

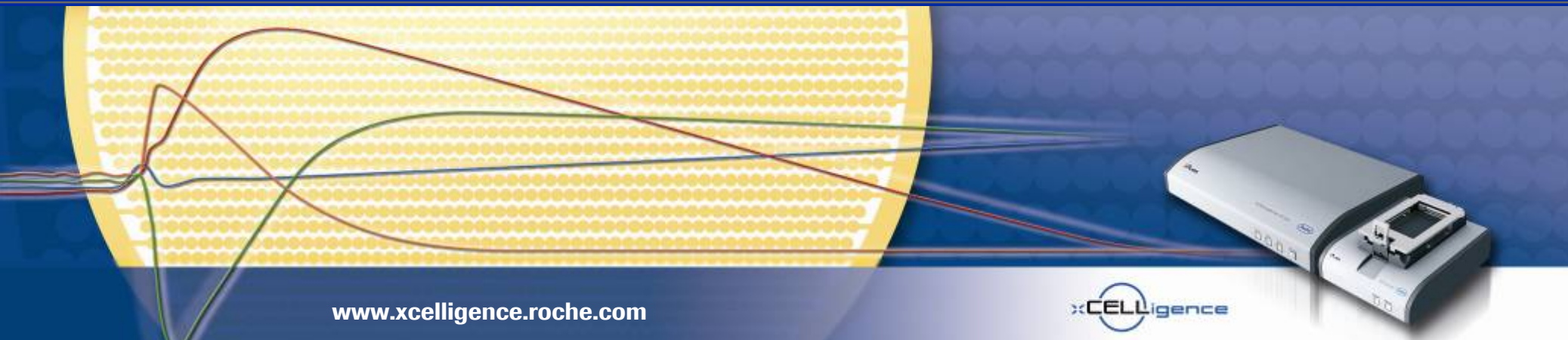
xCELLigence

Label-free, real-time cell monitoring technology

Ang Kok Long, PhD

APAC Regional Product Manager, Cell Biology

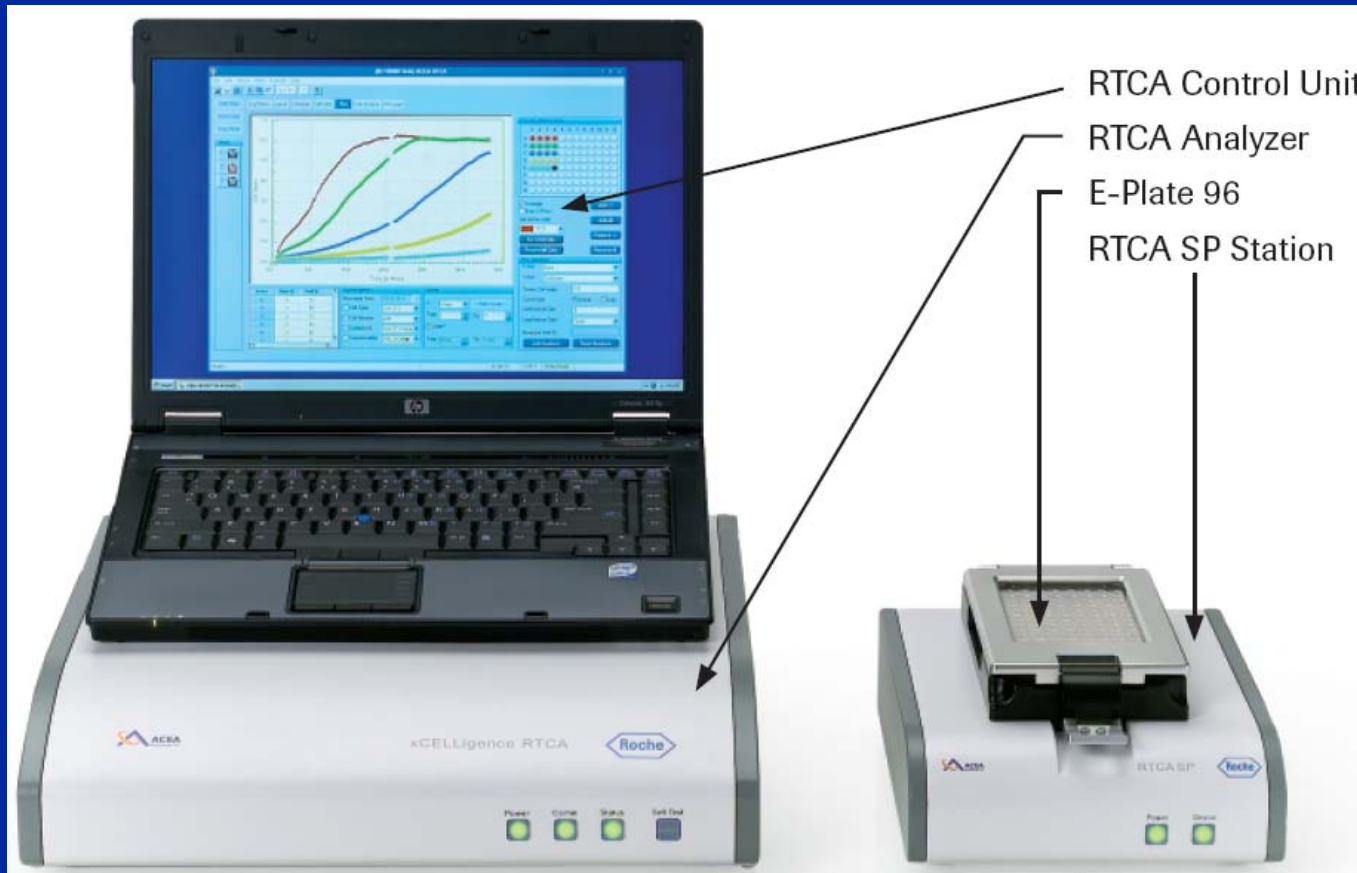
Roche Diagnostics



xCELLigence - Real-Time Cell Analyzer

Partnership of Roche and ACEA

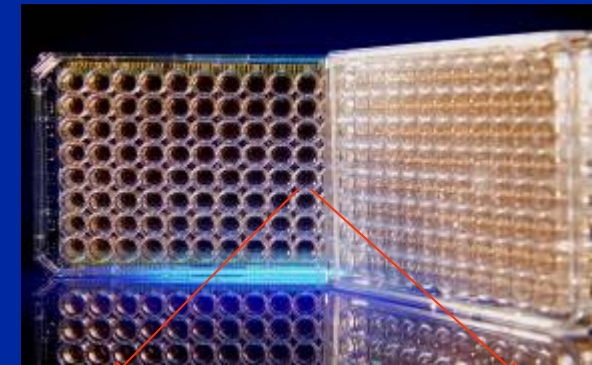
RTCA SP system



on benchtop

in cell incubator

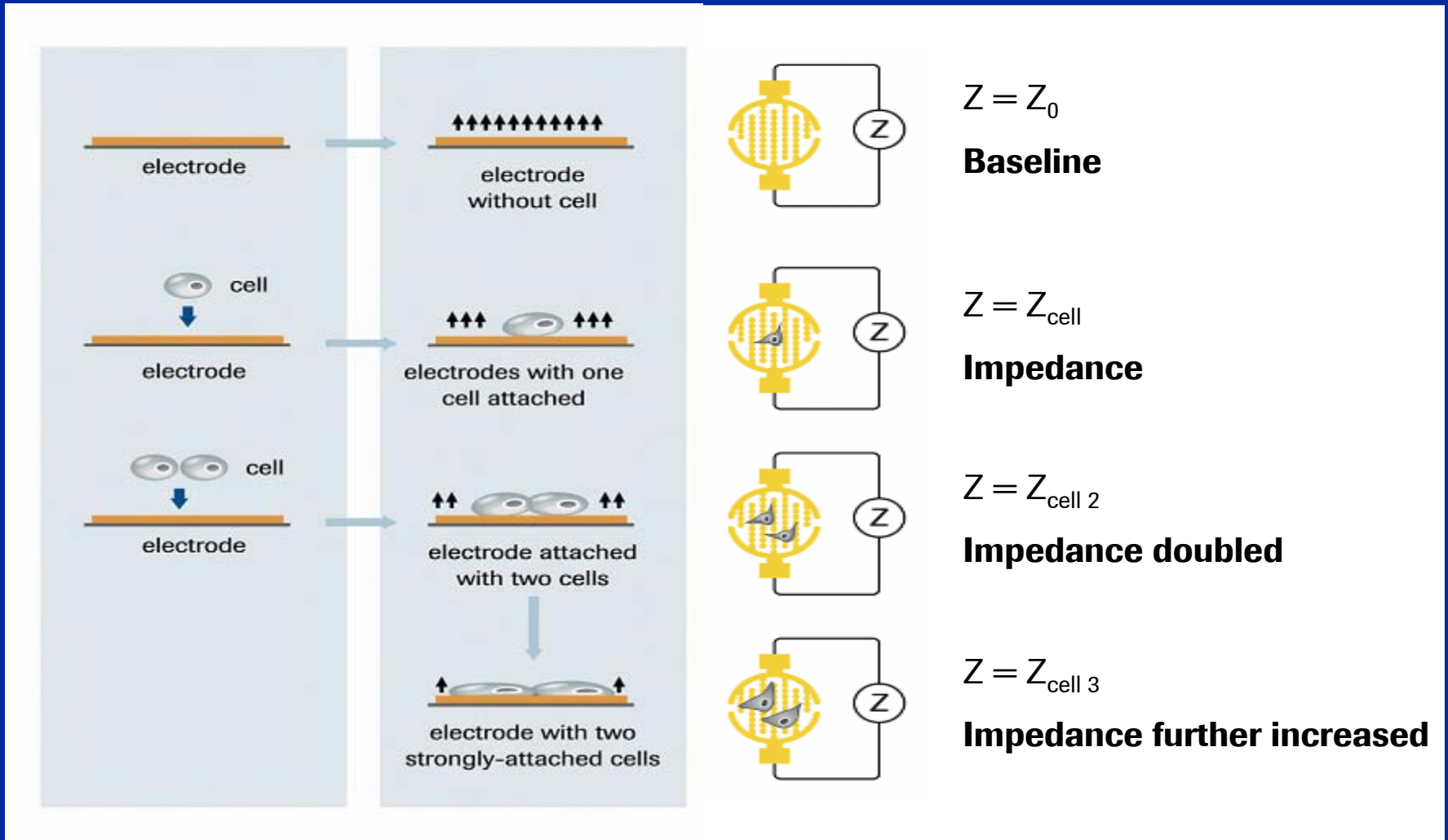
E-Plate 96



Gold Microelectrode Array
(covers ~ 80% of the area on the bottom of a well)

