Webinar Series

Brought to you by ACEA Biosciences

xCELLigence® Technical Webinar

Evaluating Drug Mediated Cytotoxicity in Real Time – Protocol, Tips, Tricks and Data Analysis

Date: Thursday June 15, 2017

Time: 9AM PDT- 12PM EDT - 5PM London Time

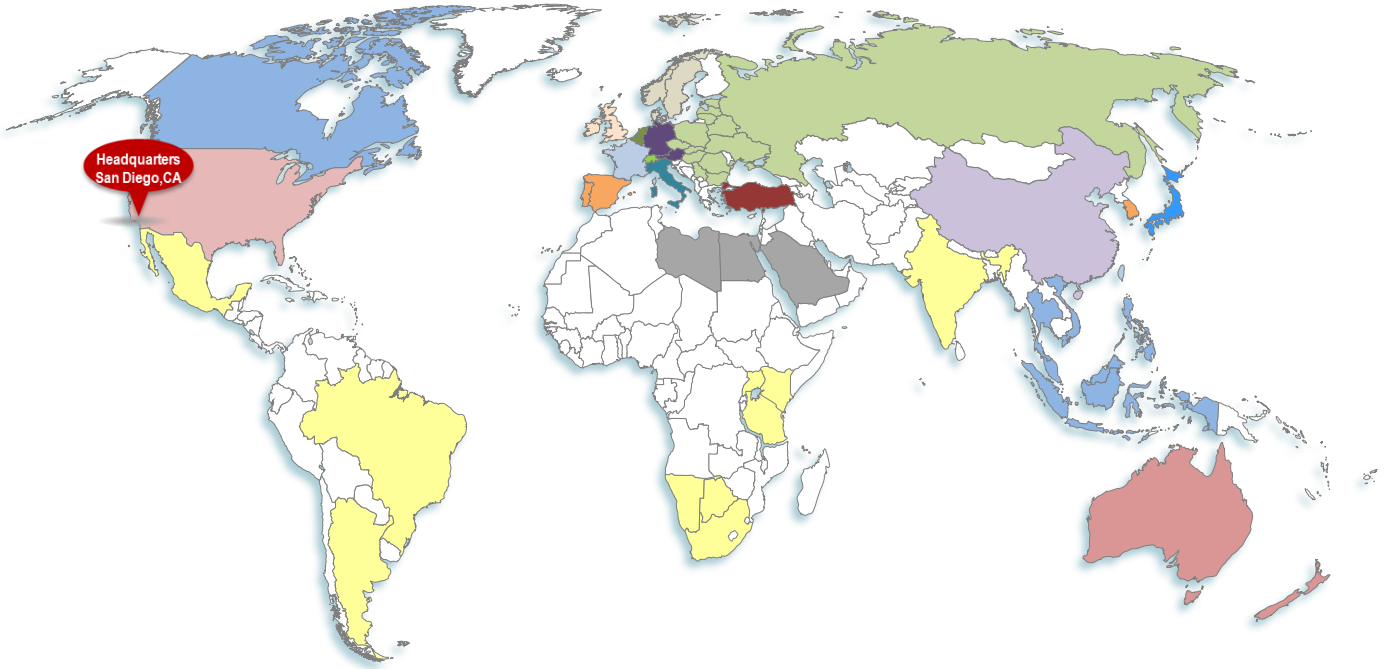
Duration: 45 min

Speaker: Leyna Zhao, Ph.D. ACEA Biosciences

Outline

1. **Introduction to ACEA Biosciences and xCELLigence technology**
2. The utility of the xCELLigence technology for quantitatively monitoring drug mediated cytotoxicity in real-time
3. The protocols, tips, and tricks for conducting drug mediated cytotoxicity assays
4. Real-time demonstration of data analysis and plotting for publications
5. Trouble shoot and Maintenance

ACEA Biosciences, Inc.



- Founded 2002
- Headquarters: San Diego, CA. USA
- Personnel 300+ FTEs, 40+ PhDs
- The technology inventor of the xCELLigence® Real-Time Cell Analysis Systems
- 29 Distributors Worldwide, Direct Sales/Support in the US, Canada & UK

https://aceabio.com/support/distributors/

ACEA Biosciences, Inc.

ABOUT PRODUCTS APPLICATIONS PUBLICATIONS RESOURCES NEWS & EVENTS SUPPORT

Support

- Contact Us
- Distributors
- xCELLigence Support
- NovoCyte Support
- Warranty Policy

Distributors

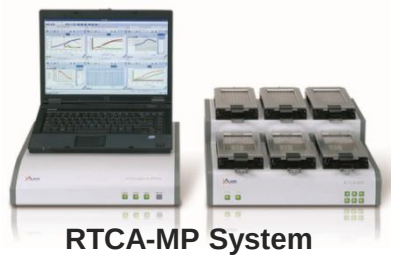
Regional Contacts

Region	Location	United States of America
North America	United States	ACEA Biosciences
Europe	Canada	6779 Mesa Ridge Road #100
Asia Pacific		San Diego, CA 92121 USA
Latin America		Tel: +1 858-724-0928
Middle East and Africa		Toll-Free: +1 866-308-2232
		Fax: +1 858-724-0927
		info@aceabio.com

Partnership with Roche (2007-2012)

xCELLigence® Real-Time Cell Analyzer

Roche



RTCA-MP System

RTCA-HT System

RTCA-SP System

RTCA-DP System

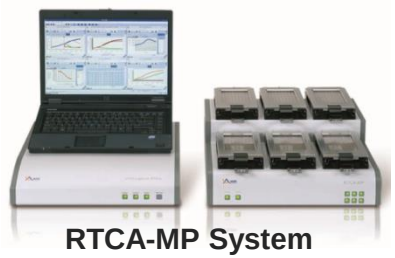
RTCA-Cardio System

June 2008 | Sep 2008 | March 2009 | Nov 2010 | Nov 2010 | 2012

Innovation and Excellence

xCELLigence® Real-Time Cell Analyzer

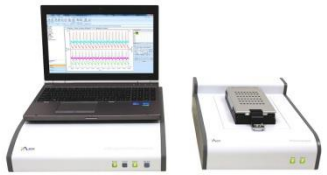
Roche



RTCA-MP System

RTCA-HT System

RTCA-iCELLigence System



RTCA-CardioECR System

RTCA-SP System

RTCA-DP System

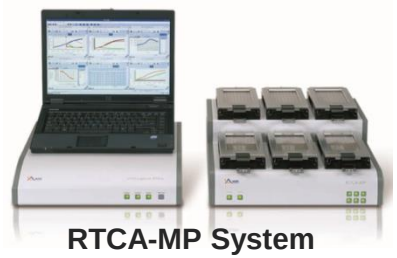
RTCA-Cardio System

June 2008 | Sep 2008 | March 2009 | Nov 2010 | Nov 2010 | July 2012 | October 2014

Innovation and Excellence

xCELLigence® Real-Time Cell Analyzer

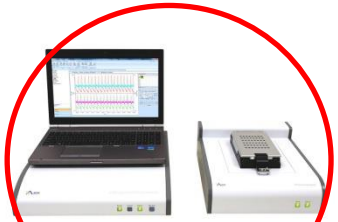
NovoCyte®
Flow Cytometer



RTCA-MP System

RTCA-HT System

RTCA-iCELLigence System



RTCA-CardioECR System

RTCA-SP System

RTCA-DP System

RTCA-Cardio System

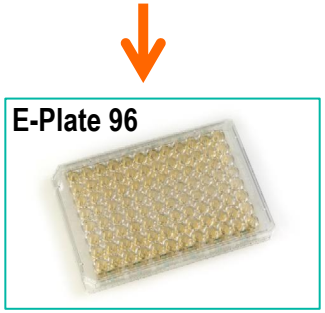
NovoCyte

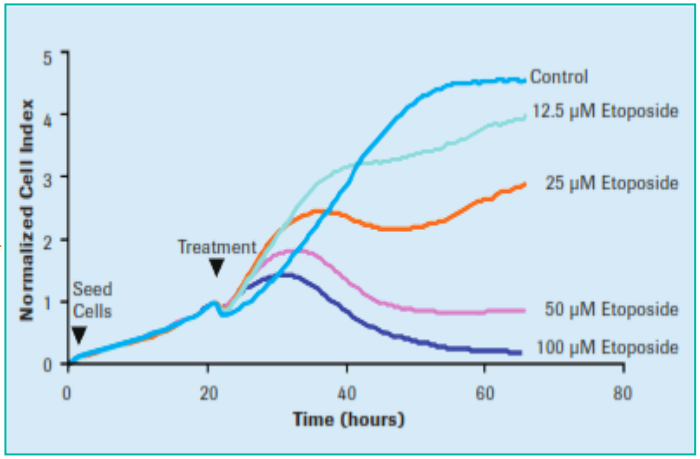
June 2008 | Sep 2008 | March 2009 | Nov 2010 | Nov 2010 | July 2012 | April 2014 | October 2014

Simple workflow

No cell labeling required, fully automated, physiological conditions

E-Plate 96



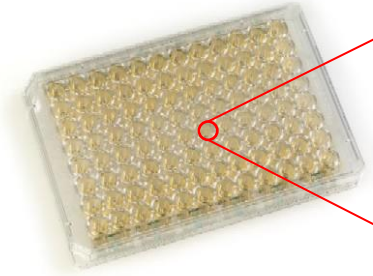


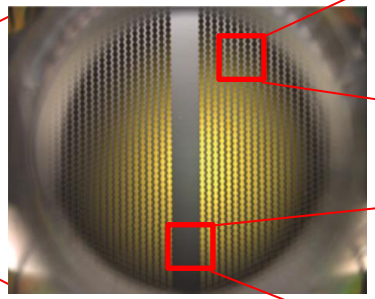
Seed Cells

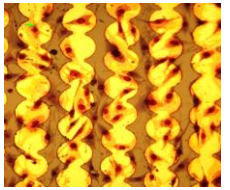
Real-time monitoring at physiological conditions

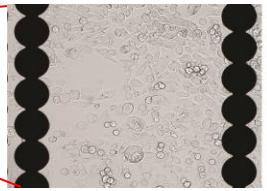
Kinetic cell response curve

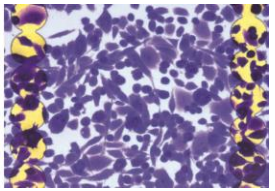
Principle of Operation: Impedance Biosensor









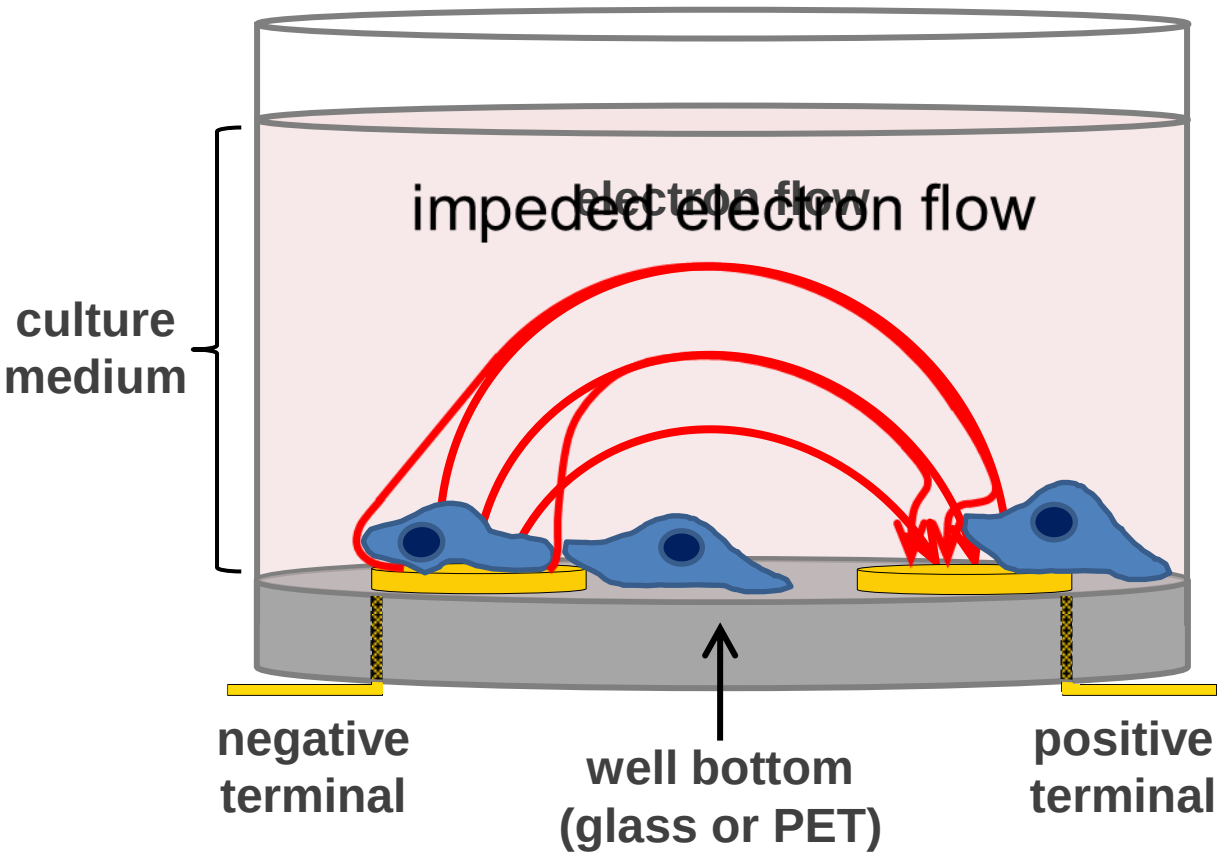


negative terminal

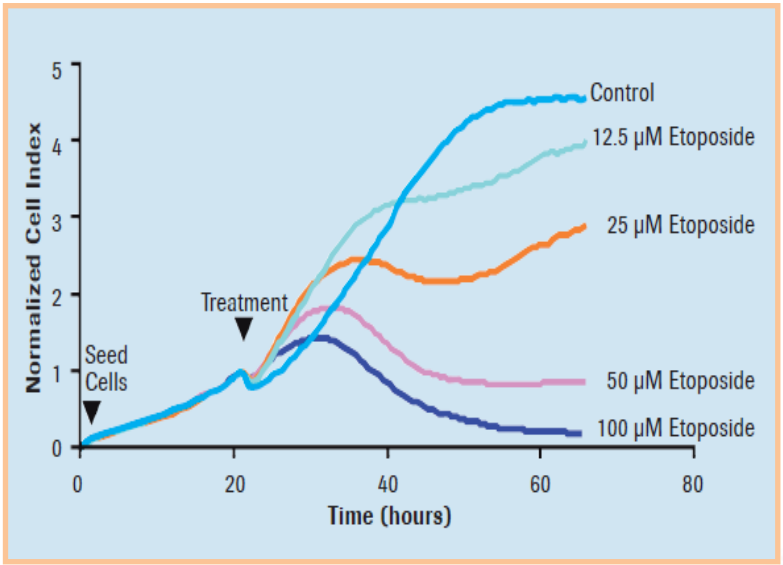
Positive terminal

Principle of Operation: Impedance Biosensor

Single Well
(side view)

