

High Content Screening for evaluation of nanoparticles interaction with cells

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Introduction

- High Content Screening (HCS):
 - Automated Imaging & Quantitative Analysis
- Principle
 - Combination of flow cytometry & ELISA or confocal microscope
- Technology
 - Automated fluorescent microscopy imaging

Core Facility at the IMM TCD

- InCell 1000
 - GE Healthcare
 - Image acquisition then analysis
- KineticScan
 - Cellomics
 - Image acquisition & analysis
 - Live cell chamber
 - Liquid handling
- Also
 - Liquid Handling
Deerac, Hydra
 - Robotics

HCS hardware



Thermo Fisher (Cellomics)
KineticScan HCS Reader



GE InCell Analyser 1000

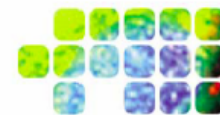
- **High Resolution cellular and sub-cellular Imaging with uncompromised resolution.**
- **Kinetic Assay scheduling software -fully automated, unattended execution of experimental protocols.**
- **Integrated Liquid Handling for reagent delivery, mixing and cell washing during live cell experiments.**
- **Onboard Environmental Control.**
- **Flexible Optical System for a variety of fluorescent reporters, including FRET and ratiometric imaging.**

The Kinetic Scan HCS Reader

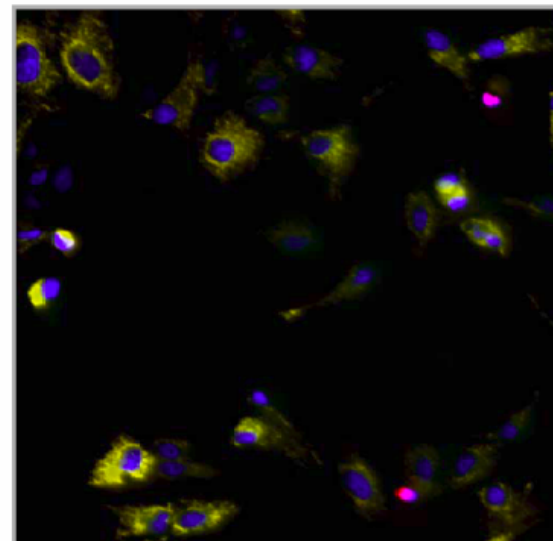


1. Live-cell Environmental Sample Chamber
 - Pipettor Access Port
2. Pipetting Capability with 1-to-8 Channel Liquid Handling
3. Inverted Fluorescence Microscope
 - 5X, 10X, 20X, 40X Objective Lenses
4. High-resolution, CCD Camera
5. Open System for Integration with Robotics
6. Software for acquiring and analysing images

High Content Screening Benefits: Broad Excitation White Light Source

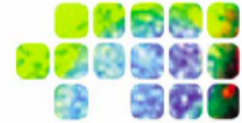


- Ability to use a wide variety of fluorescent indicators
- Excitation from 350-700 nm
- Decreased photodamage
- Decreased phototoxicity
- Low cost
- Low maintenance
- No daily alignment required

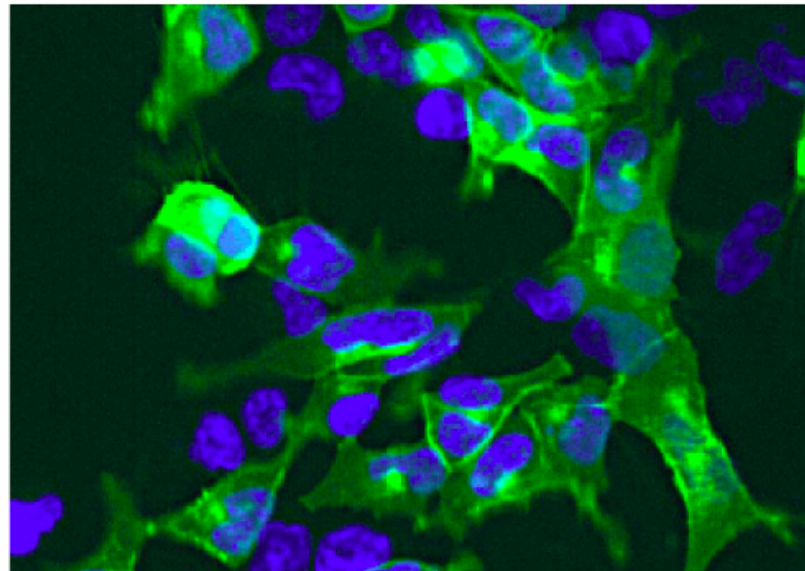


- Blue: Nuclear Morphology
- Green: Calcium Homeostasis
- Yellow: Mitochondrial Transmembrane Potential
- Red: Membrane Permeability

