of the nonconducting substrate that can be exposed to cells during the use of a device of the present invention is preferably biocompatible. Substrate materials that are not biocompatible can be made biocompatible by coating with another material, such as polymer or biomolecular coating.

All or a portion of the surface of a substrate can be chemically treated, including but not limited to, modifying the surface such as by addition of functional groups, or addition of charged or hydrophobic groups.

Descriptions of electrode arrays used for impedance measurement that apply to the devices of the present invention are described in parent U.S. Patent Application No. 10/705,447, herein incorporated by reference for all disclosure relating to electrode arrays (or structural units), electrode structures, electrode materials, electrode dimensions, and methods of manufacturing electrodes on substrates.

Preferred electrode arrays for devices of the present invention include arrays comprising two electrode structures, such as, for example, spiral electrode arrays and interdigitated arrays. In some preferred devices of the present invention, electrode arrays are fabricated on a substrate, in which the arrays comprises two electrode structures, each of which comprises multiple circle-on-line electrode elements, in which the electrode elements of one structure alternate with the electrode elements of the opposite electrode structure. For example, **Figure 1B** depicts such an array.

Preferably, the electrode elements (or electrode structures) of an array of the present device of the present invention are of approximately equal widths. Preferably the electrode elements (or electrode structures) of an array of the present device of the present invention are greater than 30 microns in width, more preferably from about 50 to about 300 microns in width, and more preferably yet about 90 microns in width.

Preferably, the electrode elements (or electrode structures) of an array of the present device of the present invention are approximately evenly spaced. Preferably, the

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gap between electrode elements (or electrode structures) of an array of the present device of the present invention is less than 50 microns in width, more preferably from about 5 to about 30 microns in width, and more preferably yet about 20 microns in width.

A device of the present invention can include one or more fluid-impermeable receptacles which serve as fluid containers. Such receptacles may be reversibly or irreversibly attached to or formed within the substrate or portions thereof (such as, for example, wells formed as in a microtiter plate). In another example, the device of the present invention includes microelectrode strips reversibly or irreversibly attached to plastic housings that have openings that correspond to electrode structure units located on the microelectrode strips. Suitable fluid container materials comprise plastics, glass, or plastic coated materials such as ceramics, glass, metal, etc. Descriptions and disclosure of devices that comprise fluid containers can be found in parent U.S. Patent Application No. 10/705,447, herein incorporated by reference for all disclosure of fluid containers and fluid container structures that can engage a substrate comprising electrodes for impedance measurements, including their dimensions, design, composition, and methods of manufacture.

In preferred embodiments, each electrode array on the substrate of a device of the present invention is associated with a fluid-impermeable container or receptacle. Preferably, the device of the present invention is assembled to a bottomless, multiwell plastic plate or strip with a fluid tight seal, as shown, for example, in **Figure 2** and **Figure 3**. The device is assembled such that a single array of the substrate is at the bottom of a receptacle or well. Preferably, each array of a device is associated with a well of a multiwell plate. In some preferred embodiments, a multiwell device for cell-substrate impedance measurement has "non-array" wells that are attached to the substrate but not associated with arrays. Such wells can optionally be used for performing non-impedance based assays, or for viewing cells microscopically.

The design and assembly of multiwell impedance measurement devices is described in parent U.S. Patent Application No. 10/705,447, herein incorporated by reference for disclosure of multiwell impedance measurement devices, including their design, composition, and manufacture. A device of the present invention preferably has between 2 and 1,536 wells, more preferably between 4 and 384 wells, and even more

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preferably, between 16 and 96 wells, all or less than all or which are associated with electrode arrays.

In some preferred embodiments, commercial tissue culture plates can be adapted to fit a device of the present invention. Bottomless plates may also be custom-made to preferred dimensions. Preferably, well diameters are from about 1 millimeter to about 20 millimeters, more preferably from about 2 millimeters to about 8 millimeters at the bottom of the well (the end disposed on the substrate). The wells can have a uniform diameter or can taper toward the bottom so that the diameter of the container at the end in contact with the substrate is smaller than the diameter of the opposing end.

Preferred Devices

The following descriptions of devices are not intended to limit the invention in any way.

Figure 2 is an image of one design of a 16x device with a glass substrate (**201**) having 16 microfabricated electrode arrays mounted to a printed-circuit-board (**208**) via metal clips (**209**). The metal clips (**209**) are engaged onto connection pads (**204**) on the substrate and are soldered to the connection lines (**210**) on the printed-circuit-board (**208**). The connection lines (**210**) on the printed-circuit-board (**208**) in turn are connected to the connection pins (**211**) located on the edge of the device. A bottomless plastic 16 well strip (**207**) is bonded to the substrate (**201**) via a double-sided pressure sensitive adhesive, forming 16 individual fluid containers. In this device, the bottom diameter of the fluid containers is about 5 mm.

Figure 3 shows an image of one design of a 96 x device with six glass substrates each having either 15 or 16 microfabricated electrode arrays. **Figure 3A** shows the bottom side of the device with open wells facing downwards and **Figure 3B** shows the device with a plastic lid with wells open well facing upwards. The six substrates (**301**) are mounted and sealed to a bottomless 96 well plate (**307**) so that 95 of 96 wells comprise an electrode array on the bottom surface of the wells (when the open wells face upwards). One of 96 wells does not have electrodes (as indicated (**312**) in **Figure 3A**). The well bottom diameter is about 5 mm. Metal clips are engaged onto the connection pads on the substrate and are soldered to the connection lines on the top side (when the

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96-well plate is placed with open wells facing upward, as shown in **Figure 3B**) of small printed-circuit-boards (**308**) located on the ends of the substrates (**301**). The printed-circuit-boards (**308**) are double-sided, and same number of connection lines exists on both sides of the boards at positions opposite to each other. The connection lines on the top side of the board are connected through conductive vias to connection lines on the bottom side, where the electronic connections to a device station are made.

Methods of Use

The present invention also includes methods of using a device of the present invention that comprises fluid containers situated over electrode arrays to measure cell-substrate impedance. Such methods include: providing a device of the present invention that comprises fluid containers situated over electrode arrays, attaching an impedance analyzer to a device of the present invention, adding cells to one or more fluid containers of the device, and measuring impedance over one or more arrays of the device. Methods of performing cell assays using impedance measurement devices can be found in parent U.S. Patent Application 10/705,447, herein incorporated by reference for its disclosure of methods of using impedance measurement devices, as well as in Sections D and E of the present application.

Cell-Substrate Impedance Measurement Systems

In another aspect, the present invention is directed to a cell-substrate impedance measurement system comprising a) at least one multiple-well cell-substrate impedance measuring device, in which at least two of the multiple wells comprise an electrode array at the bottom of the well; b) an impedance analyzer electronically connected to the multiple-well cell-substrate impedance measuring device; c) a device station capable of engaging the one or more multiple-well devices and comprising electronic circuitry capable of selecting and connecting electrode arrays within any of the multiple wells to the impedance analyzer; and d) a software program connected to the device station and impedance analyzer to control the device station and perform data acquisition and data analysis from the impedance analyzer.

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In a cell-substrate impedance measurement system of the present invention, the impedance analyzer engages connection pads of one or more multi-well devices to measure impedance. A cell-substrate measurement system can be used to efficiently and simultaneously perform multiple assays by using circuitry of the device station to digitally switch from recording from measuring impedance over an array in one well to measuring impedance over an array in another well.

A multiple-well cell-substrate impedance measuring device in a system of the present invention can be any multiple-well cell-substrate impedance measuring device in which at least two of the multiple wells comprise an electrode array at the bottom of the well, and in which at least two of the multiple wells comprise an electrode array are individually addressed. A device used in a system of the present invention, when connected to an impedance analyzer, can measure differences in impedance values that relate to cell behavior. For example, a cell-substrate impedance measuring device used in a system of the present invention can measure differences in impedance values when cells are attached to the electrode array and when cells are not attached to the electrode array, or can detect differences in impedance values when the number, type, activity, adhesiveness, or morphology of cells attached to the electrode-comprising surface of the apparatus changes.

Preferred devices that can be part of a cell-substrate impedance monitoring system can be those described in parent U.S. Patent Application No. 10/705,447, herein incorporated by reference for disclosure of cell-substrate impedance monitoring devices that comprise electrode arrays, including disclosure of their design, composition, and manufacture. Preferred devices that can be part of a cell-substrate impedance monitoring system can also be those described in the present application.

Preferably a multi-well device of a system of the present invention comprises between 4 and 1,536 wells, some or all of which can comprise electrode arrays. In some embodiments of the present invention, a device station can comprise one or more platforms or one or more slots for positioning one or more multiwell devices. The one or more platforms or one or more slots can comprise sockets, pins or other devices for electrically connecting the device to the device station. The device station preferably can be positioned in a tissue culture incubator during cell impedance measurement assays. It

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can be electrically connected to an impedance analyzer and computer that are preferably located outside the tissue culture incubator.

The device station comprises electronic circuitry that can connect to an impedance monitoring device and an impedance analyzer and electronic switches that can switch on and off connections to each of the two or more electrode arrays of the multiwell devices used in the system. The switches of the device station are controlled by a software program. The software program directs the device station to connect arrays of the device to an impedance analyzer and monitor impedance from one or more of the electrode arrays. During impedance monitoring, the impedance analyzer can monitor impedance at one frequency or at more than one frequency. Preferably, impedance monitoring is performed at more than one time point for a given assay, and preferably, impedance is monitored at at least three time points. The device station can connect individual arrays of a device to an impedance analyzer to monitor one, some, or all of the arrays of a device for a measurement time point. The switches of the device station allow the selected individual arrays to be monitored in rapid succession for each desired monitoring time point. Each monitoring time point is in fact a narrow time frame (for example from less than one second to minutes) of measurement in the assay during which impedance monitoring is performed. In some preferred embodiments of the present invention, the device station software is programmable to direct impedance monitoring of any of the wells of the device that comprise arrays at chosen time intervals.

The software of the impedance monitoring system can also store and display data. Data can be displayed on a screen, as printed data, or both. Preferably the software can allow entry and display of experimental parameters, such as descriptive information including cells types, compound concentrations, time intervals monitored, etc.

Preferably, the software can also analyze impedance data. In preferred embodiments, the software can calculate a cell index for one or more time points for one or more wells of the multiwell device.

Figure 4 shows one design of a 16x device station with 6 16 well devices connected to the station. The device station has six individual slots for six devices, in which each slot comprises a zero-insertion force socket (**416**) with connection indicators (**418**). When a device is properly engaged with the device station, a light (418) will be on

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because the light indicator (**418**) will be connected through circuit lines on the PCB of the device to an electrical power source. The station comprises electronic switches that can be switched on (connected) or off (disconnected) digitally to connect electrode arrays in individual wells to an impedance analyzer.

Figure 5 shows a 96 well device station with that can engage the 96-well device depicted in **Figure 3**. The station uses POGO pins (**520**) to connect to the connection lines on small printed-circuit-boards (the device has small PCBs connected to connection pads on the edges of the device with metal clips. The POGO-pins (**520**) are connected a circuitry inside the device station. The circuitry comprises electronic switches that can be switched on (connected) or off (disconnected) digitally to connect electrode arrays in those electrode-containing wells to an impedance analyzer.

Figure 6 shows different pages from real-time cell electronic sensing software, illustrating the entry of experimental parameters that can be entered and the display of results of data analysis. A) An experimental note page allows the recording of key information about the experiment by the experimenter, such as the goals and procedures of the experiment. (B) An experimental layout page allows the recording of cells, cell number, compound and compound concentration added into each well. (C) A test time setting page allows for the recording and control of time intervals used for performing cell-substrate impedance measurement and multiple experimental steps each having different time interval values and different length times can be setup. (D) A cell index page is a result page where the software automatically update the measured and derived cell index values for all wells that are under test after the completion of each measurement at predetermined time interval as setup by the Test time setting page. (E) An experimental data plot page allows for flexible plotting and organization of experimental data.

C. Methods for Calculating Cell Index

Based on the dependent relationship between the measured impedance, cell number (more accurately, the viable cell number, or attached cell number) and cell attachment status, it is possible to derive a so-called "cell number index" or "cell index" from the measured impedance frequency spectra that provides a useful index for

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quantitating and comparing cell behavior in the impedance-based assays of the present invention. In some applications of the present invention, "cell index" in the present application is the same as "cell number index" in PCT Application No. PCT/US03/22557,entitled "IMPEDANCE BASED DEVICES AND METHODS FOR USE IN ASSAYS", filed on July 18, 2003 and in United States patent application No. 10/705,447,entitled "IMPEDANCE BASED DEVICES AND METHODS FOR USE IN ASSAYS," Attorney Docket No. ACE-00101.P.1.1-US, filed on November 10, 2003. U.S. Patent application 10/705,447 and PCT Application No. PCT/US03/22557 are hereby incorporated by reference for the discussions and disclosures of cell index and cell number index they contain.

Various methods for calculating such a cell number index can be used, some of which are novel methods disclosed herein.

The present invention provides several methods of calculating cell index numbers for cells attached to two or more essentially identical arrays of a cell-substrate impedance device, where the cells are monitored for impedance changes. In preferred embodiments of the present invention, the methods calculate cell index number with better accuracy than previous methods of calculating cell index for cells on two or more arrays of a cell-substrate monitoring device. In some preferred methods of the present invention, methods of calculating a cell index rely on novel methods for calculating the resistances of electrical traces leading to two or more essentially identical arrays. The present invention therefore also includes methods of calculating resistances of electrical traces leading to two or more essentially identical arrays on a substrate.

By "essentially identical electrode arrays" or "essentially identical arrays" is meant that the dimensions and arrangement of electrodes, electrode structures, and electrode elements is the same for the referenced arrays. Thus, two essentially identical electrode arrays will have electrode structures of the same dimensions (length, width, thickness), where the electrode structures have the same number of electrode elements, and the arrangement of electrode structures and electrode elements in each array are the same. By arrangement is meant the distance between structures or elements (gap width), their physical position with respect to one another, and their geometry (angles, degree of curvature, circle-on-line or castellated geometries, etc.), including the same features of

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any electrode buses that may be connected to electrode structures or electrode elements. Electrodes of essentially identical arrays also comprise the same materials. For the purposes of calculating trace resistances and cell index number, a substrate can have any number of essentially identical arrays.

The following discussion provides novel methods of calculating cell index of cells adhered to arrays of a cell-substrate impedance monitoring device and novel methods for the calculation of the resistances of the electrical connection traces leading to two or more electrode arrays of a cell-substrate impedance monitoring device.

Impedance (Z) has two components, namely the resistance Rs and reactance Xs. Mathematically, the impedance Z is expressed as follows,

$$Z = Rs + j\,Xs,$$

where $j = \sqrt{-1}$, depicting that for the (serial) reactance component Xs, the voltage applied over it is 90 degree phased-out from the current going through it. For the (serial) resistance, the voltage applied over it is in phase with the current going through it. As it is well-known in electronic and electrical engineering, the impedance can also be expressed in terms of parallel resistance Rp and parallel reactance Xp, as follows,

$$Z = Rp*(j\,Xp)/(Rp+j\,Xp),$$

where $j = \sqrt{-1}$. Nevertheless, these expressions (serial resistance and serial reactance, or parallel resistance and parallel reactance) are equivalent. Those who are skilled in electrical and electronic engineering can readily derive one form of expression from the parameter values in the other expression. For the sake of clarity and consistency, the description and discussion in the present invention utilizes the expression of serial resistance and serial reactance. For simplicity, serial resistance and serial reactance are simply called resistance and reactance.

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As described in US patent application no. 10/705,447, entitled "Impedance based devices and methods for use in assays", filed on November 10, 2003 and PCT application number PCT/US03/22557, entitled "Impedance based devices and methods for use in assays", filed on July 18, 2003, both of which are herein incorporated by reference for disclosures relating to cell-substrate impedance monitoring, monitoring cell-substrate impedance for detection or measurement of change in impedance can be done by measuring impedance in any suitable range of frequencies. For example, the impedance can be measured in a frequency range from about 1 Hz to about 100 MHz. In another example, the impedance can be measured in a frequency range from about 100 Hz to about 2 MHz. The impedance is typically a function of the frequency, i.e., the impedance values change as frequency changes. Monitoring cell-substrate impedance can be done either in a single frequency or multiple frequencies. If the impedance measurement is performed at multiple frequencies, then a frequency-dependent impedance spectrum is obtained – i.e., there is an impedance value at each measured frequency. As mentioned above, the impedance has two components – a resistance component and a reactance component. A change in either resistance component or reactance component or both components can constitute a change in impedance.

As described in the US patent application no. 10/705,447,entitled "Impedance based devices and methods for use in assays", filed on November 10, 2003 and PCT application number PCT/US03/22557,entitled "Impedance based devices and methods for use in assays", filed on July 18, 2003, herein incorporated by reference for disclosure of methods of measuring electrical impedance, the method for the measurement of electrical (or electronic) impedance is achieved by , (1) applying a voltage between or among said electrodes at a given frequency (or multiple frequencies, or having specific voltage waveform) and monitoring the electrical current through said electrodes at the frequency (or multiple frequencies, or having specific waveform), dividing the voltage amplitude value by the current amplitude value to derive the impedance value; (2) applying an electric current of a single frequency component (or multiple frequencies or having specific current wave form) through said electrodes and monitoring the voltage resulted between or among said electrodes at the frequency (or multiple frequencies, or having specific waveform), dividing the voltage amplitude value by the current amplitude value

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to derive the impedance value; (3) other methods that can measure or determine electric impedance. Note that in the description above of "dividing the voltage amplitude value by the current amplitude value to derive the impedance value", the "division" is done for the values of current amplitude and voltage amplitude at same frequencies. As it is well-known in electrical and electronic engineering, in such calculations (e.g. divisions mentioned above), the current amplitude and voltage amplitude are expressed in the form of complex numbers, which take into account of how big the current and the voltage are and what the phase difference between the sinusoidal waves of the current and the voltage is. Similarly, the impedance value is also expressed in a complex form, having both resistance and reactance component, as shown in equations above.

As described in the US patent application no. 10/705,447, entitled "Impedance based devices and methods for use in assays", filed on November 10, 2003 and PCT application number PCT/US03/22557,entitled "Impedance based devices and methods for use in assays", filed on July 18,. 2003, both incorporated herein by reference for disclosure relating to Cell Index or Cell Number Index, the measured cell-substrate impedance can be used to calculate a parameter termed Cell Index or Cell Number Index. Various methods for calculating such a cell number index can be used based on the changes in resistance or reactance when cells are attached to the electrode structures with respect to the cases no cells are attached to the electrode structures. The impedance (resistance and reactance) of the electrode structures with no cell attached but with same cell culture medium over the electrode structures is sometimes referred as baseline impedance. The baseline impedance may be obtained by one or more of the following ways: (1) the impedance measured for the electrode structures with a cell-free culture medium introduced into the well containing the electrode structures, wherein the culture medium is the same as that used for the impedance measurements for the condition where the cell attachment is monitored; (2) the impedance measured shortly (e.g. 10 minutes) after the cell-containing medium was applied to the wells comprising the electrode structures on the well bottom (during the short period after cell-containing medium addition, cells do not have enough time to attach to the electrode surfaces. The length of this short-period may depend on cell type and/or surface treatment or modification on the electrode surfaces); (3) the impedance measured for the electrode structures when all the cells in

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the well were killed by certain treatment (e.g. high-temperature treatment) and/or reagents (e.g. detergent) (for this method to be used, the treatment and/or reagents should not affect the dielectric property of the medium which is over the electrodes).

In one example (**A**), the cell index or cell number index can be calculated by:

(**A1**) at each measured frequency, calculating the resistance ratio by dividing the resistance of the electrode arrays when cells are present and/or attached to the electrodes by the baseline resistance,

(**A2**) finding or determining the maximum value in the resistance ratio over the frequency spectrum,

(**A3**) and subtracting one from the maximum value in the resistance ratio.

Using a mathematically formula, Cell Index is derived as

$$\text{Cell Index} = \max_{i=1,2,\ldots N}\left(\frac{R_{cell}(f_i)}{R_b(f_i)} - 1\right) \qquad (4)$$

Where N is the number of the frequency points at which the impedance is measured. For example, if the frequencies used for the measurements are at 10 kHz, 25 kHz and 50 kHz, then N=3, f_1= 10 kHz, f_2= 25 kHz, f_3= 50 kHz. $R_{cell}(f_i)$ is the resistance (cell-substrate resistance) of the electrode arrays or electrode structures when the cells are present on the electrodes at the frequency f_i and $R_b(f_i)$ is the baseline resistance of the electrode array or structures at the frequency f_i.

In this case, a zero or near-zero "cell index or cell number index" indicates that no cells or very small number of cells are present on or attached to the electrode surfaces. A higher value of "cell number index" indicates that, for same type of the cells and cells under similar physiological conditions, more cells are attached to the electrode surfaces. A higher value of "cell index" may also indicate that, for same type of the cells and same number of the cells, cells are attached better (for example, cells spread out more, or cell adhesion to the electrode surfaces is stronger) on the electrode surfaces.

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In another example (**B**), the cell number index can be calculated by:

(**B1**) at each measured frequency, calculating the reactance ratio by dividing the reactance of the electrode arrays when cells are present on and/or attached to the electrodes by the baseline reactance,

(**B2**) finding or determining the maximum value in the reactance ratio over the frequency spectrum,

(**B3**) and subtracting one from the maximum value in the resistance ratio.

In this case, a zero or near-zero "cell number index" indicates that no cells or very small number of cells are present on or attached to the electrode surfaces. A higher value of "cell number index" indicates that, for same type of the cells and cells under similar physiological conditions, more cells are attached to the electrode surfaces.

In yet another example (**C**), the cell index can be calculated by:

(**C1**) at each measured frequency, subtracting the baseline resistance from the resistance of the electrode arrays when cells are present or attached to the electrodes to determine the change in the resistance with the cells present relative to the baseline resistance;

(**C2**) then finding or determining the maximum value in the change of the resistance.

In this case, "cell-number index" is derived based on the maximum change in the resistance across the measured frequency range with the cells present relative to the baseline resistance. This cell index would have a dimension of ohm.

In yet another example (**D**), the cell index can be calculated by:

(**D1**) at each measured frequency, calculating the magnitude of the impedance (equaling to $\sqrt{R_s^2 + X_s^2}$, where R_s and X_s are the serial resistance and reactance, respectively).

(**D2**) subtracting the magnitude of the baseline impedance from the magnitude of the impedance of the electrode arrays when cells are present or attached to the electrodes

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to determine the change in the magnitude of the impedance with the cells present relative to the baseline impedance;

(**D3**) then finding or determining the maximum value in the change of the magnitude of the impedance.

In this case, "cell-number index" is derived based on the maximum change in the magnitude of the impedance across the measured frequency range with the cells present relative to the baseline impedance. This cell index would have a dimension of ohm.

In yet another example (**E**), the index can be calculated by:

(**E1**) at each measured frequency, calculating the resistance ratio by dividing the resistance of electrode arrays when cells are present or attached to the electrodes by the baseline resistance,

(**E2**) then calculating the relative change in resistance in each measured frequency by subtracting one from the resistance ratio,

(**E3**) then integrating all the relative-change value (i.e., summing together all the relative-change values at different frequencies).

In this case, "cell-number index" is derived based on multiple-frequency points, instead of single peak-frequency like above examples. Again, a zero or near-zero "cell number index" indicates that on cells are present on the electrodes. A higher value of "cell number index" indicates that, for same type of the cells and cells under similar physiological conditions, more cells are attached to the electrodes.

In yet another example (**F**), the cell index can be calculated by:

(**F1**) at each measured frequency, subtracting the baseline resistance from the resistance of the electrode arrays when cells are attached to the electrodes to determine the change in the resistance with the cells present relative to the baseline impedance; (here the change in the resistance is given by $\Delta R(f_i) = R_{s-cell}(f_i) - R_{s-baseline}(f_i)$ for the frequency f_i, R_{s-cell} and $R_{s-baseline}$ are the serial resistances with the cells present on the electrode array and the baseline serial resistances, respectively);

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(**F3**) analyzing the frequency dependency of the change of the resistance to derive certain parameters that can quantify such dependency. In one example, such parameters can be calculated as $\sqrt{\sum_i [\Delta R(f_i)]^2}$. In another example, such parameter can be calculated as $\sum_i |\Delta R(f_i)|$. The parameter(s) are used as cell index or cell number index.

In this case, "cell-number index" is derived based on the analysis of the frequency spectrum of the change in the resistance. Depending how the parameters are calculated, the cell index may have a dimension of ohm.

In yet another example (**G**), the cell index can be calculated by:

(**G1**) at each measured frequency, calculating the magnitude of the impedance (equaling to $\sqrt{R_s^2 + X_s^2}$, where R_s and X_s are the serial resistance and reactance, respectively).

(**G2**) subtracting the magnitude of the baseline impedance from the magnitude of the impedance of the electrode arrays when cells are attached to the electrodes to determine the change in the magnitude of the impedance with the cells present relative to the baseline impedance; (here, the change in the magnitude of the impedance is given by $\Delta Z(f_i) = |Z_{cell}(f_i)| - |Z_{baseline}(f_i)|$ for the frequency f_i, $|Z_{cell}(f_i)| = \sqrt{R_{s-cell}(f_i)^2 + X_{s-cell}(f_i)^2}$, R_{s-cell} and X_{s-cell} being the serial resistance and reactance with the cells present on the electrode arrays, respectively, $|Z_{cell}(f_i)|$ is the magnitude of the impedance of the electrode array with cells present on the electrode arrays, $|Z_{baseline}(f_i)|$ is the magnitude of the baseline impedance of the electrode array);

(**G3**) analyzing the frequency dependency of the change of the magnitude of the impedance to derive certain parameters that can quantify such dependency. In one example, such parameters can be calculated as $\sqrt{\sum_i [\Delta Z(f_i)]^2}$. In another example,

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such parameter can be calculated as $\sum_i |\Delta Z(f_i)|$. The parameter(s) are used as cell index or cell number index.

In this case, "cell-number index" is derived based on the analysis of the frequency spectrum of the change in the magnitude of the impedance. Depending how the parameters are calculated, the cell index may have a dimension of ohm.

As described in the US patent application no. 10/705,447, entitled "Impedance based devices and methods for use in assays", filed on November 10, 2003 and PCT application number PCT/US03/22557,entitled "Impedance based devices and methods for use in assays", filed on July 18,. 2003, both herein incorporated by reference for disclosure of Cell Index or Cell Number Index and its calculation, there are different methods for calculating the parameter termed Cell Index or Cell Number Index from the measured cell-substrate impedance (resistance or reactance). Cell Index or Cell Number Index is a quantitative measure of cells in the wells under cell-substrate impedance measurement.

It is worthwhile to point out that it is not necessary to derive such a "cell number index" for utilizing the impedance information for monitoring cell conditions over the electrodes. Actually, one may choose to directly use impedance values (e.g., at a single fixed frequency; or at a maximum relative-change frequency, or at multiple frequencies) as an indicator of cell conditions.

Still, deriving "cell index" or "cell number index" and using such index to monitor cell conditions may have advantages. There are several advantages of using "cell number index" to monitor cell growth and/or attachment and/or viability conditions.

First, one can compare the performance of different electrode geometries by utilizing such cell number index.

Secondly, for a given electrode geometry, it is possible to construct "calibration curve" for depicting the relationship between the cell number and the cell number index by performing impedance measurements for different number of cells added to the electrodes (in such an experiment, it is important to make sure that the seeded cells have well-attached to the electrode surfaces). With such a calibration curve, when a new impedance measurement is performed, it is then possible to estimate cell number from the newly-measured cell number index.

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Thirdly, cell number index can also be used to compare different surface conditions. For the same electrode geometry and same number of cells, a surface treatment given a larger cell number index indicates a better attachment for the cells to the electrode surface and/or better surface for cell attachment.

As shown above, for some methods of calculating cell index or cell number index, it is important to know the impedance (resistance and/or reactance) of the electrode structures with and without cells present on them. Based on the equation (1), the impedance of the electrode array (with or without cells present on the electrodes) is given by

$$Z_{electrode\text{-}array} = Z_{total} - Z_{trace} - Z_{switch} \qquad (5)$$

Where Z_{switch} is the impedance of electronic switch at its on stage, Z_{trace} is the impedance of the electrical connection traces (or electrical conductive traces) on the substrate between the connection pads and the electrode buses, Z_{total} is the total impedance measured at the impedance analyzer. By choosing electronic switches with good quality, it is possible to have all the electronic switches have a consistent on-impedance (mainly resistance). For example, the on-resistance of electronic switches can be about 3 ohm (+/- 10%) with the on reactance being negligible (for example, less than 0.2 ohm in the frequency range of interest). Thus, if the trace impedance is determined or calculated, then formula (5) can be used to calculate the impedance of the electrode arrays with or without cells present.

A method is invented in the present application to determine the impedance of electrical conductive (electrical connection) traces (mainly trace resistance, trace reactance is very small for the thin conductive film trace) based on the relationships among two or more essentially identical arrays on a cell-substrate impedance monitoring device. In the following, the four electrode arrays A, B, C and D as indicated in **Figure 1A**, are used to illustrate this method. The electrical reactance (serial reactance) of the electronic switches and the electrical reactance (serial reactance) of the electrical connection traces are small as compared with the corresponding electrical resistances (serial resistances). Thus, we focus on the analysis of the resistance of the electrical

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connection traces. The impedance determined from the impedance analyzer does contain both resistance (serial resistance, R_{total}) and reactance (serial reactance). For the electrode arrays A – D, the measured total resistance R_{total}, the resistance (R_{trace}) of electrical conductive (connection) trace, the switch resistance (R_{switch}) and the resistance ($R_{e-array}$) of the electrode array satisfy the following equations:

$$R_{e-array-A} = R_{total-A} - R_{trace-A} - R_{switch-A} \quad (6A)$$

$$R_{e-array-B} = R_{total-B} - R_{trace-B} - R_{switch-B} \quad (6B)$$

$$R_{e-array-C} = R_{total-C} - R_{trace-C} - R_{switch-C} \quad (6C)$$

$$R_{e-array-D} = R_{total-D} - R_{trace-D} - R_{switch-D} \quad (6D)$$

With chosen electronic switches having consistent switch-on resistance, $R_{switch-A}$, $R_{switch-B}$, $R_{switch-C}$ and $R_{switch-D}$ have very similar values and can be assumed to be the same, R_{switch}. Thus, in above equations, the known parameters are $R_{total-A}$, $R_{total-B}$, $R_{total-C}$, and $R_{total-D}$, and $R_{switch-A}$, $R_{switch-B}$, $R_{switch-C}$ and $R_{switch-D}$, and there are eight unknown parameters $R_{e-array-A}$, $R_{e-array-B}$, $R_{e-array-C}$, and $R_{e-array-D}$, and $R_{trace-A}$, $R_{trace-B}$, $R_{trace-C}$ and $R_{trace-D}$. It is impossible to solve these equations for the eight unknown variables from these four equations directly. Additional relationships between these variables are needed to solve for them. Each trace resistance ($R_{trace-A}$, $R_{trace-B}$, $R_{trace-C}$ and $R_{trace-D}$) depends on the metal film type used, and the geometry of the trace such as the how many rectangular segments the trace has, the film thickness(es) of the segments, the width(s) of the segments, the length(s) of the segment(s). For example,

$$R_{trace-A} = \sum_{i=1}^{N} \rho \frac{L_{A-i}}{t_{A-i} * d_{A-i}} \quad (7)$$

where N is the number of the segments of the trace-A, t_{A-i}, d_{A-i} and L_{A-i} is the thickness, width and length of the i-th segment of the traces for the electrode array A, and ρ is the resistivity of the thin film. The equation here applies to the film comprising a single type of metal. The equation can be readily modified to be applicable to the film comprising two or more metal types (e.g. gold film over chromium adhesion layer).

