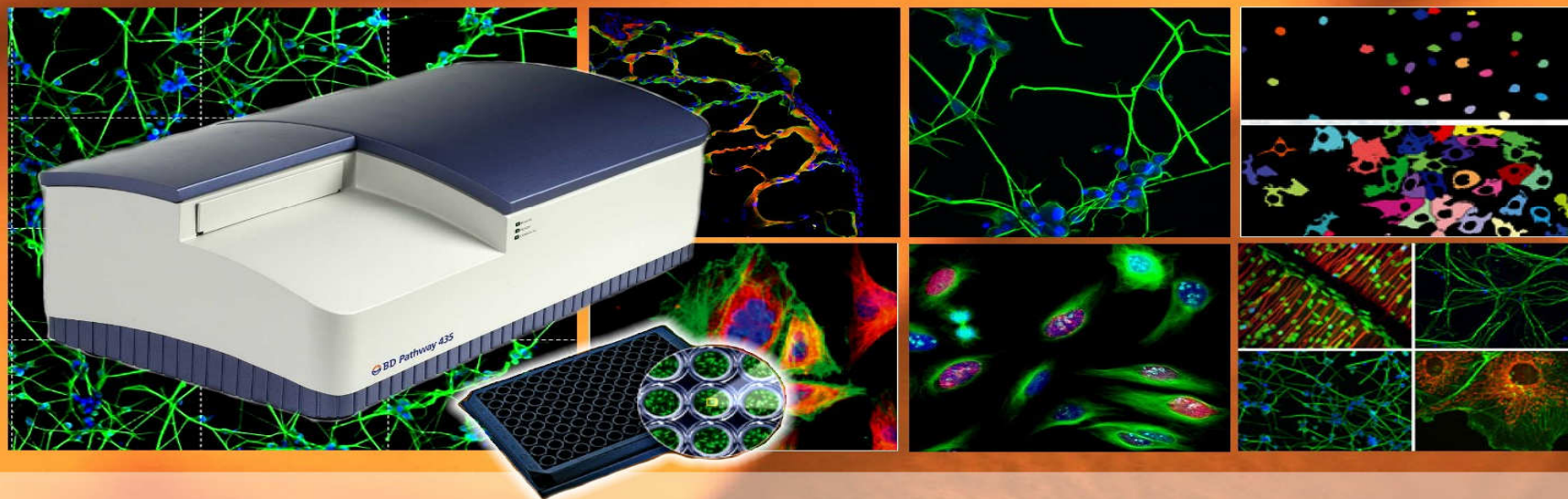


高通量、高內涵、自動化 共軛焦細胞影像分析系統的原理與應用



Helping all people
live healthy lives



主題：Principle , Application and Current state of Confocal base
High Throughput / High Content Bioimaging Sytem
高通量、高內涵、自動化 共軛焦細胞影像分析系統的原理與應用

時間：98年7月29(星期三) 上午 10:00-11:30

下午 14:00-16:00 (兩場課程相同請同仁任選一場參加)

地 點：教學研究部 (研究大樓第三會場)

演講者：楊利君 Glenn Yang - 尚博生物科技有限公司 產品部主任

Glenn Yang.

主辦單位：臺中榮民總醫院 教學研究部

協辦單位：尚博生物科技有限公司

TEL:8862-27855860

CELL:0953062485

Email:apoptosistw@gmail.co

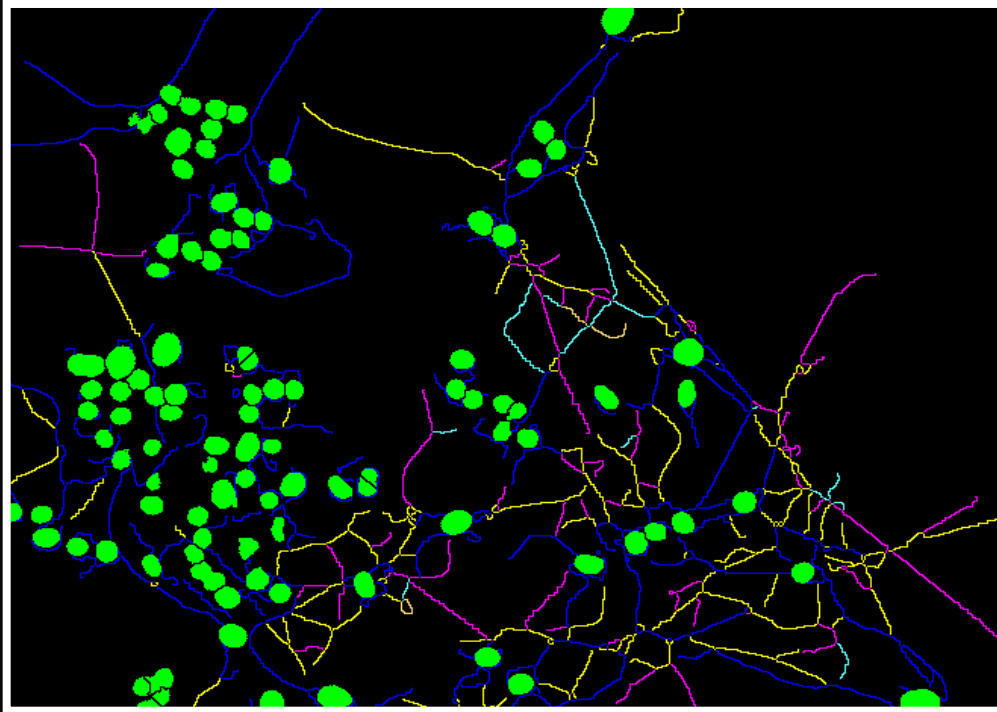


尚博生物科技有限公司

電話: (02)2785-5860

What's from Image to Analysis?

PC12 Neurite Growth



How many cell body ?

Um...pretty much ...

124 cell body are counted

How many branch?

Um...several I guess...

16 branch are found

How long they grow?

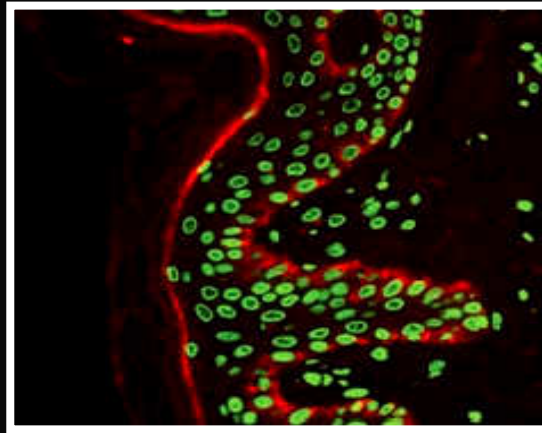
Um...Pretty long I guess...

48 pixel average of each cell

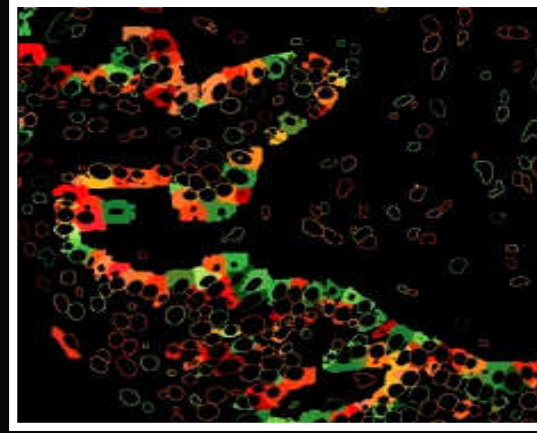
What's from Image to Analysis?

Effect of therapy on the density of antigen expression in human skin

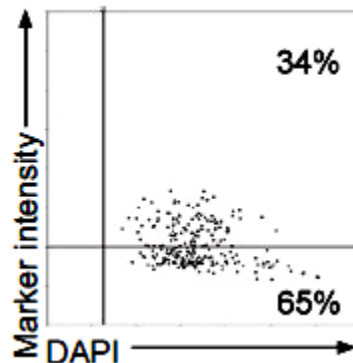
Normal skin before therapy



Normal skin after therapy

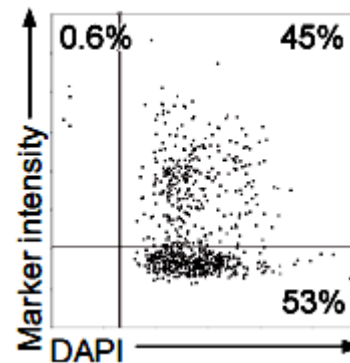


Marker expression before therapy



Before therapy the percentage of cells that react with the marker was 34% and the mean intensity was 39.

Marker expression after therapy

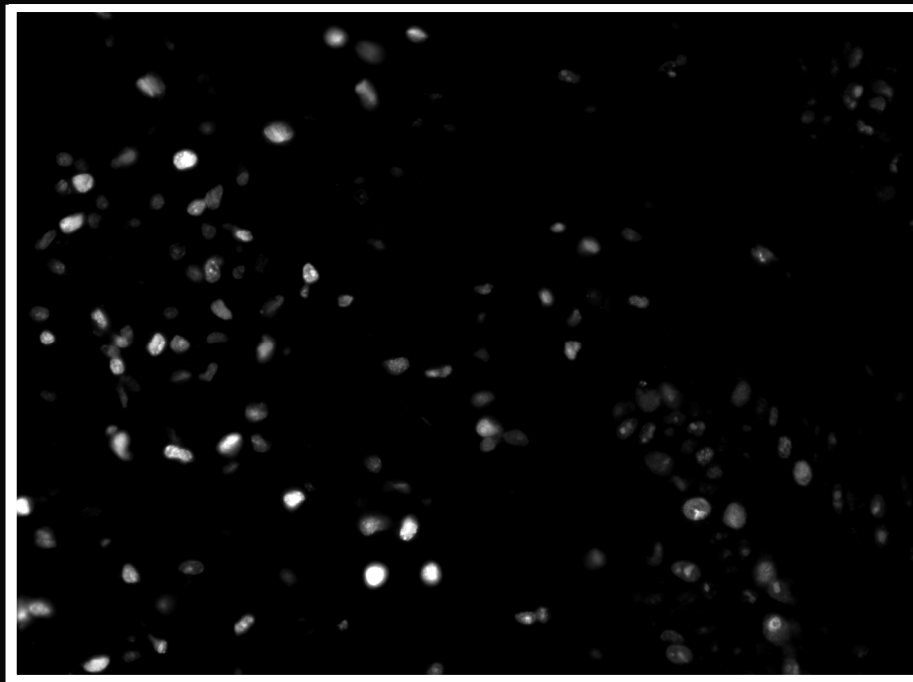


After therapy the percentage of cells that react with the marker was 45 and the mean intensity was 51.

Why from Image to Analysis?

REASON :

BECAUSE SEEING IS NOT REALLY BELIEVING!!



*How many of the blue nuclei
are also stained in red (in %)?
(Blue:DAPI , Red:Ki67)*

1. $\leq 5\%$

2. 5~10%

3. 10~15%

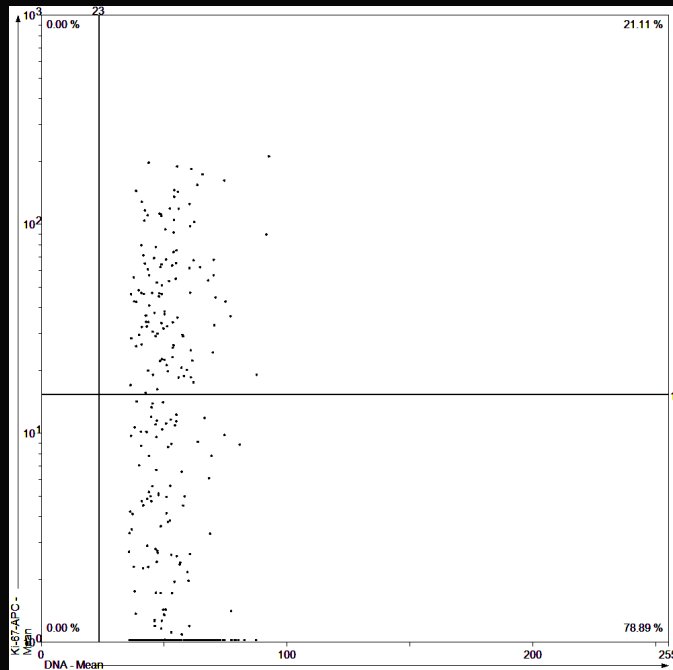
4. 15~20%

5. $>20\%$

Why from Image to Analysis?

REASON :

BECAUSE SEEING IS NOT REALLY BELIEVING!!



*How many of the blue nuclei
are also stained in red (in %)?
(Blue:DAPI , Red:Ki67)*

Expert's estimations:

0,5% - 18% (n=20)

Observer independent

measurement:

21,11%

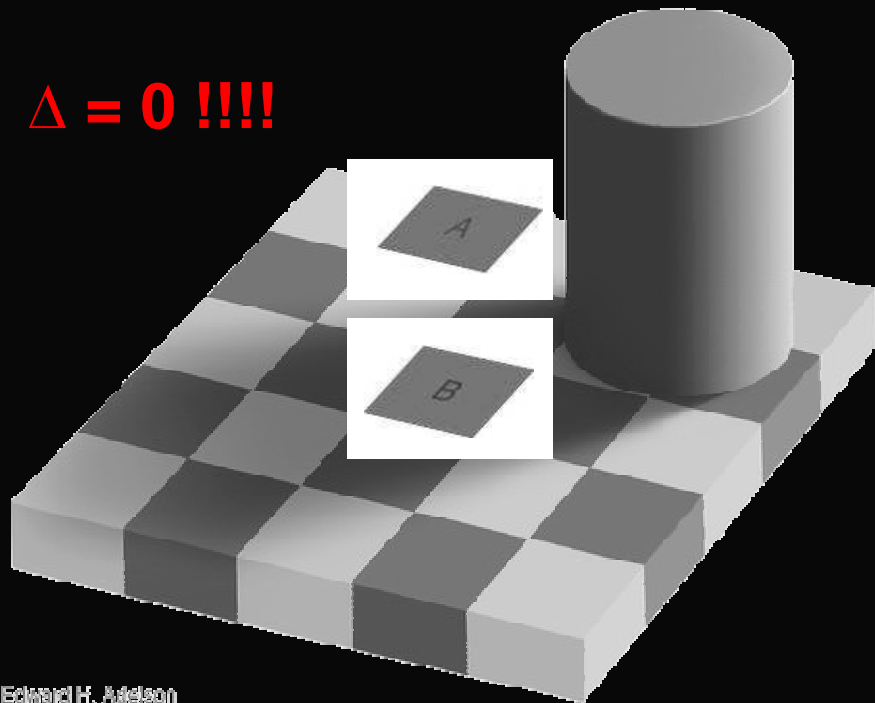
Why from Image to Analysis?

REASON :

BECAUSE SEEING IS NOT REALLY BELIEVING!!



$\Delta = 0$!!!!



GV = 0: BLACK



GV = 255: WHITE



What is the difference in GV
between A and B?

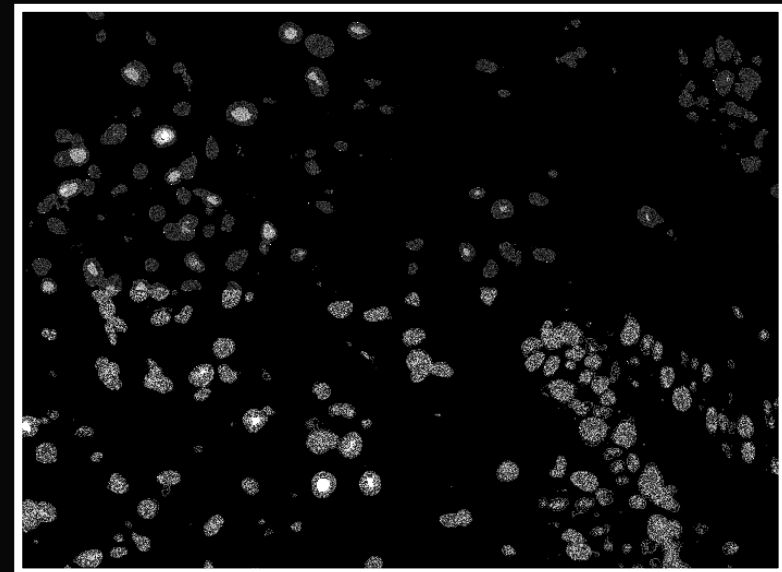
1. $\Delta \leq 20$

2. $\Delta \leq 50$

3. $\Delta \leq 100$

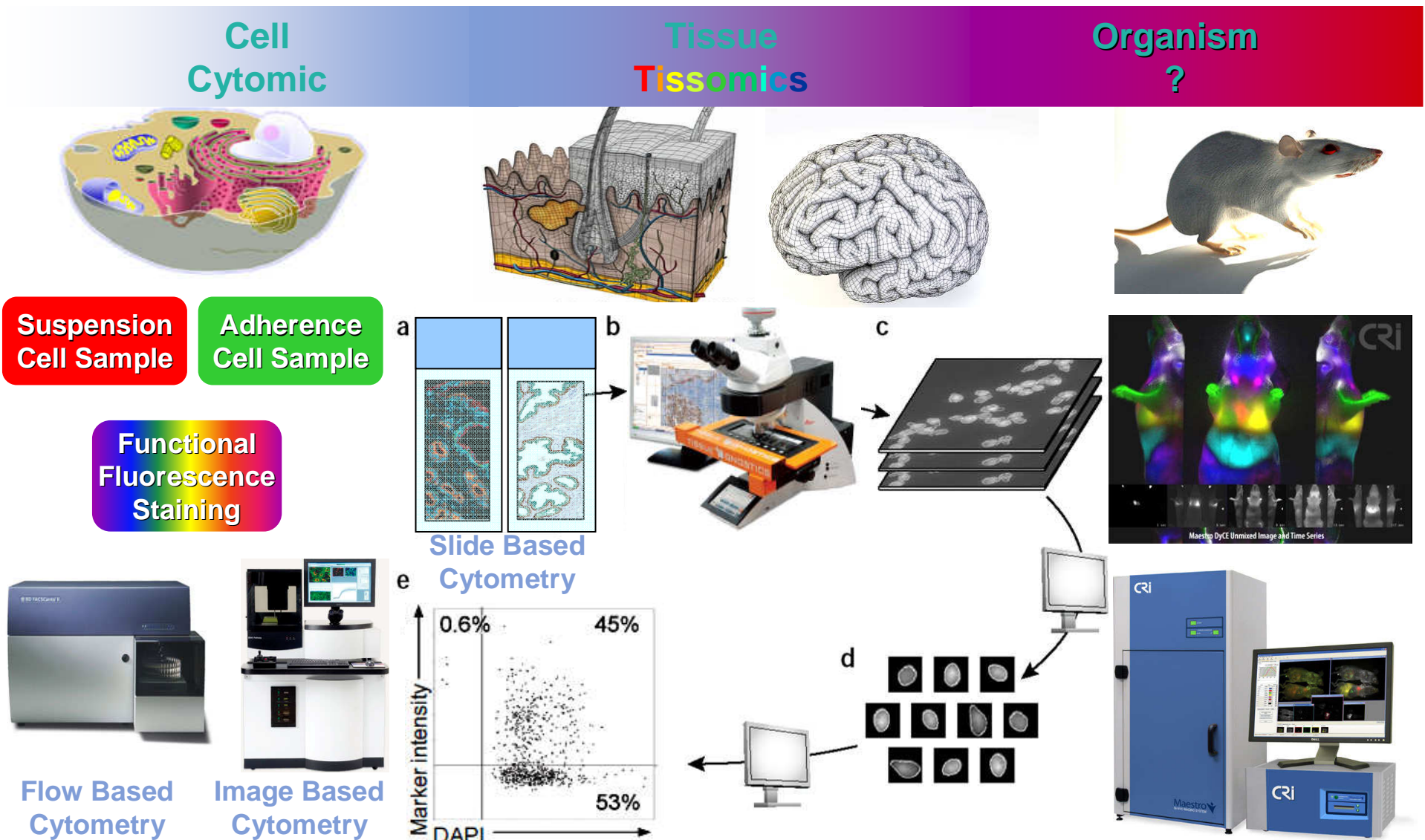
4. $\Delta > 100$

**REASON :
BECAUSE SEEING IS NOT REALLY BELIEVING!!**



*How to judgement the different of
each cell ?
What if this judgement is cancer
marker ,prlifereation marker ?*

Quantitative Tool from Cell to Organic



Introducing Cellbio biotechnology ltd

From Cellular level to Animal Assay

專業技術、代客服務

- ✿ 流式細胞儀
- ✿ 全光譜共軛焦影像分析儀
- ✿ 高內含共軛焦生物影像分析儀

✿ 組織細胞儀



CRi

全鼠螢光定量儀



MAESTRO

IN-VIVO
IMAGING SYSTEM



Goals of this Presentation...