High Content Screening Benefits: High Resolution CCD Imaging

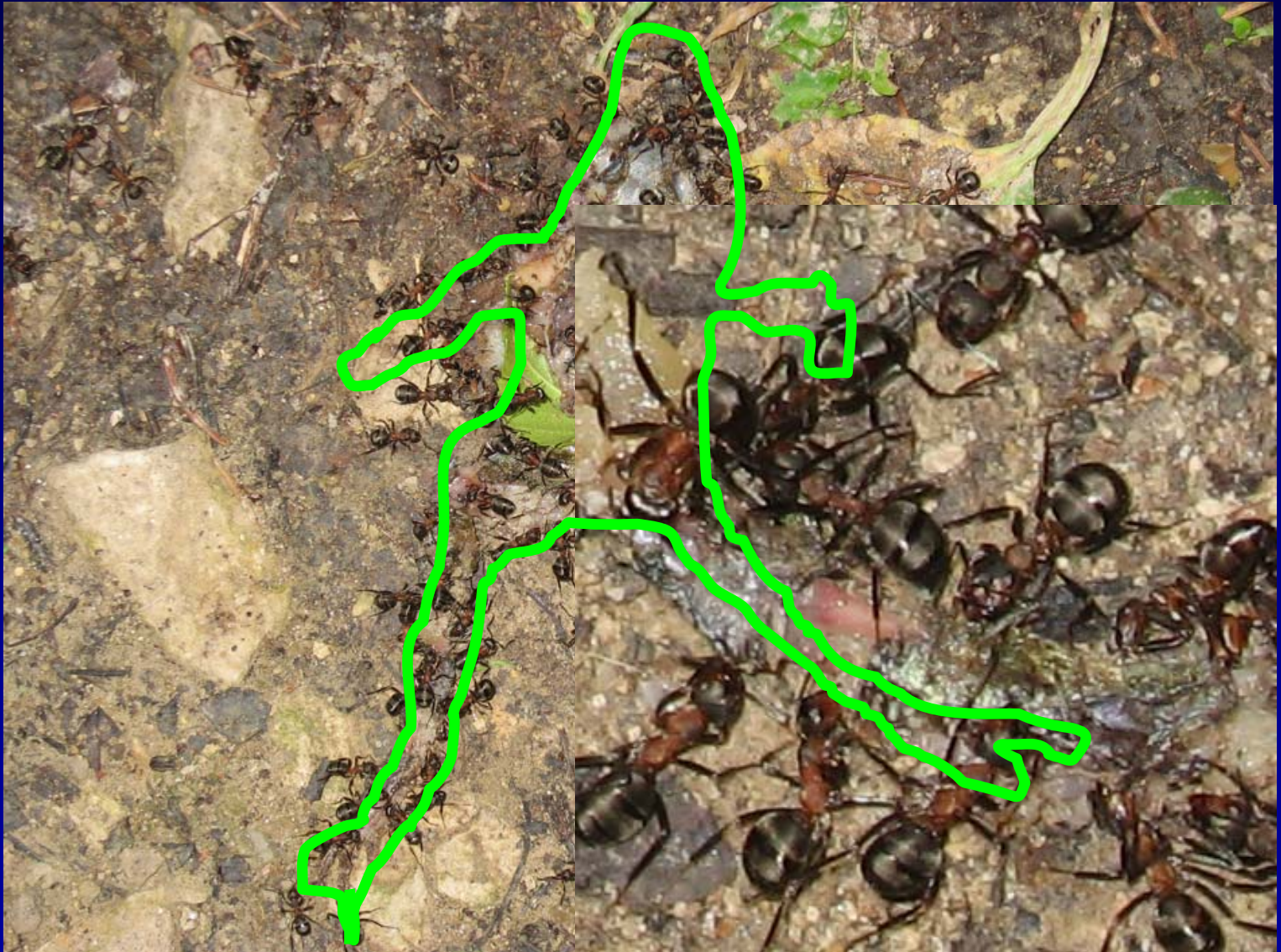


- Sub-micron detection capability
- Identify single organelles
- Track targets within the cell
- Measure target co-localization
- High dynamic range
- Sub-cellular imaging capability



How is it done?