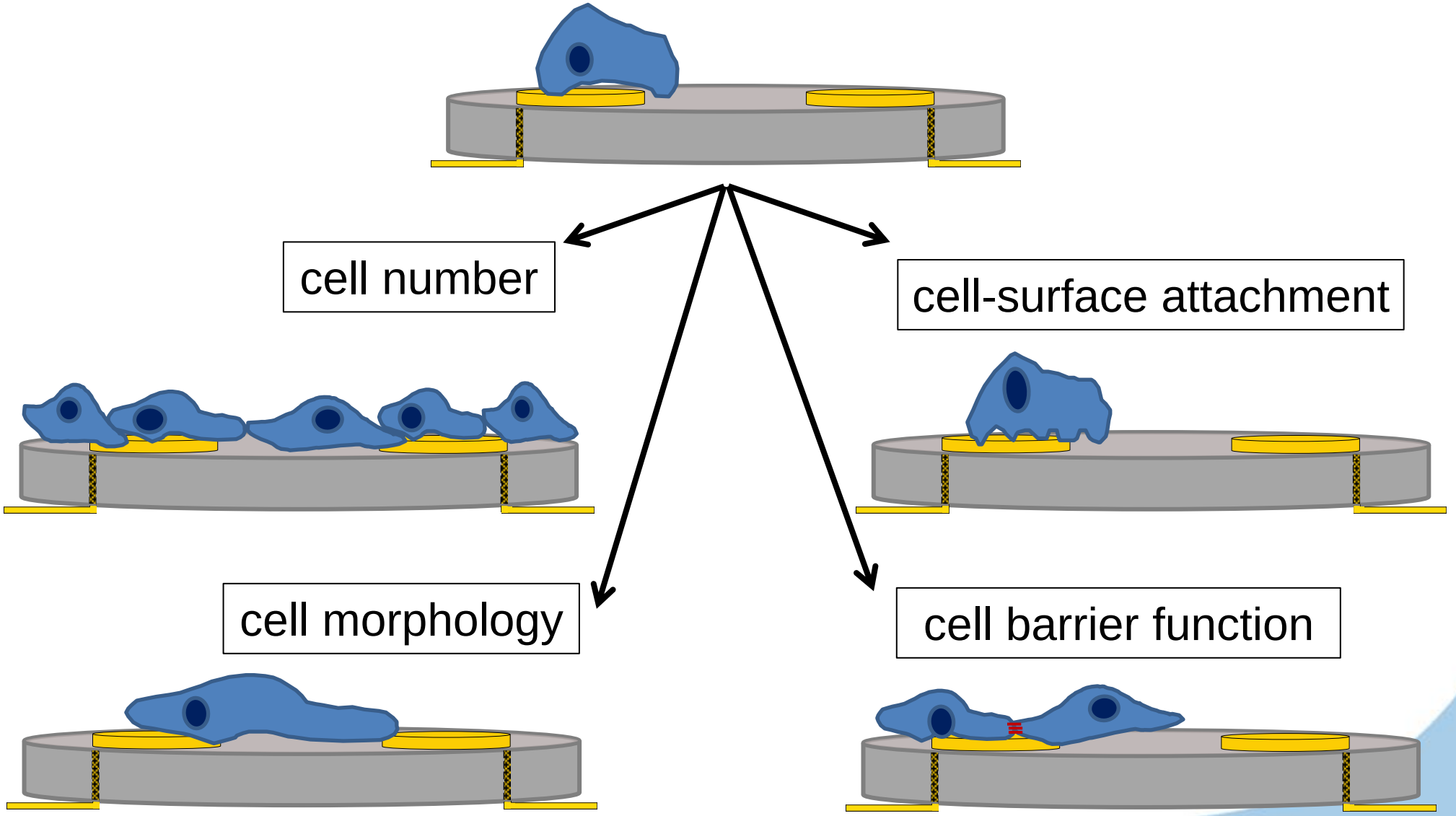


- Label-free
- Real-time and kinetic readout
- Non-invasive measurements



Cell Index = $(Imp_{cell} - Imp_{BG})/ 15\ \Omega$

What Can Impedance Monitor?



xCELLigence® RTCA Key Applications List

1.Cell Adhesion and Spreading

2. Quality Control of Cells

3. Drug-Mediated Cytotoxicity/ Apoptosis

4. Cell Invasion and Migration

5. Cell-Mediated Cytotoxicity & ADCC

6. Virus-Mediated Cytopathogenicity

7. Receptor-Mediated Signaling: GPCR RTK Ion channels NHR

8. Cell-Response Profiling (Small Molecule and siRNA)

9. Cell Barrier Function

10. Immune Cell Activation

11. Cardiac safety assessment & cardiovascular disease model research

12. Stem Cells and Differentiation

13. Parasite Motility Assay

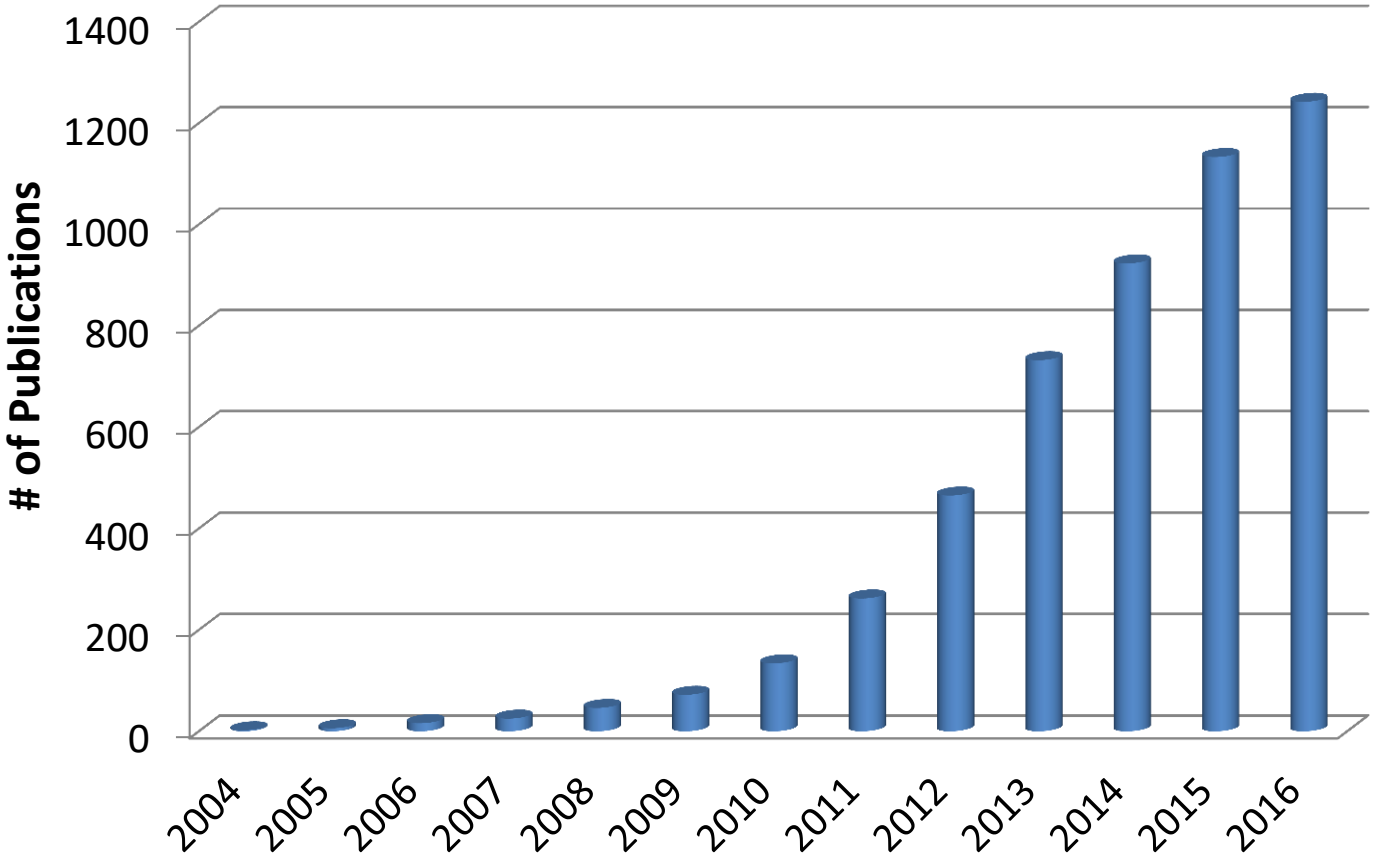
14. Cell-Cell Interactions

15. Biofilms

- ✓Cancer
- ✓Regenerative medicine
- ✓Immunotherapy
- ✓Inflammation
- ✓Microbial infection
- ✓Toxicology

Solid Publication Track Record

Over 1200 peer-reviewed publications citing ACEA's Real-Time Cell Analysis Technology

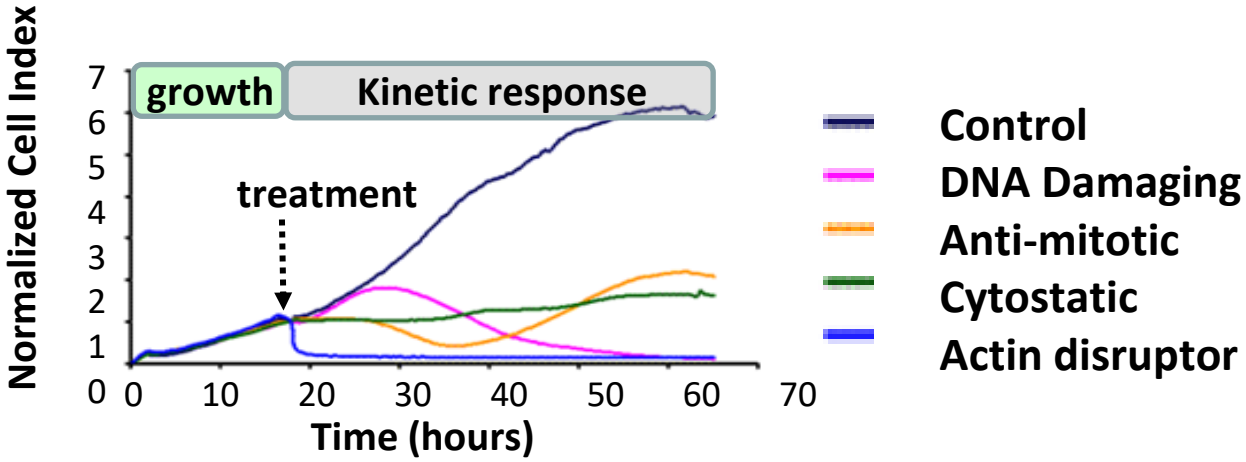


Outline

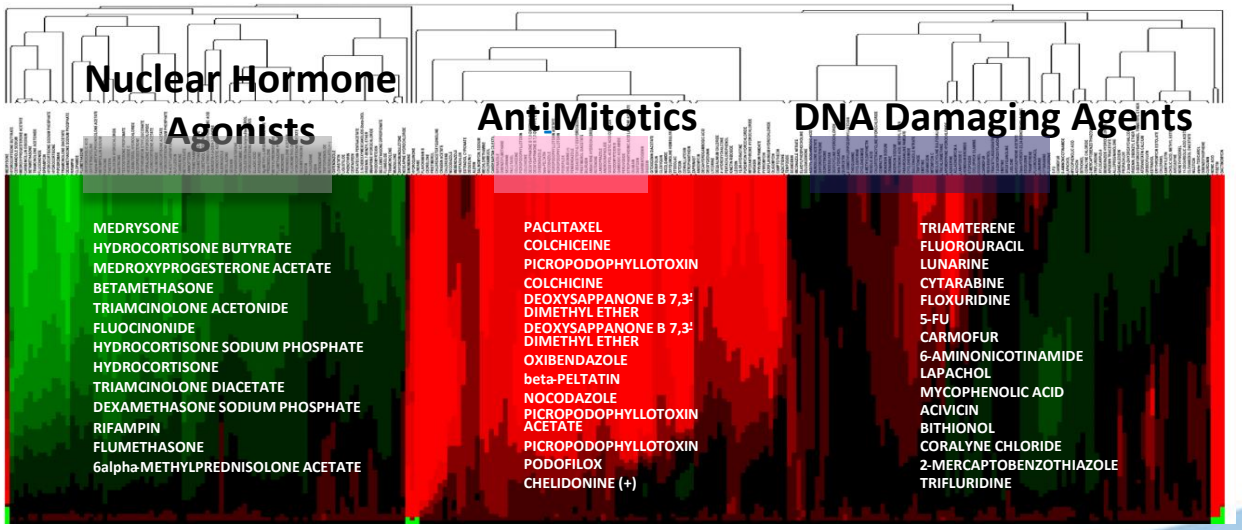
1. Introduction to ACEA Biosciences and xCELLigence technology
2. **The utility of the xCELLigence technology for quantitatively monitoring drug mediated cytotoxicity in real-time**
    1) Drug discovery: small mol., big mol., and cell therapy
    2) Biocompatibility
    3) Environmental Toxicology:
    4) Drug safety tests: predictive tox