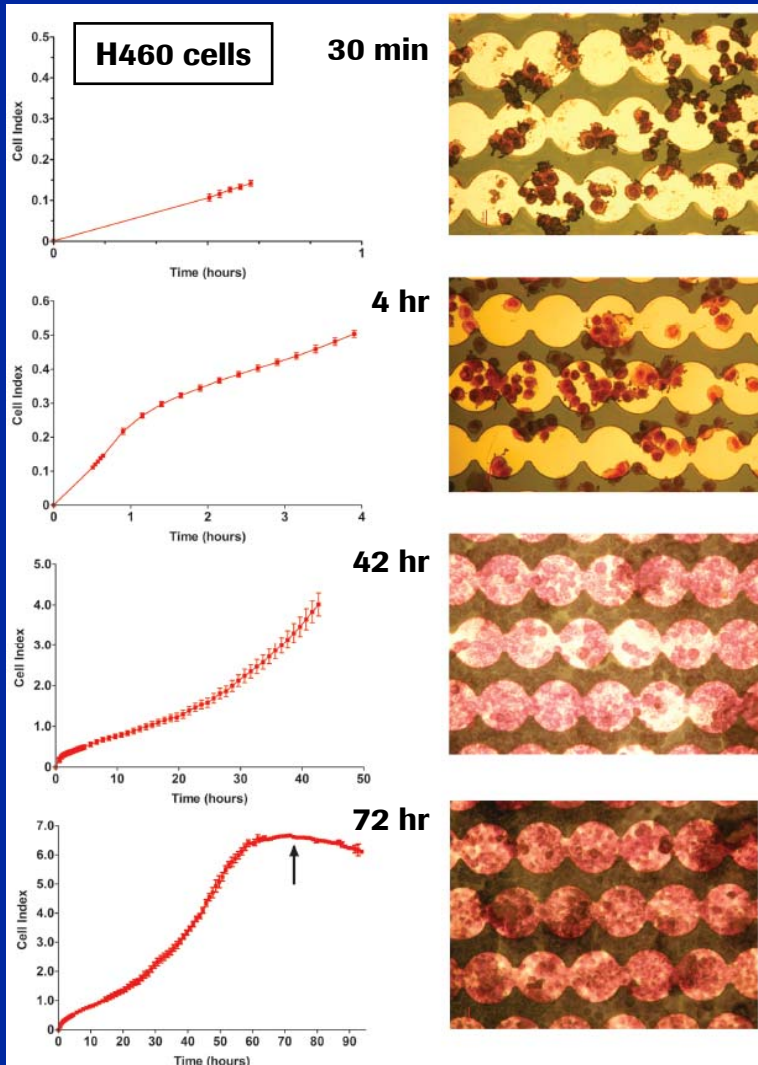
xCELLigence - Real-Time Cell Analyzer

Principle – Electric Cell Impedance Sensing

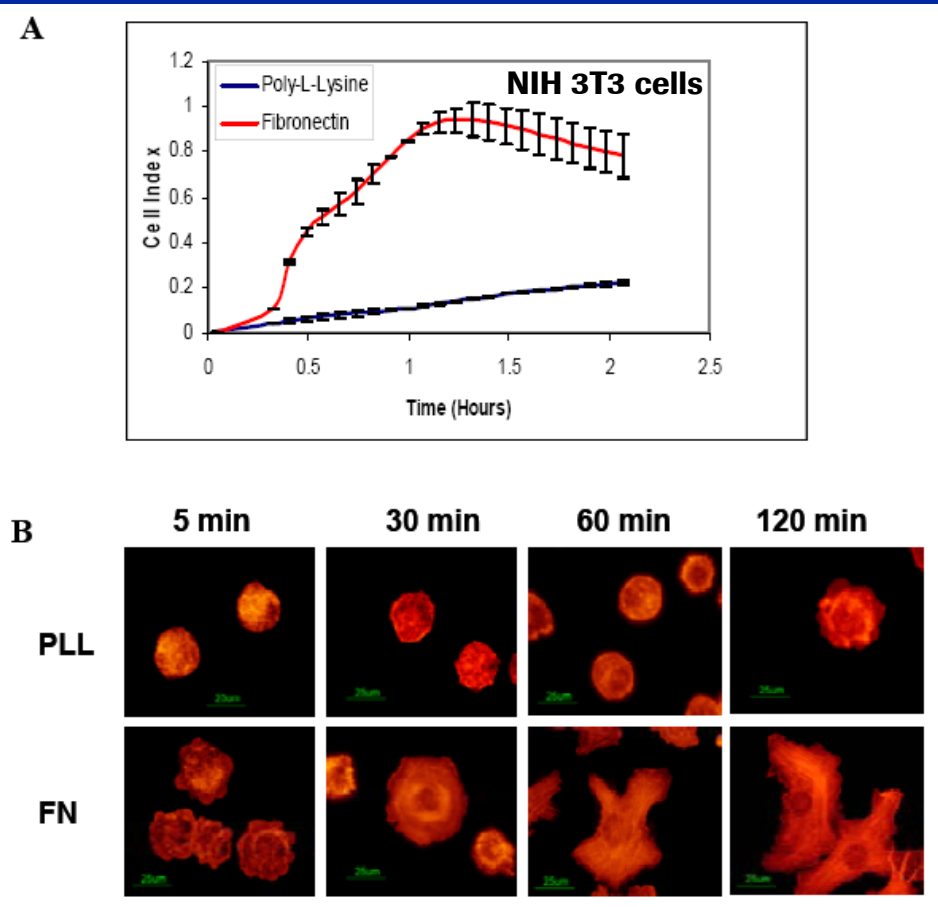


xCELLigence - Real-Time Cell Analyzer

Detect changes in cell number or cell adhesion/morphology



Kirstein_SL et al., 2006

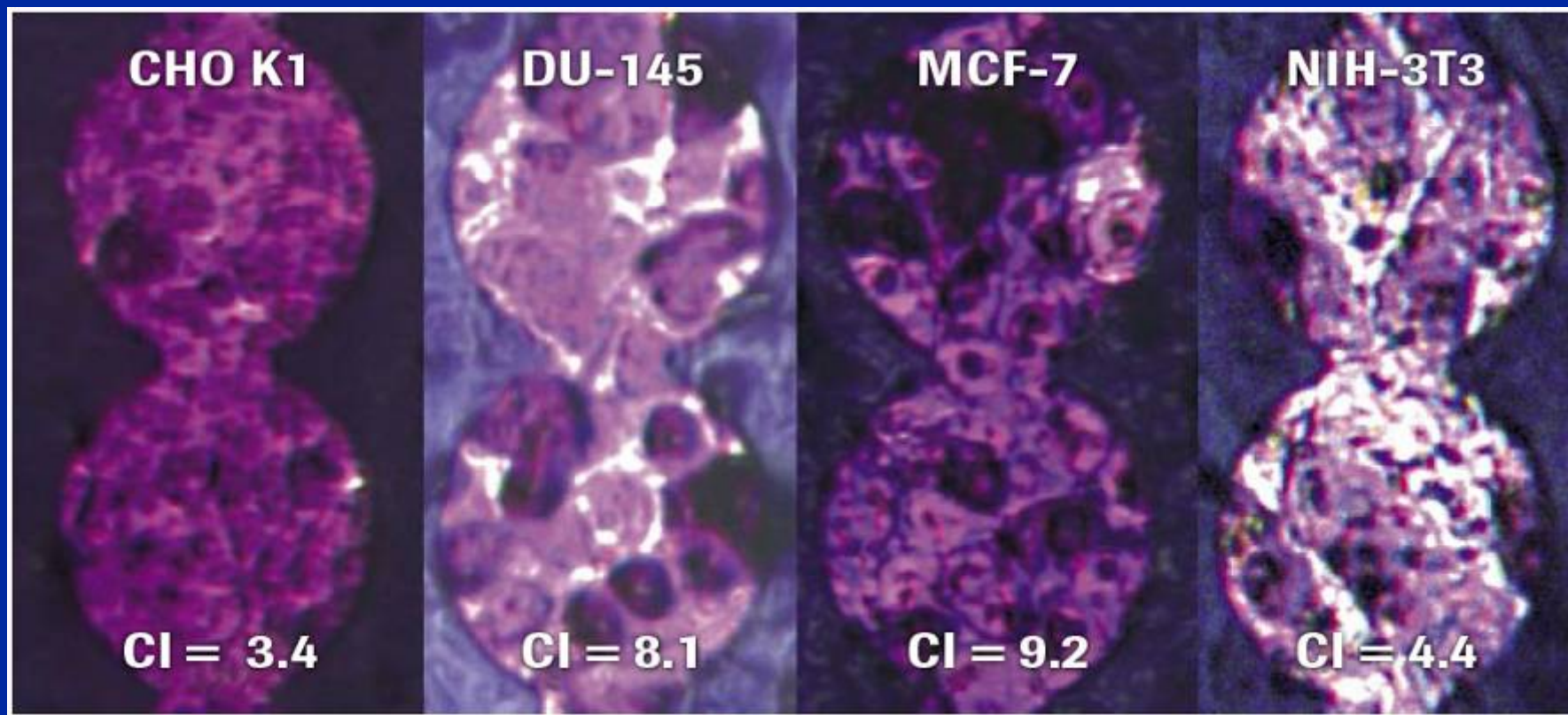


Atienza_JM et al., 2005

A parameter termed **Cell Index** (CI) is derived to represent cell status based on the measured electrical impedance.

xCELLigence - Real-Time Cell Analyzer

Detect adhesion strength



xCELLigence - Real-Time Cell Analyzer

Key features and advantages

Label-free detection

- Cells are assayed in more physiological conditions
- Avoid artifacts that can be introduced by sample handling/preparation
- Avoid compound interference (which can be common in optical-based assays)

Non-invasive, real-time monitoring

- Enables long term assay of live cells
- Use of internal control for each well (before and after treatment)
- Less cells required, important for the use of primary cells/disease-relevant cells from patient biopsy

Automated continuous measurement

- Enables data collection for the entire duration of the experiment with no hands-on time
- More consistent results (by avoiding operator-related variations)

Obtain distinctive cytological profiles of predictive value

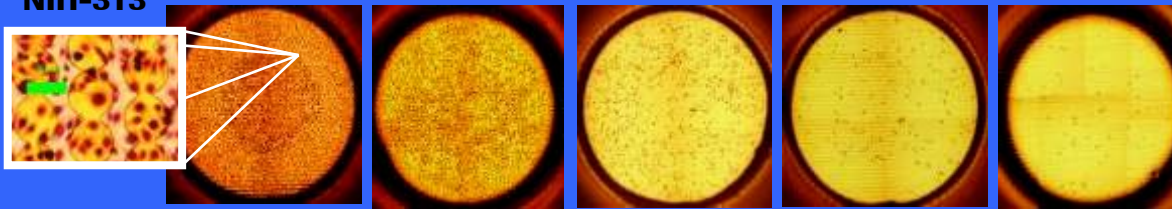
