

Agilent Technologies, Inc.
v.
Axion Biosystems, Inc.

Case No. 23-cv-00198-CJB

Technology Tutorial

Asserted Patents

'752 Patent

(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**

(56) **References Cited**
U.S. PATENT DOCUMENTS

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

2,656,508 A 10/1953 Coulter
3,259,842 A 7/1996 Coulter et al.

(Continued)

FOREIGN PATENT DOCUMENTS

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

EP 1195432 B1 9/2004
WO 96/01836 1/1996

(Continued)

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

OTHER PUBLICATIONS
Arawata et al. A generally engineered cell-based biosensor for functional classification of agents. *Biosensors & Bioelectronics* 16:571-577 (2001).

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

(Continued)

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

Primary Examiner—David Redding
(74) Attorney, Agent, or Firm—David R. Preston & Associates; David Preston

(65) **Prior Publication Data**

(57) **ABSTRACT**

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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 effects of compounds on cells, such as cytotoxicity assays. In other preferred aspects, the assays are designed to investigate the compounds that effect ligand-mediated responses of cells to antigens.

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, and a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003.

(66) Provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/397,349, filed on Jul. 20, 2002.

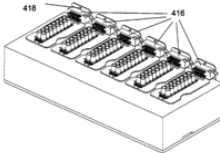
(51) Int. Cl. **C12N 1/00** (2006.01)

(52) U.S. Cl. **435/288.4; 435/285.5; 204/403.03**

(58) **Field of Classification Search** **435/285.2; 435/287.2; 288.4; 305.2; 305.3; 204/403.03; 204/403.02**

See application file for complete search history.

27 Claims, 26 Drawing Sheets



'080 Patent

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

(10) Patent No.: **US 8,026,080 B2**
(45) Date of Patent: ***Sep. 27, 2011**

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

(56) **References Cited**
U.S. PATENT DOCUMENTS

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

5,187,096 A 2/1993 Guener et al.
5,218,212 A 6/1993 Mann
5,247,827 A 9/1993 Shah et al.
5,276,606 A 1/1994 Paves et al.
5,284,733 A 2/1994 Goodwin
5,315,454 A 9/1996 Springer et al.
5,363,067 A 10/1996 Stephens et al.
5,601,097 A 7/1997 Taylor et al.
5,622,872 A 4/1997 Riba
5,625,734 A 9/1997 Dsouza et al.
5,643,542 A 7/1997 Maita et al.
5,766,534 A 6/1999 Guenep-Fle
5,801,015 A 9/1998 Bushman
5,810,175 A 9/1998 Bushman
5,811,480 A 12/1998 Ward et al.
5,901,206 A 11/1999 Kovacs et al.
6,011,422 A 8/2000 Senoo et al.
6,132,883 A 10/2000 Stephens et al.
6,169,204 B1 8/2001 Frazer et al.

(73) Assignee: **ACEA Biosciences, Inc., 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 1159 days.

(Continued)

(21) Appl. No.: **11/725,049**

FOREIGN PATENT DOCUMENTS

(22) Filed: **Mar. 15, 2007**

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(65) **Prior Publication Data**

(Continued)

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OTHER PUBLICATIONS

Related U.S. Application Data

(60) Division of application No. 10/987,732, filed on Nov. 12, 2004, now Pat. No. 7,192,752, which is a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003, now Pat. No. 7,470,533, said application No. 10/987,732 is a continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, now Pat. No. 7,459,503.

(66) Provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004.

(51) Int. Cl. **C12Q 1/00** (2006.01)

(52) U.S. Cl. **435/288.4; 435/285.5; 204/403.03**

(58) **Field of Classification Search** **435/285.2; 435/287.2; 288.4; 305.2; 305.3; 204/403.03; 204/403.02**

See application file for complete search history.

27 Claims, 26 Drawing Sheets

Primary Examiner—William H. Belser
(74) Attorney, Agent, or Firm—Hochstetler Beach Law Group PC; Raymond Wagonkhuwe

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 effects of compounds on cells, such as cytotoxicity assays. In other preferred aspects, the assays are designed to investigate the compounds that effect ligand-mediated responses of cells to antigens.

7/1992 Camp

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

5,187,096 A 2/1993 Guener et al.

5,218,212 A 6/1993 Mann

5,247,827 A 9/1993 Shah et al.

5,276,606 A 1/1994 Paves et al.

5,284,733 A 2/1994 Goodwin

5,315,454 A 9/1996 Springer et al.

5,363,067 A 10/1996 Stephens et al.

5,601,097 A 7/1997 Taylor et al.

5,622,872 A 4/1997 Riba

5,625,734 A 9/1997 Dsouza et al.

5,643,542 A 7/1997 Maita et al.

5,766,534 A 6/1999 Guenep-Fle

5,801,015 A 9/1998 Bushman

5,810,175 A 9/1998 Bushman

5,811,480 A 12/1998 Ward et al.

5,901,206 A 11/1999 Kovacs et al.

6,011,422 A 8/2000 Senoo et al.

6,132,883 A 10/2000 Stephens et al.

6,169,204 B1 8/2001 Frazer et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP 1 138 788 A1 4/2001

(Continued)

OTHER PUBLICATIONS

Wagner et al. Electric Cell-Substrate Impedance Sensing (ECIS) as a Noninvasive Means to Monitor the Kinetics of Cell Spreading to Artificial Surfaces. *Experimental Cell Research* 259: 158-166 (2000).

Arawata et al. A generally engineered cell-based biosensor for functional classification of agents. *Biosensors & Bioelectronics* 16:571-577 (2001).

Barnett et al. Microelectronic sensor system for microphysiological application on living cells. *Sensors & Actuators B55:77-89* (1999).

Berger et al. Separation of human breast cancer cells from blood by differential dielectric affinity. *Cell Biology* 92:960-964 (1993).

Brown et al. The role of extracellular matrix in human astrocyte migration and proliferation studied in a microfluidic assay. *Cell Eng. Materials* 12:405-415.

Bergveld. A critical evaluation of direct electrical protein detection methods. *Biosensors & Bioelectronics* 6:55-72 (1991).

(Continued)

Primary Examiner—William H. Belser

(74) Attorney, Agent, or Firm—Hochstetler Beach Law Group

PC; Raymond Wagonkhuwe

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 effects of compounds on cells, such as cytotoxicity assays. In other preferred aspects, the assays are designed to investigate the compounds that effect ligand-mediated responses of cells to antigens.

7/1992 Camp

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

7/1991 Taylor et al.

'255 Patent

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

(10) Patent No.: **US 7,468,255 B2**
(45) Date of Patent: **Dec. 23, 2008**

(54) **METHOD FOR ASSAYING FOR NATURAL KILLER, CYTOTOXIC LYMPHOCYTE AND NEUTROPHIL-MEDIATED KILLING OF TARGET CELLS USING REAL-TIME MICROELECTRONIC CELL SENSING TECHNOLOGY**

(56) **References Cited**
U.S. PATENT DOCUMENTS

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

2,656,508 A 10/1953 Coulter
3,259,842 A 7/1996 Coulter et al.
3,743,581 A 7/1973 Cady et al.
3,800,301 A 6/1975 Cady
4,072,378 A 2/1978 Cady et al.
4,225,410 A 9/1980 Paves
4,686,190 A 8/1987 Cramer et al.
4,920,847 A 4/1990 Guener et al.
5,091,048 A 3/1991 Taylor et al.
5,134,070 A 7/1992 Camp
5,187,096 A 2/1993 Guener et al.
5,218,212 A 6/1993 Mann
5,247,827 A 9/1993 Shah et al.

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

(Continued)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 315 days.

OTHER PUBLICATIONS

(21) Appl. No.: **11/197,994**

(Continued)

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Primary Publication Data

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Related U.S. Application Data

(63) Continuation-in-part of application No. 11/055,639, filed on Feb. 9, 2005, which is a continuation-in-part of application No. 10/987,732, filed on Nov. 12, 2004, now Pat. No. 7,192,752, and a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003, said application No. 10/987,732 is a continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, application No. 11/197,994, which is a continuation-in-part of application No. PCT/US2004/004481, filed on Feb. 9, 2005, and a continuation-in-part of application No. PCT/US2004/037696, filed on Nov. 12, 2004.

(66) Provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb

Asserted Patents

'752 Patent



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(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

(56) References Cited
U.S. PATENT DOCUMENTS

(75) Inventors: Xiao Xu, San Diego, CA (US); Yama Akashi, San Diego, CA (US); Xiaohu Wang, San Diego, CA (US); Jiangbo Guo, San Diego, CA (US)

2,656,508 A 10/1953 Coulter
3,259,842 A 7/1966 Coulter et al.

FOREIGN PATENT DOCUMENTS

(73) Assignee: ACCE Biosciences, San Diego, CA (US)

EP 0594323 B1 9/2004
WO 99/03036 1/1999

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

OTHER PUBLICATIONS

(21) Appl. No.: 10/980,732

Primary Examiner—David Redding
(74) Attorney, Agent, or Firm—David R. Preston & Associates; David Preston

(22) Filed: Nov. 12, 2004

(65) Prior Publication Data
US 2005/015423 A1 Jul. 14, 2005

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/705,675, filed on Nov. 10, 2003, and a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003.

(60) Provisional application No. 60/542,927, filed on Feb. 9, 2004; provisional application No. 60/548,713, filed on Feb. 2, 2004; provisional application No. 60/460,572, filed on May 9, 2003; provisional application No. 60/435,400, filed on Dec. 26, 2002; provisional application No. 60/397,349, filed on Jul. 20, 2002.

(51) Int. Cl. C12N 1/10 (2006.01)

(52) U.S. Cl. 435/287.2, 435/287.4, 435/287.5, 435/287.6, 435/287.7, 435/287.8, 435/287.9, 435/288.1, 435/288.2, 435/288.3, 435/288.4, 435/288.5, 435/288.6, 435/288.7, 435/288.8, 435/288.9, 435/289.1, 435/289.2, 435/289.3, 435/289.4, 435/289.5, 435/289.6, 435/289.7, 435/289.8, 435/289.9, 435/290.1, 435/290.2, 435/290.3, 435/290.4, 435/290.5, 435/290.6, 435/290.7, 435/290.8, 435/290.9, 435/291.1, 435/291.2, 435/291.3, 435/291.4, 435/291.5, 435/291.6, 435/291.7, 435/291.8, 435/291.9, 435/292.1, 435/292.2, 435/292.3, 435/292.4, 435/292.5, 435/292.6, 435/292.7, 435/292.8, 435/292.9, 435/293.1, 435/293.2, 435/293.3, 435/293.4, 435/293.5, 435/293.6, 435/293.7, 435/293.8, 435/293.9, 435/294.1, 435/294.2, 435/294.3, 435/294.4, 435/294.5, 435/294.6, 435/294.7, 435/294.8, 435/294.9, 435/295.1, 435/295.2, 435/295.3, 435/295.4, 435/295.5, 435/295.6, 435/295.7, 435/295.8, 435/295.9, 435/296.1, 435/296.2, 435/296.3, 435/296.4, 435/296.5, 435/296.6, 435/296.7, 435/296.8, 435/296.9, 435/297.1, 435/297.2, 435/297.3, 435/297.4, 435/297.5, 435/297.6, 435/297.7, 435/297.8, 435/297.9, 435/298.1, 435/298.2, 435/298.3, 435/298.4, 435/298.5, 435/298.6, 435/298.7, 435/298.8, 435/298.9, 435/299.1, 435/299.2, 435/299.3, 435/299.4, 435/299.5, 435/299.6, 435/299.7, 435/299.8, 435/299.9, 435/300.1, 435/300.2, 435/300.3, 435/300.4, 435/300.5, 435/300.6, 435/300.7, 435/300.8, 435/300.9, 435/301.1, 435/301.2, 435/301.3, 435/301.4, 435/301.5, 435/301.6, 435/301.7, 435/301.8, 435/301.9, 435/302.1, 435/302.2, 435/302.3, 435/302.4, 435/302.5, 435/302.6, 435/302.7, 435/302.8, 435/302.9, 435/303.1, 435/303.2, 435/303.3, 435/303.4, 435/303.5, 435/303.6, 435/303.7, 435/303.8, 435/303.9, 435/304.1, 435/304.2, 435/304.3, 435/304.4, 435/304.5, 435/304.6, 435/304.7, 435/304.8, 435/304.9, 435/305.1, 435/305.2, 435/305.3, 435/305.4, 435/305.5, 435/305.6, 435/305.7, 435/305.8, 435/305.9, 435/306.1, 435/306.2, 435/306.3, 435/306.4, 435/306.5, 435/306.6, 435/306.7, 435/306.8, 435/306.9, 435/307.1, 435/307.2, 435/307.3, 435/307.4, 435/307.5, 435/307.6, 435/307.7, 435/307.8, 435/307.9, 435/308.1, 435/308.2, 435/308.3, 435/308.4, 435/308.5, 435/308.6, 435/308.7, 435/308.8, 435/308.9, 435/309.1, 435/309.2, 435/309.3, 435/309.4, 435/309.5, 435/309.6, 435/309.7, 435/309.8, 435/309.9, 435/310.1, 435/310.2, 435/310.3, 435/310.4, 435/310.5, 435/310.6, 435/310.7, 435/310.8, 435/310.9, 435/311.1, 435/311.2, 435/311.3, 435/311.4, 435/311.5, 435/311.6, 435/311.7, 435/311.8, 435/311.9, 435/312.1, 435/312.2, 435/312.3, 435/312.4, 435/312.5, 435/312.6, 435/312.7, 435/312.8, 435/312.9, 435/313.1, 435/313.2, 435/313.3, 435/313.4, 435/313.5, 435/313.6, 435/313.7, 435/313.8, 435/313.9, 435/314.1, 435/314.2, 435/314.3, 435/314.4, 435/314.5, 435/314.6, 435/314.7, 435/314.8, 435/314.9, 435/315.1, 435/315.2, 435/315.3, 435/315.4, 435/315.5, 435/315.6, 435/315.7, 435/315.8, 435/315.9, 435/316.1, 435/316.2, 435/316.3, 435/316.4, 435/316.5, 435/316.6, 435/316.7, 435/316.8, 435/316.9, 435/317.1, 435/317.2, 435/317.3, 435/317.4, 435/317.5, 435/317.6, 435/317.7, 435/317.8, 435/317.9, 435/318.1, 435/318.2, 435/318.3, 435/318.4, 435/318.5, 435/318.6, 435/318.7, 435/318.8, 435/318.9, 435/319.1, 435/319.2, 435/319.3, 435/319.4, 435/319.5, 435/319.6, 435/319.7, 435/319.8, 435/319.9, 435/320.1, 435/320.2, 435/320.3, 435/320.4, 435/320.5, 435/320.6, 435/320.7, 435/320.8, 435/320.9, 435/321.1, 435/321.2, 435/321.3, 435/321.4, 435/321.5, 435/321.6, 435/321.7, 435/321.8, 435/321.9, 435/322.1, 435/322.2, 435/322.3, 435/322.4, 435/322.5, 435/322.6, 435/322.7, 435/322.8, 435/322.9, 435/323.1, 435/323.2, 435/323.3, 435/323.4, 435/323.5, 435/323.6, 435/323.7, 435/323.8, 435/323.9, 435/324.1, 435/324.2, 435/324.3, 435/324.4, 435/324.5, 435/324.6, 435/324.7, 435/324.8, 435/324.9, 435/325.1, 435/325.2, 435/325.3, 435/325.4, 435/325.5, 435/325.6, 435/325.7, 435/325.8, 435/325.9, 435/326.1, 435/326.2, 435/326.3, 435/326.4, 435/326.5, 435/326.6, 435/326.7, 435/326.8, 435/326.9, 435/327.1, 435/327.2, 435/327.3, 435/327.4, 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Presentation Roadmap

- 1 Field of Invention**
- 2 Problems in the Art**
- 3 Solutions to the Problems**

Presentation Roadmap

1 **Field of Invention**

2 **Problems in the Art**

3 **Solutions to the Problems**

Field Of Invention

- **Bioelectronics** is a progressing interdisciplinary research field that involves the integration of biomaterials with electronic devices. Bioelectronic methods have been used for analyzing cells and assaying biological molecules and cells.
- **Cell analysis / Cell-based assays**
 - Measures the impact of a drugs on living cells
- **Cell-based assays provide valuable diagnostic data regarding:**
 - Drug efficacy
 - Drug safety
 - Drug toxicity

Field Of Invention

Cell-based assay applications in drug discovery and life science research:

- Cell-based assays are used in various stages of drug development. It is a prerequisite to test a drug candidate in defined cell-based *in vitro* experiments before moving on to live animals or later stage clinical trials.
- Cell-based assays unravel both normal and abnormal biochemical processes. Cells are essential tools for mirroring real-life conditions and crucial for testing purposes in drug discovery.

Presentation Roadmap

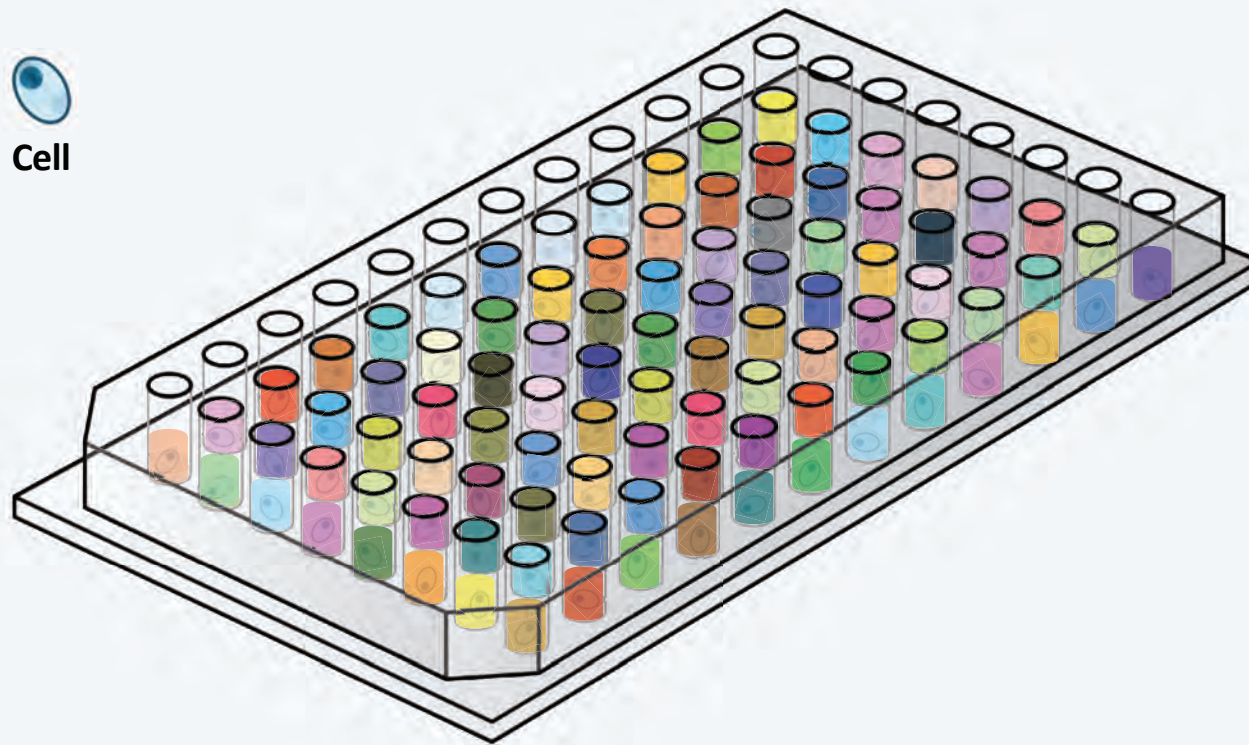
1 Field of Invention

2 Problems in the Art

3 Solutions to the Problems

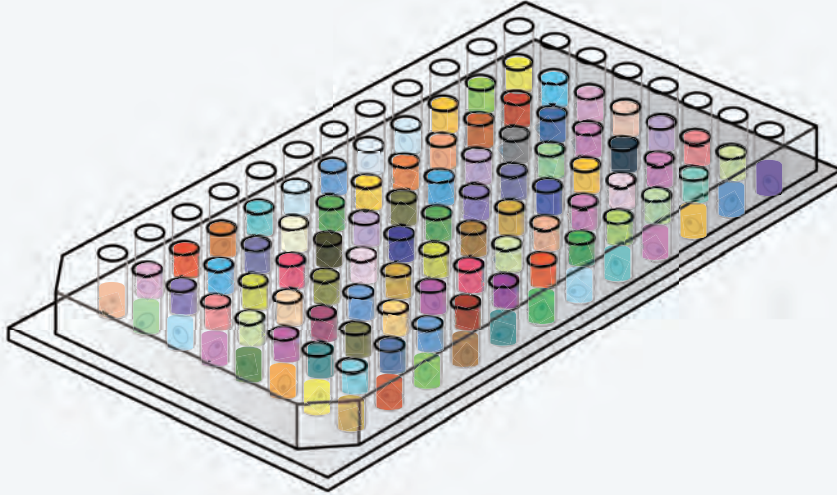
Problems In The Art

Endpoint Assays



Problems In The Art

Endpoint Assays



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02: 0.723	26: 0.228	50: 0.288	74: 0.723
03: 0.621	27: 0.213	51: 0.285	75: 0.621
04: 0.598	28: 0.198	52: 0.281	76: 0.598
05: 0.570	29: 0.184	53: 0.278	77: 0.570
06: 0.535	30: 0.167	54: 0.274	78: 0.535
07: 0.501			79: 0.501
08: 0.467			80: 0.467
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Problems In The Art

Endpoint Assays

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-

'752 Patent at 2:65-3:3

Problems In The Art

Endpoint Assays

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.

'752 Patent at 3:14-21, '080 Patent at 3:21-28

Problems In The Art

Endpoint Assays

studies used end-point single-measurement assays. Each time point for a dose concentration of drug or compound applied to the cultured cells required 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.

'752 Patent at 3:34-42

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 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.

'080 Patent at 3:39-47

Problems In The Art

Endpoint Assays

based endpoint assays. Data obtained by the RT-CES system clearly shows that cytolytic activity can reach up to 70%, which occurs after 12 hours of adding the mNK cells. Such cytolytic activity which occurs after the standard incubation time of the assay can be easily missed by existing label-based, endpoint assays. Therefore, the RT-CES system not only offers label free detection but, more importantly, also allows more accurate assessment of cytolytic activity by dynamically monitoring of the entire history of the cytotoxicity.

'255 Patent at 89:44-53

Presentation Roadmap

1 Field of Invention

2 Problems in the Art

3 Solutions to the Problems

The '752 Patent

US Patent Number 7,192,752

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

- **Inventors:** Xiao Xu, Yama Abassi, Xiaobo Wang, Jiangbo Gan
- **Assignee:** Agilent Technologies, Inc.
 - **Issue Date:** Mar. 20, 2007
 - **Filing Date:** Nov. 12, 2004



US007192752B2

(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

(65) **Prior Publication Data**
US 2005/0153425 A1 Jul. 14, 2005

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, and a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003.