(1) Drug discovery

Small Molecule: Mode Of Action (MOA) Identification

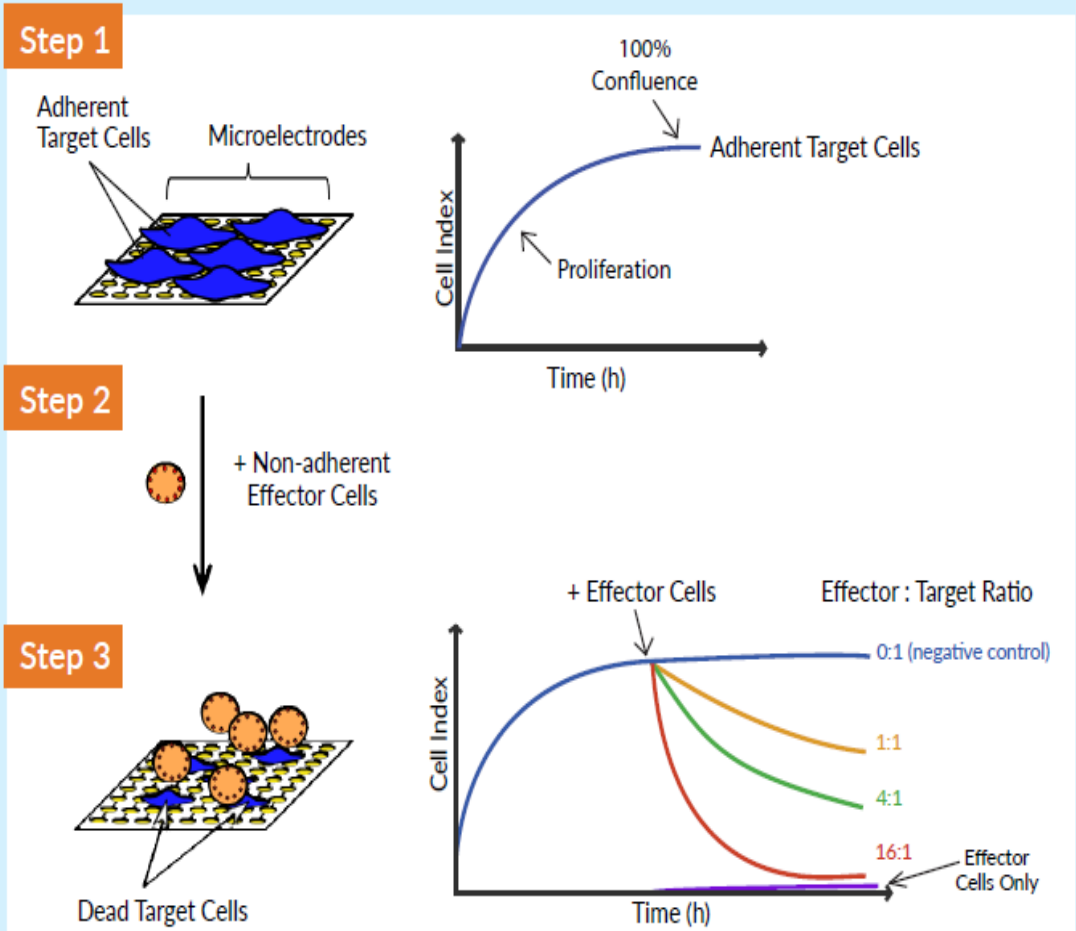


Abassi YA et al. (2009) Kinetic Cell-Based Morphological Screening: Prediction of Mechanism of Compound Action and Off-Target Effects. **Chemistry & Biology 16, 712–723.**



(1) Drug discovery

Big molecule (ADCC) and Cell therapy: CAR-T, CTL, NK…

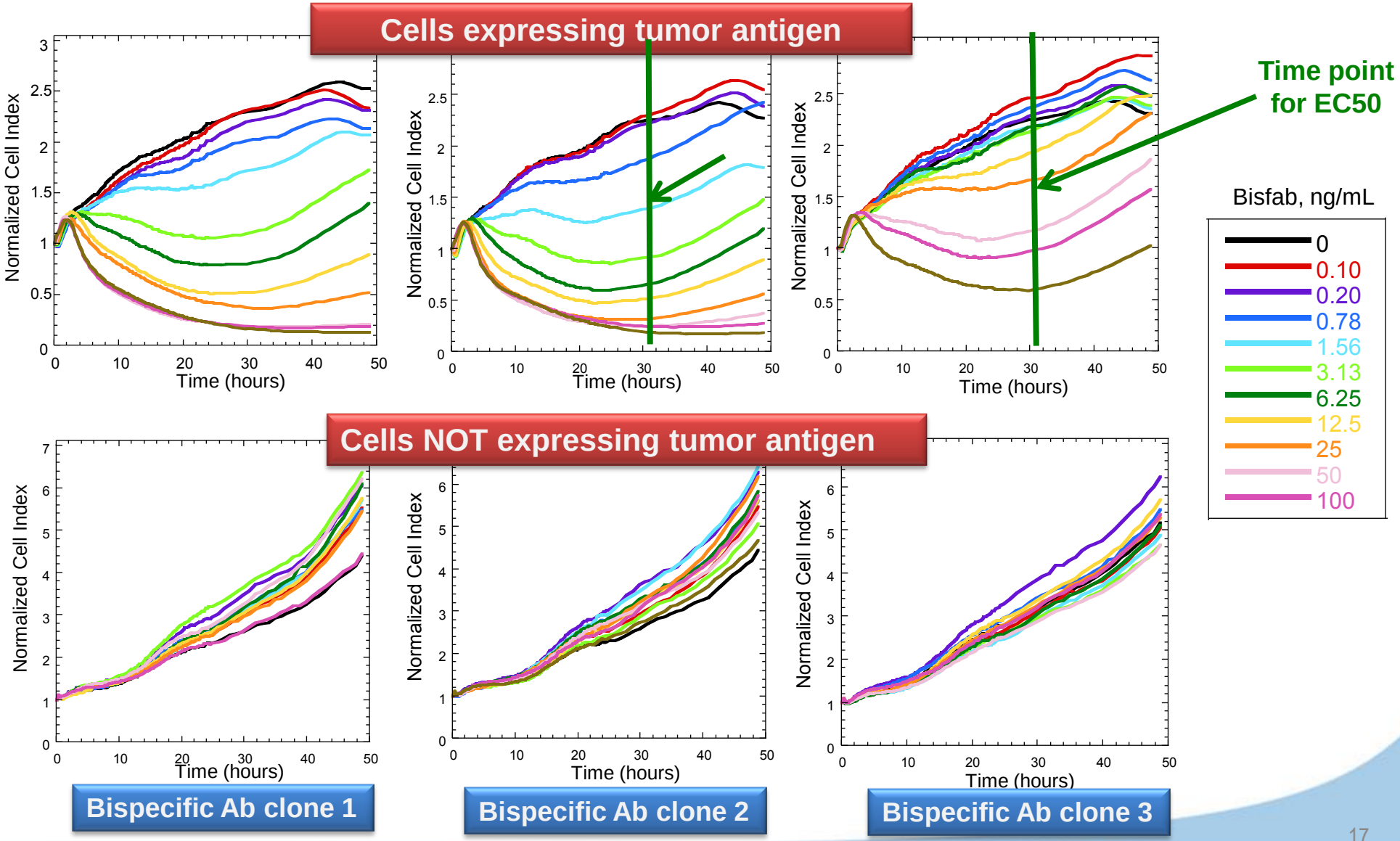


Key benefits of using xCELLigence to monitor immune cell-mediated killing

1. **Label-Free**: Allowing for more physiological assay conditions; labeling or secondary assays aren't required.
2. **Real-Time:** Quantitative monitoring of both fast (hours) and slow (days) killing kinetics.
3. **Sensitive:** Capable of evaluating low effector cell to target cell ratios that are physiologically relevant.
4. **Simple Workflow:** Requires only the addition of effector cells to target cells (in the presence or absence of antibodies);homogeneous assay without additional sample handling.
5. **Automatic Data Plotting:** RTCA software enables facile data display and objective analysis, precluding the subjective data vetting that is common to imaging-based assays.

(1) Drug discovery

Big Molecule: Bispecific Antibody Screening at Genentech



Slide Decks Courtesy of Dr. Judy Young (Genentech), USA

(1) Drug discovery

Big Molecule: Bispecific Antibody Screening at Genentech

End Point Methods:

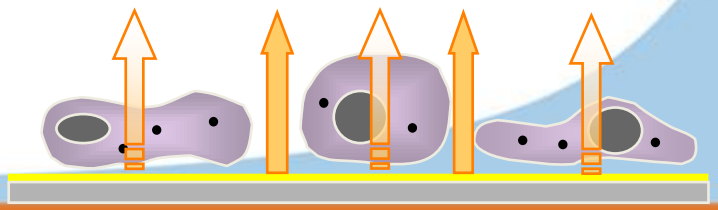
1) FACS	Laborious, Cell Removal Artifacts Possible
2) Radioisotope Release	Need to Label Target Cells
3) Enzyme Release	Dying Effector Cells Could Confound Reading
4) ATP Production	Need to Wash Effectors Out of Well First

Impedance xCELLigence assay:

1) Measurements in Real Time

2) No Labels or Secondary Readout Assays

3) Non-Labor Intensive Assay Development and Performance



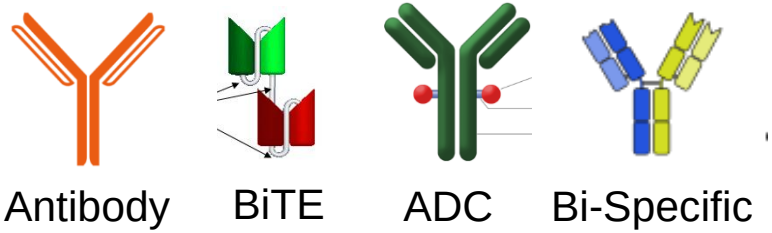
Slide Decks Courtesy of Dr. Judy Young (Genentech), USA

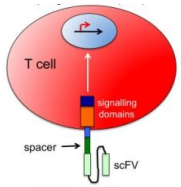
(1) Drug discovery

Cancer immunotherapy studies citing xCELLigence

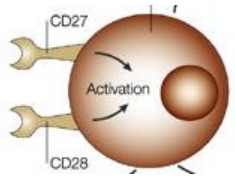
Antibody-Based Applications

Cell-Based Applications

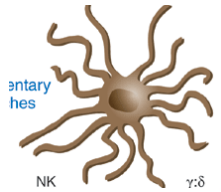




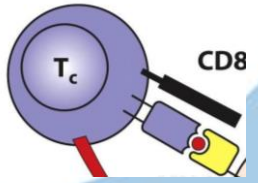
CART



NK



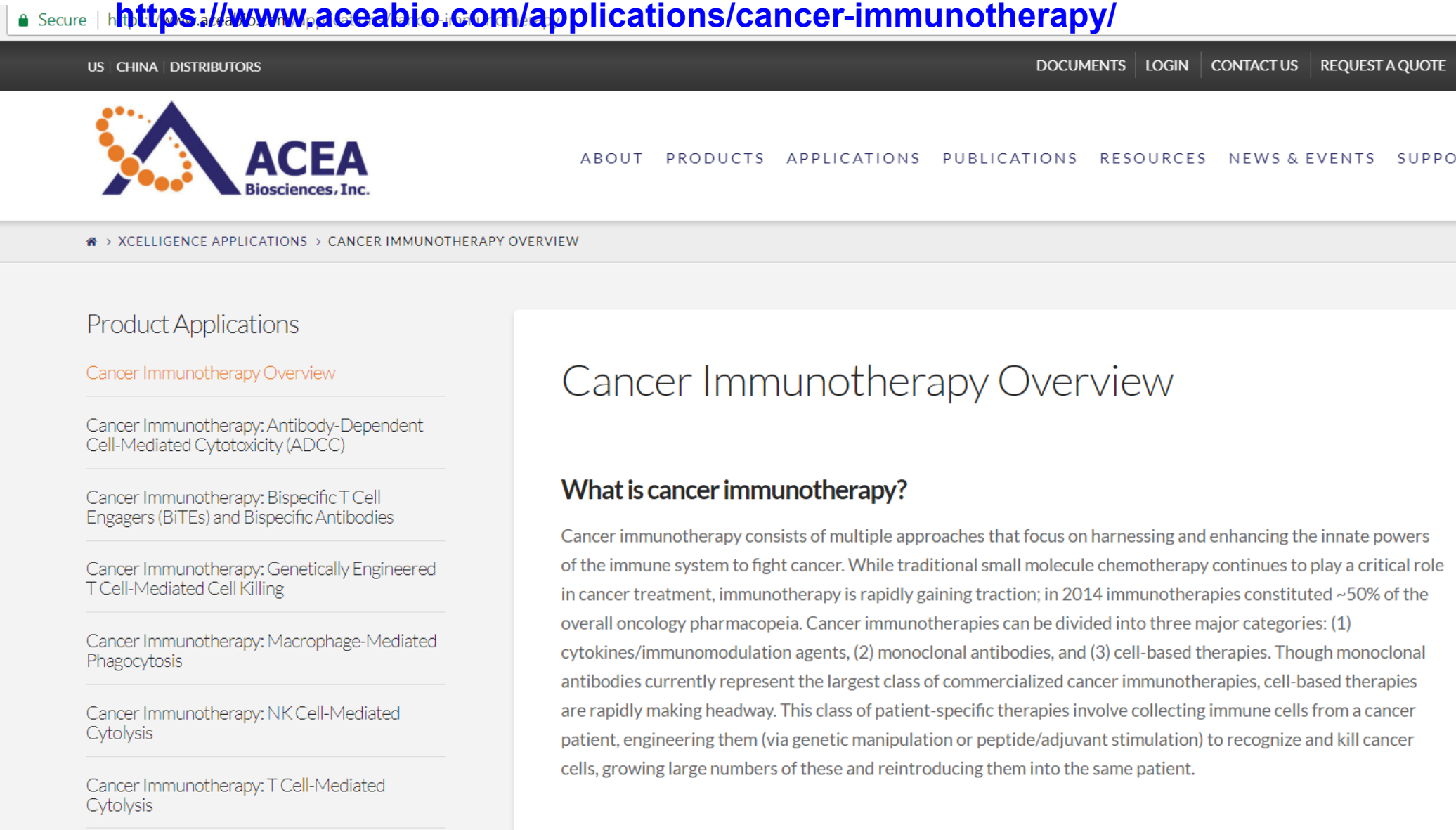
Dendritic Cells



CTL

For more information, visit our website

https://www.aceabio.com/applications/cancer-immunotherapy/



(2) Biocompatibility:
A case study from KCI (part of Acelity)

(2) Biocompatibility:

Visual assessment is described in ISO 10993-5 for the analysis of in vitro cytotoxicity of medical devices