Broad range of applications on a single platform

- *Enable experiments not possible or very difficult to perform with other technologies*
e.g. Long time-course, rapid kinetics and long-term effect, multiple sequential treatments
- *Capture the whole picture and don't miss the effect you want to analyze!*

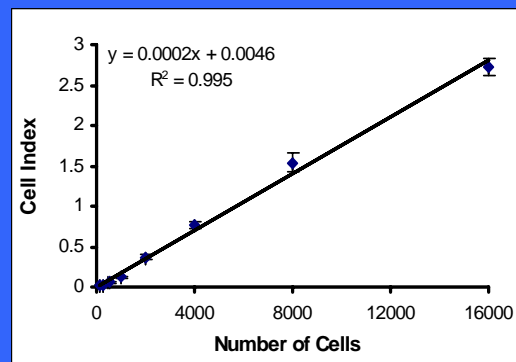
xCELLigence - Real-Time Cell Analyzer

Cell Index is a quantitative measure of cell number

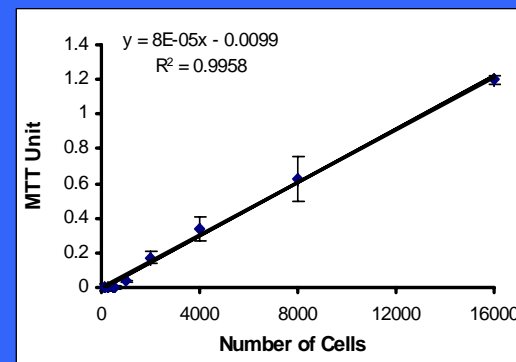
NIH-3T3



Cell number	16,000	4,000	1,000	250	125
Cell index	2.723	0.759	0.121	0.025	0.014
SD	0.103	0.04	0.011	0.004	0.003
CV %	3.8	6.0	8.8	15.2	24.8



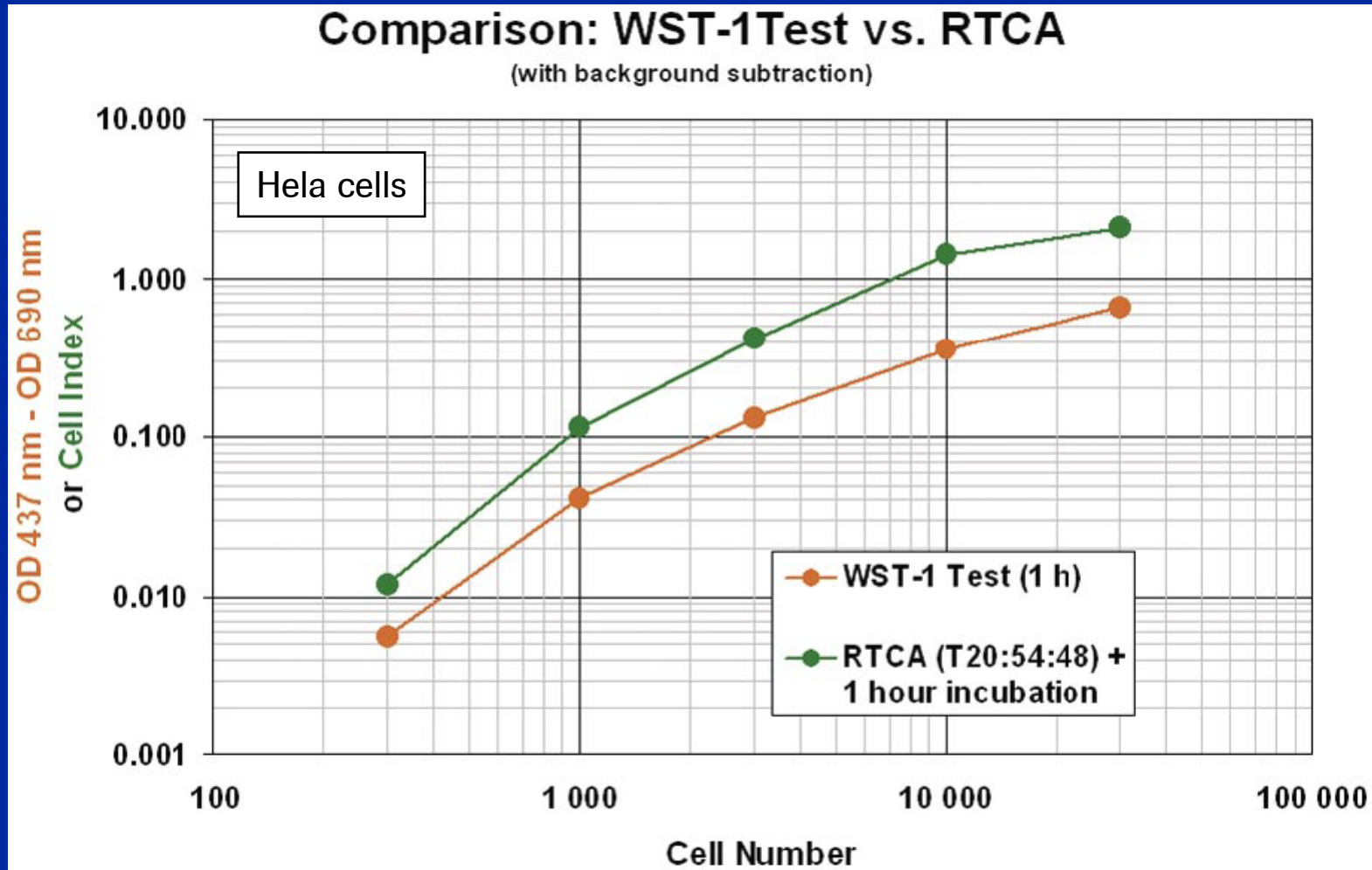
RTCA Assay



MTT Assay

xCELLigence - Real-Time Cell Analyzer

Cell Index is a quantitative measure of cell number



xCELLigence - Broad Applications

Major application areas

Cell culture management and QC

- Cell growth curve, cytological profiles

Comprehensive cytotoxicity assays

- Compound & RNAi screening
- Cell-mediated cytotoxicity
- Virus-mediated cytotoxicity
 - virus titration, virus neutralization
 - minimize exposure risk to pathogen
- Toxicology
 - Cancer drug discovery in pharma
 - *in vitro* predictive toxicology
 - Environment health
 - Agents with unknown MOA
- Profiling tumor cell response to treatment
 - Less cells required, important for use of primary cells/patient biopsy