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If the film thickness is reasonably uniform (for example, less than 10% in thickness variation) across the substrate, then the relationship among the trace resistances is simply determined by the pre-determined geometrical shapes (e.g. the length, width of the segments). For example, it would be straightforward to calculate the ratio α_{A-D} between the resistance of the electrically conductive traces for the electrode array A to the resistance of the electrically conductive traces for the electrode array D as below, where the film thickness is assumed to be the same everywhere on these traces and the resistivity is also the same everywhere on these traces,

$$\alpha_{A-D} = \frac{R_{trace\_A}}{R_{trace\_D}} = \frac{\sum_{i=1}^{N} \rho \frac{L_{A-i}}{t_{A-i} * d_{A-i}}}{\sum_{i=1}^{M} \rho \frac{L_{D-i}}{t_{D-i} * d_{D-i}}} = \frac{\sum_{i=1}^{N} \frac{L_{A-i}}{d_{A-i}}}{\sum_{i=1}^{M} \frac{L_{D-i}}{d_{D-i}}} . \quad (8)$$

Similarly, one can determine the ratio α_{B-D} and α_{C-D} based on the pre-determined geometrical relationships for the traces of the electrode arrays B, C and D. Note that above equations can be similarly derived for the cases where the thin film in these traces comprises more than one metal type. Thus, based on the equalities

$$R_{switch-A} = R_{switch-B} = R_{switch-C} = R_{switch-D} = R_{switch}, \quad (9A)$$

$$R_{trace-A} = \alpha_{A-D} \bullet R_{trace-D}, \quad (9B)$$

$$R_{trace-B} = \alpha_{B-D} \bullet R_{trace-D}, \quad (9C)$$

and $$R_{trace-C} = \alpha_{C-D} \bullet R_{trace-D} \quad (9D)$$

equations (6A)-(6D) can be re-written in the following format:

$$R_{e-array-A} = R_{total-A} - \alpha_{A-D} \bullet R_{trace-D} - R_{switch} \quad (10A)$$

$$R_{e-array-B} = R_{total-B} - \alpha_{B-D} \bullet R_{trace-D} - R_{switch} \quad (10B)$$

$$R_{e-array-C} = R_{total-C} - \alpha_{C-D} \bullet R_{trace-D} - R_{switch} \quad (10C)$$

$$R_{e-array-D} = R_{total-D} - R_{trace-D} - R_{switch-D} \quad (10D)$$

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For equations (10A) through (10D), there are five unknown variables, $R_{e-array-A}$, $R_{e-array-B}$, $R_{e-array-C}$, and $R_{e-array-D}$ and $R_{trace-D}$. Mathematically, these unknown variables cannot be determined from these equations. Additional information is needed to solve for these variables $R_{e-array-A}$, $R_{e-array-B}$, $R_{e-array-C}$, and $R_{e-array-D}$ and $R_{trace-D}$.

One approach is invented and described in the present invention. In this approach, same biological or chemical solutions or suspensions are applied to the electrode-arrays A through D. Because the electrode arrays A through D have essentially identical electrode structures, the electrode array resistances $R_{e-array-A}$, $R_{e-array-B}$, $R_{e-array-C}$ and $R_{e-array-D}$ should be of same, or very similar value for such a condition when all the electrode arrays are exposed to the same biological or chemical solutions or suspensions, i.e.: $R_{e-array-A} \approx R_{e-array-B} \approx R_{e-array-C} \approx R_{e-array-D}$. If we assume the averaged electrode array resistance is $R_{e-array}$, then these approximate relationship exists $R_{e-array-A} \approx R_{e-array-B} \approx R_{e-array-C} \approx R_{e-array-D} \approx R_{e-array}$. Thus, equations (10A – 10D) can be changed to the following:

$$R_{e-array} \approx R_{total-A} - \alpha_{A-D} \bullet R_{trace-D} - R_{switch} \qquad (11A)$$

$$R_{e-array} \approx R_{total-B} - \alpha_{B-D} \bullet R_{trace-D} - R_{switch} \qquad (11B)$$

$$R_{e-array} \approx R_{total-C} - \alpha_{C-D} \bullet R_{trace-D} - R_{switch} \qquad (11C)$$

$$R_{e-array} \approx R_{total-D} - R_{trace-D} - R_{switch-D} \qquad (11D)$$

Thus, we would need to find $R_{trace-D}$ and $R_{e-array}$ that satisfy the above approximate equality as close as possible. One mathematical approach is to find $R_{trace-D}$ and $R_{e-array}$ that would result in the minimum value for the following expression – an expression that quantifies the differences between the two sides of the approximate equality in (11A, 11B, 11C and 11D),

$$F(R_{trace-D}, R_{e-array}) = \left[R_{e-array} - \left(R_{total-A} - \alpha_{A-D} R_{trace-D} - R_{switch}\right)\right]^2 +$$

$$\left[R_{e-array} - \left(R_{total-B} - \alpha_{B-D} R_{trace-D} - R_{switch}\right)\right]^2 +$$

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$$\left[R_{e-array}-\left(R_{total-C}-\alpha_{C-D}R_{trace-D}-R_{switch}\right)\right]^2+$$

$$\left[R_{e-array}-\left(R_{total-D}-R_{trace-D}-R_{switch}\right)\right]^2 \qquad (12)$$

The expression $F(R_{trace-D}, R_{e-array})$ is the sum of the squared-differences between the two-sides of the approximate equality in (11A, 11B, 11C and 11D). The smaller $F(R_{trace-D}, R_{e-array})$, the closer the two sides of the approximate equality (11A, 11B, 11C and 11D). Thus, values of $R_{trace-D}$ and $R_{e-array}$ that result in the minimum value of $F(R_{trace-D}, R_{e-array})$ should be determined. Mathematical approach involves in the calculation of the first order derivative of $F(R_{trace-D}, R_{e-array})$ to $R_{trace-D}$ and to $R_{e-array}$ and let such first order derivatives equal to zero. The values of $R_{trace-D}$ and $R_{e-array}$ that result in zero for these first-order-derivatives are those that result in the minimum value of $F(R_{trace-D}, R_{e-array})$. The first order derivatives are as follows:

$$\frac{\partial\left[F(R_{trace-D,}R_{e-aaray})\right]}{\partial\ R_{trace-D}}=2\bullet\alpha_{A-D}\bullet\left[R_{e-array}-\left(R_{total-A}-\alpha_{A-D}R_{trace-D}-R_{switch}\right)\right]+$$

$$2\bullet\alpha_{B-D}\bullet\left[R_{e-array}-\left(R_{total-B}-\alpha_{B-D}R_{trace-D}-R_{switch}\right)\right]+$$

$$2\bullet\alpha_{C-D}\bullet\left[R_{e-array}-\left(R_{total-C}-\alpha_{C-D}R_{trace-D}-R_{switch}\right)\right]+$$

$$2\bullet\left[R_{e-array}-\left(R_{total-D}-R_{trace-D}-R_{switch}\right)\right]$$

$$=0; \qquad (13A)$$

$$\frac{\partial\left[F(R_{trace-D,}R_{e-aaray})\right]}{\partial\ R_{e-array}}=2\bullet\left[R_{e-array}-\left(R_{total-A}-\alpha_{A-D}R_{trace-D}-R_{switch}\right)\right]+$$

$$2\bullet\left[R_{e-array}-\left(R_{total-B}-\alpha_{B-D}R_{trace-D}-R_{switch}\right)\right]+$$

$$2\bullet\left[R_{e-array}-\left(R_{total-C}-\alpha_{C-D}R_{trace-D}-R_{switch}\right)\right]+$$

$$2\bullet\left[R_{e-array}-\left(R_{total-D}-R_{trace-D}-R_{switch}\right)\right]$$

$$=0. \qquad (13B)$$

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Equations (13A) and (13B) can be re-written as

$$R_{e-array} \bullet [\alpha_{A-D} + \alpha_{B-D} + \alpha_{C-D} + 1] + R_{trace-D} \bullet [\alpha_{A-D}{}^2 + \alpha_{B-D}{}^2 + \alpha_{C-D}{}^2 + 1] =$$
$$\alpha_{A-D} \bullet [R_{total-A} - R_{switch}] + \alpha_{B-D} \bullet [R_{total-B} - R_{switch}] +$$
$$\alpha_{C-D} \bullet [R_{total-C} - R_{switch}] + [R_{total-D} - R_{switch}] \qquad (14A)$$

$$4 \bullet R_{e-array} + R_{trace-D} \bullet [\alpha_{A-D} + \alpha_{B-D} + \alpha_{C-D} + 1] =$$
$$[R_{total-A} - R_{switch}] + [R_{total-B} - R_{switch}] + [R_{total-C} - R_{switch}] + [R_{total-D} - R_{switch}] \qquad (14B)$$

Thus, we can solve for $R_{trace-D}$ as follows:

$$R_{trace-D} = \frac{4 \bullet S_1 - A_{11} \bullet S_2}{4 \bullet A_{12} - A_{11} \bullet B_{12}} \qquad (15)$$

where $A_{11} = [\alpha_{A-D} + \alpha_{B-D} + \alpha_{C-D} + 1]$;

$A_{12} = [\alpha_{A-D}{}^2 + \alpha_{B-D}{}^2 + \alpha_{C-D}{}^2 + 1]$;

$S_1 = \alpha_{A-D} \bullet [R_{total-A} - R_{switch}] + \alpha_{B-D} \bullet [R_{total-B} - R_{switch}] +$
$\alpha_{C-D} \bullet [R_{total-C} - R_{switch}] + [R_{total-D} - R_{switch}]$;

$B_{12} = [\alpha_{A-D} + \alpha_{B-D} + \alpha_{C-D} + 1]$;

$S_2 = [R_{total-A} - R_{switch}] + [R_{total-B} - R_{switch}] + [R_{total-C} - R_{switch}] + [R_{total-D} - R_{switch}]$.

Thus, with the determined $R_{trace-D}$, the trace resistances of $R_{trace-A}$, $R_{trace-B}$, and $R_{trace-C}$ can be calculated using equations (9B), (9C) and (9D). Furthermore, the electrode array resistance $R_{e-array-A}$, $R_{e-array-B}$, $R_{e-array-C}$ and $R_{e-array-D}$ can be calculated from the measured resistance $R_{total-A}$, $R_{total-B}$, $R_{total-C}$ and $R_{total-D}$ respectively using equations (10A), (10B), (10C) and (10D).

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Thus, one aspect of the present invention is directed to a method of calculation of the resistances of the electrical connection traces s from the measured, total resistances for two or more essentially identical electrode arrays (such as, for example arrays A-D in **Figure 1A)**, comprising the following steps:

(1) exposing the electrode arrays to the solutions having same or similar solutions or suspensions;

(2) with an impedance analyzer or impedance measurement circuit, measuring the resistance (serial resistance) for each electrode array, such resistance being the sum of the resistance of electronic switches, the resistance of the electrical connection traces between the connection pads and the electrode structures (for example, between the connection pads and the electrode buses, for the electrode structures in **Figure 1A**), and the resistance of the electrode array with the solutions or suspensions present;

(3) solving for the resistances of electrical connection traces using equation (15) and equations (9B), (9C) and (9D), noting in the calculation with equation (15), the geometrical relationships between the electrode arrays are used to determine the factor α_{A-D}, α_{B-D} and α_{C-D}.

Another aspect of the present invention is directed to a method of calculating the resistance of the electrode arrays from the measured, total electrode resistances for two ro more essentially identical electrode arrays (such as, for example arrays A-D in **Figure 1A**) if the same or similar solutions or suspensions are added to be in contact with the electrode assays, comprising the following steps:

(1) exposing the electrode arrays to the solutions having same or similar solutions or suspensions;

(2) with an impedance analyzer or impedance measurement circuit, measuring the resistance (serial resistance) for each electrode array, such resistance being the sum of the resistance of electronic switches, the resistance of the electrical connection traces between the connection pads and the electrode structures (for example, between the connection pads and the electrode

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buses, for the electrode structures in **Figure 1A**) and the resistance of the electrode arrays with the solutions or suspensions present;

(3) solving for the resistances of electrical connection traces using equation (15) and equations (9B), (9C) and (9D), noting in the calculation with equation (15), the geometrical relationships between the electrode arrays are used to determine the factor α_{A-D}, α_{B-D} and α_{C-D};

(4) calculating the resistances of the electrode arrays using equations (10A, 10B, 10C and 10D)).

In many applications, the solutions or suspensions (for example, cell suspension) applied to each electrode array may have different compositions. For example, cell suspensions of different cell numbers may be used so that the suspensions applied to each electrode array are quite different. Under such cases, the determination of the resistance of the electrode arrays with the cells present would require the determination of the resistance of the electrical connection traces by performing a "reference run" or "calibration run" in which the electrode arrays are exposed to a same, reference solution. From the "reference run", the resistances of the electrical connection traces can be determined. In a separate test, the electrode arrays are exposed to the solutions or cell suspensions of interest and the resistances for the electrode arrays under such conditions are measured with an impedance analyzer or impedance measuring circuit. The resistance of the electrode arrays with such cell suspensions present can be determined (or continuously determined) from the measured resistance by subtracting the sum of the resistance of the electronic switches and the resistance of the electrical connection traces for corresponding electrode arrays from the measured resistances.

Thus, another aspect of the present invention is directed to a method of calculating the resistance of the electrode arrays from the total electrical resistances measured at an impedance analyzer for essentially identical electrode arrays (such as electrode arrays A-D in **Figure 1A** used as an example) if different solutions or suspensions of interest are applied to the electrode assays, comprising the following steps:

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(1) exposing the electrode arrays to the solutions having same or similar solutions or suspensions (reference solutions);

(2) with an impedance analyzer or impedance measurement circuit, measuring the resistance (serial resistance) for each electrode array, such resistance being the sum of the resistance of electronic switches, the resistance of the electrical connection traces between the connection pads and the electrode structures (for example, between the connection pads and the electrode buses, for the electrode structures in **Figure 1A**) and the resistance of the electrode arrays with the reference solutions present;

(3) solving for the resistances of electrical connection traces using equation (15) and equations (9B), (9C) and (9D), noting in the calculation with equation (15), the geometrical relationships between the electrode arrays of **Figure 1A** are used to determine the factor α_{A-D}, α_{B-D} and α_{C-D};

(4) applying the solutions or suspensions of interest to each electrode array; and with an impedance analyzer or impedance measurement circuit, measuring the resistance (serial resistance) of each electrode array, such resistance being the sum of the resistance of electronic switches, the resistance of the electrical connection traces between the connection pads and the electrode structures, the resistance of the electrode arrays with the solutions or suspensions of the interest present,

(5) Calculating the resistance of the electrode arrays using equations (10A), (10B), (10C) and (10D) by subtracting the electronic switch resistances and the resistances of electrical connection traces from the measured resistances in the step (4).

Note that in above method, the steps of exposing the electrode arrays to reference solutions for the determination of the resistances of electrically conductive traces (step (1), (2) and (3)) may be performed before or after the steps of applying the solutions or suspensions of interest to the electrode arrays and measuring the total electrical resistance (step (4)). For example, step (4) may be performed first. After that, the solutions or suspensions of the interest may be removed from the electrode array. The reference

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solutions can then be added to the electrode arrays (step (1)). Step (2) and step (3) can be then performed to determine the resistances of electrical connection traces. Finally, Step (5) can be done.

In another approach, step (1) and (2) can be performed ahead of step (4).

Another aspect of the present invention is directed to a method of determining the resistance of the electrode arrays with the cells present for a cell-based assay based on the total electrical resistance measured at an impedance analyzer for essentially identical electrode arrays. In this method, the electrode arrays are exposed to a same, reference solution (for example, a same cell culture medium that does not contain any cells) and electrical measurement is conducted to determine the resistance of electrical connection traces. With the resistances of the electrical connection traces determined, electrical resistances of the electrode arrays with cell suspensions added to electrode arrays can be calculated from the total electrical resistances measured at an impedance analyzer. Such total electrical resistance would include the resistance of the electrode arrays with cells present, the resistance of electronic switches and the resistance of electrical connection traces. The method comprises following steps

(1) exposing the electrode arrays to the solutions having same or similar solutions or suspensions (reference solutions);

(2) with an impedance analyzer or impedance measurement circuit, measuring the resistance (serial resistance) for each electrode array, such resistance being the sum of the resistance of electronic switches, the resistance of the electrical connection traces between the connection pads and the electrode structures (for example, between the connection pads and the electrode buses, for the electrode structures in **Figure 1A**) and the resistance of the electrode arrays with the reference solutions present;

(3) solving for the resistances of electrical connection traces using equation (15) and equations (9B), (9C) and (9D), noting in the calculation with equation (15), the geometrical relationships between the electrode arrays in **Figure 1A** are used to determine the factor α_{A-D}, α_{B-D} and α_{C-D};

(4) applying the cell suspensions of interest to each electrode array; and with an impedance analyzer or impedance measurement circuit, measuring the

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resistance (serial resistance) of each electrode array, such resistance being the sum of the resistance of electronic switches, the resistance of the electrical connection traces between the connection pads and the electrode structures, the resistance of the electrode arrays with the cell suspensions of the interest present,

(5) Calculating the resistance of the electrode arrays using equations (10A), (10B), (10C) and (10D) by subtracting the electronic switch resistances and the resistances of electrical connection traces from the measured resistances in step (4).

Note that in above method, the steps of exposing the electrode arrays to reference solution for the determination of the electrical resistance of electrically conductive traces (step (1), (2) and (3)) may be performed before or after the steps of applying the solutions of interest or cell suspensions of interest to the electrode arrays and measuring the total electrical resistance (step (4)). For example, step (4) may be performed first, followed by steps (1) and (2). In one approach, after step (4), the cell suspensions of the interest may be removed from the electrode array. Then reference solutions can be added to the electrode arrays. In another approach, after step (4), the cells are all lysed with some cell lysis solutions so that the electrodes are exposed to the same, reference solutions for the measurement and calculation of step (2) and (3). And then, step (5) is performed to determine the electrical resistance of electrode arrays with the cell suspensions of interest present.

The determination of the resistances of the electrical conductive traces for the electrode arrays that essentially identical electrode arrays may be, or may not be, part of the monitoring of cell-substrate impedance for cell-based assays. It depends on how the impedance data (measured at a single frequency or multiple frequencies, measured at multiple time points) of the electrode arrays is analyzed.

In some assays, one is interested in the relative change in the resistance or impedance of the electrode arrays with the cells present relative to the baseline resistance or impedance. For such cases, it is preferred to determine the resistance (or impedance) of the electrode arrays from the total, measures resistance (or impedance) by subtracting

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the resistance of the electrical conductive traces and the resistance of electronic switches. Thus, determination of the resistances or impedance of the electrically conductive traces may be required.

In some other assays, one is interested in the absolute changes in the resistance (or impedance) of the electrode arrays with cells present relative to the baseline resistance (or impedance). In these cases, one can directly subtract the measured resistance or impedance for the baseline condition from the measured resistance or impedance for the condition that the cells are present on the electrode arrays. The contribution of the resistance (or impedance) of the electronic switches and the resistance (or impedance) of the electrically conductive traces to the total measured resistance (or impedance) values is cancelled out in such subtractions. Thus, there is no need for determining the resistances of the electrically conductive traces.

In some assays, one is interested in calculating the Cell Index or Cell Number Index based on the monitored impedance values. Depending on which method is used for calculating the Cell Index, it may, or may not, be necessary to determine the resistances of the electrically conductive traces. For example, for the Cell Index calculation method (**A**) described above, the resistances of the electrically conductive traces are needed, in order to remove the effect of the resistance of the electrically conductive traces on the analysis of the relative change of the resistance or impedance. In another example, for the Cell Index calculation method (**F**) described above, there is no need to determine the resistances of the electrically conductive traces since the effect of the resistance of the electrically conductive traces is canceled out in the calculations.

The monitoring of the cell-substrate impedance may be or may not be based on the change with respect to the baseline impedance (or resistance). For example, a cell-based assay is performed to assess the effect of a test compound on the cells. One method in performing such an assay is by monitoring of the cell-substrate impedance and determining the change in the cell-substrate impedance before and after the addition of the test compound to the cells. The monitoring of cell-substrate impedance can be performed at a single frequency point or multiple frequency points, at a single time point or multiple time points after drug addition. For example, the impedance is first measured at a single frequency or multiple frequencies for the electrode arrays with the cells

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present just before addition of test compound. The test compound is then added to the cells. The impedance is then measured again at the same single frequency or multiple frequencies for the electrode arrays with the cells after the addition of test compound. Such post-compound addition measurement may be performed for many time points continuously in a regular or irregular time intervals. The change in the cell-substrate impedances can be determined or quantified by subtracting the impedance(s) (resistance and/or reactance) measured before addition of the test compound from the impedance(s) (resistance and/or reactance) measured after addition of the test compound. If the measurement is done at multiple frequencies, a single parameter or multiple parameters may be further derived for each time point after compound addition based on the calculated change in the cell-substrate impedances. Such parameters are used to quantify the cell changes after compound addition. Such approaches can be used further to analyze the responses of the cells to a test compound at multiple concentrations to derive dose-dependent response curves.

D. Methods for performing real-time cell-based assays

The present invention provide cell-based assays that can be performed in real time to assess cell proliferation, cell growth, cell death, cell morphology, cell membrane properties (for example, size, morphology, or composition of the cell membrane) cell adhesion, and cell motility. Thus the assays can be cytotoxicity assays, proliferation assays, apoptosis assays, cell adhesion assays, cell activation assays, anti-cancer compound efficacy assays, receptor-ligand binding and signal transduction analysis, assays of cytoskeletal changes, assays of cell structural changes (including but not limited to, changes in cell membrane size, morphology, or composition), assays of cell differentiation or de-differentiation, assays of cell adhesivity, assays of cell-cell interactions, analysis of microbial and environmental toxins, etc. The assays are real-time assays in the sense that cell behavior or cell condition being assayed can be assessed continuously at regular or irregular intervals. Depending on the applications, cell behaviors, cell responses, or cell conditions being assayed can be within seconds to minutes of their occurrence. The cell response during an assay can be monitored essentially continuously over a selected time period. For example, a culture can be

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monitored every five to fifteen minutes for several hours to several days after addition of a reagent. The interval between impedance monitoring, whether impedance monitoring is performed at regular or irregular intervals, and the duration of the impedance monitoring assay can be determined by the experimenter.

Thus, the cell-based impedance assays of the present invention avoid inadvertently biased or misleading evaluation of cell responses due to the time point or time points chosen for sampling or assaying the cells. In addition, the assays do not require sampling of cell cultures or addition of reagents and thus eliminate the inconvenience, delay in obtaining results, and error introduced by many assays.

Descriptions of cell-substrate monitoring and associated devices, systems and methods of use have been provided in United States provisional application number 60/379,749, filed on July 20, 2002; United States provisional application number 60/435,400, filed on December 20, 2002; United States Provisional application 60/469,572, filed on May 9, 2003, PCT application number PCT/US03/22557,entitled "Impedance based devices and methods for use in assays", filed on July 18, 2003; PCT application number PCT/US03/22537,entitled "Impedance based apparatuses and methods for analyzing cells and particles", filed on July 18, 2003; United States patent application number 10/705,447,entitled "Impedance based devices and methods for use in assays", filed on November 10, 2003; United States patent application number10/705,615,entitled "Impedance based apparatuses and methods for analyzing cells and particles", filed on November 10, 2003, all incorporated herein by reference for their disclosure of cell-substrate impedance devices, systems, and methods of use. Additional details of cell-substrate impedance monitoring technology is further disclosed in the present invention.

In brief, for measurement of cell-substrate or cell-electrode impedance using the technology of the present invention, cell-substrate impedance monitoring devices are used that have microelectrode arrays with appropriate geometries fabricated onto the bottom surfaces of wells such as microtiter plate wells, or have a similar design of having multiple fluid receptacles (wells) having electrodes fabricated on their bottom surfaces facing into the wells. Cells are introduced into the wells of the devices, and make contact with and attach to the electrode surfaces. The presence, absence or change of properties

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of cells affects the electronic and ionic passage on the electrode sensor surfaces.

Measuring the impedance between or among electrodes provides important information about biological status of cells present on the sensors. When there are changes to the biological status of the cells analogue electronic readout signals can be measured automatically and in real time, and can be converted to digital signals for processing and for analysis. In a system of the present invention, a cell index can be automatically derived and provided based on measured electrode impedance values. The cell index obtained for a given well reflects : 1) how many cells are attached to the electrode surfaces in this well, 2) how well (tightly or extensively) cells are attached to the electrode surfaces in this well. Thus, the more the cells of same type in similar physiological conditions attach the electrode surfaces, the larger the cell index. And, the better the cells attach to the electrode surfaces (e.g., the cells spread-out more to have larger contact areas, or the cells attach tighter to electrode surfaces), the larger the cell index.

In one aspect of the present invention, a method is provided for performing cell-based assays, comprising: a) providing a cell-substrate impedance monitoring system of the present invention; b) introducing cells into one or more wells of a device of the system, wherein at least one of the one or more wells comprises an electrode array; and d) monitoring cell-substrate impedance of at least one of the wells that comprise an electrode array and cells.

The method can be used to assay cell status, where cell status includes, not limited to, cell attachment or adhesion status (e.g. the degree of cell spread, the attachment area of a cell, the degree of tightness of cell attachment, cell morphology) on the substrate including on the electrodes, cell growth or proliferation status; number of viable cells and/or dead cells in the well; cytoskeleton change and re-organization and number of cells going through apoptosis and/or necrosis. The cell-based assays that be performed with above methods include, but are not limited to, cell adhesion, cell apoptosis, cell differentiation, cell proliferation, cell survival, cytotoxicity, cell morphology detection, cell quantification, cell quality control, time-dependent cytotoxicity profiling, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes and cell death, detection and quantification of

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neutralizing antibodies, specific T-cell mediated cytotoxic effect, cell-based assay for screening and measuring ligand-receptor binding.

In preferred embodiments of this aspect of the present invention, cells are added to at least two wells of a device, each of which comprises an electrode array, and impedance is monitored from at least two wells that comprise cells and an electrode array.

The cells used in the assay can be primary cells isolated from any species or cells of cell lines. The cells can be engineered cells. In some embodiments, different cell types are added to different wells and the behavior of the cells is compared.

Impedance can be monitored at regular or irregular time intervals. Preferably, impedance is monitored at three or more time points, although this is not a requirement of the present invention. In one embodiment of the above cell-based assay, the cell-substrate impedance is monitored at regular time intervals. Impedance can be monitored at one frequency or at more than one frequency. For example, in some preferred embodiments, impedance is monitored over a range of frequencies for each time point at which impedance is monitored. Preferably, impedance is monitored at at least one frequency between about 1 Hz and about 100 MHz, more preferably at at least one frequency between about 100 Hz and about 2 MHz.

Figure 7 depicts results of the use of methods of the present invention to monitor cell proliferation. In this experiment, H460 cells were introduced into wells of a 16 well device of a cell-substrate impedance monitoring system of the present invention, with different wells receiving different initial cell seeding numbers. The device was engaged with a device station of the system that was in a tissue culture incubator that kept a temperature of 37 degrees C and an atmosphere of 5% CO_2. Cell-substrate impedance was monitored at 15 minute intervals for 125 hours. The cell index was calculated by the system for each time point and displayed as a function of time to give cell growth (proliferation) curves for each cell seeding number. The cell growth curves were plotted on a log scale showing exponential growth phases and stationary phases.

Figure 8 depicts results of real-time monitoring of cell attachment and spreading of NIH3T3 cells. The cells were seeded onto cell-substrate impedance monitoring devices of the present invention that were coated with either poly-L-lysine or fibronectin. The

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device was engaged with a device station that was in a tissue culture incubator that kept a temperature of 37 degrees C and an atmosphere of 5% CO_2. Cell attachment and cell spreading on the difference coating surfaces were monitored by measuring impedance on the cell-substrate monitoring system. Impedance was monitored in real time every 3 minutes for 3 hours. The cell index for each time point was calculated by the impedance monitoring system and plotted as a function of time.

Figure 9 shows the results of an experiment monitoring morphological changes in Cos-7 cells in response to stimulation with epidermal growth factor (EGF). Cells were seeded in wells of a 16 well monitoring device of the present invention that engaged a device station of a cell-substate monitoring system. The device station was positioned in an incubator held at 37 degrees C and 5% CO_2. The cells were serum starved for 8 hours and then stimulated with 50 nanograms/mL of EGF. Control cells did not receive EGF. Impedance was monitored at 3 minute intervals for 2 hours and then at 1 hour intervals for 14 hours. The cell index was calculated by the system and plotted as a function of time. An initial jump in cell index is seen in EGF-treated cells due to membrane ruffling and actin dynamics in response to EGF. The arrow indicated the point of EGF addition.

D.1. Cell-based assays to test the effects of compounds on cells

In yet another aspect, the present invention provides a method for performing a cell-based assay investigating the effect of one or more test compounds on cells, comprising: a) providing a cell-substrate impedance monitoring system of the present invention; b) introducing cells into at least one of the well of the device that comprises an electrode array; c) adding at least one test compound to one or more of the wells comprising cells and an electrode array; and d) monitoring cell-substrate impedance of the one or more wells before and after adding the compound, in which changes in impedance can provide information about cell responses to the one or more compounds.

Information about cell responses to the one or more compounds includes, but is not limited to, information about cell attachment or adhesion status (e.g. the degree of cell spread, the attachment area of a cell, the degree of tightness of cell attachment, cell morphology) on the substrate including on the electrodes, cell growth or proliferation status; number of viable cells and/or dead cells in the well; cytoskeleton change and re-

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organization and number of cells going through apoptosis and/or necrosis. Information about cell status may also include any compound-cell interaction leading to any change to one or more of above cell status indicators. For example, if the compound binds to a receptor on the cell surface and such binding leads to a change in cell morphology, then the binding of compound to the receptor can be assayed by the monitored cell-substrate impedance. The cell-based assays that be performed with above methods include, but not limited to, cell adhesion, cell apoptosis, cell differentiation, cell proliferation, cell survival, cytotoxicity, cell morphology detection, cell quantification, cell quality control, time-dependent cytotoxicity profiling, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes and cell death, detection and quantification of neutralizing antibodies, specific T-cell mediated cytotoxic effect, cell-based assay for screening and measuring ligand-receptor binding.

The cells used in the assay can be primary cells isolated from any species or can be cells of cell lines. The cells can be genetically engineered cells (For example, cells from a genetically modified organism, such as for example from a "gene knockout" organism, or cells that have been engineered to overexpress an endogenous gene or a transgene, or cells whose normal gene expression has been manipulated by use of antisense molecules or silencing RNA.) In some embodiments, different cell types are added to different wells and the behavior of the different cell types in response to one or more compounds is compared.

