

***Axion BioSystems, Inc. v.
Agilent Technologies, Inc.***

**IPR2024-01467
U.S. Patent No. 11,906,508**

Petitioner's Demonstratives

December 12, 2025

**Axion Ex. 1029
Axion v. Agilent
IPR2024-01467**

Procedural History

- Petition (Paper 1) – Challenging claims 1-30 over Grounds 1 and 2:

Ground	Claims	Basis
Ground 1	23-30	§ 102 and § 103 based on U.S. Patent No. 7,192,752 to Xu et al. (“Xu-752”)
Ground 2	1-22	§ 103 based on Xu-752 and U.S. Patent Application Publication No. 2007-0057680 Jones et al. (“Jones”)

Procedural History (cont'd)

- **PO Preliminary Response (Paper 6)** – Filed with statutory disclaimer disclaiming claims 9, 10, and 23-30 (EX2001)
- **DI (Paper 9)** – Instituting IPR as to claims 1-8 and 11-22
- **PO Response (Paper 12)** – Filed with statutory disclaimer disclaiming claims 16-22 (EX2020)

Remaining issue: The obviousness of claims 1-8 and 11-15 over Xu-752 and Jones

Impedance-Based Cell Analysis

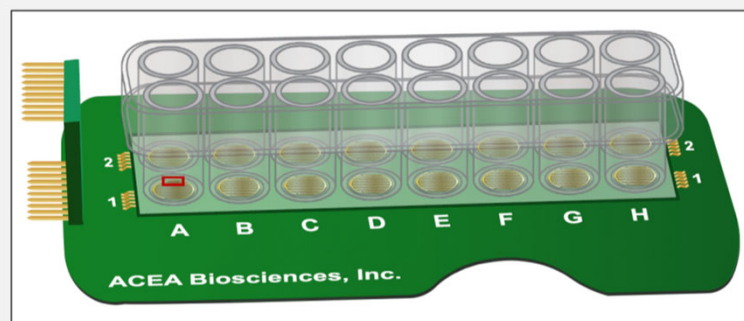
41. Impedance-based cell analysis systems generally work by applying a voltage or current to electrode(s) in the bottom of a well in a multiwell microtiter plate so that the impedance of the well can be measured. As examples, 16-well and 96-well

EX1003 (Declaration of Dr. Bruce Kim), ¶41; Pet., 3.

43. In impedance-based systems, biological cells present in a well of a plate will block some of the current and thus act as an impedance. As a result, the behavior of cells can be observed by monitoring their impedance. Prior-art impedance-

EX1003, ¶43; Pet., 4.

Figure 2 Illustration From The '752 and '080 Patents



EX1012, 53; Pet., 3.

Giaever and Keese (1984)

Monitoring fibroblast behavior in tissue culture with an applied electric field

(cell locomotion/cytochalasin B)

I. GIAEVER AND C. R. KEESE

General Electric Research and Development Center, Schenectady, NY 12301

Contributed by I. Giaever, March 2, 1984

EX1025; Pet., 2.

40. As early as 1984, Ivar Giaever and Charles Keese described using electrical cell-substrate impedance sensing for real-time analysis of cellular processes. See Ex. 1025; Ex. 1015 at 3:44-52 (referencing Giaever and Keese's work); Ex. 1016 at 2:16-21 (same).

EX1003, ¶40; Pet., 2.

method for cell monitoring and sensing. Adherent cells are cultured on the surface of microelectrode structures located on a solid substrate. The presence and absence of cells on the electrode surface affect sensitively the electronic and ionic passage between cell culture media and the electrode structures (see, for example, Giaever I. and Keese C. R., "Monitoring fibroblast behavior in tissue culture with an applied electric field", Proc. Natl. Acad. Sci. (USA), 1984, vol. 81, pp 3761-3764). Thus, interrogating the electrode impedance

EX1015 (Agilent's USP 7,459,303), 3:44-52; see also EX1016 (Agilent's USP 7,470,533) at 2:16-21; Pet., 2.

Ghosh (1993) – Impedance-Based Cell Analysis at Millisecond Time Resolution

Monitoring electroporation in the plasma membrane of adherent mammalian cells

Paramita M. Ghosh, Charles R. Keese, and Ivar Giaever
School of Science, Rensselaer Polytechnic Institute, Troy, New York 12180-3590 USA

EX1017 (Ghosh).

Circuit and electrode setup. Gold electrodes were evaporated on the bottom of a tissue culture dish as described in earlier work (15). The dish containing the electrodes was placed in an incubator and connected to a lock-in amplifier interfaced with a computer as shown in Fig. 1. The amplifier measures the in- and out-of-phase (real and imaginary) voltages across the small electrode and these are converted by the computer into a resistance and capacitance in series taking the external circuit into consideration. The amplifier can take readings as fast as once every 5 milliseconds, and the setup can be used to monitor the impedance of the sample. The electrolyte used is the cell growth medium (DMEM + 10% FBS).

EX1017, 1604; Pet., 37.

A short time prior to pulsation, the amplifier was set to take data once every 10 milliseconds, and the impedance was followed with a 3.5 mV signal applied by the oscillator. Next, the amplifier settings were changed to avoid overload, and the high voltage (1 V–5 V) pulse was applied for 200 milliseconds. The voltage was then dropped back to 3.5 mV, and the amplifier settings were changed back to normal. Because of these amplifier adjustments the impedance of the cell layer for a short time before and after the pulse could not be measured.

EX1017, 1604; Pet., 37.