(60) Provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/397,749, filed on Jul. 20, 2002.

(51) **Int. Cl.** C12N 1/00 (2006.01)

(52) **U.S. Cl.** 435/288.4; 435/288.5; 204/403.03; 435/285.2; 204/403.01; 204/403.02

(58) **Field of Classification Search** 435/287.2, 288.4, 305.2, 305.3; 204/403.01, 204/403.02

See application file for complete search history.

References Cited

U.S. PATENT DOCUMENTS

2,656,508 A 10/1933 Coulter
3,259,842 A 7/1966 Coulter et al.
(Continued)

FOREIGN PATENT DOCUMENTS

EP 1195432 B1 9/2004
WO 96/01836 1/1996
(Continued)

OTHER PUBLICATIONS

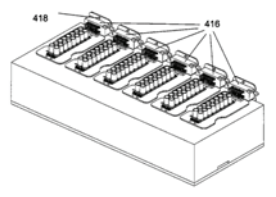
Arrais et al. A genetically engineered cell-based biosensor for functional classification of agents. *Biosensors & Bioelectronics* 16:571-577 (2001).
(Continued)

Primary Examiner—David Redding
(74) **Attorney, Agent, or Firm**—David R. Preston & Associates; David Preston

(57) **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 effects 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.

27 Claims, 26 Drawing Sheets

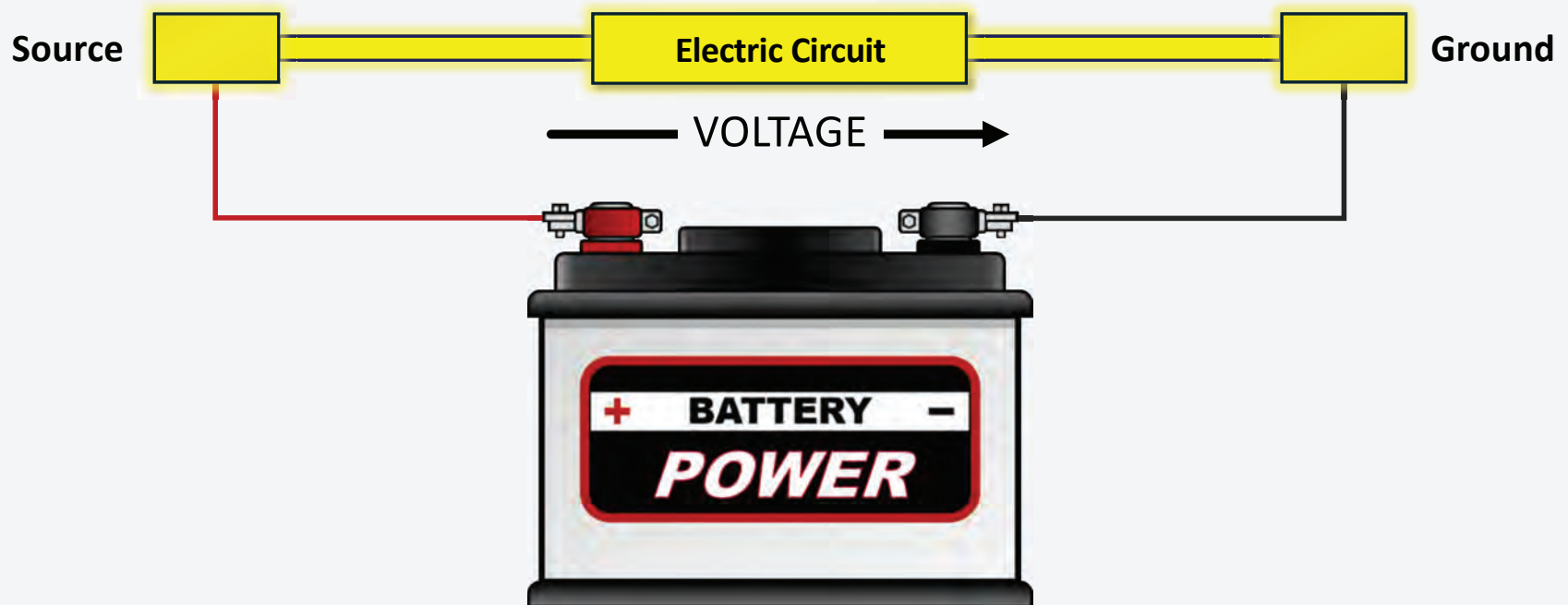


(57)

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 effects 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.

Circuitry Basics

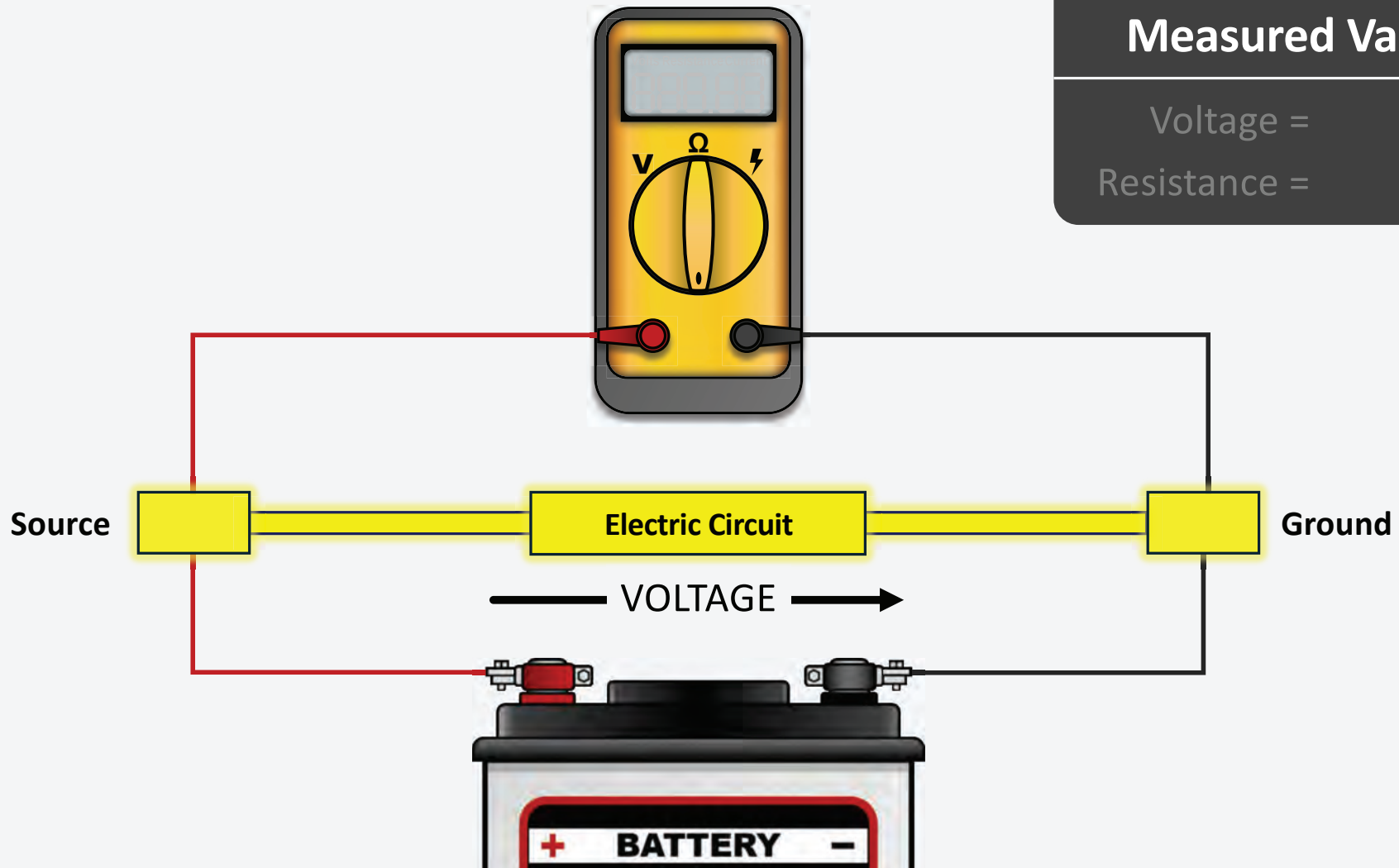


Circuitry Basics

Measured Values

Voltage =

Resistance =

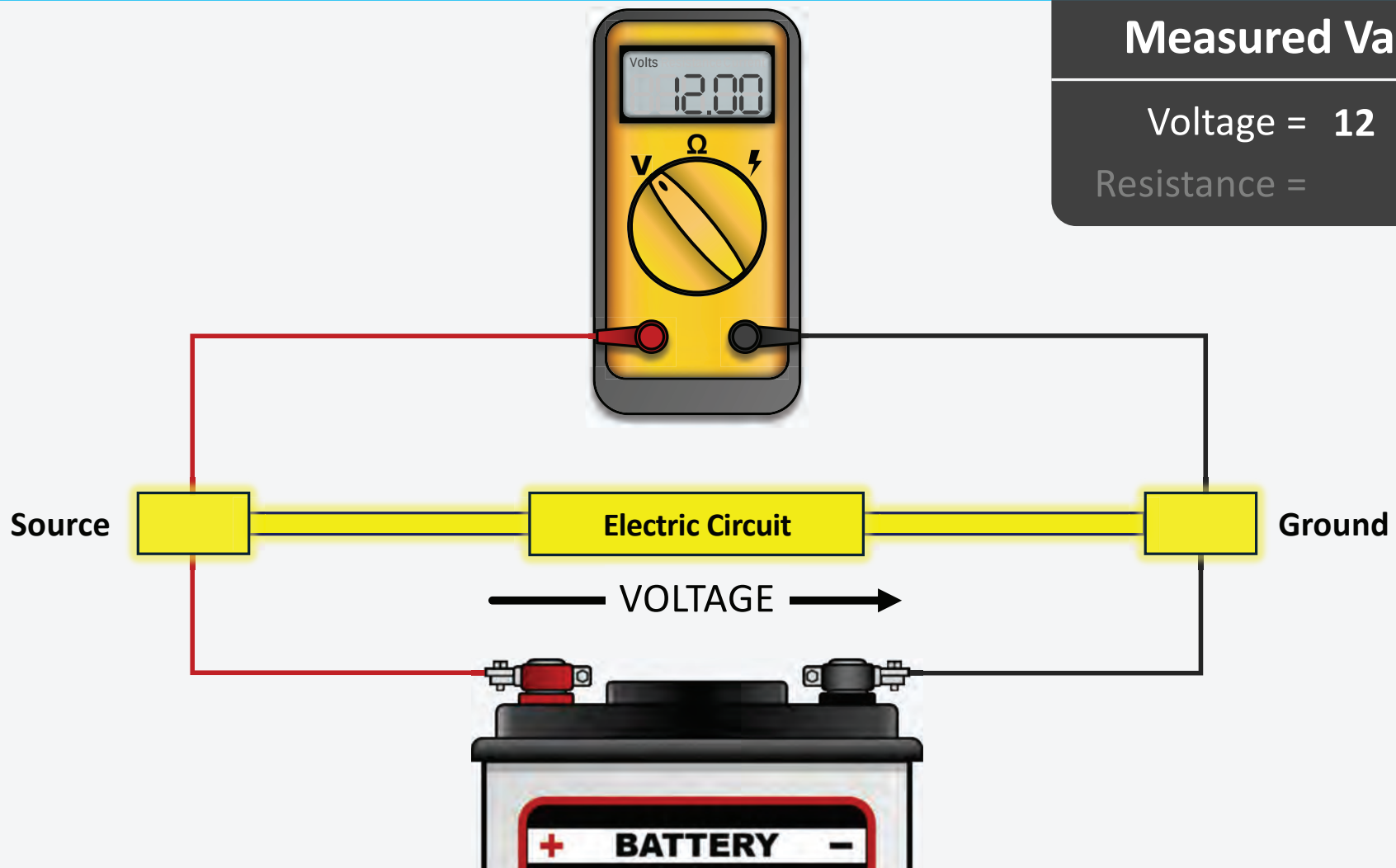


Circuitry Basics

Measured Values

Voltage = 12 Volts

Resistance =



Circuitry Basics

Resistance: Measure of the opposition to the flow of electric current

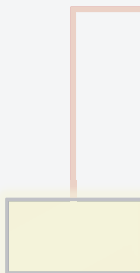


Measured Values

Voltage = 12 Volts

Resistance =

Source



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.