Algorithms of pattern recognition



Assay Preparation

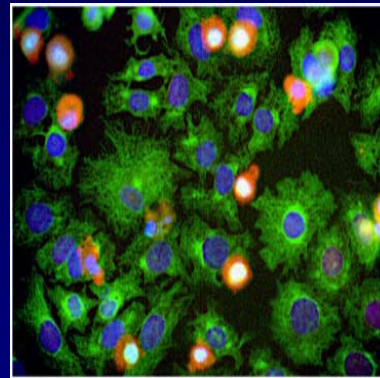
- Plate: - 6, 24, 96, 384, 1536 well plates
 - Microtitre dimensions
 - Any good optical quality plate
- Cells: - monolayer 100uls of 10^4 - 10^5 /mL (depends on assay)
- Add test material
 - Drugs, cytokines, enzymes, siRNA, Quantum dots,
- Staining: - nuclei or cells clearly stained
- Wells should be initially examined by microscope to ascertain cell numbers, staining and distribution.
- Rubbish in, Rubbish out

Cellomics Platform Workflow

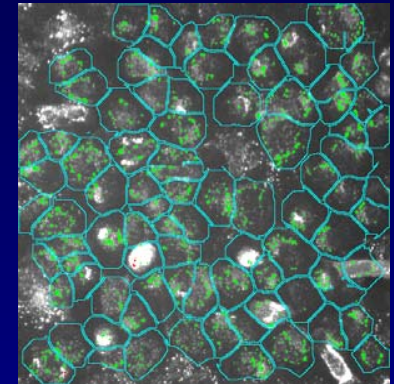
Automated Plate Delivery



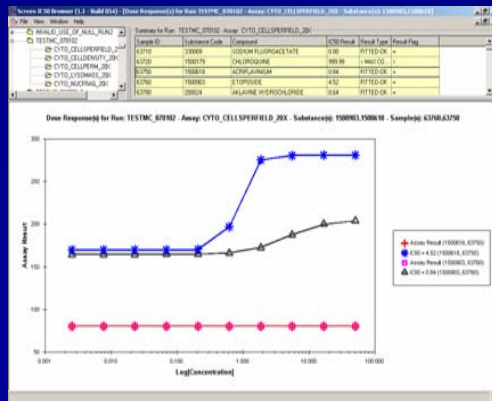
Auto-focus, Expose & Acquire



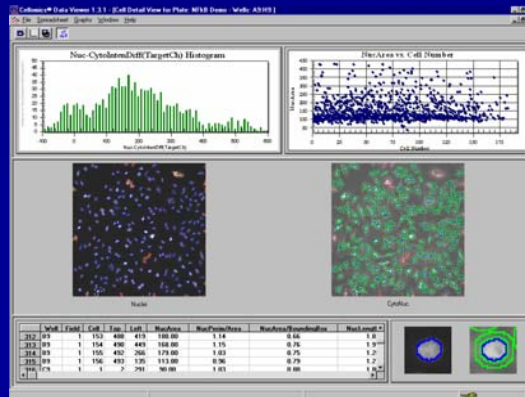
Automated Image Analysis



Analysis of Results



Instantaneous Data Display

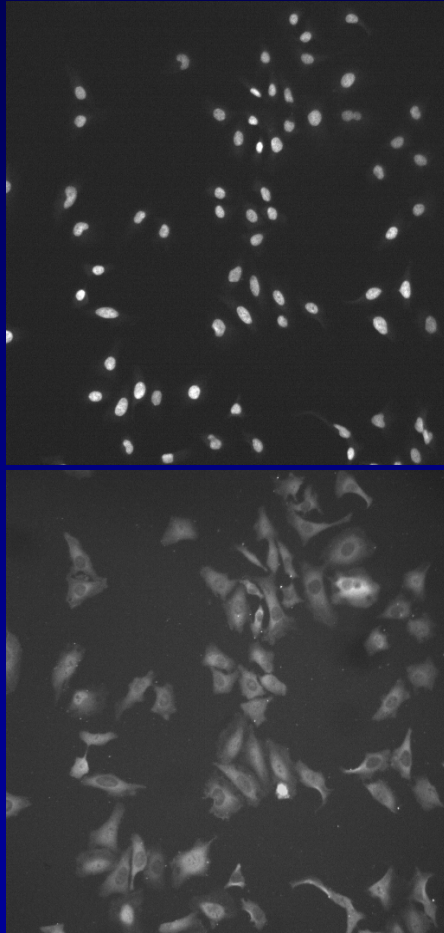


Automatic Data Archival

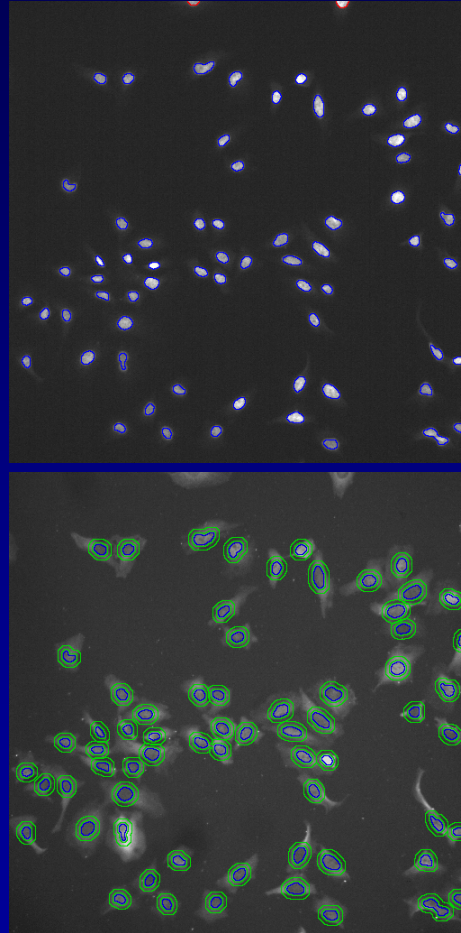
Plate ID / Bar Code	Plate	Location	Creation Date	Assay	Creator	Scan Type
Apoptosis DR	KOL	LOCAL	2001/06/13	Apoptosis MP-1	CELL	Active Scan
ROSCOP	KOL	LOCAL	2002/06/01	GPCR Signaling Cell	CELL	Active Scan
RSAR-LPH Demo	KOL	LOCAL	2002/03/11	GPCR Signaling Cell	CELL	Active Scan