Leslie Gutierrez

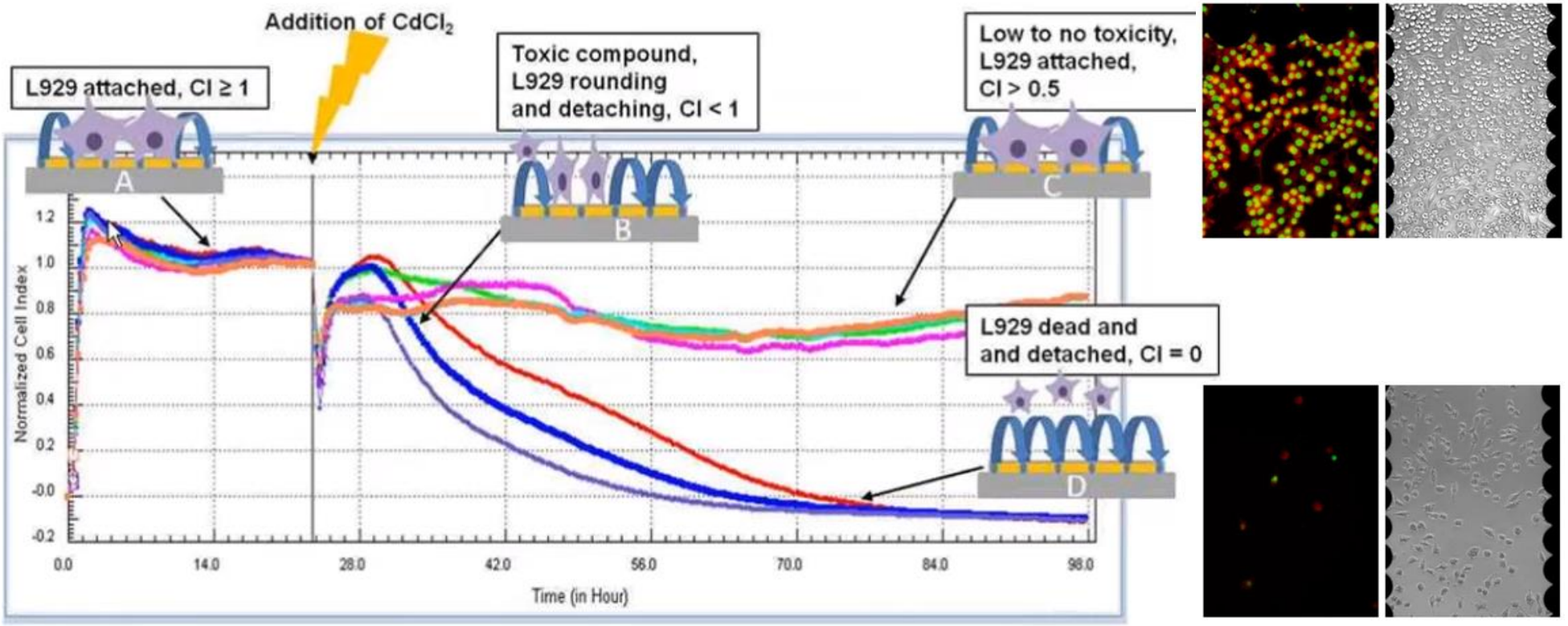
Score	Reactivity	Condition of Cells based on morphology
0	None	No Cell Lysis
1	Slight	Not more than 20% of the cells are loosely attached, and without intracytoplasmic granules, occasionally lysed cells are present
2	Mild	Not more than 50% of the cells are loosely attached, and without intracytoplasmic granules, occasionally lysed cells are present
3	Moderate	Not more than 70% of the cell layers contain loosely attached rounded cells and/or are lysed
4	Severe	Nearly complete destruction of the cell layers

J Biomed Mater Res A. 2013 Jul;101(7):2097-106

Real-time cellular analysis as a novel approach for in vitro cytotoxicity testing of medical device extracts.

(2) Biocompatibility:

The xCELLigence assay for biocompatibility studies

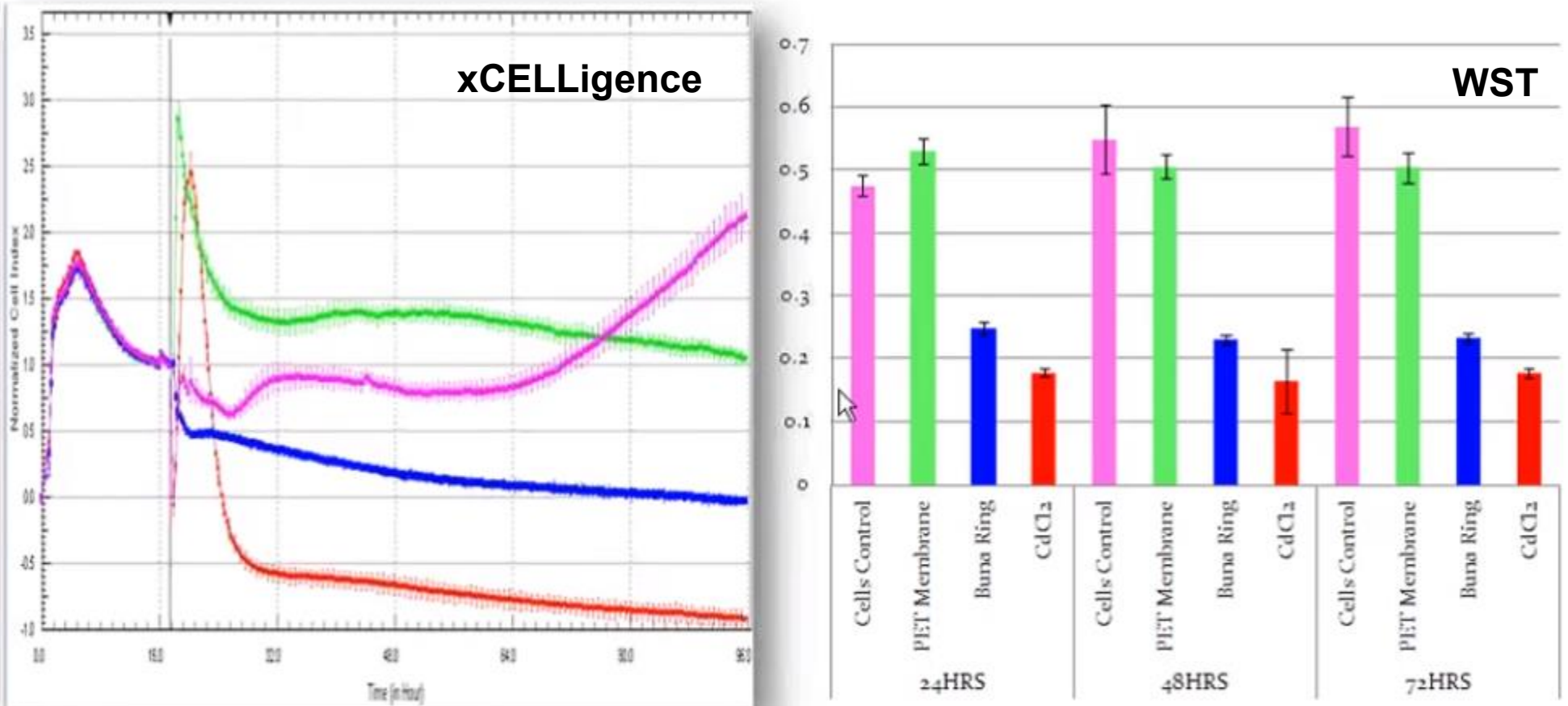


J Biomed Mater Res A. 2013 Jul;101(7):2097-106

Real-time cellular analysis as a novel approach for in vitro cytotoxicity testing of medical device extracts.

(2) Biocompatibility:

Good correlation between xCELLigence and WST-1 assays



J Biomed Mater Res A. 2013 Jul;101(7):2097-106

Real-time cellular analysis as a novel approach for in vitro cytotoxicity testing of medical device extracts.

(2) Biocompatibility:

Good correlation between xCELLigence and microscopic analyses defined by the ISO 10993-5:2009 at ISO/IEC 17025:2005-Accredited Labs

Reactivity Scores for Various Medical Device Materials from ISO/IEC 17025:2005-Accredited Laboratories Versus xCELLigence RTCA at KCl

Test Article and Controls	Cytotoxicity Score 24 h	48 h	72 h	Location
Urethane Cast Coated Stretch Knit	4	4	NT	MicroMed Laboratories[a]
Urethane Cast Coated Stretch Knit	4	4	4	Kinetic Concepts Inc.[b]
Double-sided hydrogel adhesive	0	0	0	WuXi AppTec[c]
Double-sided hydrogel adhesive	0	0	0	Kinetic Concepts Inc.
Absorbent Pouch Composite	0	0	0	WuXi AppTec
Absorbent Pouch Composite	0	0	0	Kinetic Concepts Inc.

J Biomed Mater Res A. 2013 Jul;101(7):2097-106

Real-time cellular analysis as a novel approach for in vitro cytotoxicity testing of medical device extracts.

(3) Environmental Tox:

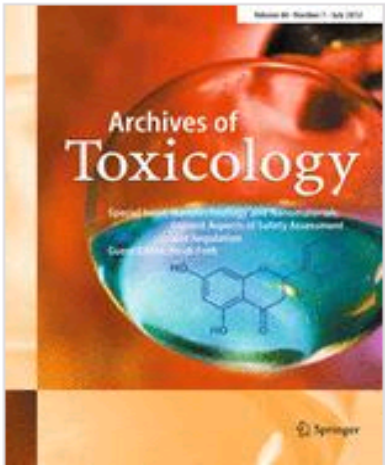
A case study: ACEA Contract Research with EPA ToxCast

Springer

Archives of Toxicology
July 2012, Volume 86, Issue 7, pp 1123-1136

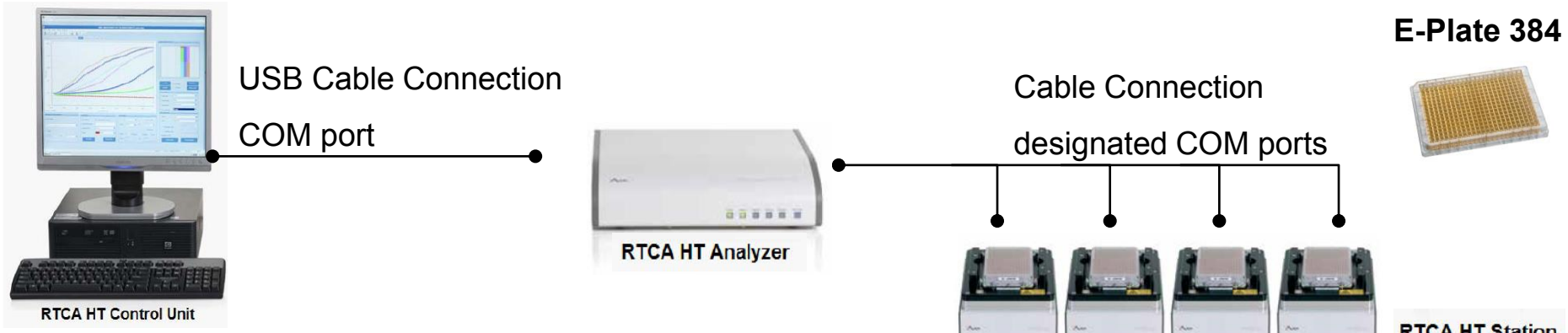
Interference of engineered nanoparticles with in vitro toxicity assays

A. Kroll · M. H. Pillukat · D. Hahn · J. Schnekenburger (✉)
Biomedical Technology Center, Westfälische Wilhelms-Universität, Albert-Schweitzer-Campus 1 A14, 48149 Münster, Germany
e-mail: schnekenburger@uni-muenster.de



(3) Environmental Tox:

ACEA Contract Service using xCELLigence HT systems

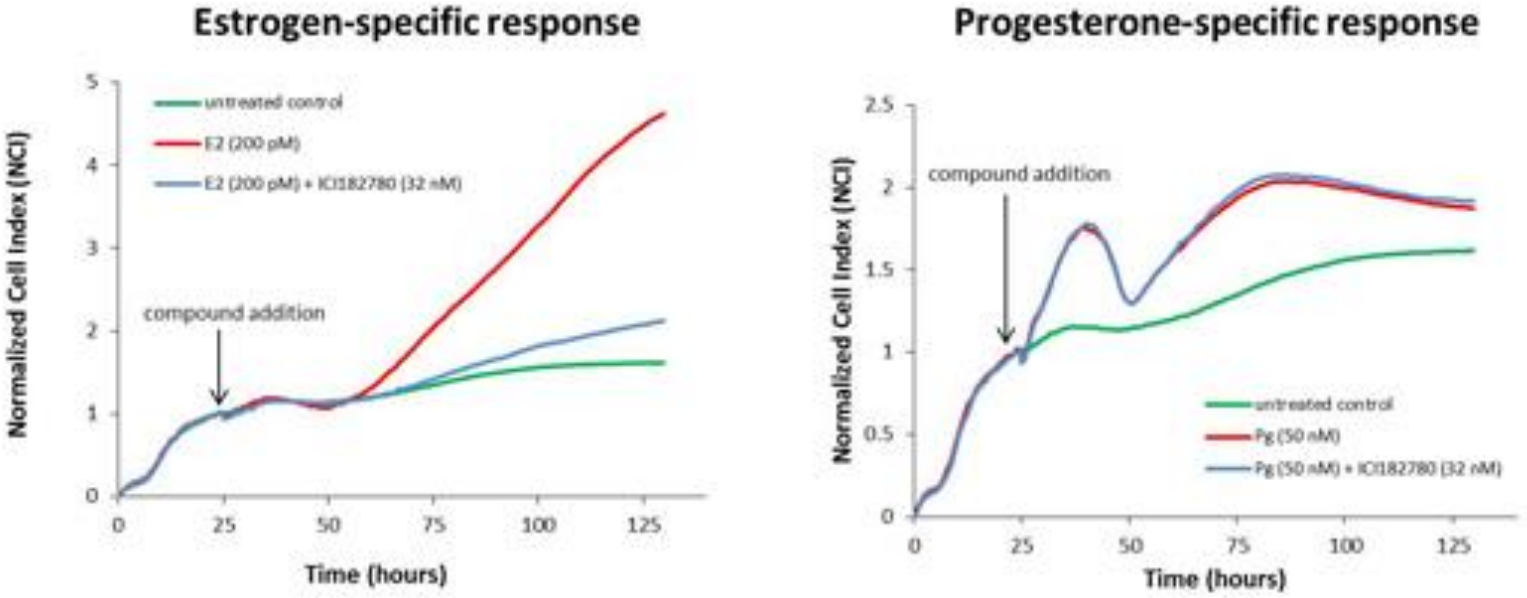


- ✓ US Environmental Protection Agency ToxCast™ Program
- ✓ Collaboration with the Alberta Centre for Toxicology (ACFT)

(3) Environmental Tox:

ACEA Contract Service using xCELLigence HT systems

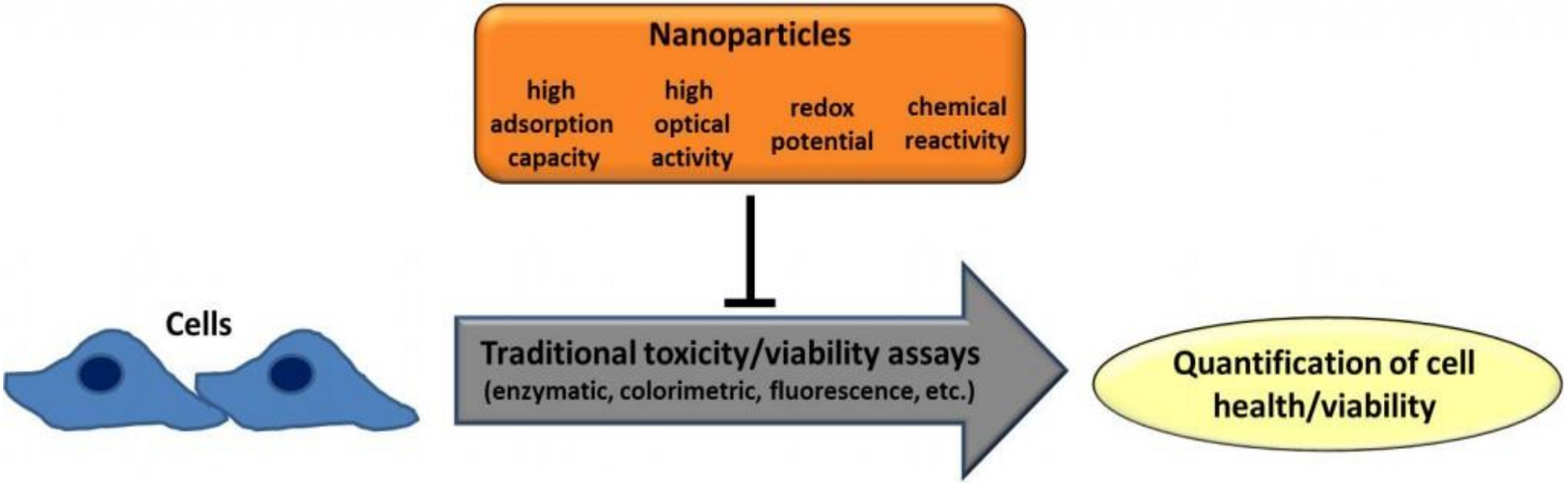
EPA ToxCast: Cytotoxicity and estrogen/progesterone mimics screening



- The data from these cytotoxicity and estrogen/progesterone mimics screening are available in the **ToxCast database**(http://www.epa.gov/ncct/toxcast/),
- Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T-47D human ductal carcinoma cells. Rotroff et al (2013) *Chem Res Toxicol.* 26:1097-107.