Ground

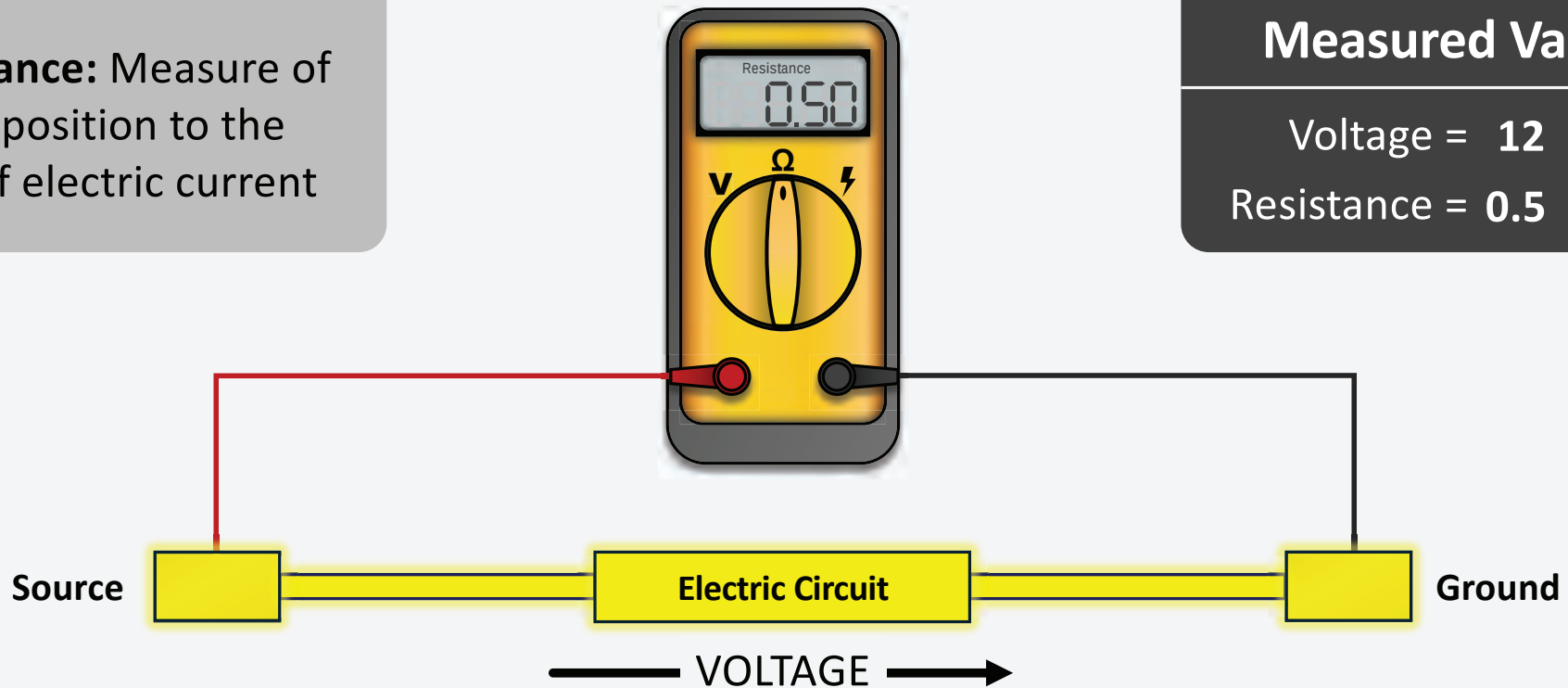
'752 Patent at Col 13:11-33

Circuitry Basics

Resistance: Measure of the opposition to the flow of electric current

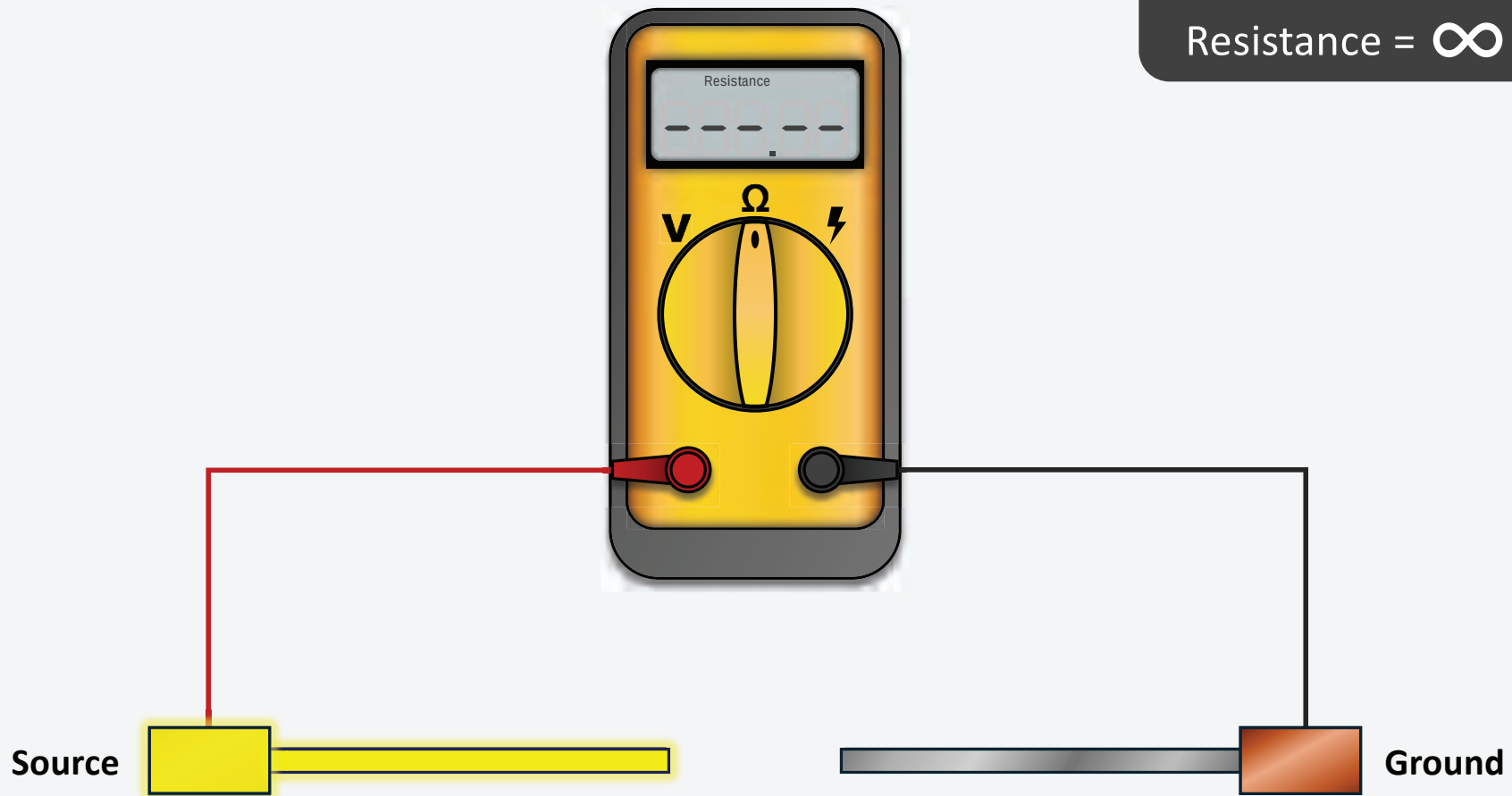
Measured Values

Voltage = 12 Volts
Resistance = 0.5 Ohms



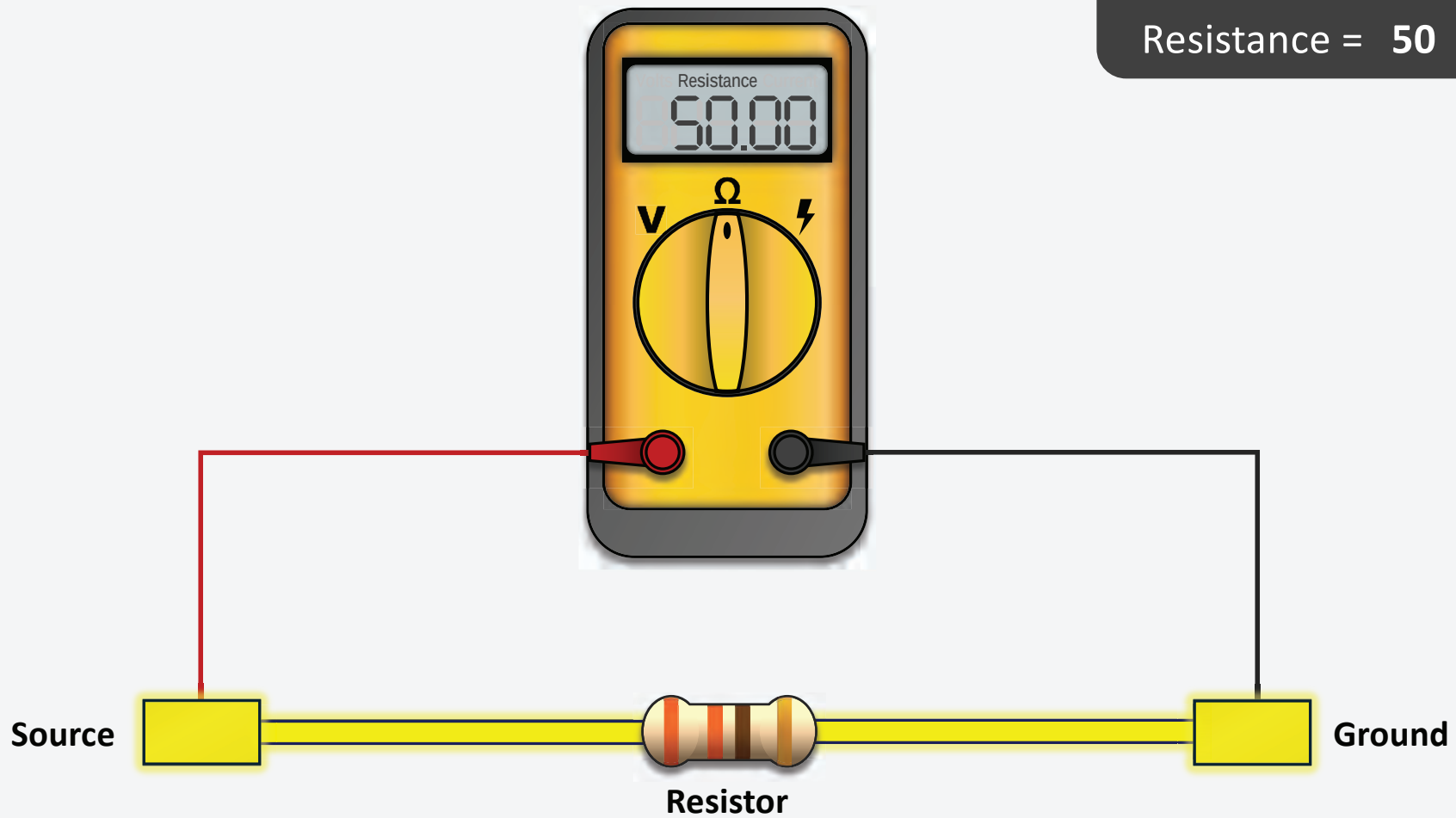
Circuitry Basics

Resistance = ∞ Ohms



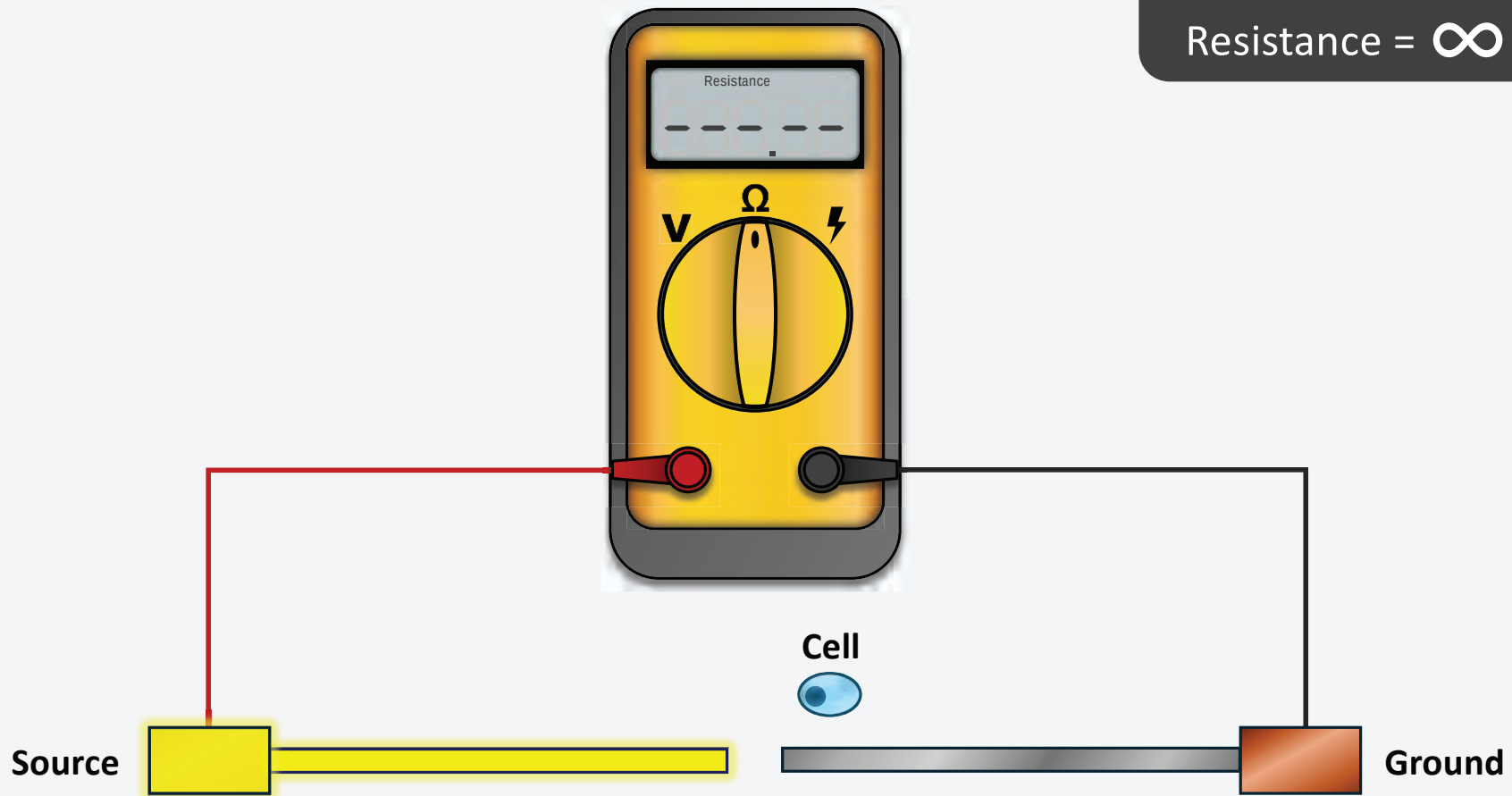
Circuitry Basics

Resistance = 50 Ohms



Circuitry Basics

Resistance = ∞ Ohms



Circuitry Basics

Resistance = 1 Ohm

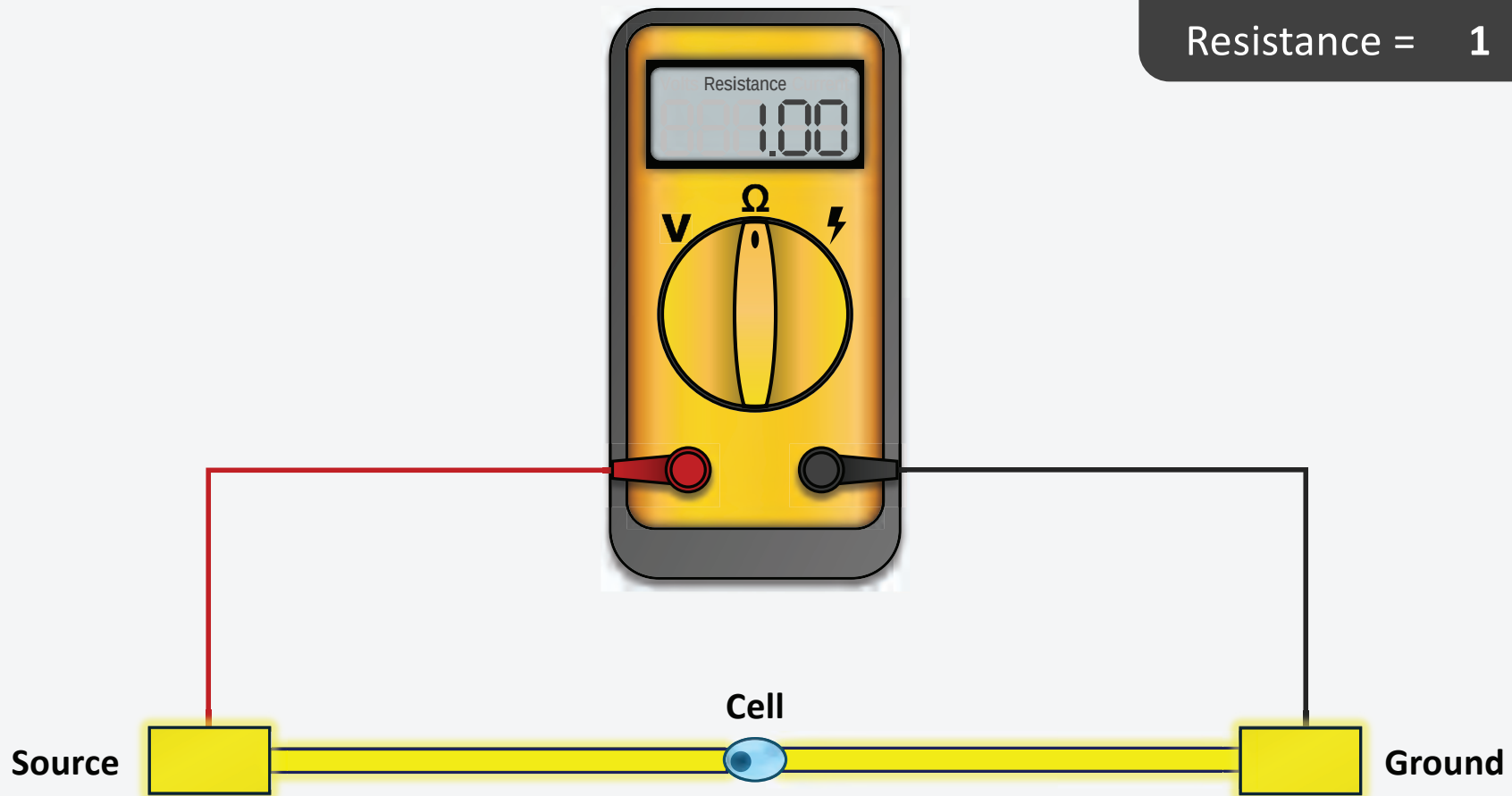
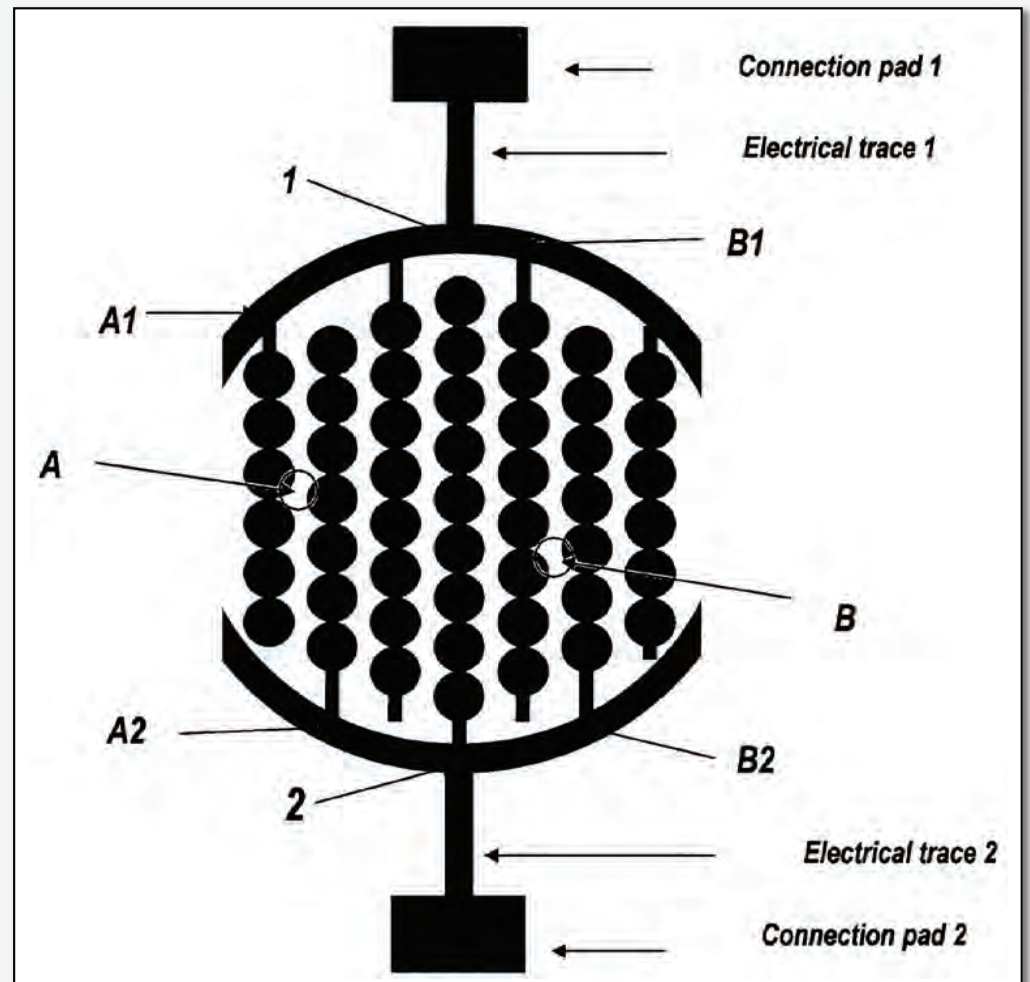
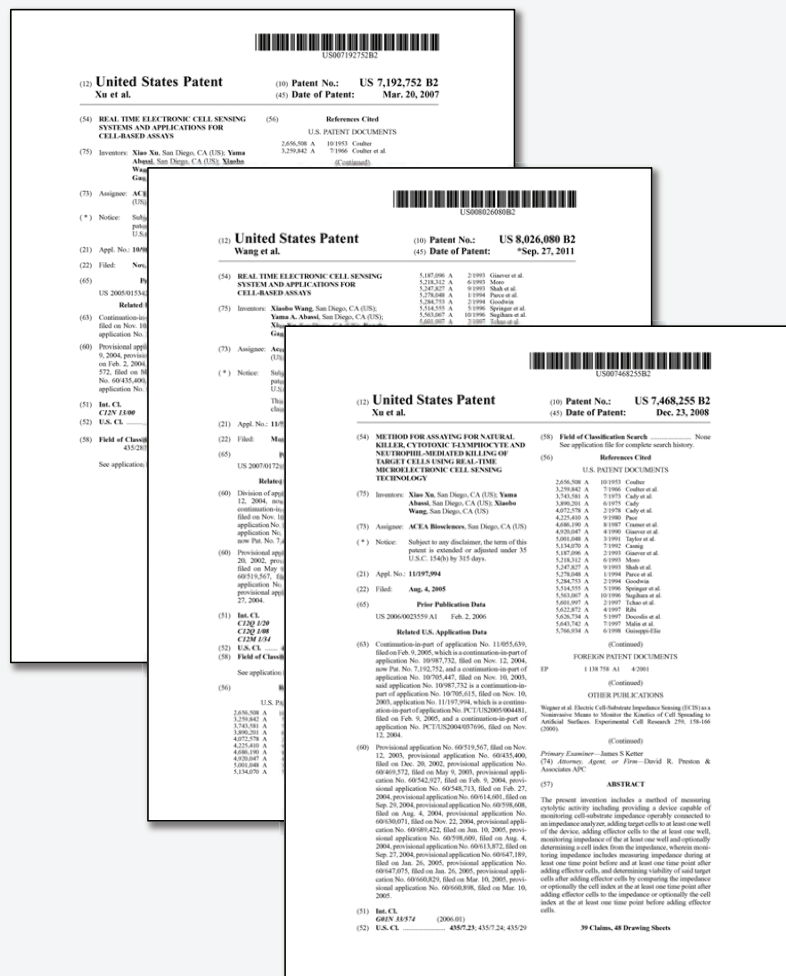


Figure 1C From The '752, '080, and '255 Patents



'752 Patent at Fig. 1C

Figure 1C From The '752, '080, and '255 Patents

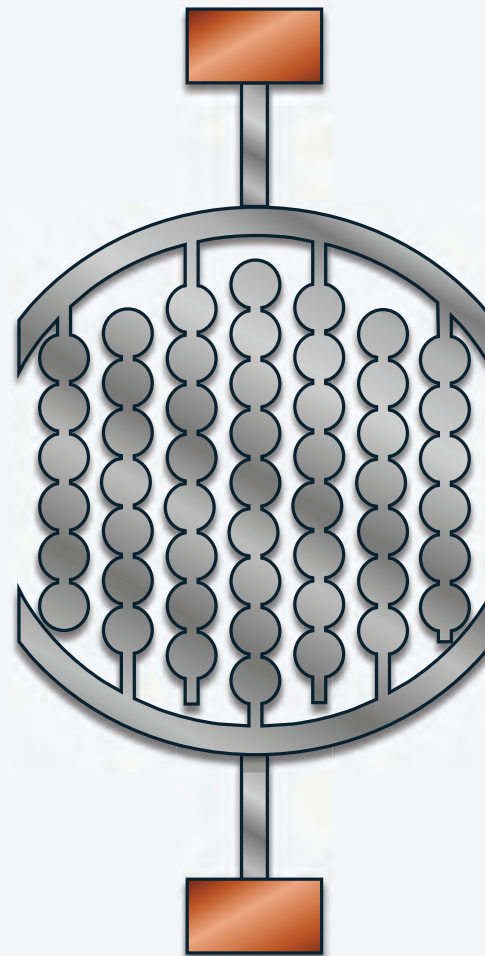
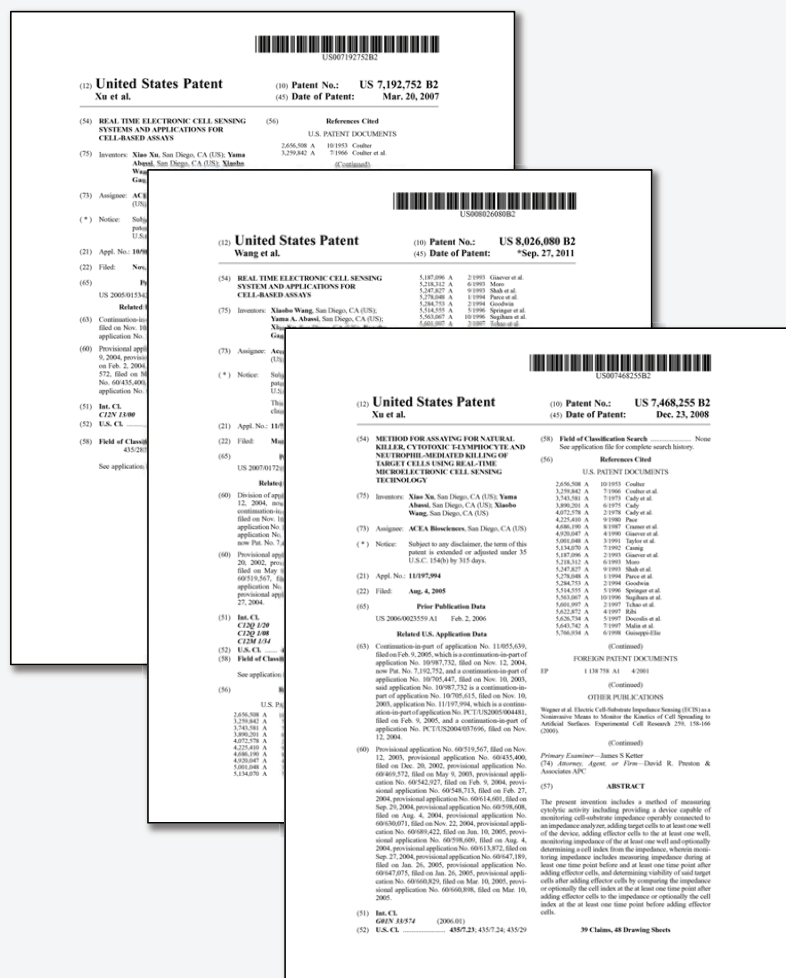