Ghosh (1993) – Impedance-Based Cell Analysis at Millisecond Time Resolution (cont'd)

The observations made in Fig. 2 can now be explained in the light of the above discussion. When a voltage pulse is applied, the permeability of the membrane increases, because of pore formation. These pores start resealing as soon as the high voltage is turned off, and therefore, recovery of initial membrane resistance is observed. This is in accordance with the observations of others (6, 9, 10) who have determined that the pores form within 100 μ s after the application of the pulse and start resealing within a few milliseconds. It has been proposed that pore

EX1017, 1608; Pet., 37.

For clarity, we have only talked about the measured changes in resistance due to pore formation. As is evident from Eq. 2, very similar changes in the measured reactance take place due to formation of pores. During these experiments, we have tracked the changes in reactance as well, and these measurements support and confirm the conclusions drawn in this paper. Finally, the method can be improved by using an amplifier that has a faster response time and can take data more frequently. This will allow us to look at further details in the formation and resealing of the membrane pores.

EX1017, 1609; Pet., 37.

“Ghosh describes the use of millisecond-time resolution between two measurements for a well to monitor impedance while studying electroporabilization ... Thus, at least as early as the 1990s, applications of impedance-based cell analysis systems using fast, millisecond-time resolution between measurements were known to POSAs, and further, even for such applications, POSAs were seeking faster sampling times to obtain data more frequently.” Pet., 36-38.

Early 2000s – ACEA Entered the Market; Xu-752 Issued in 2007

(12) **United States Patent** **Xu et al.**

(10) **Patent No.:** **US 7,192,752 B2**
(45) **Date of Patent:** **Mar. 20, 2007**

(54) **REAL TIME ELECTRONIC CELL SENSING SYSTEMS AND APPLICATIONS FOR CELL-BASED ASSAYS**

(75) **Inventors:** **Xiao Xu**, San Diego, CA (US); **Yama Abassi**, San Diego, CA (US); **Xiaobo Wang**, San Diego, CA (US); **Jiangbo Gan**, San Diego, CA (US)

(73) **Assignee:** **ACEA BioSciences**, San Diego, CA (US)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **10/987,732**

(22) **Filed:** **Nov. 12, 2004**

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(Continued)

Primary Examiner—David Redding

(74) *Attorney, Agent, or Firm*—David R Preston &

EX1005 (Xu-752).

44. Xu-752 issued in 2007 and is described in further detail below with respect to the unpatentability of the '508 patent's claims. Xu-752 states that its "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." Ex. 1005 at 3:49-53. Xu-752's system includes four main components: (a) a multiwell plate or device with an electrode array in the bottom of each well (*id.* at 4:17-19, 22:50-52, 23:4-8); (b) an impedance analyzer (*id.* at 4:19-20, 22:62-23:3, 23:49-60); (c) electronic circuitry that connects the electrode arrays to the impedance analyzer (*id.* at 22:44-52, 23:44-52); and (d) a software program that performs data acquisition and data analysis on data from the impedance analyzer (*id.* at 4:24-26, 22:58-61, 23:66-24:12). *See also id.* at claim 1.

EX1003, ¶44; Pet., 5.

Pet., 5 ("Almost 20 years after Giaever and Keese[,] ... [ACEA] entered the market.").

The '508 Patent

(12) **United States Patent**
Wang et al.

(10) **Patent No.:** **US 11,906,508 B2**
(45) **Date of Patent:** **Feb. 20, 2024**

(54) **LABEL-FREE MONITORING OF
EXCITATION-CONTRACTION COUPLING
AND EXCITABLE CELLS USING
IMPEDANCE BASED SYSTEMS WITH
MILLISECOND TIME RESOLUTION**

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(73) Assignee: **AGILENT TECHNOLOGIES, INC.**,
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(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(58) **Field of Classification Search**
CPC G01N 33/4836; G01N 27/02; G01N
33/4833; G01N 33/48728; G01N 33/5438
See application file for complete search history.

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(Continued)

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(Continued)

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ACEA Biosciences, Inc. Jan. 1, 2014, 37 pages