Monitoring cellular signaling/event

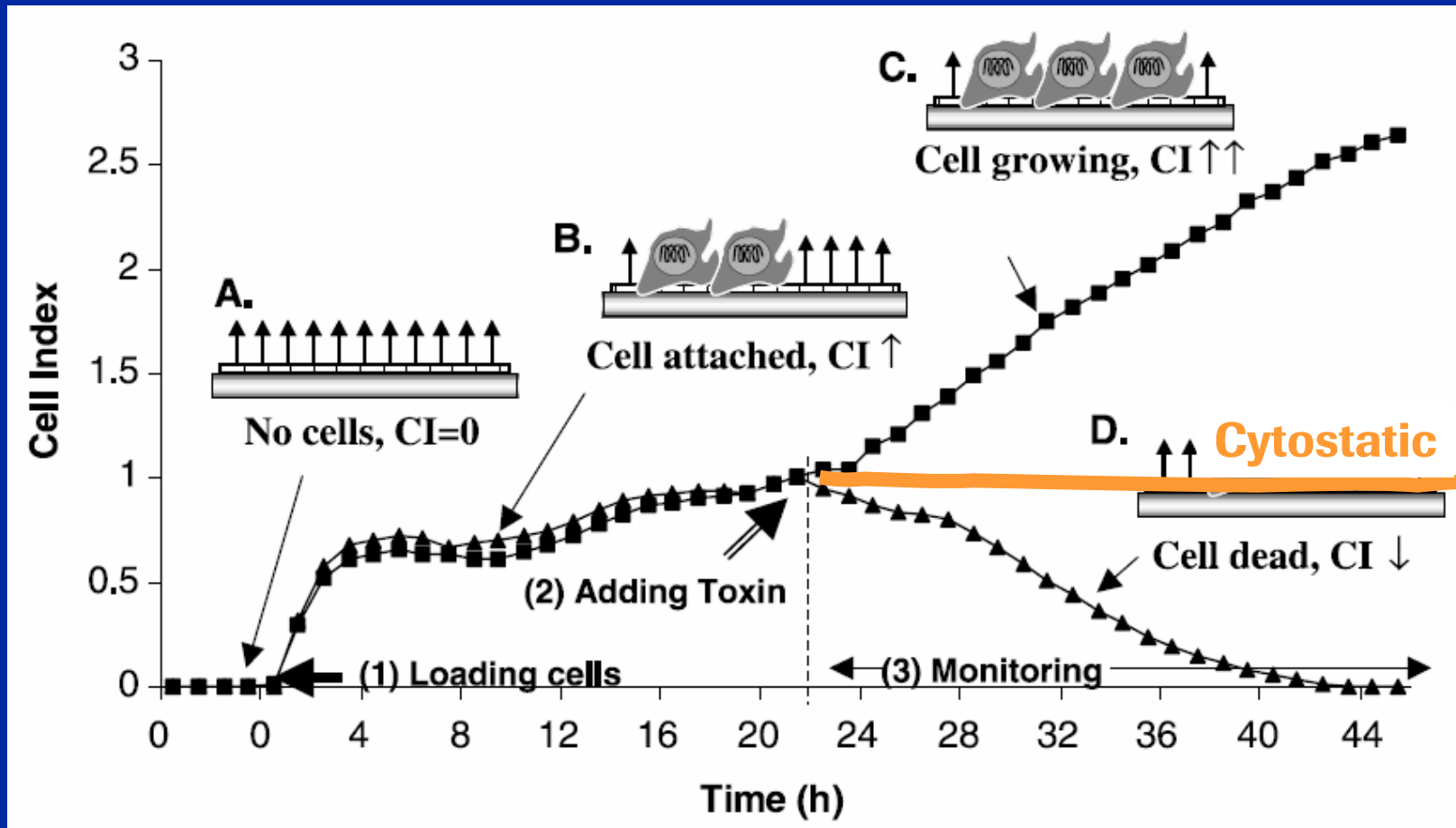
- Cell proliferation, cell death
- Cell differentiation
- Cell adhesion, migration and invasion
- Receptor activation/inhibition (e.g. GPCRs, RTKs)

Facilitate cell-based assay development

- Shorten assay development time
- Simplify optimization of assay conditions
- Define when to do what objectively

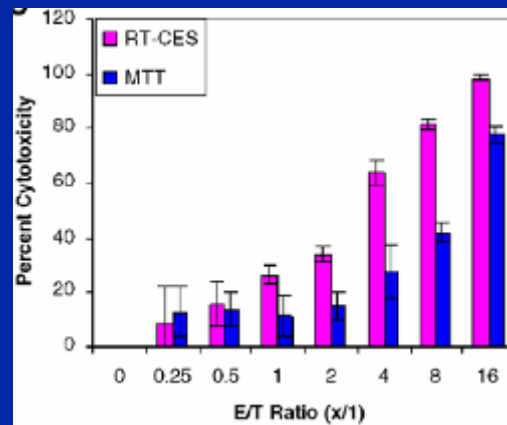
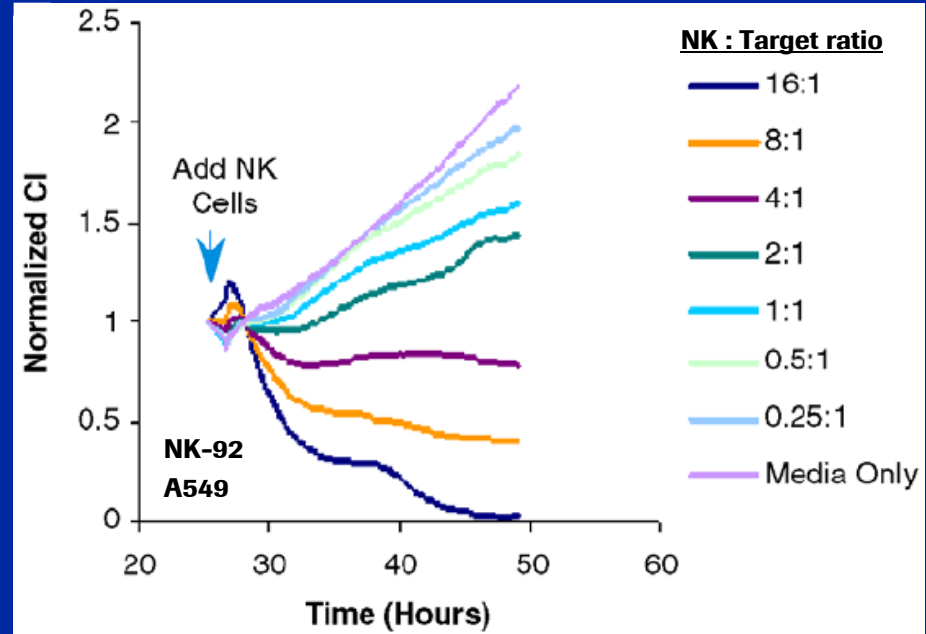
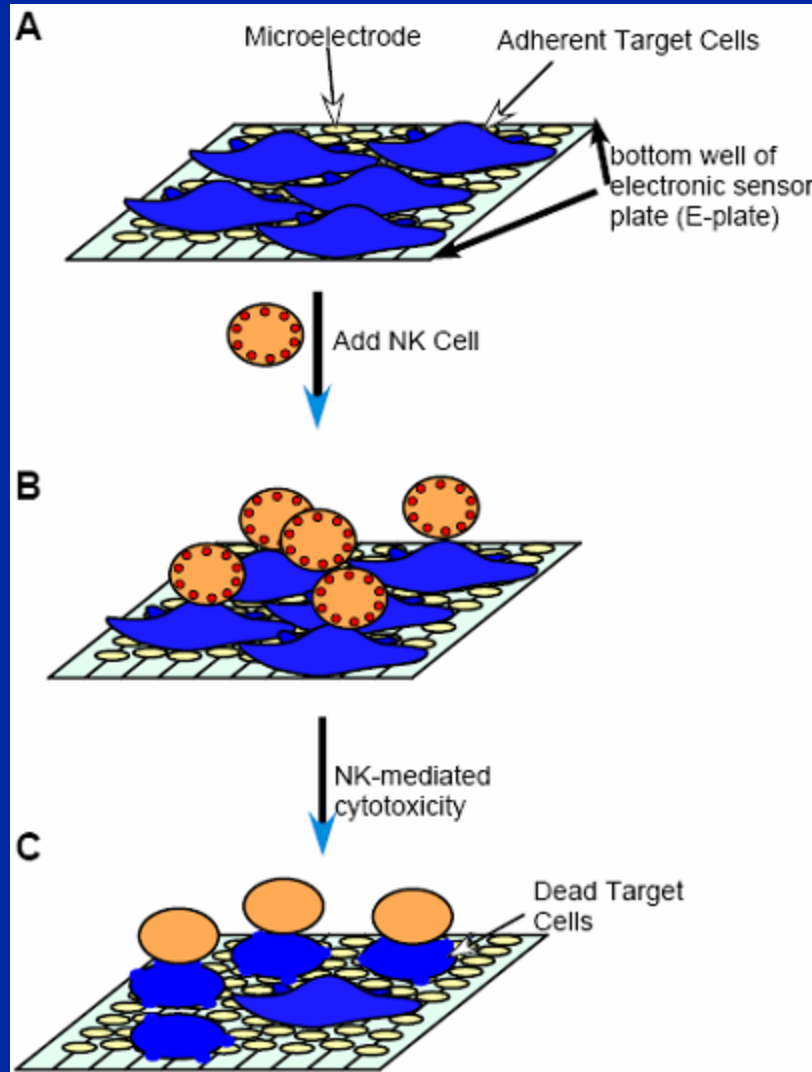
xCELLigence – Cytotoxicity Assays