Figure 1C From The '752, '080, and '255 Patents

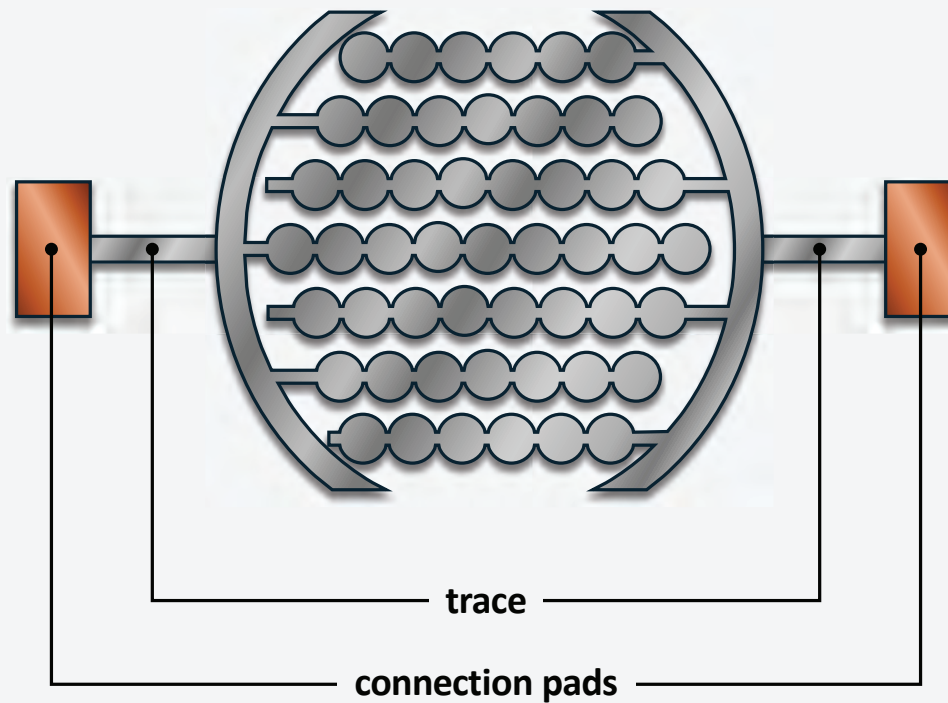


Figure 1C From The '752, '080, and '255 Patents

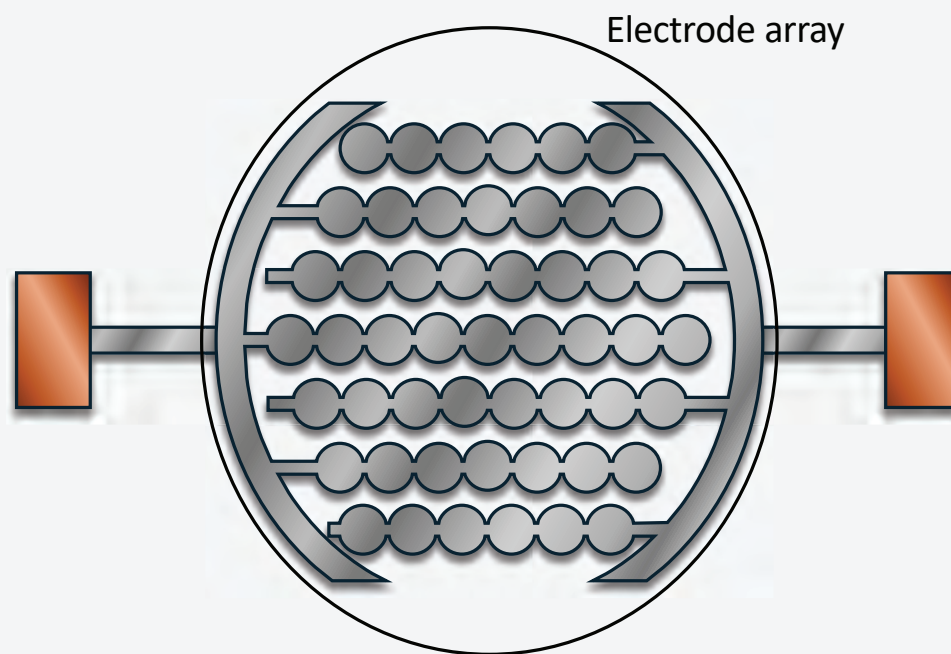


Figure 1C From The '752, '080, and '255 Patents

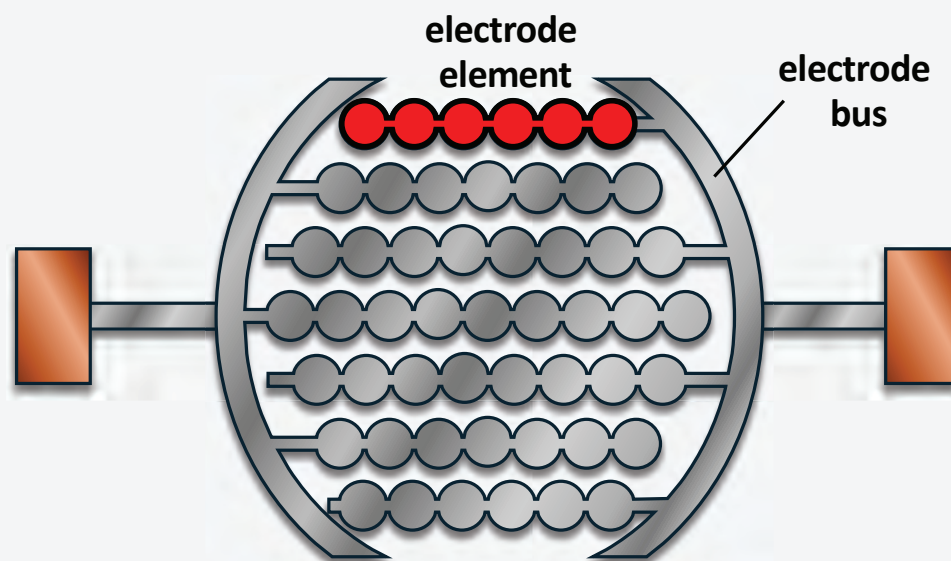


Figure 1C From The '752, '080, and '255 Patents

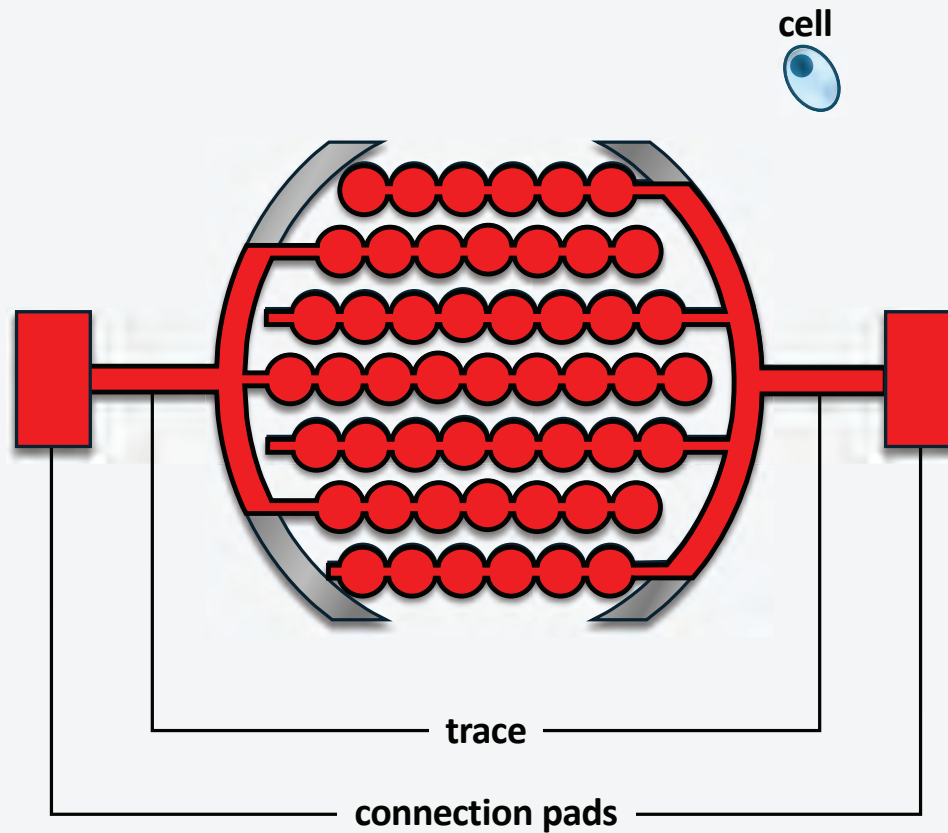


Figure 1C From The '752, '080, and '255 Patents

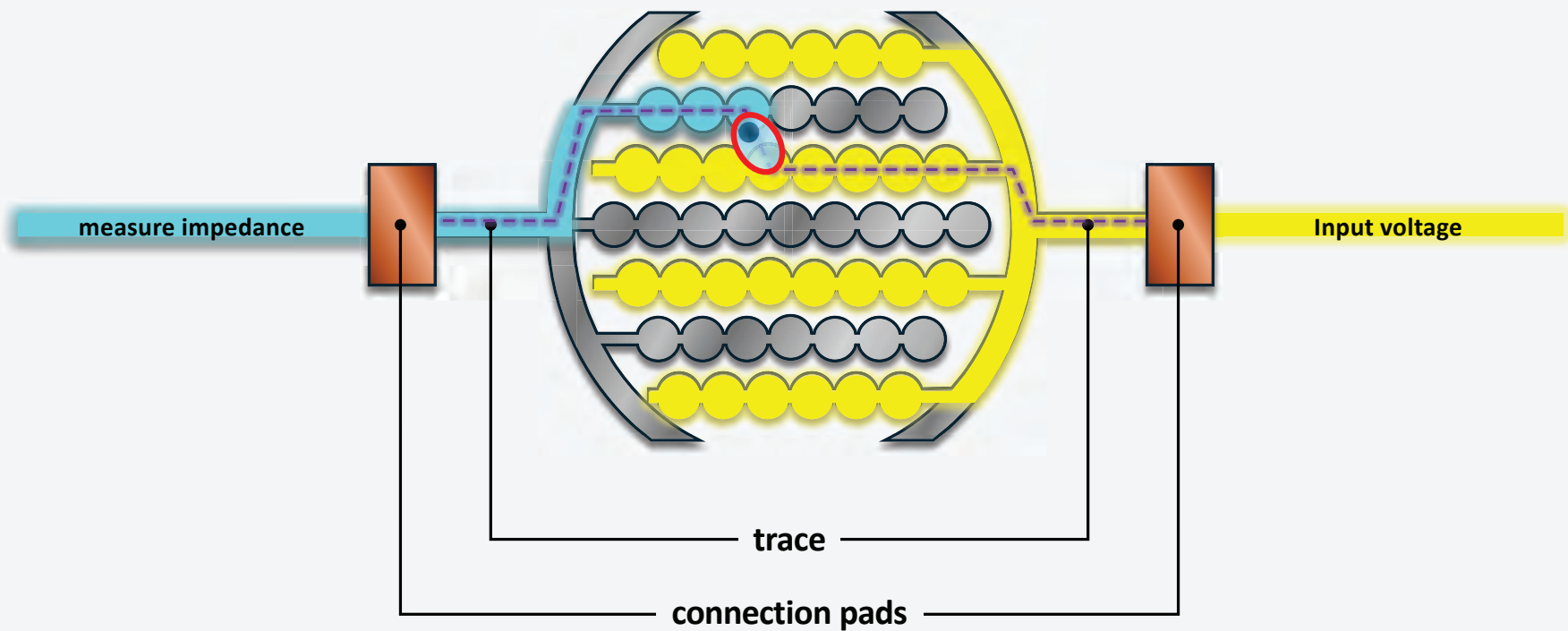


Figure 1C From The '752, '080, and '255 Patents

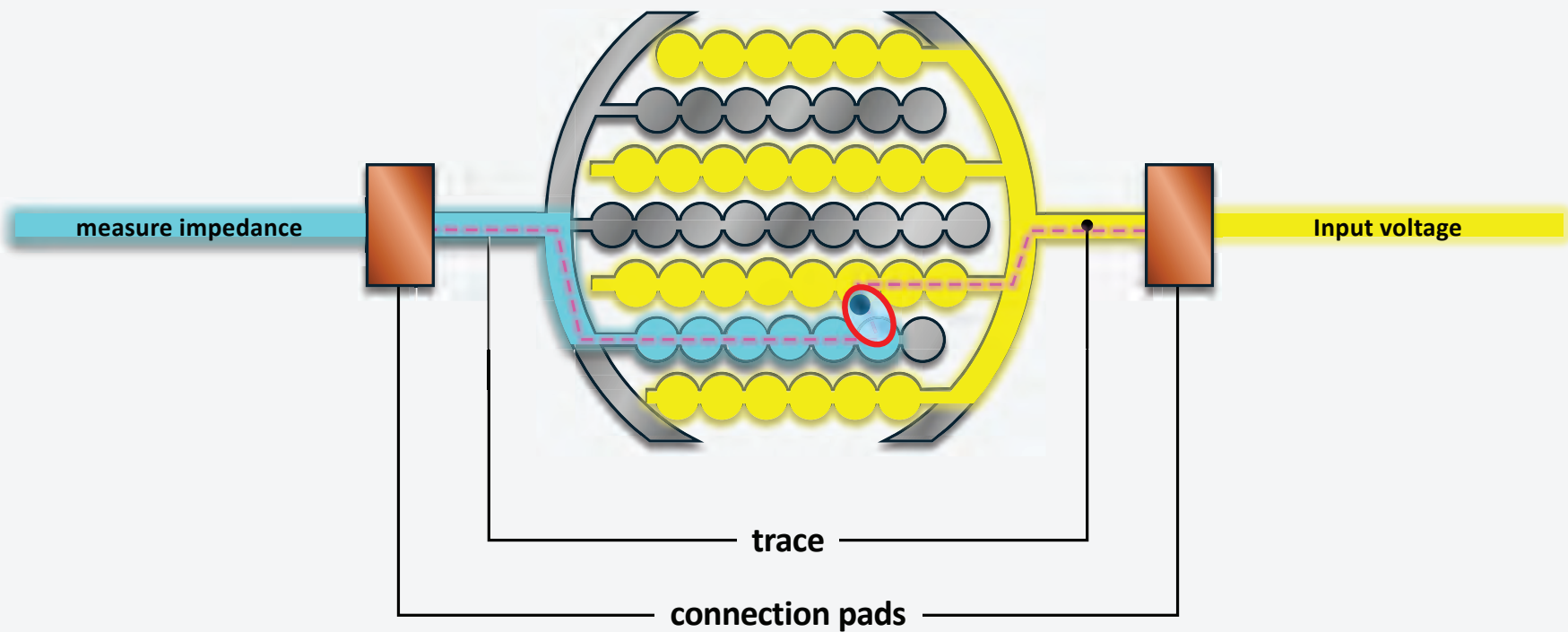


Figure 1C From The '752, '080, and '255 Patents

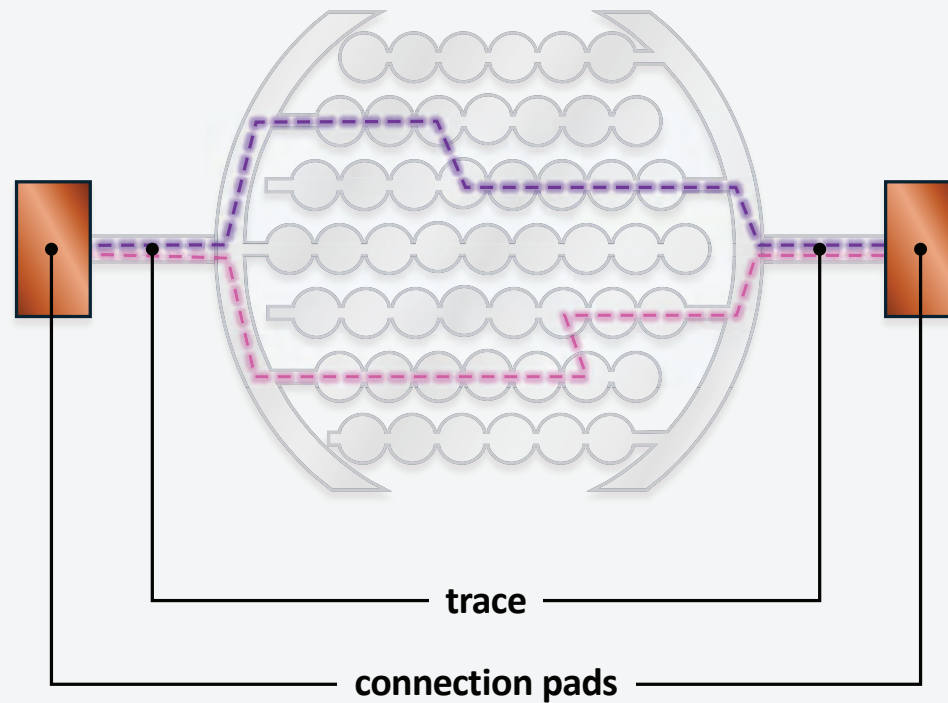


Figure 1A Illustration From The '752, '080, and '255 Patents

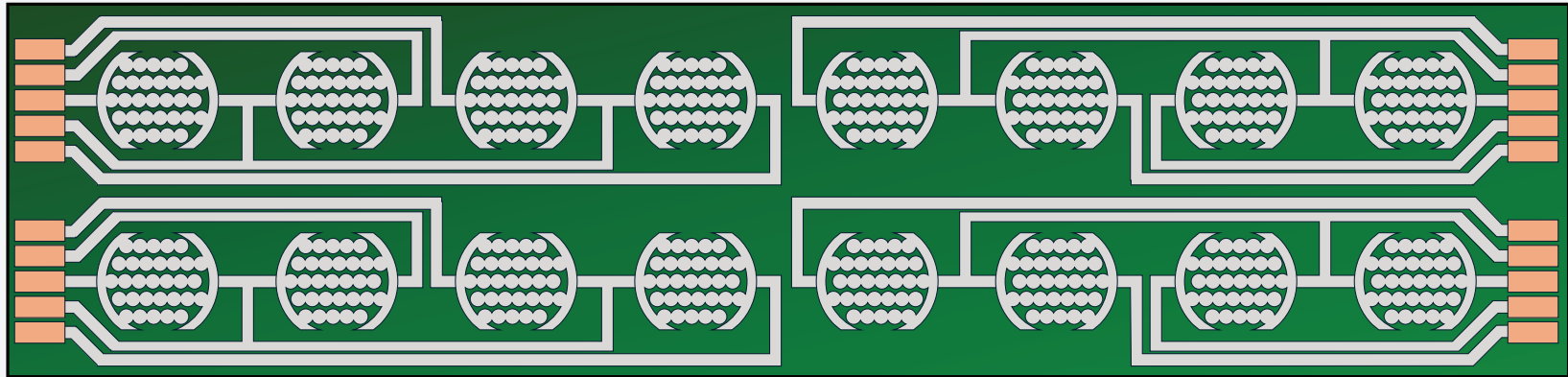
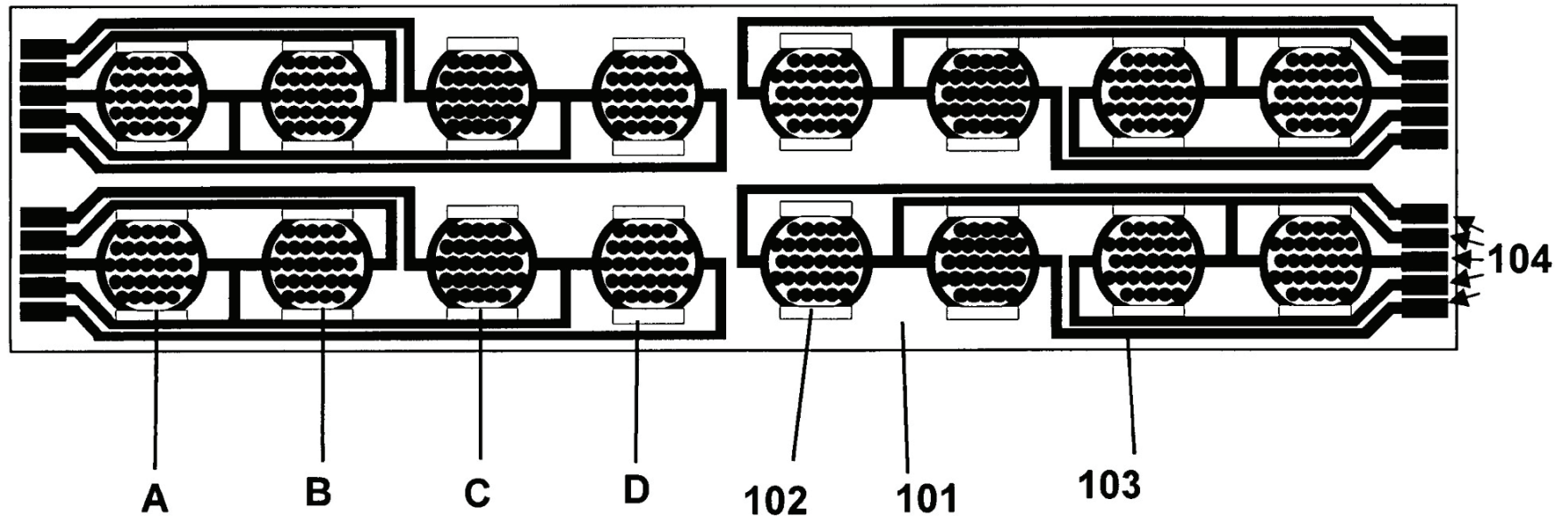