A test compound can be any compound, including a small molecule, a large molecule, a molecular complex, an organic molecule, an inorganic molecule, a biomolecule such as but not limited to a lipid, a steroid, a carbohydrate, a fatty acid, an amino acid, a peptide, a protein, a nucleic acid, or any combination of these. A test compound can be a synthetic compound, a naturally occurring compound, a derivative of a naturally-occurring compound, etc.

In preferred methods of the present invention, cells are added to at least two wells of the cell-substrate impedance monitoring device that comprise an electrode array, and at least one well that comprises an electrode array and comprises cells does not receive a test compound. A control well that does not receive a test compound can be monitored,

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and its impedance data can be compared with that of wells that do receive compound to determine the effect of the one or more test compounds on cells.

Impedance can be monitored at regular or irregular time intervals. Preferably, impedance is monitored at three or more time points, at least one of which is prior to the addition of one or more test compounds. In one embodiment of the above cell-based assay, the cell-substrate impedance is monitored at at least one time point prior to addition of the test compound, and at regular time intervals thereafter. For example, impedance can be measured at one or more intervals before adding the compound and at a regular 2 hour, 1 hour, 30 min or 15 min time intervals after adding the compound. Preferably, impedance is measured at three or more time points spaced at regular intervals. In the present application, a real-time assay means allows one to perform the measurement on cell-substrate impedance with various time resolutions, for example, measurement taking place at a longer time interval such as every hour or every two hours, or at a shorter time interval every minute or a few minutes.

Impedance can be monitored at one frequency or at more than one frequency. For example, in some preferred embodiments, impedance is monitored over a range of frequencies for each time point at which impedance is monitored. Preferably, impedance is monitored at at least one frequency between about 1 Hz and about 100 MHz, more preferably at at least one frequency between about 100 Hz and about 2 MHz.

Preferably, data from impedance monitoring of a well that comprises cells and a test compound is compared with data from impedance monitoring of a well that comprises cells in the absence of a test compound, however, this is not a requirement of the present invention. For example, it is also possible to compare impedance measurements from one or more time points prior to the addition of compound to compare impedance measurements from one or more time points after the addition of compound. Such comparisons can be used directly to assess the cells' response to a compound. It is also possible to calculate a cell index (or cell number index) using the impedance values obtained. Methods of calculating a cell index (cell number index) are disclosed herein as well as in parent application U.S. Patent Application 10/705,447, herein incorporated by reference for disclosures relating to cell number index and its calculation. The cell index calculated from impedance measurements of wells receiving

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compound can be compared with the cell index calculated from impedance measurements of control wells to assess the effect of a compound on cells. Alternatively, cell index calculated from impedance measurements of wells from one or more time points after the addition of a compound can be compared with the cell index calculated from impedance measurements of wells from one or more time points prior to the addition of a compound to assess the effect of a compound on cells. In some preferred embodiments, the cell index can be used as an indicator of cytotoxicity.

The present invention includes assays in which different concentrations of a compound are added to wells of a device. The method includes: a) providing a cell-substrate impedance monitoring system of the present invention; b) introducing cells into at least two wells of the device that each comprise an electrode array; c) adding to at least one well of the device comprising cells and an electrode array a first concentration of a test compound; d) adding to at least one other well of the device comprising cells and an electrode array a second concentration of a test compound; and e) monitoring cell-substrate impedance of the two or more wells before and after adding the compound, in which changes in impedance can provide information about cell responses to the compound.

Information about cell responses to the compound includes, but is not limited to, information about cell attachment or adhesion status (e.g. the degree of cell spread, the attachment area of a cell, the degree of tightness of cell attachment, cell morphology) on the substrate including on the electrodes, cell growth or proliferation status; number of viable cells and/or dead cells in the well; cytoskeleton change and re-organization and number of cells going through apoptosis and/or necrosis. Information about cell status may also include any compound-cell interaction leading to any change to one or more of above cell status indicators. For example, if the compound binds to a receptor on the cell surface and such binding leads to a change in cell morphology, then the binding of compound to the receptor can be assayed by the monitored cell-substrate impedance. The cell-based assays that be performed with above methods include, but not limited to, cell adhesion, cell apoptosis, cell differentiation, cell proliferation, cell survival, cytotoxicity, cell morphology detection, cell quantification, cell quality control, time-dependent cytotoxicity profiling, IgE-mediated cell activation or stimulation, receptor-ligand

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binding, viral and bacterial toxin mediated cell pathologic changes and cell death, detection and quantification of neutralizing antibodies, specific T-cell mediated cytotoxic effect, cell-based assay for screening and measuring ligand-receptor binding.

The cells and test compound used in the assay can be as described above for assays testing effects of test compounds. In preferred methods of the present invention, cells are introduced into at least three wells of the device that each comprise an electrode array, and at least one well that comprises an electrode array and comprises cells does not receive test compound. A control well that does not receive a test compound can be monitored, and its impedance data can be compared with that of wells that do receive compound to determine the effect of the one or more test compounds on cells.

Impedance monitoring can be as described immediately above for assays testing effects of test compounds.

Preferably, data from impedance monitoring of wells that comprise cells and different concentrations of test compounds are compared. Such comparisons can be used directly to assess the cells' response to increasing concentrations of a compound. It is also possible to calculate a cell index (or cell number index) using the impedance values obtained. Methods of calculating a cell index (cell number index) are disclosed herein as well as in parent application U.S. application No. 10/705,447, herein incorporated by reference for disclosure of cell index number and method of calculating cell index number. In some preferred embodiments, the cell index can be used as an indicator of cytotoxicity.

The cell index calculated from impedance measurements of wells receiving different concentrations of compound can be compared to assess the effect of a compound on cells. Alternatively, cell index calculated from impedance measurements of wells comprising different concentrations of a compound can be compared. Dose response relationships can be derived from such comparisons. In some preferred embodiments, time dependent IC50 values may be calculated from cell index values for compounds that exhibit cytotoxicity or inhibit particular cell responses.

In one embodiment of the method, analyzing the cytotoxicity response may include derivation of the slope of change in the time dependent cytotoxicity response at a given compound concentration. In yet another embodiment of the method, analyzing

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real-time cytotoxicity response may include derivation of high-order derivatives of the time dependent cytotoxicity response with respect to time at a given compound concentration.

Cell-based Assays with More Than One Compound

In yet another aspect, the present invention provides a method for performing a cell-based assay investigating the effect of two or more test compounds on cells. The method includes: a) providing a cell-substrate impedance monitoring system of the present invention; b) introducing cells into at least two wells of the device that each comprise an electrode array; c) adding to at least one well of the device comprising cells and an electrode array a first test compound; d) adding to at least one other well of the device comprising cells and an electrode array a second test compound; and e) monitoring cell-substrate impedance of at least one well comprising cells and a first compound and at least one well comprising cells and a second compound, in which changes in impedance can provide information about cell responses to the first and second compounds.

Preferably, time-dependent responses of cells to the first compound and the second compound are compared to see how similar or different the responses from the two compounds are. In one preferred embodiment of this method, time-dependent cytotoxic responses are compared.

The cells and test compound used in the assay can be as described above for assays testing effects of test compounds. In preferred methods of the present invention, cells are introduced into at least three wells of the device that each comprise an electrode array, and at least one well that comprises an electrode array and comprises cells does not receive a test compound. A control well that does not receive a test compound can be monitored, and its impedance data can be compared with that of wells that receive a compound to determine the effect of the test compounds on cells.

Impedance monitoring can be as described immediately above for assays testing effects of test compounds.

Preferably, data from impedance monitoring of wells that comprise different test compounds are compared. In one preferred embodiment impedance monitoring is

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performed for the first compound at multiple dose concentrations. In another embodiment, time-dependent cellular responses are determined for the second compound at multiple dose concentrations. In yet another embodiment, time-dependent cellular responses are determined for both first compound and second compound at multiple dose concentrations.

In another embodiment of above method, the first compound is a compound with a known mechanism for its cytotoxic effect and the second compound is a compound with an unknown mechanism for its cytotoxic effect. If the time dependent cytotoxic responses from the second compound are similar to that of the first one, the second compound may follow a similar mechanism for its cytotoxic effect to the first compound.

Various approaches may be used in comparing the cytotoxic responses of the compounds. A cell index (or cell number index) can optionally be calculated using the impedance values obtained. In one embodiment of the method described above, time dependent IC50 may be derived for the compounds and comparison between their cytotoxic responses is done by comparing their time dependent IC50 curves based on cell index values. If the IC50 curves follow a similar time-dependent trend, the two compounds may follow a similar mechanism for inducing cytotoxicty effects. In another embodiment of the method described, direct comparison of time-dependent cytotoxic responses of two compounds are done where the concentrations for the two compounds may be the same or may be different. Direct comparison between time-dependent cytotoxic responses may be done by analyzing the slope of change in the measured responses (that is equivalent to the first order derivative of the response with respect to time) and comparing the time-dependent slopes for the two compounds. In another approach, the time-dependent cytotoxic responses may be analyzed for their higher order derivatives with respect to time. Comparing such high order derivatives may provide additional information as for the mechanisms of compound-induced cytotoxicity.

Cell-based Assays with More Than One Cell Type

In yet another aspect, the present invention provides a method for cytotoxicity profiling for a compound on multiple cell types, comprising: a) providing a cell-substrate impedance monitoring system of the present invention; b) introducing a first type of cells

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into at least one well of the device that comprises an electrode array; c) introducing a second type of cells into at least one other well of the device that comprises an electrode array; d) adding a test compound to at least one well comprising cells of a first type and to at least one well comprising cells of a second type; and e) monitoring cell-substrate impedance of at least one well comprising cells of a first type and test compound and of at least one well comprising cells of a second type and test compound, in which changes in impedance can provide information about cell responses to the first and second compounds.

Preferably, time-dependent responses of the first and second types of cells are compared to see how similar or different the responses from the two types of cells are. In one preferred embodiment of this method, time-dependent cytotoxic responses are compared.

The cell types used in the assay can be primary cells isolated from any species or can be cells of cell lines. In some preferred embodiments, the different cell types are the same type of cell from different individuals, and thus have different genotypes. One or more of the cell types can be genetically engineered (For example, cells from a genetically modified organism, such as for example from a "gene knockout" organism, or cells that have been engineered to overexpress an endogenous gene or a transgene, or cells whose normal gene expression has been manipulated by use of antisense molecules or silencing RNA.) In these cases, genetically modified cells can be compared with control cells. In some embodiments, three or more different cell types are added to different wells and the behavior of the three or more different cell types in response to one or more compounds is compared.

The test compound or compounds used in the assay can be as described above for assays testing effects of test compounds. In preferred methods of the present invention, cells are introduced into at least three wells of the device that each comprise an electrode array, and at least one well that comprises an electrode array and comprises cells does not receive a test compound. A control well that does not receive a test compound can be monitored, and its impedance data can be compared with that of wells that receive a compound to determine the effect of the test compounds on cells. In preferred

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embodiments of the present invention, for each cell type tested there is a control performed in which the control does not receive test compound.

Impedance monitoring can be as described immediately above for assays testing effects of test compounds.

Preferably, data from impedance monitoring of wells that comprise different cell types are compared. In one preferred embodiment impedance monitoring is performed for different cell types exposed to multiple dose concentrations of a compound. In some embodiments, multiple compounds can be tested with multiple cell types. In some embodiments, multiple compounds at multiple concentrations can be tested with multiple cell types.

In one embodiment of the method, analyzing real-time cytotoxicity response may include the derivation of time-dependent IC50 values for the compound on the multiple cell types. In another embodiment of the method, analyzing real-time cytotoxicity response may include derivation of the slope of change in the time dependent cytotoxicity response at a given compound concentration. In yet another embodiment of the method, analyzing real-time cytotoxicity response may include derivation of high-order derivatives of the time dependent cytotoxicity response with respect to time at a given compound concentration.

In one embodiment of the method, analyzing real-time cytotoxicity responses may include the derivation of time-dependent IC50 values for the compound on the multiple cell types. In yet another embodiment, the above methods are applied to perform cytotoxicity profiling of multiple compounds on multiple cell types.

In another embodiment of the method, analyzing real-time cytotoxicity response may include derivation of the slope of change in the time dependent cytotoxicity response at a given compound concentration. In yet another embodiment of the method, analyzing real-time cytotoxicity response may include derivation of high-order derivatives of the time dependent cytotoxicity response with respect to time at a given compound concentration.

Some examples of compound assays that can be performed using a cell-substrate impedance system of the present invention are provided by way of illustration with reference to the figures. In these examples, cell index is calculated using the same method

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as the Cell Index calculation method (A) as described in Section C of the present application. In some of the figures of the present application, Normalized Cell Index was plotted. The Normalized Cell Index at a given time point is calculated by dividing the Cell Index at the time point by the Cell Index at a reference time point. Thus, the Normalized Cell Index is 1 at the reference time point.

As described in the present application, if the cell attachment conditions remain unchanged or exhibit little change over the course of an assay that uses impedance monitoring, then the larger the cell index, the larger the number of the cells in the wells. A decrease in cell index suggests that some cells are detaching from the substrate surface or dying under the influence of the compound. An increase in cell index suggests that more cells are attaching to the substrate surfaces, indicating an increase in overall cell number.

Figure 10 shows curves that represent the time-dependent cell index for H460 cells treated with different concentrations of the anticancer drug paclitaxel. In this experiment, H460 cells were introduced into wells of a 16X cell-substrate impedance monitoring device. The device was positioned on a device station that was located in an incubator maintaining conditions of 37 degrees C and 5% CO_2. The cells were cultured and treated at their exponential growth phase with different concentrations of paclitaxel. The dynamic response of the cells to different doses of paclitaxel was monitored by monitoring cell-substrate impedance in real time every 15 minutes for 50 hours after treatment using a cell-substrate impedance monitoring system. The cell-substrate impedance monitoring system calculated the cell index at each time point monitored and plotted the cell index as a function of time. For paclitaxel concentrations between 67 nanomolar and 500 nanomolar, H460 cells exhibited a gradual decrease in cell index after compound addition. However, the cell index reached a minimum at a time dependent on the compound concentration, between about 15 hours and 20 hours after compound addition. After that point, there was a gradual increase in cell index in these wells. The cell index for compound concentration of 33 nanomolar exhibited a near-constant value for up to about 15 hours after compound addition. After 15 hours following compound addition, the cell index exhibited a gradual increase.

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Figure 11 shows curves that represent the time-dependent cell index for H460 cells treated with anticancer drug AC101103. H460 cells were introduced into wells of a 16X cell-substrate impedance monitoring device. The device was positioned on a device station that was located in an incubator maintaining conditions of 37 degrees C and 5% CO_2. The cells were cultured and treated at their exponential growth phase with different concentrations of AC101103. The dynamic response of the cells to different doses of AC101103 was monitored by measuring impedance in real time every 30 minutes for about 20 hours after treatment on the cell-substrate monitoring system.

Notably, the time-dependent cell index in **Figure 11** is significantly different from those shown in **Figure 10**. For compound concentrations at 3.125 microgram/ml, 6.25 microgram /ml and 12.5 microgram /ml, the cell index exhibited a near-constant value for about 5 hrs, about 15 hrs and > 20 hrs respectively. For compound concentrations at 3.125 microgram /ml and 6.25 microgram/ml, the cell index started to increase after about 5 hrs and about 15 hrs following compound addition. For the compound concentration of 25 microgram/ml, there was a gradual, yet slow decrease in the cell index after compound addition. For the compound concentration of 50 microgram /ml, there was an about 10 hr time period over which the cell index remained near-constant, and after that, the cell index decreased steadily.

Figure 12 shows dynamic drug response curves of A549 cells treated with doxorubicin. 10,000 A549 cells were seeded into each well of a 16 X device. The device was positioned on a device station that was located in an incubator maintaining conditions of 37 degrees C and 5% CO_2. Cell attachment and cell growth were monitored on a cell-substrate impedance system in real time before treatment by monitoring impedance at regular intervals. When the cells were in exponential growth phase, doxorubicin at different concentrations was added to the wells. The same volume of the solvent used to dissolve the drug was added to some wells as a control. The time, and drug dose dependent cell response (calculated as cell index) to doxorubicin was recorded in real time on the cell-substrate impedance monitoring system as shown in this figure.

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E. Cell-Substrate Impedance Assays to Monitor IgE-Mediated Cell Activation

The present invention also includes methods of monitoring cell-substrate impedance of cells stimulated by IgE. The method is based on quantifying in real time the cytoskeletal changes that result from antigen binding to the IgE-Fc(epsilon)RI complex on the surface of responsive cells, such as, but not limited to, mast cells. The electronic assays provided rely on cytoskeletal dynamics that are an intrinsic mast cell response to antigen and an essential part of the mast cell activation program, and precludes the need for establishing reporter cell lines or using other assay reagents. Furthermore, since the assay is performed in real time, both antigen-dependent and antigen-independent response to IgE-mediated activation of mast cells can be monitored in the same assay.

The assays described herein that monitor cell-substrate impedance of cells stimulated by IgE can use any cell-substrate impedance measuring device, including but not limited to those described in parent U.S. Patent Application 10/705,447 and herein. A cell-substrate impedance device useful in the methods of the present invention refers to any device that has a surface suitable for cells and has electrodes that cells can settle on and interact with. The measurement on the electrode impedance can reflect the cell status such as cell number, cell morphology, or cell adhesion. In preferred embodiments, a cell-substrate impedance device useful in the methods of the present invention comprises a substrate that comprises one or more electrode arrays on its surface, in which each of the one or more electrode arrays comprises two electrodes or electrode structures, where the two electrodes or electrode structures have substantially the same surface area. In operation, a cell-substrate impedance device used in the methods of the present invention can detect impedance changes at one or more frequencies due to changes in cell number, cell size, cell morphology, cell attachment to the substrate, and the quality of cell attachment to the substrate.

Preferably a cell-substrate impedance device used in the methods of the present invention comprises at least one fluid container that surrounds an electrode array of the device and provides a fluid-impermeable container for cells being monitored. In preferred embodiments, a device comprises at least two arrays and at least two receptacles in the form of wells, where each array of the device is encompasses by a well. More preferably, a device used in the screening methods described in the present application comprises at

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least 8 wells (for example, devices comprising 16 or 96 wells), of which the majority comprise electrode arrays, so that assays can be performed in a high-throughput fashion. Cells can optionally be assayed in multiple multi-well devices simultaneously (for example, connected to the same impedance analyzer, engaged with a device station that can engage more than one multi-well device, or connected to separate impedance analyzers) to increase high-throughput capacity.

Systems for monitoring cell-substrate impedance that comprise one or more multiwell devices, an impedance analyzer, and a device station are described herein and are preferred but not required for use in the methods of the present invention.

The cells used in the IgE stimulation assays can be any cells, isolated from one or more organisms or from cell lines. Cells can be isolated from blood or other tissues of one or more organisms. Preferably the cells are mammalian cells. Preferably the cells are mast cells, basophils, or eosinophils, but that is not a requirement of the present invention. The cells can be genetically engineered cells of any type that express components of the IgE response pathway. Cells can be engineered to inappropriately express or overexpress components of the IgE response pathway. Cells can also be engineered to express dominant negative or other altered versions of components of the IgE response pathway, or to ablate expression of components of the IgE response pathway (for example, by use of homologous recombination knockouts, antisense or silencing RNA technologies). As used herein, "components of the IgE response pathway" also includes molecules suspected of being components of the IgE response pathway.

In some embodiments of some aspects of the present invention, RBL-2H3 rat mast cells can be used investigate the IgE-mediated signaling mechanism and also to assess the effect of various inhibitors on the signaling pathways leading to mast cell degranulation and mediator release. RBL-2H3 cells offer the advantage of being maintained and expanded in culture and a large body of literature which has utilized this cell line as a model system for IgE-mediated mast cell activation. However, the methods and systems of the present invention can readily be adapted to other cells that may be of academic and pharmaceutical interest. The cells include but are not limited to mouse bone marrow mast cells, human lung mast cells, human skin mast cells, and mast cells and basophils from other mammalian species.

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The following subsections disclose assays that use impedance monitoring devices that can measure both IgE binding and antigen-mediated IgE-Fc(epsilon)RI cross-linking leading to mast cell morphological changes and degranulation. The assays can be performed in high-throughput format, in real time and without the need for any other reagents or cellular manipulation.

E.1. Methods for monitoring changes in cell-substrate impedance in response to IgE stimulation

IgE-mediated cell stimulation has been shown to lead to dramatic morphological changes in cells such as mast cells. For example, RBL-2H3 mast cells are transformed from spindle shape morphology to flattened and fibroblastic cell morphology. Cell-substrate impedance monitoring devices of the present invention can be used to detect cell shape changes and changes in cell-substratum interaction that result from IgE-mediated stimulation of responsive cells.

The method comprises: a) providing a device for cell-substrate impedance monitoring that comprising at least one well that comprises at least one electrode array; b) connecting an impedance analyzer to the device; c) introducing cells into one or more wells of the device that comprise an electrode array; d) adding IgE to the one or more wells comprising cells; e) adding at least one antigen, at least one allergen, or at least one IgE crosslinker to the one or more wells comprising cells; and e) monitoring cell-substrate impedance of the one or more wells comprising cells.

In the methods of the present invention, a device for cell-substrate impedance monitoring is a device that comprises a substrate having one or more electrode arrays on its surface each of which is encompassed by a fluid container in the form of a well. Each of the one or more electrode arrays comprises two electrodes or electrode structures, where the two electrodes or electrode structures have substantially the same surface area , in which the device, when connected to an impedance analyzer, can detect impedance changes at one or more frequencies due to changes in cell number, cell size, cell morphology, cell attachment to the substrate, or the quality of cell attachment to the substrate.

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The cells can be any cells whose response or possible response to IgE stimulation is of interest. Preferred cells are mast cells, eosinophils, basophils, or genetically engineered cells. In some preferred embodiments, the cells are of mammalian origin.

Ig E can be added before, after, or simultaneous with the addition of the antigen, allergen, or IgE crosslinker. IgE can be added to wells at a concentration of from about 10 nanograms per milliliter to about 1 microgram per milliliter. Preferably, one well of the device that comprises an electrode array and cells does not receive IgE to provide at least one control well, but this is not a requirement of the present invention.

Antigens, allergens, or crosslinkers can be known or suspected antigens, allergens, or crosslinkers. The concentration added to the wells can be from about 1 nanogram per milliliter to about 1 microgram per milliliter. Different wells may receive different concentrations of an antigen, allergen, or crosslinker. Or, for replica purposes, multiple wells may receive same concentrations of an antigen, allergen or crosslinker. Preferably, one well of the device that comprises an electrode array and cells does not receive antigen, allergen, or crosslinker to provide at least one control well, but this is not a requirement of the present invention.

The method can be used to assess and quantify the morphological changes that occur in mast cells as a result of *IgE* stimulation and *IgE* cross-linking with an antigen by using cell-substrate impedance technology. Impedance is preferably monitored during two or more phases of the experiment. For example, in one embodiment, impedance is monitored before and after the addition of IgE to the wells. . In another embodiment, impedance is monitored before and after the addition of antigen, allergen, or crosslinker to the wells. In yet another embodiment, impedance can be monitored before and after the addition of IgE plus antigen, allergen, or crosslinker to the wells. . Impedance can be also be monitored after the addition of IgE to the wells and before the addition of antigen, allergen, or crosslinker to the wells, as well as after the addition of antigen, allergen, or crosslinker to the wells. In some preferred embodiments, impedance is monitored before addition of IgE to the wells, after the addition of IgE to the wells and before the addition of antigen, allergen, or crosslinker to the wells, and after the addition of antigen, allergen, or crosslinker to the wells.

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Impedance can be monitored at two or more time points. Preferably, impedance is monitored at at least three time points. Preferably, impedance is monitored at at least three time points at two or more phases of the assay. Impedance at each time point can be monitored at one or at more than one frequency. Data obtained from impedance monitoring can be used to assess morphological changes to cells that result from IgE stimulation. For example, impedance before and after addition of IgE, and/or before and after addition of antigen, allergen, or crosslinker can be compared for each well. Impedance data can be compared for wells that receive IgE and control wells that do not receive IgE. Impedance data can be compared for wells that receive antigen, allergen, or crosslinker and control wells that do not receive antigen, allergen, or crosslinker. Impedance data can be used to calculate a cell index, which can be used for comparisons.

One exemplary protocol is as follows:

(1) Add a predetermined number of mast cells to the wells of either a 16-well or 96 well cell-substrate impedance measurement device that is attached to an impedance analyzer.

(2) Allow the cells to attach and grow for 16-20 hours while recording the IgE anywhere from 10 nanogram/mL to 1 microg/mL final concentration and continue recording the cellular response using a cell-substrate impedance monitoring system as described herein.

(3) At 16-20 hours after IgE stimulation, change media to serum free media. Allow the cells to recover for 30 minutes and then stimulate with antigen. Continue monitoring the cellular response to antigen on the impedance monitoring system.

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E.2. Methods of screening for compounds that can modulate a cellular response to IgE stimulation

The present invention also includes methods of assessing the effect of one or more compounds on one or more cellular responses to IgE-mediated stimulation. The methods comprise: a) providing a device for cell-substrate impedance monitoring that comprising at least one well that comprises an electrode array; b) connecting an impedance analyzer to the device; c) introducing cells into one or more wells of the device that comprise an electrode array; d) adding at least one test compound to one or more of the one or more wells that comprise cells; e) adding IgE to the one or more wells comprising cells and at least one test compound; e) adding at least one antigen, at least one allergen, or at least one IgE crosslinker to the one or more wells comprising cells and at least one test compound; and e) monitoring cell-substrate impedance of the one or more wells comprising cells and at least one test compound.

In the methods of the present invention, a device for cell-substrate impedance monitoring is a device that comprises a substrate having one or more electrode arrays on its surface, each of which is encompassed by a fluid container in the form of a well. Each of the one or more electrode arrays comprises two electrodes or electrode structures, where the two electrodes or electrode structures have substantially the same surface area . The device, when connected to an impedance analyzer, can detect impedance changes at one or more frequencies due to changes in cell number, cell size, cell morphology, cell attachment to the substrate, or the quality of cell attachment to the substrate.

Preferably, a device used in the methods of the present invention is part of a cell-substrate impedance monitoring system as described herein.

The cells can be any cells that have a detectable response to IgE stimulation. Preferred cells are mast cells, eosinophils, basophils, or genetically engineered cells. In preferred embodiments, the cells are of mammalian origin.

Ig E can be added before, after, or simultaneous with the addition of the antigen, allergen, or IgE crosslinker. IgE can be added to wells at a concentration of from about 10 nanograms per milliliter to about 1 microgram per milliliter. Optionally, one well of

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the device that comprises an electrode array and cells does not receive IgE to provide a control well.

Antigens can be known or suspected antigens, allergens, or crosslinkers. The concentration added to the wells can be from about 1 nanogram per milliliter to about 1 microgram per milliliter. Different wells may receive different concentrations of an antigen, allergen, or crosslinker. Or, for replica purposes, multiple wells may receive same concentrations of an antigen, allergen or crosslinker. Optionally, one well of the device that comprises an electrode array and cells does not receive antigen, allergen, or crosslinker to provide a control well.

A test compound can be any compound, including a small molecule, a large molecule, a molecular complex, an organic molecule, an inorganic molecule, a biomolecule such as but not limited to a lipid, a steroid, a carbohydrate, a fatty acid, an amino acid, a peptide, a protein, a nucleic acid, or any combination of theses. A test compound can be a synthetic compound, a naturally occurring compound, a derivative of a naturally-occurring compound, etc. The structure of a test compound can be known or unknown. One or many compounds can be assayed in a single experiment. Compounds can be tested in combination. Different concentrations of a given compound can be assayed using the methods of the present invention.

The advent of combinatorial chemistry has allowed for the generation of large and diverse compound libraries that can be used to screen for potential inhibitors of *IgE*-mediated mast cell activation. Assays can be performed in cell-substrate impedance monitoring systems of the present invention to screen compound libraries for potential inhibitors in high-throughput manner.

Test compounds can be compounds that interfere with IgE-mediated signaling through the Fc(epsilon)RI receptor expressed on the surface of mast cells and basophils. For example, a test compound can be an antibody raised against the IgE. or a recombinant protein comprising all or a portion of the antigen binding domain of Fc(epsilon)RI that can be tested for its ability to specifically bind to the IgE and prevent it from interacting with the endogenous Fc(epsilon)RI. Test compounds can also be candidates for small molecule inhibitor compounds that can bind to the IgE-binding pocket of Fc(epsilon)RI and block its interaction with the IgE molecule. Test compounds

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can also be siRNAs that can be tested for their ability to down-regulate the expression of Fc(epsilon)RI on the surface of mast cells.

Test compounds can also be screened to identify compounds that can act intracellularly to inhibit cellular response to IgE stimulation. For example, test compounds can be screened to identify inhibitors of kinases or lipases that have a role in cell signaling in response to IgE stimulation. For example, the methods of the present invention can be used to screen for compounds that can inhibit protein kinase C, SRC, Syk, or PLC(gamma).

A test compound is preferably added to wells prior to the addition of IgE, but can also be added after the addition of IgE and before the addition of antigen, or after the addition of IgE and antigen. In embodiments where IgE and antigen are added at the same time, a test compound can be added after but is preferably added after the addition of IgE and antigen.

The method can be used to assess and quantify the effects of a compound on the response of cells, such as but not limited to mast cells, as a result of IgE by using cell-substrate impedance technology. Impedance is preferably monitored during two or more phases of the experiment. Depending on the experimental protocol, impedance is preferably monitored after adding IgE to the one or wells, and is preferably monitored before and after adding IgE to the one or wells. Impedance is preferably monitored after the addition of test compound, before and after the addition of IgE. Impedance can also be monitored after the addition of test compound and IgE, before and after addition antigen. In some preferred embodiments, impedance is monitored after the addition of test compound and before addition of IgE to the wells, and after the addition of IgE to the wells and before the addition of antigen, allergen, or crosslinker to the wells, and after the addition of antigen, allergen, or crosslinker to the wells.

Impedance is preferably monitored at two or more time points. Preferably, impedance is monitored at at least three time points. Preferably, impedance is monitored at at least three time points at two or more phases of the assay. Impedance at each time point can be monitored at one or at more than one frequency. Data obtained from impedance monitoring can be used to assess the effects of a test compound on the morphological changes to cells that result from IgE stimulation. For example,

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impedance before and after addition of test compound, and/or before and after addition of IgE , and/or before and after addition of antigen, allergen, or crosslinker can be compared for each well. Impedance data can be compared for wells that receive test compound and control wells that do not receive test compound Impedance data can be used to calculate a cell index, which can be used for comparisons. Such comparisons can be used to identify compounds that inhibit the response of cells such as mast cells to IgE stimulation. For example, IC50s can be calculated from recorded impedance values for one or more test compounds that inhibit a cellular response to IgE stimulation

One exemplary protocol for compound screening is as follows:

(1) A predetermined number of mast cells is added to the wells of either a 16-well or 96 well cell-substrate impedance measurement device that is part of a cell impedance monitoring system.

103. The attachment and growth of the cells is monitored using the impedance monitoring system.

104. The cells are pre-incubated with increasing concentrations of one or more potential inhibitors of interest (anti-*IgE* antibody, recombinant fragment of the Fc(epsilon)RI antigen binding domain or small molecular compounds) for a defined length of time.