Compound-mediated cytotoxicity



xCELLigence – Cytotoxicity Assays

Cell-mediated cytotoxicity

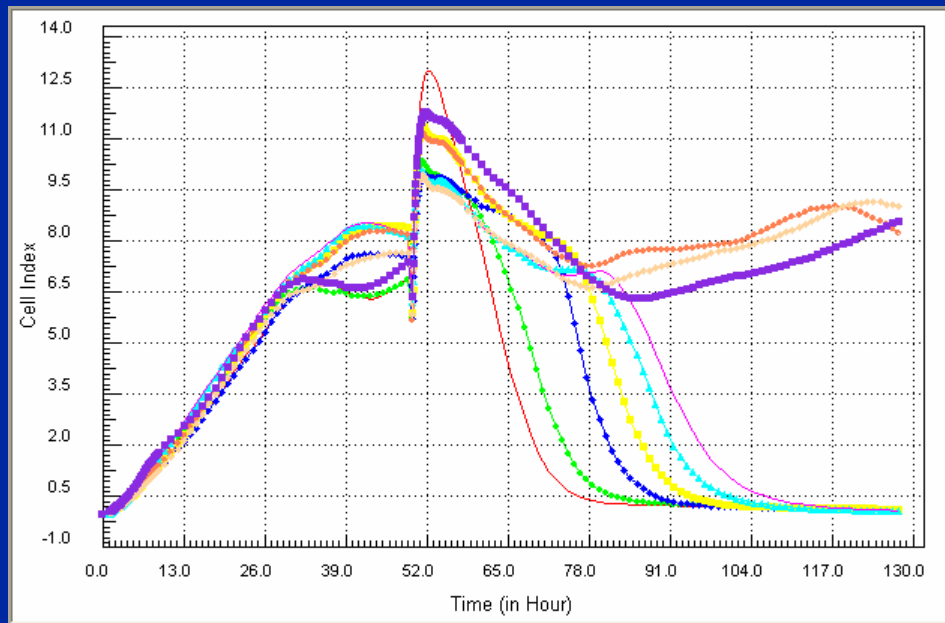


Zhu et al., 2006

xCELLigence – Cytotoxicity Assays

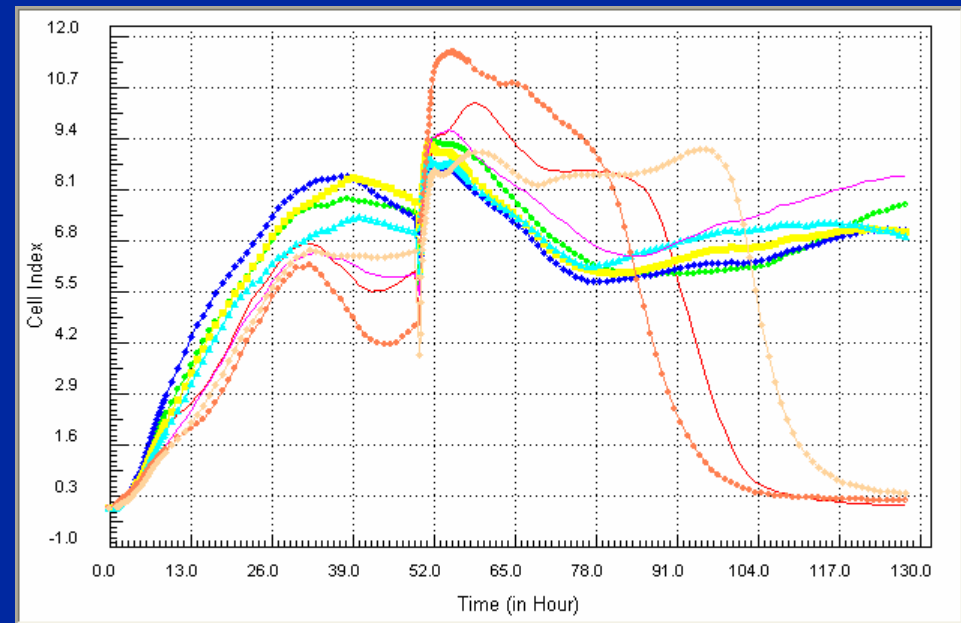
Virus-mediated cytopathic effects (CPE)

Virus titration



MDCK cells + H1N1 virus

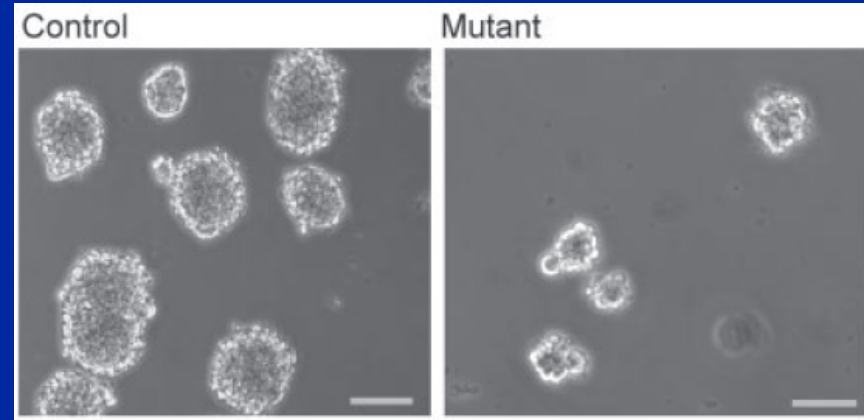
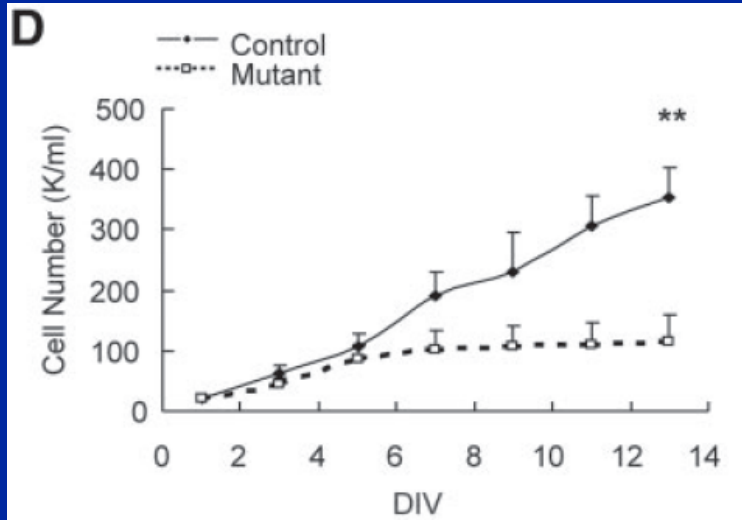
Virus neutralization