(22) Filed: **Nov. 23, 2022**

(65) **Prior Publication Data**

US 2023/0130050 A1 Apr. 27, 2023

Related U.S. Application Data

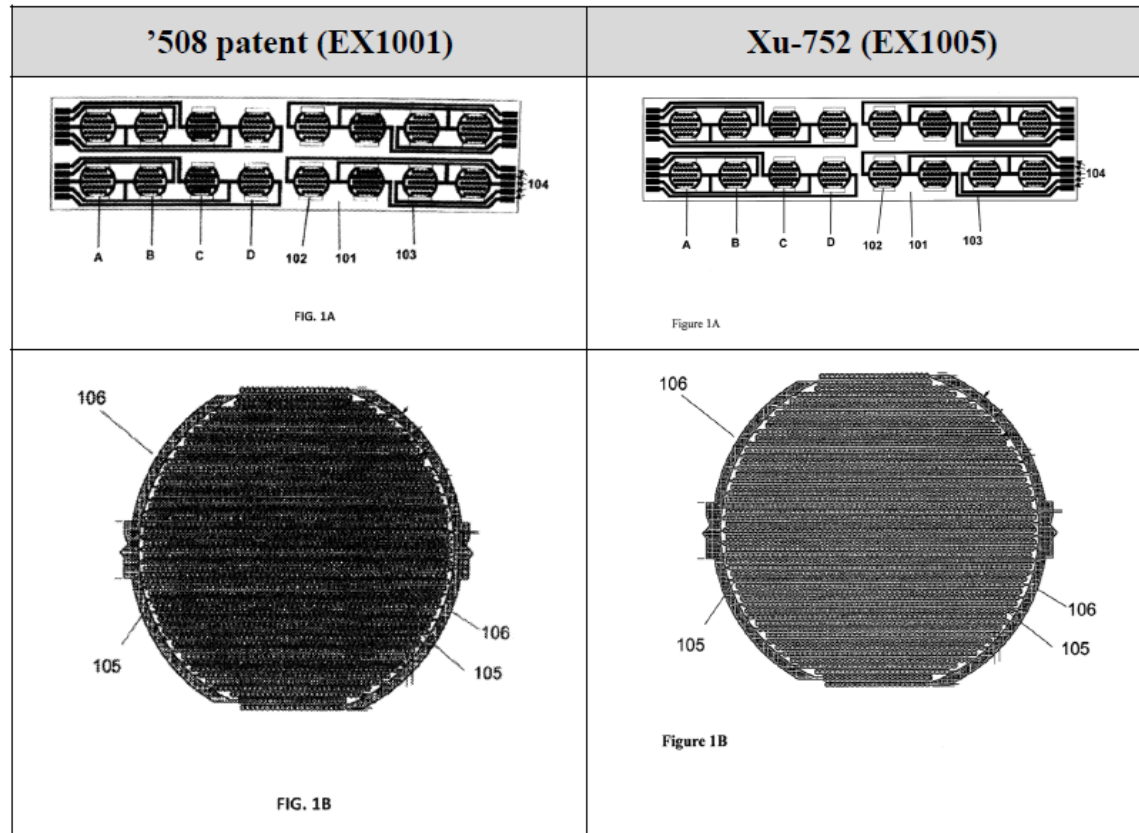
(63) Continuation of application No. 17/824,782, filed on
May 25, 2022, which is a continuation of application
No. 16/733,781, filed on Jan. 3, 2020, now Pat. No.
11,360,072, which is a continuation of application
No. 16/020,679, filed on Jun. 27, 2018, now Pat. No.
10,533,985, which is a continuation of application
No. 15/651,882, filed on Jul. 17, 2017, now Pat. No.
10,012,636, which is a continuation of application
No. 12/435,569, filed on May 5, 2009, now Pat. No.
9,709,548.

(60) Provisional application No. 61/191,684, filed on Sep.
11, 2008, provisional application No. 61/126,533,
filed on May 5, 2008.

EX1001; Pet., 10.

EX1001; Pet., 10.

The '508 Patent Shares Disclosure With Xu-752



Pet., 12.

The Alleged Improvements of the '508 Patent

(57)

ABSTRACT

A system for monitoring cells, which includes a device for monitoring cell-substrate impedance, the device having a plurality of wells on a nonconductive substrate, where each of the plurality of wells has an electrode array fabricated on the substrate for measurement of cell-substrate impedance; an impedance analyzer that measures cell-substrate impedance from the plurality of wells; electronic circuitry with multiple analogue-to-digital conversion channels, where the electronic circuitry electrically connects the electrode arrays to the impedance analyzer such that the electrode arrays are electrically monitored at millisecond time resolution; and a software program that analyzes the measured cell-substrate impedance.

EX1001, Abstract.

A number of improvements ... can be used to achieve such millisecond time resolution. [1] For instance, improvement likely requires the use of *fast processing electronic chips for analogue-to-digital conversion*, for parallel digital signal processing and data calculation with field-programmable gate array (FPGA) and for fast communication between the impedance measurement circuitry and software. [2] Another example of improvements includes the use of multiple analogue-to-digital (AD) conversion channels so that analog electronic signals from multiple channels can be converted to digital signals simultaneously. *Such parallel AD conversion is important*, particular for the system having multiple wells, each of which's measurement time resolution is required to be in the millisecond resolution. [3] And, a very important improvement is to replace previous working mode of "measurement of one-well's impedance at a time" with *a mode of "measurement of multiple-wells' impedances at a time"*.

Id. at 24:53-25:44 (emphases and bracketed numbers added).

EX1003, ¶53 (quoting EX1001, 24:53-25:44);
Pet., 12-13.

Independent Claims of the '508 Patent

Claim	'508 Patent Features Not Disclosed in Xu-752	Status
1	"at least two analogue-to-digital conversion channels coupled to the plurality of electrode arrays" and "wherein the impedance analyzer monitors the electrode arrays at millisecond time resolution"	At issue in this IPR
16	"a plurality of analog-to-digital (A2D) signal converters electrically arranged in parallel to one another"	Disclaimed (EX2020)
23	None (Xu-752 anticipates claim 23)	Disclaimed (EX2001)

1. An apparatus for continuous, label free, real-time monitoring of cells using impedance-based analysis, the apparatus comprising:

- a) a multiwell plate comprising a plurality of wells on a nonconductive substrate, wherein each of the plurality of wells comprises an electrode array of a plurality of electrode arrays that is disposed on the nonconductive substrate;
- b) an impedance analyzer that independently measures a cell-substrate impedance at each well, the cell-substrate impedance being measured between a first electrode structure and a second electrode structure of the electrode array and across a cellular sample comprising a plurality of cells disposed within each well, the impedance analyzer including
at least two analogue-to-digital conversion channels coupled to the plurality of electrode arrays, wherein the impedance analyzer monitors the electrode arrays at millisecond time resolution between first measurements of the cell-substrate impedance and second measurements of the cell-substrate impedance for each well; and
- c) a software program that analyzes the first measurements and the second measurements to produce a time period analysis of the cellular sample.

EX1001, claim 1.