- Introduce Image based Cytometry
- Current state technologies of HTS/HCS Image Cytometry
- Several Applications of HTS/HCS Image Cytometry



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Concept of Image Based Cytometry

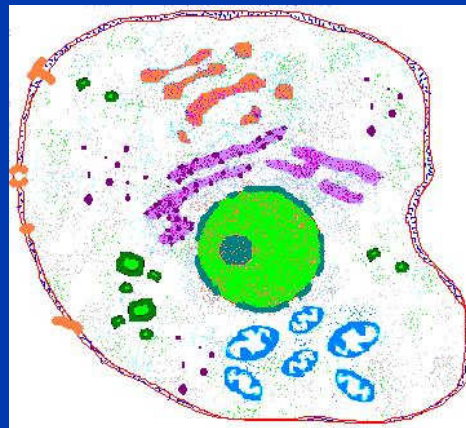
The cell is the ultimate functional endpoint

- *Cytomics* is going to be important because it is the cell that is the ultimate functional endpoint. The cell is the **minimal functional unit** within our physiology and thus the functional unit that can be manipulated.
- Complexity of cell function is only part of why *Cytomics* will become a major field of study. Every cell is different. By studying each cell's unique function, that cell type can be further modeled for subsequent analysis using statistical techniques.
- As the field of tissue engineering explodes, it will not be long before **cellular engineering** will be a most important component of which an essential element will be a full understanding of *Cytomics*.

gene

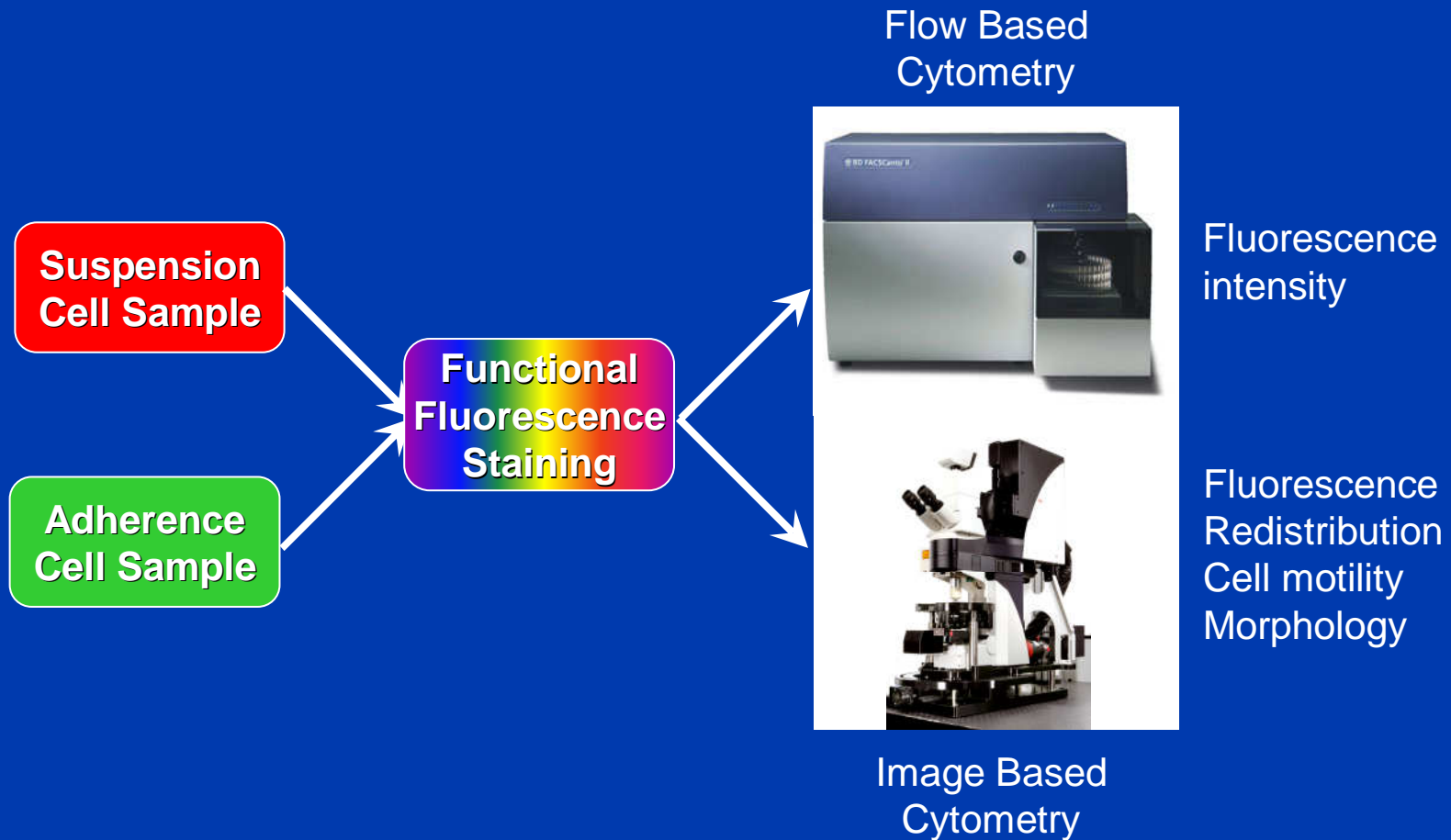
protein

cell

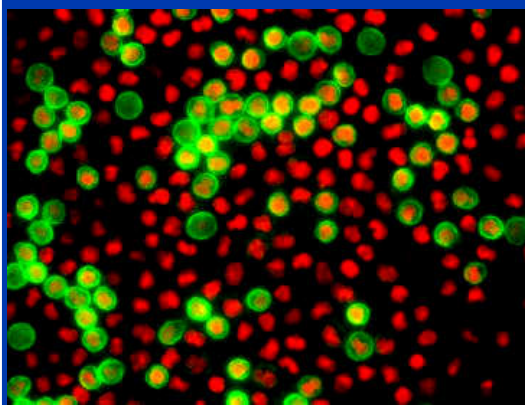


Hey buddy...
Don't you know you
genes, proteins and
organelles are in
my territory now!!

2 Major systems for Cell Functional Assay...



Flow Base Cytometry



**Detach Cells and
Resuspend in Fluid**

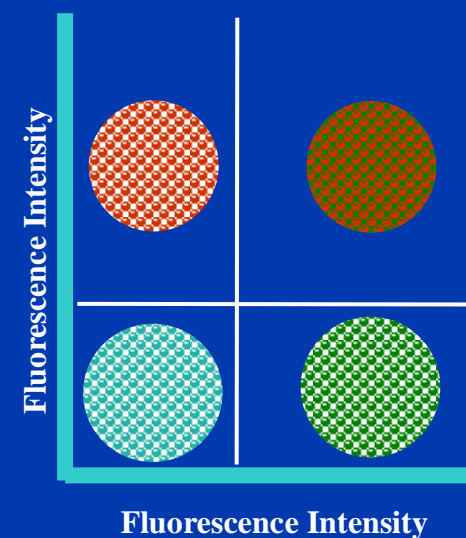
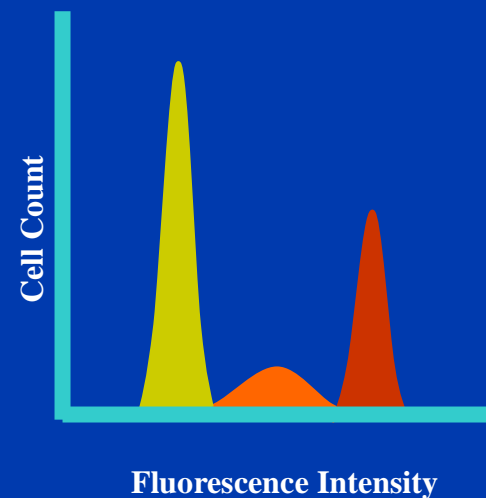
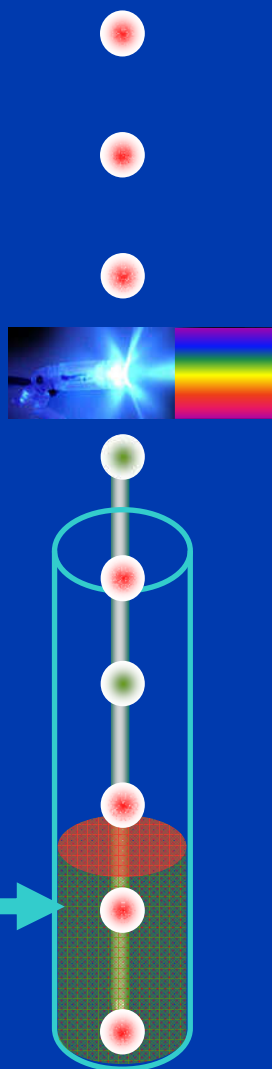
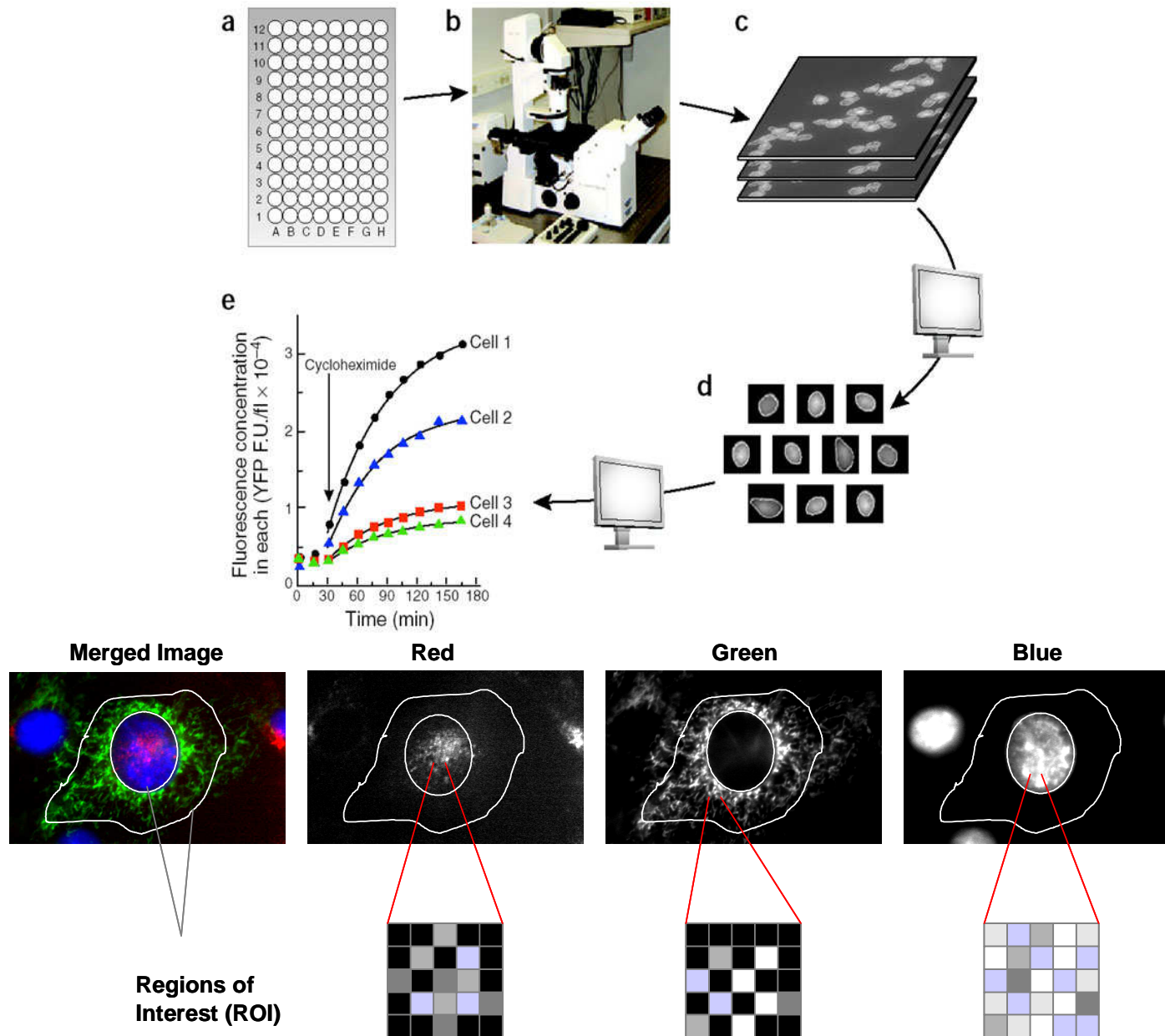


Image Base Cytometry



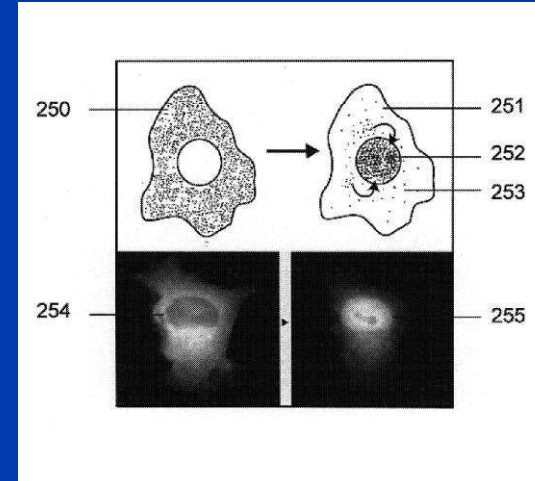
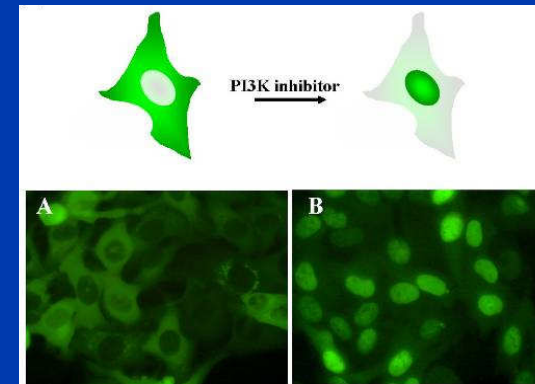
Why Image?

There something FACS limitation:

- Limit morphological Information
- Limit distribution information
- Not very suit for adherence cell
- Not very suit for kinetic assay
- Not very suit for long time assay (Re-analysis)

Advantage of Image cytometry:

- Rich Information (High Content)
- Perfect for heterogeneous population
- Very versatile technology
- Very suit for adherence cell
- Very suit for kinetic assay
- Very suit for long time assay

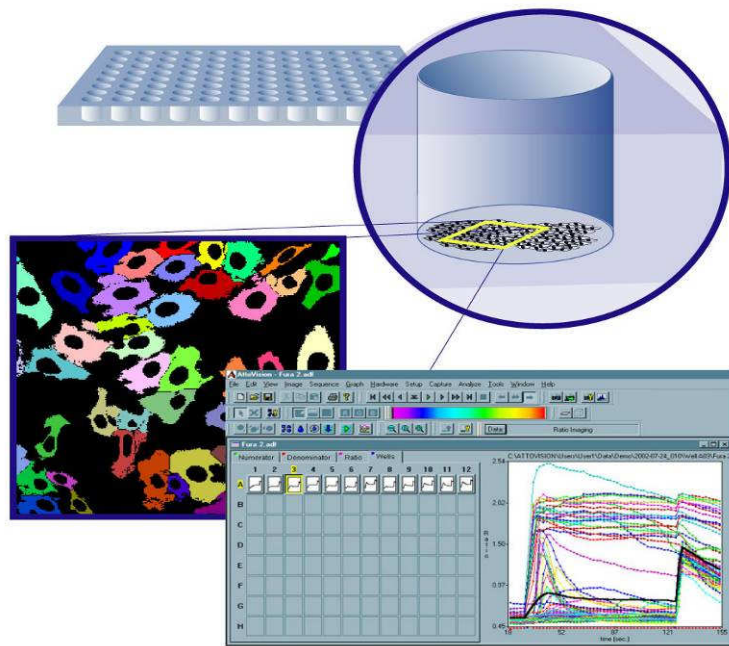
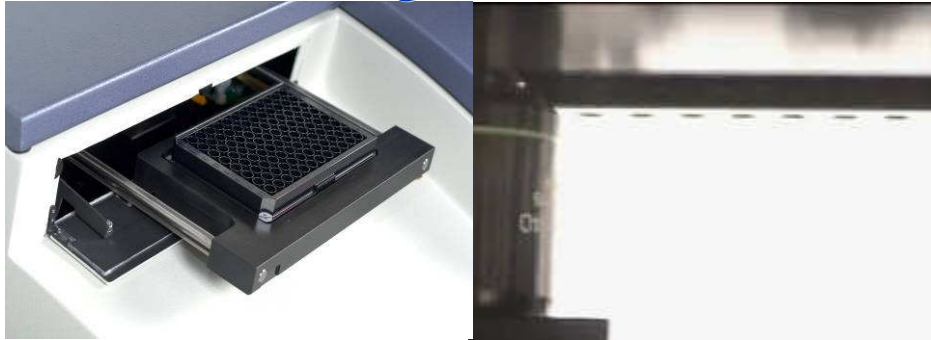


**Images data contain a lot of information ,
but it is *time consuming and difficult to extract it...***

Current state technologies of Image based Cytometry

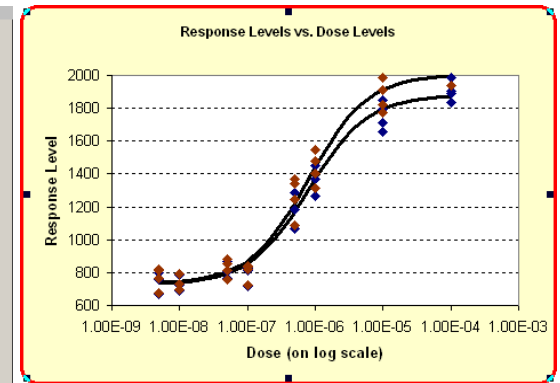
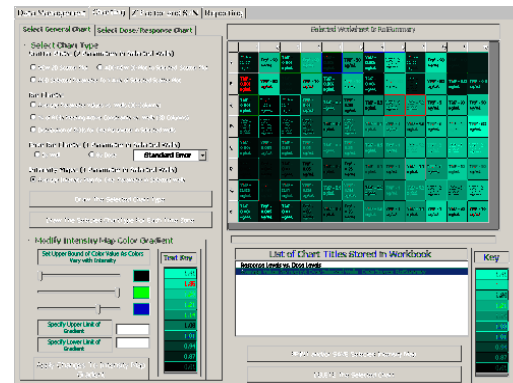
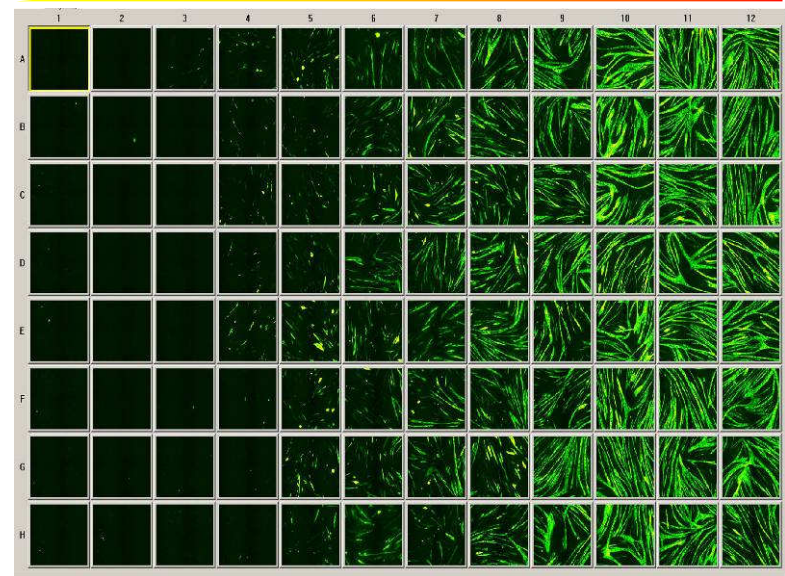
HCS/HTS Analysis Process

BD 435/855 Bioimager



Lo

Hi



Biology

Image Acquisition

Image Analysis

Data Analysis

Summary: Imaging from a Flow Cytometry Perspective

Flow Cytometry

Fluorescence intensity

Single cell resolution using PMT

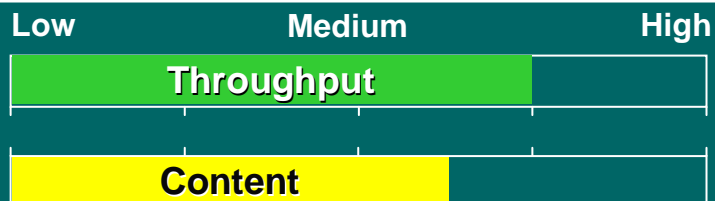
Thousands to hundreds of thousands of suspended cells

Multiplexible – 12+ colors

Cells can be damaged or lost after flow

Strengths:

- Cell preparation (sorting)
- Statistical population analysis
- Low abundance events



Automated Imaging

Fluorescence intensity

Redistribution

Cell motility

Morphology

Subcellular resolution using CCD

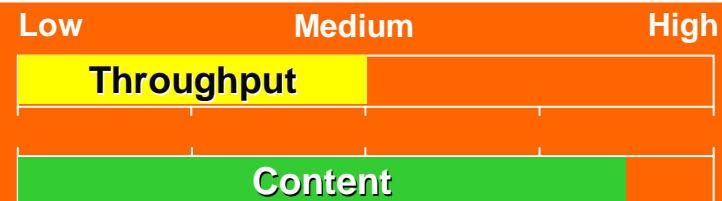
One to thousands of suspension or adherent cells