105. IgE specific for the cell line used is administered and the transient morphological changes of the cells due IgE stimulation is electronically monitored by the system. The extent of inhibition and IC-50 determination is assessed by comparing the peak response of the recording of the cells in the presence of increasing concentrations of the inhibitor.

In another example of the methods of the present invention, mast cells or other cells of interest are dispensed into a 96 well device either by liquid handling station or by multi-channel pipette. Cell attachment and growth is monitored as above. To assess the effect of the inhibitors on IgE-alone mediated signaling, inhibitors at a single

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concentration or multiple concentrations are pre-incubated with the cells prior to IgE application. The cellular response to IgE in the presence of the drug is then monitored as described above. Alternatively, if the effect of the drugs are to be assessed in response to the antigen cross-linking of the IgE-Fc(epsilon)RI complex, the inhibitors are preincubated with the cells just prior to the addition of the antigen as described above.

E.3 Methods for target validation of enzymes and proteins involved in the signaling pathway initiated by engagement of the high-affinity Fc(epsilon)RI receptor in the presence or absence of IgE cross-linking by antigen using the cell-substrate impedance technology

The intracellular signaling pathway that is stimulated by engagement of the Fc(epsilon)RI by IgE involves the activation of key enzymes such as but not limited to kinases, phosphatases and phospholipases. These downstream mediators of the cellular response to IgE are potential targets for pharmaceutical drug discovery. However, prior to screening for potential inhibitors of these target proteins and enzymes, they must be validated to ascertain that they can interfere with IgE-mediated signaling. This can be achieved by introducing into responsive cells by transfection, electroporation or viral infection the DNA encoding for the dominant negative versions of suspected target proteins or one or more siRNAs that target and reduce the expression of these proteins. Alternatively, antisense reagents can be used to reduce or ablate expression of a suspected target.

The method includes: a) providing a device for cell-substrate impedance monitoring that comprising at least one well that comprises an electrode array; b) connecting an impedance analyzer to the device; c) introducing cells into one or more wells of the device that comprise an electrode array, wherein said cells are genetically modified to alter the function of, or reduce or ablate the expression of, a suspected target molecule; d) adding IgE to the one or more wells comprising cells; e) adding at least one antigen, at least one allergen, or at least one IgE crosslinker to the one or more wells comprising cells; and e) monitoring cell-substrate impedance of the one or more wells comprising cells.

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In the methods of the present invention, a device for cell-substrate impedance monitoring is a device that comprises a substrate having one or more electrode arrays on its surface, each of which is encompassed by a fluid container in the form of a well. Each of the one or more electrode arrays comprises two electrodes or electrode structures, where the two electrodes or electrode structures have substantially the same surface area . The device, when connected to an impedance analyzer, can detect impedance changes at one or more frequencies due to changes in cell number, cell size, cell morphology, cell attachment to the substrate, or the quality of cell attachment to the substrate.

Devices for measuring cell-substrate impedance are described in parent U.S. Patent Application 10/705,447 and in the present application. Preferably a cell-substrate impedance device used in the methods of the present invention comprises at least one fluid container that surrounds an electrode array of the device and provides a fluid-impermeable container for cells being monitored. In preferred embodiments, a device comprises at least two arrays and at least two receptacles in the form of wells, where each array of the device is encompassed by a well. More preferably, a device used in the screening methods described in the present application comprises at least 8 wells (for example, devices comprising 16 or 96 wells), of which the majority comprise electrode arrays, so that assays can be performed in a high-throughput fashion. Cells can optionally be assayed in multiple multi-well devices simultaneously (for example, connected to the same impedance analyzer, engaged with a device station that can engage more than one multi-well device, or connected to separate impedance analyzers) to increase high-throughput capacity.

Systems for monitoring cell-substrate impedance that comprise one or more multiwell devices, an impedance analyzer, and a device station are described herein and are preferred but not required for use in the methods of the present invention.

The genetically modified cells can be any type of cells that is responsive to IgE stimulation. Preferably, mast cells are used, such as RBL-2H3 rat mast cells. Methods of genetically modifying cells are well known in the art, and include introducing expression constructs that direct the expression of altered versions of the supsected target protein (for example, dominant negative versions of a protein), introducing expression constructs that direct the expression of antisense RNAs that can reduce or ablate the expression of a

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target protein, and introducing expression constructs that direct the expression of silencing RNAs that can reduce of ablate the expression of a target protein. Antisense or gene silencing reagents can also be added to cultured cells before or after adding the cells to a device of the present invention.

Preferably, control cells that have not been genetically modified are also assayed alongside genetically modified cells.

Ig E can be added before, after, or simultaneous with the addition of the antigen, allergen, or IgE crosslinker. IgE can be added to wells at a concentration of from about 10 nanograms per milliliter to about 1 microgram per milliliter. Optionally, one well of the device that comprises an electrode array and cells does not receive IgE to provide a control well.

Antigens used in the assays can be antigens, allergens, or crosslinkers. The concentration added to the wells can be from about 1 nanogram per milliliter to about 1 microgram per milliliter.

The method can be used to assess and quantify the effects of a compound on the response of cells, such as but not limited to mast cells, as a result of IgE by using cell-substrate impedance technology. Impedance is preferably monitored during two or more phases of the experiment. Depending on the experimental protocol, impedance is preferably monitored after adding IgE to the one or wells, and is preferably monitored before and after adding IgE to the one or wells. Impedance is preferably monitored after the addition of test compound, before and after the addition of IgE. Impedance can also be monitored after the addition of test compound and IgE, before and after addition antigen. In some preferred embodiments, impedance is monitored after the addition of test compound and before addition of IgE to the wells, and after the addition of IgE to the wells and before the addition of antigen, allergen, or crosslinker to the wells, and after the addition of antigen, allergen, or crosslinker to the wells.

Impedance is preferably monitored at two or more time points. Preferably, impedance is monitored at at least three time points. Preferably, impedance is monitored at at least three time points at two or more phases of the assay. Impedance at each time point can be monitored at one or at more than one frequency. For example, impedance values before and after addition of IgE , and/or before and after addition of antigen can

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be compared for each well. Impedance data can be compared for wells that comprise genetically manipulated cells and control wells comprise cells that are not genetically manipulated. Impedance data can be used to calculate a cell index, which can be used for comparisons. Such comparisons can be used to identify genes that affect the response of cells to IgE stimulation.

Data obtained from impedance monitoring can be used to assess the effects of a genetic manipulation (such as expression knockdown, expression knockout, or dominant negative expression) on the morphological changes to cells that result from IgE stimulation. Identification of a genetic manipulation that affects the morphological changes to cells that result from IgE stimulation is used to identify a gene, and thus a gene product, that has a role in the IgE response.

One example of an assay of the present invention for validating a target is as follows:

(1) Provide cells having either the DNA for the dominant negative version of a protein of interest or for siRNA targeting of a protein of interest.

(2) Transfer cells to wells of a device of an impedance monitoring system and monitor the attachment and growth of the cells as described above. Alternatively, gene interfering reagents can be directly introduced into the cells in the wells of the device.

(3) Cells are stimulated with IgE in the presence or absence of antigen and the cellular response is recorded by the impedance monitoring system as previously discussed. The ability of a genetic construct or reagent to interfere with the response of cells to IgE stimulation by antigen will allow identification of the molecule the construct or reagent acts against as a potential target for drug discovery.

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E.4 Method for screening genetic markers that determine or influence engagement of high-affinity Fc(epsilon)RI cross-linking and subsequently mast cell activation

It is well known that host genetic background determines the types and severity of allergic response to similar antigens. The present invention also includes methods of comparing the responses of cell of different genotypes to antigen-mediated IgE stimulation. The responses of cells of different genotypes to antigen-mediated IgE stimulation can be correlated with any of a number of genetic markers that the cells of different genotypes display.

The method includes: a) providing a device for cell-substrate impedance monitoring that comprising at least one well that comprises an electrode array; b) connecting an impedance analyzer to the device; c) introducing cells of a first genotype into at least one well of the device; d) introducing cells of a second genotype into at least one other well of the device; e) adding IgE to at least one well comprising cells of a first genotype and at least one well comprising cells of a second genotype; e) adding at least one antigen to the one or more wells comprising cells of a first genotype and to the one or more wells comprising cells of a second genotype; e) monitoring cell-substrate impedance of the one or more wells comprising cells of a first genotype and .of the one or more wells comprising cells of a second genotype; and f) comparing the cell-substrate impedance values of the one or more wells comprising cells of a first genotype with the cell-substrate impedance values of the one or more wells comprising cells of a second genotype.

Preferably, the method further includes: correlating at least one genetic marker with the cell substrate impedance values obtained from monitoring cells of said first genotype and cells of said second genotype.

In the methods of the present invention, a device for cell-substrate impedance monitoring is a device that comprises a substrate having one or more electrode arrays on its surface, each of which is encompassed by a fluid container in the form of a well. Each of the one or more electrode arrays comprises two electrodes or electrode structures, where the two electrodes or electrode structures have substantially the same surface area . The device, when connected to an impedance analyzer, can detect impedance changes at

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one or more frequencies due to changes in cell number, cell size, cell morphology, cell attachment to the substrate, or the quality of cell attachment to the substrate.

Devices for measuring cell-substrate impedance are described in parent U.S. Patent Application No. 10/705,447 and in the present application. Preferably a cell-substrate impedance device used in the methods of the present invention comprises at least one fluid container that surrounds an electrode array of the device and provides a fluid-impermeable container for cells being monitored. In preferred embodiments, a device comprises at least two arrays and at least two receptacles in the form of wells, where each array of the device is encompassed by a well. More preferably, a device used in the screening methods described in the present application comprises at least 8 wells (for example, devices comprising 16 or 96 wells), of which the majority comprise electrode arrays, so that assays can be performed in a high-throughput fashion. Cells can optionally be assayed in multiple multi-well devices simultaneously (for example, connected to the same impedance analyzer, engaged with a device station that can engage more than one multi-well device, or connected to separate impedance analyzers) to increase high-throughput capacity.

Systems for monitoring cell-substrate impedance that comprise one or more multiwell devices, an impedance analyzer, and a device station are described herein and are preferred but not required for use in the methods of the present invention.

The cells used in the methods of the present invention can be any IgE responsive cells, but are preferably mast cells isolated from different individuals. In addition to impedance monitoring, mast cells isolated from individuals can be analyzed for genetic markers. These genetic markers include, as nonlimiting examples, SNPs, mutations, alternative RNA splicing variants, gene expression profiles, and protein expression profiles. Methods of analyzing genetic markers using cell isolated from individuals is well-known in the art.

In the assay procedure, Ig E can be added before, after, or simultaneous with the addition of the antigen. IgE can be added to wells at a concentration of from about 10 nanograms per milliliter to about 1 microgram per milliliter. Optionally, one well of the device that comprises an electrode array and cells does not receive IgE to provide a control well.

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Antigens used in the assays can be antigens, allergens, or crosslinkers. The concentration added to the wells can be from about 1 nanogram per milliliter to about 1 microgram per milliliter. Antigens can be chosen for their characteristic propensity to be identified as allergens among members of a population.

The method can be used to correlate a genetic marker with the antigen-IgE response of cells, such as but not limited to mast cells, as a result of by using cell-substrate impedance technology. Impedance is preferably monitored during two or more phases of the experiment. Depending on the experimental protocol, impedance is preferably monitored after adding IgE to the one or wells, and is preferably monitored before and after adding IgE to the one or wells. Impedance is preferably monitored after the addition of test compound, before and after the addition of IgE. Impedance can also be monitored after the addition of test compound and IgE, before and after addition antigen. In some preferred embodiments, impedance is monitored after the addition of test compound and before addition of IgE to the wells, and after the addition of IgE to the wells and before the addition of antigen, allergen, or crosslinker to the wells, and after the addition of antigen, allergen, or crosslinker to the wells.

Impedance is preferably monitored at two or more time points. Preferably, impedance is monitored at at least three time points. Preferably, impedance is monitored at at least three time points at two or more phases of the assay. Impedance at each time point can be monitored at one or at more than one frequency. For example, impedance values before and after addition of IgE, and/or before and after addition of antigen can be compared for each well. Impedance data can be compared for wells that comprise genetically manipulated cells and control wells comprise cells that are not genetically manipulated. Impedance data can be used to calculate a cell index, which can be used for comparisons. Such comparisons can be used to identify genetic markers that affect the response of cells to IgE stimulation.

Data obtained from impedance monitoring can be used to correlate genetic markers assigned to the cells with the responses of the cells to IgE stimulation. Identification of a genetic marker that correlates with the responsiveness of cells to IgE can be used to develop strategies for developing therapies that moderate the IgE response.

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An exemplary assay is as follows:

1) Isolate mast cells from two or more individuals potent to allergic reactions
2) Identify genetic markers of interest in the individuals
3) Incubate the mast cells (or differentiate stem cells to mast cells in the presence of specific differentiation factors)
4) Transfer the cells to impedance monitoring devices and monitor the cell growth
5) Add IgE (human) with or without an allergen
6) Quantify mast cell impedance response
7) Correlate genetic markers with mast cell response

Genetic analysis can be conducted using standard RFLP and SNP analysis, RNA and protein expression profiling, and RNA splicing detection methods as they are known in the art. Preferably, assays are performed in a high throughput manner using cells from a very large number of individuals to provide robust correlations of genetic markers and IgE responses.

E.4 Method for screening, discovering, and validating chemical structures of antigen (allergen) or half antigen binding the IgE receptor leading to receptor cross-linking

The present invention also provides methods of identifying antigens or half-antigens that can cause an allergic response. The method comprises: a) providing a device for cell-substrate impedance monitoring that comprising at least one well that comprises at least one electrode array; b) connecting an impedance analyzer to the device; c) introducing cells into one or more wells of the device that comprise an electrode array; d) adding IgE to the one or more wells comprising cells; e) adding at least one suspected antigen or at least one suspected allergen or half-allergen to the one or more wells comprising cells; and e) monitoring cell-substrate impedance of the one or more wells comprising cells.

In the methods of the present invention, a device for cell-substrate impedance monitoring is a device that comprises a substrate having one or more electrode arrays on its surface each of which is encompassed by a fluid container in the form of a well. Each

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of the one or more electrode arrays comprises two electrodes or electrode structures, where the two electrodes or electrode structures have substantially the same surface area , in which the device, when connected to an impedance analyzer, can detect impedance changes at one or more frequencies due to changes in cell number, cell size, cell morphology, cell attachment to the substrate, or the quality of cell attachment to the substrate.

The cells can be any cells whose response or possible response to IgE stimulation is of interest. Preferred cells are mast cells, eosinophils, basophils, or genetically engineered cells. In some preferred embodiments, the cells are of mammalian origin. In some preferred embodiments, the cells are mast cells isolated from individuals.

Ig E can be added before, after, or simultaneous with the addition of the antigen, allergen, or half-allergen. IgE can be added to wells at a concentration of from about 10 nanograms per milliliter to about 1 microgram per milliliter. Preferably, one well of the device that comprises an electrode array and cells does not receive IgE to provide at least one control well, but this is not a requirement of the present invention.

Antigens, allergens, or half-allergens can be known or suspected antigens, allergens, or allergens. The concentration added to the wells can be from about 1 nanogram per milliliter to about 1 microgram per milliliter. Different wells may receive different concentrations of an antigen, allergen, or half-allergens. Or, for replica purposes, multiple wells may receive same concentrations of an antigen, allergen or crosslinker. Preferably, one well of the device that comprises an electrode array and cells does not receive antigen, allergen, or half-allergen to provide at least one control well, but this is not a requirement of the present invention.

The method can be used to assess and quantify the morphological changes that occur in mast cells as a result of *IgE* stimulation and *IgE* cross-linking with an allergen by using cell-substrate impedance technology. Impedance is preferably monitored during two or more phases of the experiment. For example, in one embodiment impedance is monitored before and after the addition of antigen, allergen, or half-allergen to the wells. In another embodiment, impedance is additionally monitored before and after the addition of IgE to the wells. In yet another embodiment, impedance can be monitored before and after the addition of IgE plus antigen, allergen, or half-

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allergen to the wells. Impedance can be also be monitored after the addition of IgE to the wells and before the addition of antigen, allergen, or half-allergen to the wells, as well as after the addition of antigen, allergen, or half-allergen to the wells. In some preferred embodiments, impedance is monitored before addition of IgE to the wells, after the addition of IgE to the wells and before the addition of antigen, allergen, or half-allergen to the wells, and after the addition of antigen, allergen, or half-allergen to the wells.

Impedance can be monitored at two or more time points. Preferably, impedance is monitored at at least three time points. Preferably, impedance is monitored at at least three time points at two or more phases of the assay. Impedance at each time point can be monitored at one or at more than one frequency. Data obtained from impedance monitoring can be used to assess morphological changes to cells that result from IgE stimulation. For example, impedance before and after addition of IgE , and/or before and after addition of antigen, allergen, or half-allergen can be compared for each well. Impedance data can be compared for wells that receive IgE and control wells that do not receive IgE. Impedance data can be compared for wells that receive antigen, allergen, or half-allergen and control wells that do not receive antigen, allergen, or crosslinker. Impedance data can be used to calculate a cell index, which can be used for comparisons.

One exemplary protocol is as follows:

(1) Mast cells are seeded into wells of an impedance monitoring device.
(2) The cells are monitored electronically using an impedance monitoring system for a given amount of time.
(3) The IgE that is specific for the allergen or the half allergen will be added to the cells.
(4) Cellular response will be monitored by using the system
(5) After a pre-determined increment of time, the antigen or half antigen that is specific for the IgE in step (3) will be added.
(6) Cellular response will continue to be monitored using the system.

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Example 1

As an example, we describe here the use of the use of a cell-substrate impedance monitoring system of the present invention to measure and monitor the morphological changes that occur as a result of IgE-mediated stimulation of RBL-2H3 cells (ATCC) in the presence or absence of an antigen. The cell-substrate impedance monitoring system has a device station that can engage 6 16X devices as depicted in **Figure 4**. It also has an impedance analyzer, and software that directs impedance measurement and recording and analysis of impedance data.

RBL-2H3 cells were seeded in the 16X device chamber (depicted in **Figure 2**) at 20,000 cells/well and the attachment and growth of the cells in the 37°C tissue culture incubator were monitored in real-time using the cell-substrate monitoring system. After 22 hours mouse monoclonal anti-dinitrophenyl (DNP) antibody (Clone SPE-7, Sigma) was added at final concentration of 1 microgram/mL. As a control, a non-specific mouse IgG was added at a final concentration of 1 microgram/mL. The device chambers were returned to the incubator and recording was resumed. At 24 hours post-IgE stimulation the media in the chamber was aspirated and 150 microliters of fresh media was added to the cells. Recording was resumed for 1 hour followed by the addition of DNP-albumin (Sigma) at a final concentration of 1 microgram/ml. The recording was continued for an additional 4 hours.

RBL-2H3 mast cells have been used extensively as a model system to investigate the signaling pathways that are initiated as a result of IgE-mediated binding to and stimulation of the high affinity Fc(epsilon)RI receptors located on the membrane. Mast cell degranulation is accompanied by distinct morphological changes which involve the actin cytoskeleton (Pfeiffer et al. J Cell Biol. 1985 Dec; 101(6):2145-55.). Engagement of Fc(epsilon)RI by IgE, even in the absence of cross-linking by an antigen, leads to dose-dependent degranulation and morphological changes (Oka et al. Am J Physiol Cell Physiol. 2003 Sep 17). This IgE-mediated dose-dependent phenomenon is also observed using the impedance system as shown in **Figure 13**. It is observed as an abrupt shift in the trace of the cell index number at 22 hours, immediately after the administration of the

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IgE. The duration of the signal for the IgE concentrations that illicit a change lasts for approximately 6 hours. Such dynamic changes are consistent with morphological dynamics observed by microscopy and degranulation detected by enzymatic assays. Importantly, the shift in the trace does not occur in those wells which have IgG, indicating that the response is specific for IgE. The peak amplitude of the recording is directly dependent on the concentration of the IgE used to stimulate the cells. The duration of the signal for the IgE concentrations that illicit a change lasts for approximately 6 hours.

Once RBL-2H3 mast cells have been sensitized with IgE, further degranulation and actin-mediated morphological dynamics can be elicited by the administration of the antigen to which the IgE has been raised against. In RBL-2H3 cells this has been observed by immunofluorescence microscopy as extensive membrane ruffling and lamellapodia formation on the apical surface of the cells. The phenomenon of antigen-mediated IgE-dependent mast cell degranulation and morphological dynamics can also be monitored and measured using cell-impedance monitoring system. 20 hours after IgE addition the media is removed and replaced by fresh media. The change in media also induces a non-specific transient change in the signal which immediately returns to baseline after 30 minutes. Application of DNP-albumin at this point induced an abrupt shift in the recording which peaks at about 15-20 minutes and returns to baseline about 1 hour later. The duration of the shift in the signal is much more transient under these conditions, when compared to IgE alone stimulation (3 hours vs. 6 hours).

Example 2

The following example is provided to show how a real-time cell electronic sensing system and methods can be used in real-time monitoring of IgE-mediated mast cell activation. The methods and devices of the present invention are not limited to those described in the Examples. Indeed, within the scope of the present invention, there are other specific methods and approaches to conduct assays for monitoring IgE-mediated mast cell signaling and activation.

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Introduction

The work presented here describes an assay for IgE-mediated mast cell activation using measurements of cell-substrate impedance. The method is based on quantification in real time of the morphological, cytoskeletal and cell adhesion changes that arise as a response to multivalent antigen aggregation of the IgE-Fc(epsilon)RI complex on the surface of mast cells. Because the electronic assay readout relies on morphological, cytoskeletal and adhesive dynamics, which are intrinsic mast cell responses to the antigen and an essential part of the mast cell activation program, it precludes the need for establishing reporter cell lines or using any other reagent or cellular manipulation. Since the assay is performed in real time, both antigen-dependent and independent responses to IgE-mediated activation of mast cells can be monitored in the same assay. The assay is readily adaptable to a 96 well format and as shown here can be used to assay for pharmacological inhibitors of mast cell activation in high throughput manner.

Materials and Methods

Cells and reagents

The RBL-2H3 cell line was purchased from American Type Culture Collection (ATCC) and maintained in DMEM containing 10 % fetal bovine serum at 37°C and 5 % CO_2. Mouse monoclonal anti-dinitrophenyl IgE antibody (Clone SPE-7) and DNP-HSA were purchased from Sigma Aldich (St. Louis, MO). The Src-specific inhibitor SU6656 , MEK-specific inhibitor PD98059 and the PLC-specific inhibitor U73122 were purchased from Calbiochem (La Jolla, CA). The PKC-specific inhibitor Bisindolylmaleimide and the Syk inhibitor Piceatannol were purchased from Sigma. Rhodamine phalloidin was obtained from Molecular Probes (Eugene, OR). Lab-Tek chamber slides were purchased through VWR Scientific.

Cell-substrate impedance Monitoring System

The real-time cell-substrate impedance monitoring system comprises three components, an impedance analyzer, a device station, and one or more 16X microtiter devices. Microelectrode sensor arrays were fabricated on glass slides using lithographical microfabrication methods and the electrode-containing slides were

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assembled to plastic trays to form 16 electrode-containing wells. The device station of the system receives the 16X microtiter devices and is capable of electronically switching any one of the wells to the sensor (impedance) analyzer for impedance measurement. In operation, the devices with cells cultured in the wells are placed into a device station that is located inside an incubator. Electrical cables connect the device station to the sensor (impedance) analyzer. Under the impedance monitoring system's software control, the impedance analyzer can automatically select wells to be measured and continuously conduct impedance measurements. The impedance data from the analyzer is transferred to a computer, analyzed and processed by integrated software.

Impedance measured between electrodes (electrode elements) in an individual well depends on electrode geometry, ionic concentration in the well and whether there are cells attached to the electrodes. In the absence of cells, electrode impedance is mainly determined by the ion environment both at the electrode/solution interface and in the bulk solution. In the presence of cells, cells attached to the electrode sensor surfaces will alter the local ionic environment at the electrode/solution interface, leading to an increase in the impedance. The more cells there are on the electrodes, the larger the increase in cell-electrode impedance. Furthermore, the impedance change also depends on cell morphology and the extent to which cells attach to the electrodes.

To quantify cell status based on the measured cell-electrode impedance, a parameter termed Cell Index is derived, according to

$$CI = \max_{i=1,\ldots,N}\left(\frac{R_{cell}(f_i)}{R_b(f_i)} - 1\right)$$

where $R_b(f)$ and $R_{cell}(f)$ are the frequency dependent electrode resistances (a component of impedance) without cells or with cell present, respectively. N is the number of the frequency points at which the impedance is measured. Thus, Cell Index is a quantitative measure of the status of the cells in an electrode-containing well. Under the same physiological conditions, more cells attached on to the electrodes leads to larger $R_{cell}(f)$ value, leading to a larger value for Cell Index. Furthermore, for the same number of cells present in the well, a change in the cell status such as morphology will lead to a change in the Cell Index. For example, an increase in cell adhesion or cell

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spreading leads to larger cell-electrode contact area which will lead to an increase in $R_{cell}(f)$ and thus a larger value for Cell Index.

Fluorescence microscopy

RBL-2H3 cells were seeded in 16 well Lab-Tec chamber slides and allowed to attach and spread for 6 hours. The cells were stimulated with anti-DNP IgE at a final concentration of 100 ng/mL or a non-specific mouse IgG at 100 ng/mL and then 16 hours later, the media was aspirated, replaced with fresh media and treated with 100 ng/mL DNP-BSA for the indicated time and then fixed with 4 % parafarmaldehyde. The cells were washed 3X with PBS, permeablized in PBS containing 0.2% TX-100 and blocked in PBS containing 0.5 % BSA. The cells were then stained with rhodamine-phalloidin for 30 minutes, washed 3X with PBS and visualized and imaged using the tritc filter on a Nikon E400 epi-fluorescence microscope and Nikon ACT software.

2.5. Beta-hexosaminidase assay

RBL-2H3 cells growing in 96 well plates were washed and incubated in Tyrode buffer (10 mM Hepes, pH 7.4, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl2, 1 mM MgCl2, 5.6 mM Glucose, and 0.1 % BSA) and stimulated with 100 ng/mL anti-DNP IgE. After 2 hours the supernatant was removed and the cell monolayer was lysed in Tyrode buffer containing 0.5 % TX-100. Hexosaminidase activity was measured in both supernatant and the cell monolayer using the substrate 4-nitrphenyl-2acetamido-2-deoxy-b-D-glucopyranoside (1 mg/mL). After 1 hour incubation at 37 ^{o}C, the reaction was stopped by the addition of 2 volumes of 0.4 M glycine pH 10.7. The absorbance at 405 nm was read in Molecular Devices ELISA reader.

Impedance Assay

20,000 RBL-2H3 cells were seeded per well of a 16X microtiter device and monitored by the impedance measuring system. The cells were allowed to attach and spread for 5-24 hours prior to the addition of IgE at the indicated final concentration. The cell-electrode impedance was continuously measured and the corresponding, time dependent Cell-Index values were derived and recorded.

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Impedance monitoring of RBL-2H3 mast cell activation

IgE-mediated RBL-2H3 mast cell activation in the presence of antigen leads to initiation of signaling cascade resulting in degranulation of secretory vesicles which contain mediators of allergic reaction such as histamine. In addition, IgE-mediated stimulation through Fc(epsilon)RI also leads to dramatic remodeling of the actin cytoskeleton (Oliver et al., 1997). Since monitoring of cell-electrode impedance provides information about the parameters of cell morphology and adhesion we sought to determine the impedance response of RBL-2H3 mast cells that were pre-sensitized with IgE in the presence of antigen application.

RBL-2H3 mast cells were seeded onto the surface of 16X microtiter plate devices having integrated microelectronic sensor arrays in the bottom of each well. The cells were allowed to adhere to the surface of the sensors and 18 hours later were sensitized with anti-DNP *Ig*E (**Figure 14**). Approximately 24 hours later DNP-BSA at a final concentration of 100 ng/mL was applied to the cells to induce oligomerization of the *Ig*E-bound Fc(epsilon)RI receptor and induce mast cell activation. The cell-electrode impedance measurements were continuously monitored using the impedance monitoring system. As shown in **Figure 14**, DNP-BSA application induced an immediate and transient increase in the impedance value which was detectable within 5 minutes of DNP-BSA application, maximal by 30 minutes and returned to baseline in approximately 2.5 hours. IgE-mediated activation of mast cells not only led to dramatic morphological changes (Pfeiffer et al., 1985) but also to augmentation of integrin-mediated cell adhesion (Wyczolkowska et al., 1994), both of which contributed to cell-electrode impedance measurements using the impedance monitoring system.

Impedance measurement of mast cell activation correlates with cytoskeletal dynamics and degranulation

To determine if the IgE-mediated cell-electrode impedance increase correlates with RBL-2H3 mast cell activation, both IgE-mediated morphological dynamics and mediator release were monitored.

RBL-2H3 mast cells were sensitized and activated as described above and at the indicated time points, fixed with parafarmaldehyde and stained with rhodamine-

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phalloidin to visualize the actin cytoskeleton (**Figure 15A**). As seen in **Figure 15A** and shown previously (Pfeiffer et al., 1985; O'Luanaigh et al., 2002; Powner et al., 2002), DNP-BSA-mediated cross-linking of the Fc(epsilon)RI receptor leads to time-dependent remodeling of the actin cytoskeleton. The cells undergo extensive ruffling which is apparent as early as 2.5 minutes post IgE stimulation, followed by morphological changes which lead to cell spreading and formation of lamellapodia. The peak cytoskeletal reorganization is observed at 30-45 minutes post-IgE stimulation which correlates directly with the peak cell-electrode impedance response using the impedance monitoring system. As a control, RBL-2H3 mast cells were also sensitized with an irrelevant IgG and subsequently cross-linked with DNP-BSA. No obvious cytoskeletal and morphological changes were observed.

As an additional marker for RBL-2H3 mast cell activation, beta-hexosaminidase activity was also measured in response to IgE stimulation in the presence of antigen cross-linking. The enzyme beta-hexosaminidase is stored within the secretory vesicles and is a marker for mast cell degranulation. It has been shown that beta-hexosaminidase is released into the culture media in response to antigen-mediated cross-linking of IgE-bound Fc(epsilon)RI on the surface of mast cells (Razin et al., 1983). RBL-2H3 cells were sensitized with anti-DNP IgE, activated by application of DNP-BSA and beta-hexoseaminidase activity was measured as described in materials and methods section. Antigen cross-linking leads to a two and a half to 4 fold increase in beta-hexosaminidase depending on the experiment (**Figure 15B**). Taken together, IgE-mediated mast cell-electrode impedance increase correlates directly with morphological changes and degranulation which is characteristic of mast cell activation. Therefore, cell-electrode impedance measurements can be used as readout for mast cell activation.