Obviousness of the Remaining Claims (1-8 and 11-15) over Xu-752 and Jones

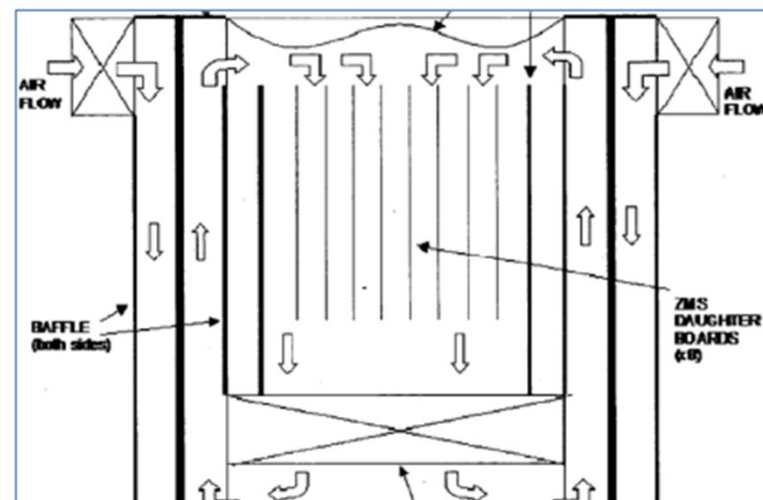
(19) **United States**
(12) **Patent Application Publication** (10) Pub. No.: US 2007/0057680 A1
Jones et al. (43) Pub. Date: Mar. 15, 2007

(54) IMPEDANCE MEASUREMENT SYSTEM WITH INCORPORATED INTERNAL MEASUREMENT DRIFT COMPENSATION NETWORKS
(21) Appl. No.: 11/224,354
(22) Filed: Sep. 9, 2005
Publication Classification

EX1006.

188. Jones discloses that its impedance measurement system includes a plurality of ADCs electrically arranged in parallel to one another. Jones describes, as shown in Figure 4 below, eight daughter boards electrically arranged in parallel to one another. Ex. 1006, ¶¶[0002]-[0003], [0012], [0014], [0018], [0020], Figs. 2, 4.

EX1003, ¶188; Pet., 67.



EX1006, Fig. 4; Pet., 67.

Jones's Disclosure

(57)

ABSTRACT

The present technology provides an Impedance Measurement System (ZMS) for measuring the complex impedance of each well of a single well or multiwell microtiter plate utilizing a novel combination of parallel impedance measurement channels, multiplexing, and reference impedance networks, allowing internal correction of impedance measurement drift, maximized measurement accuracy, and minimized system size.

EX1006, Abstract.

BACKGROUND OF INVENTION

[0002] The present technology is novel in that it provides an impedance measurement system with massive parallelism, high temporal sampling, and a means for interconnecting multiple microtiter plate sensors to impedance measurement channels. The technology also includes a method for internal compensation of impedance measurement drift due to temperature changes or component aging.

EX1006, ¶[0002].

Jones's Disclosure (cont'd)

[0030] Each well is subject to a stepped sweep (a series of single frequency bursts-one burst for each selected frequency). The bursts consist of an integral number of cycles. In one embodiment, the number of cycles is typically 15. The number of cycles for additional embodiments are chosen to be sufficient for well and gain multiplexer switching transients to settle and to obtain the necessary number of sample points. From preliminary modeling and testing, no more than 68.2 ms per well for all frequencies is required to obtain sufficient samples. For an embodiment in which a 96 well plate with 12 wells per row plus four reference network channels, calculations to determine sufficient samples suggest a 1091 mS measurement period, leaving 409 mS for multiplexer switching and signal settling within the target 1.5 second update period.

EX1006, ¶[0030].

SUMMARY OF THE INVENTION

[0003] The present technology provides an Impedance Measurement System (ZMS) for measuring the complex impedance of each well of a single well or multiwell microtiter plate. The ZMS utilizes a novel combination of parallel impedance measurement channels and the multiplexing of impedance measurement channels for interconnecting multiple microtiter plate sensors and reference impedance networks to the channels, allowing internal correction of impedance measurement drift, maximized measurement accuracy, and minimized system size. The system is self-contained, thermally controlled, provides well excitation and measurement, and allows the measurement data to be stored or communicated to an external host computer. The system can be used for impedance measurements of varying time periods, single or multiple frequencies, and various measurement rates. Additionally, the ZMS employs parallelism to obtain the required throughput at the repeatability required and incorporates novel calibration, self-check, and in-run compensation schemes as well as internal temperature control to ensure increased measurement accuracy and stabilization against thermal drift.

EX1006, ¶[0003].

Jones's Disclosure (cont'd)

[0025] In an additional embodiment, each column of sensors, rather than each row of sensors, on a microtiter plate is addressed by measurement circuitry on a single daughter board with the system arrangement of the boards remaining the same.

EX1006, ¶[0025].

[0042] In-run auto compensation is performed by including high stability RC networks as reference channels which are treated as an additional well and applying compensation derived from the reference data set to the well data set. For in-run compensation, three precision RC networks are included in the system. In one embodiment, three precision RC networks are included. An alternative embodiment includes four reference networks. Additional embodiments may include any number greater than three. The principal reference network has values set to match the gross characteristics of a well with cells. The other networks have values that provide an impedance characteristic both above and below the primary network to allow checking and compensation for system non-linearity. These networks are also used with the self-checking feature of the ZMS.

EX1006, ¶[0042].