(3) Environmental Tox:

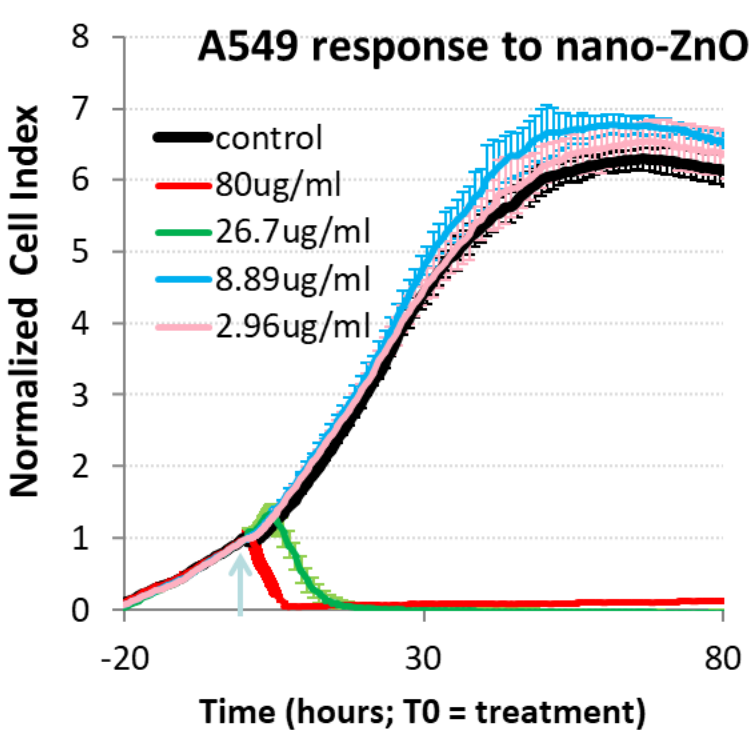
Existing Challenges in Accurately Predict Nanotoxicity

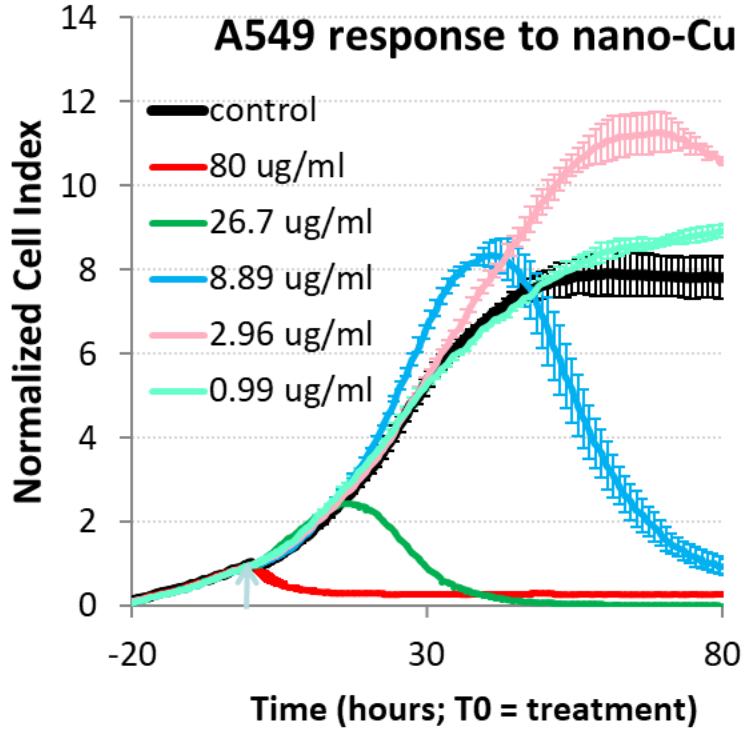


(3) Environmental Tox:

ACEA Contract Service using xCELLigence HT systems

EPA ToxCast: xCELLigence Nanotoxicity Assay For MOA Study

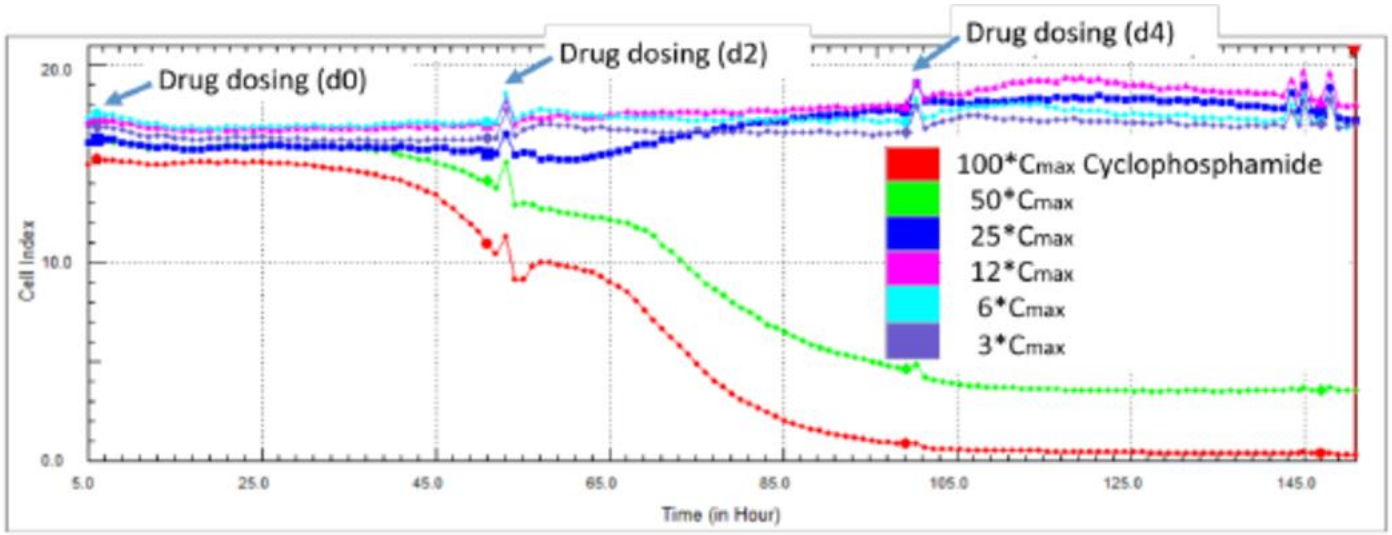




Different Nano-material Cause Different Response Profiles

(4) Drug safety tests

A Case Study: Repeat-dose Hepatotoxic Screening on Primary Hepatocyte – CRO Service offered by Hurel



Outline

1. Introduction to ACEA Biosciences and xCELLigence technology
2. The utility of the xCELLigence technology for quantitatively monitoring drug mediated cytotoxicity in real-time
3. **The protocols, tips, and tricks for conducting drug mediated cytotoxicity assays**

Workflow: Drug Mediated Cytotox Assay

DAY-1

1

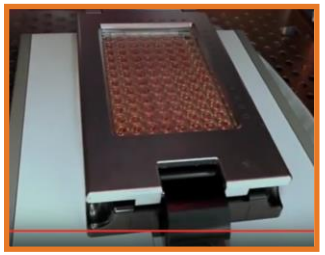
Add 80µL Media

2

30 min

RT

3



Background Read

4

Add Cells (100 µL)

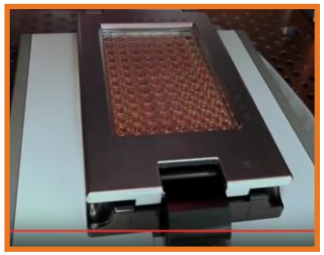
typically 2.5-5K/well

5

30 min

RT

6



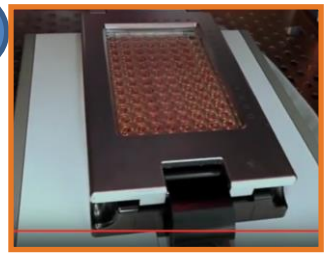
Monitor Cell Proliferation

DAY-2

7

Add 20 µL Drug Containing Media

8



Monitor Cell Killing Kinetics

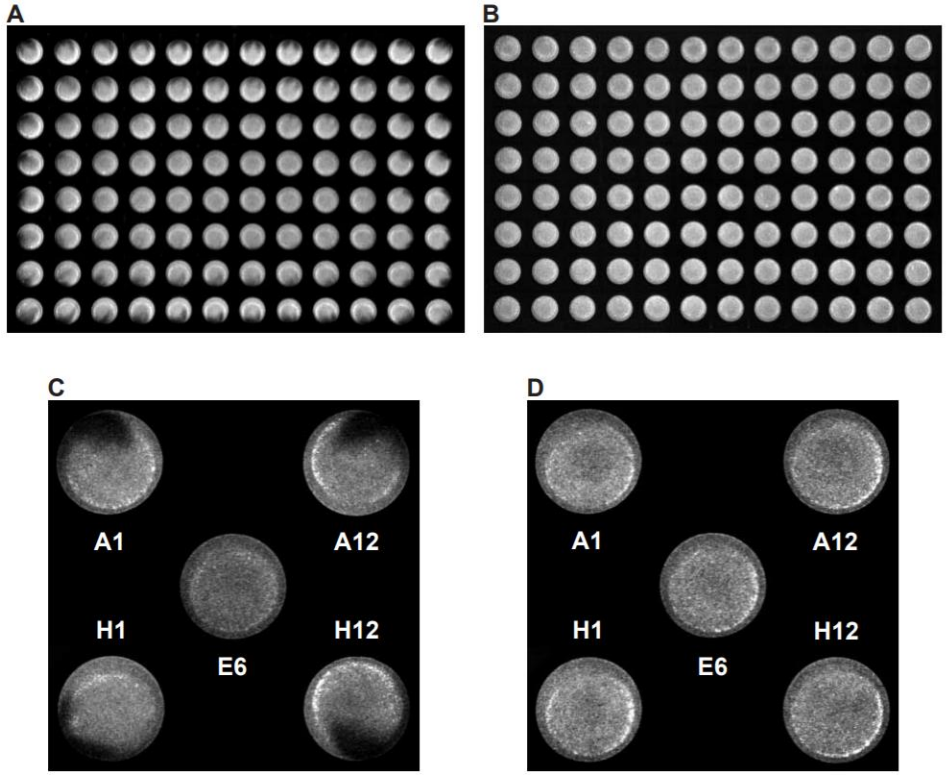
Final concentration of DMSO should be less than 0.1%

- For typical drugs, where long term effect needs to be monitored, sampling rate =15 min after drug addition.
- For receptor activation (e.g., GPCR agnoist/antagonist) , sample rate =30 sec after drug addition for 2 hour, then every 1 hr

Tip #1: how to reduce CV across the plate?

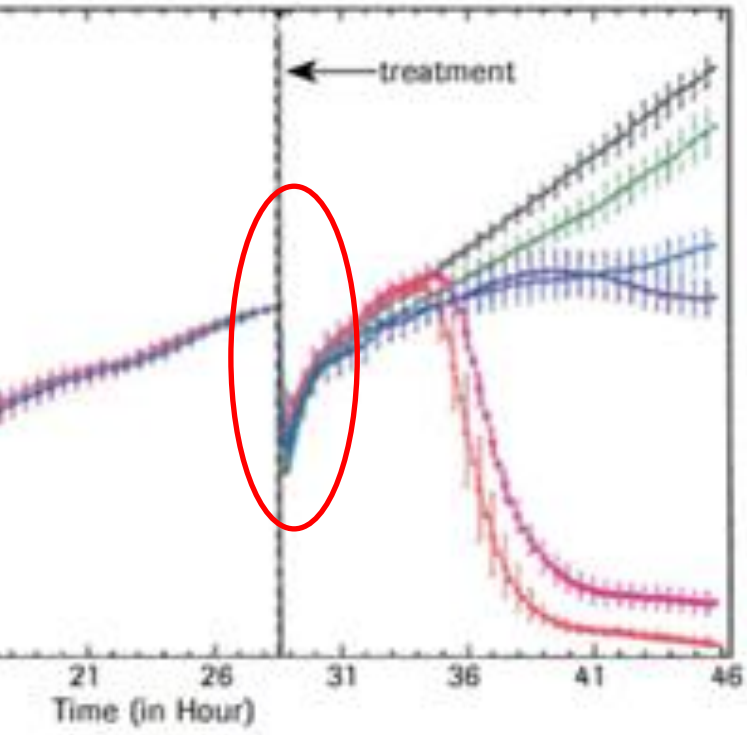
1. Gently rock the cell reservoir, or pipette up and down to mix the cell suspension

2. Pre-incubation of newly seeded plates in room temperature resulted in even distribution of the cells in each well



Journal of Biomolecular Screening 2003:566-570
A Simple Technique for Reducing Edge Effect in Cell-Based Assays

Tip #2: how to get rid of the “dip” on the curve?



1. Limit vehicle (DMSO) concentration (<0.1%)
2. Maintain the same vehicle concentration in each dose
3. Pre-equilibrate the drug stock (media containing drugs) in the tissue culture incubator prior to the drug addition.