Figure 1A Illustration From The '752, '080, and '255 Patents



'752 Patent at Fig. 1A

Figure 1A Illustration From The '752, '080, and '255 Patents

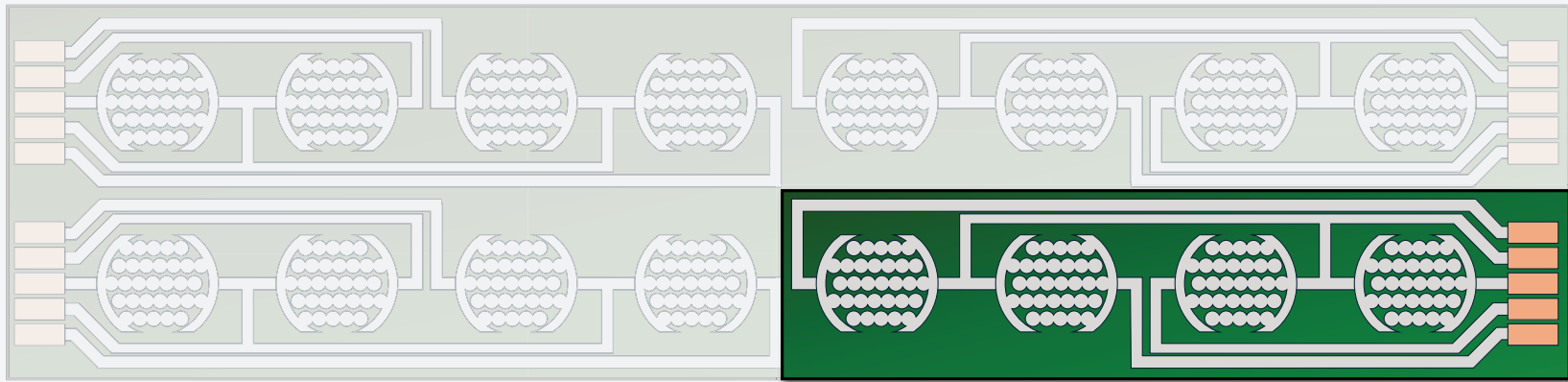


Figure 1A Illustration From The '752, '080, and '255 Patents

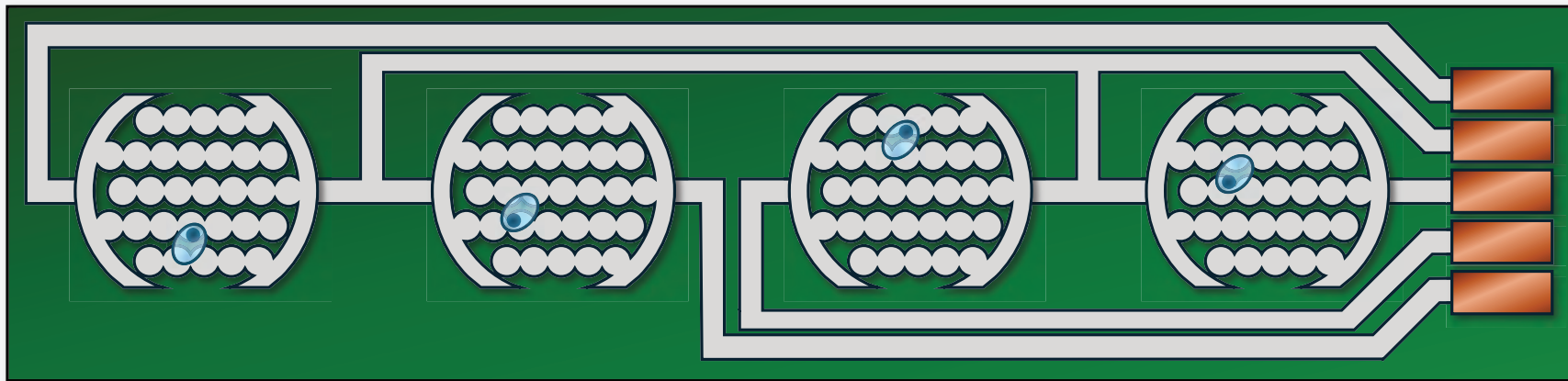


Figure 1A Illustration From The '752, '080, and '255 Patents

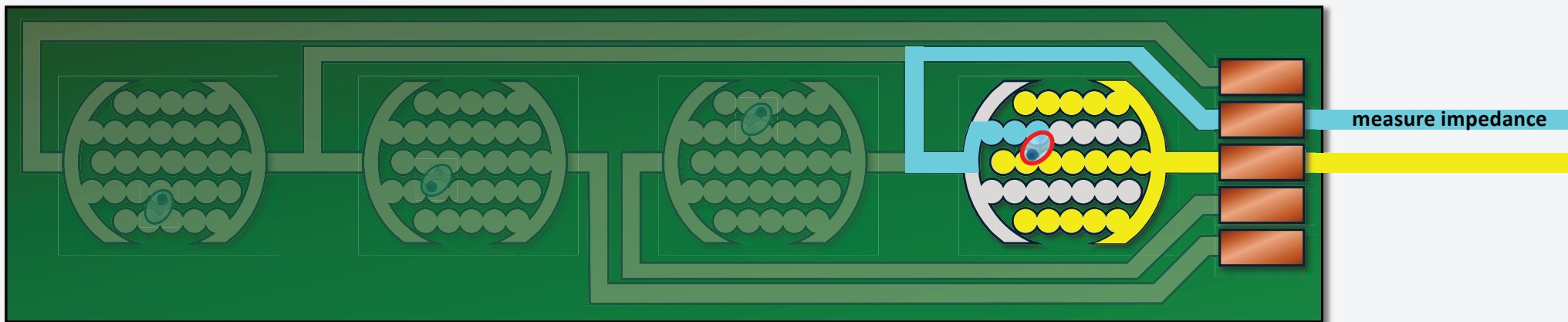


Figure 1A Illustration From The '752, '080, and '255 Patents

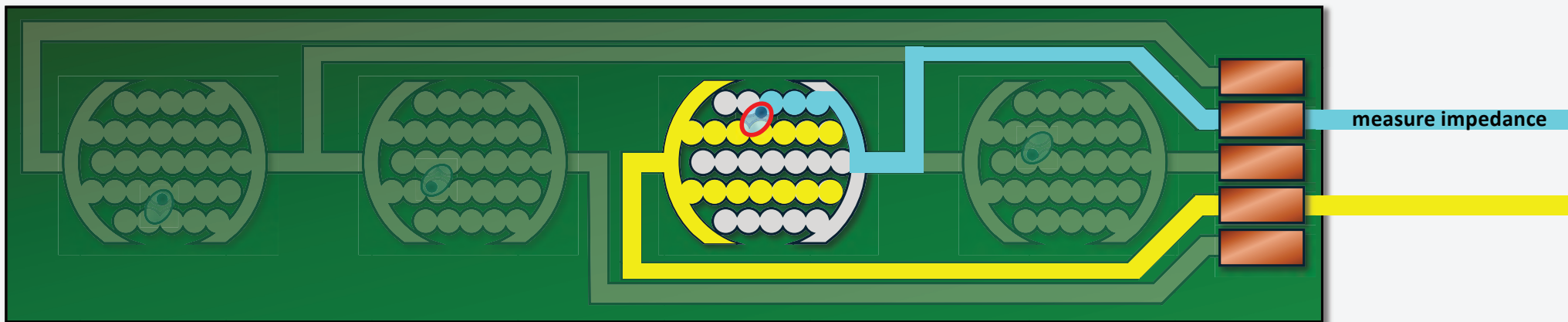


Figure 1A Illustration From The '752, '080, and '255 Patents

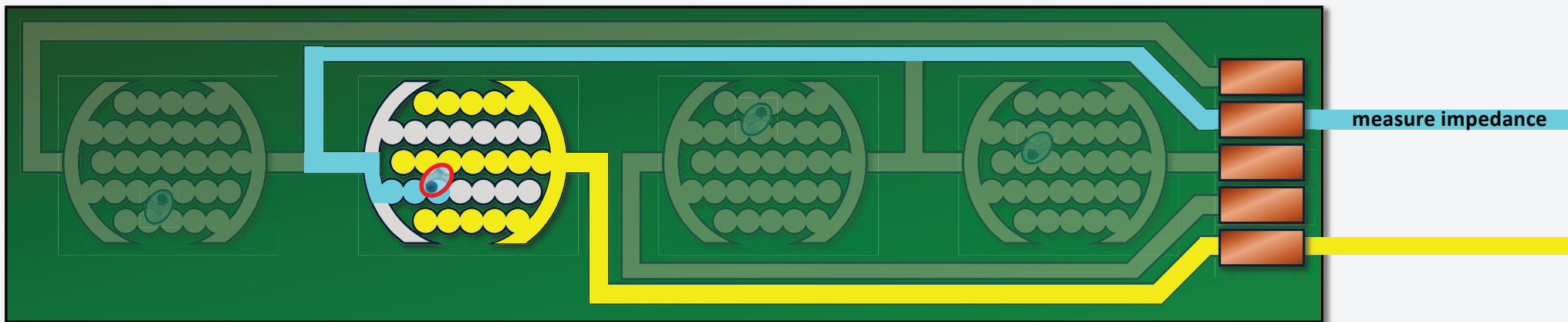


Figure 1A Illustration From The '752, '080, and '255 Patents

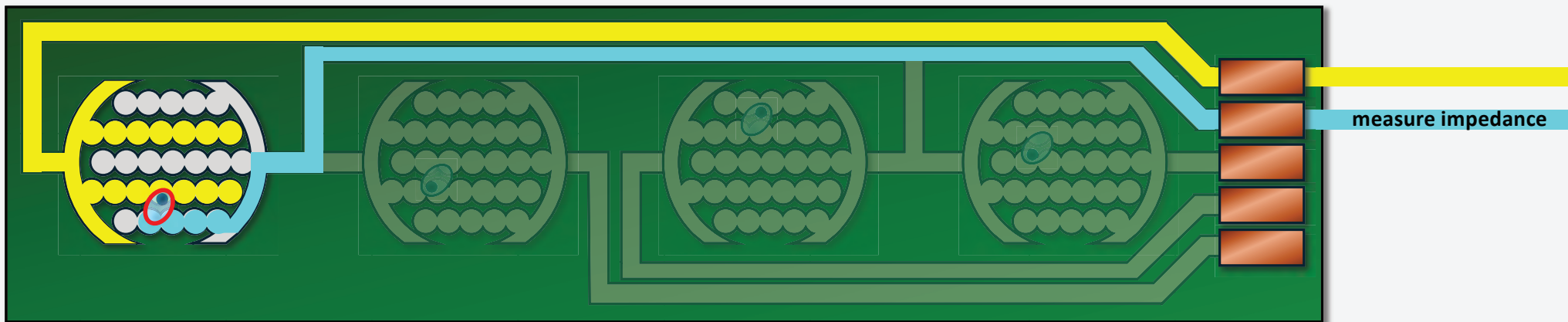


Figure 1A Illustration From The '752, '080, and '255 Patents

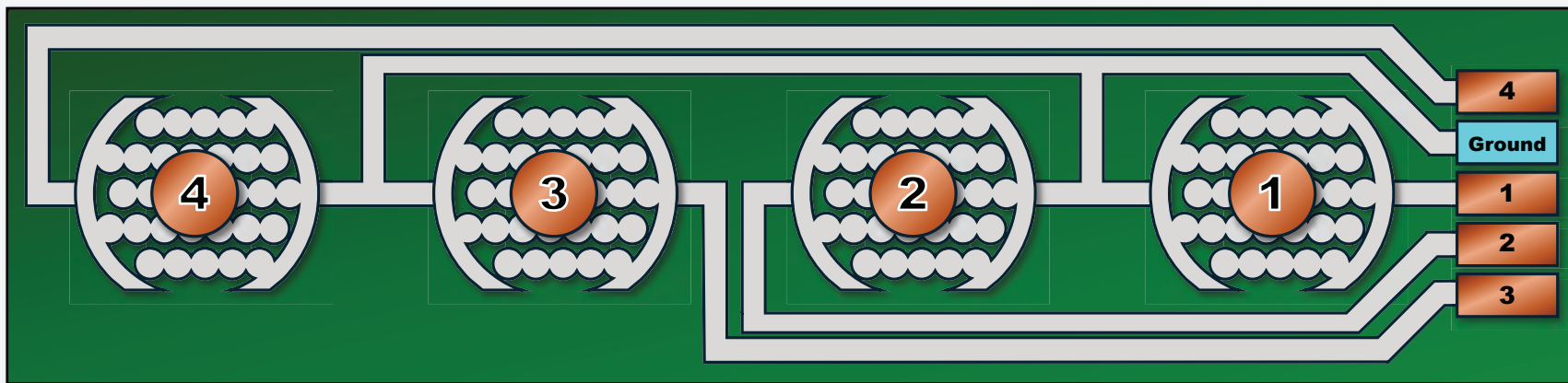
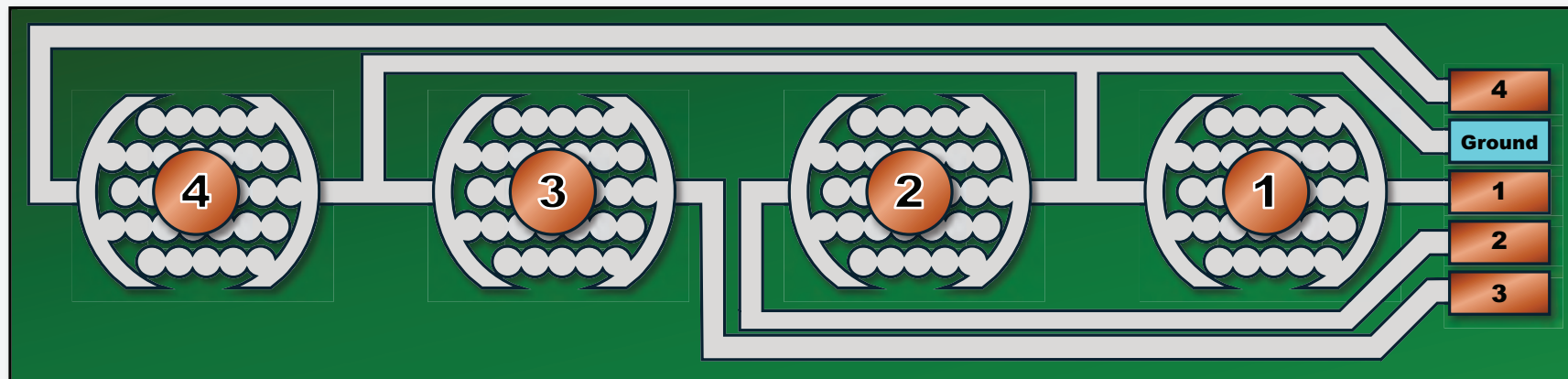


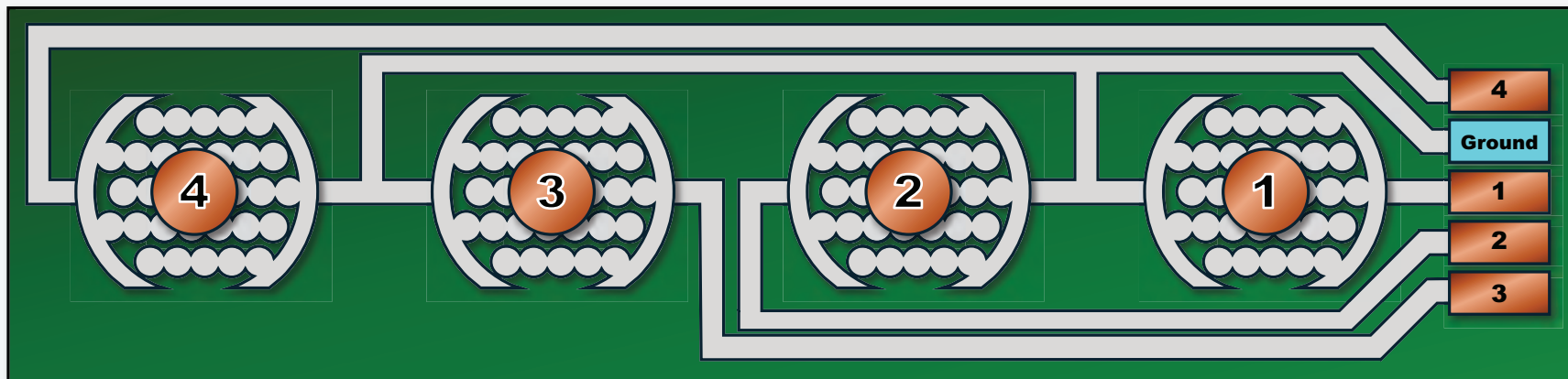
Figure 1A Illustration From The '752, '080, and '255 Patents



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

'752 Patent at Col 18:65-19:11

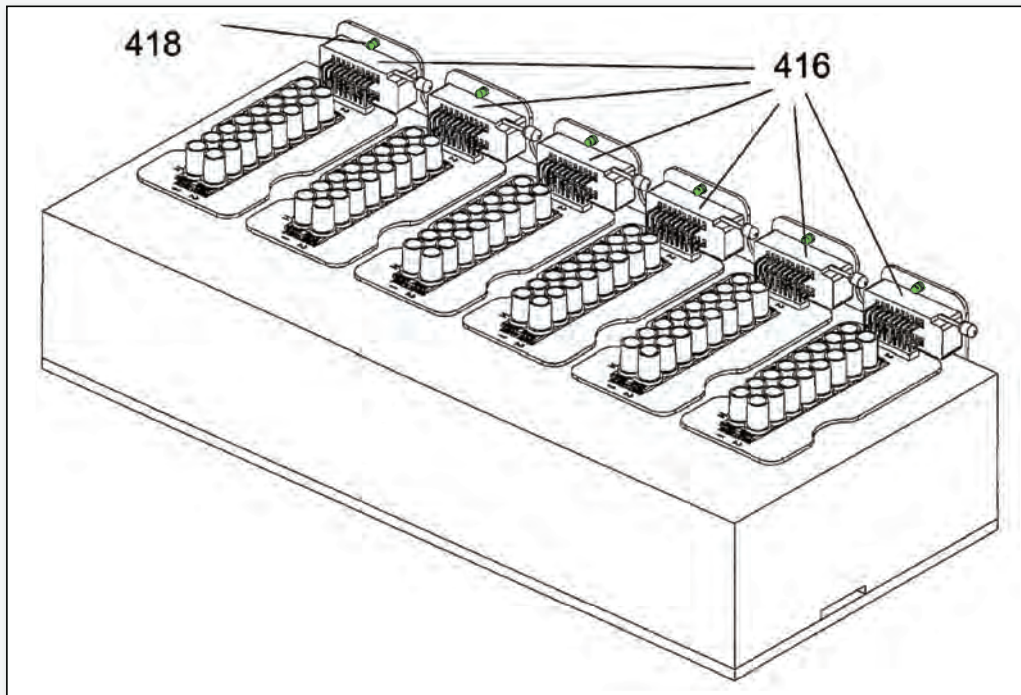
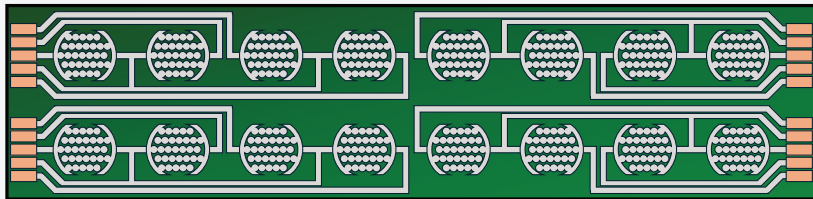
Figure 1A Illustration From The '752, '080, and '255 Patents



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

'752 Patent at Col 23:49-60

Figure 1A Illustration From The '752, '080, and '255 Patents



'752 Patent, Fig. 4

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

'752 Patent at Col 4:20-23

FIG. 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 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.

'752 Patent at Col 24:13-24

Figure 1A Illustration From The '752, '080, and '255 Patents

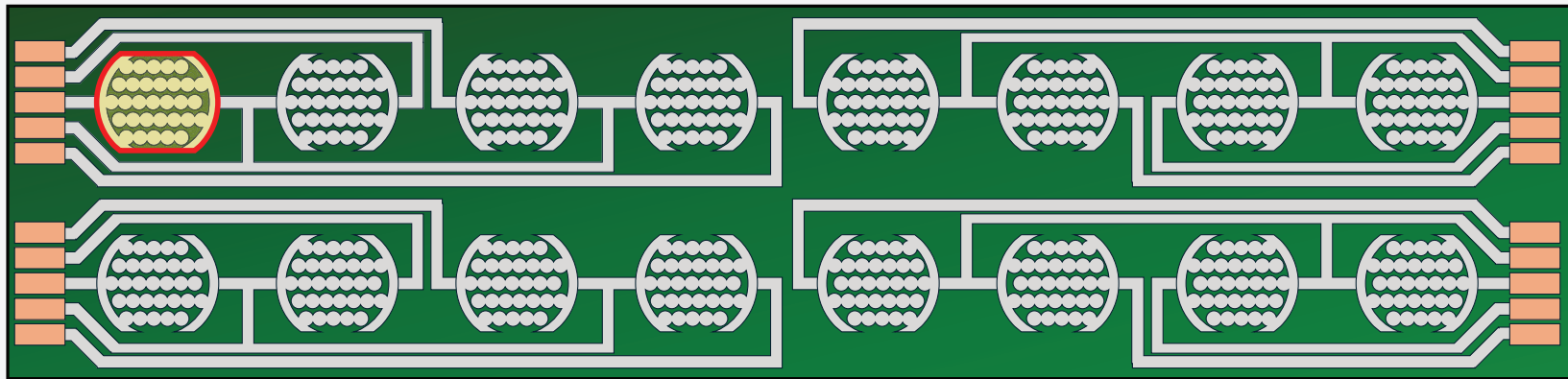
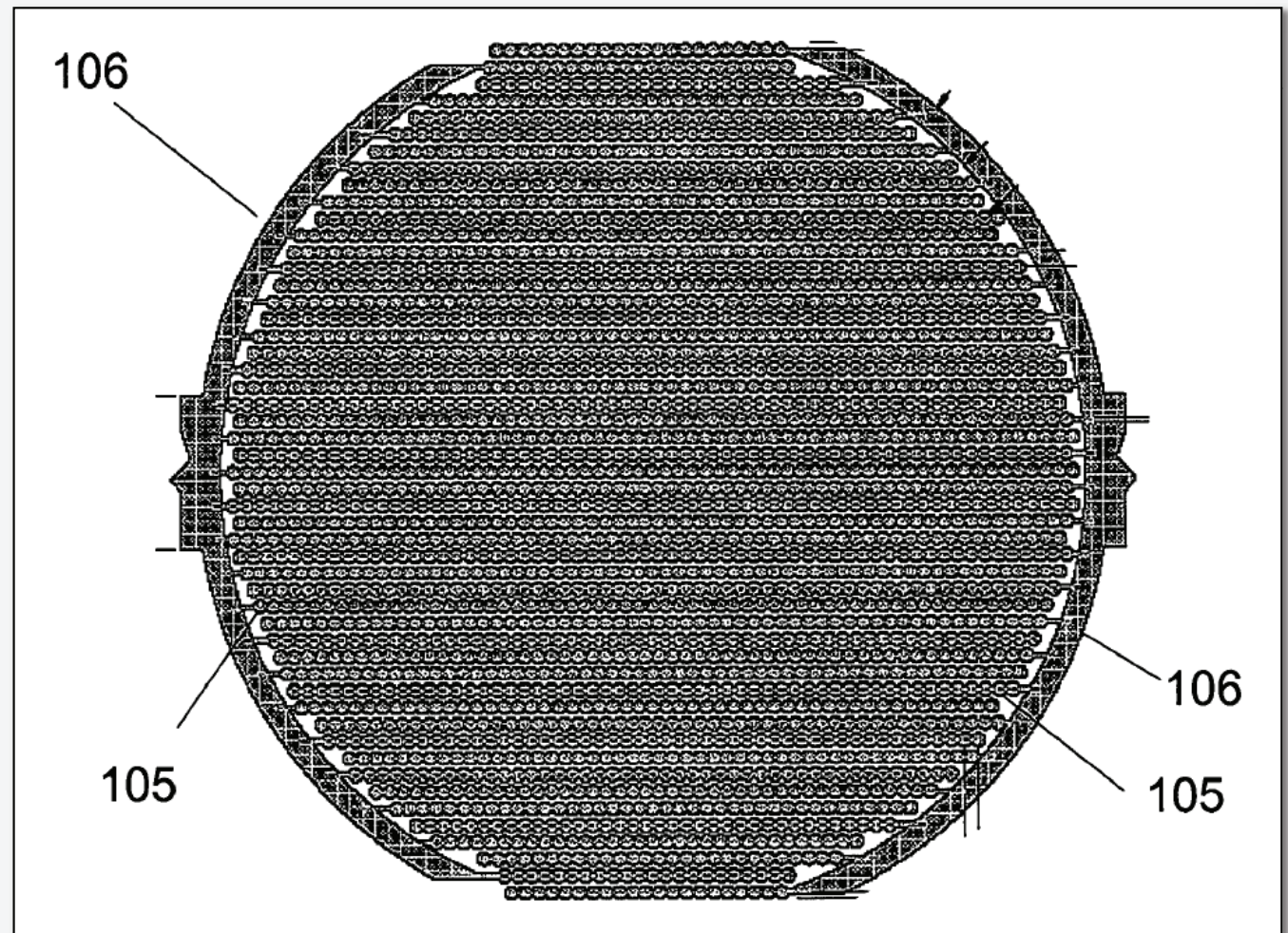
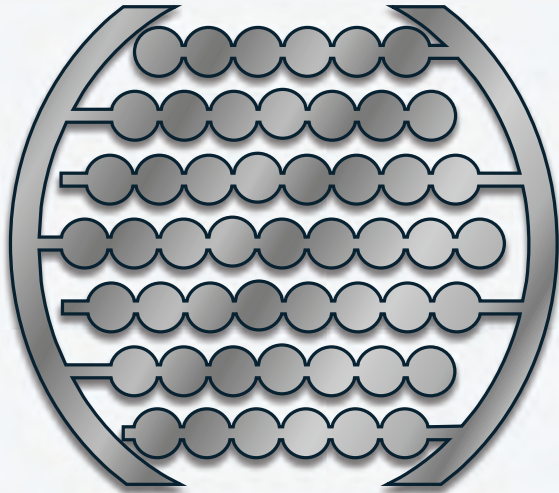