CRISPR-FCO	KOL	LOCAL	2001/07/09	Heated/Targeted Assay/21	CELL	Active Scan
CRISPR-FCO	KOL	LOCAL	2002/02/27	Apoptosis MP-1	CELL	Active Scan
GPCR Signaling	KOL	LOCAL	2002/02/20	GPCR Signaling Cell	CELL	Active Scan
GPCR Signaling	KOL	LOCAL	2002/02/20	GPCR Signaling Cell	CELL	Active Scan
Nucleus Demo	KOL	LOCAL	2001/06/06	Nucleus Translocation	CELL	Active Scan
NFkB Demo	KOL	LOCAL	2002/05/01	Nucleus Translocation	CELL	Active Scan
NFkB Responders	KOL	LOCAL	2002/05/01	Nucleus Translocation	CELL	Active Scan

Processing of Images on the Cellomics® HCS Systems

Images Acquired



Auto-Threshold & Algorithm Applied



Data Extracted

Avg CytoNuc Diff	66.0
Cell Count	148.5
Avg Nuclear Intensity	495.0
Avg Nuclear Area	154.2
Std Dev Nuclear Area	43.6
Std Dev Nuclear Stain Intensity	65.5
Std Dev CytoNuc Diff	205.7
Std Err Nuclear Area	5.4
Std Err Nuclear Intensity	8.1
Std Err CytoNuc Diff	25.3
COV Nuclear Area	0.3
COV Nuclear Intensity	0.1
COV CytoNuc Diff	1.3
Avg Nucs Per Field	66.0
Avg Cyto Stain Over Nucleus	1126.9
Avg Cyto Stain Over Ring	972.7
StdDev Cyto Stain Over Nucleus	207.1
StdDev Cyto Stain Over Ring	100.1
Number of valid fields	1.0

Where do I begin? Create Protocol ...