Quick Start and data acquisition guides

xCELLigence® Real-Time Cell Analysis (RTCA) DP I

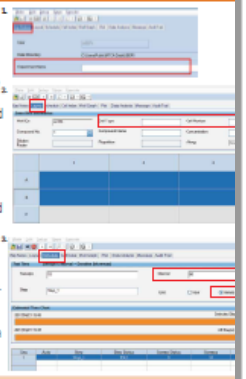
QUICK START AND DATA ACQU

LAUNCH THE RTCA SOFTWARE

1. Double click the RTCA software icon to launch the software
2. Sign in as USER1/USER2 (no password necessary)

DEFINE EXPERIMENTAL SETUP

1. Click *Exp Notes* tab
   - Fill in "Experiment Name"
2. Click *Layout* tab
   - Within the plate map, left click and drag to highlight the wells of interest. Then add the "Cell Type" and "Cell Number". Click "Apply".
   - Highlight wells that will receive a compound treatment and add the following:
     - Compound Name
     - The highest (final) compound "Concentration" that will be used
     - The concentration "Unit"
     - The "Dilution Factor" to be applied to serial dilutions
     - Click "Apply"
3. Click *Schedule* tab
   - Click the icon to add a step. By default, the 1st step is the background reading step. No change is needed.
   - Click the icon to add another step. A typical step would be 15 min "Interval" for a "Duration" of 96 hours.
   - Click "Apply" to confirm entries.

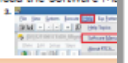


RUN THE RTCA EXPERIMENT

1. **Take a background reading:** Add growth media (50-100 µL/well) to the E-Plate, engage the E-P icon to take the background reading.
2. **Seed cells:** Add cell suspension (100 µL/well) to the E-Plate wells. This gives a total volume of **at room temperature for 30 min.** This step allows cells to settle in an evenly distributed pattern drastically minimizing edge effects and CVs.*Exception: this step may be omitted for short-ter
3. **Engage the E-Plate in the cradle, then click the** icon to monitor cell adhesion and prolifera

DISPLAY AND ANALYZE THE RTCA DATA

1. **Open file:** Click the icon and choose the PLT file to open.
2. **Plot the wells of interest:** In the *Plot* tab: Left click and drag to highlight the wells of interest, C button to visualize the Cell Index curves. Display the average of replicates, and the associated deviation error bars, by checking "Average" and "STD DEV". To zoom into an area of the d click and drag a box around the region of interest within the plot.
3. **Data Analysis:** Within the *Data Analysis* tab, select the desired type of data output from the se "Analysis Option". *For detailed information on data analysis please download the Software M "Help" dropdown menu.



SAVE AND EXPORT RTCA DATA

1. Click the icon to save any changes made to the experimental notes or layout.
2. Click "Plate" and then choose "Export Experimental Info..." from the drop down menu to expo
3. Click "Plate" and then choose "Release" from the drop down menu to close the current RTCA

xCELLigence® Real-Time Cell Analysis (RTCA) SP

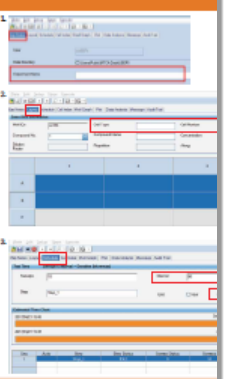
QUICK START AND DATA ACQ

LAUNCH THE RTCA SOFTWARE

1. Double click the RTCA software icon to launch the software
2. Sign in as USER1/USER2 (no password necessary)

DEFINE EXPERIMENTAL SETUP

1. Click *Exp Notes* tab
   - Fill in "Experiment Name"
2. Click *Layout* tab
   - Within the plate map, left click and drag to highlight the wells of interest. Then add the "Cell Type" and "Cell Number". Click "Apply".
   - Highlight wells that will receive a compound treatment and add the following:
     - Compound Name
     - The highest (final) compound "Concentration" that will be used
     - The concentration "Unit"
     - The "Dilution Factor" to be applied to serial dilutions
     - Click "Apply"
3. Click *Schedule* tab
   - Click the icon to add a step. By default, the 1st step is the background reading step. No change is needed.
   - Click the icon to add another step. A typical step would be 15 min "Interval" for a "Duration" of 96 hours.
   - Click "Apply" to confirm entries.

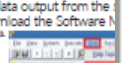


RUN THE RTCA EXPERIMENT

1. **Take a background reading:** Add growth media (50-100 µL/well) to the E-Plate, engage the E icon to take the background reading.
2. **Seed cells:** Add cell suspension (100 µL/well) to the E-Plate wells. This gives a total volume o **at room temperature for 30 min.** This step allows cells to settle in an evenly distributed patte drastically minimizing edge effects and CVs.*Exception: this step may be omitted for short-te
3. **Engage the E-Plate in the cradle, then click the** icon to monitor cell adhesion and prolife
4. **To add compounds or cells to the E-Plate:** Pause the experiment by clicking the icon befo cradle.

DISPLAY AND ANALYZE THE RTCA DATA

1. **Open files:** Click the icon and choose the PLT file to open.
2. **Plot the wells of interest:** In the *Plot* tab: Left click and drag to highlight the wells of interest, button to visualize the Cell Index curves. Display the average of replicates, and the associate deviation error bars, by checking "Average" and "STD DEV". To zoom into an area of the click and drag a box around the region of interest within the plot.
3. **Data Analysis:** Within the *Data Analysis* tab, select the desired type of data output from the "Analysis Option". *For detailed information on data analysis please download the Software M "Help" dropdown menu.



SAVE AND EXPORT RTCA DATA

1. Click the icon to save any changes made to the experimental notes or layout.
2. Click "Plate" and then choose "Export Experimental Info..." from the drop down menu to exp
3. Click "Plate" and then choose "Release" from the drop down menu to close the current RTC

xCELLigence® Real-Time Cell Analysis (RTCA) MP Instrument

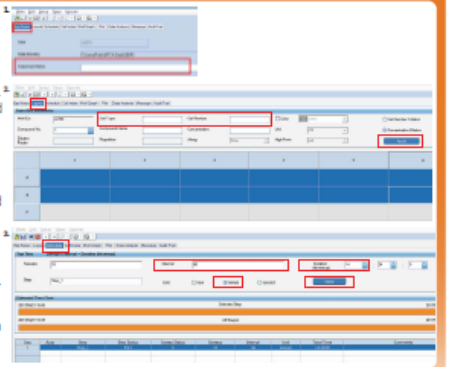
QUICK START AND DATA ACQUISITION GUIDE

LAUNCH THE RTCA SOFTWARE

1. Double click the RTCA software icon to launch the software
2. Sign in as USER1/USER2 (no password necessary)

DEFINE EXPERIMENTAL SETUP

1. Click *Exp Notes* tab
   - Fill in "Experiment Name"
2. Click *Layout* tab
   - Within the plate map, left click and drag to highlight the wells of interest. Then add the "Cell Type" and "Cell Number". Click "Apply".
   - Highlight wells that will receive a compound treatment and add the following:
     - Compound Name
     - The highest (final) compound "Concentration" that will be used
     - The concentration "Unit"
     - The "Dilution Factor" to be applied to serial dilutions
     - Click "Apply"
3. Click *Schedule* tab
   - Click the icon to add a step. By default, the 1st step is the background reading step. No change is needed.
   - Click the icon to add another step. A typical step would be 15 min "Interval" for a "Duration" of 96 hours.
   - Click "Apply" to confirm entries.

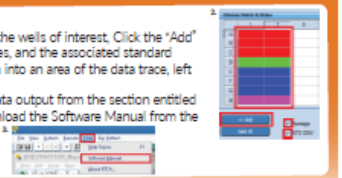


RUN THE RTCA EXPERIMENT

1. **Take a background reading:** Add growth media (50-100 µL/well) to the E-Plate, engage the E-Plate in the cradle, then click the icon to take the background reading.
2. **Seed cells:** Add cell suspension (100 µL/well) to the E-Plate wells. This gives a total volume of 150-200 µL/well. **Leave the E-Plate at room temperature for 30 min.** This step allows cells to settle in an evenly distributed pattern at the bottom of the wells, drastically minimizing edge effects and CVs.*Exception: this step may be omitted for short-term cell adhesion assays.
3. **Engage the E-Plate in the cradle**, then click the icon to monitor cell adhesion and proliferation.
4. **To add compounds or cells to the E-Plate:** Pause the experiment by clicking the icon and unlock the plate by clicking the icon before removal of the E-Plate from the cradle.

DISPLAY AND ANALYZE THE RTCA DATA

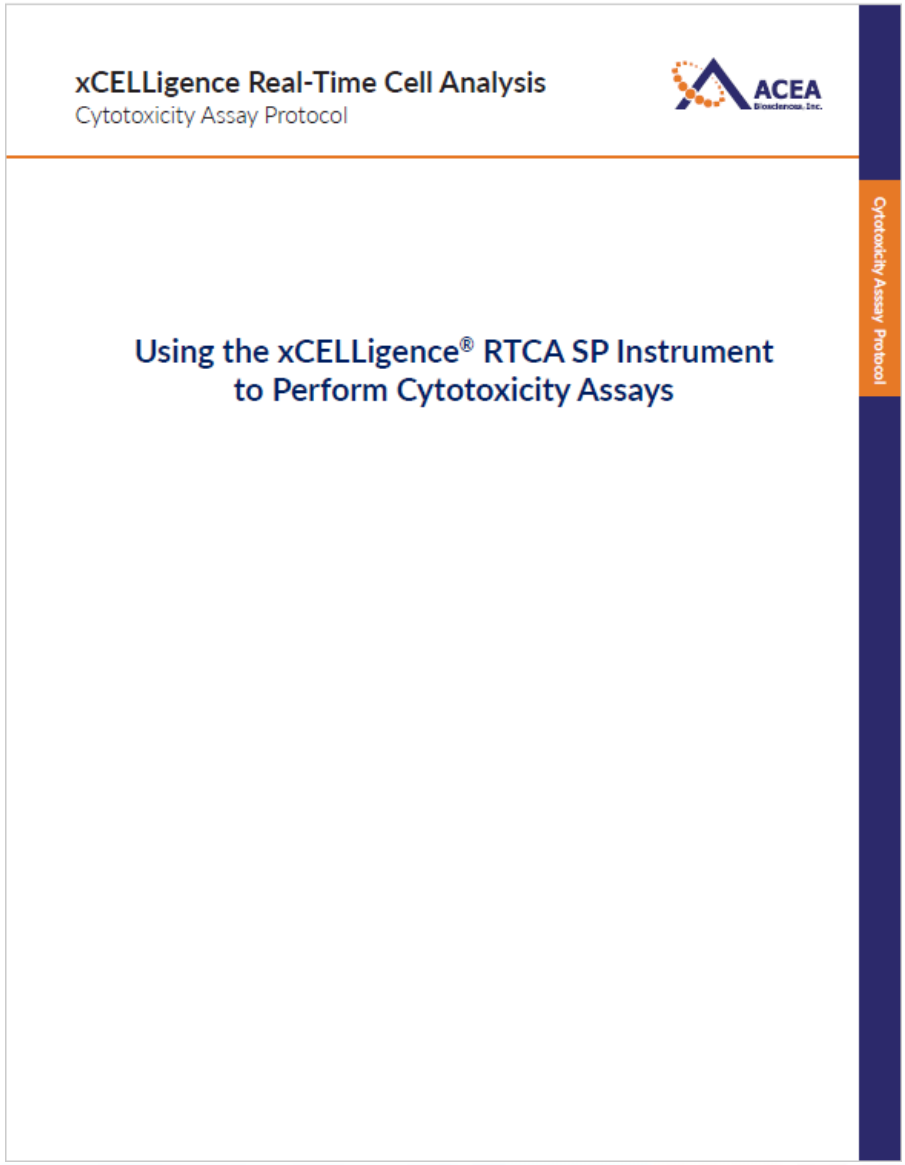
1. **Open file:** Click the icon and choose the PLT file to open.
2. **Plot the wells of interest:** In the *Plot* tab: Left click and drag to highlight the wells of interest, Click the "Add" button to visualize the Cell Index curves. Display the average of replicates, and the associated standard deviation error bars, by checking "Average" and "STD DEV". To zoom into an area of the data trace, left click and drag a box around the region of interest within the plot.
3. **Data Analysis:** Within the *Data Analysis* tab, select the desired type of data output from the section entitled "Analysis Option". *For detailed information on data analysis please download the Software Manual from the "Help" dropdown menu.



SAVE AND EXPORT RTCA DATA

1. Click the icon to save any changes made to the experimental notes or layout.
2. Click "Plate" and then choose "Export Experimental Info..." from the drop down menu to export data in Excel format.
3. Click "Plate" and then choose "Release" from the drop down menu to close the current RTCA experiment and start a new one.

Protocols:


xCELLigence Real-Time Cell Analysis
Cytotoxicity Assay Protocol
ACEA
Using the xCELLigence® RTCA SP Instrument
to Perform Cytotoxicity Assays
Cytotoxicity Assay Protocol

Outline

1. Introduction to ACEA Biosciences and xCELLigence technology
2. The utility of the xCELLigence technology for quantitatively monitoring drug mediated cytotoxicity in real-time
3. The protocols, tips, and tricks for conducting drug mediated cytotoxicity assays
4. **Real-time demonstration of data analysis and plotting for publications**

1006151503P5_repeatsforH1993.plt

Outline

1. Introduction to ACEA Biosciences and xCELLigence technology
2. The utility of the xCELLigence technology for quantitatively monitoring drug mediated cytotoxicity in real-time
3. The protocols, tips, and tricks for conducting drug mediated cytotoxicity assays
4. Real-time demonstration of data analysis and plotting for publications
5. **Trouble shoot and Maintenance**

Trouble shoot and Maintenance

1. Big variations between replications at times

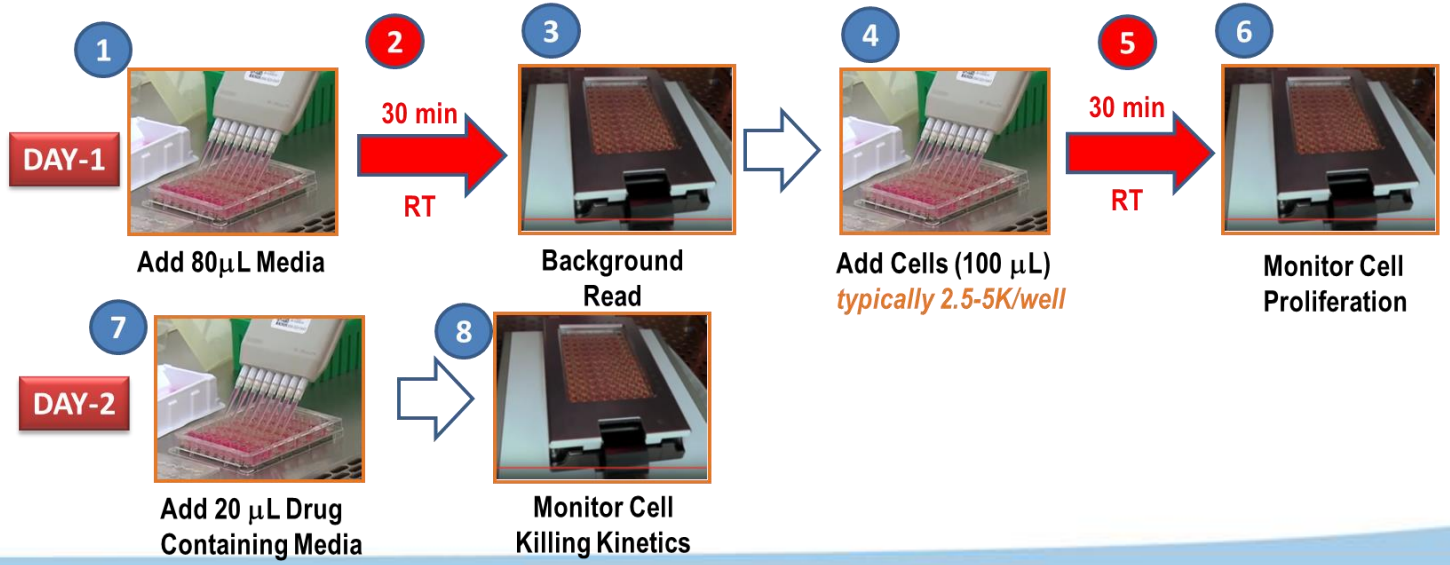
- Didn't leave the E-Plate at room temperature for 30 min after cell seeding.