Multiplexible – 4+ colors

Cells can be revisited and reanalyzed over time

Strengths:

- Morphological measurements
- Spatial analysis
- Images can be reanalyzed
- Visual data





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Current state technologies of HTS/HCS Image Cytometry

Current state technologies of Image based Cytometry

New idea of Image based Cytometry :

I. Fast and Automatic

--- *High Throughput* (96 well / 384 well format)

II. Multi-information

--- *High Content* (Multi-color image)

III. High Image quality and precise analysis

--- *Confocal Image* (LSCM / Spinning Disk / OptiGrid

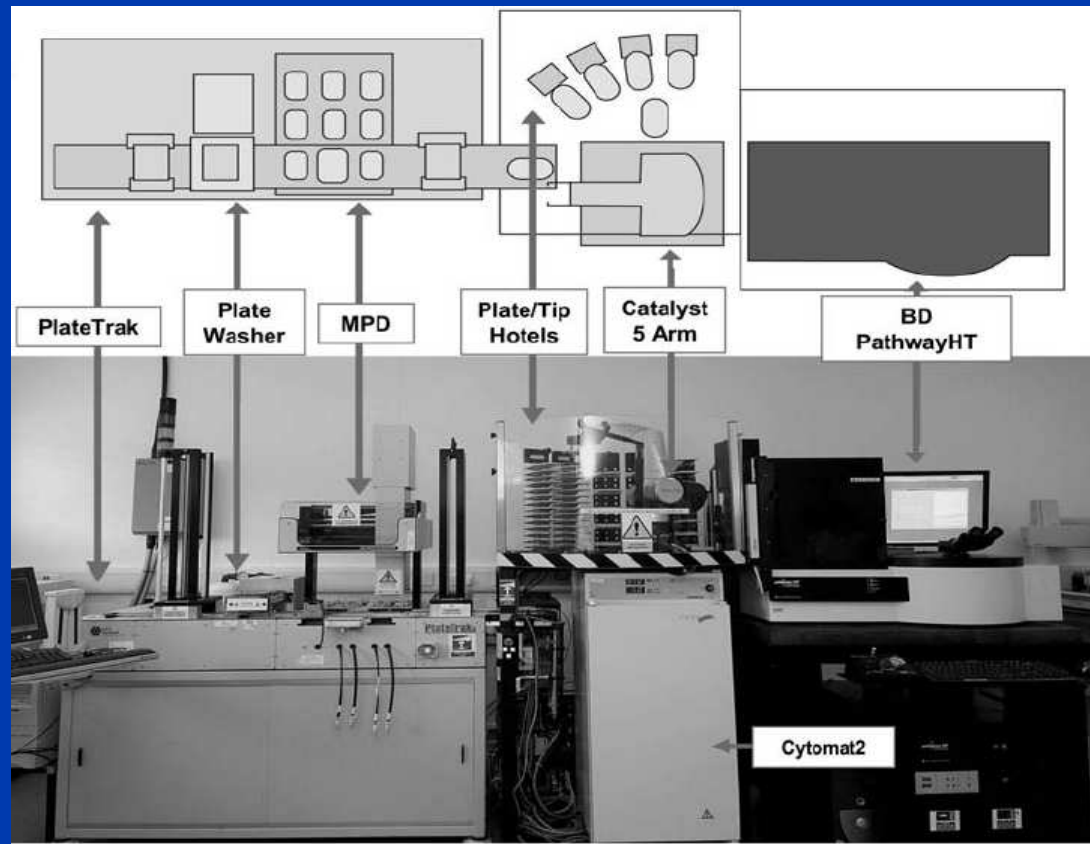
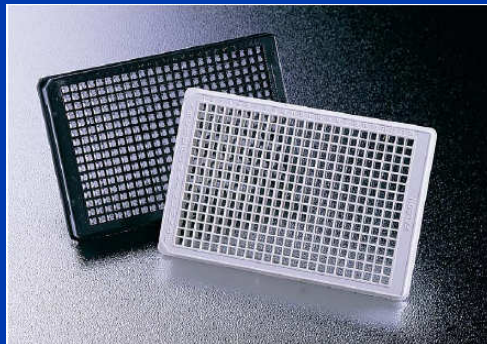
IV. Quantification / Qualification / Coordination

--- *Powerful software* (segmentation)

New idea of Image based Cytometry :

I. Fast and Automatic---High Throughput

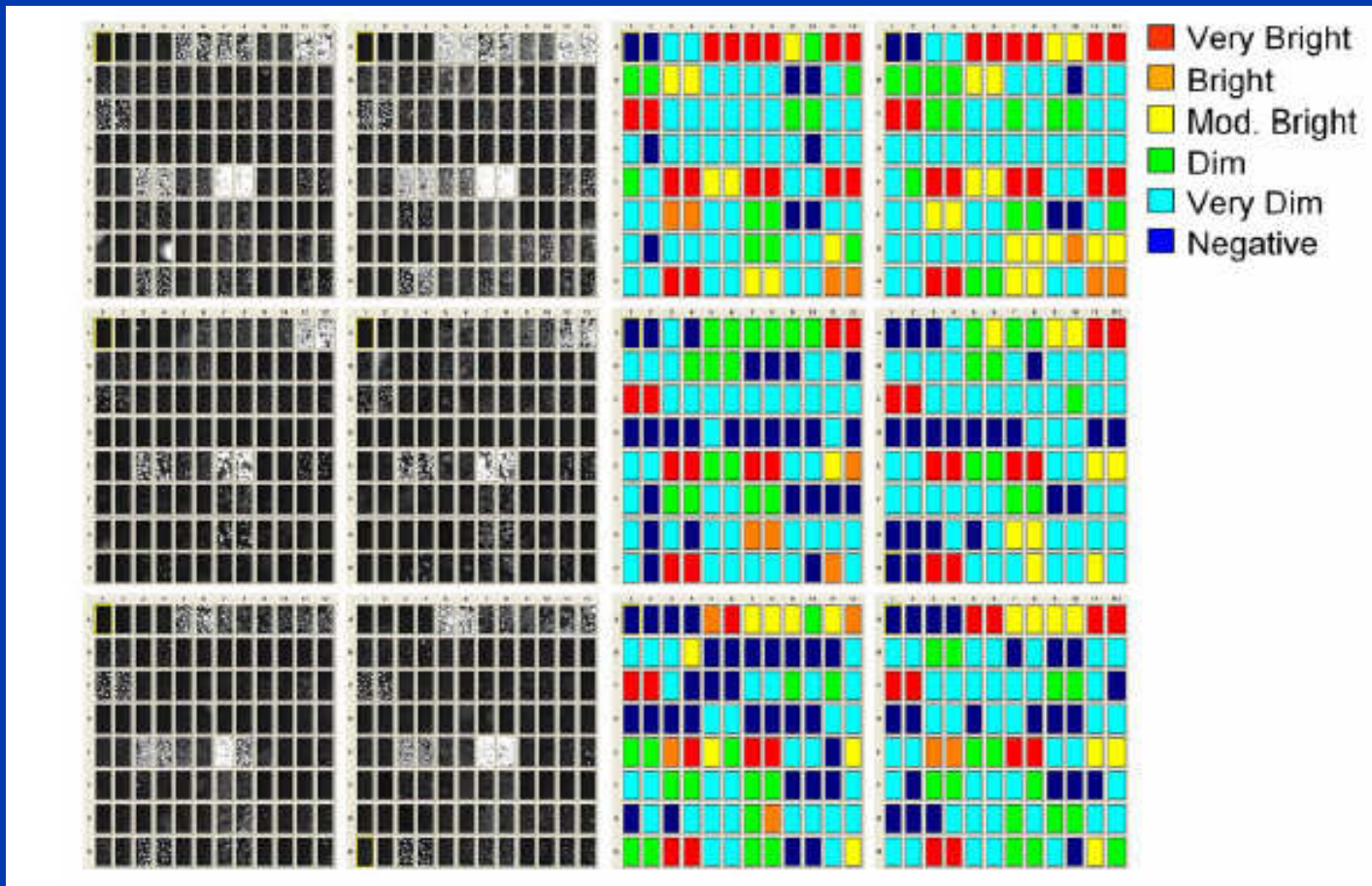
96 well / 384 well format , Fully Automation data acquire system



New idea of Image based Cytometry :

I. Fast and Automatic---High Throughput

96 well / 384 well format , Fully Automation data acquire system



New idea of Image based Cytometry :

II. Multi-Information---High Content

高內涵細胞分析技術（High Content Screening, HCS）是在保持細胞結構和功能完整性的前提下，利用螢光試劑標定目標物質，在細胞內呈現的功能，與時間和空間相對應之資訊。因此高內涵細胞分析技術（HCS）整合了以細胞為基礎的分析，高解析螢光顯微鏡和細胞及次細胞內自動分析之影像處理演算技術，使得以往缺乏較客觀有效測量細胞生理現象，例如：細胞形態改變、細胞分化、細胞架構改變、細胞與細胞間之交互作用、趨化性、移動性和空間的分布改變等，得以有效的以數據呈現

- Huge number of Variable & Parameters
- Very High Speed



- Huge data sets
- Opportunity for Rapid classification



Rapid
Identification
Or Diagnostics

Much of this can become
Real-Time decision making

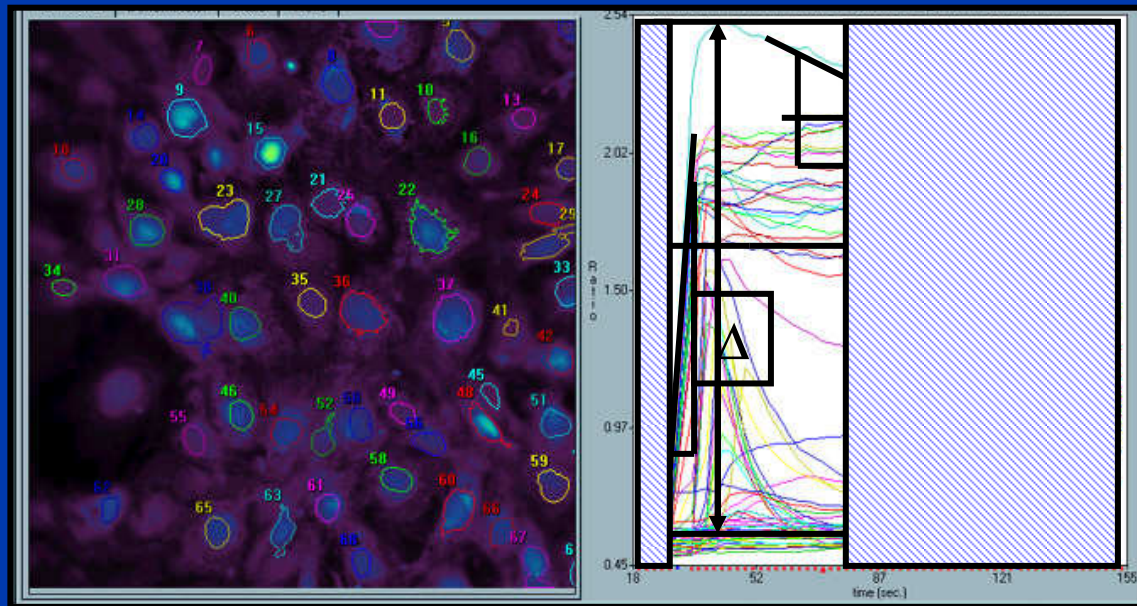
New idea of Image based Cytometry :

II. Multi-Information---High Content

Time Information With Kinetic Mode

Single-cell Kinetic Imaging Reveals Heterogeneous Calcium Response

- Maximal response from baseline
- Rate of rise
- Rate of fall
- Treatment zones
- Difference in 2 peaks



New idea of Image based Cytometry :

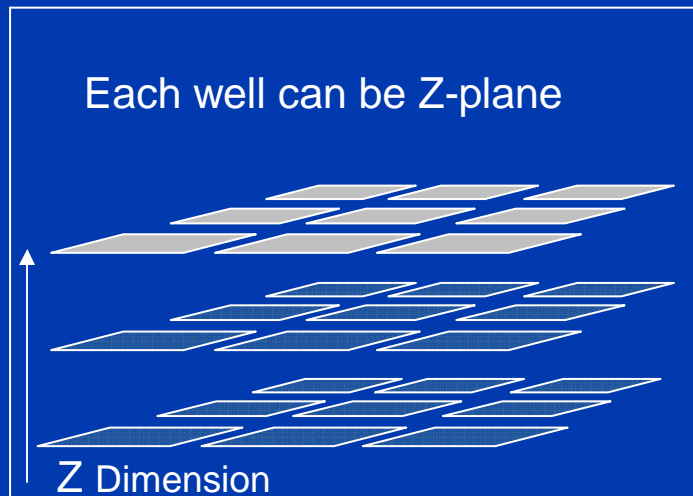
II. Multi-Information---High Content

Spatial Information with 3D Image



Single Plane

- Image peripheral events
- Improve resolution
- Image specific cell layer
- Remove background fluorescence



Collapsed Z

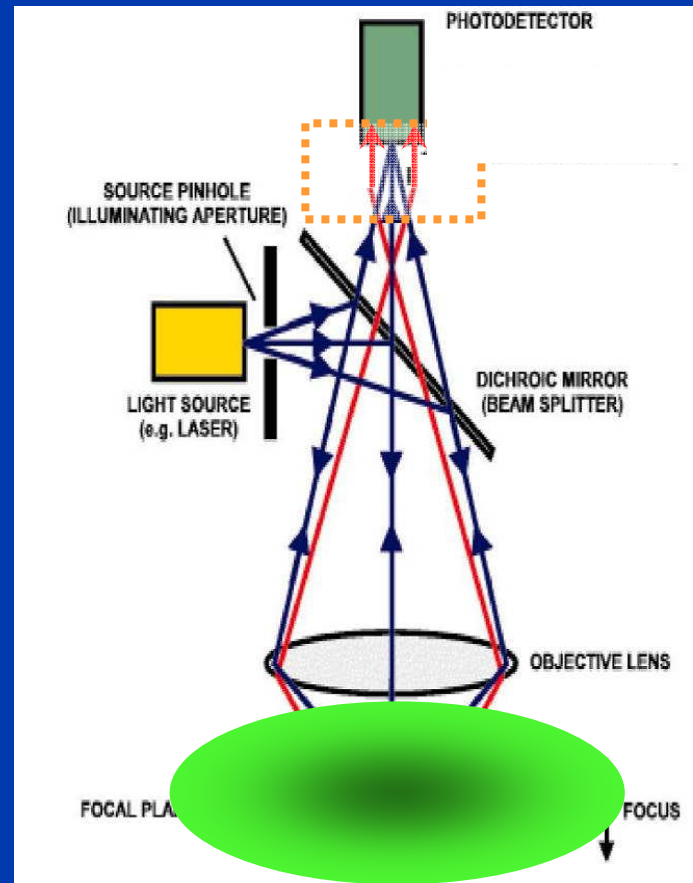
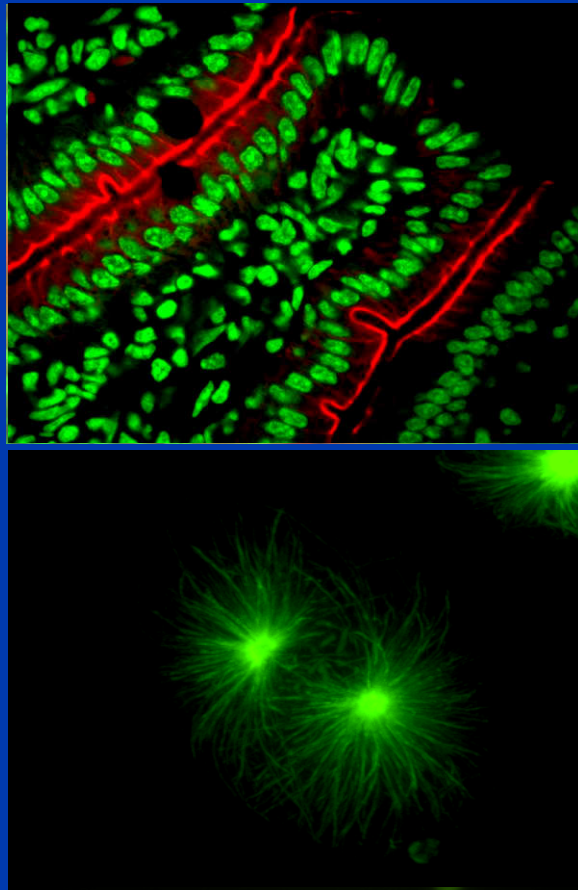
- Collect all fluorescence In sample
- Improve resolution
- Quantities fluorescence

...can also capture over time in kinetic mode!

New idea of Image based Cytometry :

III.High Image Quality and Precise Analysis---Confocal

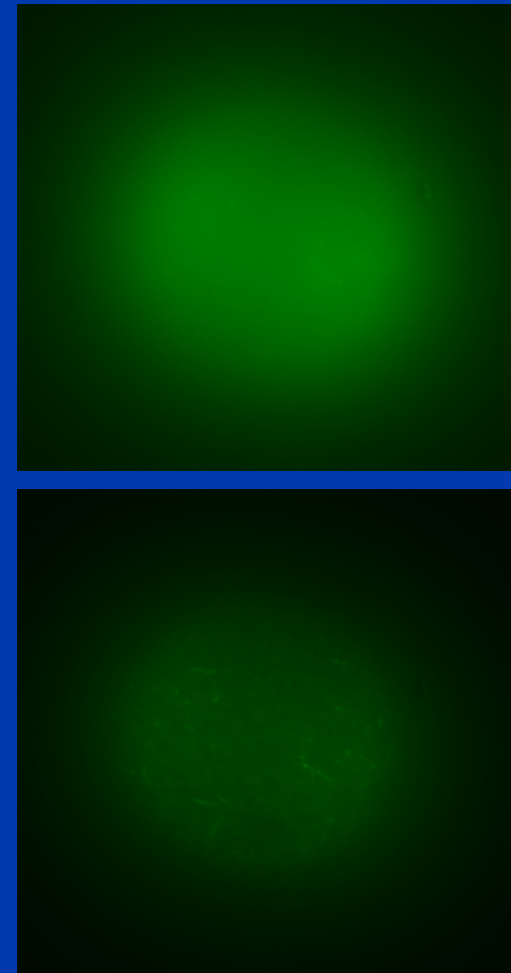
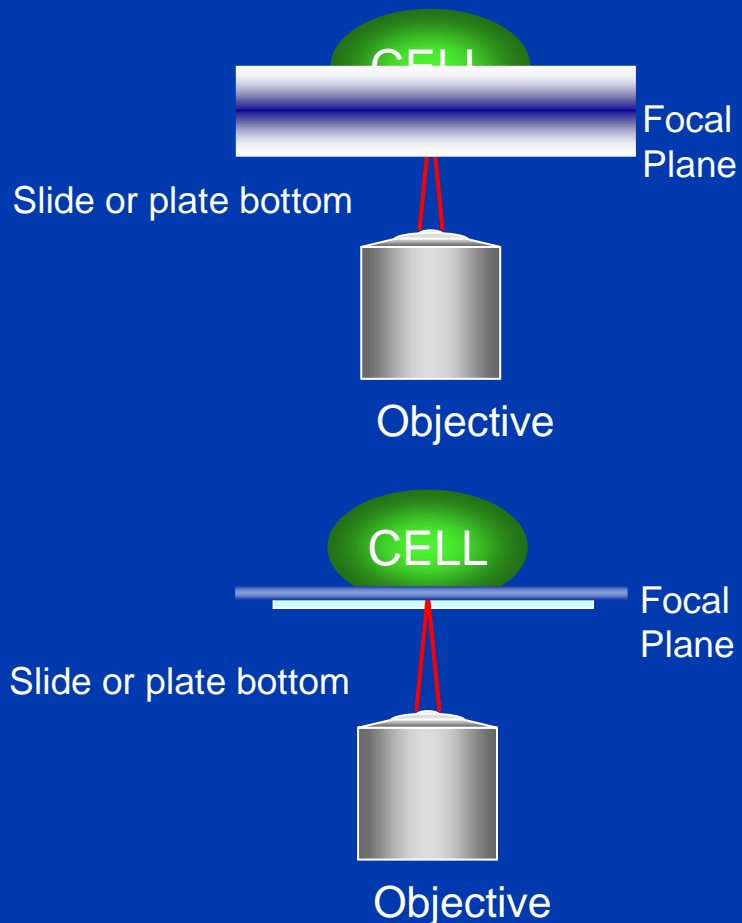
Benefit of Confocal Image 1: Clear single plane of image



New idea of Image based Cytometry :