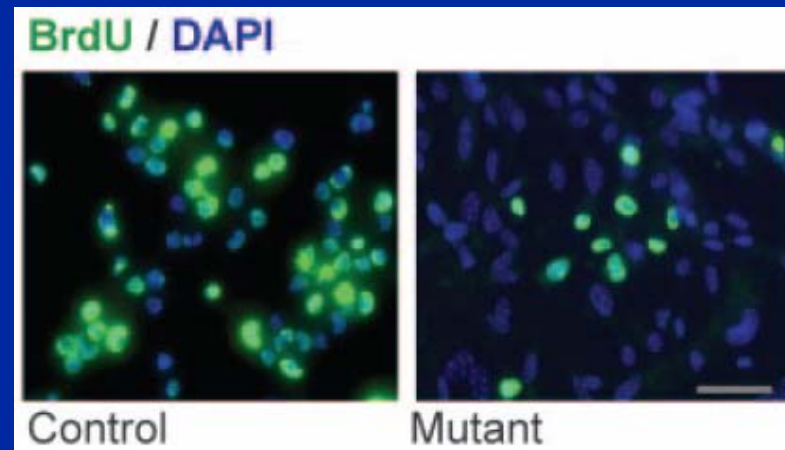
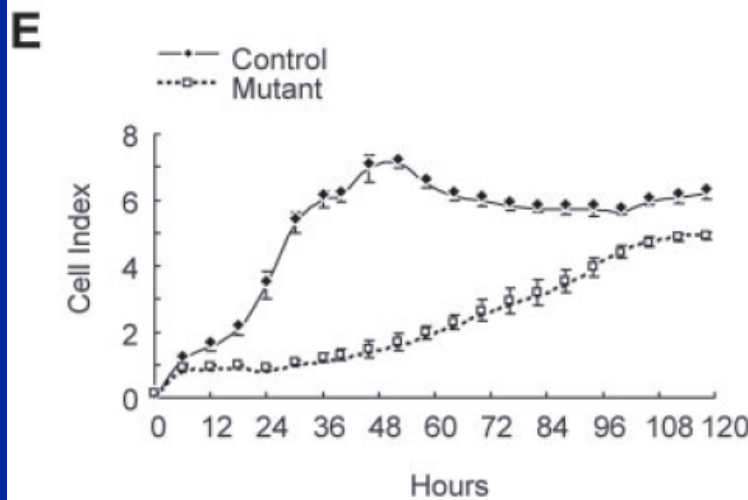
MDCK cells + H1N1 virus + antiserum

Neural Stem Cell (NSC)

Shp2 is required for self-renewal and proliferation of NSCs

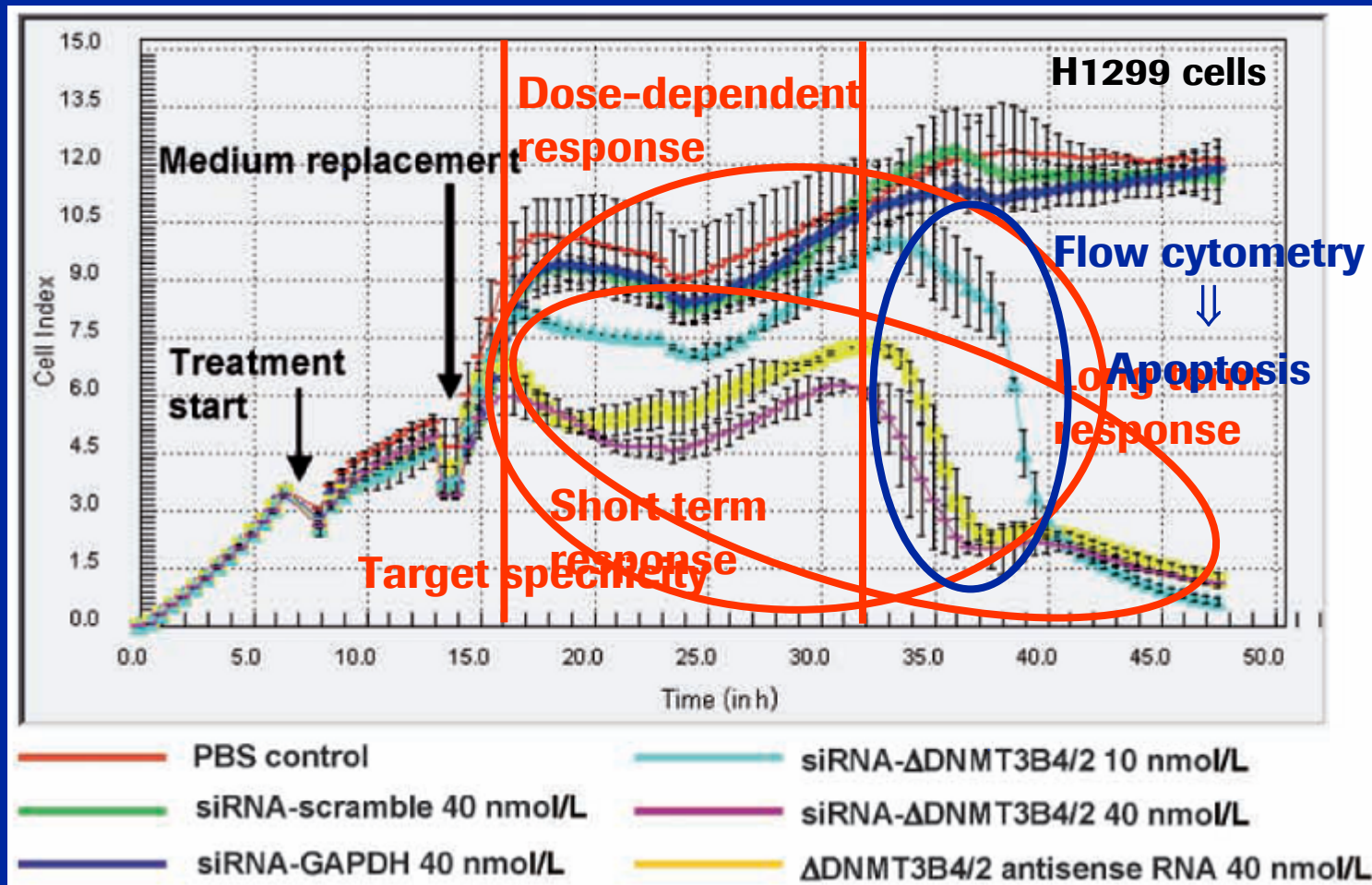


Control: Normal NSC
Mutant: Shp2-deficient NSC



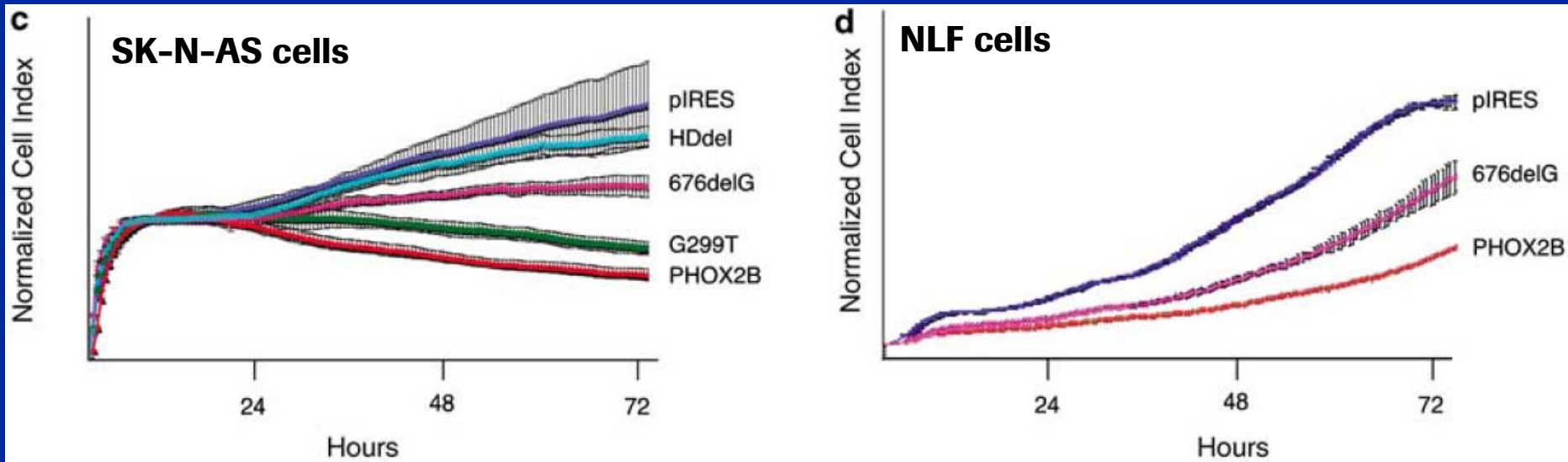
Capture the whole picture and don't miss any effect!