KineticScan v2.2.0.0 Build 19 - [Assay Protocol]

File Options View Help AP Load AP Unload

Assay Protocol: No Plate Protocol Loaded
Assay Protocol: Demo_Cellomics_NucTrans.V2_10X_p2.0

1 Assay Protocol

2 Assay Parameters

3 Channel Specific Parameters

4 Image Acquisition

5 Scan Limits

6 Well Features

6 Image Display Options

Assay Parameters Table:

Parameter	Value
TargetCircModifierCh2	-1.00
TargetRingDistanceCh2	1.00
TargetRingWidthCh2	2.00
TargetCircRingDiffLevelHighCh2	65.00
TargetCircRingDiffLevelLowCh2	-15.00

Channel Specific Parameters Table:

Name	Min	Max
NucArea	20.00	1,000.00
NucShapeP2A	0.50	2.00
NucShapeLWR	1.00	2.43
NucAvgIntenCh1	1.00	4,095.00
NucTotalIntenCh1	1.00	10,000,000,000.00

Image Acquisition Table:

Parameter	Value
Camera	Quantix.Camera32.1
Acquisition Mode	Standard
Focus	AutoFocus
Autofocus Preset	10x

Scan Limits Table:

Parameter	Value
Maximum Fields Per Well	1
Min. Objects For Well	100

Well Features Table:

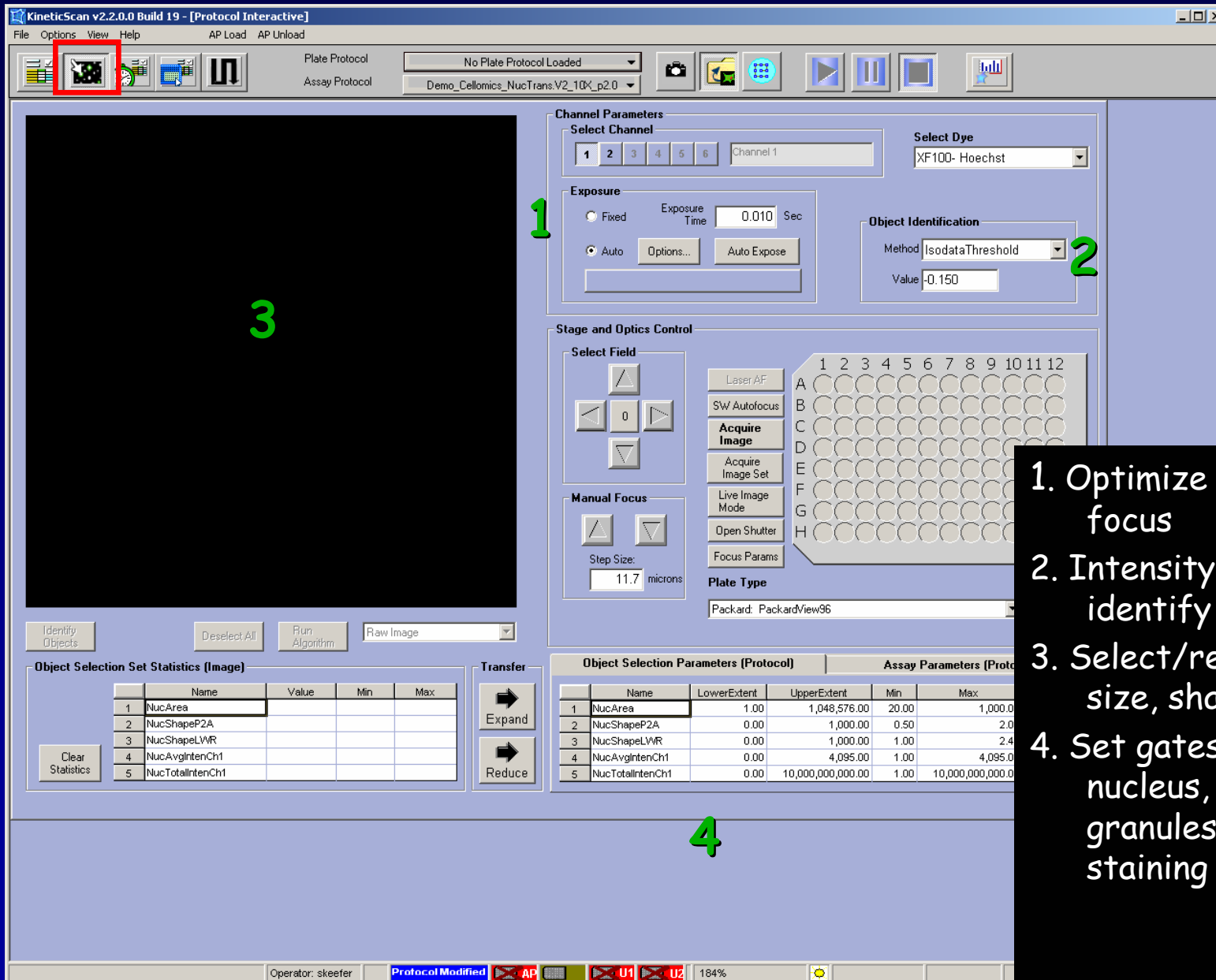
Parameter	Value
Default Well Feature	ValidCellCount