III.High Image Quality and Precise Analysis---Confocal

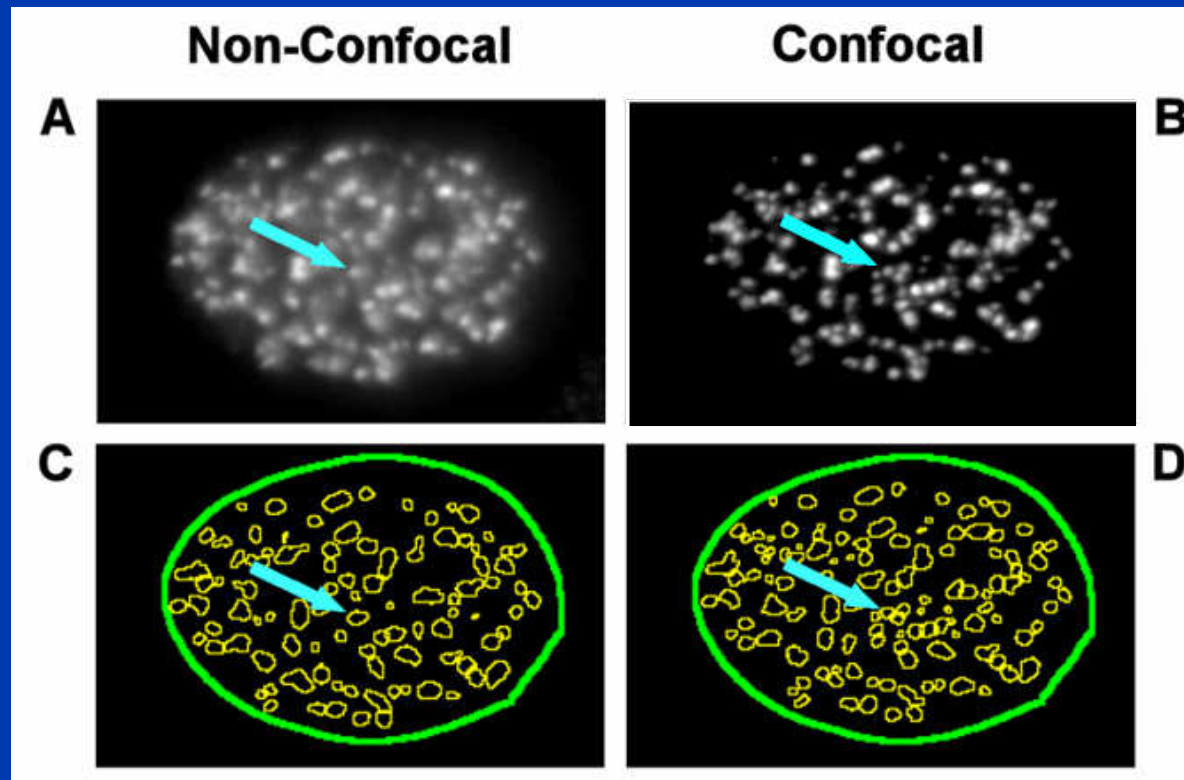
Benefit of Confocal Image 2: Better 3D Structure



New idea of Image based Cytometry :

III.High Image Quality and Precise Analysis---Confocal

Benefit of Confocal Image 3: accurate counting and analyze result



H2AX foci

Segmentation

94 foci identified

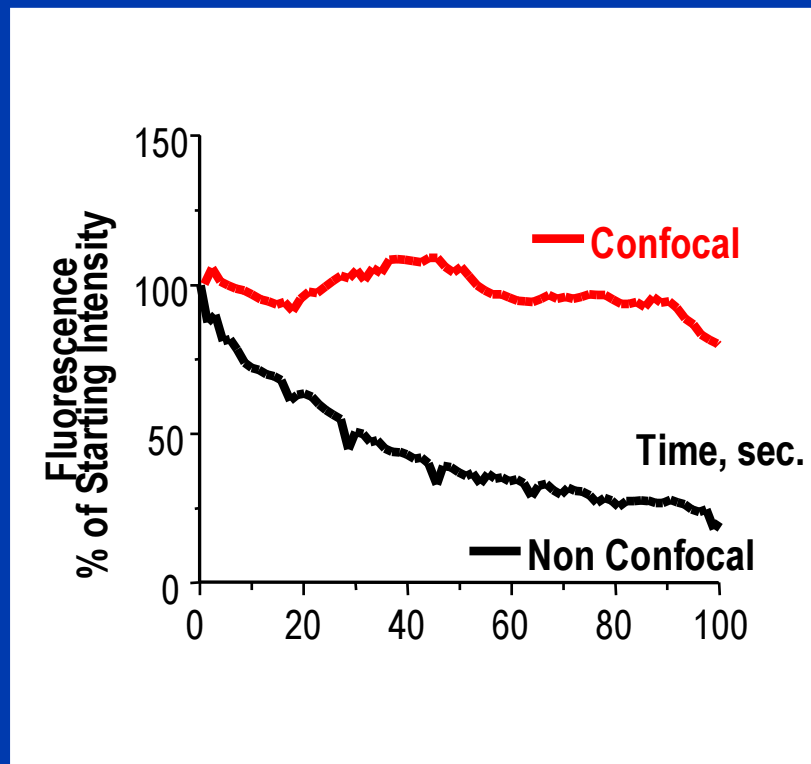
118 foci identified

25% increase in foci count

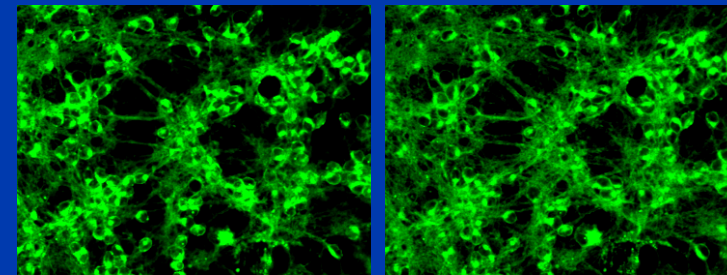
New idea of Image based Cytometry :

III.High Image Quality and Precise Analysis---Confocal

Benefit of Confocal Image 4: Reduce photobleach



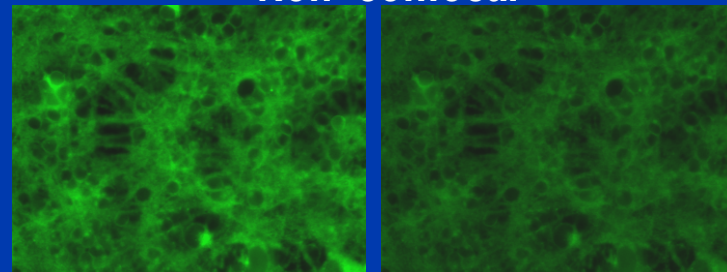
Confocal



t = 0 sec

t = 100 sec

Non- confocal



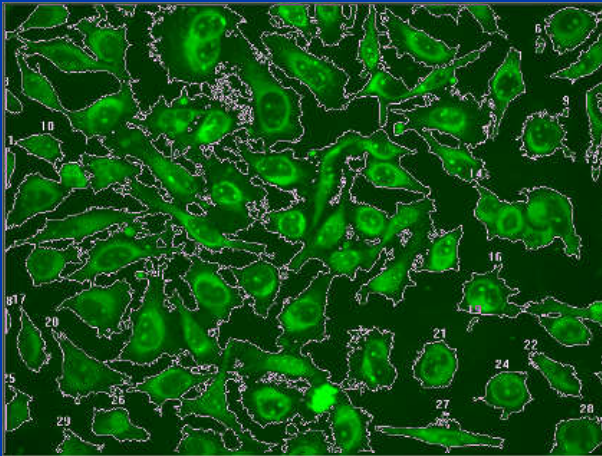
t = 0 sec

t = 100 sec

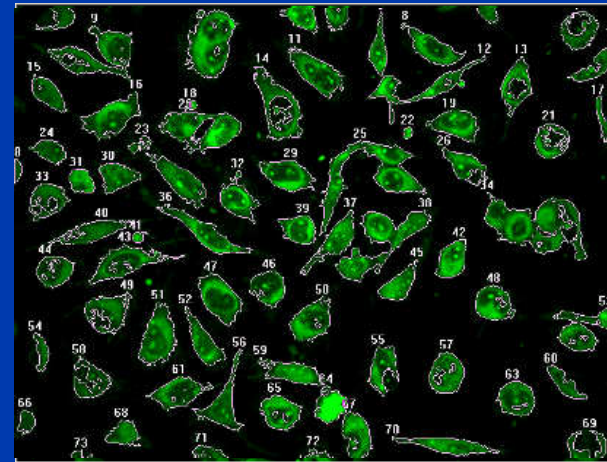
New idea of Image based Cytometry :

IV.Quantification / Qualification /Coordination

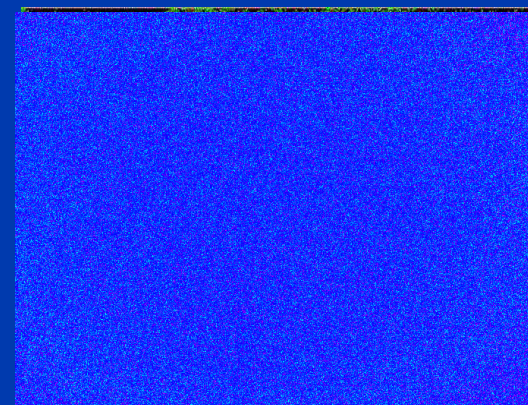
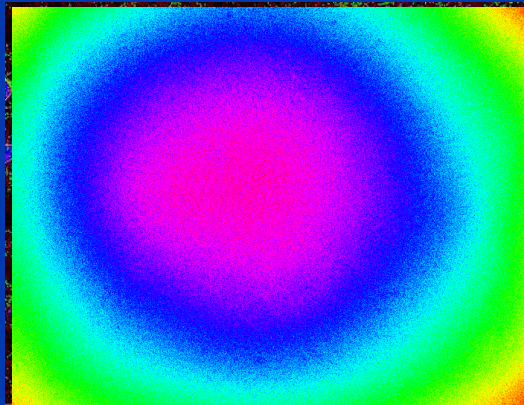
Powerful Software: Image Process



Unprocessed Image



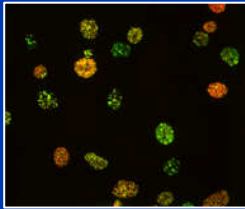
Background Subtracted Image



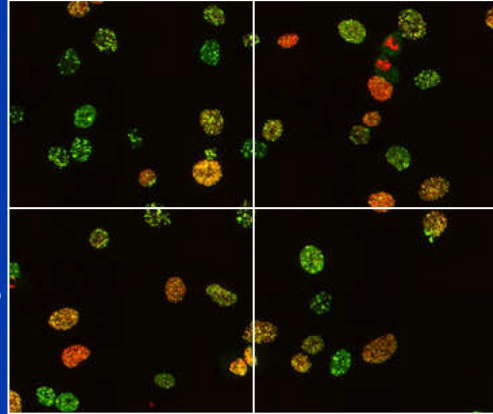
New idea of Image based Cytometry :

IV. Quantification / Qualification / Coordination

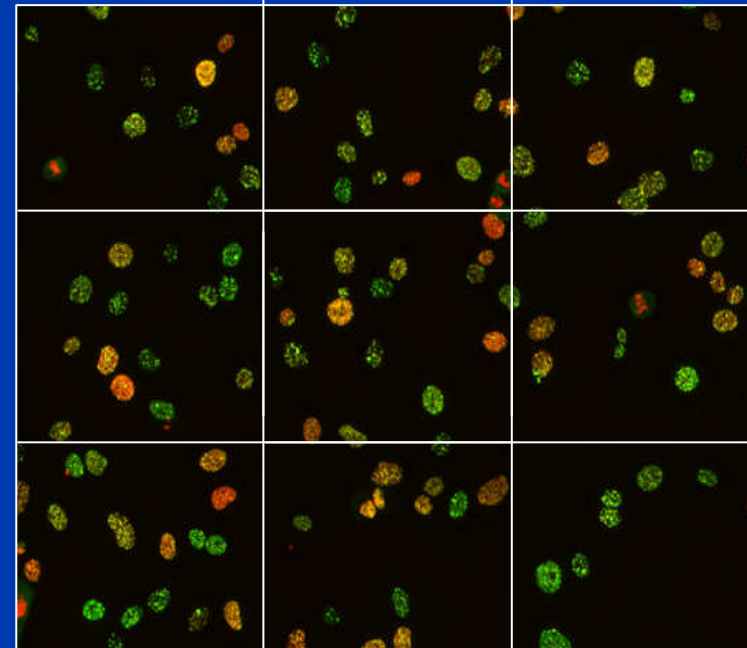
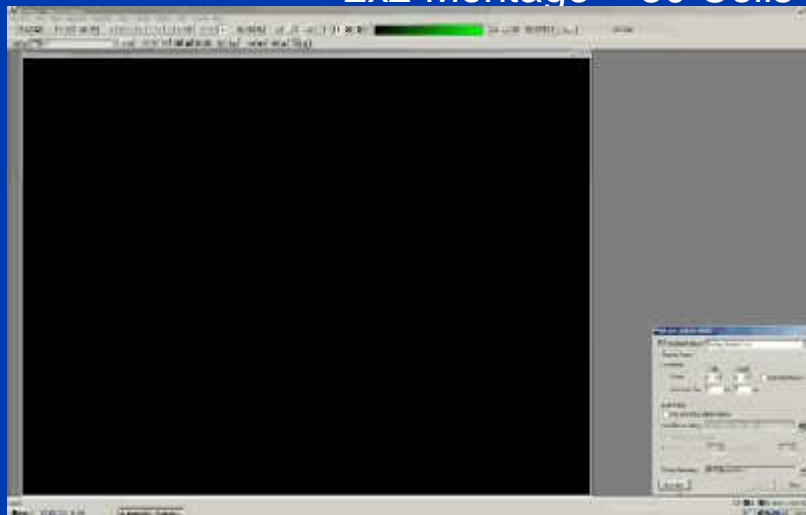
Enlarge event number : Montage



Single Image
Field ~ 20 Cells



2x2 Montage ~ 80 Cells



3x3 Montage ~ 180 cells

New idea of Image based Cytometry :

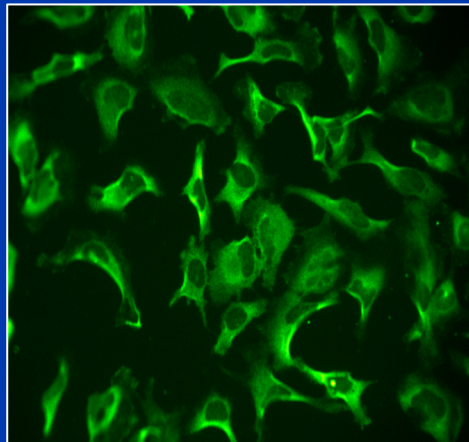
IV. Quantification / Qualification / Coordination

Identification : Cell Segmentation (Region Of Interest) and Quantification, Qualification

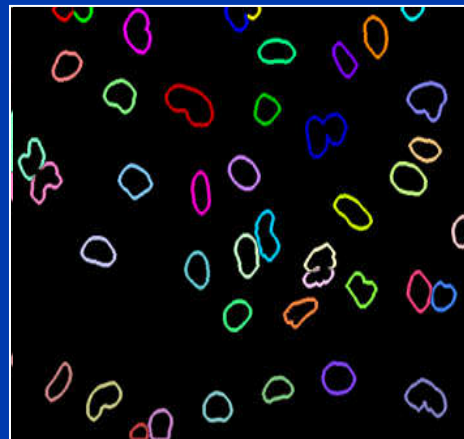
- Dual output ROIs used in translocation assays
- Masked dilation to secondary channel
- Many possibilities from common interface



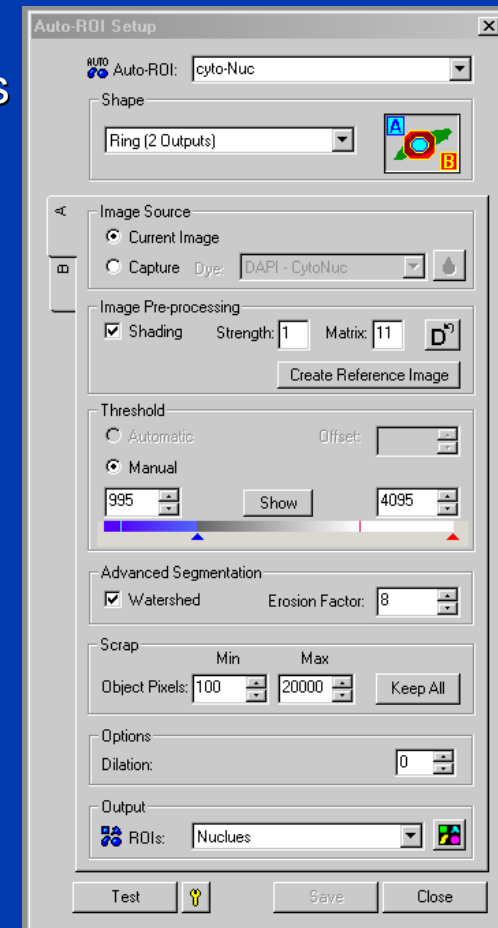
- Automatic thresholding



Cytoplasm



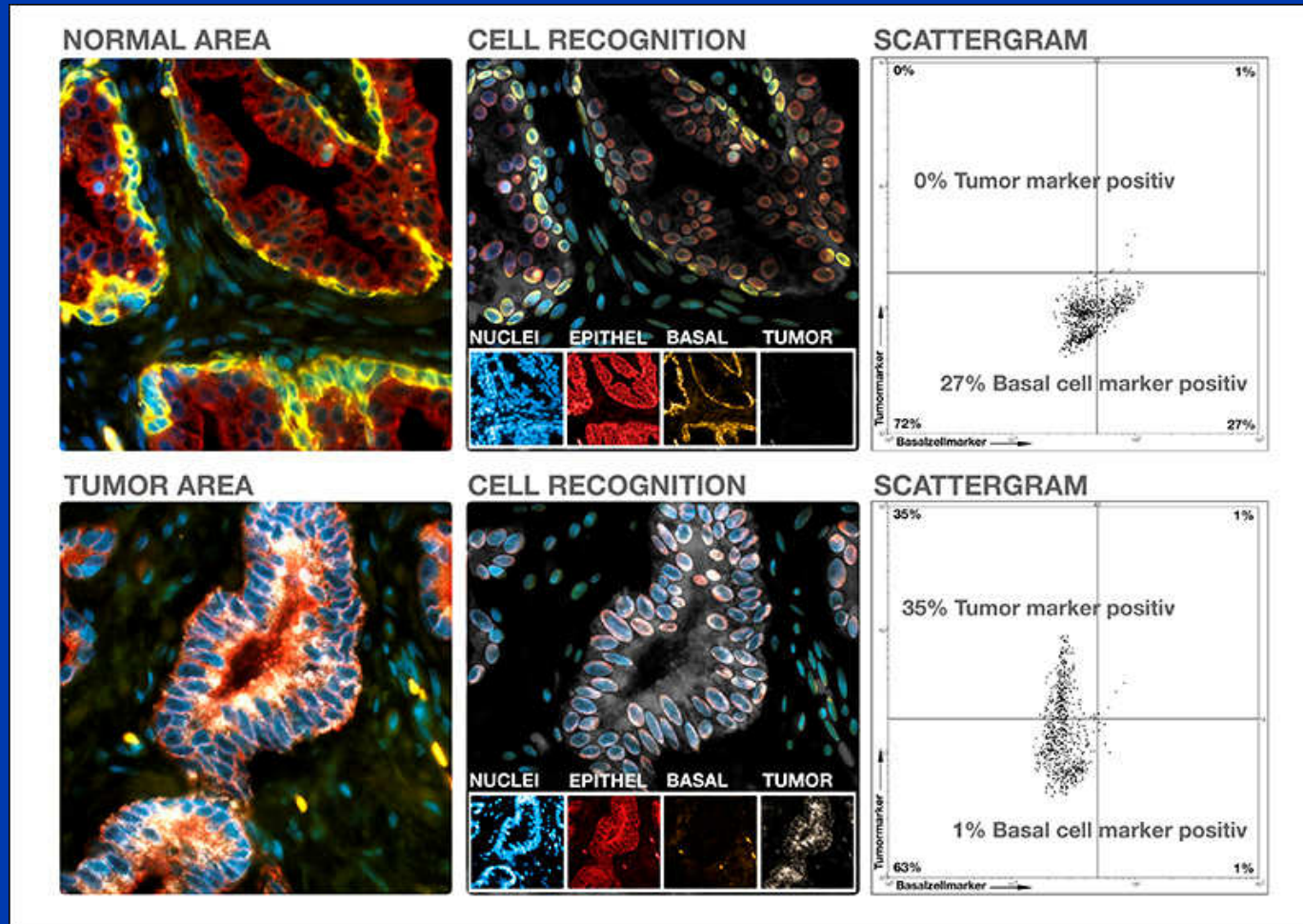
Plasma Membrane Mask



New idea of Image based Cytometry :

IV. Quantification / Qualification / Coordination

Data Coordination and Data Export



New idea of Image based Cytometry :

IV.Quantification / Qualification /Coordination

Intelligent Interface and Data Coordination

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Summary:

Current state technologies of Image based Cytometry

--- *High Throughput*

Fully automatic hardware system
Working with microtiter plate or multiwell plate
Strait forward assay protocol