Knockdown of DNMT3B4/2 inhibits cell growth and promotes apoptosis



Gene Overexpression

Functional consequence of exogenous wild-type and mutant PHOX2B



Real-Time Assessment of Cytotoxicity

Easier, less laborious, time-dependent IC50 values

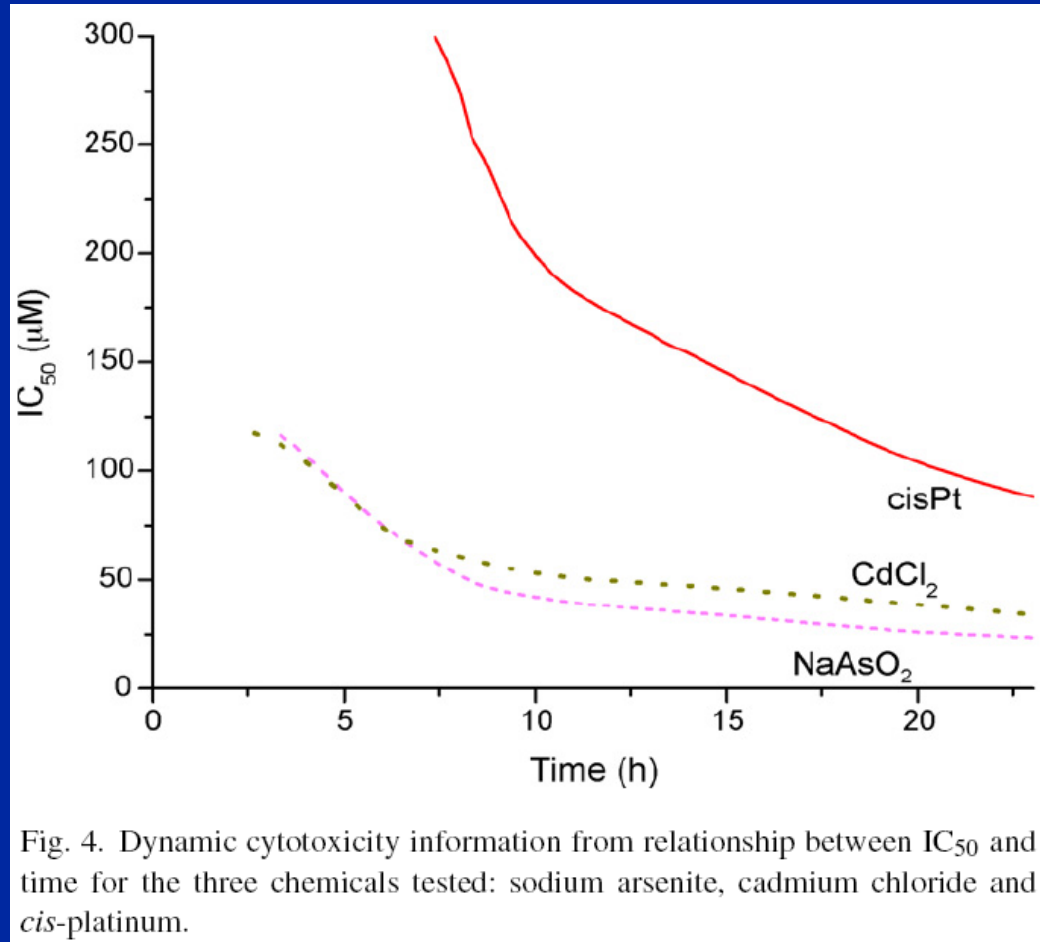
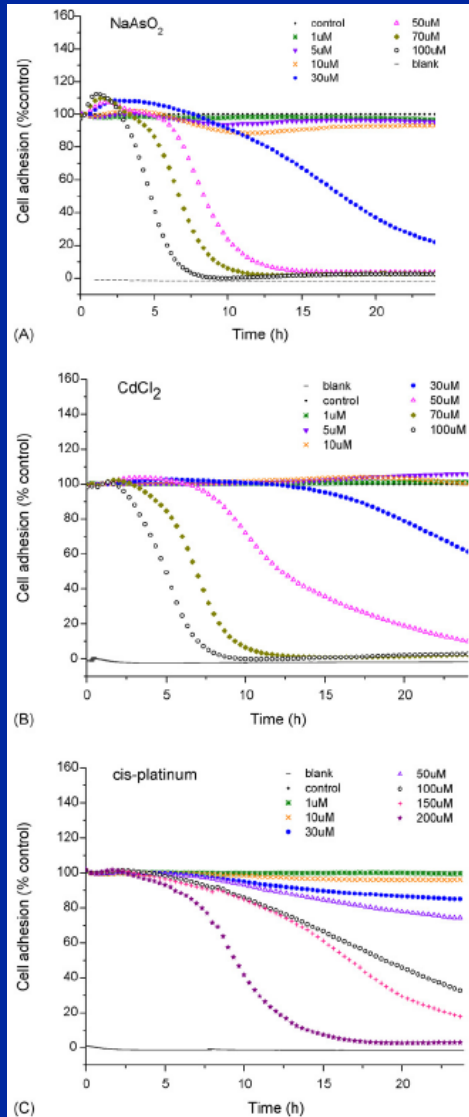
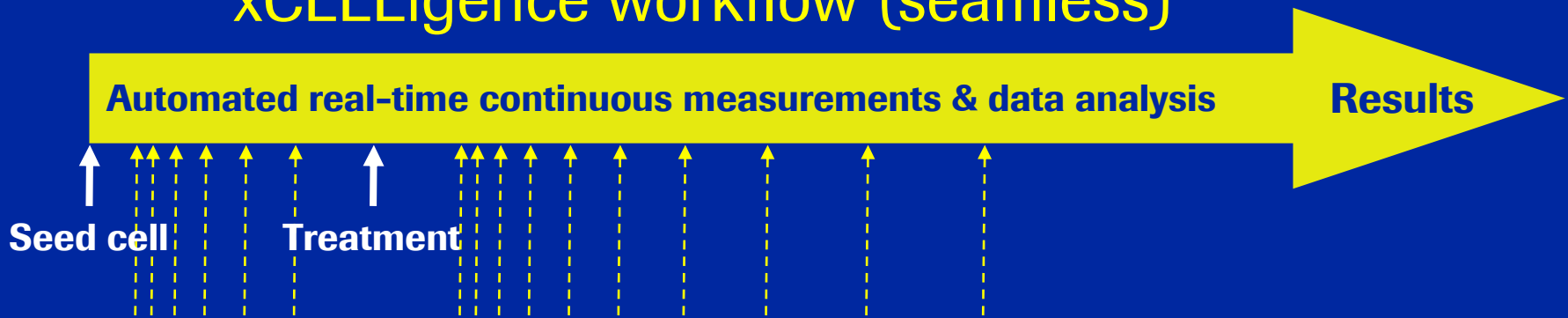


Fig. 4. Dynamic cytotoxicity information from relationship between IC₅₀ and time for the three chemicals tested: sodium arsenite, cadmium chloride and *cis*-platinum.

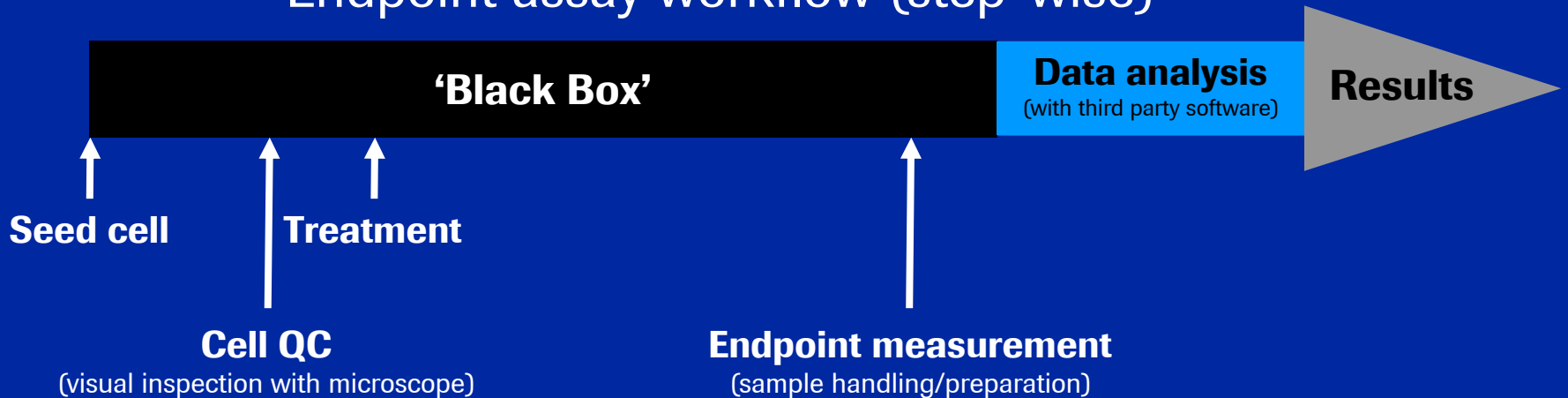
xCELLigence - Real-Time Cell Analyzer

Improve cell-based assay workflow

xCELLigence workflow (seamless)