Undisputed: Jones's Impedance Analyzer Includes “At Least Two [ADC] Channels”

135. Jones discloses that its impedance analyzer utilizes at least two ADC channels, and that such channels are coupled to the sensors at the bottom of each well of the plate. Jones describes “parallel impedance measurement channels” and, more specifically, using multiple daughter boards (e.g., eight daughter boards, one for each row of wells of a plate) where each daughter board has multiple ADCs and a digital signal processor to process impedance data. Ex. 1006, ¶¶[0003], [0012], [0018], [0035]. Jones states that “[t]he interconnection of each sensor from a particular well in

EX1003, ¶135.

Obviousness of Using Xu-752/Jones System with Parallel ADCs to Monitor Wells at Millisecond Time Resolution

- A POSA would have understood that Jones's impedance analyzer with parallel ADCs is capable of millisecond time resolution measurements. (Pet., 34-36, 53-55, 63-64; EX1003, ¶¶69-75, 137-140, 168-182; Reply, 2-9)
- A POSA would have been motivated to configure the Xu-752/Jones system for millisecond time resolution measurements. (Pet., 37-42, 64-65; EX1003, ¶¶178-182; Reply, 10-15)

“Millisecond Time Resolution” Claim Limitation

Accordingly, for purposes of this IPR, Agilent proposes that the term “millisecond time resolution” be construed to mean the time between two consecutive impedance measurements for each and every well being evaluated in the claimed apparatus or system, which can be performed in less than 500 milliseconds from one another. Ex. 2009 ¶ 33.

PO Response, 15.

² As explained in the Petition (at 13-14) and in Section III below, there are indefiniteness issues with “millisecond time resolution” in claim 1. For purposes of this proceeding, Axion adopts the POR’s position (at 12-15) that claim 1’s “millisecond time resolution” includes up to 500 milliseconds. The claims would also be unpatentable under a narrower “less than 300 milliseconds” construction, as shown in the Petition (at 36-47, 53-55, 63-65).

Reply, 5 n.2.

Jones is Capable of Millisecond Time Resolution Measurements

The system can be used for impedance measurements of varying time periods, single or multiple frequencies, and various measurement rates. Additionally, the ZMS employs parallelism to obtain the required throughput at the repeatability required and incorporates novel calibration, self-check, and in-run compensation schemes as well as internal temperature control to ensure increased measurement accuracy and stabilization against thermal drift.

EX1006, ¶[0003].

75. As noted above, Jones states that its system “can be used for impedance measurements of varying time periods, single or multiple frequencies, and various measurement rates.” *Id.*, ¶[0003]. The measurement rate for a single-frequency sampling (rather than a stepped sweep) in Jones for the entire 96-well plate would be significantly less than 1.5 seconds, as explained above and summarized in Table 2:

EX1003, ¶75.

Belden Inc. v. Berk-Tek LLC, 805 F.3d 1064, 1076 (Fed. Cir. 2015) (A “reference must be considered for everything it teaches.”).

Bradium Techs. LLC v. Iancu, 923 F.3d 1032, 1049 (Fed. Cir. 2019) (A reference must be considered “not only for what it expressly teaches, but also for what it fairly suggests” to a POSA.) (quotation omitted).

Reply, 4.

Jones is Capable of Millisecond Time Resolution Measurements (cont'd)

[0025] In an additional embodiment, each column of sensors, rather than each row of sensors, on a microtiter plate is addressed by measurement circuitry on a single daughter board with the system arrangement of the boards remaining the same.

EX1006, ¶[0025].

74. In addition, Jones explains that its parallel daughter boards may be used in different numbers and configurations. Jones states, for example, embodiments could utilize one daughter board for each column, rather than each row, of wells. *Id.*, ¶[0025]. Thus, for an 8 row, 12 column configuration for a 96-well plate, twelve daughter boards would be used in this alternative embodiment described by Jones, lowering the time needed to measure impedance in each well when compared to the configuration using only eight daughter boards.

EX1003, ¶74.

Jones is Capable of Millisecond Time Resolution Measurements (cont'd)

derived from the reference data set to the well data set. For in-run compensation, three precision RC networks are included in the system. In one embodiment, three precision RC networks are included. An alternative embodiment includes four reference networks. Additional embodiments may include any number greater than three. The principal

EX1006, ¶[0042].

between two measurements in each well.” EX1003, ¶75. And Dr. Kim’s calculations show that using three reference channels instead of four, as described in Jones ¶[0042], would result in a measurement rate of less than 1.5 seconds. See EX1003, ¶¶70, 137, 139.

Reply, 5.

Jones is Capable of Millisecond Time Resolution Measurements (cont'd)

EX1003	Number of Wells	Number of Frequencies	Daughter Boards	Reference Channels	Update Period
¶72	96-well	Single freq. (10, 50, or 100 kHz)	8	4	412-433 ms
¶137	16-well	Multi-freq. sweep	8	3	470 ms
¶139	16-well	Single freq. (10 kHz)	8	3	136 ms
¶173	16-well	Single freq. (10 kHz)	16	0	1.5 ms
¶175	16-well	Single freq. (10 kHz)	2	0	55 ms

Reply, 6.