Cell-substrate monitoring of RBL-2H3 sensitization step

It has recently been shown that high concentrations of monomeric IgE induce mast cell activation in the absence of antigen cross-linking (Oka et al., 2004). Accordingly, we wanted to determine if high concentrations of IgE alone may induce an increase in cell-electrode impedance response. RBL-2H3 mast cells growing on the surface of 16X microtiter cell-substrate impedance monitoring devices were stimulated

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with increasing amounts of IgE-ranging from 15 nanogram/mL to 1 micrograms/mL and their impedance response was continuously monitored (**Figure 16A**). IgE application alone leads to a dose-dependent increase in cell-electrode impedance response which correlated with morphological dynamics and mediator release. To determine if the initial concentration of IgE used to sensitize the cells influences the subsequent antigen-mediated response, RBL-2H3 cells were sensitized with the indicated concentrations of anti-DNP IgE and after 16 hours were incubated with 100 nanograms/mL final concentration of DNP-BSA. As shown in **Figure 16B**, the DNP-BSA-mediated response is inversely proportional to the initial anti-DNP IgE concentration used to sensitize the cells. Sensitizing with 1 microgram/ml anti-DNP IgE leads to negligible increase in cell-electrode impedance response when cross-linked with the multi-valent antigen DNP-BSA while sensitization with 15 nanograms/mL anti-DNP IgE resulted in a robust increase in cell-electrode (cell-substrate) impedance in the presence of DNP-BSA aggregation. Furthermore, if the increment of time between High IgE application and DNP-BSA addition is increased to 24 hours, then the cell will undergo antigen-mediated activation.

In conclusion these experiments illustrate that IgE concentration and the timing between IgE sensitization and subsequent antigen stimulation is critical and should be taken into account. Additionally, these experiments further illustrate the advantage of real-time monitoring of mast cell activation on microelectronic cell-substrate impedance sensor arrays with respect to cell status and response at any given time point.

Pharmacological inhibition of mast cell activation as detected by the cell-substrate impedance monitoring system

Antigen-mediated aggregation of the IgE-bound Fc(epsilon)RI triggers a signaling cascade which involves a number of signaling proteins such as protein kinases, protein phosphatases, and phospholipases amongst others whose activity and function is indispensable for mast cell activation (Turner and Kinet, 1999). Accordingly, we were interested in determining if pharmacological inhibitory effects of some of these signaling proteins can be monitored by mast cell-electrode impedance measurement.

RBL-2H3 cells were seeded in a 16X microtiter plate device and pre-stimulated with anti-DNP-IgE were incubated for 1 hour with the indicated doses of the PKC-

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specific inhibitor Bisindolylmaleimide and subsequently treated with 100 ng/mL DNP-BSA (**Figure 17**). The dose-dependent inhibition of RBL-2H3 mast cell activation by Bisindolylmaleimide can be monitored on the cell-substrate monitoring system in real-time. A number of other pharmacological agents such as SU6656, specific for Src family kinases, U73122, specific for phospholipase C, Piceatannol, specific for Syk tyrosine kinase and PD98059 specific for MEK were also tested. The IC-50 value for these pharmacological inhibitors at peak response was calculated and is shown in **Table I**. All the inhibitors with the exception of PD 98059 dose dependently inhibited antigen-mediated RBL-2H3 mast cell activation. The PLC-specific inhibitor U73122 displayed potent inhibitory effect with an IC-50 value of around 1 microM. The Src and Syk specific inhibitors also inhibited the antigen-mediated response although at higher concentrations (**Table I**). The MEK-specific inhibitor PD 98059 had minimal effect on IgE-mediated RBL-2H3 response at the concentrations tested.

In order to ascertain that inhibitor-mediated abrogation of the cell-electrode impedance response correlates with inhibition of mast cell activation, the beta-hexosaminidase activity of IgE stimulated RBL-2H3 cells were measured in the presence of pharmacological agents. According to **Figure 18**, SU6656, U73122, Bisindolylmaleimide and Piceatannol abrogated the anti-DNP IgE mediated stimulation of beta-hexosaminidase activity in the presence of antigen aggregation whereas the MEK specific inhibitor had minimal effect. These findings are in agreement with previously published data indicating that the Src, PLC, PKC and Syk specific inhibitors completely block IgE-mediated mast cell degranulation and activation (Oliver et al., 1994; Amoui et al., 1997; Moriya et al., 1997; Tedeschi et al., 2000). In summary, the results presented here indicate that real-time monitoring of IgE-mediated mast cell activation on microelectronic cell sensor arrays offer a convenient way of assessing mast cell activation. The assay does not require any cellular manipulation such as labeling, fixation or lysis. The assay was validated by demonstrating that the cell-electrode (cell-substrate) impedance measurement correlates directly with IgE-mediated mast cell activation as measured by actin cytoskeleton dynamics and mediator release. Furthermore, previously characterized specific inhibitors of mast cell activation pathway inhibit mast cell

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activation as measured by the cell-substrate impedance monitoring system, further validating this assay.

Compound	Target	IC50±SD (μM)
Bisindolylmaleimide	**Protein Kinase C**	**2.9±1.7 (N=3)**
SU6656	**SRC**	**5.8±3.5 (N=5)**
U73122	**Phospholipase C**	**0.93±0.6 (N=5)**
Piceatannol	**Syk**	**1 (N=1)**

Table I. IC-50 determination of pharmacological agents inhibiting mast cell activation monitored by cell-substate impedance monitoring.

Discussion

Cell-electrode impedance reading is primarily influenced by three main parameters: the number of cells seeded on microelectronic sensor arrays, the shape of the cell and the strength of cell adhesion to the electrode surface. IgE-mediated RBL-2H3 mast cell activation is accompanied by an increase in effective surface area due to fusion of secretory granules, dramatic actin cytoskeleton rearrangement as well as an increase in integrin-mediated adhesion (Pfeiffer et al., 1985; Wyczolkowska et al., 1994). Morphological dynamics combined with an increase in adhesive interaction of the cell with the electrode surface leads to an increase in antigen-dependent IgE-mediated mast cell electrode impedance value. Furthermore, pharmacological inhibitors of signaling proteins which participate in IgE-mediated mast cell activation inhibit IgE-mediated mast cell activation in a dose-dependent manner (**Figure 17** and **Table I**).

According to the current paradigm for mast cell activation, antigen-mediated cross-linking of the IgE-bound Fc□RI leads to phosphorylation of critical tyrosines in the immunoreceptor tyrosine-based activation motifs (ITAM) by Src family kinase, Lyn (Turner and Kinet, 1999). Phosphorylation of the ITAMS serves as a docking site for the

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Syk protein tyrosine kinase, which subsequently leads to its activation and phosphorylation of its downstream substrates such as phospholipase C gamma (PLCgamma). PLCgamma catalyzes the hydrolysis of membrane phospholipids to generate inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mobilizes calcium from internal reserves in the endoplasmic reticulum and both calcium and DAG lead to general activation of protein kinase C (PKC) family as well as other kinases and signaling proteins. Activation of IgE-mediated kinase pathways ultimately results in mast cell degranulation and release of inflammatory mediators. Accordingly pharmacological inhibitors of Src, Syk, PLCgamma and PKC led to a dose-dependent inhibition of IgE-mediated RBL-2H3 mast activation which can be monitored by the cell-electrode impedance measurement. The cell-electrode impedance values of the mast cells treated with these pharmacological inhibitors correlated with mast cell degranulation as measured by beta-hexosaminidase activity (**Figure 18**). Utilization of these pharmacological inhibitors further validates this assay and indicates that the cell-substrate impedance monitoring system can be used to assess mast cell activation.

Several lines of recent evidence indicate that IgE-alone mediated interaction with mast cells has crucial biological functions that surpass its role as merely a sensitizer of mast cells (Kawakami and Galli, 2002). Two different groups have shown that application of monomeric IgE without antigen-mediated aggregation induces phosphorylation and activation of mitogen activated protein kinases (MAPKs) and AKT leading to enhanced mast cell survival (Asai et al., 2001; Kalesnikoff et al., 2001; Kitaura et al., 2003). However, the same papers also showed that monomeric IgE did not induce mast cell degranulation. In contrast, another recently published report indicates that high concentrations of monomeric IgE can induce mast cell activation in itself without the need for antigen-mediated ligation (Oka et al., 2004). According to the authors, IgE alone-mediated mast cell activation as measured by mediator release and actin cytoskeleton rearrangement was indistinguishable from antigen-mediated cross-linking (Oka et al., 2004). According to our results application of high concentrations of monomeric IgE alone also elicited an increase in mast cell-electrode impedance value, indicating mast cell activation (**Figure 16**). However, the duration of the response was prolonged when compared to the antigen-mediated mast cell activation. While the

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molecular mechanism of antigen-independent and antigen-dependent mast cell activation response remains to be clarified, it is clear that monomeric IgE can illicit biologically important responses. We've also determined that the initial IgE concentration used to sensitize the cells ultimately determines the amplitude and duration of the subsequent antigen-mediated mast cell activation. Sensitization with high concentrations of IgE (1 microgram/mL) resulted in very low antigen-dependent mast cell response, while sensitization with low concentrations of IgE (15 nanogram/mL) resulted in mast cell response with much higher duration and amplitude (**Figure 16B**). The lack of a significant antigen-mediated mast cell response with high concentration of IgE-sensitized mast cells more likely is due to the fact that mast cells have not had an opportunity to fully reform their secretory granules after IgE-mediated activation and this is supported by the fact that increasing the time increment between sensitization with 1 microgram/mL IgE and the cross-linking step will ultimately result in antigen-mediated mast cell activation.

In summary, the real-time and label-free IgE mediated mast cell activation assay described here should provide researchers in pharmaceutical industry as well as academia with a new and convenient tool to assess and quantify IgE-mediated mast cell activation. The adaptability of this assay to 96X microtiter plates with microelectronic sensors integrated in the bottom of the wells makes it ideal for high throughput analysis to screen large chemical compound libraries. Furthermore, microelectronic cell sensor technology in general can be used to assess other receptor ligand interactions.

All of the references cited herein, including patents, patent applications, and publications, and including references cited in the Bibliography, are incorporated by reference in their entireties.

Headings are for the convenience of the reader and do not limit the scope of the invention.

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CLAIMS

We claim:

1. A device for monitoring cell-substrate impedance, comprising:

a) a nonconducting substrate;

b) two or more electrode arrays fabricated on the substrate, wherein each of said two or more electrode arrays comprises two electrode structures; and

c) at least two connection pads, each of which is located on an edge of said substrate;

wherein for each of said two or more electrode arrays, each of said two electrode structures comprises multiple electrode elements and the first of said two electrode structures of each of said at least two electrode arrays is connected to one of said at least two connection pads, and the second of said two electrode structures of each of said at least two electrode arrays is connected to another of said at least two connection pads;

further wherein each electrode array has an approximately uniform electrode resistance distribution across the entire array; and

further wherein said substrate has a surface suitable for cell attachment or growth; wherein said cell attachment or growth on said substrate can result in a detectable change in impedance between or among said electrode structures within each electrode array.

2. The device of claim 1, wherein the electrode structures of each of said two or more electrode arrays have substantially the same surface area.

3. The device according to Claim 1, wherein at least two of said two or more electrode arrays are individually addressed.

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4. The device of claim 1, wherein the electrode resistance at a first location on any one array of said two or more electrode arrays and the electrode resistance at a second location on said any one array do not differ by more than 30%.

5. The device of claim 4, wherein the electrode resistance at a first location on any one of said two or more arrays and the electrode resistance at a second location on said any one of said two or more arrays do not differ is by more than 15%.

6. The device of claim 5, wherein the electrode resistance at a first location on any one of said two or more arrays and the electrode resistance at a second location on said any one of said two or more arrays do not differ is by more than 5%.

7. The device of claim 6, wherein the electrode resistance at a first location on any one of said two or more arrays and the electrode resistance at a second location on said any one of said two or more arrays is do not differ by more than 2%.

8. The device of claim 1, wherein for each of said two or more electrode arrays, said first electrode structure is connected to a first electrode bus that is connected to one of said at least two connection pads via at least one electrical trace, and said second electrode structure is connected to a second electrode bus that is connected to another of said at least two connection pads via at least one electrical trace.

9. The device of claim 8, wherein one electrode structure of each of at least two of said two or more electrode arrays is connected to a common connection pad.

10. The device of claim 9, wherein for each of said two or more electrode arrays, one of said two electrode structures is connected to a connection pad that also connects to an electrode structure of at least one other of said two or more electrode arrays, and the other of said two electrode structures is connected to a connection pad that is not connected to any other electrode structures.

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11. The device according to Claim 1, wherein the substrate comprises glass, sapphire, silicon dioxide on silicon, or a polymer.

12. The device according to Claim 11, wherein the substrate is configured as a flat surface.

13. The device according to Claim 12, wherein the electrode elements of each of said two or more electrode arrays are of approximately equal widths.

14. The device according to Claim 13, wherein said electrode elements have a width of greater than 30 microns.

15. The device according to Claim 14, wherein said electrode elements have a width of between about 50 microns and about 300 microns.

16. The device according to Claim 15, wherein said electrode elements have a width of about 90 microns.

17. The device according to Claim 14, wherein the electrode elements of each of said two or more electrode arrays are approximately evenly spaced.

18. The device according to Claim 17, wherein the gap between adjacent electrode elements is less than 50 microns.

19. The device according to Claim 18, wherein the gap between adjacent electrode elements is between about 5 microns and about 30 microns.

20. The device according to Claim 18, wherein the gap between adjacent electrode elements is about 20 microns.

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21. The device according to Claim 17, wherein said two or more electrode arrays comprise circle-on-line electrode elements.

22. The device according to Claim 21, wherein for each array of said two or more electrode arrays, said first electrode bus comprises an electrode that extends around at least a portion of the perimeter of said each of said each array and said second electrode bus comprises an electrode that extends around at least a portion of the perimeter of said each array, wherein said first electrode bus and said second electrode bus do not overlap.

23. The device according to claim 22, wherein electrode elements of the same electrode structure of said two or more electrode arrays are connected to the same electrode bus.

24. The device according to Claim 13, wherein each array of said two or more electrode arrays is organized in a concentric, sinusoidal, interdigitated, or castellated fashion.

25. The device according to Claim 8, wherein said at least one electrical trace is covered with an insulating layer.

26. The device according to Claim 22, wherein said at least one electrical trace is disposed in a second plane of the substrate.

27. The device according to Claim 1, further comprising a plurality of receptacles, wherein each receptacle is disposed on the nonconductive substrate in a perpendicular orientation thereto, further wherein each receptacle forms a fluid-impermeable container and each of said two or more electrode arrays on the substrate is associated with a fluid-impermeable container.

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28. The device according to Claim 27, wherein each container is shaped as a tube with opposing open ends, one end of which being in fluid-tight contact with the substrate.

29. The device according to Claim 28, further wherein the diameter of the container at the end in contact with the substrate is smaller than the diameter of the opposing end.

30. The device according to Claim 28, wherein the diameter of one or more containers is, at the container end disposed on the substrate, between about 1 millimeter and about 20 millimeters.

31. The device according to Claim 28, wherein the containers together form a multi-well bottomless microtiter plate.

32. The device according to Claim 30, wherein the number of wells present is between 2 and 1,536.

33. The device according to Claim 32, wherein the number of wells present is between 4 and 384.

34. The device according to Claim 32, wherein the number of wells present is between 16 and 96.

34. The device of claim 28, wherein less than all of the wells are associated with an electrode array.

35 The device according to Claim 31, wherein less than all of the wells are associated with an electrode array.

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36. The device of claim 34, wherein said two or more electrode arrays comprise circle-on-line elements.

37. The device of claim 36, further wherein two electrode buses are associated with each of said electrode arrays, wherein for each electrode array, multiple electrode elements of a first electrode structure connect to the first of said two electrode buses and multiple electrode elements of a second electrode structure connect to the second of said two electrode buses.

38. The device of claim 37, wherein said two electrode buses are on the perimeter of said each of said electrode arrays.

39. The device of claim 38, wherein each of said two electrode buses associated with each of said electrode arrays is connected to one of said at least two connection pads via at least one electrical trace.

40. The device of claim 39, wherein each of said electrode arrays comprises a first electrode structure comprising multiple electrode elements connected to a first electrode bus and a second electrode structure comprising multiple electrode elements connected to a second electrode bus, and said first electrode bus and said second electrode bus are connected to separate connection pads.

41. The device of claim 40, wherein a first electrode structure of each of at least two of said electrode arrays is connected to a common connection pad.

42. The device of claim 41, wherein for each of said at least two electrode arrays, a first electrode structure is connected to a connection pad that also connects to an electrode structure of at least one other of said electrode arrays, and a second of said electrode structures is connected to a connection pad that is not connected to an electrode structure of any other electrode array.

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43. The device of claim 42, further comprising at least one printed circuit board (PCB) attached to the bottom surface of said substrate.

44. The device of claim 43, further comprising metal clips that contact said at least two connection pads and said at least one PCB.

45. The device of claim 44, further comprising metal connector pins that contact said at least one PCB, wherein said connector pins can be connected to an impedance analyzer.

46. The device of claim 45, comprising 16 wells.

47. The device of claim 45, comprising 96 wells.

48. The device of claim 47, comprising 95 electrode arrays, each of which is localized to the bottom of one of said 96 wells.

49. The device according to Claim 1, further comprising an impedance analyzer electrically connected to said at least two connection pads.

50. The device according to Claim 36, wherein said impedance analyzer can measure impedance at a frequency ranging from about 1 Hz to about 1 MHz.

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51. A system for monitoring cell-substrate impedance, comprising:

a) one or more multiple well cell-substrate impedance monitoring devices, wherein at least two of the multiple wells of said device comprise an electrode array at the bottom of the well, wherein said electrode array is individually addressed, further wherein said device can be used to measure differences in impedance values that relate to cell behavior;

b) an impedance analyzer;

c) a device station comprising electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer; and

d) a software program that can said control device station and record and analyze data obtained from said impedance analyzer.

52. The system of claim 51, wherein said device station comprises electronic switches that can switch on and off connections of each of said two or more electrode arrays to said impedance analyzer.

53. The device station of claim 51, wherein said software program can direct said device station to measure impedance values at regular or irregular time intervals from one or more of said two or more electrode arrays.

54. The system of claim 51, wherein said impedance analyzer can measure impedance at one or more frequencies.

55. The device station of claim 51, wherein said software program can store said data.

56. The device station of claim 51 wherein said system can display said data.

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57. The device station of claim 51, wherein said software can calculate a cell index for one or more time points for one or more wells of said multi-well plate based on impedance values measured from said one or more electrode arrays localized to said one or more wells.

58. The device station of claim 56 wherein said system can display said cell index for said one or more time points data for said one or more wells.

59. The system of claim 51, wherein said software is programmable to direct impedance monitoring of any of said two or more electrode arrays at chosen time intervals.

60. The system of claim 55, wherein said software allows entry and display of experimental parameters.

61. The system of claim 51, further comprising at least one platform onto which said one or more devices can be positioned during operation of said system.

62. A system for monitoring cell-substrate impedance, comprising:

a) one or more devices of claim 44;

b) an impedance analyzer;

c) a device station comprising electronic circuitry
that can engage said device and selectively connect any one of said two or more electrode arrays of said device to said impedance analyzer; and

d) a software program that can said control device station and record and analyze data obtained from said impedance analyzer.

63. The system of claim 62, wherein said device station comprises electronic switches that can switch on and off connections of each of said two or more electrode arrays to said impedance analyzer.

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64. The system of claim 62, wherein said software can direct said device to measure impedance values at regular or irregular time intervals from one or more of said two or more electrode arrays.

65. The system of claim 62, wherein said software system can store said data.

66. The system of claim 62, wherein said system can display said data.

67. The system of claim 62, wherein said software can calculate a cell index for one or more time points for one or more wells of said multi-well plate based on impedance values measured from said one or more electrode arrays localized to said one or more wells.

68. The device station of claim 67 wherein said system can display said cell index for said one or more time points data for said one or more wells.

69. The system of claim 62, wherein said software is programmable to direct impedance monitoring from any of said two or more electrode arrays at chosen time intervals.

70. The system of claim 66, wherein said software allows entry and display of experimental parameters.

71. The system of claim 62, further comprising at least one platform onto which said one or more devices can be positioned during operation of said system.

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72. A method of performing an assay that monitors cell-substrate impedance, comprising:

providing the system of claim 51;

introducing cells into at least one well of said system;

monitoring cell-substrate impedance of said at least one well.

73. The method of claim 72, wherein said monitoring comprises analyzing impedance changes at three or more time points.

74. The method of claim 73, wherein at least three of said three or more time points are spaced at regular intervals.

75. A method of performing an assay that monitors cell-substrate impedance in response to one or more test compounds, comprising:

providing the system of claim 51;

introducing cells into one or more wells of said system that comprise electrode arrays;

adding at least one test compound to said one or more wells; and

monitoring cell-substrate impedance of said one or more wells before and after adding said at least one test compound.

76. The method of claim 75, wherein impedance is monitored at three or more time points.

77. The method of claim 75, further comprising analyzing impedance values before and after adding said at least one test compound.

78. The method of claim 75, further comprising introducing cells to at least one well to which a test compound is not added to create a control well.

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79. The method of claim 77, wherein said analyzing comprises calculating a cell index.

80. The method of claim 77, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a comound, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

81. The method of claim 79, wherein said cell index is used as an indicator of cytotoxicity.

82. A method of performing multiple assays that monitor cell-substrate impedance in response to one or more compounds, comprising:

providing the system of claim 51;

introducing cells into two or more wells of said system;

adding to at least one well of said two or more wells of said system a first concentration of a test compound, and adding to at least one other one well of said two or more wells of said system a second concentration of a test compound; and

monitoring cell-substrate impedance of said at two or more wells.

83. The method of claim 82, wherein said monitoring comprises monitoring impedance changes at three or more time points.

84. The method of claim 83, wherein at least one of said three or more time points is at a time prior to said adding said first concentration of a test compound to said at least one well and said second concentration of a test compound to said at least one other well.

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85. The method of claim 82, wherein introducing cells into two or more wells comprises wherein introducing cells into three or more wells and one of said three or more wells is a control well that does not receive test compound.

86. The method of claim 83, wherein at least three of said three or more time points are spaced at regular intervals.

87. The method of claim 83, further comprising analyzing the effect of same one or more compounds on said cells.

88. The method of claim 89 wherein said analyzing comprises calculating a cell index.

89. The method of claim 89, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a comound, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

90. The method of claim 88, wherein said cell index is used as an indicator of cytotoxicity.

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91. A method of performing multiple assays that monitor cell-substrate impedance in response to two or more compounds, comprising:

providing a system of claim 51;

introducing cells into two or more wells of said system;

adding a first compound to at least one well of said two or more wells of said system;

adding a second compound to at least one other well of said two or more wells of said system; and

monitoring cell-substrate impedance of said at least one well comprising said first test compound and said at least one other well comprising said second compound.

92. The method of claim 91, wherein said monitoring comprises monitoring impedance changes at three or more time points.

93. The method of claim 91, wherein at least one or said two or more wells is a control well that does not receive said test compound.

94. The method of claim 92, wherein at least one of said one or more time points is at a time prior to said adding at least one test compound to said at least one well.

95. The method of claim 91, wherein at least three of said three or more time points are spaced at regular intervals.

96. The method of claim 92, further comprising analyzing the effect of said one or more compounds on said cells.

97. The method of claim 96, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a comound, IgE-mediated cell activation or stimulation, receptor-

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ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

98. The method of claim 96, wherein said analyzing comprises calculating a cell index.

99. The method of claim 98, wherein said cell index is plotted versus time.

100. The method of claim 98, wherein said cell index is used as an indicator of cytotoxicity.

101. A method for monitoring changes in cell-substrate impedance in response to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system, wherein each of said one or more wells comprises an electrode array;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance of at least one of said one or more wells of said system.

102. The method of claim 101, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

103. The method of claim 102, wherein said cells are mast cells.

104. The method of claim 101, wherein said IgE is added at a concentration of from between about 10 nanograms per milliliter to about 1 microgram per milliliter.

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105. The method of claim 101, wherein said cell-substrate impedance is monitored after adding said IgE to said one or more wells.

106. The method of claim 105, wherein cell-substrate impedance is monitored before and after adding said IgE to said one or more wells.

107. The method of claim 105, wherein said cell-substrate impedance is monitored after adding said IgE to said one or more wells and before adding antigen to said one or more wells.

108. The method of claim 107, wherein said cell-substrate impedance is monitored monitored after adding said IgE to said one or more wells and before adding antigen to said one or more wells and after adding said antigen to said one or more wells.

109. The method of claim 108,wherein said cell-substrate impedance is monitored before adding said IgE to said one or more wells, after adding said IgE to said one or more wells and before adding antigen to said one or more wells, and after adding said antigen to said one or more wells.

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110. A method for screening for compounds that can modulate cellular responses to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system;

adding at least one test compound to at least one of said one or more wells of said system, each of which comprises an electrode array;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance from said one or more wells of said system; and

analyzing said impedance data from said one or more wells to evaluate whether said at least one test compound can modulate cellular responses to IgE stimulation.

111. The method of claim 110, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

112. The method of claim 111, wherein said cells are mast cells.

113. The method of claim 110, wherein said one or more test compounds are added to said one or more wells prior to said adding IgE to said one or more wells.

114. The method of claim 110, wherein said IgE is added at a concentration of from between about 10 nanograms per milliliter to about 1 microgram per milliliter.

115. The method of claim 110, wherein said cell-substrate impedance is monitored after adding said IgE to said one or more wells.

116. The method of claim 115, wherein cell-substrate impedance is monitored before adding said IgE to said one or more wells.

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117. The method of claim 110, wherein said cell-substrate impedance is monitored after adding said IgE to said one or more wells and before adding antigen to said one or more wells.

118. The method of claim 117, wherein said cell-substrate impedance is monitored monitored after adding said IgE to said one or more wells and before adding antigen to said one or more wells and after adding said antigen to said one or more wells.

119. The method of claim 118,wherein said cell-substrate impedance is monitored before adding said IgE to said one or more wells, after adding said IgE to said one or more wells and before adding antigen to said one or more wells, and after adding said antigen to said one or more wells.

120. The method of claim 110, wherein said at least one test compound is a compound that can bind IgE or a compound that can bind Fc(epsilon)RI.

121. The method of claim 120, wherein said at least one test compound is a compound that can bind IgE.

122. The method of claim 121, wherein said at least one test compound is an antibody that can bind IgE.

123. The method of claim 120, wherein said at least one test compound is a compound that can bind Fc(epsilon)RI.

124. The method of claim 123, wherein said at least one test compound is a compound that can bind the antigen binding domain of Fc(epsilon)RI.

125. The method of claim 120, wherein said at least one test compound is a compound that can act intracellularly.

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126. The method of claim 125, wherein said at least one test compound is a compound that can inhibit a kinase.

127. The method of claim 126, wherein said at least one compound that can inhibit a kinase can inhibit protein kinase C, SRC, of Syk.

128. The method of claim 125, wherein said at least one test compound is a compound that can inhibit a phospholipase.

129. The method of claim 125, wherein said at least one test compound is a compound that can inhibit PLC(gamma).

130. The method of claim 110, wherein said at least one test compound is added to at least two of said one or more wells, and wherein at least two of said two or more wells receive different concentrations of said at least one test compound.

131. The method of claim 120, further comprising recording cell-substrate impedance values and calculating an IC50 for said at least one test compound based on said cell substrate impedance values.

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132. A method for identifying a target for modulating cellular responses to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system, wherein said cells are genetically modified to alter the function of, or reduce or ablate expression of, a suspected target molecule;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance of said one or more wells of said system to obtain impedance data from said one or more wells, and

analyzing said impedance data to identify a target for modulation cellular responses to to IgE stimulation.

133. The method of claim 132, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

134. The method of claim 133, wherein said cells are mast cells.

135. The method of claim 132, wherein one or more compounds for genetically modifying said cells are added to said one or more wells prior to said adding IgE to said one or more wells.

136. The method of claim 132, wherein said IgE is added at a concentration of from between about 10 nanograms per milliliter to about 1 microgram per milliliter.

137. The method of claim 132, wherein cell-substrate impedance is monitored before adding said IgE to said one or more wells.

138. The method of claim 132, wherein said cell-substrate impedance is monitored after adding said IgE to said one or more wells.

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139. The method of claim 138, wherein said cell-substrate impedance is monitored after adding said IgE to said one or more wells and before adding antigen to said one or more wells.

140. The method of claim 139, wherein said cell-substrate impedance is monitored after adding said antigen to said one or more wells.

141. The method of claim 140,wherein said cell-substrate impedance is monitored before adding said IgE to said one or more wells, after adding said IgE to said one or more wells and before adding antigen to said one or more wells, and after adding said antigen to said one or more wells.

142. The method of claim 132, wherein said at least one test compound is added to at least two of said one or more wells, and wherein at least two of said two or more wells receive different concentrations of said at least one test compound.

143. The method of claim 142, further comprising recording cell-substrate impedance values of genetically modified cells and cells that are not genetically modified.

144. A method for comparing responses to IgE stimulation between cells of different genotypes, comprising:

providing the system of claim 51;

introducing cells of a first genotype into one or more wells of said system to generate one or more first genotype cell wells;

introducing cells of a second genotype into one or more wells of said system to generate one or more second genotype cell wells;

adding IgE to said one or more first genotype cell wells and said one or more second genotype cell wells;

adding an antigen to said one or more first genotype cell wells and said one or more second genotype cell wells;

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monitoring cell-substrate impedance of at least one of said one or more first genotype cell wells and at least one of said one or more second genotype cell wells; and

comparing cell-substrate impedance values of said at least one of said one or more first genotype cell wells and at least one of said one or more second genotype cell wells.

145. The method of claim 144, further comprising correlating said cell-substrate impedance values with molecular genetic data obtained from said first genotype cells and said second genotype cells.

146. The method of claim 145, wherein said molecular genetic data comprises RFLIP data, SNP data, DNA marker data, RNA expression profiling data, protein expression profiling data, or alternative splicing data.

147. The method of claim 146, further comprising adding at least one antigen to at least one of said one or more first genotype cell wells and at least one of said one or more second genotype cell wells.

148. The method of claim 144, wherein said at least one antigen is a known or suspected allergen.

149. The method of claim 144, wherein said first genotype cells and said second genotype cells are from human subjects.

150. The method of claim 144, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

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151. A method for identifying an antigen or half antigen that can cause an allergic response, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system;

adding IgE to said one or more wells of said system;

adding at least one compound suspected of being an antigen or half antigen to at least one of said one or more wells of said system;

monitoring cell-substrate impedance of said at least one of said one or more wells of said system to obtain cell-substrate impedance data of said cells treated with antigen or half antigen; and

analyzing said data to identify an antigen or half antigen.

152. The method of claim 151, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

153. The method of claim 72, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

154. The method of claim 82, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

155. The method of claim 88, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

156. The method of claim 91, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

157. The method of claim 101, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

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158. The method of claim 110, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

159. The method of claim 132, wherein said one or more multiple well cell-substrate impedance monitoring devices are positioned in an incubator.