--- *High Content*

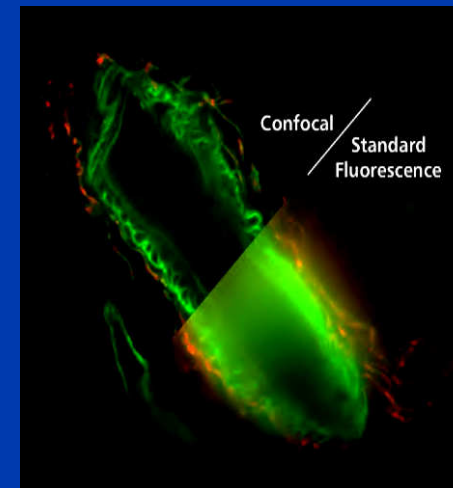
Multicolor image
Spatial and time information
Cell-cell , well-well , sample-sample comparison

--- *Confocal Image*

Better image quality for precise data analysis
Spatial information

--- *Powerful software*

Artificial Intelligent





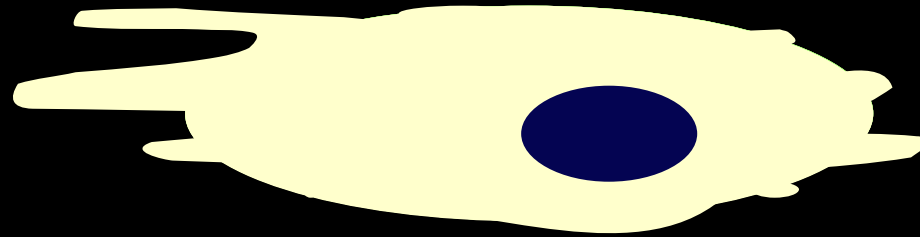
Helping all people
live healthy lives



尚博生物科技有限公司 www.cell-bio.com.tw

Applications of HTS/HCS Image Cytometry

There are 4 Basic Assay Categories of HCS Image analyzer



1. Fluorescence intensity change

- Calcium flux
- Phosphorylation
- Biomarker activation
- Protein degradation
- Image cytometry

2. Fluorescence distribution

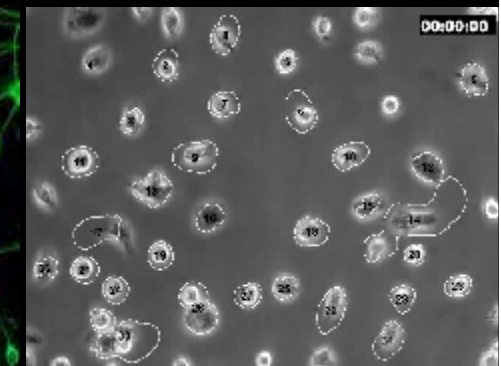
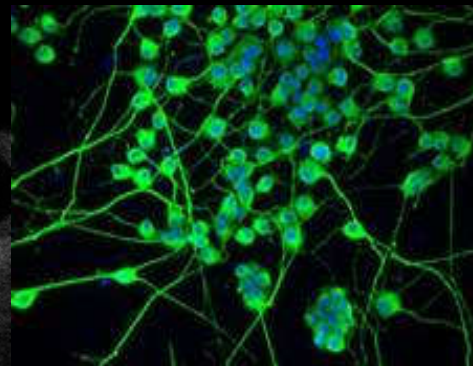
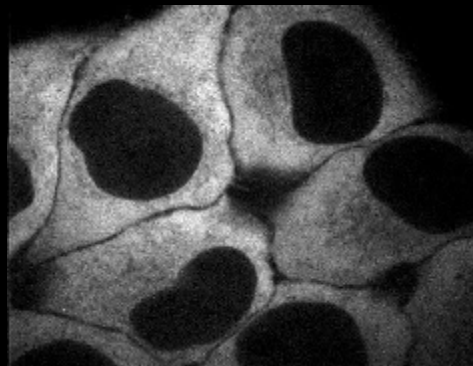
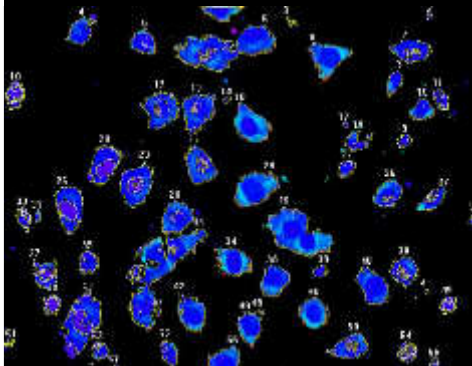
- Cytoplasm to nucleus ($\text{NF}\kappa\text{B}$)
- Cytoplasm to plasma membrane ($\text{PKC}\alpha$)
- Plasma membrane to organelle (Transfluor GPCR)
- Protein co-localization

3. Morphological change

- Neurite outgrowth
- Angiogenesis
- Cell differentiation
- Apoptosis

4. Cell Movement

- Chemotaxis/migration
- Wound healing
- Metastasis/invasion
- Long-term tracking



HCS Application

1. Fluorescence Intensity Changes

- *Investigate heterogeneous cell populations:
Identify percentage of cells responding in a population (image cytometry)*
- *Work with few cells:
Region of interest can be whole cell, structure, or whole field of view*

2. Fluorescence Distribution

- *Measure cell changes where total fluorescence intensity does not change*
- *Measure biomolecular colocalization*

3. Morphometric Measurements

- *Make measurements on cells using structural dyes/antibodies*

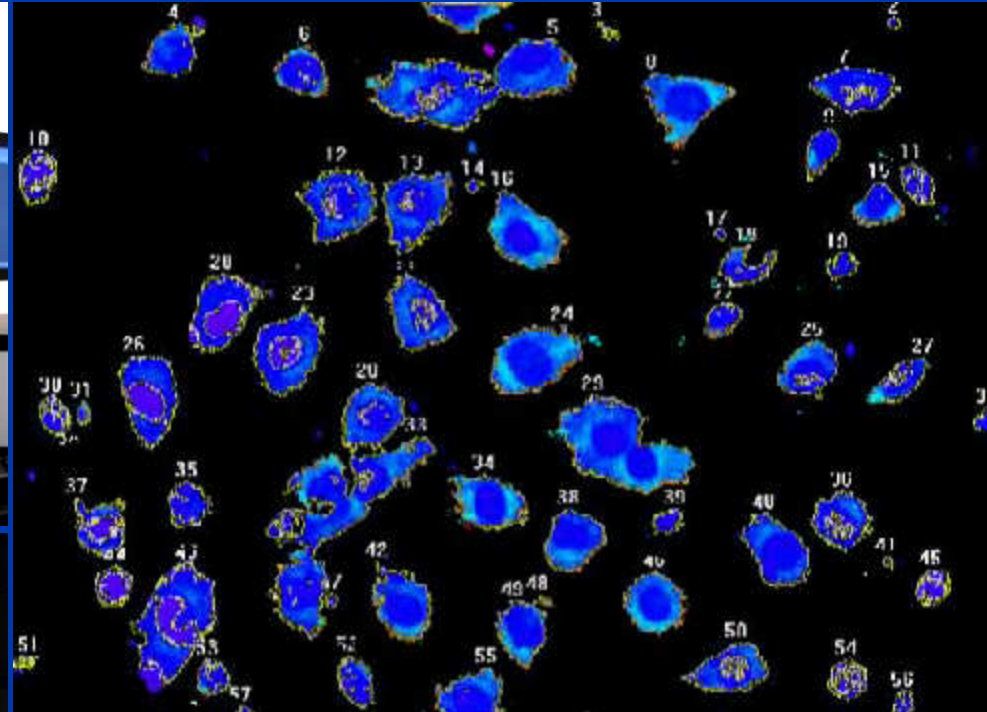
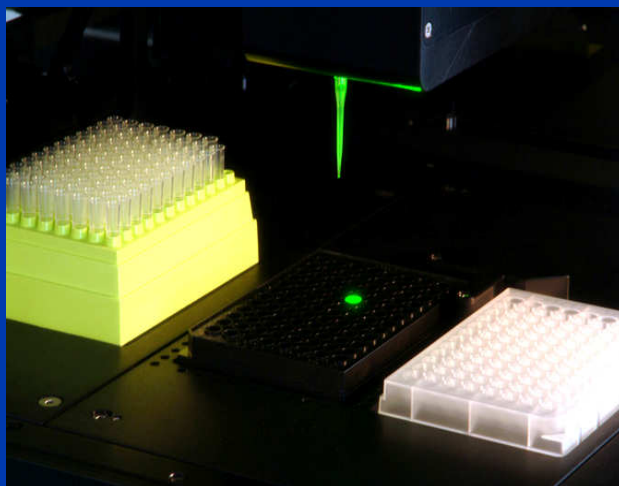
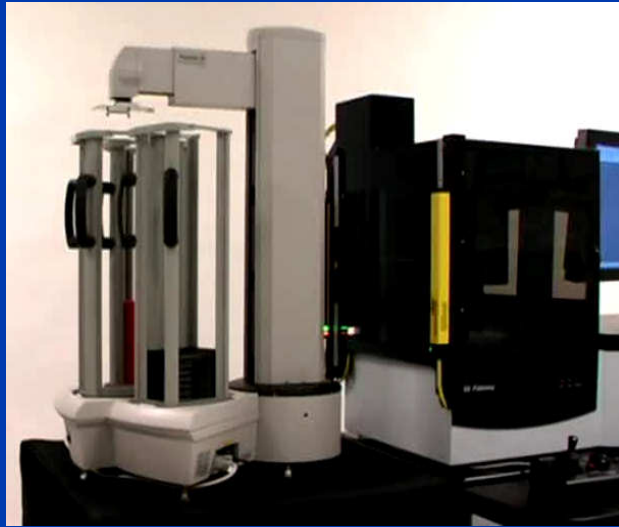
4. Cell Movement

- *Measure cellular mobility/motility and invasion*

1. Fluorescence Intensity Changes

Investigate heterogeneous cell populations :

Single-cell Kinetic Imaging Reveals Heterogeneous Calcium Response



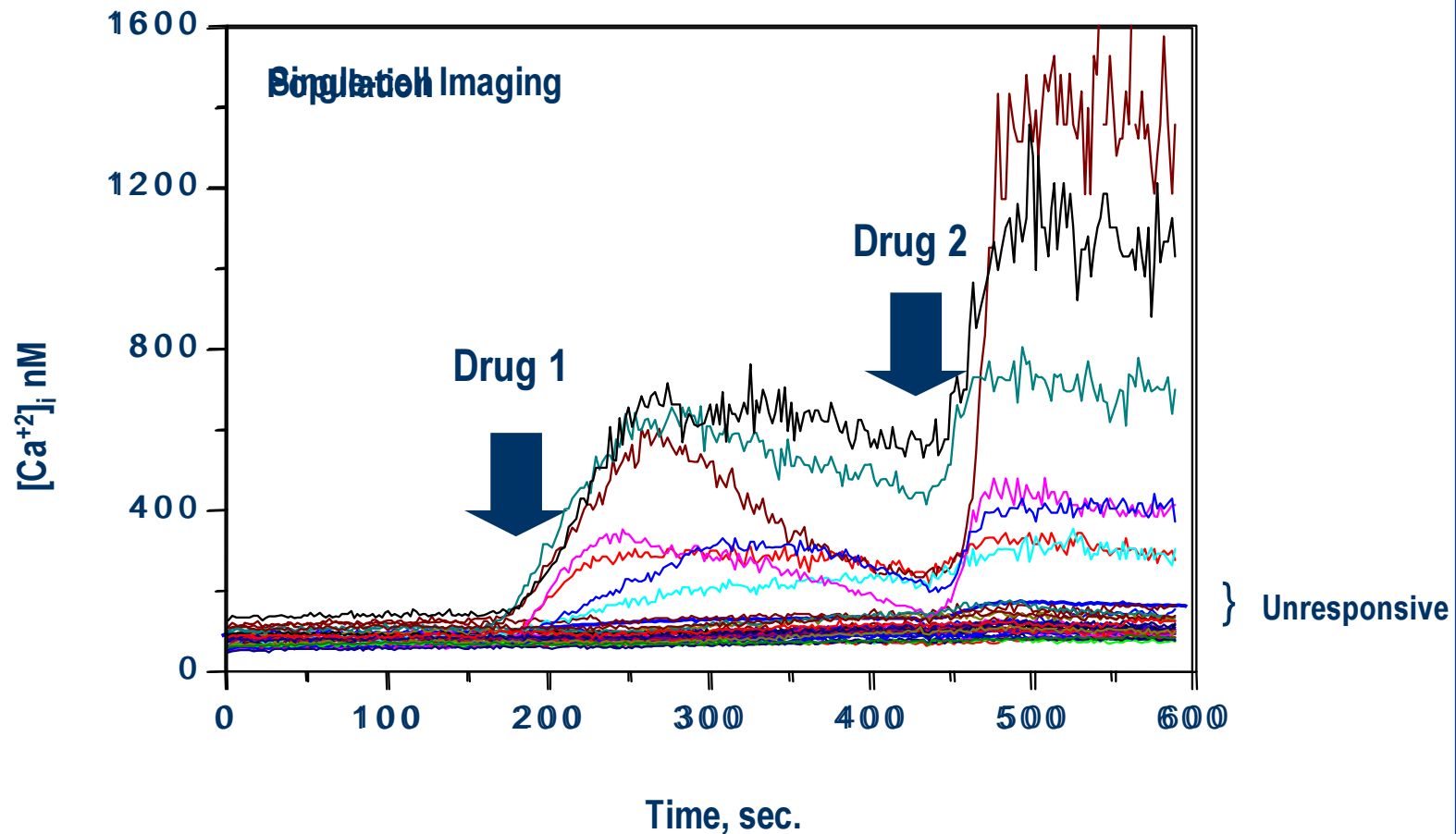
Living Cell , Kinetic Assay

***BD Pathway 855** with Environment Control &
Liquid Handling System*

1. Fluorescence Intensity Changes

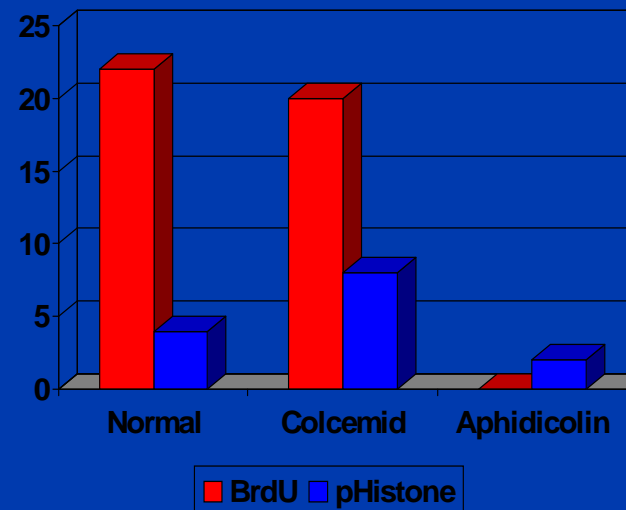
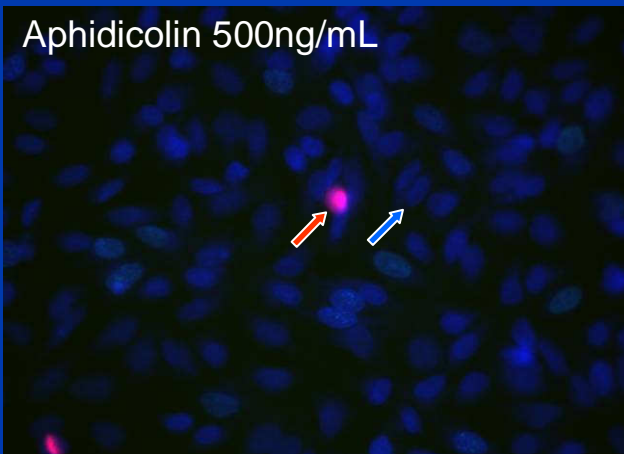
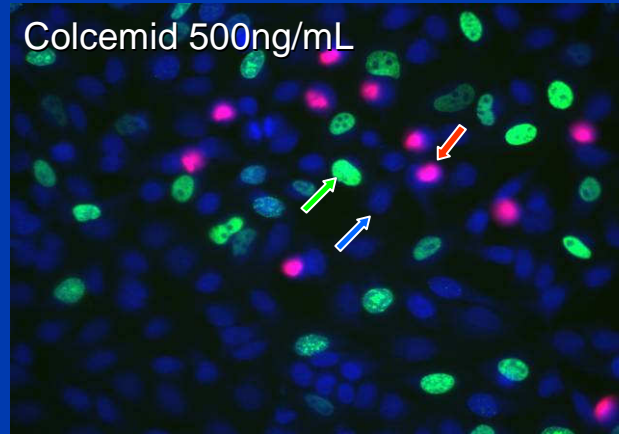
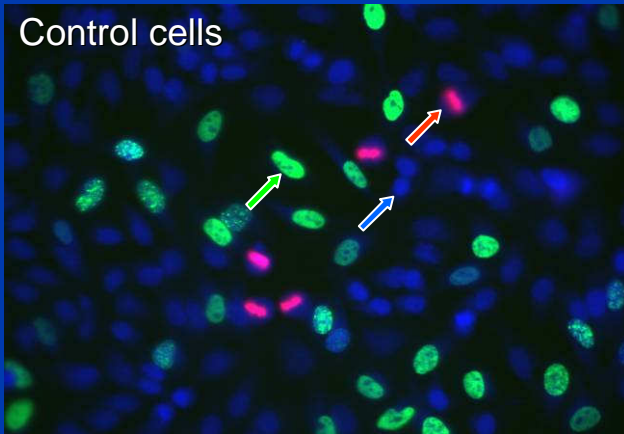
Investigate heterogeneous cell populations :

Single-cell Kinetic Imaging Reveals Heterogeneous Calcium Response



1. Fluorescence Intensity Changes

Working with few cells :
Image Based Cell Cycle Analysis



BrdU
(S phase)
Phospho-histone
(M phase)
Negative
(non cycling)

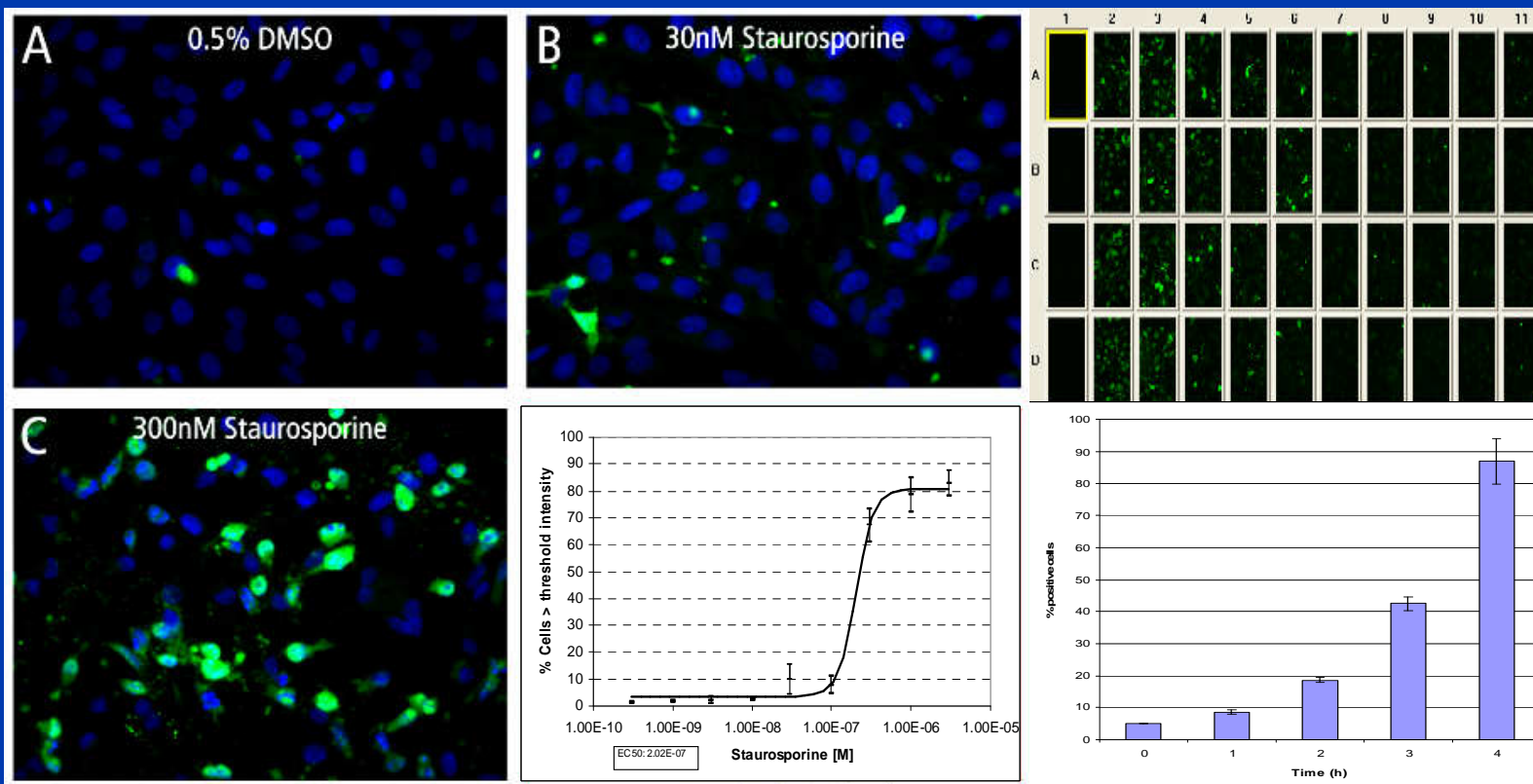
1. Fluorescence Intensity Changes

Working with few cells :