Figure 1B From The '752, '080, and '255 Patents



'752 Patent at Fig. 1B

Figure 1B Illustration From The '752, '080, and '255 Patents

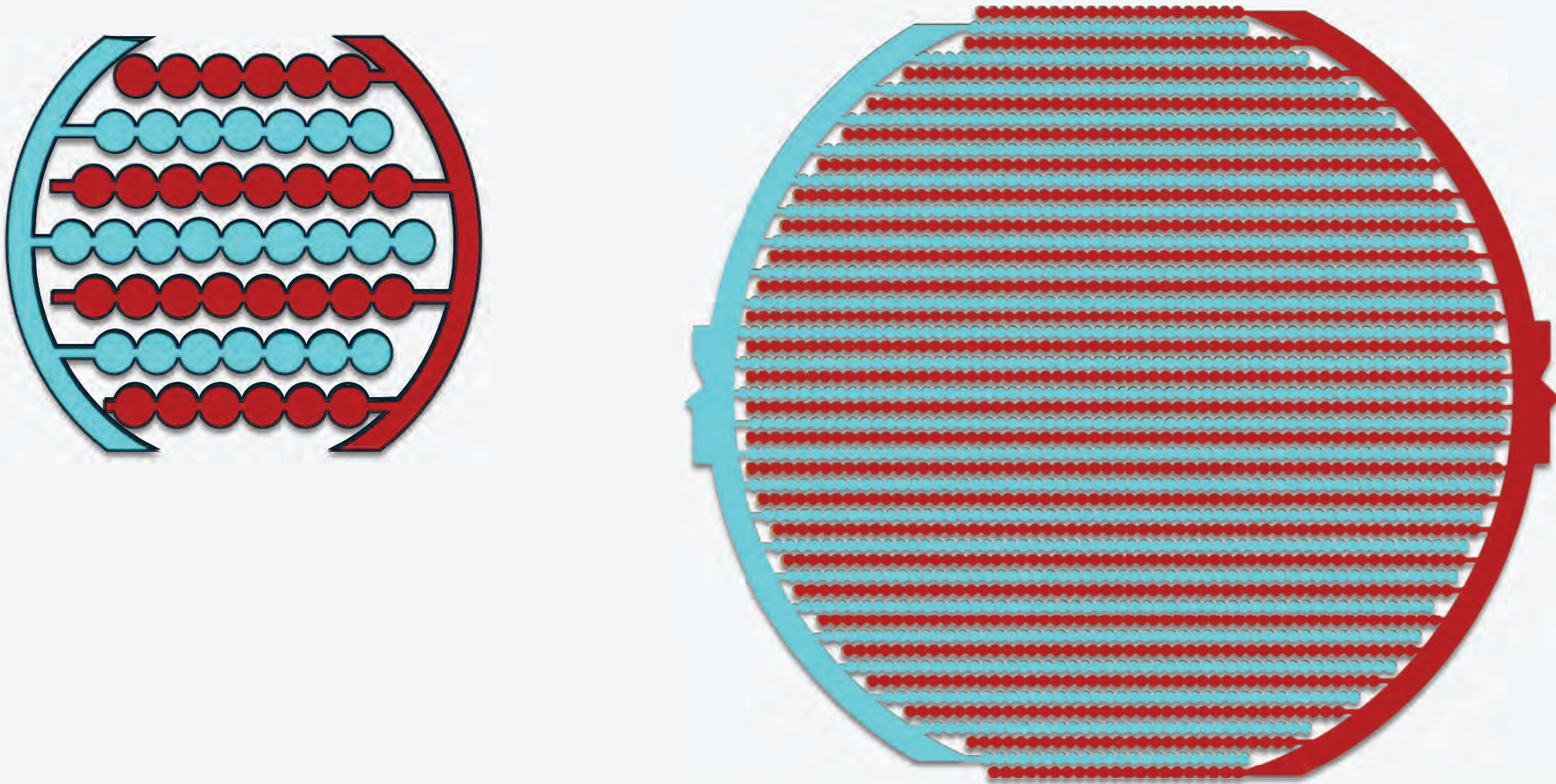


Figure 1B Illustration From The '752, '080, and '255 Patents

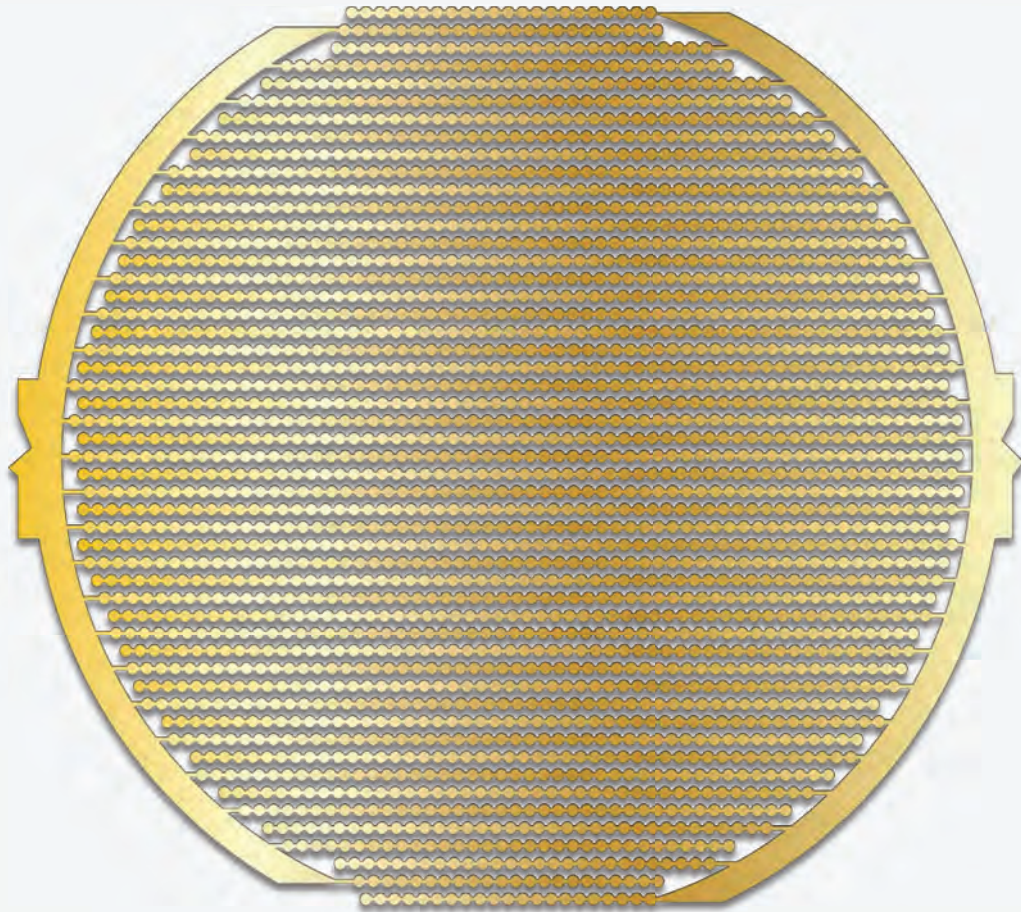
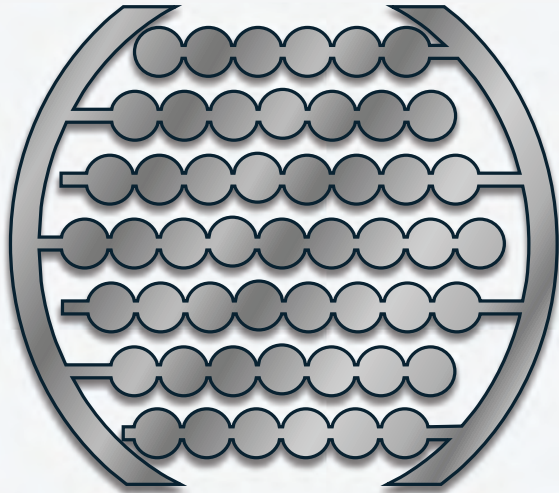
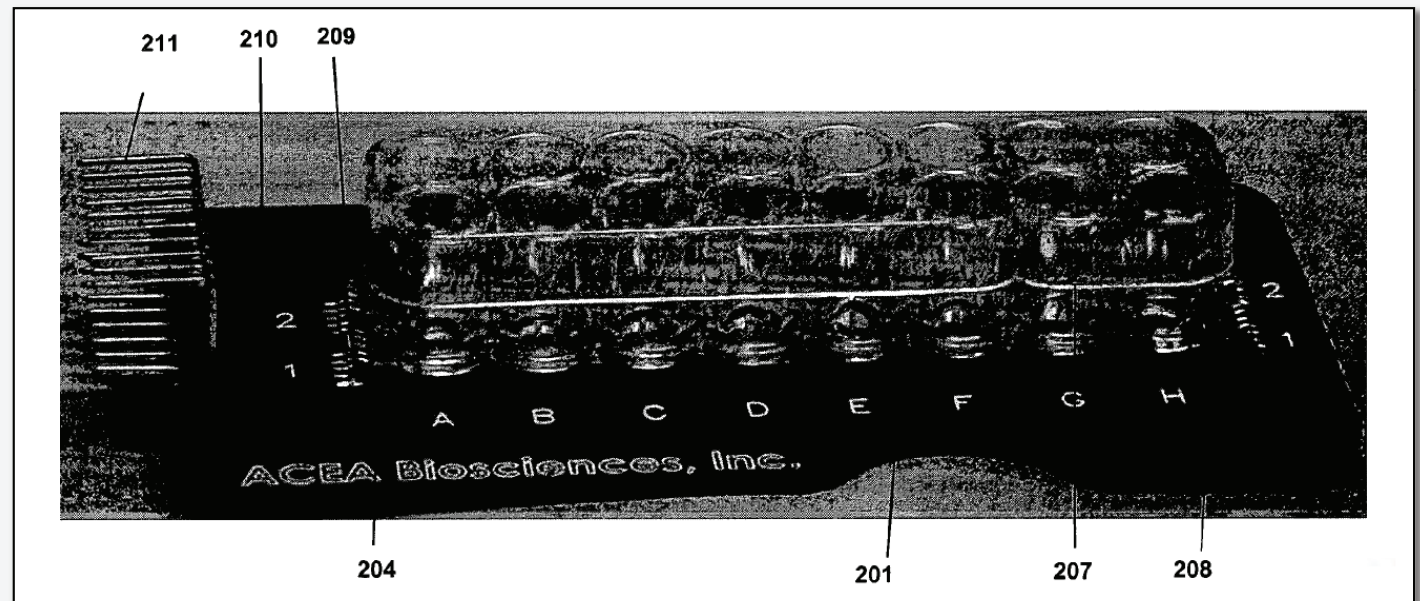
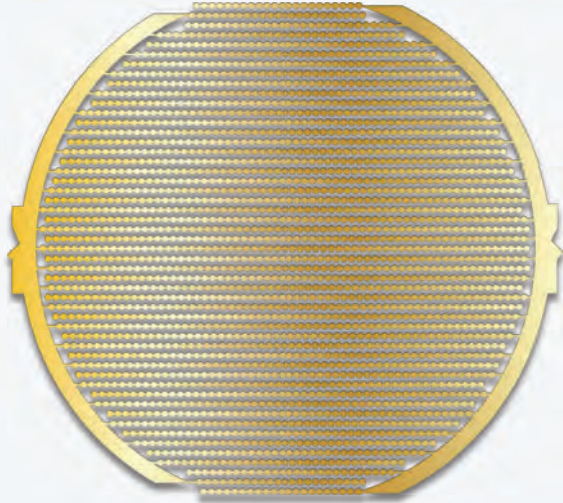
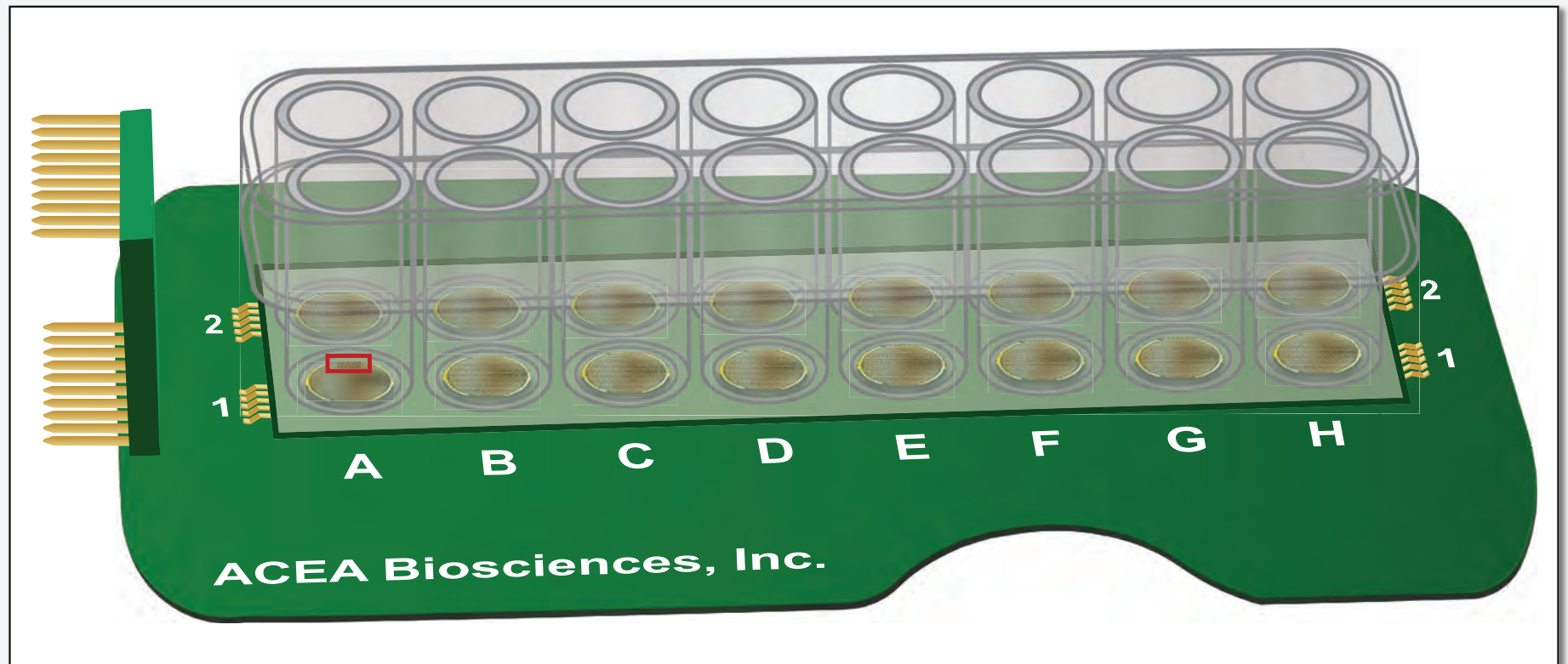