- **Xu-752 (EX1005)**
 - 16- and 96-well plates, *see, e.g.*, Figs. 2, 3A
 - Single or multiple frequencies, *see, e.g.*, 23:52-54
 - Measurement frequency 1 Hz to 100 MHz, *see, e.g.*, 26:20-24 (“For example, the impedance can be measured in a frequency range from about 1 Hz to about 100 MHz.”)
- **Jones (EX1006)**
 - Single- or multi-well plate, *see, e.g.*, ¶[0003]
 - Single or multiple frequencies, *see, e.g.*, ¶[0003]
 - Configurable number of daughter boards, *see, e.g.*, ¶[0025]
 - Three or four reference channels (*see, e.g.*, ¶[0042]), and a POSA would have been motivated to eliminate unnecessary measurements of reference channels to achieve desired measurement resolutions (EX1003, ¶¶171-175)

Jones is Capable of Millisecond Time Resolution Measurements (cont'd)

Scope of Claim 1	Jones
“plurality of wells” (<i>i.e.</i> , at least two)	“The present technology provides an Impedance Measurement System (ZMS) for measuring the complex impedance of each well of a <i>single well or multiwell microtiter plate</i> .” EX1006, ¶[0003]; <i>see also id.</i> , Abstract.
measurements at a single frequency or multiple frequencies	“The system can be used for impedance measurements of varying time periods, <i>single or multiple frequencies</i> , and various measurement rates.” <i>Id.</i> , ¶[0003].
serial or parallel measurements	“Additionally, the ZMS <i>employs parallelism to obtain the required throughput</i> ” <i>Id.</i>

Reply, 9.

capable of achieving millisecond time resolution. The '508 patent's claims are broad—they encompass the measurement of as few as *two wells* using a *single measurement frequency*. A POSA would have known that if Jones can achieve a resolution of 1.5 seconds in measuring *96 wells* via a *multi-frequency sweep*, then Jones was capable of measuring *two wells* at a *single frequency* (as described in Jones's ¶[0003]) with “millisecond time resolution.”

Reply, 1.

A POSA's Motivation to Configure the Xu-752/Jones System for Millisecond Time Resolution Measurements

Agilent concedes: There were applications of impedance-based cell analysis known to a POSA at the time of the '508 patent that required millisecond-time resolution between measurements. Pet., 31-47; EX1003, ¶¶107-126; Reply, 11-13 (“Agilent concedes this point, failing to make any counter-arguments.”).

Agilent concedes: A POSA understood that the biological cell phenomenon to be studied dictates the requisite measurement speed, and such speed must be commensurate with the cell dynamics being studied. Pet., 31-47, 63-65; EX1003, ¶¶107-126, 168-182; Reply, 13 (“Agilent does not argue otherwise,” and “Dr. Sanchez confirmed that the requisite measurement resolution is ‘driven by the application,’” quoting EX1028, 52:18-53:12).

Accordingly, a POSA seeking to employ the Xu-752/Jones system to study electroporation or other known phenomena occurring at a millisecond time scale would have used the configurations expressly disclosed in Jones to achieve a measurement resolution adequate to measure the biological phenomenon at issue. Pet., 31-47, 63-65; EX1003, ¶¶107-126, 168-182; Reply, 11.

A POSA's Motivation to Configure the Xu-752/Jones System for Millisecond Time Resolution Measurements (cont'd)

116. In sum, there were applications of impedance-based cell analysis known to a POSA at the time of the '508 patent that used millisecond-time resolution between measurements, and further there were also specific cell behaviors (*e.g.*, cardiomyocyte beating activity) that were known to a POSA to occur in short time periods (*e.g.*, milliseconds), such that millisecond-time resolution for measurements would be necessary and/or advantageous for any cell analysis system used to monitor such behaviors. Thus, it would have been obvious to a POSA to implement Xu-752's impedance-based cell analysis system using an impedance analyzer that would "monitor[] the electrode arrays at millisecond time resolution between first measurements of the cell-substrate impedance and second measurements of the cell-substrate impedance for each well," as recited in claim 1.

EX1003, ¶116; Pet., 36-41.

- **Ghosh (EX1017)** – impedance-based cell analysis at millisecond time resolution (*see* slides 6-7 above) (Pet., 36-38)
- **Yu (EX1008)** – measurements in “as little as 50 ms/well” (Pet., 38)
- **Mohr (EX1018), Pancrazio (EX1019), and Heer (EX1021)** – monitoring the beating activity of cardiomyocytes at millisecond time resolution (Pet., 38-40)
- **Yang (EX1022) and Qiu (EX1023)** – using impedance-based cell analysis to monitor cardiomyocytes (Pet., 40-41)

Prior Art: Yu's Measurements in "as little as 50 ms/well"

the pure impedance due to the presence of the cells (Z_{cell}). The addition of the agonist for different GPCRs will affect cell morphology in a very specific manner depending on the pathways being activated, and further change in impedance is a reflection of the morphological dynamics that are induced by the ligand ($Z_{\text{activated cell}}$). Because the readout can be sampled in as little as 50 ms/well, the rate of change, magnitude, and duration of the response can be precisely measured.

EX1008, 37; Pet., 38.

Prosecution of Agilent's U.S. 9,709,548 (Wang-548, EX1013), a parent of the '508 Patent:

c) monitoring cell-substrate impedance of said one or more wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate, wherein the millisecond time resolution comprises at least two consecutive impedance measurements less than 20 milliseconds apart; and

EX1014, 256 (Agilent's claim amendment to overcome Yu); Pet., 9-10.

Yu provides an approach to measuring changes in cell morphology as a result of ligand-dependent GPCR activation. Abstract. Yu indicates the readout could be sampled in "as little as 50 ms/well." Pg. 37, col. 2, para. 4.