Indicator of Apoptosis in Image-based Assays

HeLa cells

20x objective Cleaved caspase – green Nuclei - blue

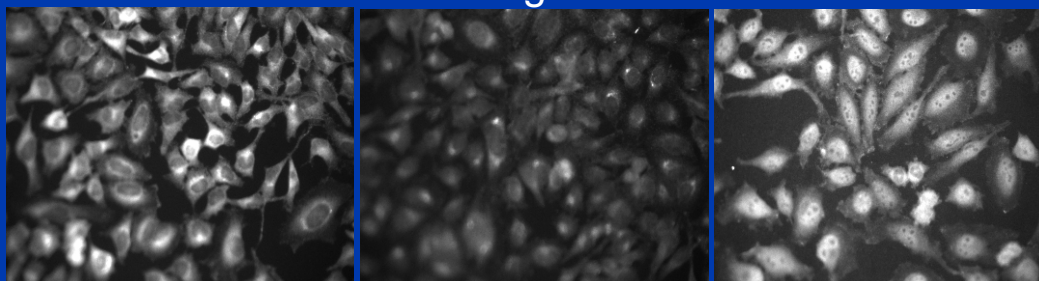


2. Fluorescence Distribution

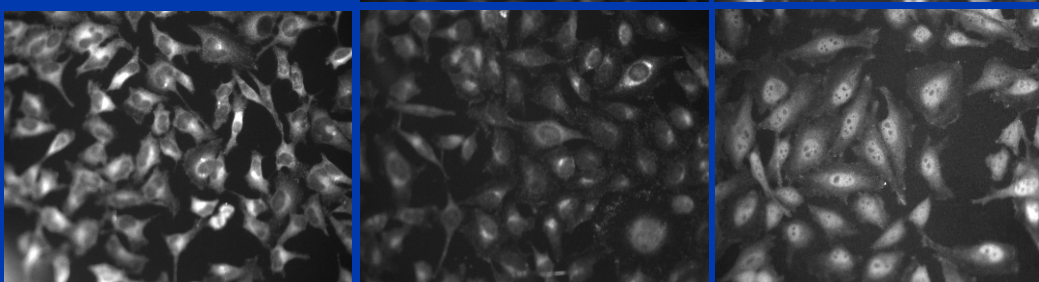
Measure cell changes where total fluorescence intensity does not change

Untreated $\text{I}\kappa\text{B}\alpha$ Degradation $\text{NF}\kappa\text{B}$ Translocation

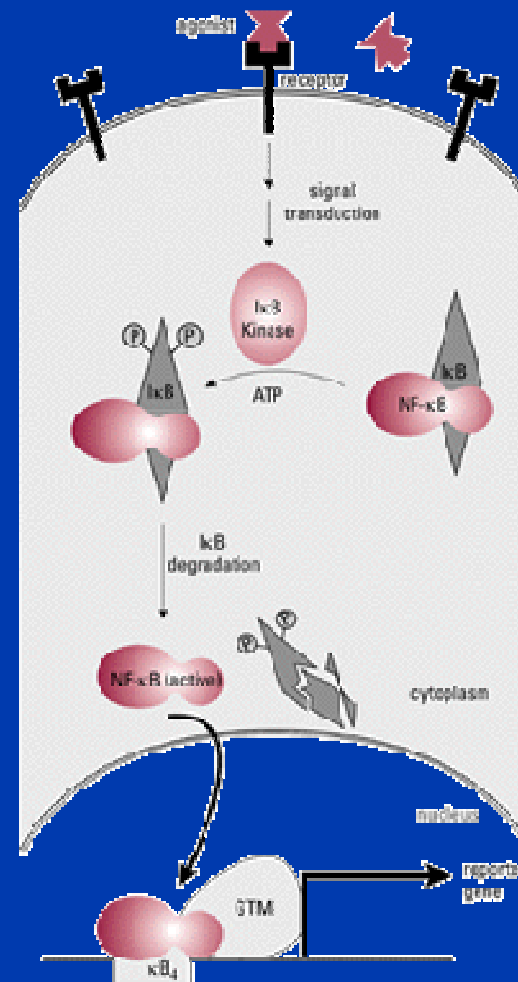
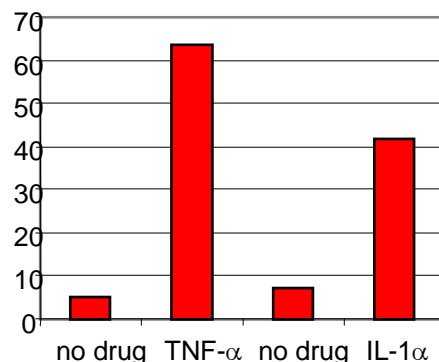
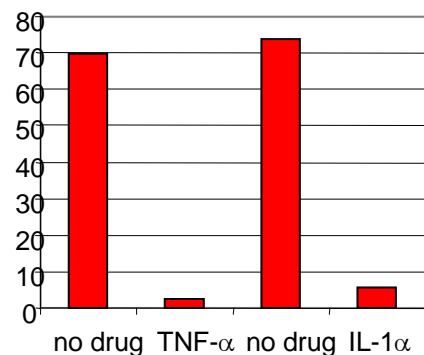
$\text{TNF-}\alpha$
25 ng/ml
30 min



$\text{IL-1}\alpha$
25 ng/ml
30 min



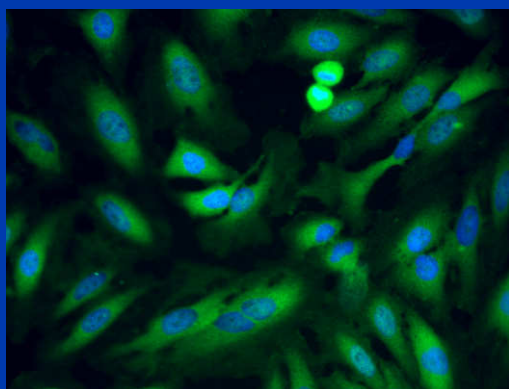
percent cells
above threshold



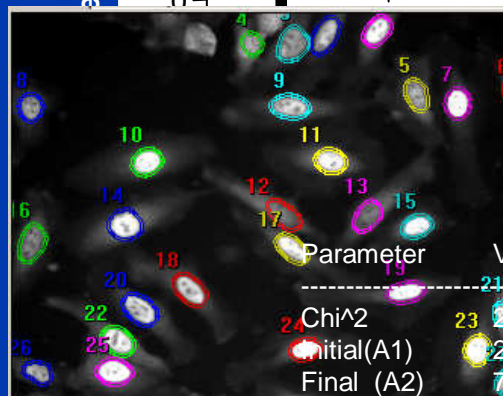
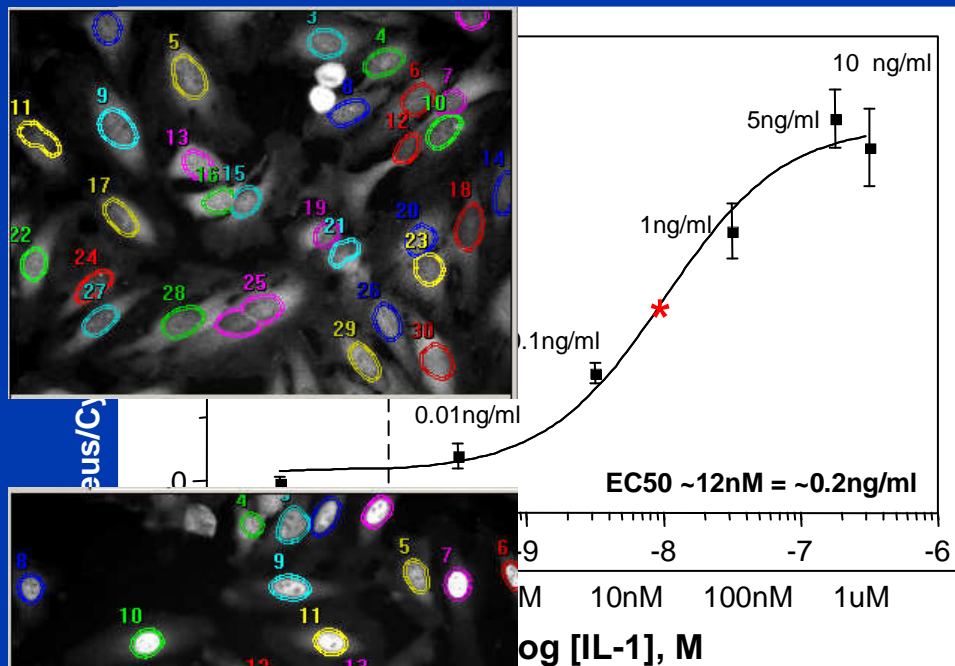
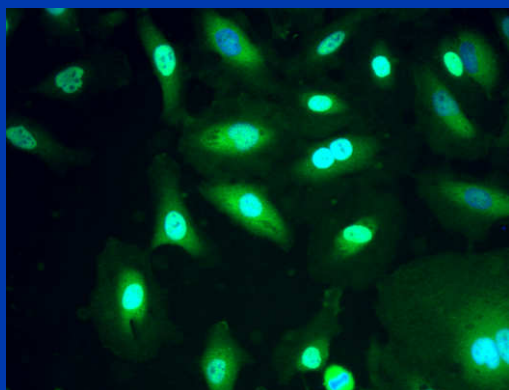
2. Fluorescence Distribution

Measure cell changes where total fluorescence intensity does not change

Overlay (Hoechst/Alexa488)



Control



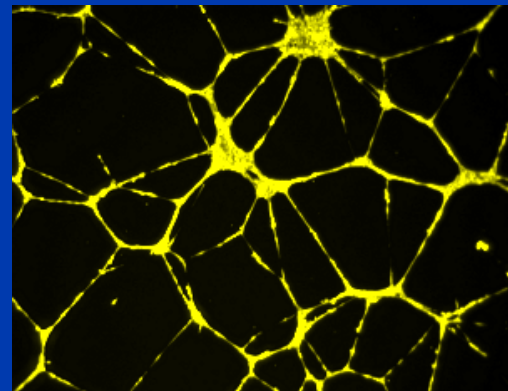
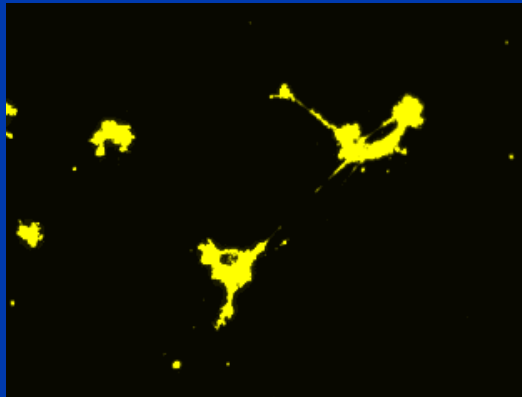
Parameter	Value	Error
Chi^2	2951.83789	
Initial(A1)	237.39175	40.15385
Final (A2)	756.02983	32.19608
EC50 (x0)	4.57328E-10	1.00818E-10
Power (p)	1.75846	0.64479

3. Morphometric Measurement

Make measurements on cells using structural dyes/antibodies:

Angiogenesis

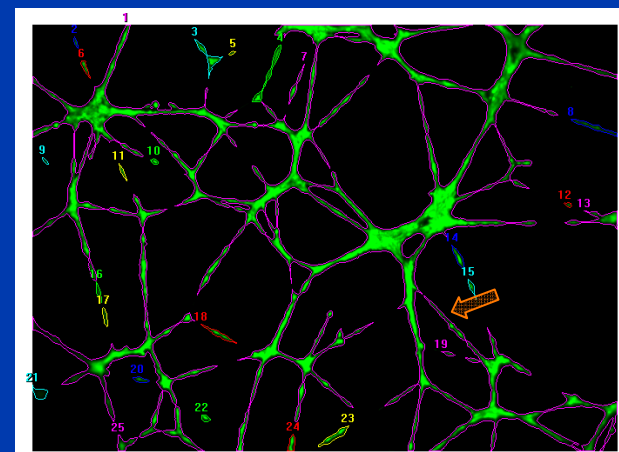
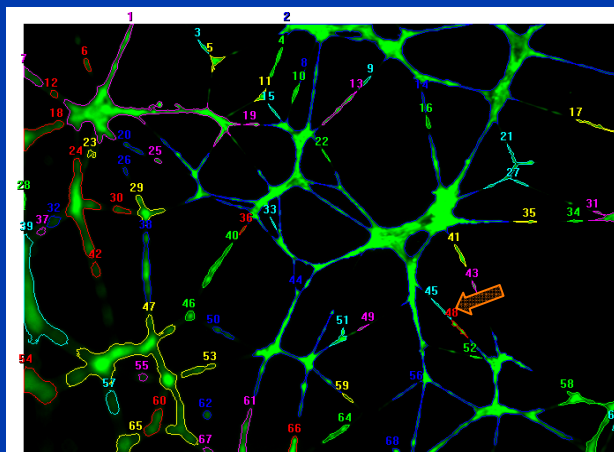
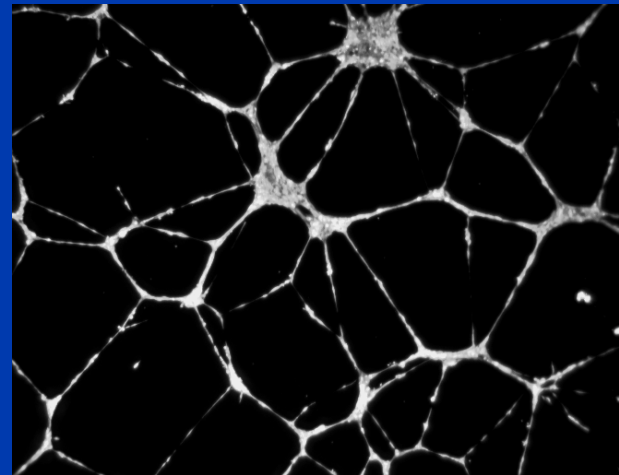
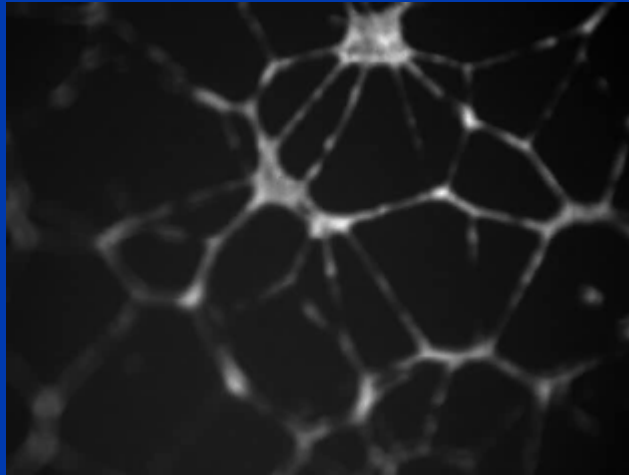
- Used to determine the +/- response of primary endothelial cells to compounds
 - Tumor formation
 - Wound healing
 - Retinopathy
 - Macular degeneration
- Model system
 - HUVEC-2 cells
 - Matrigel Matrix coated plates
 - Network of tubules formed – live cell stain & imaging
 - Suramin treatment – retards the formation of blood vessels



3. Morphometric Measurement

Make measurements on cells using structural dyes/antibodies:
Angiogenesis

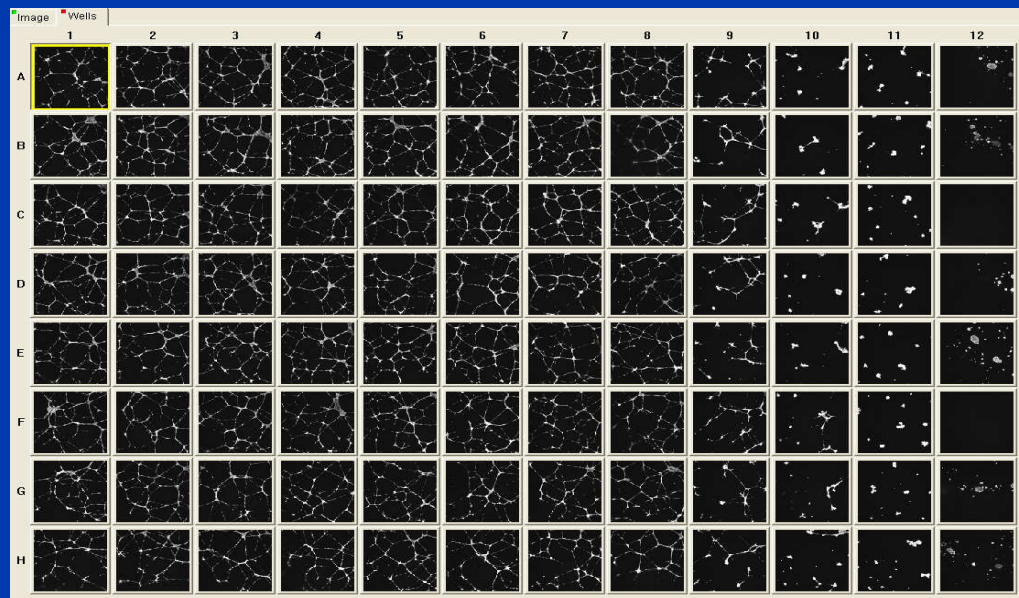
HUVEC angiogenesis on BD Matrigel™ Matrix



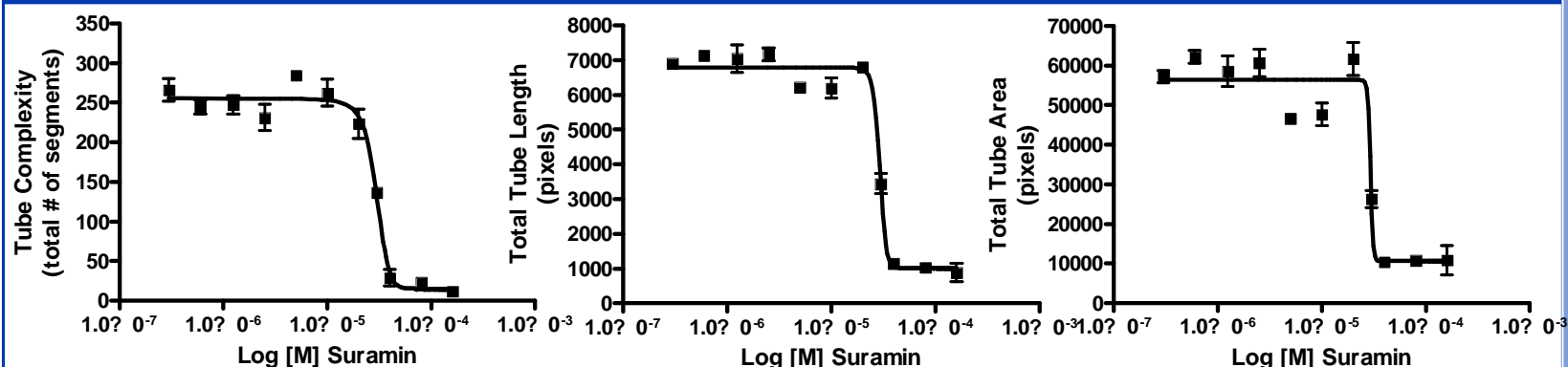
3. Morphometric Measurement

Make measurements on cells using structural dyes/antibodies:

Angiogenesis: Quantitative Data



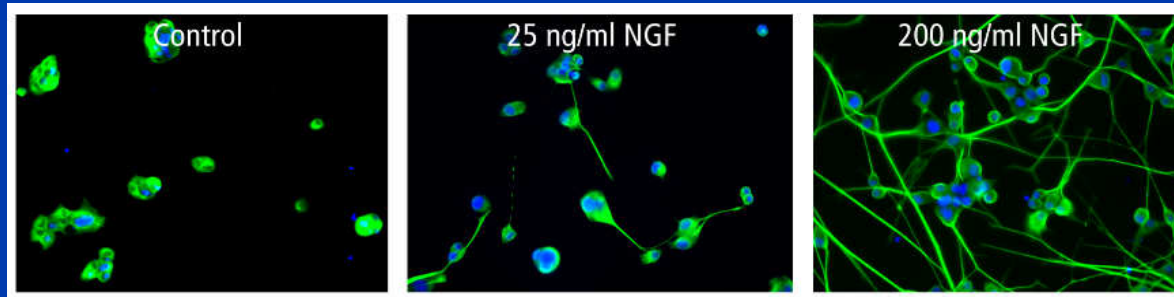
Tube Total Length
Tube Average Length
Tube Max Length
Tube Branch Count
Tube Segment Count
Tube Count
Tube Nodes Points