Figure 2 From The '752 and '080 Patents



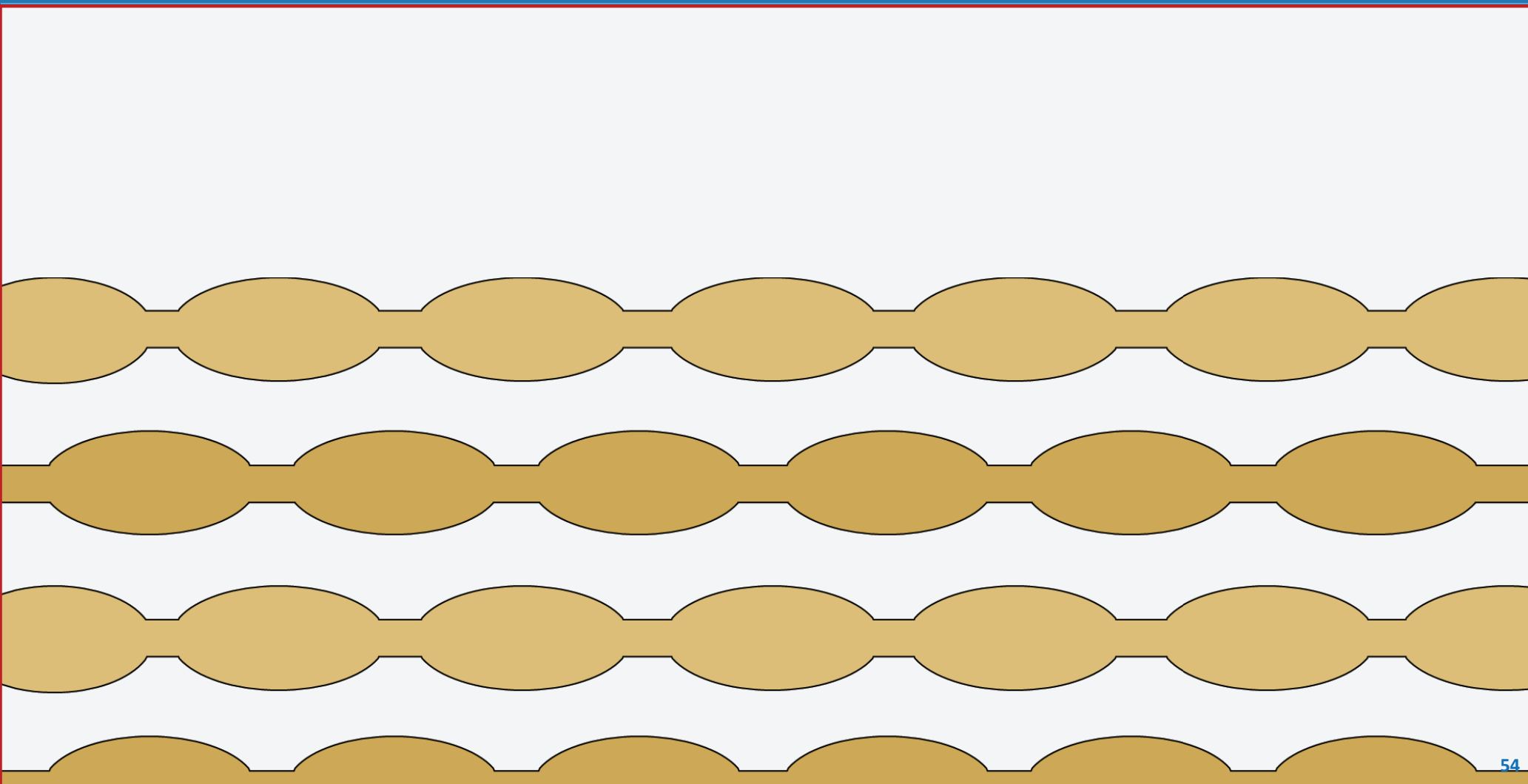
'752 Patent at Fig. 2

Figure 2 Illustration From The '752 and '080 Patents

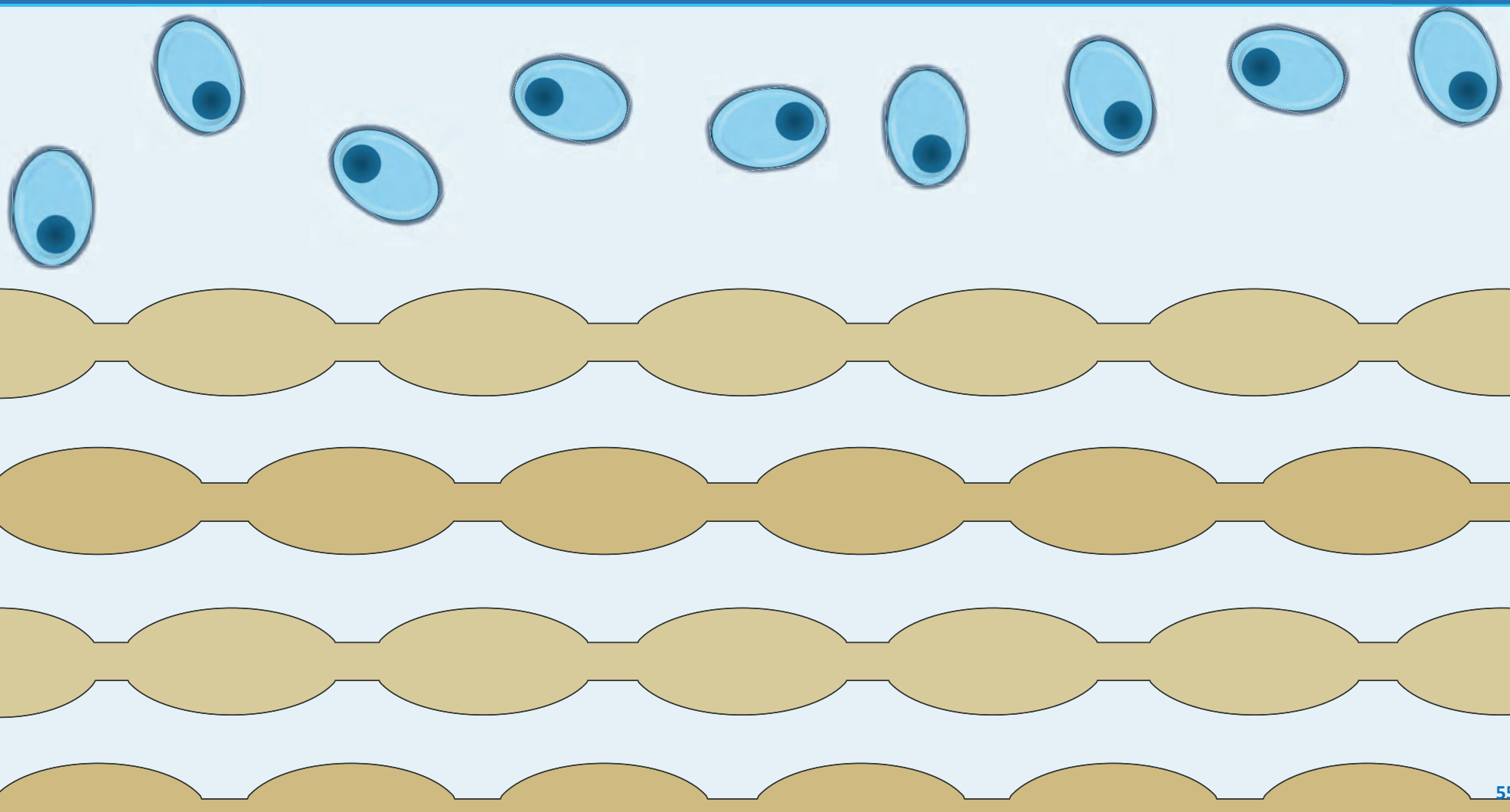


'752 Patent at Fig. 2

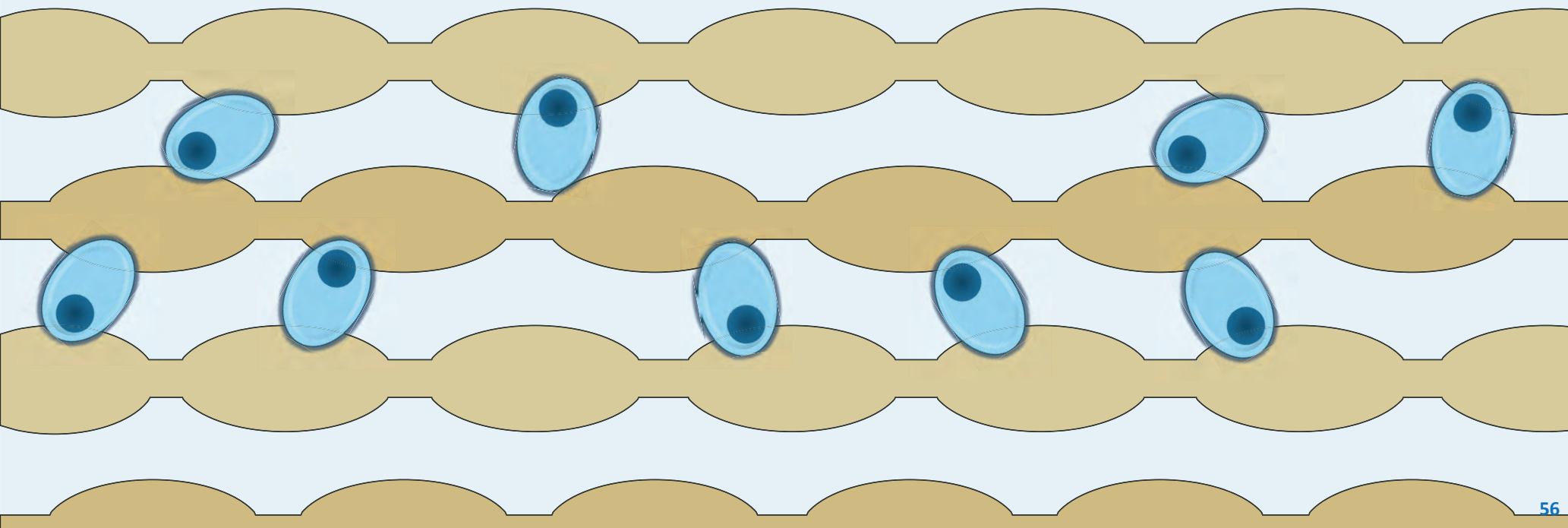
Close Up View Of Electrodes



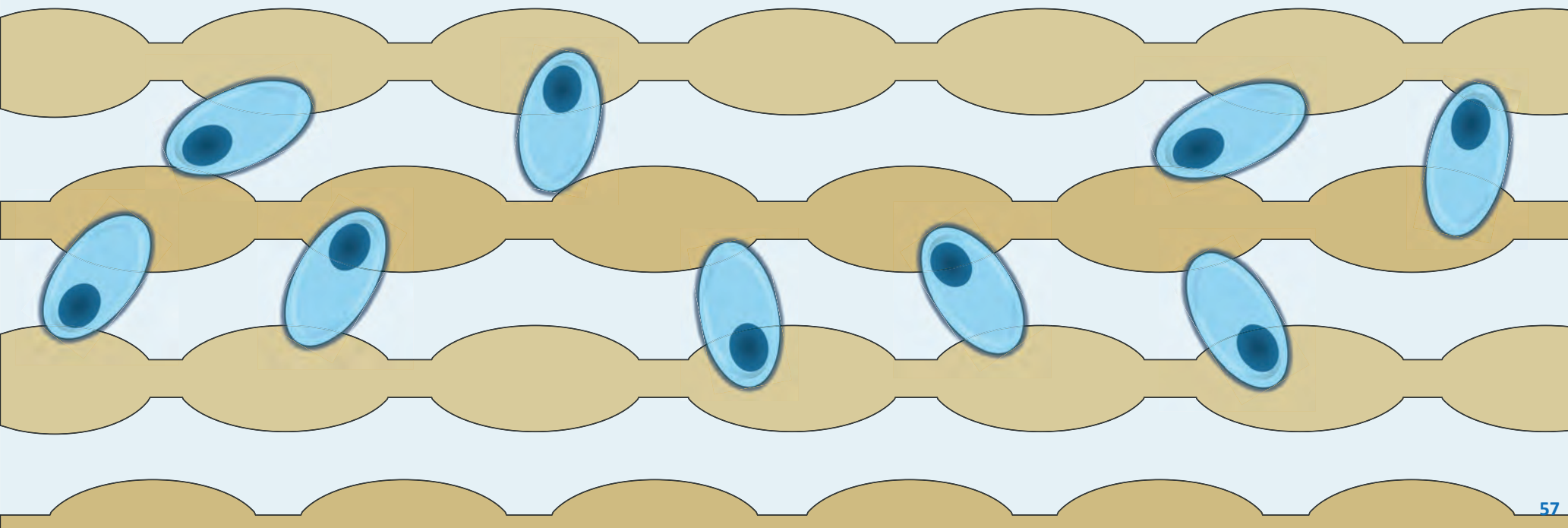
Close Up View Of Electrodes



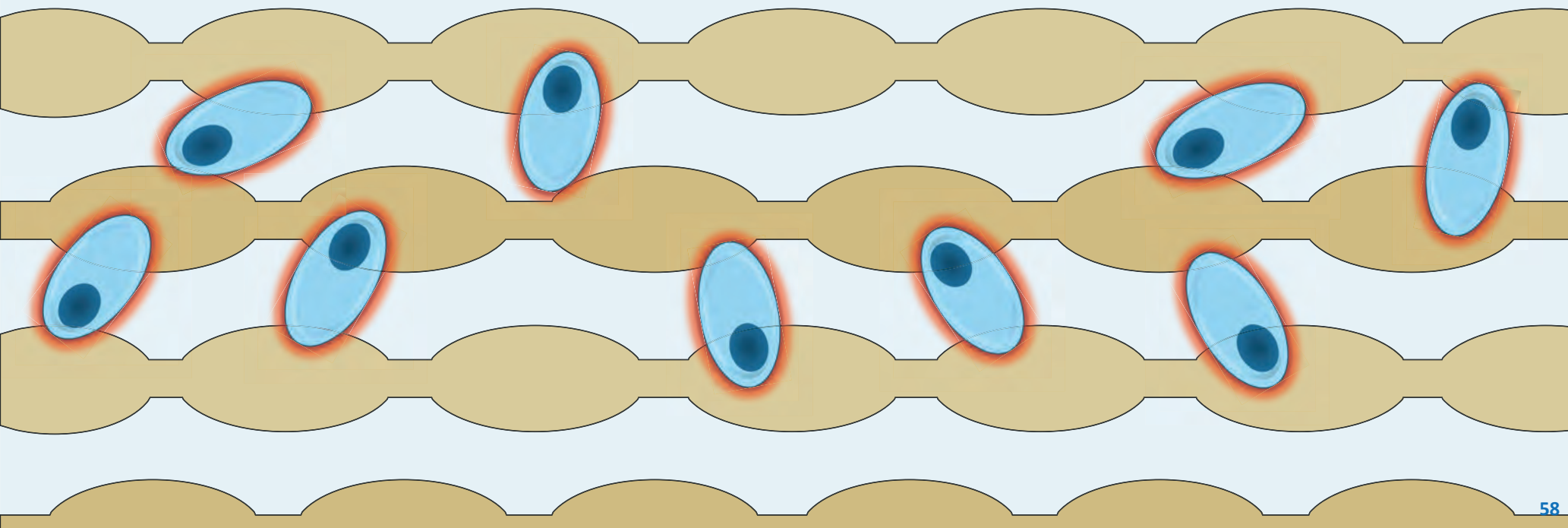
Close Up View Of Electrodes



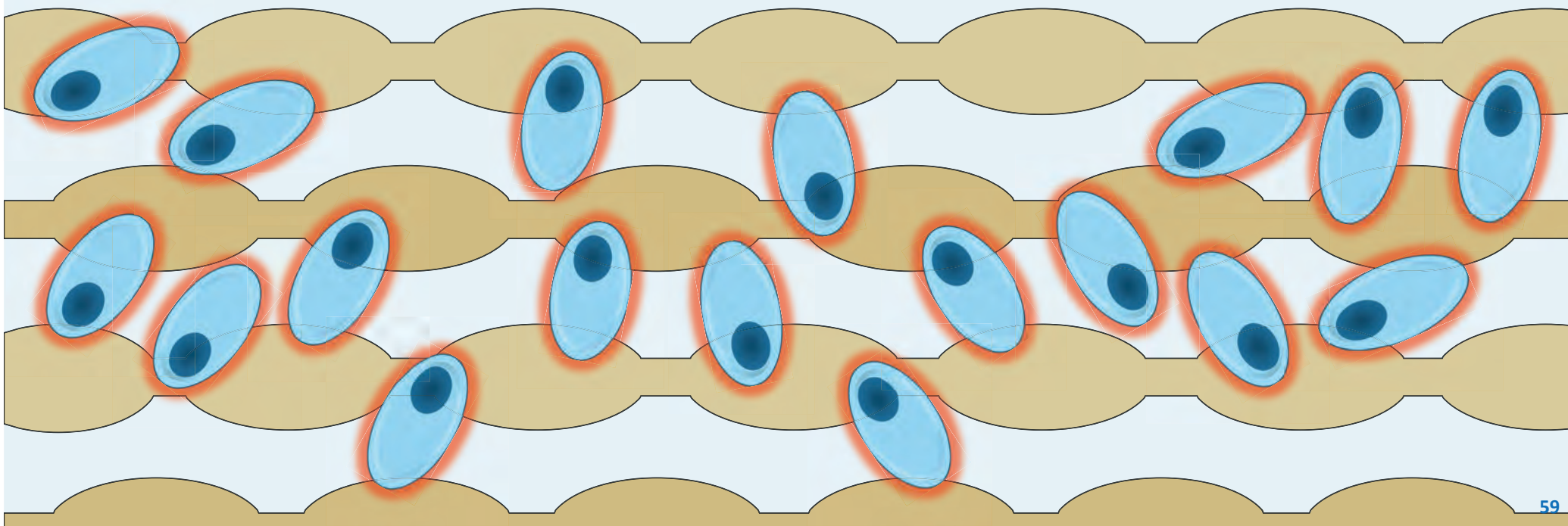
Close Up View Of Electrodes



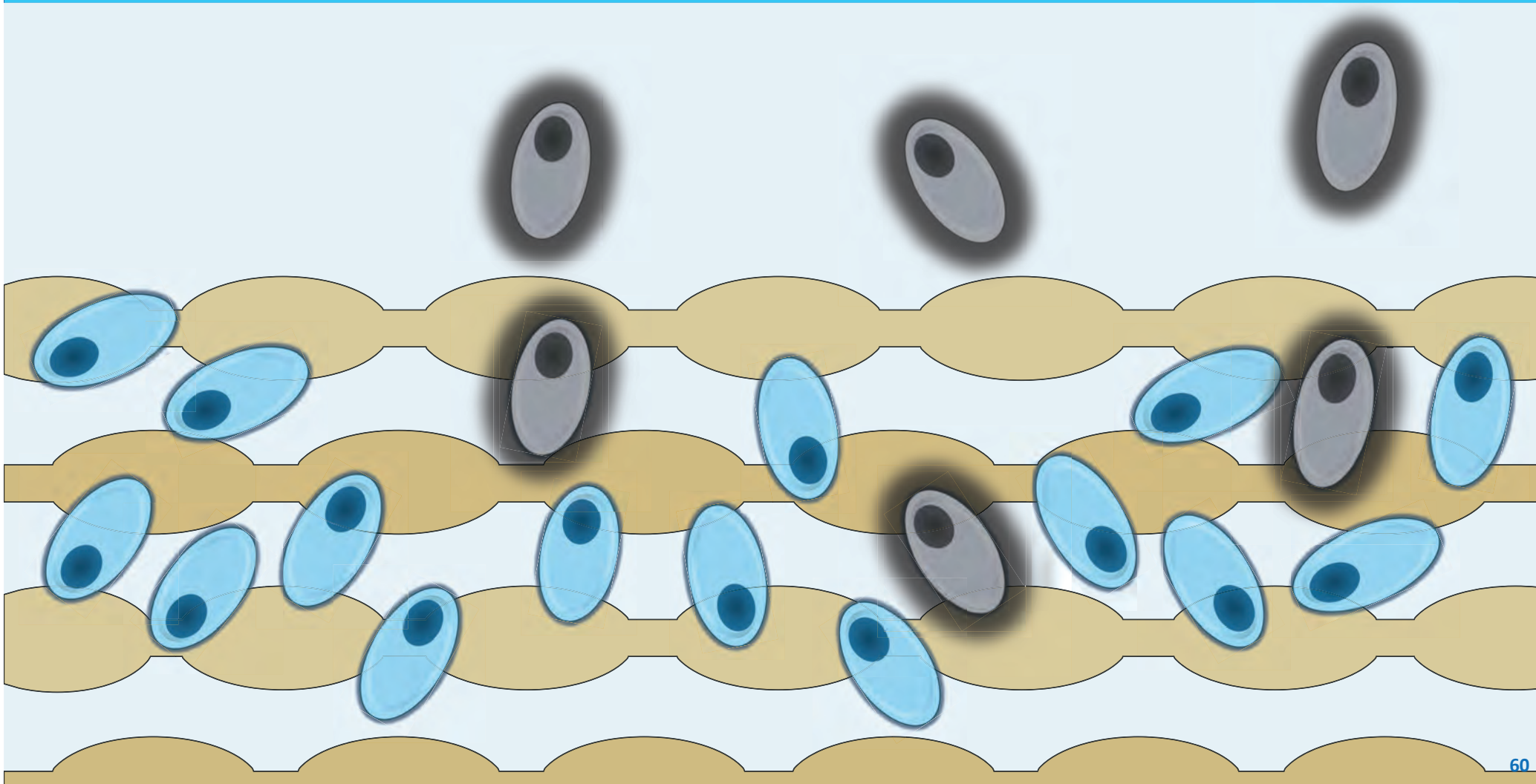
Close Up View Of Electrodes



Close Up View Of Electrodes



Close Up View Of Electrodes



Close Up View Of Electrodes

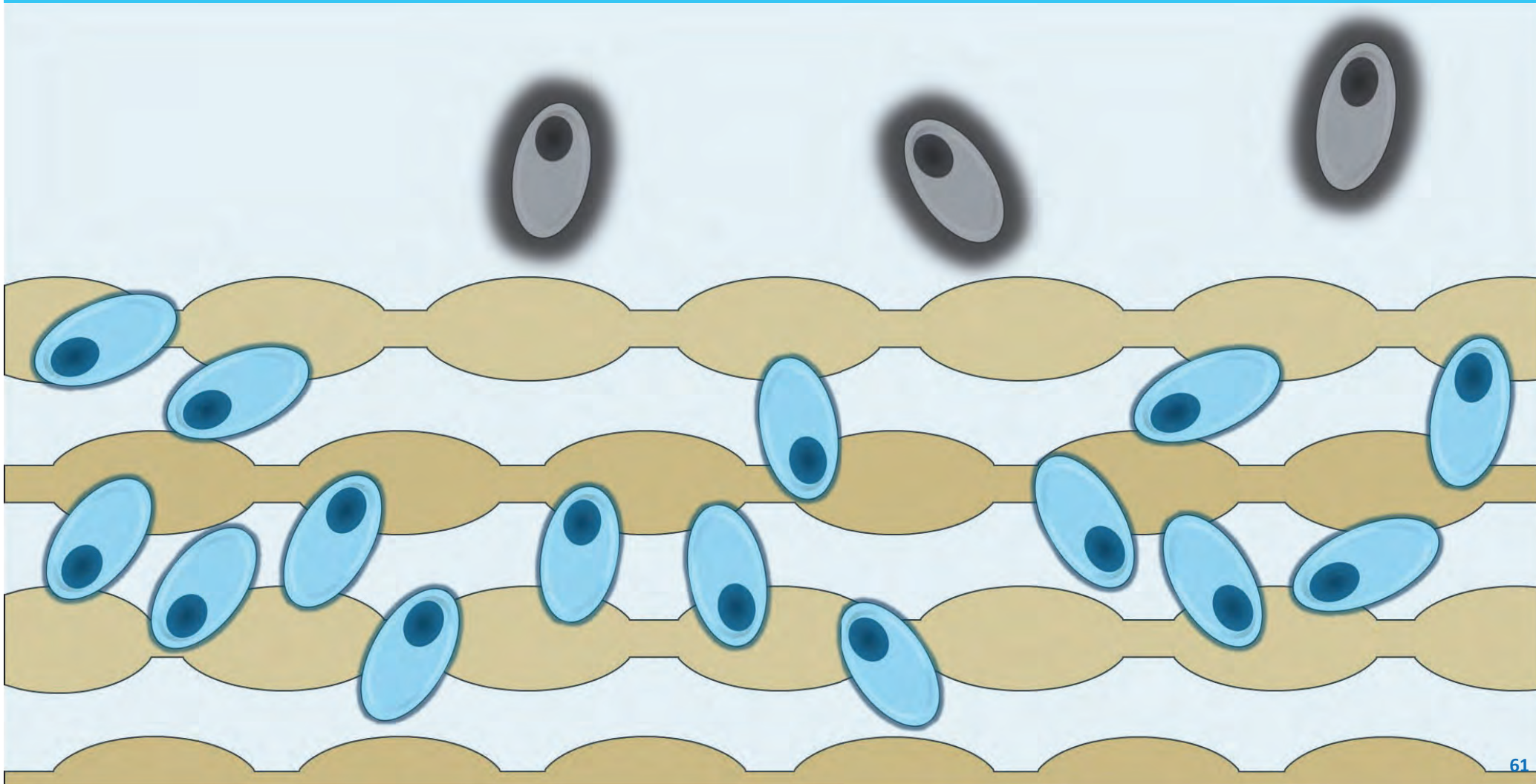


Figure 2 Illustration From The '752 and '080 Patents

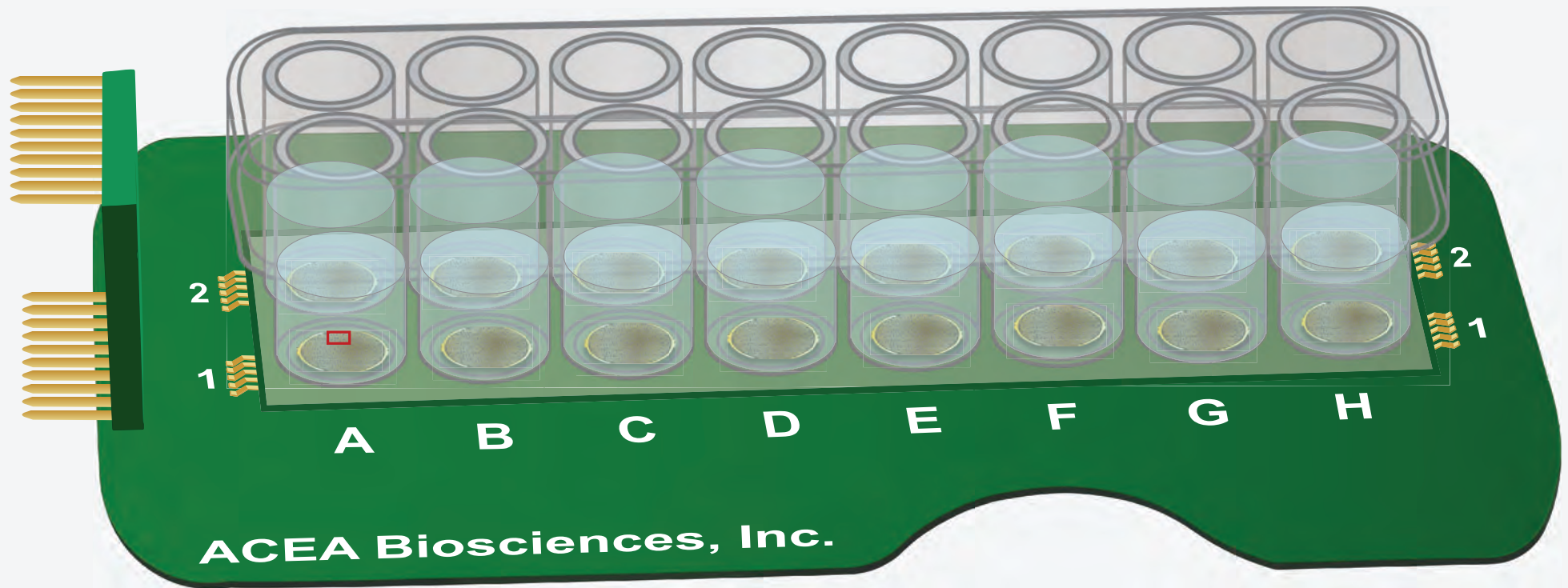


Figure 10 From The '752 and '080 Patents

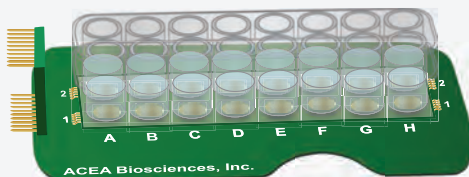
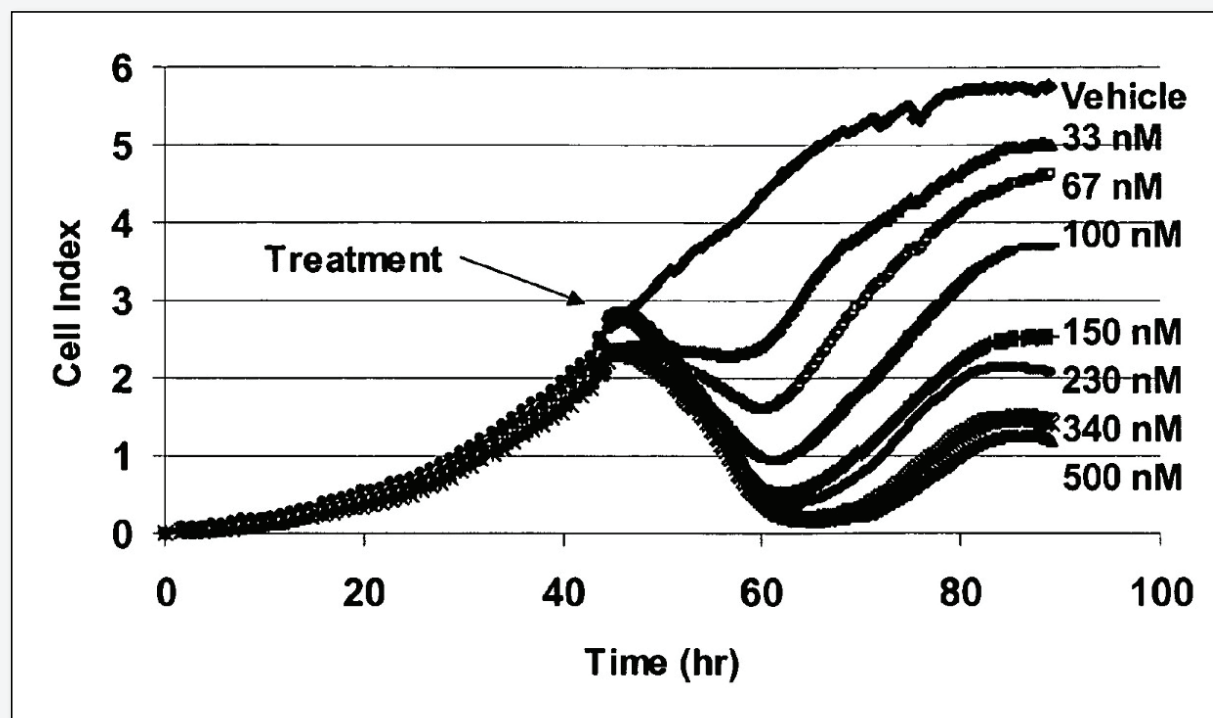


FIG. 10 shows curves that represent the time-dependent cell index for H460 cells treated with different concentrations of the anticancer drug paclitaxel. In this experiment, H460 cells were introduced into wells of a 16x cell-substrate impedance monitoring device. The device was positioned on a device station that was located in an incubator maintaining conditions of 37 degrees C. and 5% CO₂. 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.