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ABSTRACT

The present invention includes devices, systems, and methods for assaying cells using cell-substrate impedance monitoring. In one aspect, the invention provides cell-substrate impedance monitoring devices that comprise electrode arrays on a nonconducting substrate, in which each of the arrays has an approximately uniform electrode resistance across the entire array. In another aspect, the invention provides cell-substrate monitoring systems comprising one or more cell-substrate monitoring devices comprising multiple wells each having an electrode array, an impedance analyzer, a device station that connects arrays of individual wells to the impedance analyzer, and software for controlling the device station and impedance analyzer. In another aspect, the invention provides cellular assays that use impedance monitoring to detect changes in cell behavior or state. In some preferred aspects, the assays are designed to investigate the affects of compounds on cells, such as cytotoxicity assays. In other preferred aspects, the assays are designed to investigate the compounds that effect IgE-mediated responses of cells to antigens.

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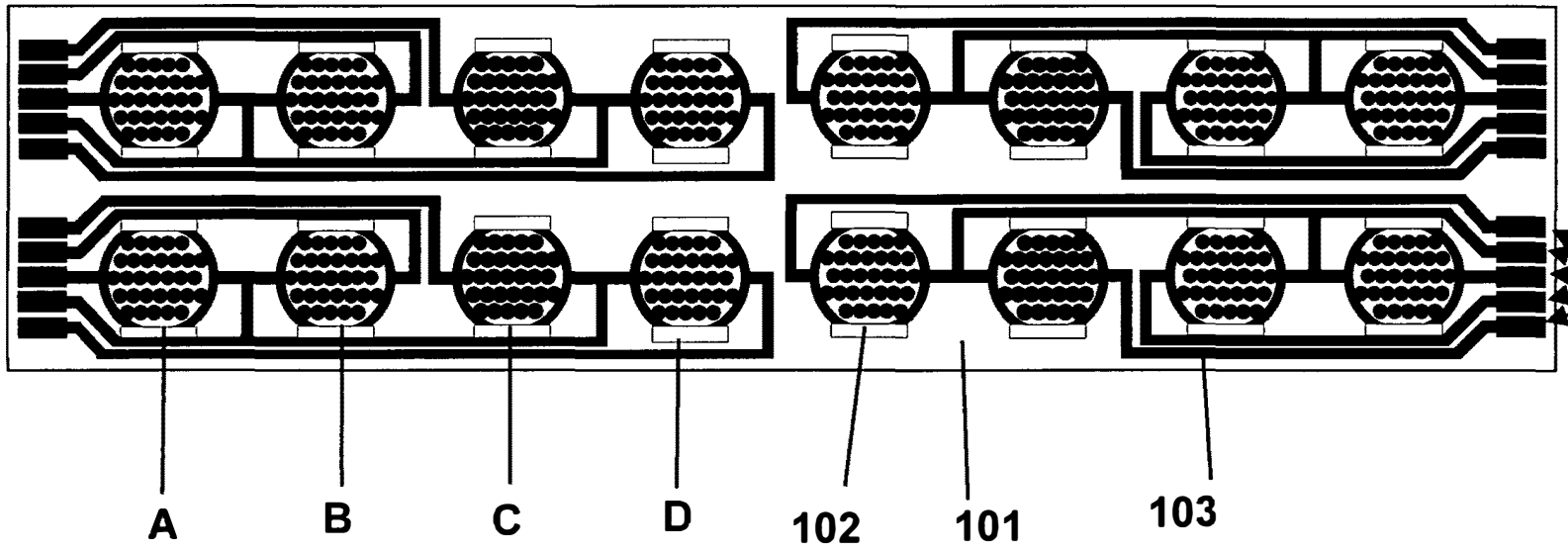


Figure 1A

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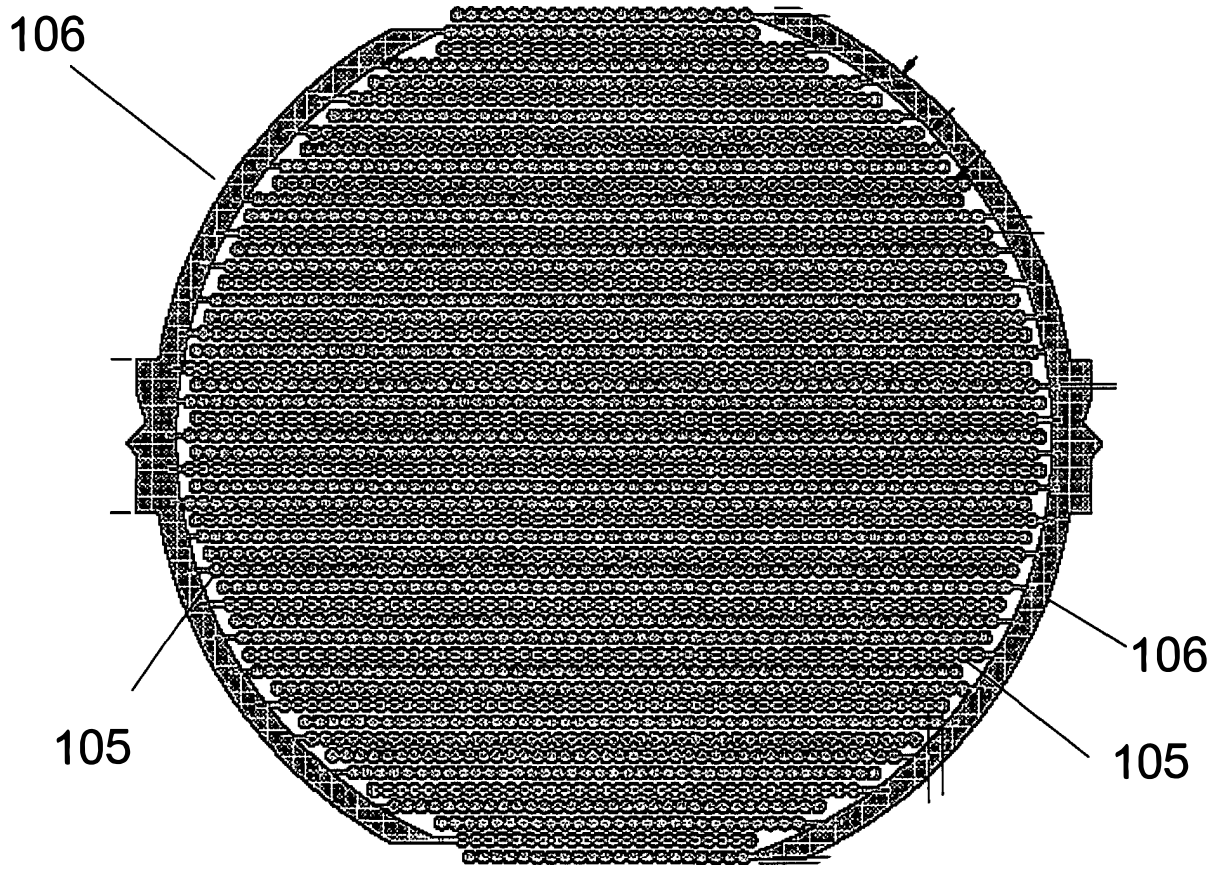


Figure 1B

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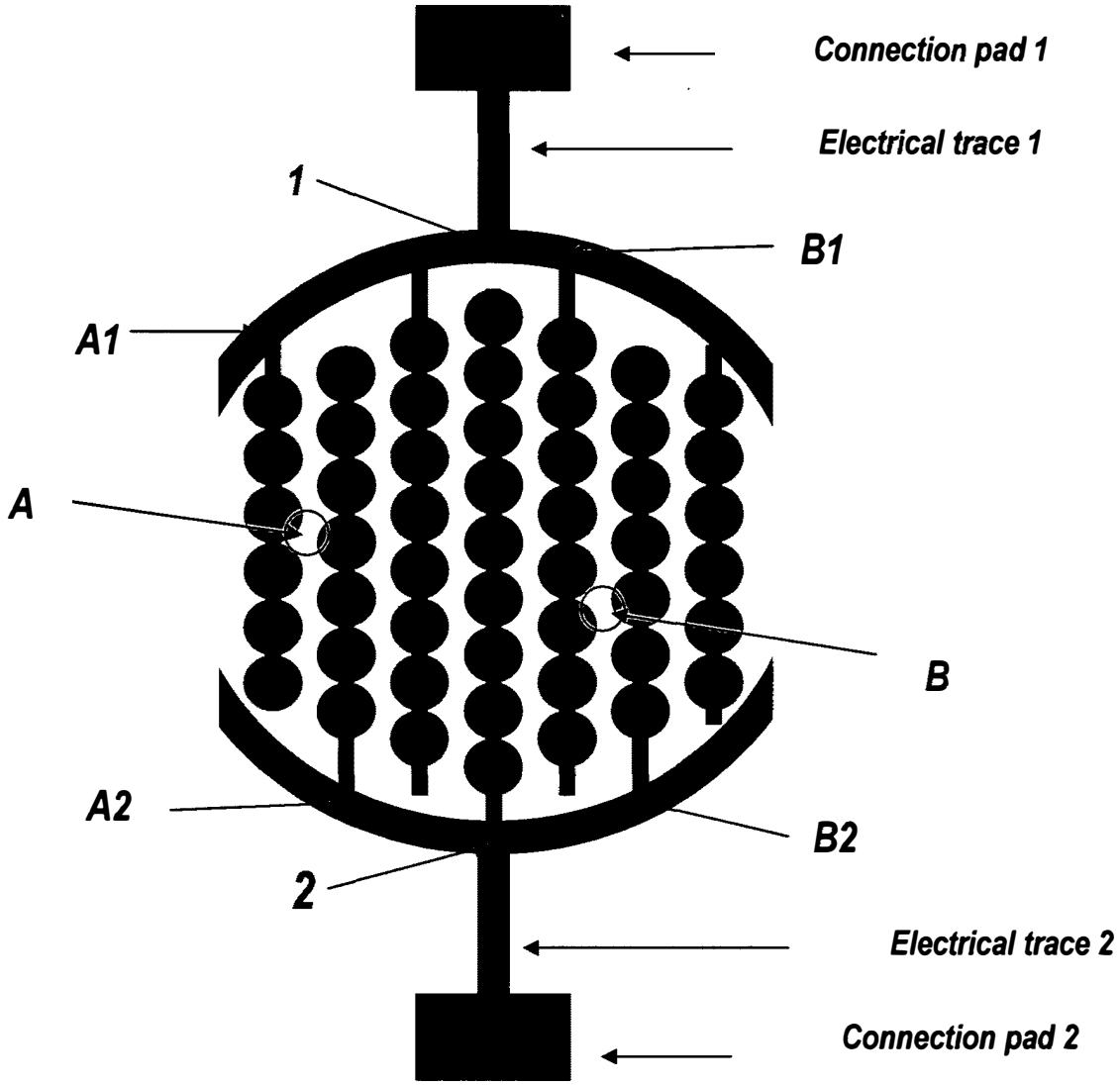


Figure 1C

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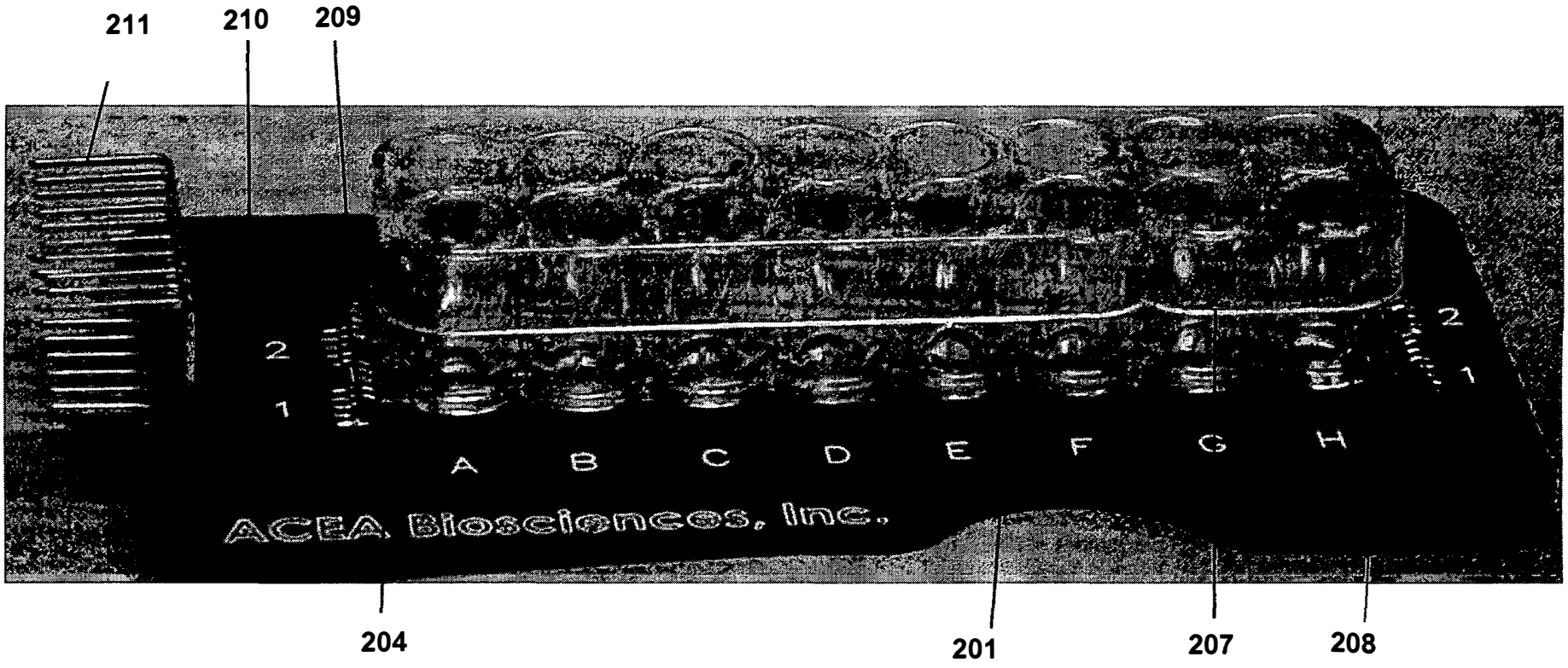


Figure 2.

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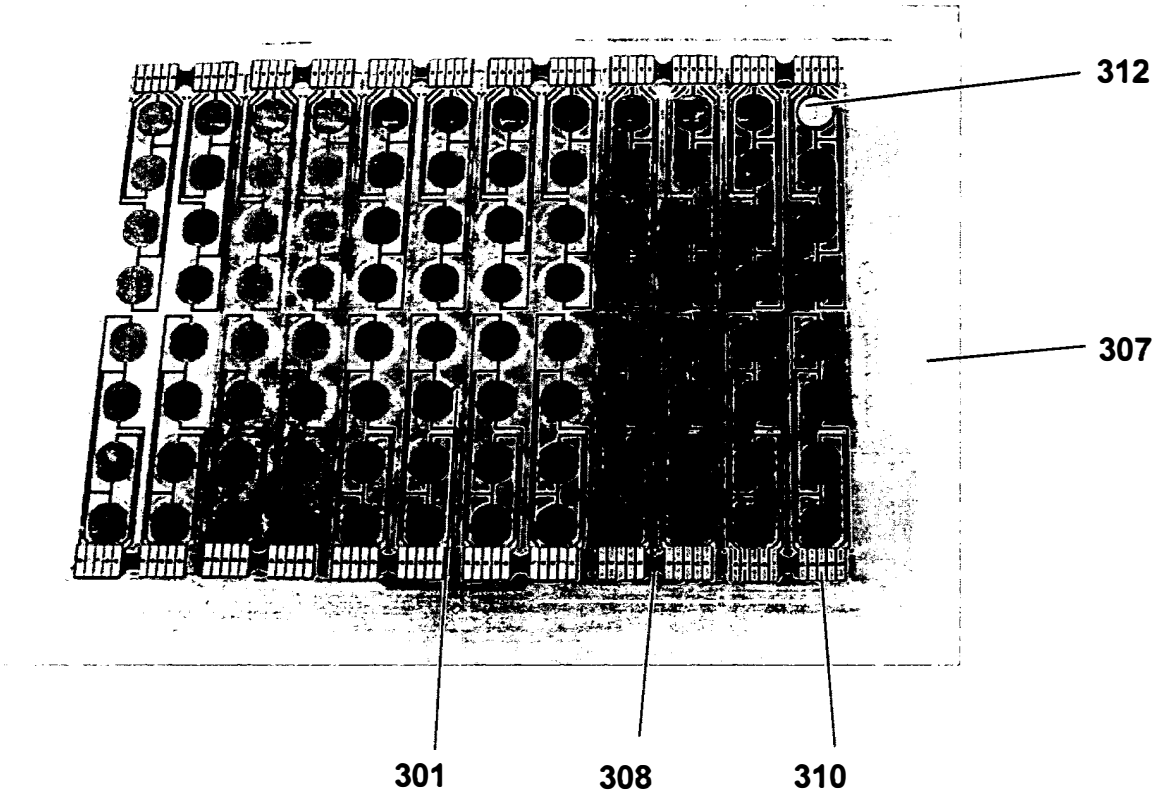


Figure 3A.

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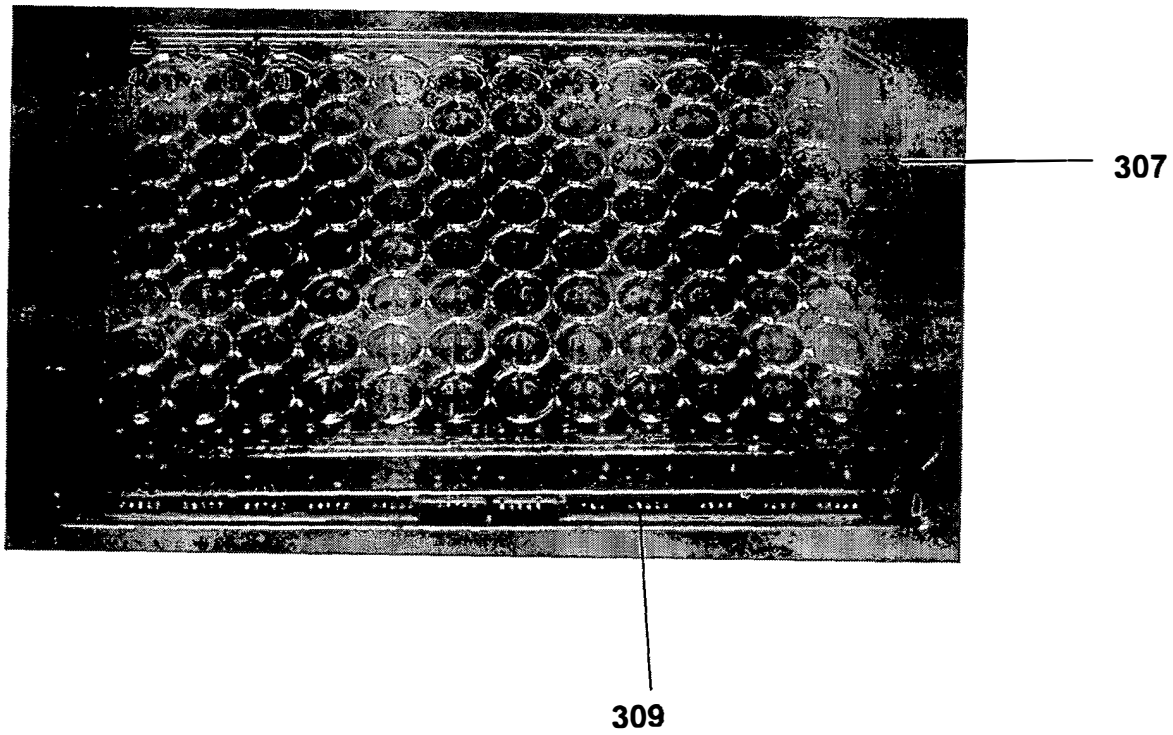


Figure 3B.

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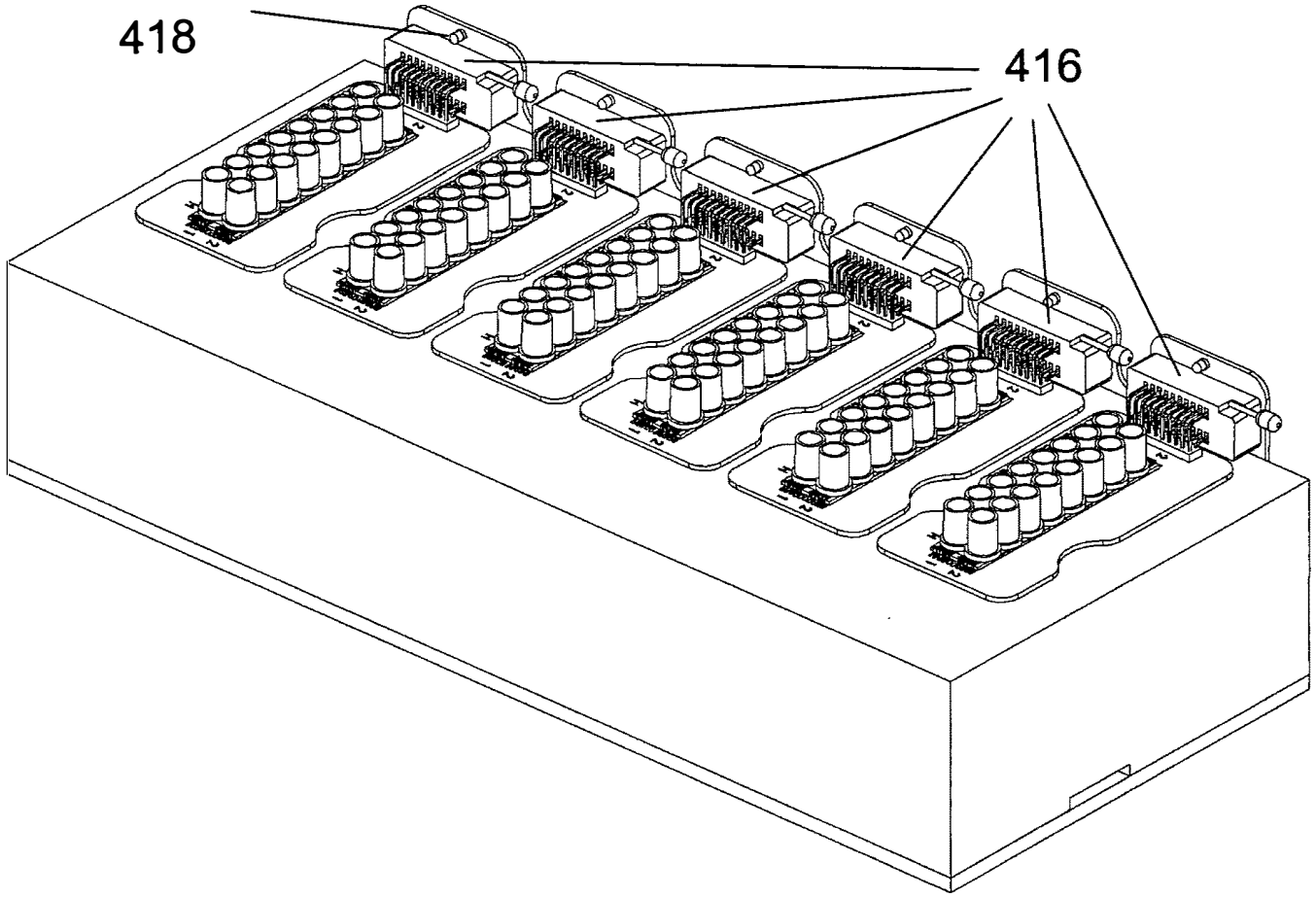


Figure 4.

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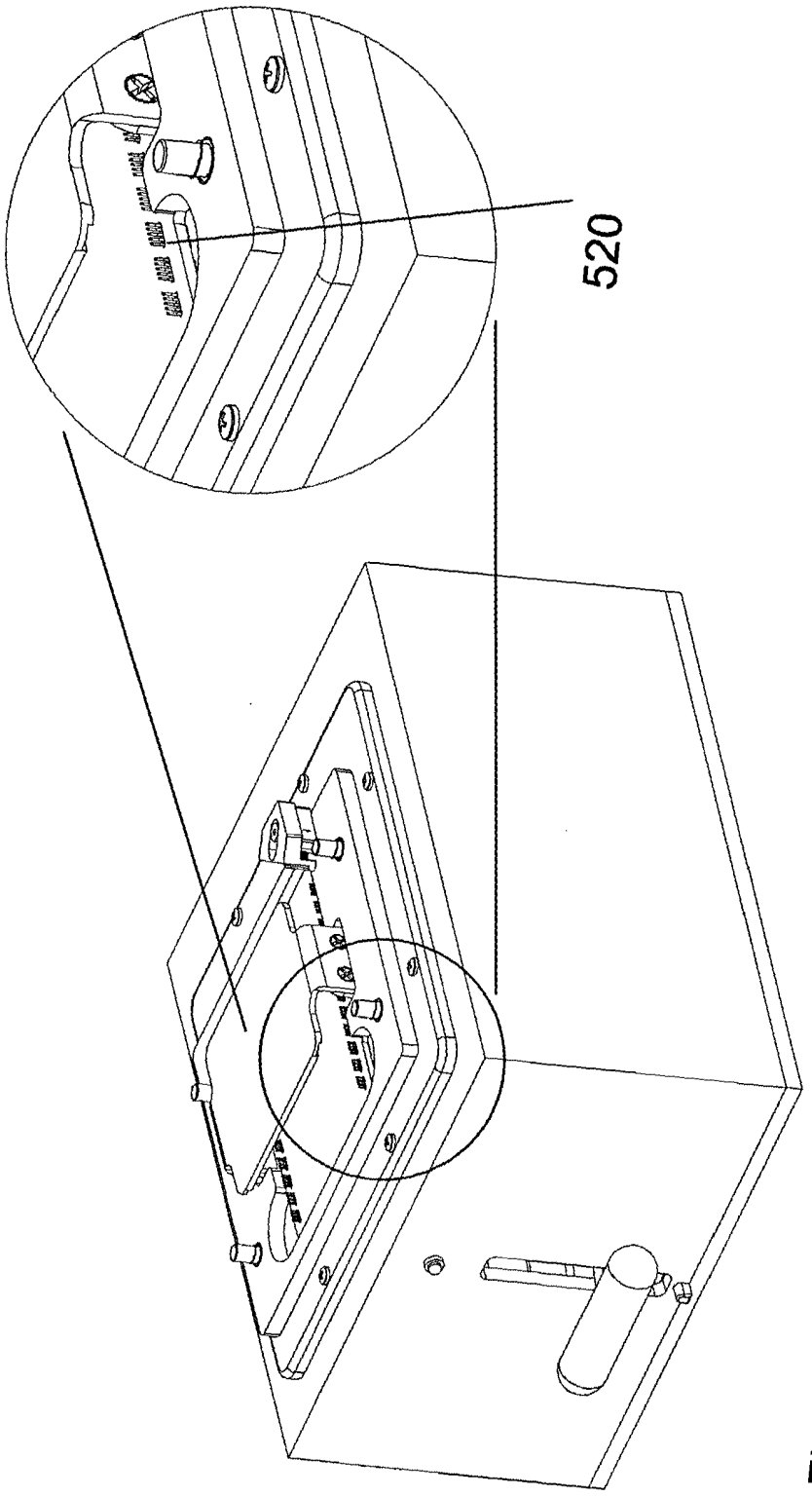


Figure 5.