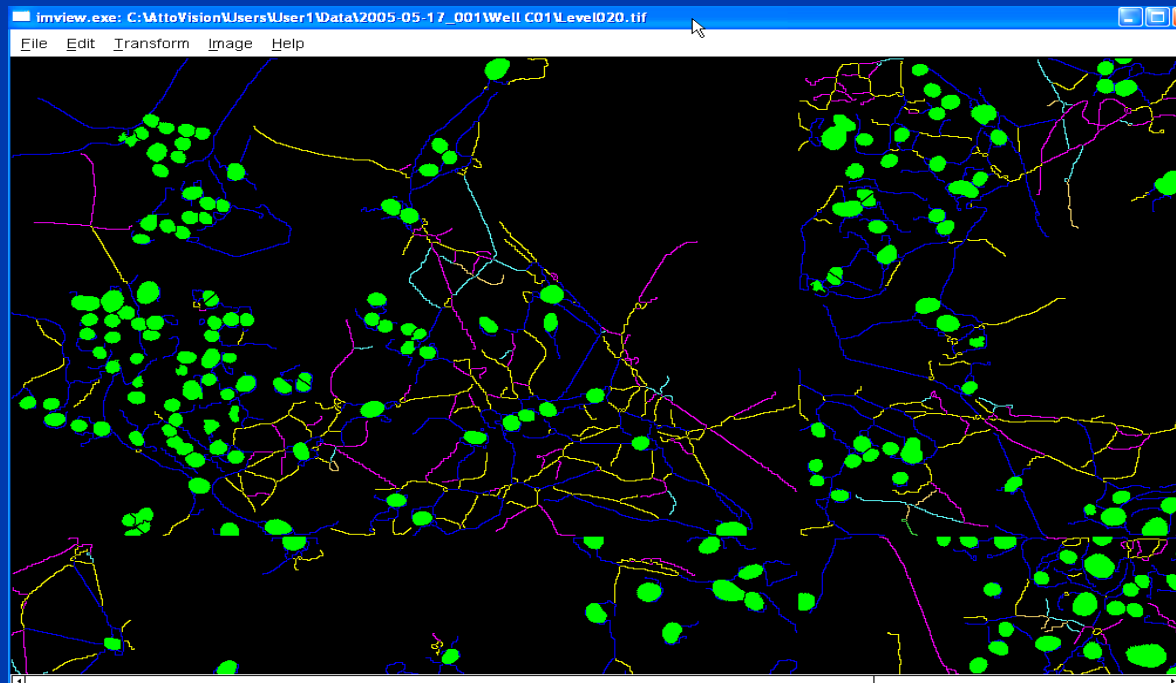
3. Morphometric Measurement

Make measurements on cells using structural dyes/antibodies:

Angiogenesis: Neurite Outgrowth



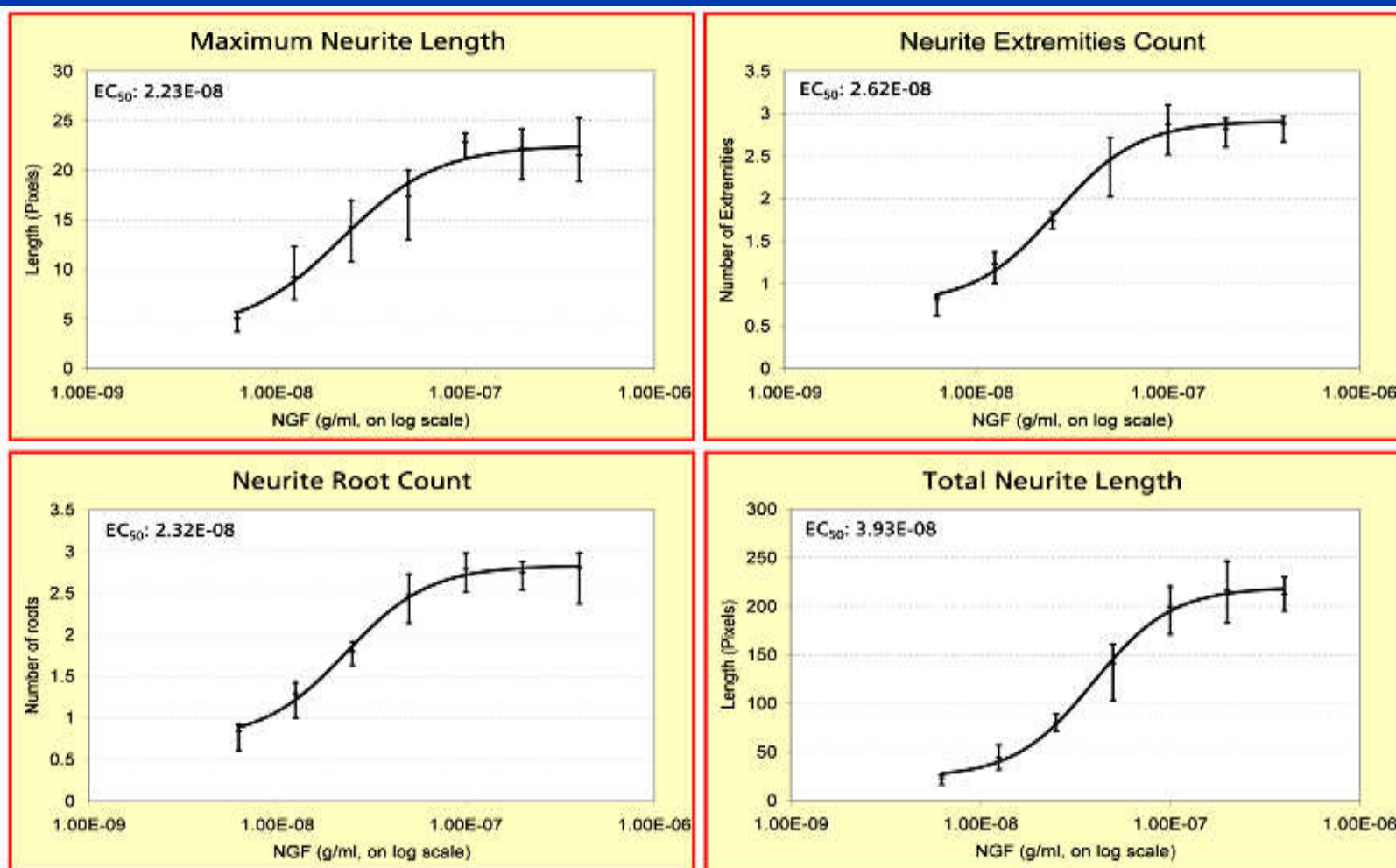
PC-12 cells
induced with NGF



- Nuclear Mask:
Cell Segment
- Nurite Mask:
Nurite Segment
- Merge:

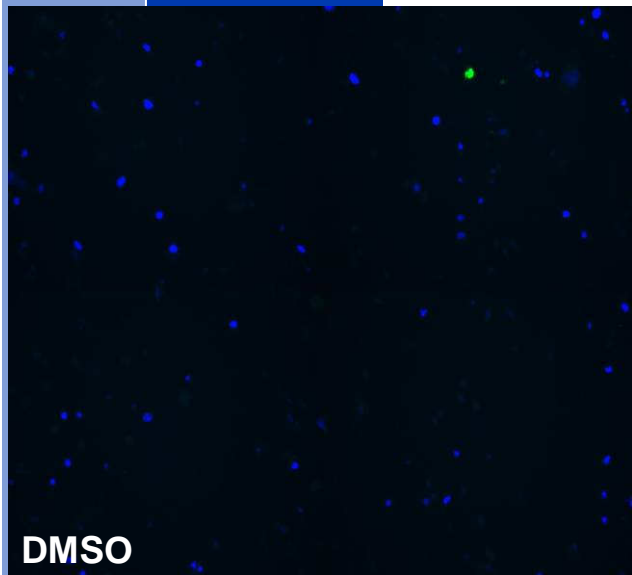
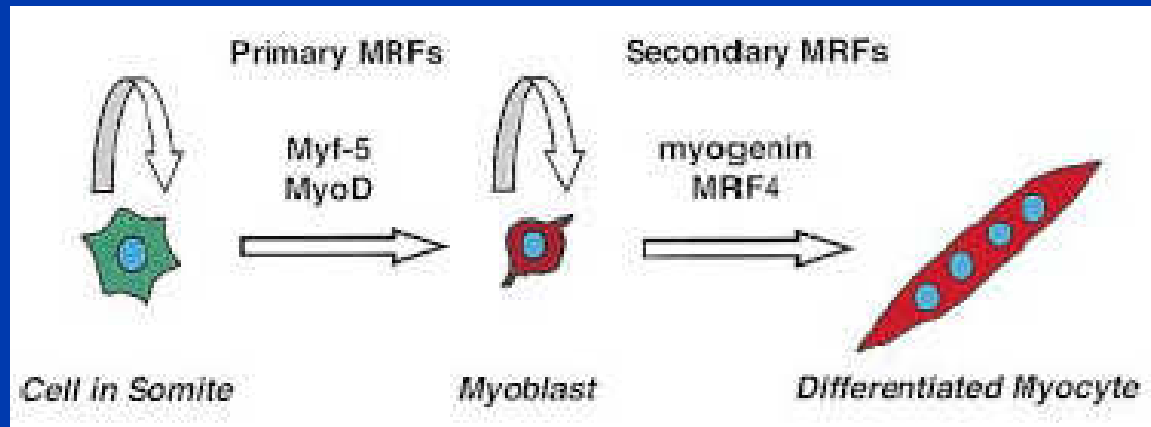
3. Morphometric Measurement

Make measurements on cells using structural dyes/antibodies:
Neurite Outgrowth

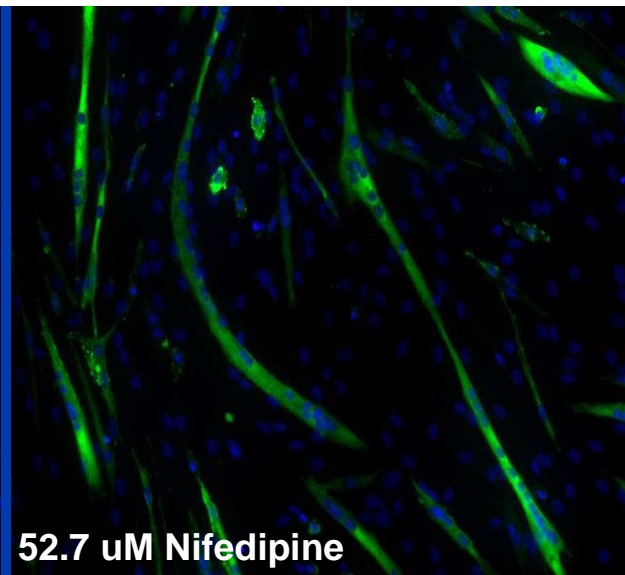


3. Morphometric Measurement

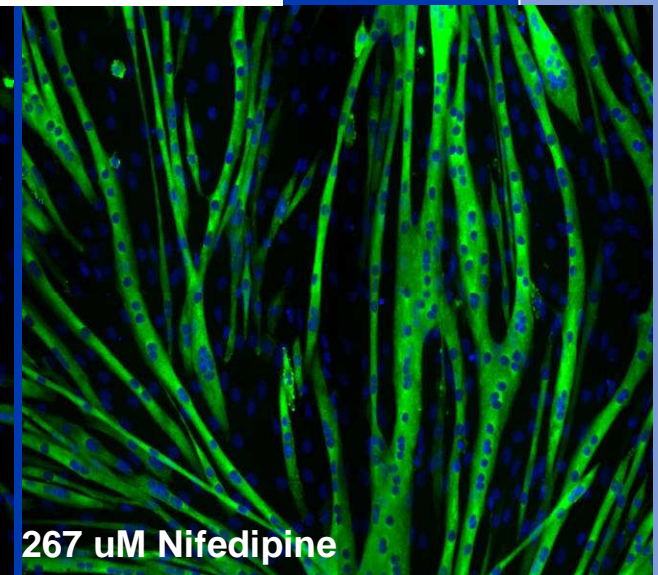
Make measurements on cells using structural dyes/antibodies:
Miotube Fusion



DMSO



52.7 uM Nifedipine

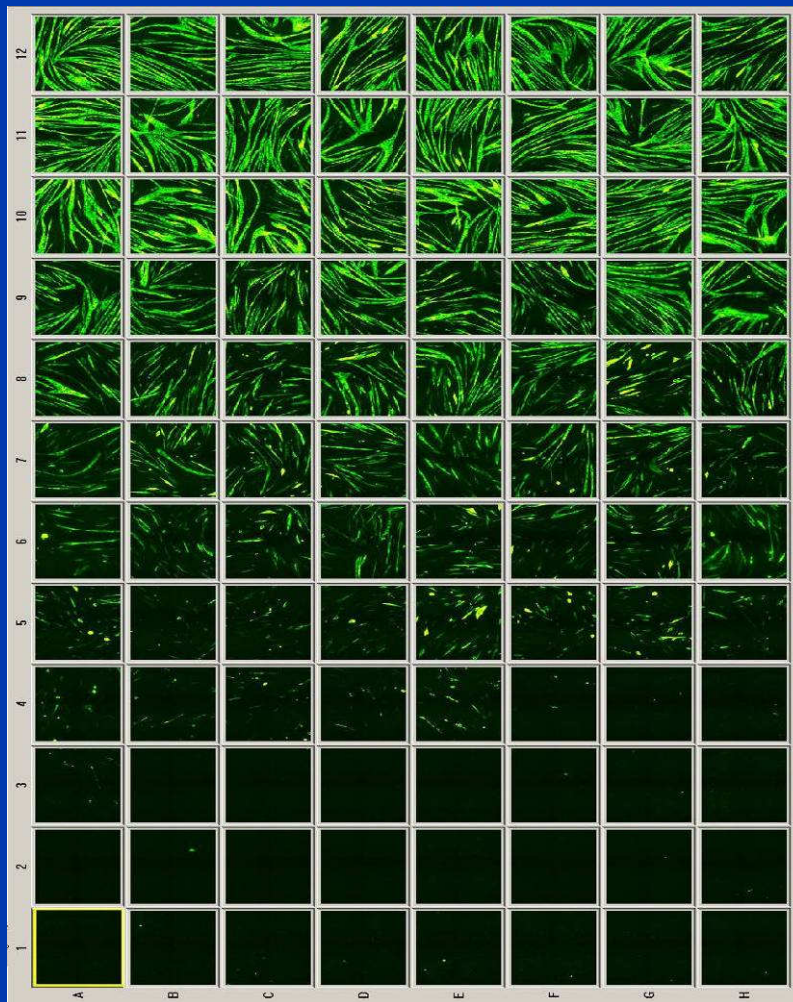


267 uM Nifedipine

3. Morphometric Measurement

Make measurements on cells using structural dyes/antibodies:

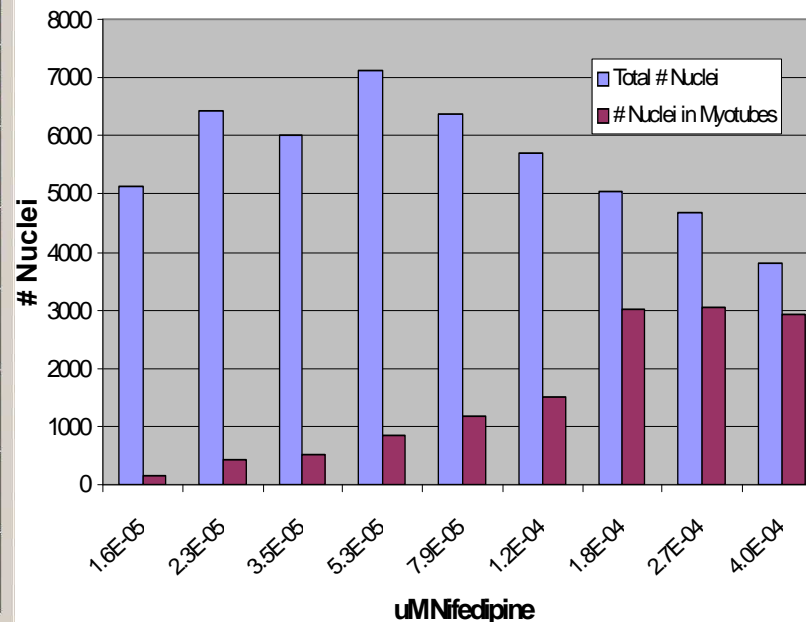
Myotube Fusion



This confocal image is using BD 96-Well Optilux Microplates to increase image quality



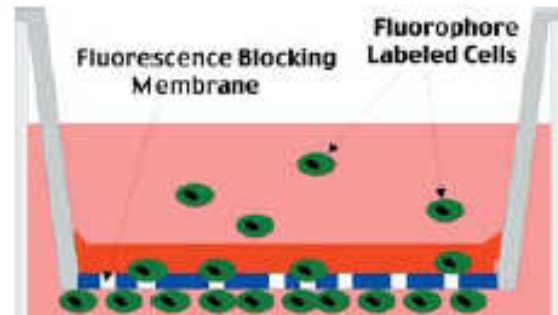
Number of Nuclei in Myotubes



4. Cell Movement

- *Measure cellular mobility/motility and invasion*
Invasion

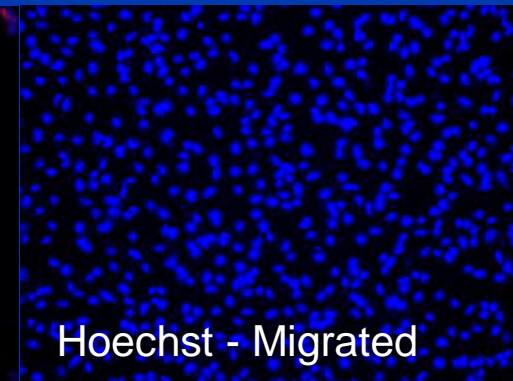
Cell Migration/Invasion Assays Using a Fluorescence Blocking Membrane



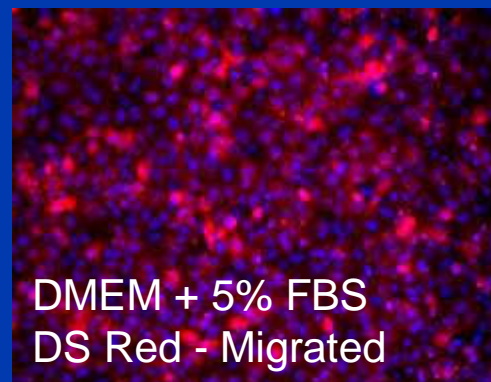
BD Falcon™ HTS FluoroBlok™ 96-Multiwell Insert System