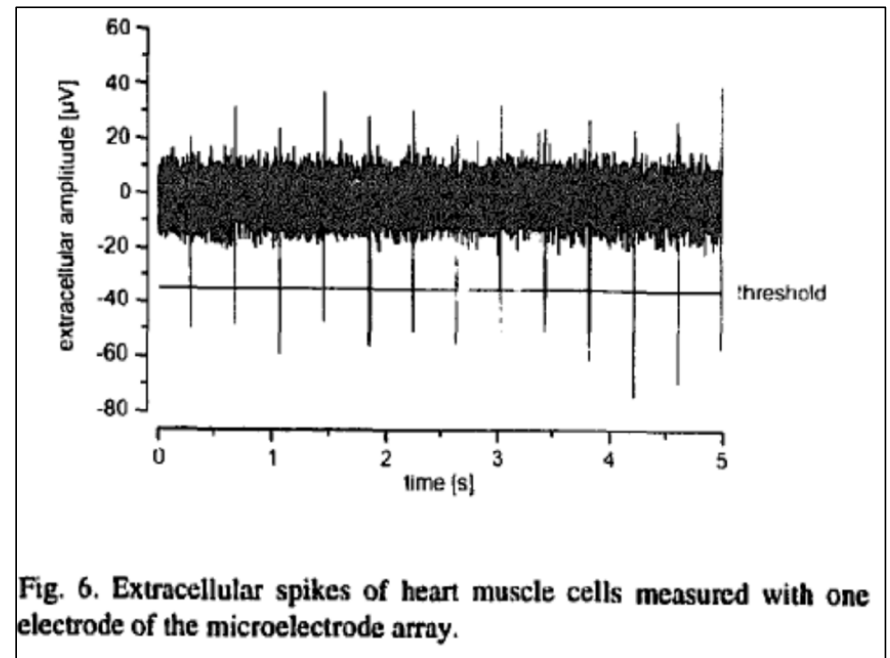
Compared to claims 16 and 24, Yu does not teach millisecond resolution, characterized by *at least two consecutive impedance measurements less than 20 milliseconds apart*. Instead, Yu indicates the readout can be *as little as 50 ms/well*. That is, Yu confirms the cell substrate measurement cannot exceed 50 millisecond resolution; whereas Applicant more than doubles this resolution.

EX1014, 268 (Agilent's remarks); Pet., 9-10.

Prior Art: Mohr (EX1018), Pancrazio (EX1019), and Heer (EX1021)

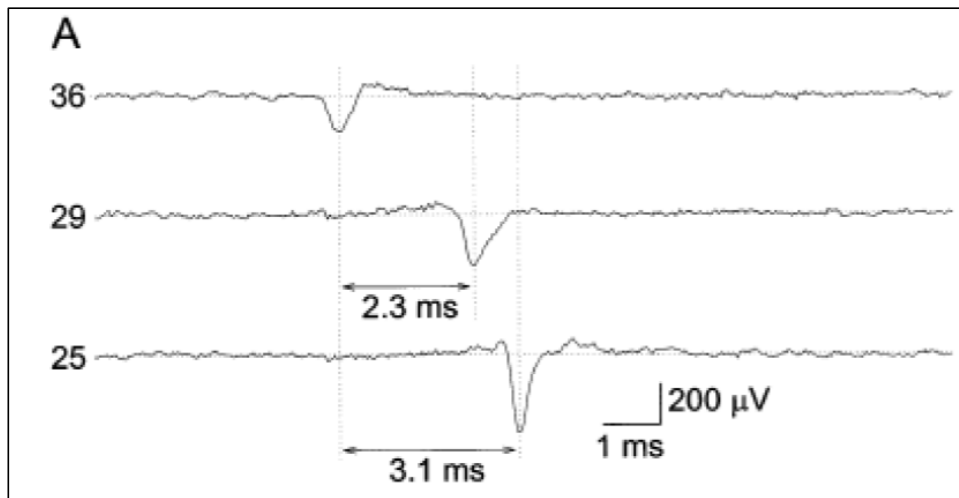
113. Another cell-analysis application known as early as the 1990s that required millisecond-time resolution between measurements was studying the beating activity of cardiomyocytes (as later described in the '508 patent). Prior art describes using various microelectrode array-based biosensors to monitor cardiomyocyte beating activity in millisecond-time resolution, including, for example, Mohr, Pancrazio, and Heer (excerpts of which are reproduced below).

EX1003, ¶113; Pet., 38-40.

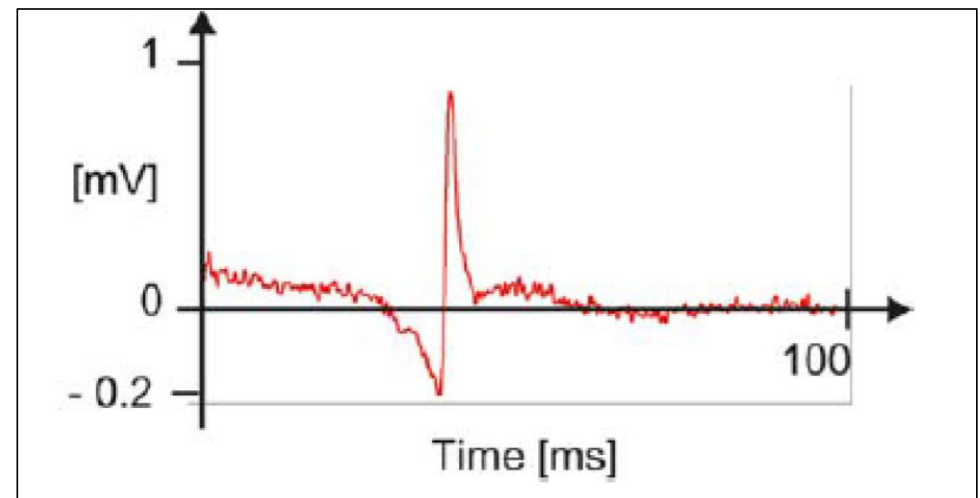


EX1018 (Mohr), 267; Pet., 39.