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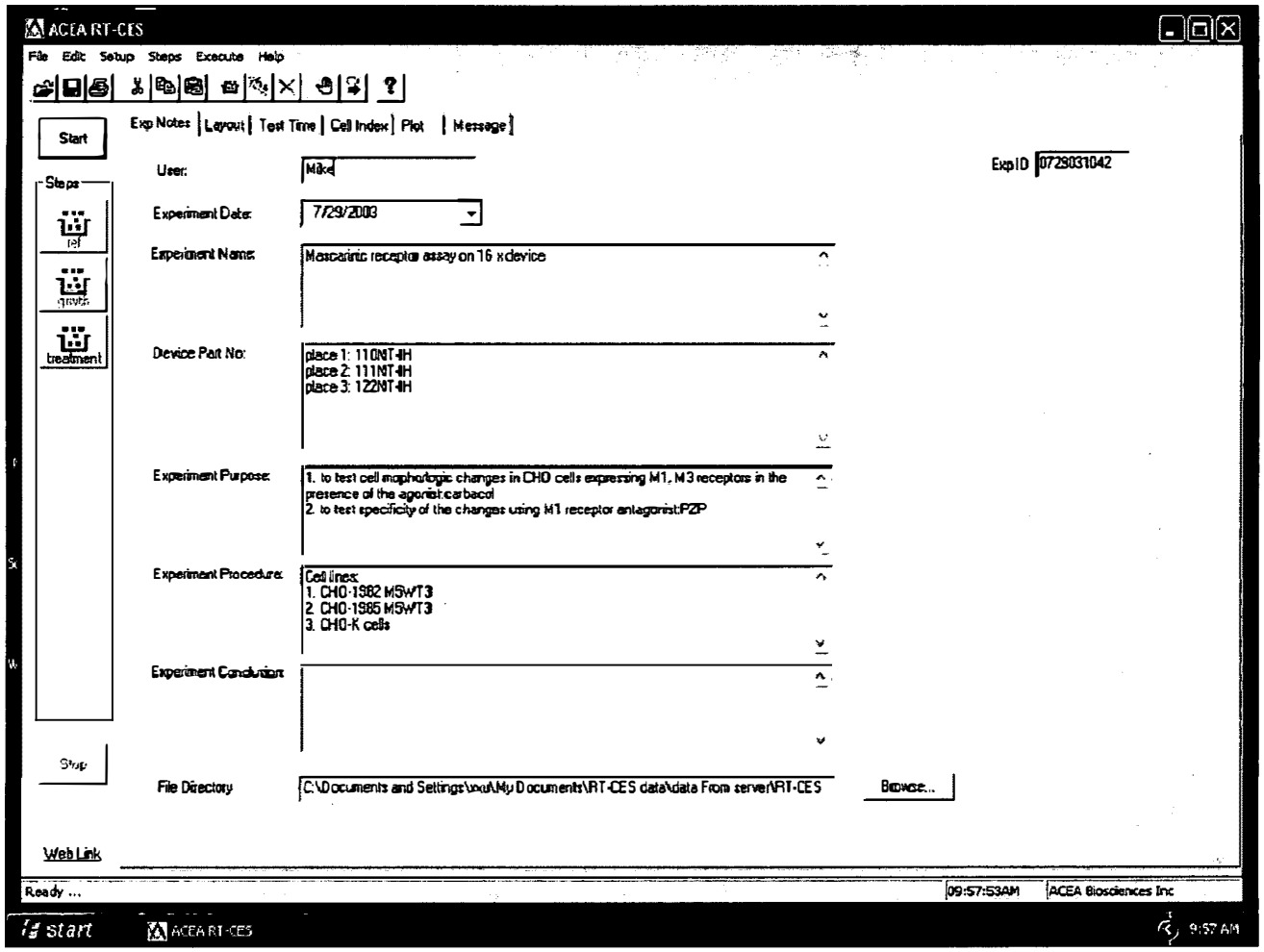


Figure 6A

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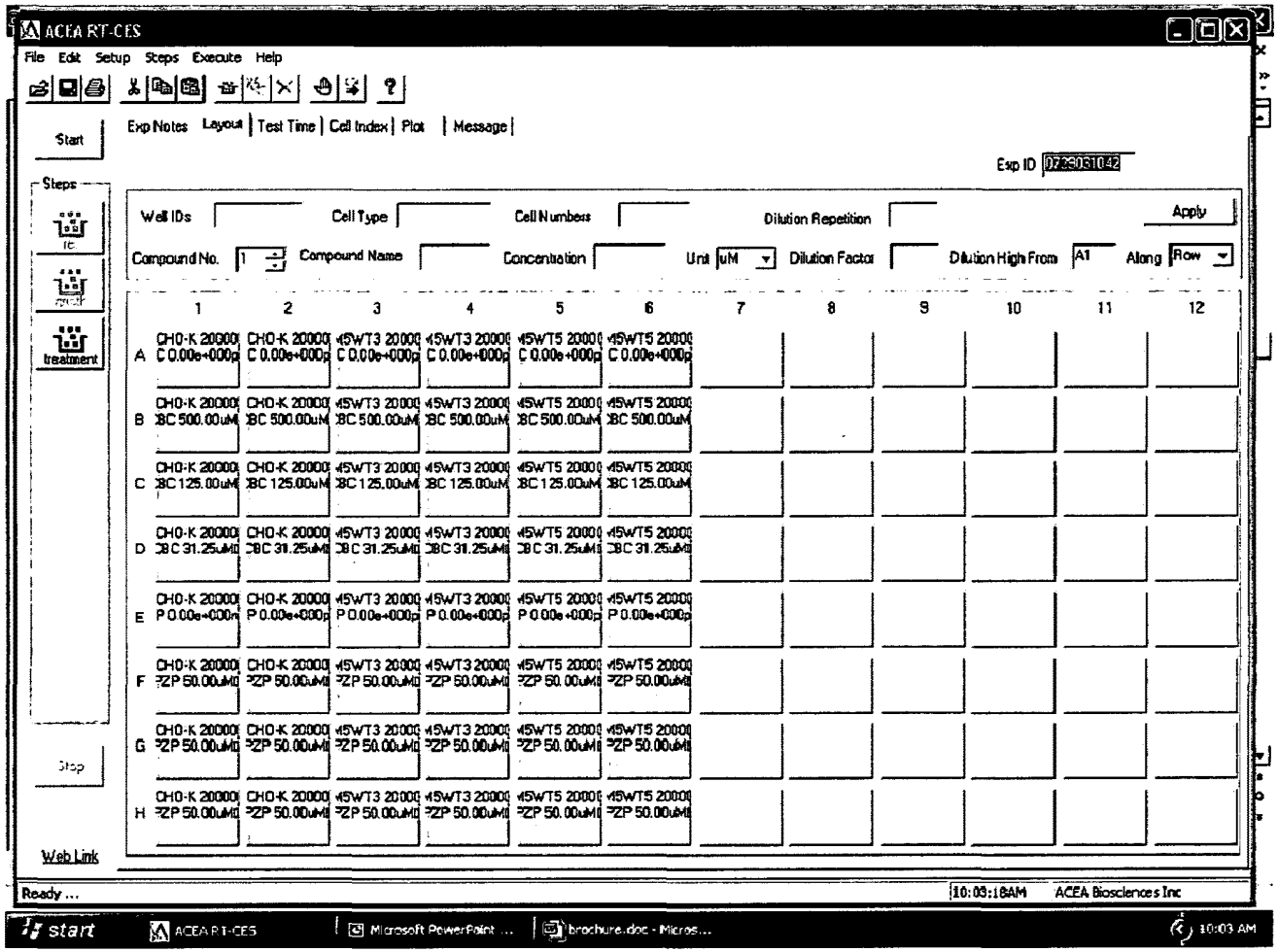


Figure 6B

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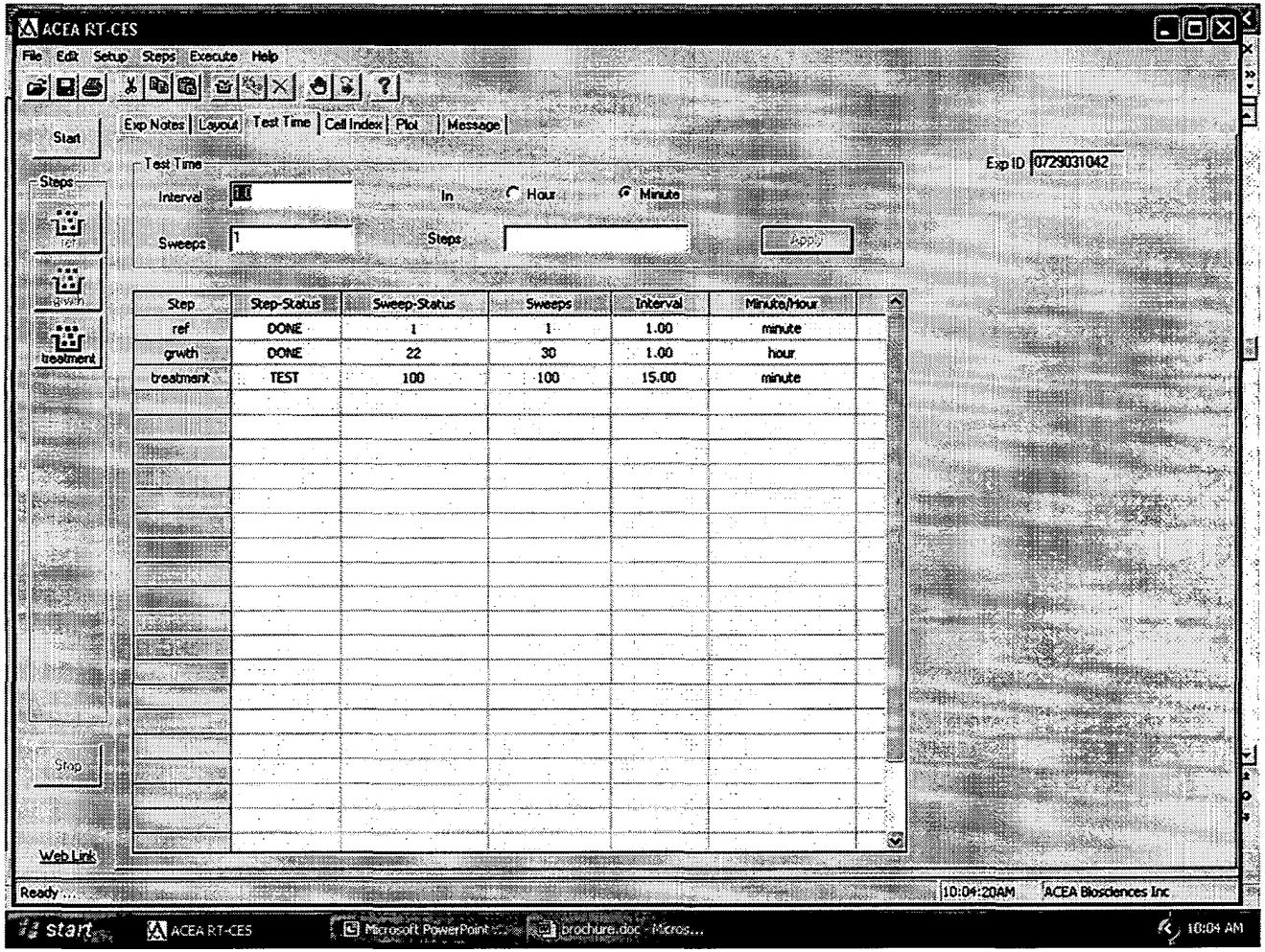


Figure 6C

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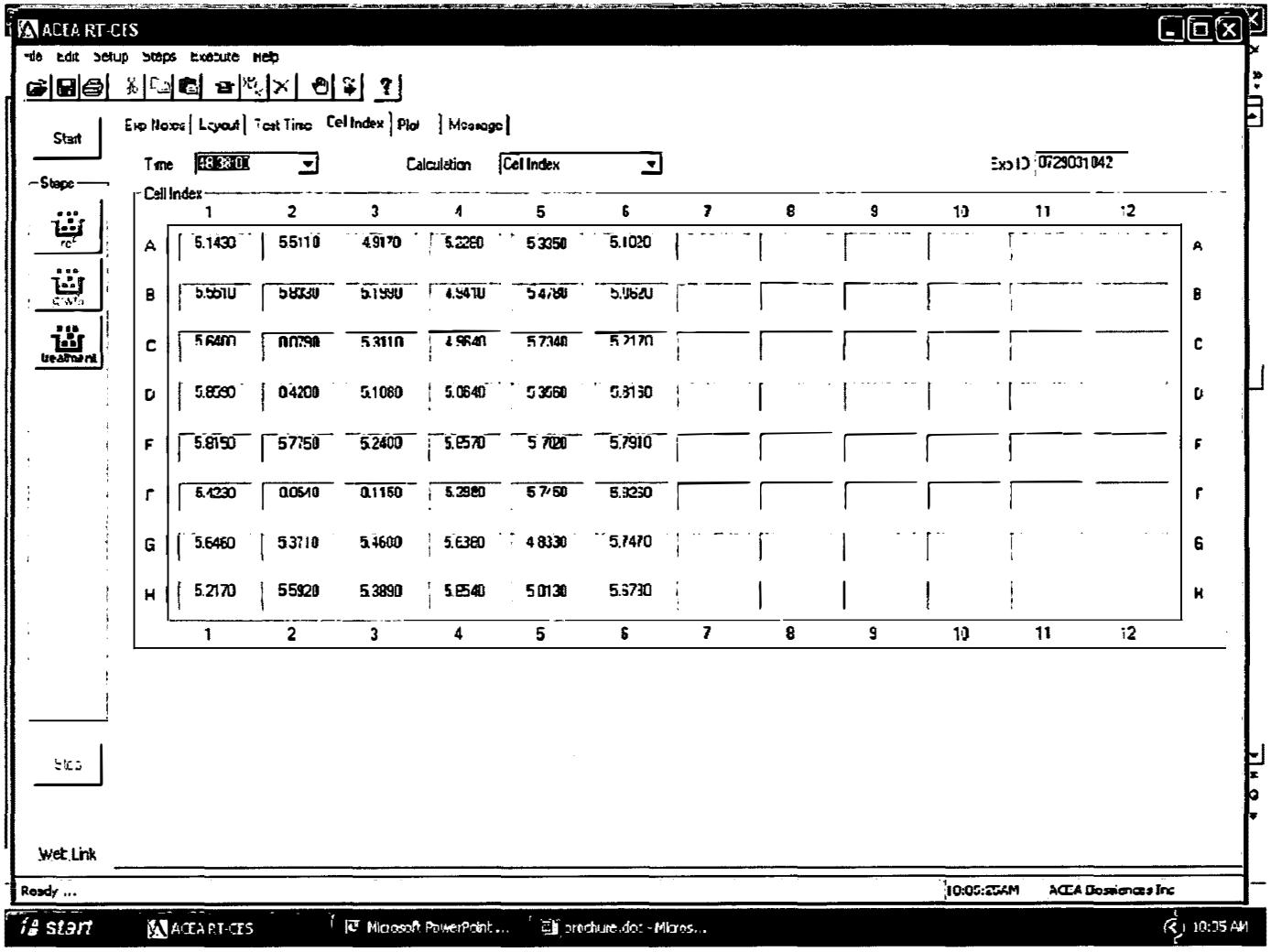


Figure 6D

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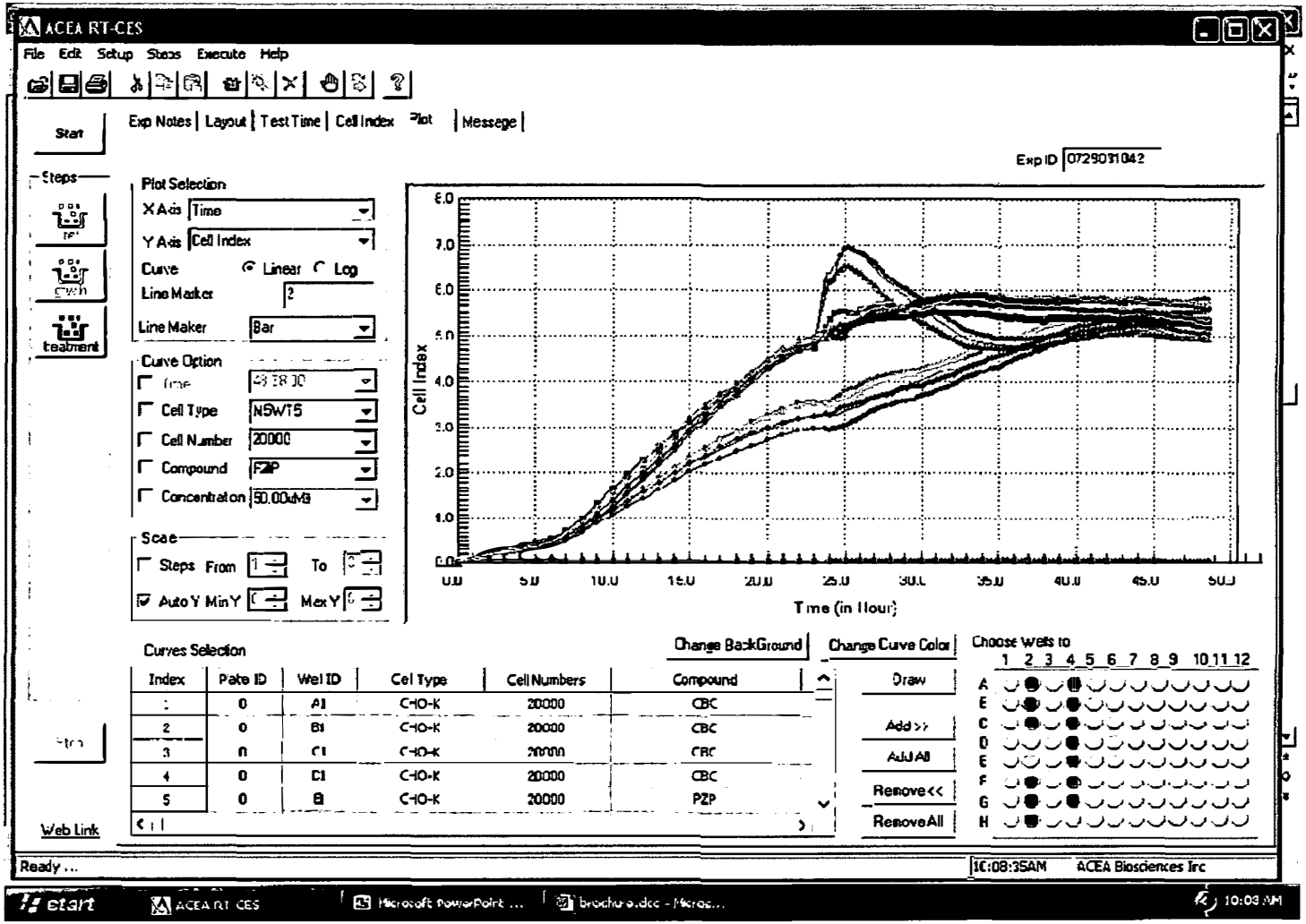


Figure 6E

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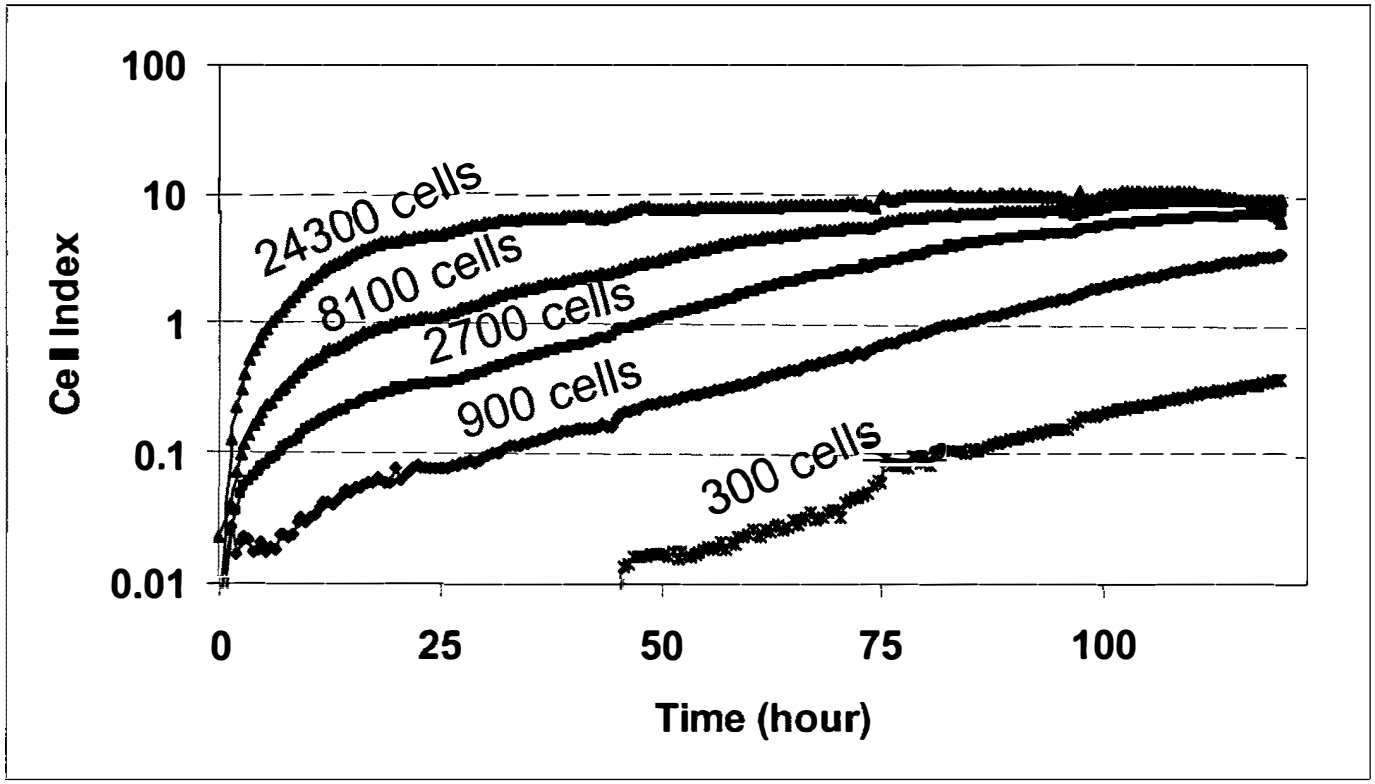


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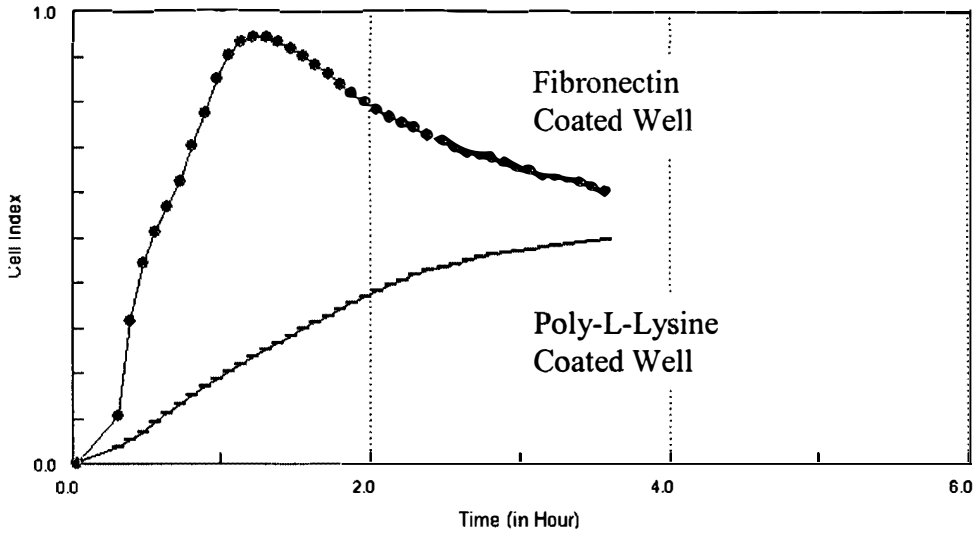


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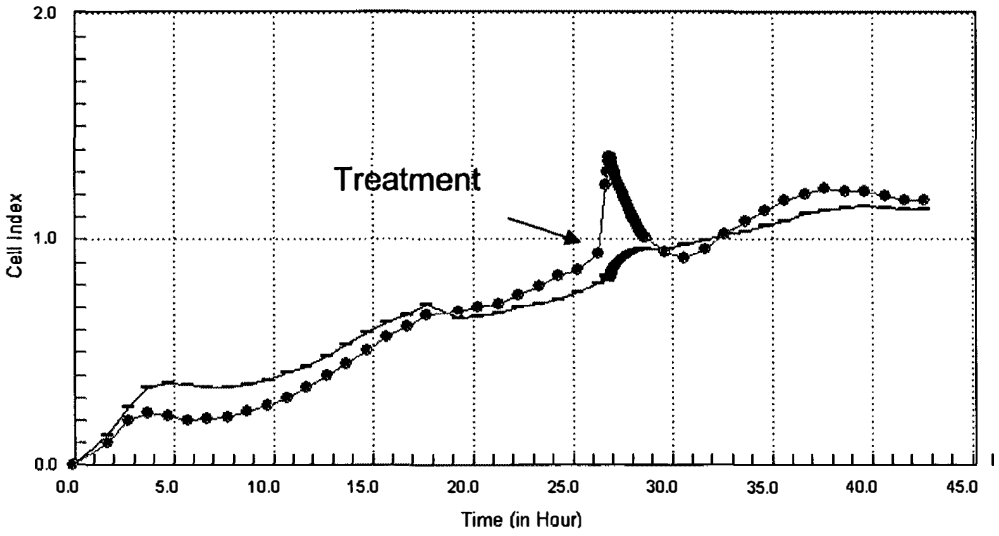


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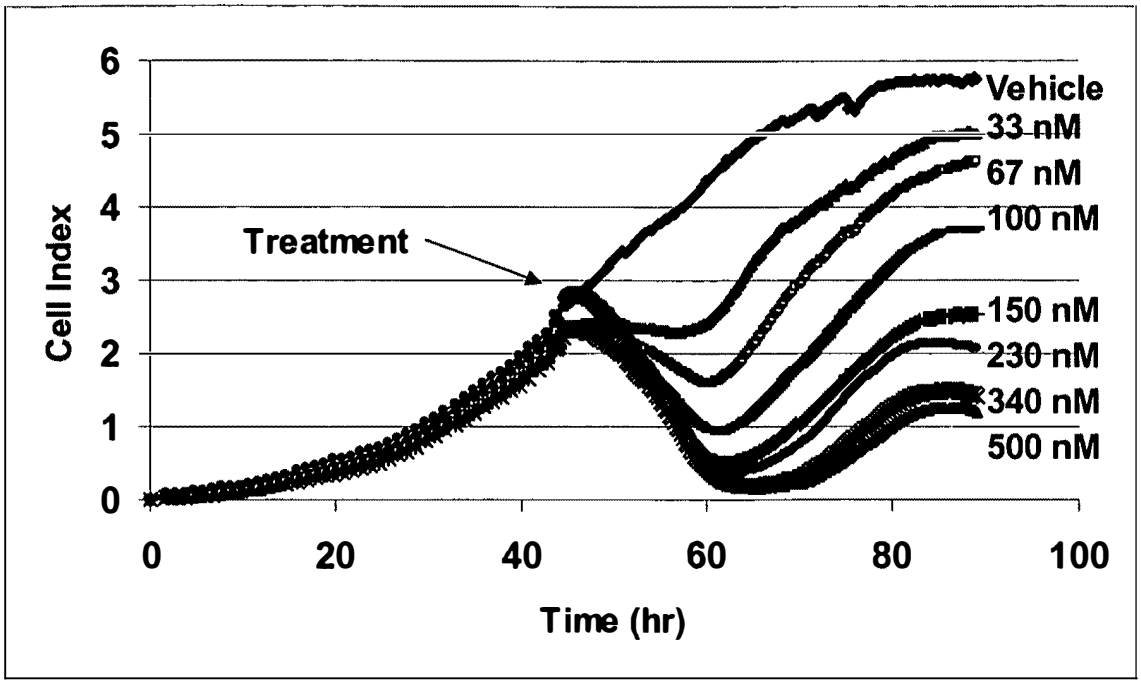


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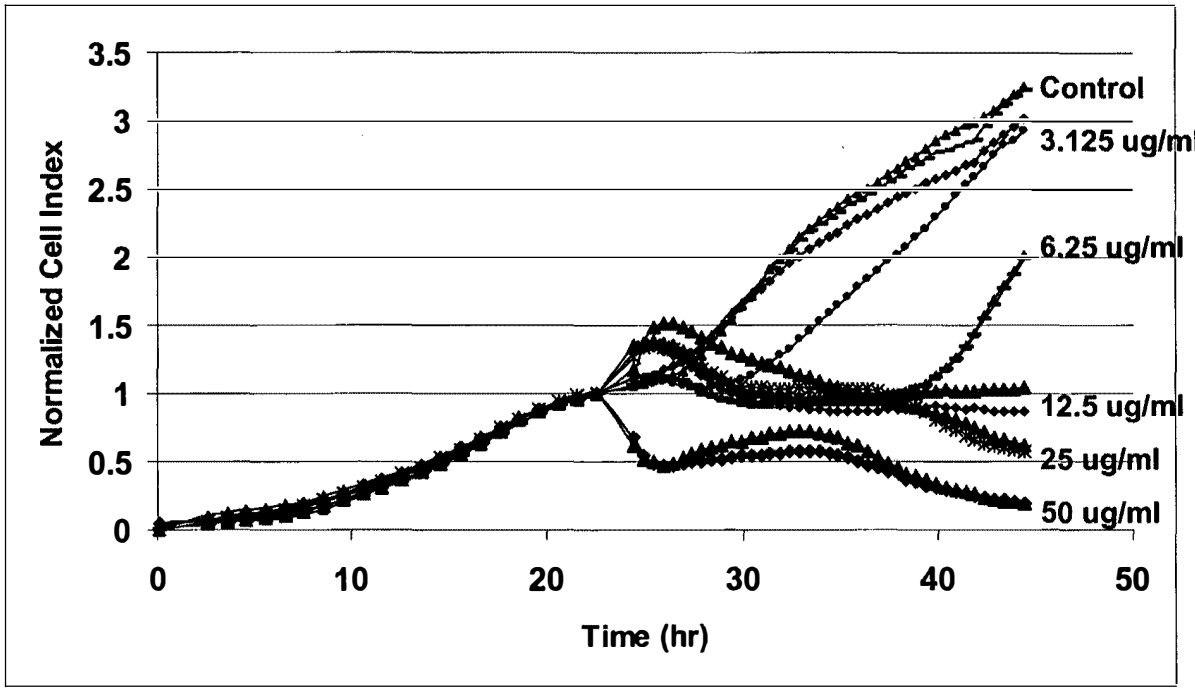


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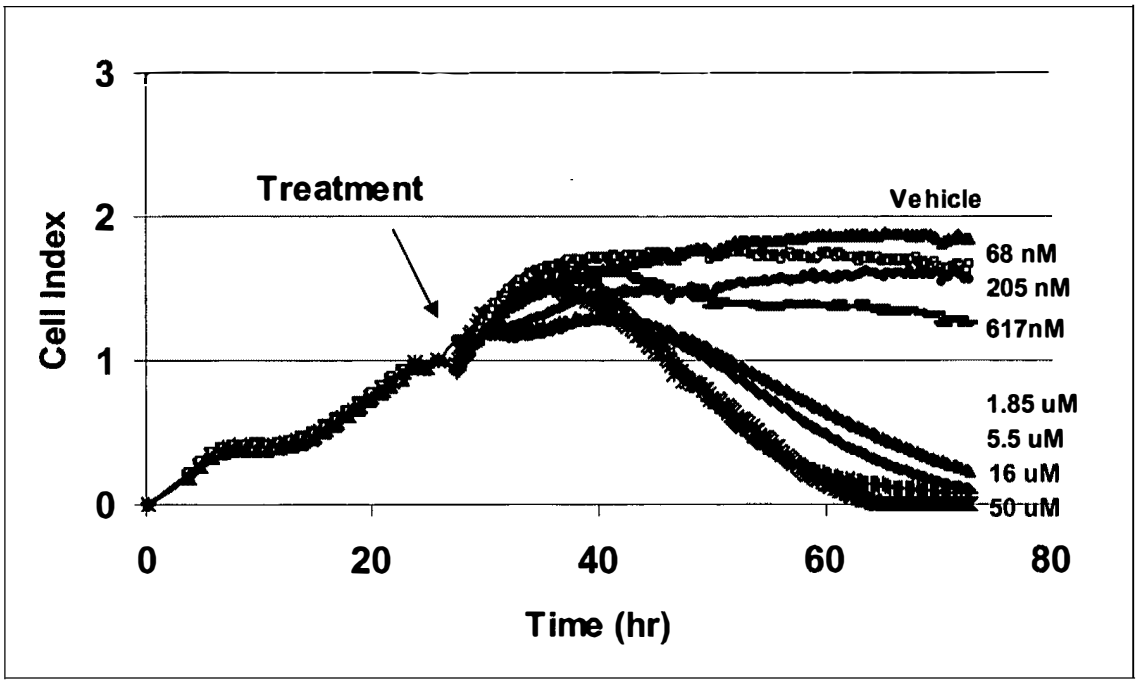
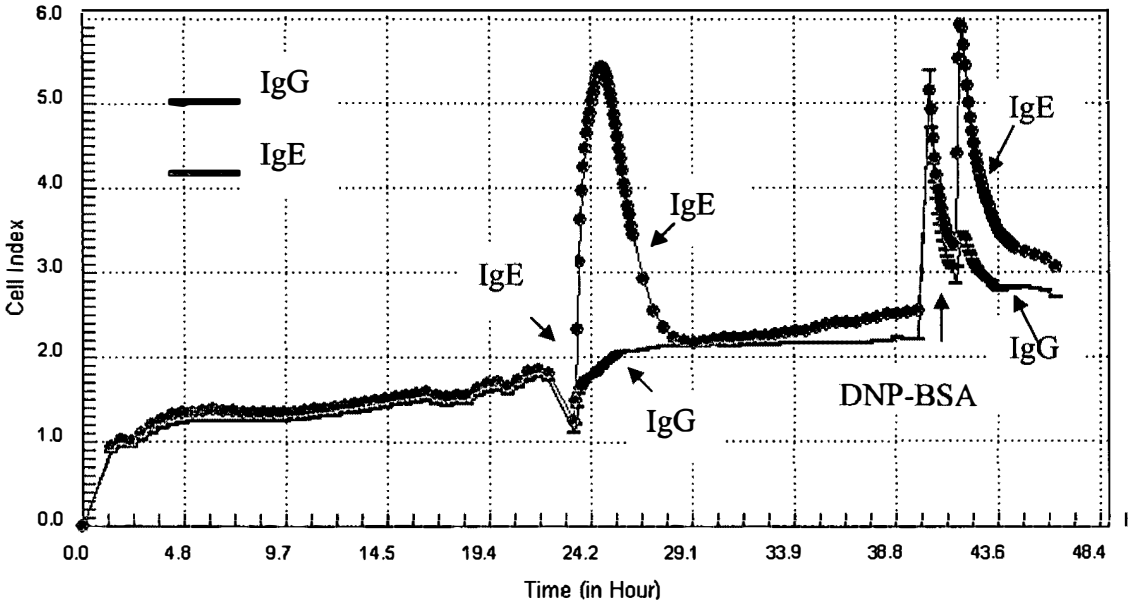


Figure 12.