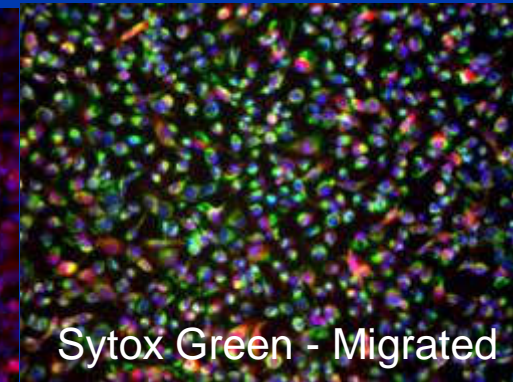
BSA Control - Migrated



Hoechst - Migrated



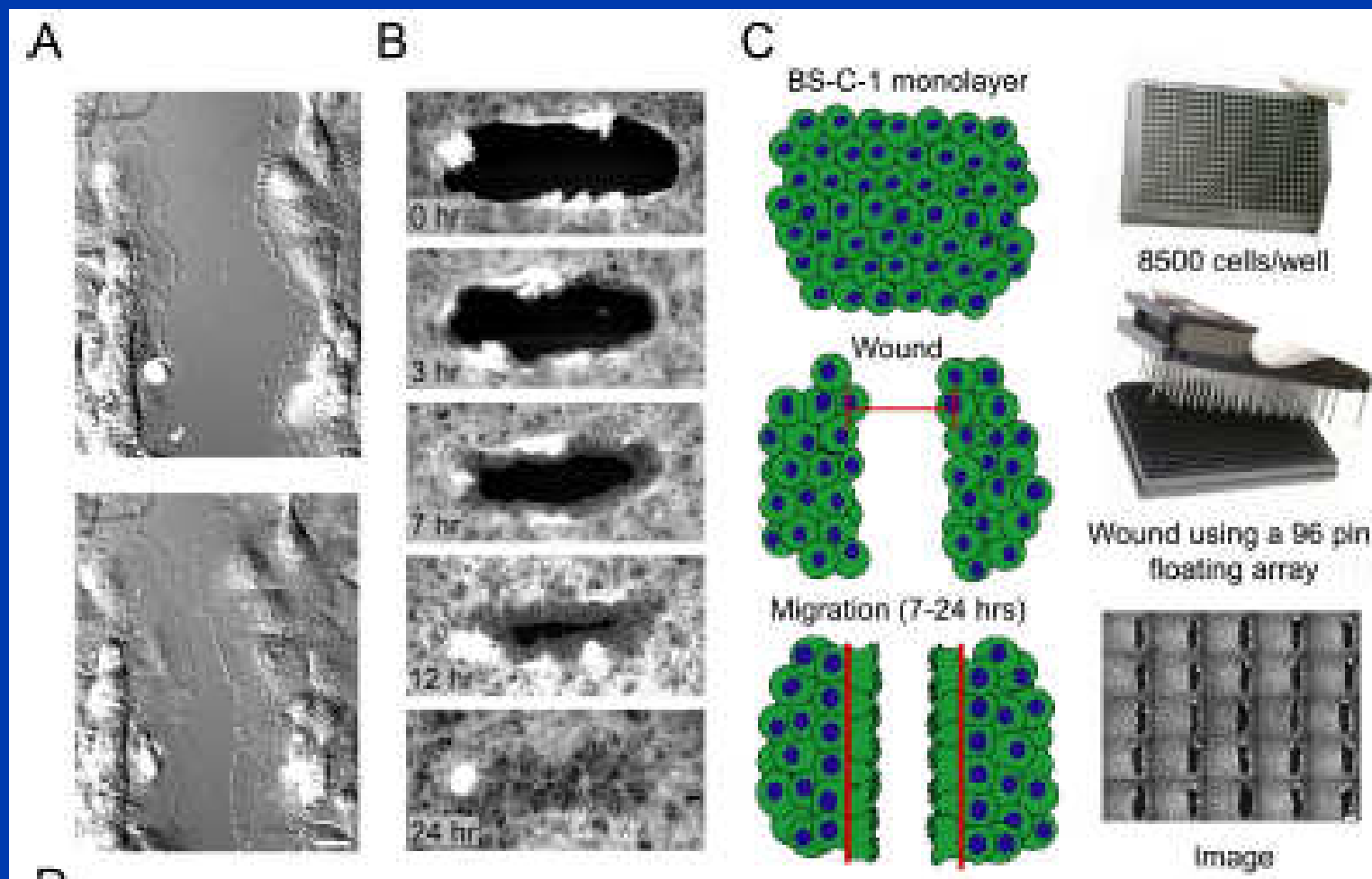
DMEM + 5% FBS
DS Red - Migrated



Sytox Green - Migrated

4. Cell Movement

- *Measure cellular mobility/motility and invasion*
Wound Healing Assay



4. Cell Movement

- *Measure cellular mobility/motility and invasion*
Wound Healing Assay

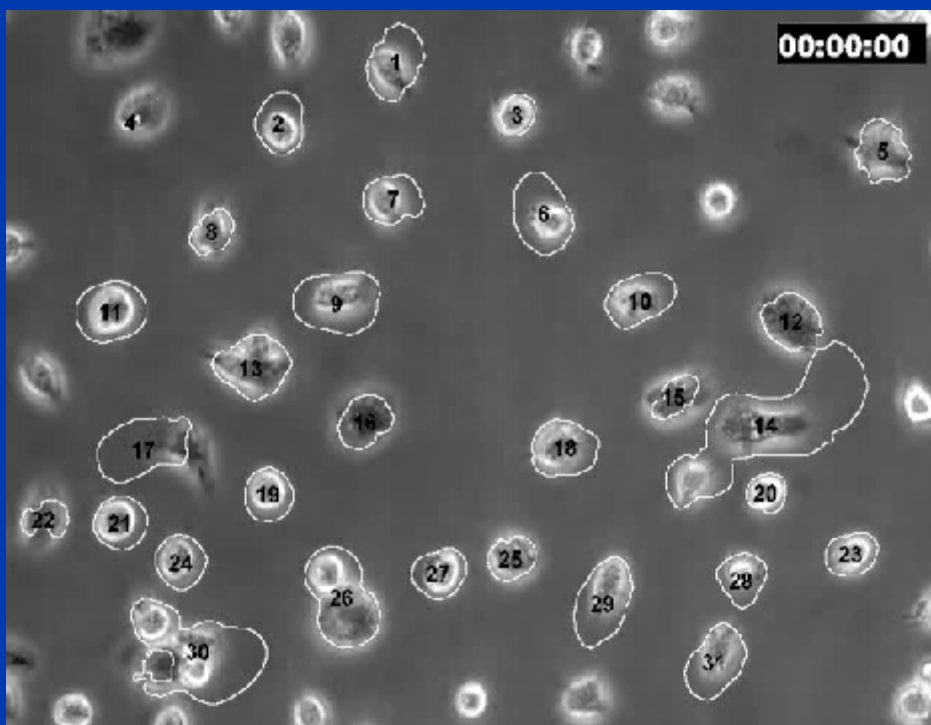


Image analysis and record by
BD IPLab V4 Software

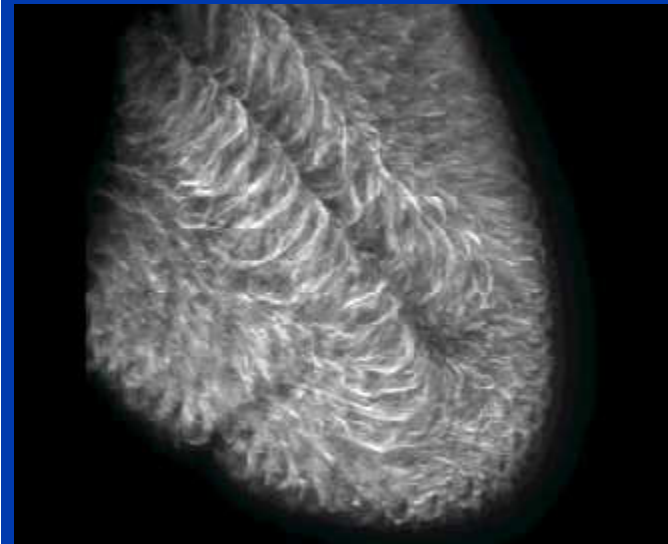
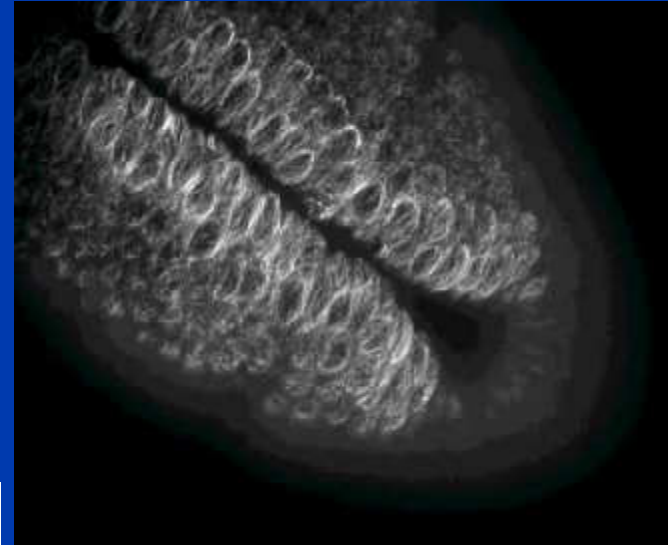
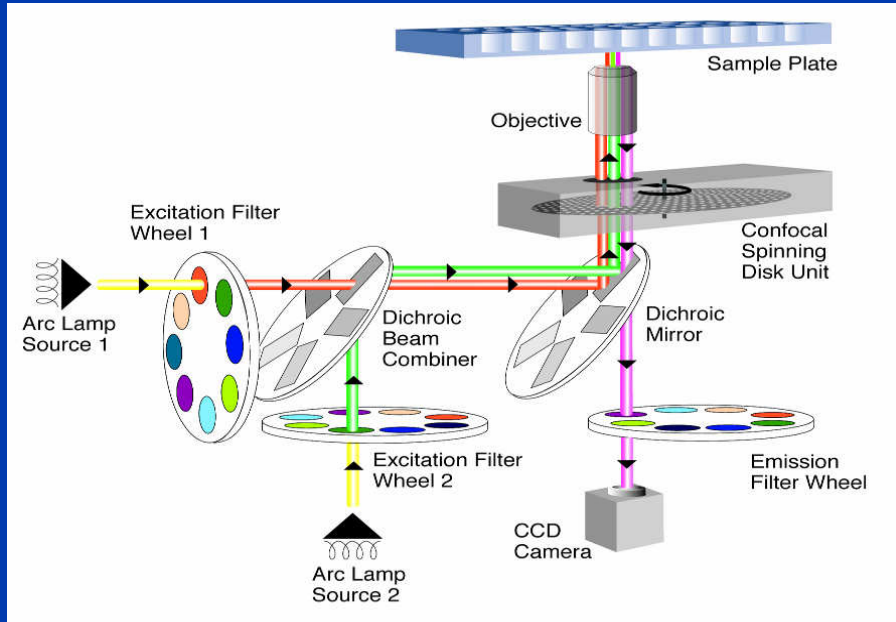
Microsoft Excel - Results Table.xls

	A	B	C	D	E
1	Meas. #	Mean	Area	Perimeter	Major Angle
2	1	1.3	48.1	86.9	70.5
3	2	1.8	39.1	71.3	86.7
4	3	1.2	27.6	50.7	63.3
5	4	1.0	8.3	18.2	22.3
6	5	1.2	44.9	81.2	-67.2
7	6	1.6	50.6	90.8	-74.1
8	7	1.3	39.8	73.1	4.2
9	8	1.3	32.3	58.0	44.7
10	9	1.4	53.6	100.2	2.6
11	10	1.5	45.1	81.0	22.7
12	11	1.8	46.4	85.1	12.4
13	12	1.2	41.9	76.4	-24.9
14	13	1.4	52.3	90.7	2.3
15	14	1.3	135.2	249.0	31.6
16	15	1.3	32.5	59.4	41.7
17	16	1.4	38.5	70.1	39.8
18	17	1.2	59.3	110.3	19.7
19	18	1.5	44.2	81.6	2.6
20	19	1.7	35.7	65.3	89.5
21	20	1.4	28.1	51.9	9.2
22	21	1.7	37.9	68.5	18.7
23	22	1.1	33.4	61.6	8.5
24	23	1.6	33.2	62.2	15.8
25	24	1.6	37.4	66.3	-77.7
26	25	1.3	33.8	60.5	29.7
27	26	1.6	64.0	116.0	-68.4
28	27	1.8	37.4	67.3	24.1
29	28	1.4	37.4	66.4	83.2
30	29	1.4	52.7	95.2	69.6
31	30	1.5	116.7	215.2	-11.4
32	31	1.5	48.9	84.7	60.3

3D Reconstruction

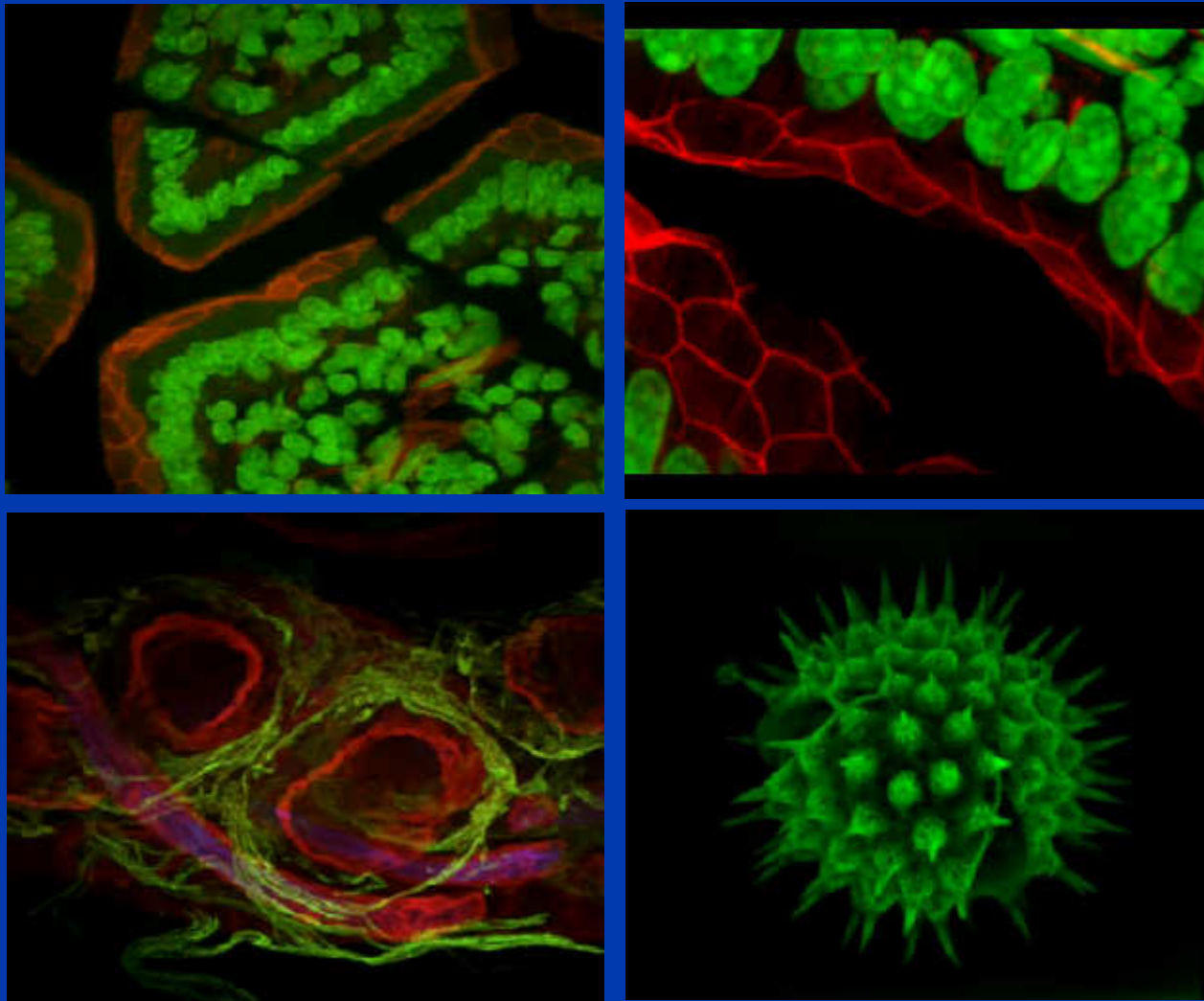
Image Acquire by **BD Pathway 855** with confocal module

- Fly embryo gastrulation
- Tubulin.
- PlanApo 60x 1.4 NA
- 0.5um steps
- xz, yz (1-256)
- George von Dassow
- Friday Harbor, UW



3D Reconstruction

Image Acquire by **BD Pathway 855** with confocal module



Time Lapse

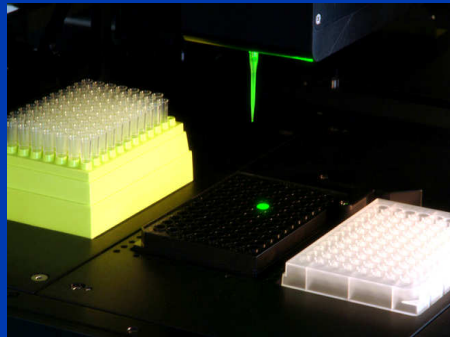
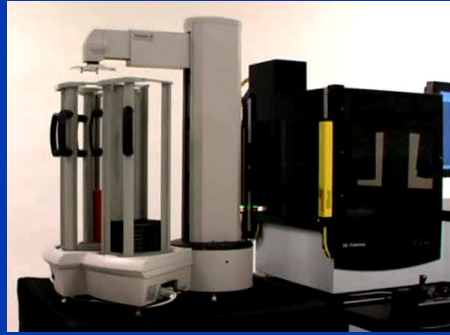
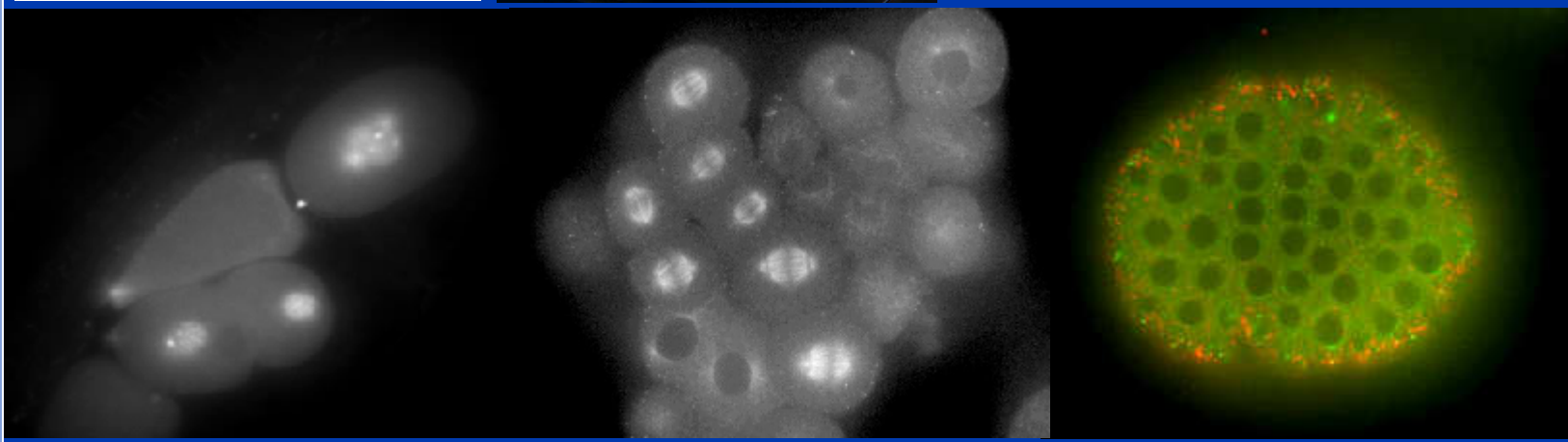


Image Acquire by
BD Pathway 855
with environment control

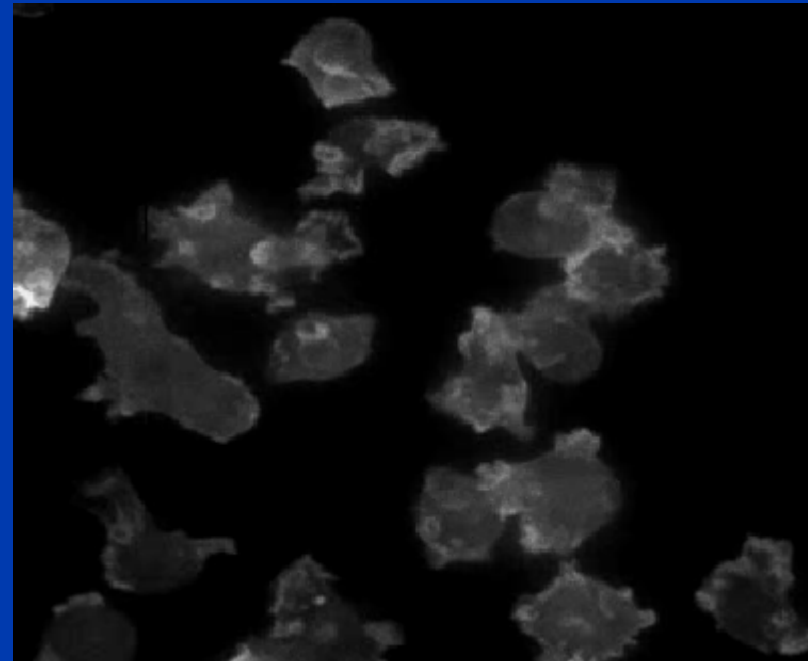
- Cell division
- Sand dollar embryos
- Injected with rhodamine-tubulin & Alexa 488 phalloidin.
- Early- mid blastula
- PlanApo 60x water lens
- George von Dassow & Bill Bement
- Friday Harbor, UW



4D Image

Image Acquire by **BD Pathway 855** with environment control

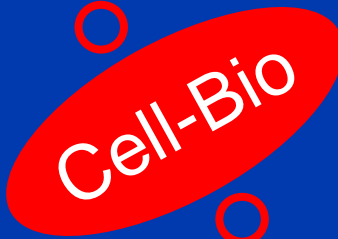
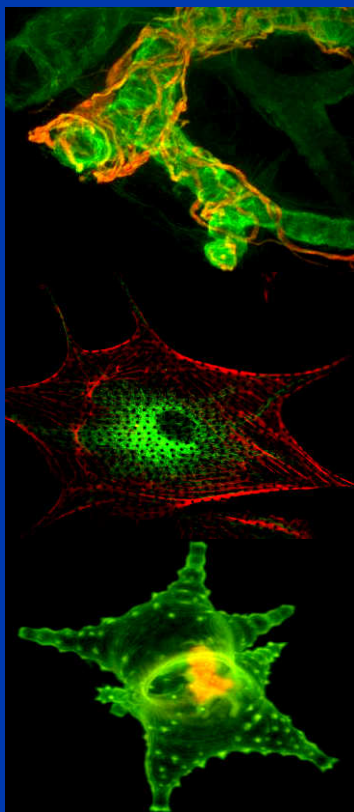
- Dictyostelium Feeding on Yeast
- Dictyostelium – GFP- cell membrane
- 150ms, 13z steps, 30min
- Cascade 512B
- David Knecht Department of Molecular and Cell Biology
University of Connecticut



Summary and Conclusions

- High content analysis provides a unique opportunity to investigate spatial and temporal events in living cells
- Kinetic measurements can provide useful information (rates of change)
- Managing kinetic data requires real-time data acquisition and data reduction capabilities
- Multiplexed kinetic experimentation requires biological events to be co-occurring

Thanks For Your Patient & Attention!!
Any Question



Cell-Bio

尚博生物科技有限公司

www.cell-bio.com.tw

Glenn Yang

apoptosistw@gmail.com

02-27855860

0953062485