Prior Art: Mohr (EX1018), Pancrazio (EX1019), and Heer (EX1021) (cont'd)



EX1019 (Pancrazio), 184; Pet., 39.



EX1021 (Heer), 364; Pet., 40.

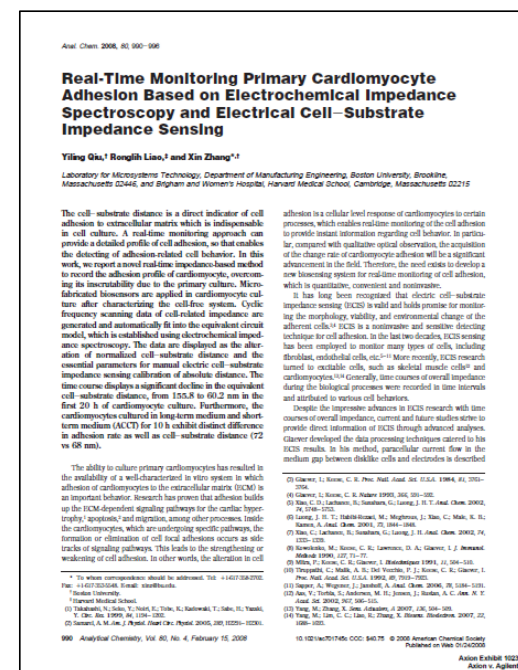
Prior Art: Using Impedance-Based Cell Analysis Systems To Monitor Cardiomyocytes (Yang, Qiu)

115. Further, and also years before the '508 patent, others in the art had reported using impedance-based cell analysis systems to monitor cardiomyocytes more generally. For example, "A novel microfluidic impedance assay for monitoring endothelin-induced cardiomyocyte hypertrophy," by Mo Yang et al., *Biosensors and Bioelectronics* (2006) ("Yang") describes an impedance-based assay for monitoring cardiomyocyte hypertrophy. See Ex. 1022. As another example, "Real Time Monitoring Primary Cardiomyocyte Adhesion Based on Electrochemical Impedance Spectroscopy and Electrical Cell-Substrate Impedance Sensing," by Yiling Qiu et al., *Analytical Chemistry*, Vol. 80, No. 4, 990-996 (February 2008) ("Qiu") describes monitoring cardiomyocyte adhesion using an impedance-based cell analysis system. See Ex. 1023.

EX1003, ¶115; Pet., 40-41.



EX1022 (Yang).



EX1023 (Qiu).

A POSA Understood That The Cellular Phenomenon To Be Monitored Dictates The Requisite Measurement Speed

A POSA would have understood, well before the '508's alleged invention, that the biological cell phenomenon to be studied drives the requisite measurement speed (*id.*, ¶178), and each of the ranges recited in claims 9-14 therefore would have been obvious. To apply the Xu-752/Jones system to study electroporabilization, for instance, where cell pores “start resealing within a few milliseconds,” it would have been obvious to configure the system to provide an adequate measurement resolution, *e.g.*, “less than 5 milliseconds” (claim 14). EX1017 (Ghosh), 1604, 1609; EX1003, ¶178. The other resolutions of claims 9-14 would have been obvious for the same reason—there is nothing inventive or non-obvious about selecting a measurement speed sufficient for the application. *Id.*

Pet., 64.

11. The apparatus of claim 1, wherein the millisecond time resolution between the first measurement and the second measurement is less than 100 milliseconds.

12. The apparatus of claim 1, wherein the millisecond time resolution between the first measurement and the second measurement is less than 40 milliseconds.

13. The apparatus of claim 1, wherein the millisecond time resolution between the first measurement and the second measurement is less than 20 milliseconds.

14. The apparatus of claim 1, wherein the millisecond time resolution between the first measurement and the second measurement is less than 5 milliseconds.

EX1001, claims 11-14.

Agilent's expert Dr. Sanchez confirmed that the requisite measurement resolution is “driven by the application,” and that the “measurement needs to be fast enough to monitor th[e] cellular activity.” EX1028, 52:18-53:12 (cited at Reply, 13).

Indefiniteness Issues With “Millisecond Time Resolution” Term

The pertinent portion of the Specification’s “Definitions” section states:

As used herein, “impedance measurement at millisecond time resolution” refers to the ability to perform a series of at least two consecutive impedance measurements within milliseconds of one another for each and every well of the system where each well is associated with an electrode array for impedance measurement. In each instance the time between two consecutive impedance measurements are less than 300 milliseconds from one another.

Ex. 1001, 10:8–15. There does not appear to be any dispute as to the definitional nature of the first sentence in the passage above. That is, DI, 9.

Q. How about 700 milliseconds, is that within the scope of Claim 1?

A. Yes.

Q. 999 milliseconds?

A. Yes.

EX1028, 50:20-51:14; Reply, 24.

As Petitioner points out, however, other portions of the intrinsic record seemingly contradict the second sentence from the “Definitions” section and support a broader interpretation of the term “millisecond time resolution,” i.e., one encompassing measurements occurring as long as 500 milliseconds apart. See Pet. 14. For example, the Specification elsewhere explains that “[i]n some embodiments 500 millisecond time resolution is sufficient.” Ex. 1001, 23:49–55; see also *id.* at 24:37–39 (similar).

Moreover, dependent claims 9 and 10, which were originally at issue but have since been disclaimed, state that “the millisecond time resolution” (referring to the term as initially recited in claim 1 for antecedent) between consecutive measurements is “less than 500 milliseconds” (claim 9) and “between 500 and 100 milliseconds” (claim 10). *Id.* at 47:46–51 (emphasis added).

DI, 10-11.