Endpoint assay workflow (step-wise)



xCELLigence - Real-Time Cell Analyzer

Distinctive cytological profiles

A new way to look at cell analysis

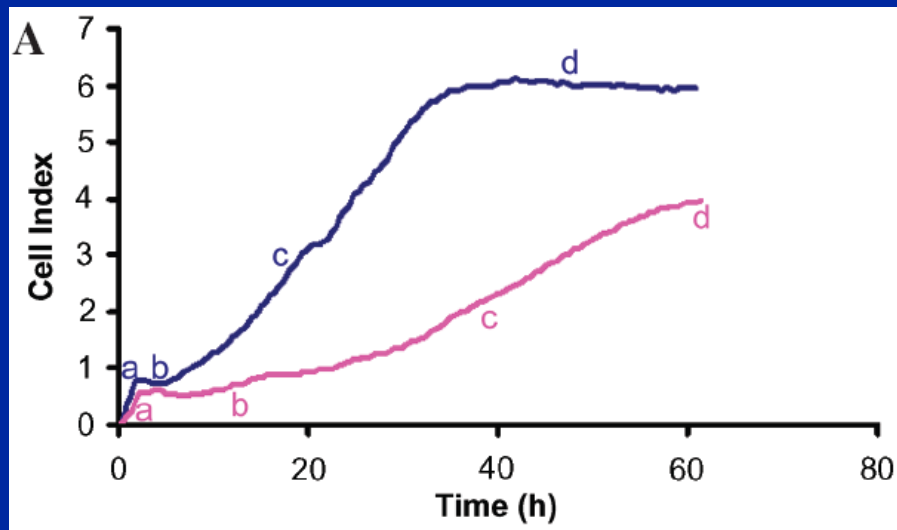
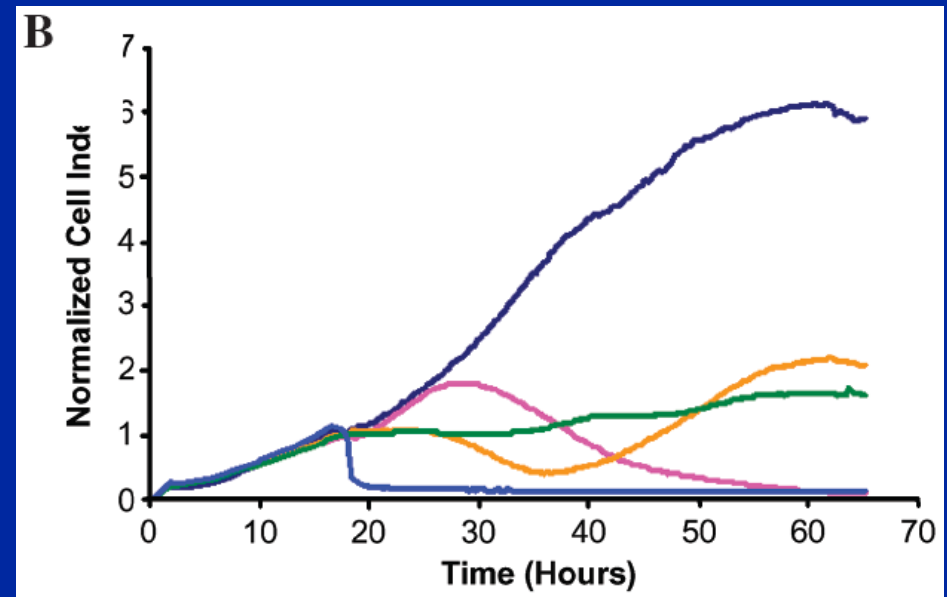


FIG. 2. (A) Dynamic proliferation curves for NIH3T3 (—) and HT1080 (—) cells seeded at 10,000 cells per well. Each cell line displays very distinct features: (a) cell attachment and spreading phase; (b) characteristic lag phase unique to each cell line; (c) logarithmic growth phase; and (d) confluent phase.



(B) Cytological profile of A549 cells treated with compounds having distinct mechanisms of action: control (—), DNA-damaging (—), antimitotic (—), cytostatic (—), and cytoskeletal (—).

xCELLigence System

Key Product Launches 2008/2009

Q3 08

Q4 08

Q1 09

RTCA SP

SP (1x96) station
W 380 analyzer
SP SW, laptop
96-well E[®]- Plate

Jul



RTCA MP

MP (6x96) station
W 380 analyzer
MP SW, laptop
96-well E[®]- Plate

Nov



RTCA DP

DP (3x16) int. station
SW, laptop
16-well E[®]- Plate







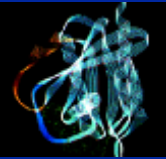




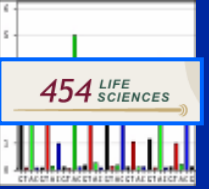
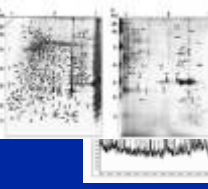
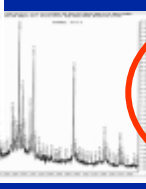

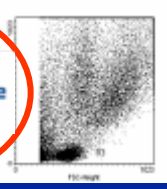
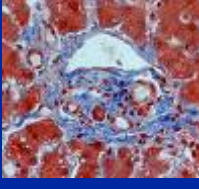
Feb



16-well CIM Device

xCELLigence – Entry into Cytomics

RAS strategy - Complete Solution Provider Inspiring Discovery

Sample	  			 				
	Subcellular	Cells	Organs	Organisms	Communities			
Target								
	DNA, RNA			Proteins	Cells	Tissues		
Detection	  			 		  		
	RT-PCR	Microarray	Sequencing	2D-GE	MS	Function	FC	IHC/ISH
	NA Isolation MagNA Pure			Protein Isolation		Cell Isolation		
	Genomics			Proteomics		Cytomics		
	Molecular Profiling				Cytological Profiling			

xCELLigence system

An essential tool in labs working with cells

Just Add Cells !

- Simple maintenance-free system, easy to set-up and use
- Direct quantitative measurement, no sample handling/preparation required

One For All !

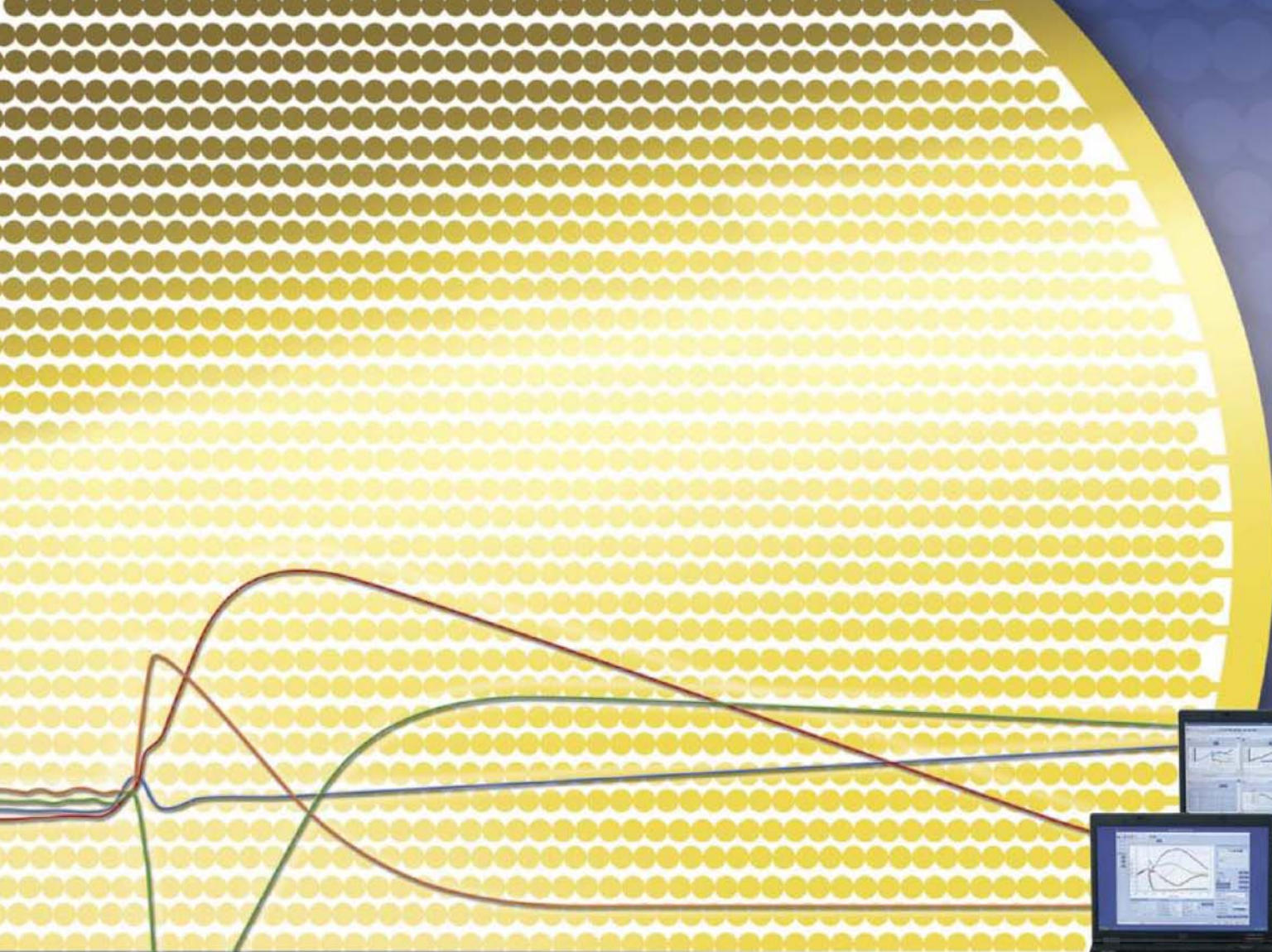
- Broad range of applications on one platform, with intuitive integrated software

Greater Insight, True Understanding !

- Non invasive, real-time continuous monitoring of live cell assays over long duration
- Capture the entire experiment and get the whole picture
- Label-free, compatible with many other assays

Obtain Results Not Possible With Endpoint Assays !

- Distinctive cytological profile - QC, troubleshooting, cell response profiling predictive of MOA
- Improve research quality, productivity and inspiring discovery



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Greater Insight, True Understanding

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