2. Cell Index profiles go below zero and come up…

- Check to see if Background reading was taken prior to cell seeding step.

3. Cell Index drop dramatically after drug addition

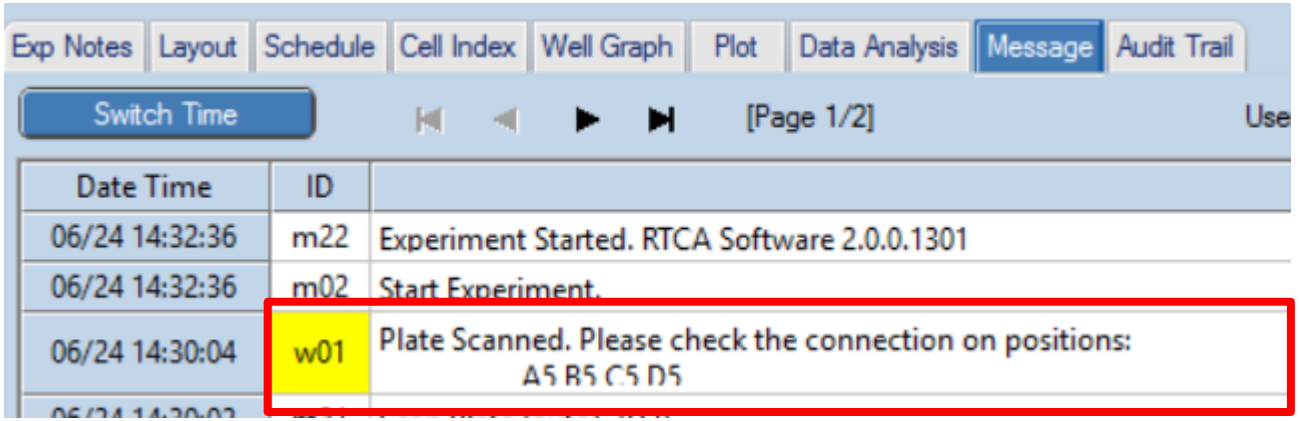
- Check to see if the solvent concentration was too high.

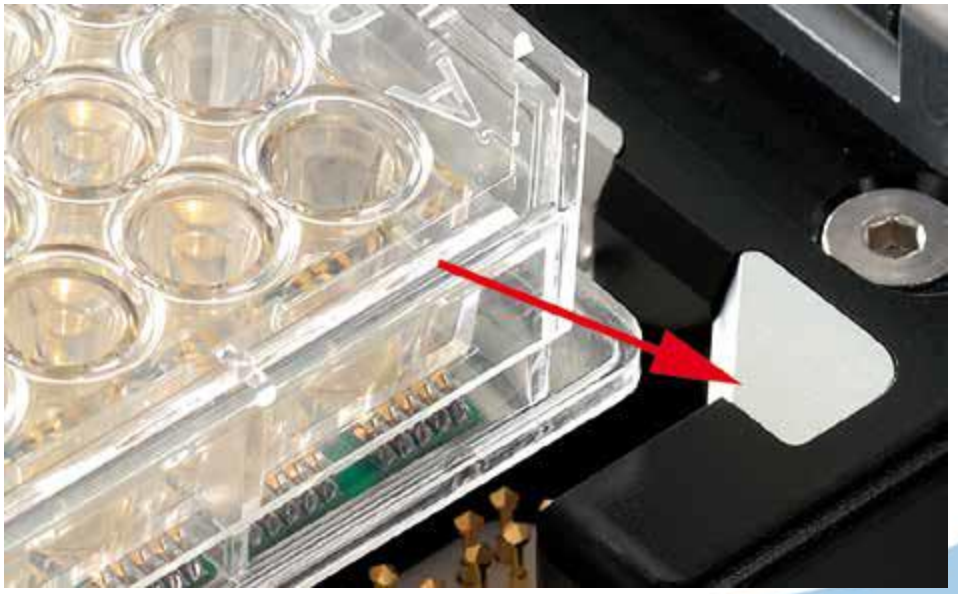


Final concentration of DMSO should be less than 0.1%

Trouble shoot and Maintenance

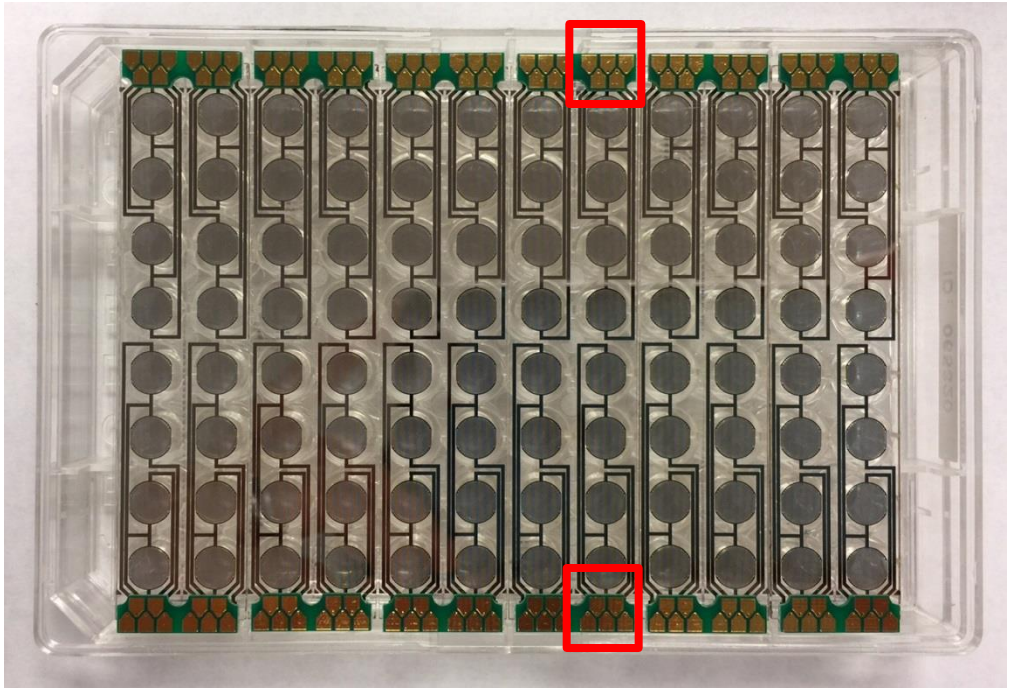
4. The Message Page indicates connection problems





Trouble shoot and Maintenance

a. Clean the contact pads on the E-Plate

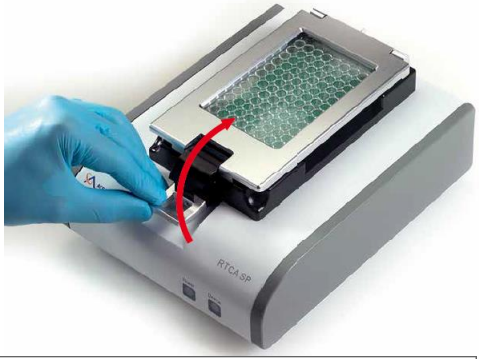


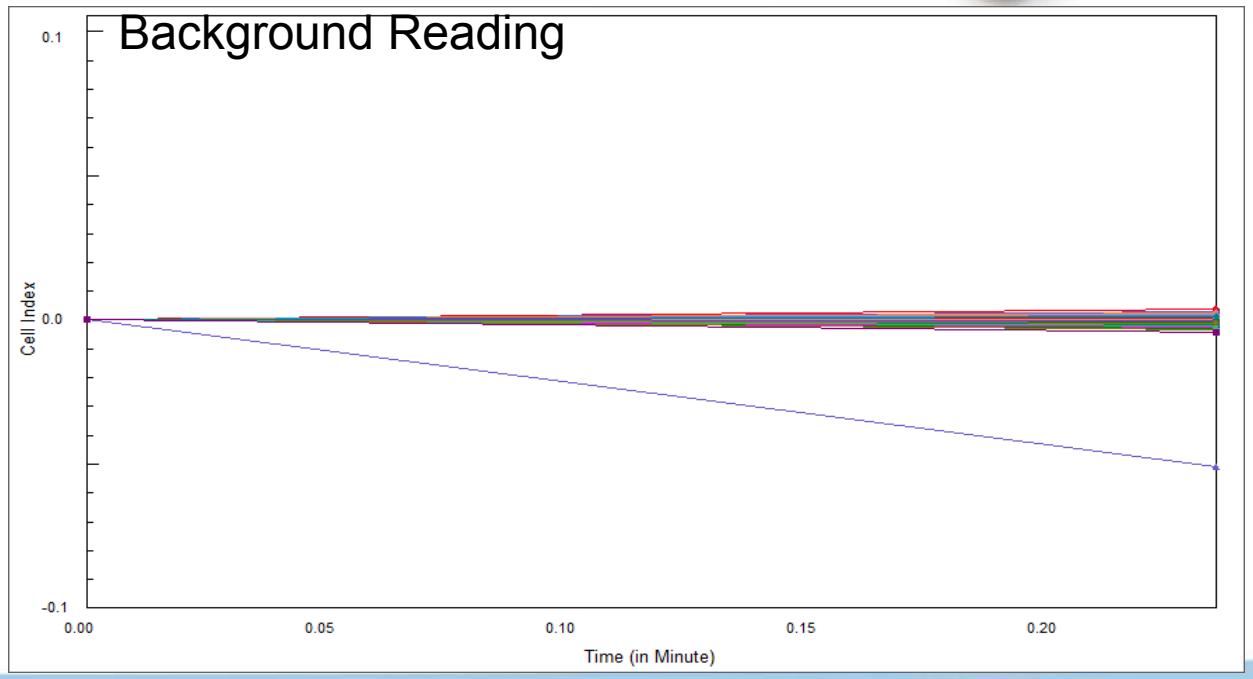
Trouble shoot and Maintenance

b. The correct handling of the E-Plates

Trouble shoot and Maintenance

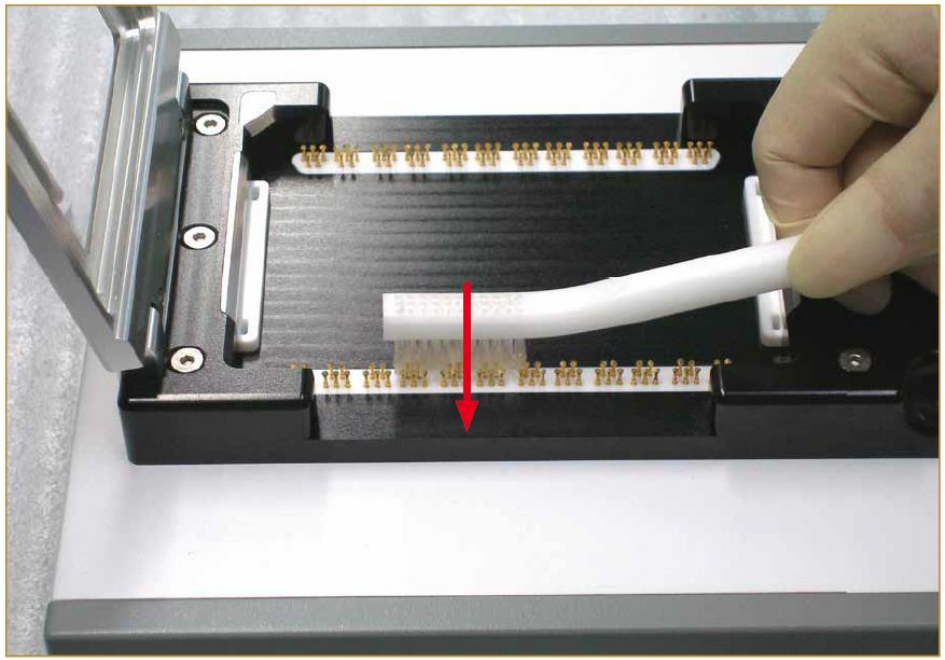
c. QC Test: Resister Plate Verification





Trouble shoot and Maintenance

d. Cleaning the RTCA Contact Pins



Trouble shoot and Maintenance

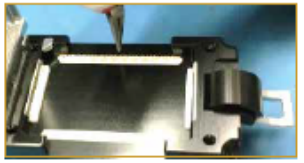
PREVENTIVE MAINTENANCE

General Cleaning Using fiber free tissue paper slightly moistened with 80% ethanol, gently wipe off any dust or contaminants from the surface of the RTCA MP Station. A small air blower can also be used to help remove any dust.

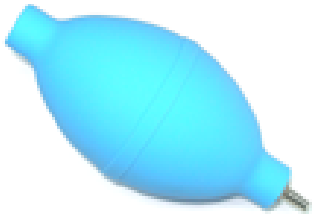
QC Test: Resistor Plate Verification Program a 5 min experiment (10 sweeps, 30 sec apart). Run the experiment with a resistor plate (Cat. No. 05232350001) in each of the instrument's six cradles. Upon completion of the test, check the "Cell Index Page". If the Cell Index values are lower than 0.063, the system has passed the QC test. If the cell Index of any well is higher than 0.063, the RTCA Contact Pins 96 (Cat. No. 05232384001) should be cleaned or replaced (see below).

Cleaning the RTCA Contact Pins To guarantee proper functioning of the RTCA MP Instrument, clean the RTCA Contact Pins regularly (every 2-3 months) by scrubbing them with the fiber-free nylon brush provided with the system. Gently brush across the tips of the Contact Pins in one direction towards the center of the cradle pocket 10 times.