'752 patent, Col 47:36-61



'752 patent at Fig.10

Figure 10 From The '752 and '080 Patents

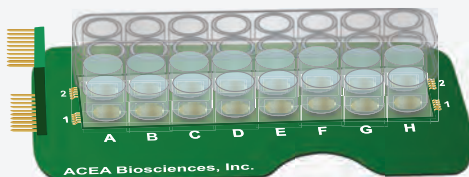
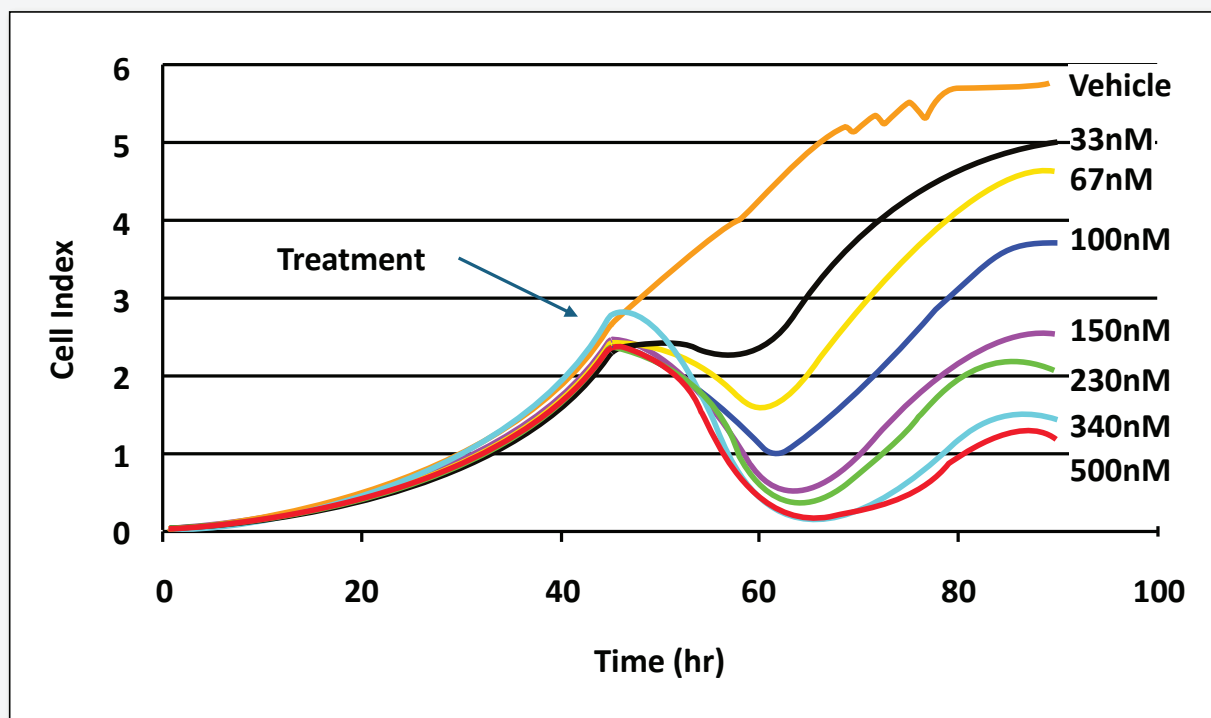


FIG. 10 shows curves that represent the time-dependent cell index for H460 cells treated with different concentrations of the anticancer drug paclitaxel. In this experiment, H460 cells were introduced into wells of a 16x cell-substrate impedance monitoring device. The device was positioned on a device station that was located in an incubator maintaining conditions of 37 degrees C. and 5% CO₂. 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.

'752 patent, Col 47:36-61



'752 patent at Fig.10

Figure 10 From The '752 and '080 Patents

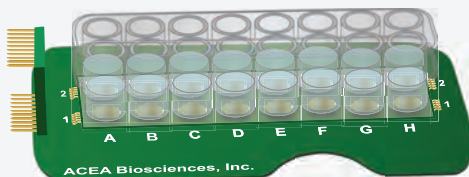
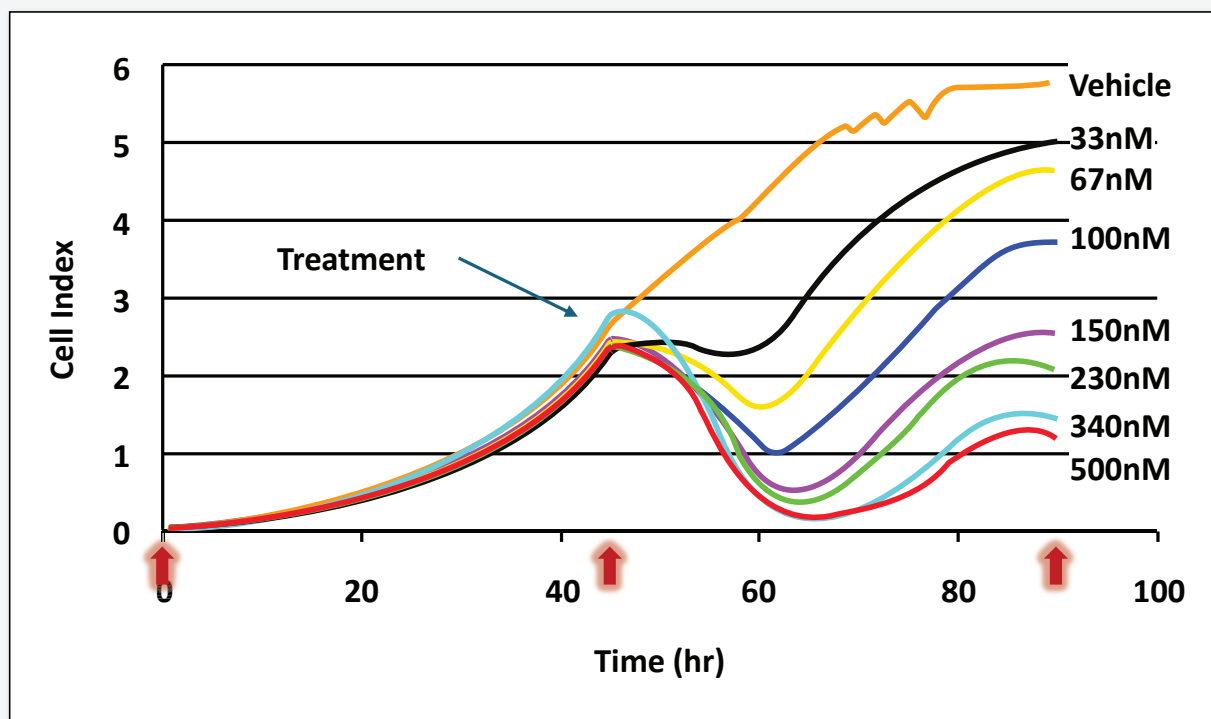


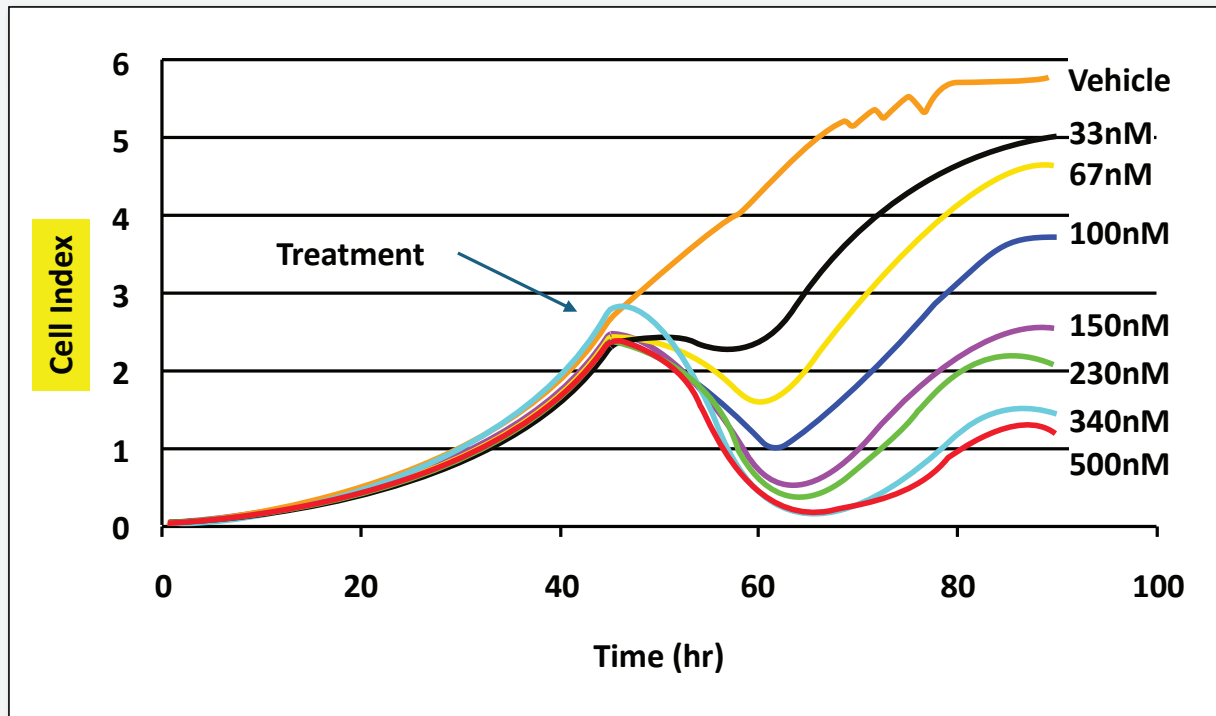
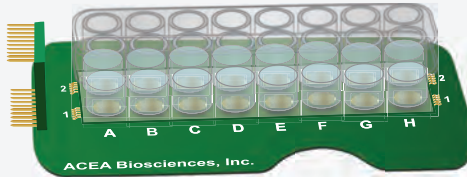
FIG. 10 shows curves that represent the time-dependent cell index for H460 cells treated with different concentrations of the anticancer drug paclitaxel. In this experiment, H460 cells were introduced into wells of a 16x cell-substrate impedance monitoring device. The device was positioned on a device station that was located in an incubator maintaining conditions of 37 degrees C. and 5% CO₂. 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.

'752 patent, Col 47:36-61



'752 patent at Fig.10

Figure 10 From The '752 and '080 Patents

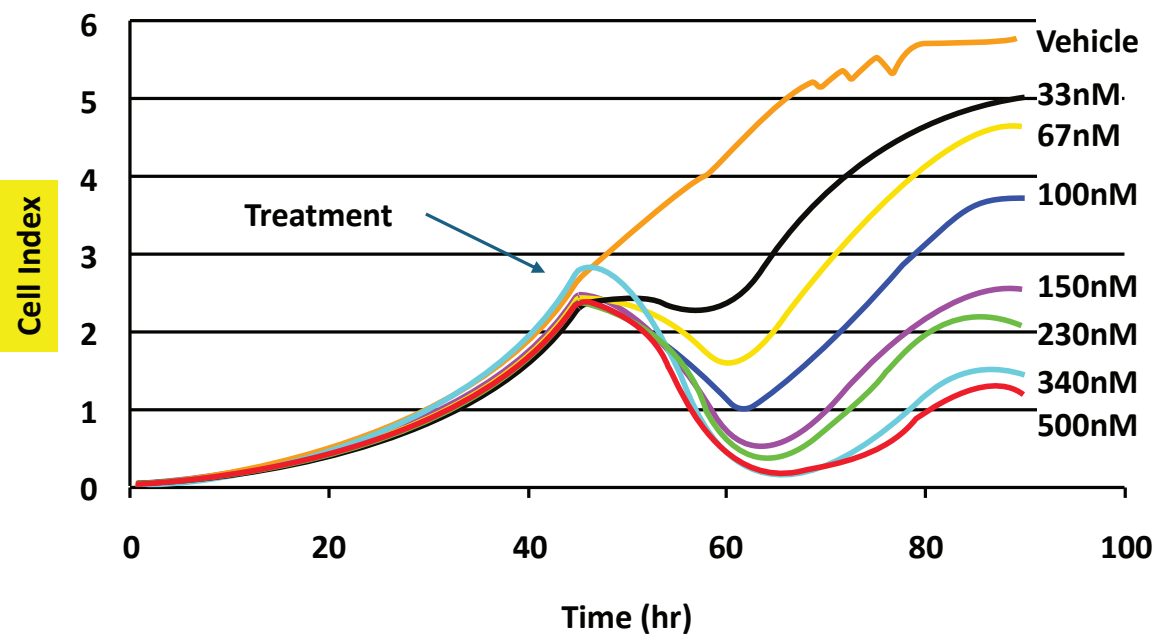
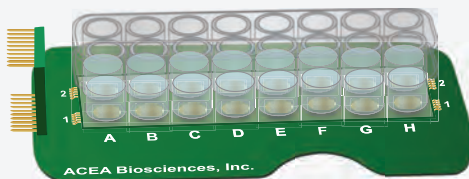


'752 patent at Fig.10

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 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,

'752 patent, Col 47:15-21

Figure 10 From The '752 and '080 Patents



'752 patent at Fig.10

C. Methods for Calculating Cell Index

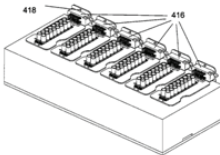
Based on the dependent relationship between the measured impedance, cell 30 number (more accurately, the viable cell number, or attached cell number) and cell attachment status, it is possible to derive a so-called "cell number index" or "cell index" from the measured impedance frequency spectra that provides a useful index for quantitating and comparing cell behavior in the impedance-based assays of the present invention. In some applications of the present

'752 patent, Col 24:56-64

Summary

'752 Patent

- (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**
- (56) **References Cited**
U.S. PATENT DOCUMENTS
2,656,508 A 10/1953 Coulter
3,259,842 A 7/1966 Coulter et al.
(Continued)
- (75) Inventors: **Xiao Xu, San Diego, CA (US); Yama Aboud, 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**
- (65) **Prior Publication Data**
US 2005/015425 A1 Jul. 14, 2005
- Related U.S. Application Data**
(63) Continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, and a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003.
(60) Provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 2, 2004, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/397,349, filed on Jul. 20, 2002.
- (51) **Int. Cl.**
C12N 1/00 (2006.01)
- (52) **U.S. Cl.**
435/288.4; 435/285.5; 204/403.03
- (58) **Field of Classification Search**
435/285.2; 435/287.2; 288.4; 305.2; 305.3; 204/403.01; 204/403.02
- See application file for complete search history.



27 Claims, 26 Drawing Sheets

'080 Patent

- (12) **United States Patent**
Wang et al.
- (10) Patent No.: **US 8,026,080 B2**
(45) Date of Patent: ***Sep. 27, 2011**
- (54) **REAL TIME ELECTRONIC CELL SENSING SYSTEM AND APPLICATIONS FOR CELL-BASED ASSAYS**
- (75) Inventors: **Xiaobo Wang, San Diego, CA (US); Yama A. Aboud, San Diego, CA (US); Xiaobo Wang, San Diego, CA (US); Jiangbo Gan, San Diego, CA (US)**
- (73) Assignee: **ACEA Biosciences, Inc., 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 1159 days.
- (21) Appl. No.: **11/725,049**
- (22) Filed: **Mar. 15, 2007**
- (65) **Prior Publication Data**
US 2007/0172939 A1 Jul. 26, 2007
- Related U.S. Application Data**
(60) Division of application No. 10/987,732, filed on Nov. 12, 2004, now Pat. No. 7,192,752, which is a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003, now Pat. No. 7,470,533, said application No. 10/987,732 is a continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, now Pat. No. 7,459,303.
(60) Provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/542,927, filed on Feb. 27, 2004, provisional application No. 60/548,713, filed on Feb. 27, 2004.
- (51) **Int. Cl.**
C12Q 1/00 (2006.01)
C12Q 1/08 (2006.01)
C12M 1/14 (2006.01)
- (52) **U.S. Cl.**
435/288.4; 435/287.1; 435/288.4
- (58) **Field of Classification Search**
435/288.4; 33, 40
- See application file for complete search history.
- (56) **References Cited**
U.S. PATENT DOCUMENTS
2,656,508 A 10/1953 Coulter
3,259,842 A 7/1966 Coulter et al.
3,541,581 A 7/1971 Cady et al.
3,980,201 A 6/1975 Cady
4,072,378 A 2/1978 Cady et al.
4,225,410 A 9/1980 Pate
4,686,190 A 8/1987 Cramer et al.
4,920,047 A 4/1990 Gauer et al.
5,091,048 A 3/1991 Taylor et al.
5,134,070 A 7/1992 Caring
5,187,099 A 2/1993 Gauer et al.
5,218,312 A 6/1993 Maw
5,247,827 A 9/1993 Shih et al.
5,278,048 A 1/1994 Pate et al.
5,345,555 A 5/1996 Springer et al.
5,563,097 A 4/1997 Rhee
5,628,734 A 5/1997 Dsouza et al.
5,643,542 A 7/1997 Maita et al.
5,766,534 A 6/1999 Gumpert-Elie
5,801,015 A 9/1998 Bushman
5,810,175 A 9/1998 Bushman
5,811,480 A 12/1998 Ward et al.
5,961,266 A 11/1999 Kovacs et al.
6,011,422 A 8/2000 Sweeney et al.
6,132,883 A 10/2000 Springer et al.
6,169,204 B1 8/2001 Frazer et al.
(Continued)
- FOREIGN PATENT DOCUMENTS**
EP 1 138 758 A1 4/2001
(Continued)
- OTHER PUBLICATIONS**
Wagner et al. *Electric Cell-Substrate Impedance Sensing (ECIS) as a Noninvasive Means to Monitor the Kinetics of Cell Spreading to Artificial Surfaces*. Experimental Cell Research 259: 158-166 (2000).
Aparicio et al. *A generally engineered cell-based biosensor for functional classification of agents*. Biosensors & Bioelectronics 16:571-577 (2001).
Barnett et al. *Microelectronic sensor system for microphysiological application on living cells*. Sensors & Actuators B55:77-89 (1999).
Bauer et al. *Separation of human breast cancer cells from blood by differential dielectric affinity*. Cell Biology 92:960-964 (1993).
Bever et al. *The role of extracellular matrix in human astrocytoma migration and proliferation studied in a microfluidic assay*. Clin Exp Metastasis 12:405-415.
Bergveld. *A critical evaluation of direct electrical protein detection methods*. Biosensors Bioelectronics 6:55-72 (1991).
(Continued)
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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 effects of compounds on cells, such as cytotoxicity assays. In other preferred aspects, the assays are designed to investigate the compounds that effect ligand-mediated responses of cells to antigens.

14 Claims, 26 Drawing Sheets

'255 Patent

- (12) **United States Patent**
Xu et al.
- (10) Patent No.: **US 7,468,255 B2**
(45) Date of Patent: **Dec. 23, 2008**
- (54) **METHOD FOR ASSAYING FOR NATURAL KILLER, CYTOTOXIC LYMPHOCYTE AND NEUTROPHIL-MEDIATED KILLING OF TARGET CELLS USING REAL-TIME MICROELECTRONIC CELL SENSING TECHNOLOGY**
- (75) Inventors: **Xiao Xu, San Diego, CA (US); Yama Aboud, San Diego, CA (US); Xiaobo Wang, 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 315 days.
- (21) Appl. No.: **11/197,994**
- (22) Filed: **Aug. 4, 2005**
- (65) **Prior Publication Data**
US 2006/0023559 A1 Feb. 2, 2006
- Related U.S. Application Data**
(63) Continuation-in-part of application No. 11/055,639, filed on Feb. 9, 2005, which is a continuation-in-part of application No. 10/987,732, filed on Nov. 12, 2004, now Pat. No. 7,192,752, and a continuation-in-part of application No. 10/705,447, filed on Nov. 10, 2003, said application No. 10/987,732 is a continuation-in-part of application No. 10/705,615, filed on Nov. 10, 2003, application No. 11/197,994, which is a continuation-in-part of application No. PCT/US2004/004481, filed on Feb. 9, 2005, and a continuation-in-part of application No. PCT/US2004/037696, filed on Nov. 12, 2004.
(60) Provisional application No. 60/519,567, filed on Nov. 12, 2003, provisional application No. 60/435,400, filed on Dec. 20, 2002, provisional application No. 60/469,572, filed on May 9, 2003, provisional application No. 60/542,927, filed on Feb. 9, 2004, provisional application No. 60/548,713, filed on Feb. 27, 2004, provisional application No. 60/514,601, filed on Sep. 25, 2004, provisional application No. 60/598,608, filed on Aug. 4, 2004, provisional application No. 60/306,071, filed on Nov. 22, 2004, provisional application No. 60/489,422, filed on Jun. 10, 2005, provisional application No. 60/598,608, filed on Aug. 4, 2004, provisional application No. 60/647,075, filed on Jan. 26, 2005, provisional application No. 60/660,826, filed on Mar. 10, 2005, provisional application No. 60/660,808, filed on Mar. 10, 2005.
- (51) **Int. Cl.**
G01N 33/574 (2006.01)
- (52) **U.S. Cl.**
435/287.23; 435/27.24; 435/29
- Field of Classification Search**
435/287.23; 435/27.24; 435/29
- References Cited**
U.S. PATENT DOCUMENTS
2,656,508 A 10/1953 Coulter
3,259,842 A 7/1966 Coulter et al.
3,541,581 A 7/1971 Cady et al.
3,980,201 A 6/1975 Cady
4,072,378 A 2/1978 Cady et al.
4,225,410 A 9/1980 Pate
4,686,190 A 8/1987 Cramer et al.
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5,091,048 A 3/1991 Taylor et al.
5,134,070 A 7/1992 Caring
(Continued)
- FOREIGN PATENT DOCUMENTS**
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(Continued)
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(Continued)
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- ABSTRACT**
The present invention includes a method of measuring cytotoxic activity including providing a device capable of measuring cell-substrate impedance operably connected to an impedance analyzer, adding target cells to at least one well of the device, adding effector cells to the at least one well, measuring impedance of the at least one well and optionally determining a cell index from the impedance, wherein monitoring impedance includes measuring impedance during at least one time point before and at least one time point after adding effector cells, and determining viability of said target cells after adding effector cells by comparing the impedance or optionally the cell index at the at least one time point after adding effector cells to the impedance or optionally the cell index at the at least one time point before adding effector cells.

39 Claims, 48 Drawing Sheets

Thank you

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AGILENT TECHNOLOGIES, INC.,	)	
	)	
Plaintiff,	)	
	)	
v.	)	C.A. No. 23-198 (CJB)
	)	
AXION BIOSYSTEMS, INC.,	)	
	)	
Defendant.	)	

NOTICE OF LODGING

PLEASE TAKE NOTICE that the undersigned counsel for Plaintiff Agilent Technologies, Inc. will cause to be lodged, via hand delivery to the Court, thumb drives containing Plaintiff Agilent Technologies, Inc.'s Technology Tutorial.

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April 5, 2024

CERTIFICATE OF SERVICE

I hereby certify that on April 5, 2024, I caused the foregoing to be electronically filed with the Clerk of the Court using CM/ECF, which will send notification of such filing to all registered participants.

I further certify that I caused copies of the foregoing document to be served on April 5, 2024, upon the following in the manner indicated:

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