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Figure 13.



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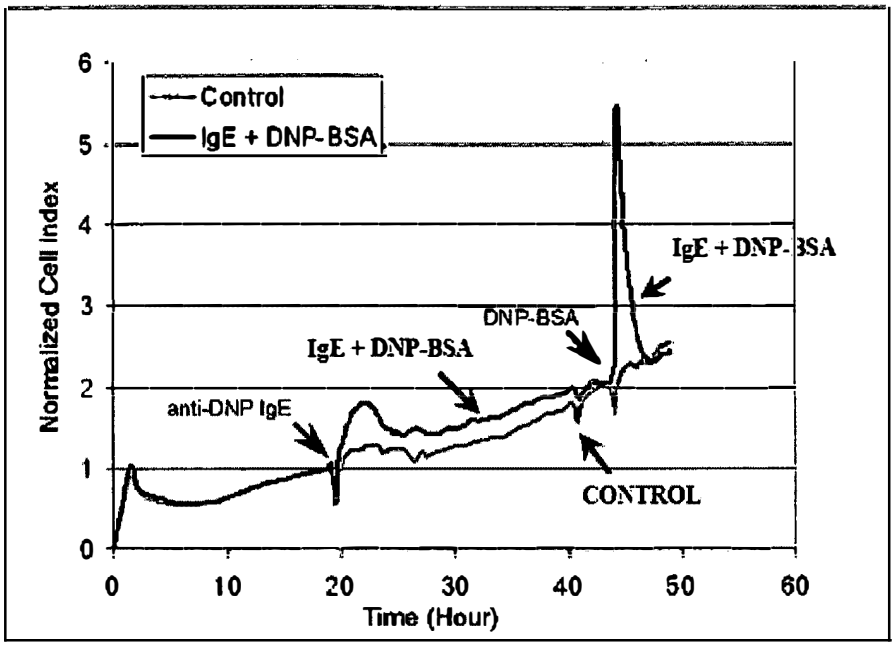
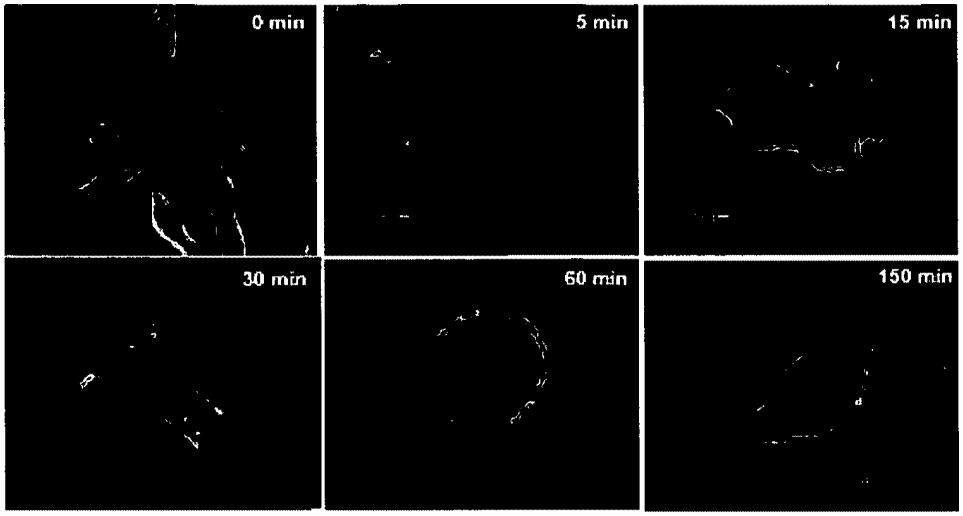


Figure 14.

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A.



B.

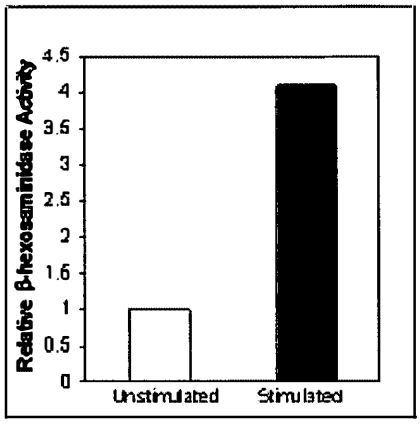


Figure 15.

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A.

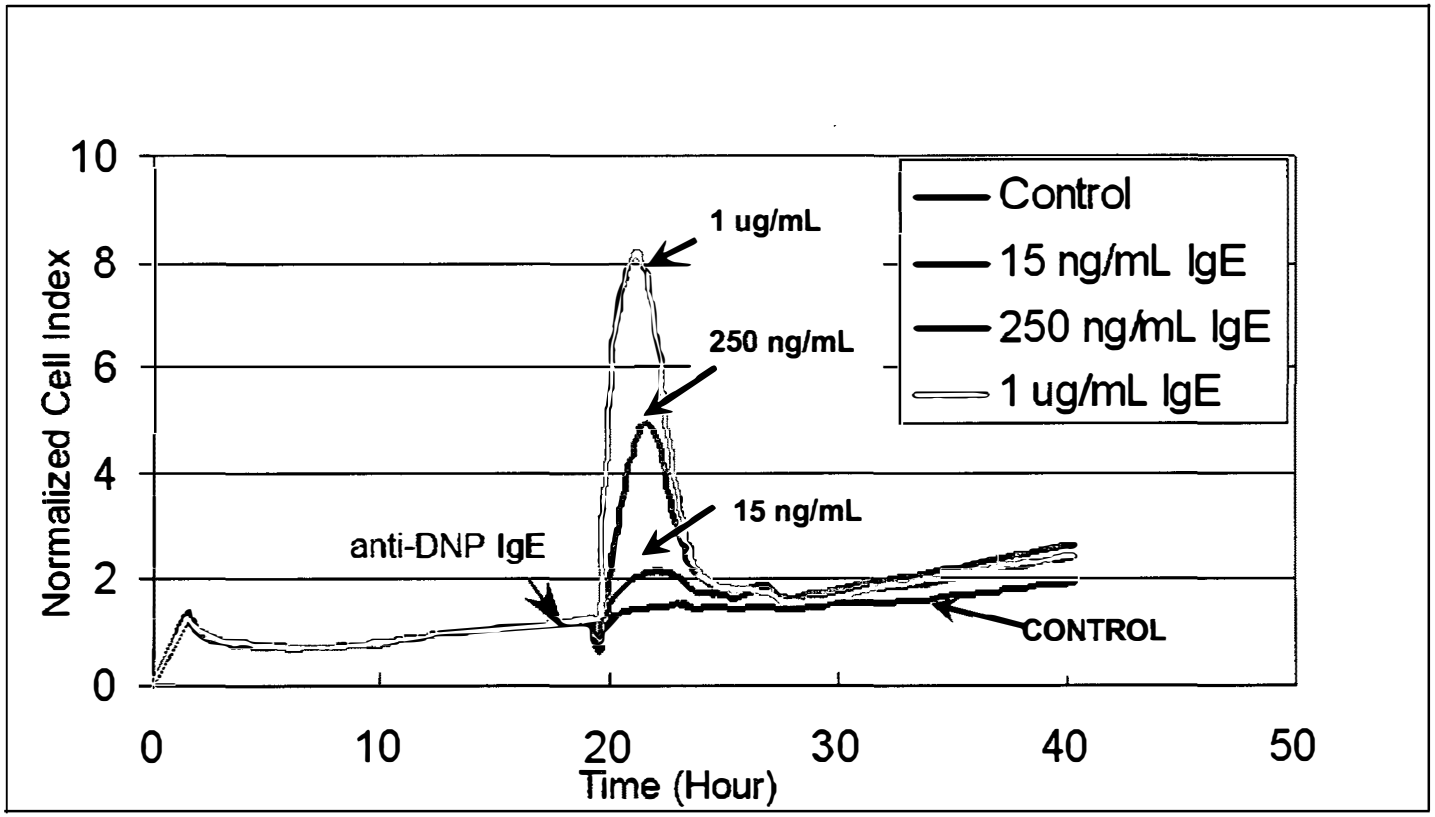


Figure 16A.

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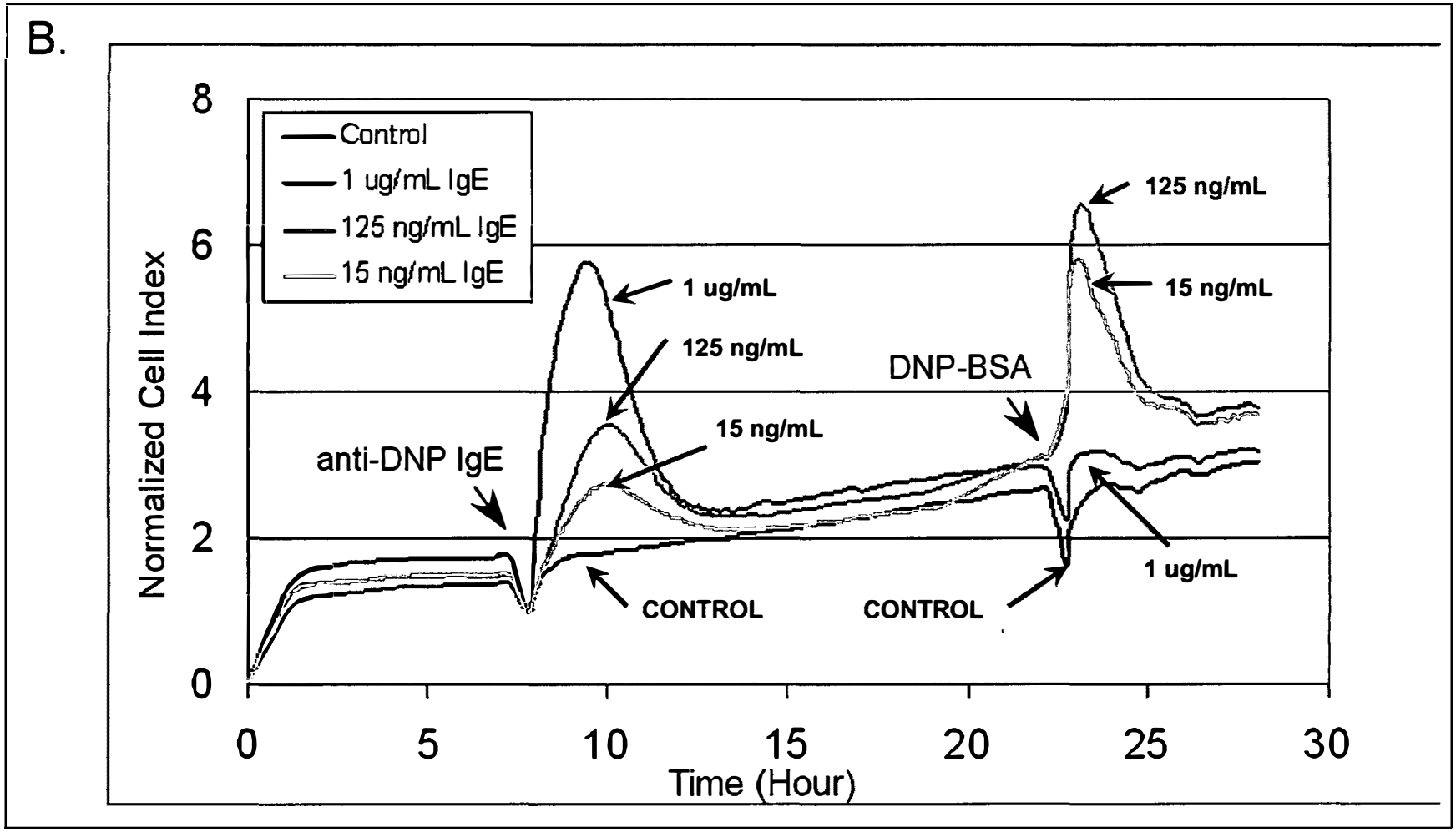


Figure 16B.

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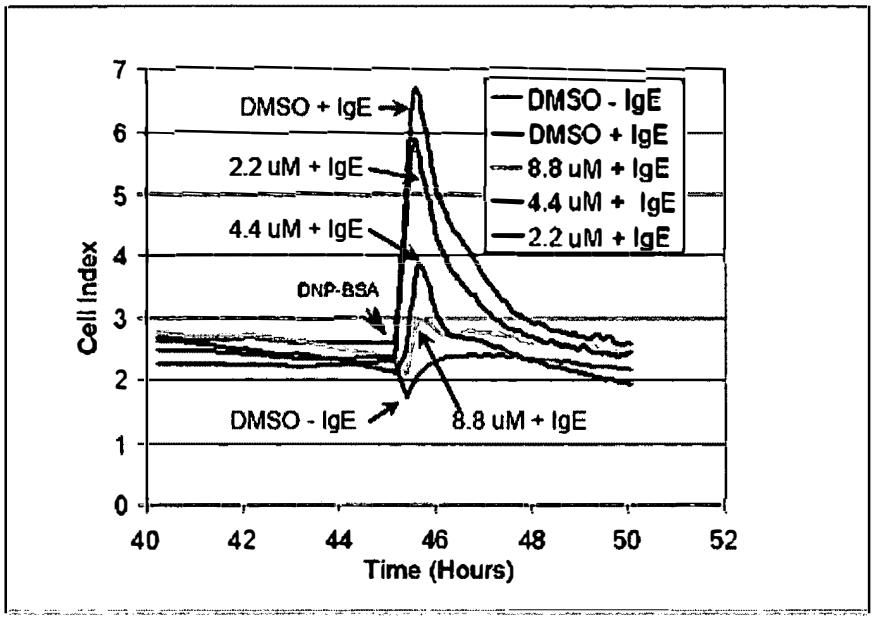


Figure 17.

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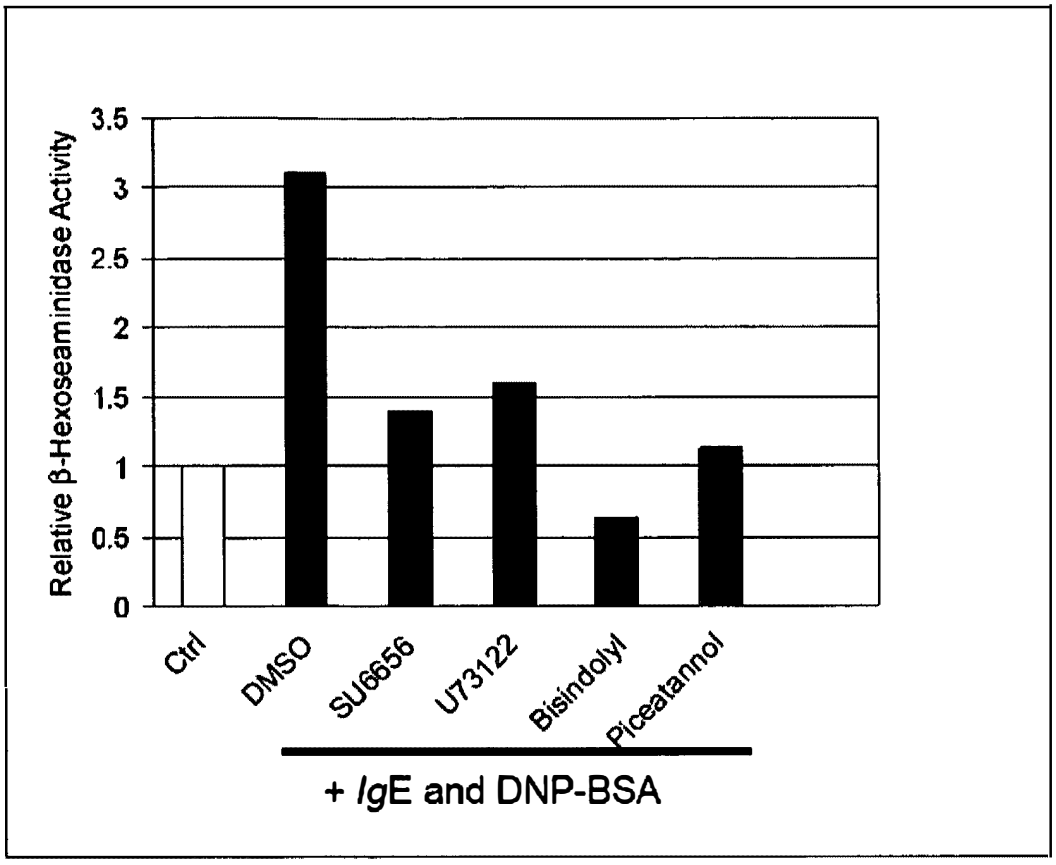


Figure 18.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number:	ACE-00102.P.1-US
	First Named Inventor:	Xiao Xu
	COMPLETE IF KNOWN	
	Application Number:	To be determined
	Filing Date:	Herewith
[X] Declaration Submitted with Initial Filing, OR	Group Art Unit:	To be determined
[ ] Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e) required)	Examiner:	To be determined

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS

the specification of which
[X] is attached hereto
OR
[ ] was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended _____ (if applicable).
I hereby state that I have reviewed and understand the contents of the above- identified specification, including the claims, as amended by any amendment specifically referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date	Priority Not Claimed	Certified Copy Attached? YES	NO

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below:		
Application Number(s)	**Filing Date**	[ ] Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto
60/397,749	July 20, 2002	
60/435,400	December 20, 2002	
60/469,572	May 9, 2003	
60/519,567	November 12, 2003	
60/542,927	February 9, 2004	
60/548,713	February 27, 2004	

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR. 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

U.S. Parent Application or PCT Parent Number	**Parent Fling Date**	**Parent Patent Number (if applicable)**
10/705,447	November 10, 2003	
10/705,615	November 10, 2003	
PCT/US03/22557	July 18, 2003	
PCT/US03/22537	July 18, 2003	

[ ] Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the United States Patent and Trademark Office connected herewith:

[X] Customer Number **24232** [Customer Number Bar Code Label]
OR
[ ] Registered practitioner(s) name/registration number listed below:

Name	**Registration Number**	**Name**	**Registration Number**
David R. Preston	38,710	Elizabeth Orr	45,937

[ ] Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: [X] Customer Number **24232** or Bar Code Label [Attached Bar Code Label] OR [ ] Correspondence address below:					
Name	David R. Preston & Associates, A.P.C.				
Address	David R. Preston				
Address	12625 High Bluff Drive, Suite 205				
City	San Diego	State	California	Zip Code	92130
Country	USA	Telephone	858.724.0375	Facsimile	858.724.0384

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR				[ ] A petition has been filed for this unsigned inventor			
Given Name (first and middle, if any)				Family Name or Surname			
Xiao				Xu			
Inventor's Signature						Date	
Residence: City	San Diego	State	California	Country	USA	Citizenship	USA
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						

[ ] Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

DECLARATION

ADDITIONAL INVENTOR(S)
SUPPLEMENTAL SHEET
PAGE _1___ OF __2___

NAME OF ADDITIONAL INVENTOR				[ ] A petition has been filed for this unsigned inventor			
Given Name (first and middle, if any)				Family Name or Surname			
Yama				Abassi			
Inventor's Signature						Date	
Residence: City	San Diego	State	CA	Country	USA	Citizenship	USA
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						
City	San Diego	State	CA	Zip Code	92121	Country	USA

Given Name (first and middle, if any)				Family Name or Surname			
Xiaobo				Wang			
Inventor's Signature						Date	
Residence: City	San Diego	State	CA	Country	USA	Citizenship	People's Republic of China
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						
City	San Diego	State	CA	Zip Code	92121	Country	USA

DECLARATION

ADDITIONAL INVENTOR(S)
SUPPLEMENTAL SHEET
PAGE 2 OF 2

NAME OF ADDITIONAL INVENTOR				[ ] A petition has been filed for this unsigned inventor			
Given Name (first and middle, if any)				Family Name or Surname			
Jiangbo				Gan			
Inventor's Signature						Date	
Residence: City	San Diego	State	CA	Country	USA	Citizenship	Peoples Republic of China
Post Office Address	ACEA Biosciences 11585 Sorrento Valley Road #103 San Diego, CA 92121						
City	San Diego	State	CA	Zip Code	92121	Country	USA

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

11/17/2004 SMINASS1 00000039 501321 10987732

01 FC:2001		395.00	OP
02 FC:2202	10.00	DA	17.00	OP

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PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2004

Application or Docket Number: 10987732

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	23	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	23 minus 20=	* 3
INDEPENDENT CLAIMS	1 minus 3 =	* 0
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	395.00	OR	BASIC FEE	790.00
X$ 9=	27	OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL	422	OR	TOTAL	

CLAIMS AS AMENDED - PART II

AMENDMENT A	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X44=		OR	X88=	
+150=		OR	+300=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Ex. 2008
Axion v. Agilent
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Patent

Attorney Docket No: ACE-00102.P.1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
XU et al	)	Examiner: To be determined
	)	
Aplication Number: To be determined	)	
	)	Group Art Unit: To be determined
Filed: Herewith	)	
	)	
For: A REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATIONS FOR CELL BASED ASSAYS	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir,

PRELIMINARY AMENDMENT NO. 1

Prior to examination, please amend the application as provided below:

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IN THE CLAIMS

Please cancel claims 1 through 50, 61-71, and 82-100, and 104-159. Accordingly, claims 51 through 60, 72-81, and 101-103 are pending upon entry of this Preliminary Amendment. The purpose of this amendment is to economize on USPTO fees.

Listing of claims:

Claims 1-50 (cancelled)

Claim 51 (previously presented) A system for monitoring cell-substrate impedance, comprising:

a) one or more multiple well cell-substrate impedance monitoring devices, wherein at least two of the multiple wells of said device comprise an electrode array at the bottom of the well, wherein said electrode array is individually addressed, further wherein said device can be used to measure differences in impedance values that relate to cell behavior;

b) an impedance analyzer;

c) a device station comprising electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer; and

d) a software program that can said control device station and record and analyze data obtained from said impedance analyzer.

Claim 52 (previously presented) The system of claim 51, wherein said device station comprises electronic switches that can switch on and off connections of each of said two or more electrode arrays to said impedance analyzer.

Claim 53 (previously presented) The device station of claim 51, wherein said software program can direct said device station to measure impedance values at regular or irregular time intervals from one or more of said two or more electrode arrays.

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Claim 54 (previously presented) The system of claim 51, wherein said impedance analyzer can measure impedance at one or more frequencies.

Claim 55 (previously presented) The device station of claim 51, wherein said software program can store said data.

Claim 56 (previously presented) The device station of claim 51 wherein said system can display said data.

Claim 57 (previously presented) The device station of claim 51, wherein said software can calculate a cell index for one or more time points for one or more wells of said multi-well plate based on impedance values measured from said one or more electrode arrays localized to said one or more wells.

Claim 58 (previously presented)The device station of claim 56 wherein said system can display said cell index for said one or more time points data for said one or more wells.

Claim 59 (previously presented) The system of claim 51, wherein said software is programmable to direct impedance monitoring of any of said two or more electrode arrays at chosen time intervals.

Claim 60 (previously presented) The system of claim 55, wherein said software allows entry and display of experimental parameters.

Claims 61-71 (cancelled)

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Claim 72 (previously presented) A method of performing an assay that monitors cell-substrate impedance, comprising:

providing the system of claim 51;

introducing cells into at least one well of said system;

monitoring cell-substrate impedance of said at least one well.

Claim 73 (previously presented) The method of claim 72, wherein said monitoring comprises analyzing impedance changes at three or more time points.

Claim 74 (previously presented) The method of claim 73, wherein at least three of said three or more time points are spaced at regular intervals.

Claim 75 (previously presented)A method of performing an assay that monitors cell-substrate impedance in response to one or more test compounds, comprising:

providing the system of claim 51;

introducing cells into one or more wells of said system that comprise electrode arrays;

adding at least one test compound to said one or more wells; and

monitoring cell-substrate impedance of said one or more wells before and after adding said at least one test compound.

Claim 76 (previously presented)The method of claim 75, wherein impedance is monitored at three or more time points.

Claim 77 (previously presented)The method of claim 75, further comprising analyzing impedance values before and after adding said at least one test compound.

Claim 78 (previously presented) The method of claim 75, further comprising introducing cells to at least one well to which a test compound is not added to create a control well.

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Claim 79 (previously presented) The method of claim 77, wherein said analyzing comprises calculating a cell index.

Claim 80 (previously presented) The method of claim 77, wherein said analyzing can provide information on cell proliferation, cell death, cell adhesion, cell apoptosis, cell differentiation, cell survival, cytotoxicity, cell morphology, cell quantity, cell quality, time-dependent cytotoxicity of a comound, IgE-mediated cell activation or stimulation, receptor-ligand binding, viral and bacterial toxin mediated cell pathologic changes, presence and quantity of neutralizing antibodies, specific T-cell mediated cytotoxic effects, presence and affinity of ligand-receptor binding.

Claim 81 (previously presented) The method of claim 79, wherein said cell index is used as an indicator of cytotoxicity.

Claims 82-100 (cancelled)

Claim 101 (previously presented) A method for monitoring changes in cell-substrate impedance in response to IgE stimulation, comprising:

providing a system of claim 51;

introducing cells into one or more wells of said system, wherein each of said one or more wells comprises an electrode array;

adding IgE to said one or more wells of said system;

adding an antigen to said one or more wells of said system; and

monitoring cell-substrate impedance of at least one of said one or more wells of said system.

Claim 102 (previously presented) The method of claim 101, wherein said cells are mast cells, eosinophils, basophils, or genetically engineered cells.

Claim 103 (previously presented) The method of claim 102, wherein said cells are mast cells.

Claims 104-159 (canceled)

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CONCLUSION

Applicants respectfully submit that the claims are ready for examination and in condition for allowance.

Respectfully submitted,

Date: Nov 12, 2004

David R. Preston
Reg. No. 38,710

David R. Preston & Associates A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, CA 92130

Telephone: 858.724.0375
Facsimile: 858.724.0384

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22713 U.S. PTO
111204

PTO/SB/05 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office. U.S. DEPARTMENT OF COMMERCE
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	ACE-00102.P.1-US
First Inventor	Xiao Xu
Title	REAL TIME ELECTRONIC CELL
Express Mail Label No.	EV 367469845 US

17510 U.S. PTO
10/987732
111204

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450**

1. ☑ Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. ☑ Applicant claims small entity status. See 37 CFR 1.27.
3. ☑ Specification [*Total Pages* 135]
   (preferred arrangement set forth below)
   - Descriptive title of the invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. ☑ Drawing(s) (*35 U.S.C. 113*) [*Total Sheets* 26]
5. Oath or Declaration [*Total Sheets* 5]
   a. ☑ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 18 completed)*
      i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☐ Application Data Sheet. See 37 CFR 1.76
7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)* ☑ Power of Attorney
11. ☐ English Translation Document *(if applicable)*
12. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
13. ☑ Preliminary Amendment
14. ☑ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. ☐ Nonpublication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☑ Other: Cover letter; Check for filing fee

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☐ Continuation ☐ Divisional ☑ Continuation-in-part (CIP) of prior application No.: 10/705,447

Prior application information: *Examiner* _____ *Art Unit:* 1632

For CONTINUATION OF DIVISIONAL APPS only; The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS

☑ Customer Number: 24232 *OR* ☐ Correspondence address below

Name	David R Preston				
Address	David Preston & Associates				
	12625 High Bluff Drive, Suite 205				
City	San Diego	*State*	CA	*Zip Code*	92130
Country	United States of America	*Telephone*	858-724-0375	*Fax*	858-724-0384

Name (Print/Type)	David R Preston	*Registration No. (Attorney/Agent)*	38,710
Signature		*Date*	Nov 12, 2004

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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Axion v. Agilent
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22713 U.S. PTO 111204

PTO/SB/17 (01-03)
Approved for use through 04/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 412.00

Complete if Known	
Application Number	To be deterrnined
Filing Date	Herewith
First Named Inventor	Xiao Xu
Examiner Name	To be determined
Art Unit	To be determined
Attorney Docket No.	ACE-00102.P.1-US

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 501321

Deposit Account Name: David R. Preston

The Commissioner is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) during the pendency of this application

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	385.00
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 385.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	Fee Paid
Total Claims	23	-20** = 3	x	9	= 27
Independent Claims	1	- 3** = 0	x	0	= 0
Multiple Dependent			x		=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 27.00

***or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($) 0.00

SUBMITTED BY				(Complete *(if applicable)*
Name (Print/Type)	David R. Preston	*Registration No. (Attorney/Agent)*	38,710	*Telephone* 858-724-0375
Signature				*Date* Nov 12, 2004

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, Washington, DC 20231.**

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Axion v. Agilent
IPR2024-01467

David R. Preston & Associates, A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, California 92130

David R. Preston
Owen Smigelski‡
Mo Savari‡
Raymond Wagenknecht‡

‡ Of Counsel

"Express Mail" Mailing Label Number: EV367469845 US
Date of Deposit: November 12, 2004

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313

Re: Utility Patent Application

Entitled:	REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS
Appl. No.:	To be determined
Filed:	Herewith
Inventor:	Xiao Xu, Yama Abassi, Xiaobo Wang and Jiangbo Gan
Our Ref.:	ACE-00102.P.1-US

Sir:

The following documents are forwarded herewith for appropriate action by the United States Patent and Trademark Office:

1. Utility Patent Application Transmittal (in duplicate);

2. Fee Transmittal (in duplicate);

3. Declaration for Utility or Design Patent Application;

4. Complete U. S. Patent Application entitled:

REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATION FOR CELL-BASED ASSAYS

and naming as inventor:

Xiao Xu, Yama Abassi, Xiaobo Wang and Jiangbo Gan

the utility application comprising:

1. Total pages of application: [161]
2. Pages of Disclosure: [110];
3. Sheets of Drawings: [26];
4. Pages of Title Page: [1];
5. Pages of Abstract: [1]; and
6. Pages of Claims: [24];

5. Preliminary Amendment;

6. One Return Post Card; and

7. Our Check number 3536 for $ 412.00 to cover the Application fee (small entity status).

It is respectfully requested that the attached postcard be stamped with the filing date and unofficial application number and returned as soon as possible.

Please apply any charges not covered, or any credits, to Deposit Account number 501321 in the name of David R. Preston & Associates, APC

The following attorneys are attorneys of record for prosecuting this application and transacting all business in the USPTO connected therewith:

David R. Preston, Esquire	Elizabeth Orr
Registration No. 38,710	Registration No. 45,937

Additional Attorneys or Agents of Record are those listed on Customer No. 24232

David R. Preston & Associates, A.P.C.
DRPNA®
www.drpna.com
phone: 858.724.0375
facsimile: 858.724.0384

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Please send all correspondence and direct all telephone calls to:

David R. Preston
David R. Preston & Associates, APC
12625 High Bluff Drive
Suite 205
San Diego, California 92130
858.724.0375

Respectfully Submitted,
DAVID R. PRESTON & ASSOCIATES, A.P.C.



David R. Preston
Attorney for Applicant
Registration No. 38,710