Changing the RTCA Contact Pins If the Cell Index value of any well in the resister plate verification test is still larger than 0.063 after the Contact Pin cleaning procedure, the Contact Pins 96 should be replaced. To identify and exchange a damaged/failed Contact Pin, refer to the **RTCA MP Instrument Operator's Manual** pages 52-53 for detailed instructions.

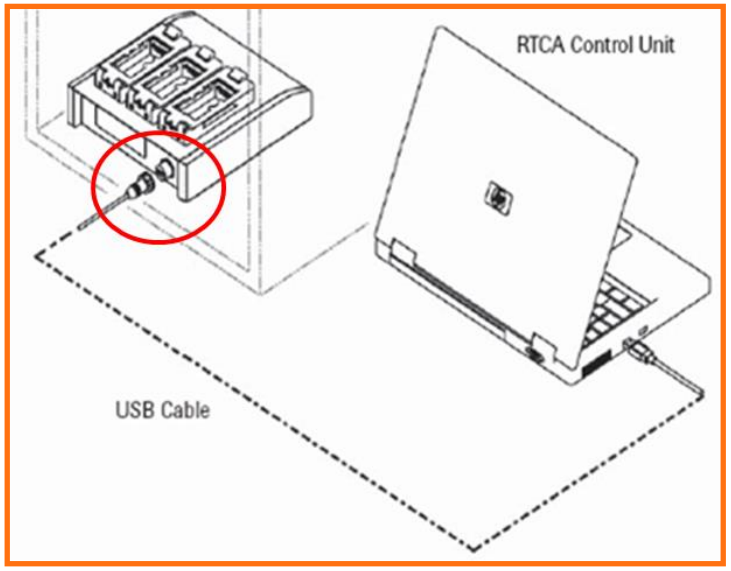


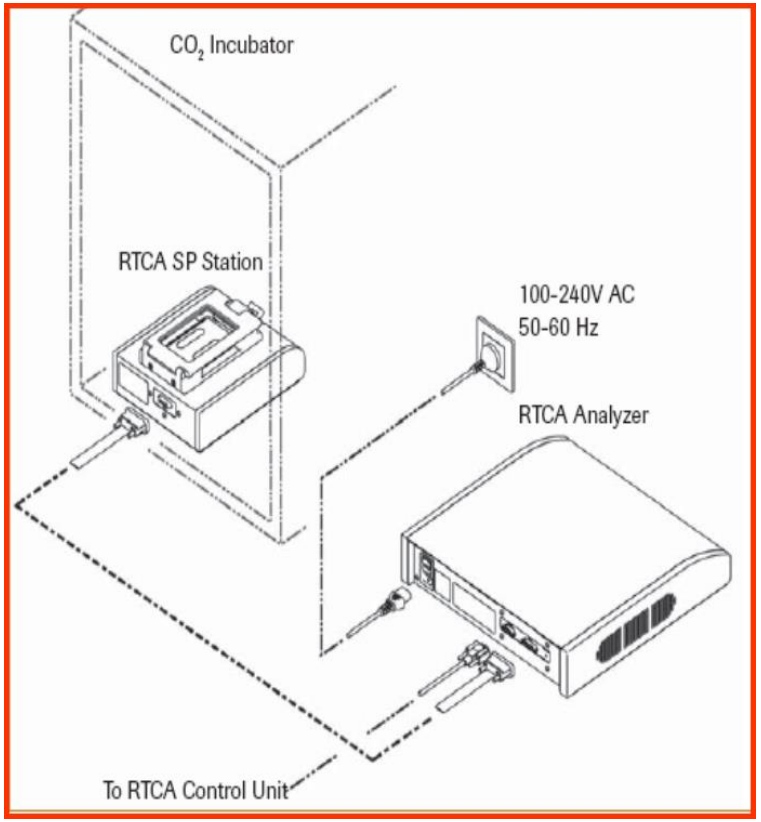
Inspection of the EPS Cable The EPS cable connecting the RTCA MP Station and the Analyzer should be inspected annually. If any sign of corrosion or breakage is observed, the cable should be replaced.



Trouble shoot and Maintenance

Inspection of the RTCA Cable





The cable connecting RTCA DP instrument and the control unit (computer) needs to be inspected annually.

Quick Maintenance Guides

ACEA Biosciences, Inc.

xCELLigence® Real-Time Cell Analysis (RT

QUICK MAINTENANC

PREVENTIVE MAINTENANCE

General Cleaning Using fiber free tissue paper slightly moistened with 80% e
or contaminants from the surface of the RTCA DP Analyzer. A small air blowe
remove any dust.

QC Test: Resistor Plate Verification Program a 3 min experiment (10 sweeps
a resistor plate (Cat. No. 05469783001) in each of the instrument's three cra
test, check the "Cell Index Page". If the Cell Index values are lower than 0.06
test. If the cell Index of any well is higher than 0.063, the RTCA Contact Pins
should be cleaned or replaced (see below).

Cleaning the RTCA Contact Pins To guarantee proper functioning of the RTC
Contact Pins regularly (every 2-3 months) by scrubbing them with the fiber-f
system. Gently brush across the tips of the Contact Pins in one direction tov
pocket 10 times.

Exchanging the RTCA Contact Pins If the Cell Index value of any well in the
still larger than 0.063 after the Contact Pin cleaning procedure, the Contact
identify and exchange a damaged/failed Contact Pin, refer to the RTCA DP I
pages 69-71 for detailed instructions.

Inspection of the RTCA USB Cable The USB cable connecting the RTCA DP
(computer) needs to be inspected annually. If any sign of corrosion or breaka
be replaced. The general life span of the USB cable is 2-3 years.

DECONTAMINATION

Decontamination of the RTCA DP Analyzer and RTCA Control Unit The follo
RTCA DP instrument and cables:

- 80% ethanol
- A mixture containing propan-1-ol (450 mg/g), propan-2-ol 250 (mg/g), and
  Chemie under the tradename Bacillol® AF. When used as described in the
  tuberculocidal, mycobactericidal, and virucidal against enveloped viruses (i
  and Rotavirus.

MAINTENANCE ITEMS

RTCA Resistor Plate 16	Cat. No. 0
RTCA Contact Pins 16 (20 units)	Cat. No. 0
USB Cable RTCA DP Analyzer	Cat No. 0
Cleaning Tool Kit	Cat No. 0

CONSUMABLES

Product	Cat No.	Descript
CIM-Plate 16	05665817001	1 x 6 pla
CIM-Plate 16	05665825001	6 x 6 pla
E-Plate 16	05469830001	1 x 6 pla
E-Plate 16	05469813001	6 x 6 pla
E-Plate VIEW 16	06324738001	1 x 6 pla
E-Plate VIEW 16	06324746001	6 x 6 pla
E-Plate VIEW 16 PET	00300600890	1 x 6 pla
E-Plate VIEW 16 PET	00300600880	6 x 6 pla
E-Plate Insert 16	06465382001	1 x 6 ins
CIM-Plate 16 Assembly Tool	05665841001	1 x CIM

6779 M

xCELLigence® Real-Time Cell Analysis (RTCA) SP

QUICK MAINTENANCE GUIDE

PREVENTIVE MAINTENANCE

General Cleaning Using fiber free tissue paper slightly moistened with 80% ethanol, gently wip
or contaminants from the surface of the RTCA SP Station. A small air blower can also be used t
remove any dust.

QC Test: Resistor Plate Verification Program a 5 min experiment (10 sweeps, 30 sec apart). Ru
experiment with a resistor plate (Cat. No. 05232350001) in the instrument's cradle. Upon com
test, check the "Cell Index Page". If the Cell Index values are lower than 0.063, the system has
QC test. If the cell Index of any well is higher than 0.063, the RTCA Contact Pins 96 (Cat. No.
05232384001) should be cleaned or replaced (see below).

Cleaning the RTCA Contact Pins To guarantee proper functioning of the RTCA SP Instrument,
RTCA Contact Pins regularly (every 2-3 months) by scrubbing them with the fiber-free nylon b
with the system. Gently brush across the tips of the Contact Pins in one direction towards the
cradle pocket 10 times.

Changing the RTCA Contact Pins If the Cell Index value of any well in the resister plate verific
still larger than 0.063 after the Contact Pin cleaning procedure, the Contact Pins 96 should be
identify and exchange a damaged/failed Contact Pin, refer to the RTCA SP Instrument Operat
pages 46-47 for detailed instructions.

Inspection of the EPS Cable The EPS cable connecting the RTCA SP Station and the Analyzer
inspected annually. If any sign of corrosion or breakage is observed, the cable should be replac

DECONTAMINATION

Decontamination of the RTCA SP Instrument The following solutions are compatible with the
and cables:

- 80% ethanol
- A mixture containing propan-1-ol (450 mg/g), propan-2-ol 250 (mg/g), and ethanol 47 (mg/g
  Chemie under the tradename Bacillol® AF. When used as described in the supplier's instruct
  tuberculocidal, mycobactericidal, and virucidal against enveloped viruses (including HBV, HIV
  and Rotavirus.

MAINTENANCE ITEMS

RTCA Resistor Plate 96	Cat No. 05232350001
RTCA Contact Pins 96 (20 units)	Cat No. 05232384001
EPS Cable, RTCA SP/MP Instrument	Cat No. 05291437001
Cleaning Tool Kits for DP/SP/MP/Cardio	Cat No. 00380101390

CONSUMABLES

Product	Cat No.	Description
E-Plate 96	05232368001	1 x 6 plates
E-Plate 96	05232376001	6 x 6 plates
E-Plate VIEW 96	06472451001	1 x 6 plates
E-Plate VIEW 96	06472460001	6 x 6 plates
E-Plate VIEW 96 PET	00300600910	1 x 6 plates
E-Plate VIEW 96 PET	00300600900	6 x 6 plates
E-Plate Insert 16	06465382001	1 x 6 inserts with receiver
E-Plate Insert 96	06465412001	6 x 6 inserts with receiver
E-Plate Insert 96 Accessories	06465455001	6 x Receiver Plate 96

6779 Mesa Ridge Road Ste 100

xCELLigence® Real-Time Cell Analysis (RTCA) MP Instrument

QUICK MAINTENANCE GUIDE

PREVENTIVE MAINTENANCE

General Cleaning Using fiber free tissue paper slightly moistened with 80% ethanol, gently wipe off any dust or contaminants from the surface of the RTCA MP Station. A small air blower can also be used to help remove any dust.

QC Test: Resistor Plate Verification Program a 5 min experiment (10 sweeps, 30 sec apart). Run the experiment with a resistor plate (Cat. No. 05232350001) in each of the instrument's six cradles. Upon completion of the test, check the "Cell Index Page". If the Cell Index values are lower than 0.063, the system has passed the QC test. If the cell Index of any well is higher than 0.063, the RTCA Contact Pins 96 (Cat. No. 05232384001) should be cleaned or replaced (see below).

Cleaning the RTCA Contact Pins To guarantee proper functioning of the RTCA MP Instrument, clean the RTCA Contact Pins regularly (every 2-3 months) by scrubbing them with the fiber-free nylon brush provided with the system. Gently brush across the tips of the Contact Pins in one direction towards the center of the cradle pocket 10 times.

Changing the RTCA Contact Pins If the Cell Index value of any well in the resister plate verification test is still larger than 0.063 after the Contact Pin cleaning procedure, the Contact Pins 96 should be replaced. To identify and exchange a damaged/failed Contact Pin, refer to the RTCA MP Instrument Operator's Manual pages 52-53 for detailed instructions.

Inspection of the EPS Cable The EPS cable connecting the RTCA MP Station and the Analyzer should be inspected annually. If any sign of corrosion or breakage is observed, the cable should be replaced.

DECONTAMINATION

Decontamination of the RTCA MP Instrument The following solutions are compatible with the surfaces of the RTCA MP instrument and cables:

- 80% ethanol
- A mixture containing propan-1-ol (450 mg/g), propan-2-ol 250 (mg/g), and ethanol 47 (mg/g). This mixture is available from Bode Chemie under the tradename Bacillol® AF. When used as described in the supplier's instructions, this mixture is fungicidal, tuberculocidal, mycobactericidal, and virucidal against enveloped viruses (including HBV, HIV, HCV), Adenovirus, FCV, Papovavirus, and Rotavirus.

MAINTENANCE ITEMS

RTCA Resistor Plate 96	Cat. No. 05232350001
RTCA Contact Pins 96 (20 units)	Cat. No. 05232384001
EPS Cable, RTCA SP/MP Instrument	Cat No. 05291437001
Cleaning Tool Kits for DP/SP/MP/Cardio	Cat No. 00380101390

CONSUMABLES

Product	Cat No.	Description
E-Plate 96	05232368001	1 x 6 plates
E-Plate 96	05232376001	6 x 6 plates
E-Plate VIEW 96	06472451001	1 x 6 plates
E-Plate VIEW 96	06472460001	6 x 6 plates
E-Plate VIEW 96 PET	00300600910	1 x 6 plates
E-Plate VIEW 96 PET	00300600900	6 x 6 plates
E-Plate Insert 16	06465382001	1 x 6 inserts with receiver plate 16
E-Plate Insert 96	06465412001	6 x 6 inserts with receiver plate 96
E-Plate Insert 96 Accessories	06465455001	6 x Receiver Plate 96

ACEA Biosciences, Inc.

6779 Mesa Ridge Road Ste 100 San Diego, CA 92121 | 858.300.2232 | www.aceabio.com

Summary

1. Introduction to ACEA Biosciences and xCELLigence technology
2. The utility of the xCELLigence technology for quantitatively monitoring drug mediated cytotoxicity in real-time
3. The protocols, tips, and tricks for conducting drug mediated cytotoxicity assays
4. Real-time demonstration of data analysis and plotting for publications
5. Trouble shoot and Maintenance

Please feel free to write to us appsupport@aceabio.com; techsupport@aceabio.com

thank you
danke
рахмат
謝謝
ngiyabonga
teşekkür ederim
Баярлалаа
спасибо
faafetai lava
mersi
barka
welalin
tack
spas
dank je
misaotra
matondo
paldies
grazzi
mahalo
tapadh leat
yinaka
спасибі
blagodaram
kiitos
dankie
nanni
nandri
dhanyavad
mauruuru
köszönöm
gracias
хвала
asante
manana
obrigada
tenki
bedankt
bayarlalaa
gracie
hvala
dankon
akun
djiere dieuf
tau
mochchakkeram
murakoze
дякую
mamnun
shokrane
enkosi
dziękuję
chnorakaloutioun
gratias ago
gracies
sulpay
taiku
go raibh maith agat
sobodi
dėkuji
obrigado
sagolun
sukriya
kop khun krap
ありがとう
arigatô
takk
dakujem
trugarez
mèsi
didi madloba
najis tuke
tanemirt
grazie
rahmet
kam sah hamnida
rahmat
terima kasih
diolch
dhanyavadagalu
shukriya
merce
мерси
감사합니다
xiexie
ευχαριστώ
merci