Image Display Options Table:

Parameter	Value
Include This Channel In Composite	Yes
Composite Color	Yes
Selected Cell	Yes
Rejected Cell	Yes
Target Circ	Yes
Target Ring	Yes

1. Assay Protocol
 - Acquire only, Target Activation, Compartmental analysis
2. No of Channels (one for each fluorochrome), focusing channel
3. Assign filter, thresholds, gates, exposure time
4. Objective lens (x5 - x40)
5. No of Fields & cells
6. Pseudocolours

Next... interacting with your sample. Interactive Protocol



1. Optimize exposure and focus
2. Intensity threshold to identify objects
3. Select/reject cells by size, shape, intensity
4. Set gates to pick out nucleus, cytoplasm, granules, extracellular staining

KineticScan v2.2.0.0 Build 19 - [Kinetics Protocol]

File Options View Help AP Load AP Unload

Kinetic Protocol: No Plate Protocol Loaded / Kinetic Protocol: Blank Kinetics Protocol

Kinetic Protocol Name: [] Make Protocol ReadOnly

Kinetics Mode

☒ Well Mode
☐ Column Mode
☐ Plate Mode

Process a different well on each kinetic cycle.

☐ Use initial scan autofocus values for all subsequent scans
☐ Reset autofocus plate geometry model every acquisition cycle
☐ Reset autofocus plate geometry model every kinetic cycle

Pipettor Door Position
☐ Door Open While Imaging (Well Mode)
☐ Door Open Between Pipettor Operations
☐ Door Closes Between Every Operation

1. ☐ Perform Baseline Scan

2. Pre-Scan Pipetting

[1] ASPIRATE: Asp. Operation

New Operations: Transfer, Mix, Aspirate, TipWash, Dispense, Delay

3. [0] Async Tip Wash Cycles

4. Pre-Scan Delay: [0] min [0] sec

5. Well Scanning

Kinetics Type: Kinetic Cycles Time Points: [0]
Cycle Timing: As Fast As Possible Periodic Time: [0] min [0] sec

Acquire Images On Every Nth Cycle
CH1 CH2 CH3 CH4 CH5 CH6
[1] [1] [1] [1] [1] [1]

Store Image Set For: Every Kinetic Cycle
☐ Do Image Analysis as a Post-Processing Step (increases memory requirements) where N is: [0]

6. Post-Scan Delay: [0] min [0] sec

7. Post-Scan Pipetting

New Operations: Transfer, Mix, Aspirate, TipWash, Dispense, Delay

Repeat for next well

Kinetic Acquisition Cycle

On each kinetic cycle, a Well is scanned. Kinetic cycles will be repeated until the Kinetics Event Time is exceeded. The kinetic cycles can be evenly spaced, or performed as fast as possible. On each kinetic cycle, the same assay algorithm is performed. There are several image storage options.

There are six types of pipetting operations available: Asp, Disp, Trans, Mix, Wash, and Delay. As part of the Well Mode, these operations are performed sequentially on a single well.

There are six types of pipetting operations available: Asp, Disp, Trans, Mix, Wash, and Delay. As part of the Well Mode, these operations are performed sequentially on a single well.

• End point
• Kinetic
- read at set interval
- length of time
• Addition of reagents

Operator: skeefer

- Addition of reagents

Plate Protocol puts everything together...

KineticScan v2.2.0.0 Build 19 - [Plate Protocol]

File Options View Help AP Load AP Unload

Plate Protocol Blank Plate Protocol

General Properties

Plate Protocol Name:

Selected Assay Protocol: **1**

Selected Plate Type: **2**

Kinetic Feature Calculations

☐ Use All Cells in Calculations

Over the course of a scan, the software may lose track of some cells. If this box is checked, all cells will be used in calculating kinetic features. Otherwise, the system will calculate features using only cells that were tracked during the entire scan.

Kinetic Steps

Available Kinetic Protocols:

Scan Area

3

4

Kinetic Protocol List:

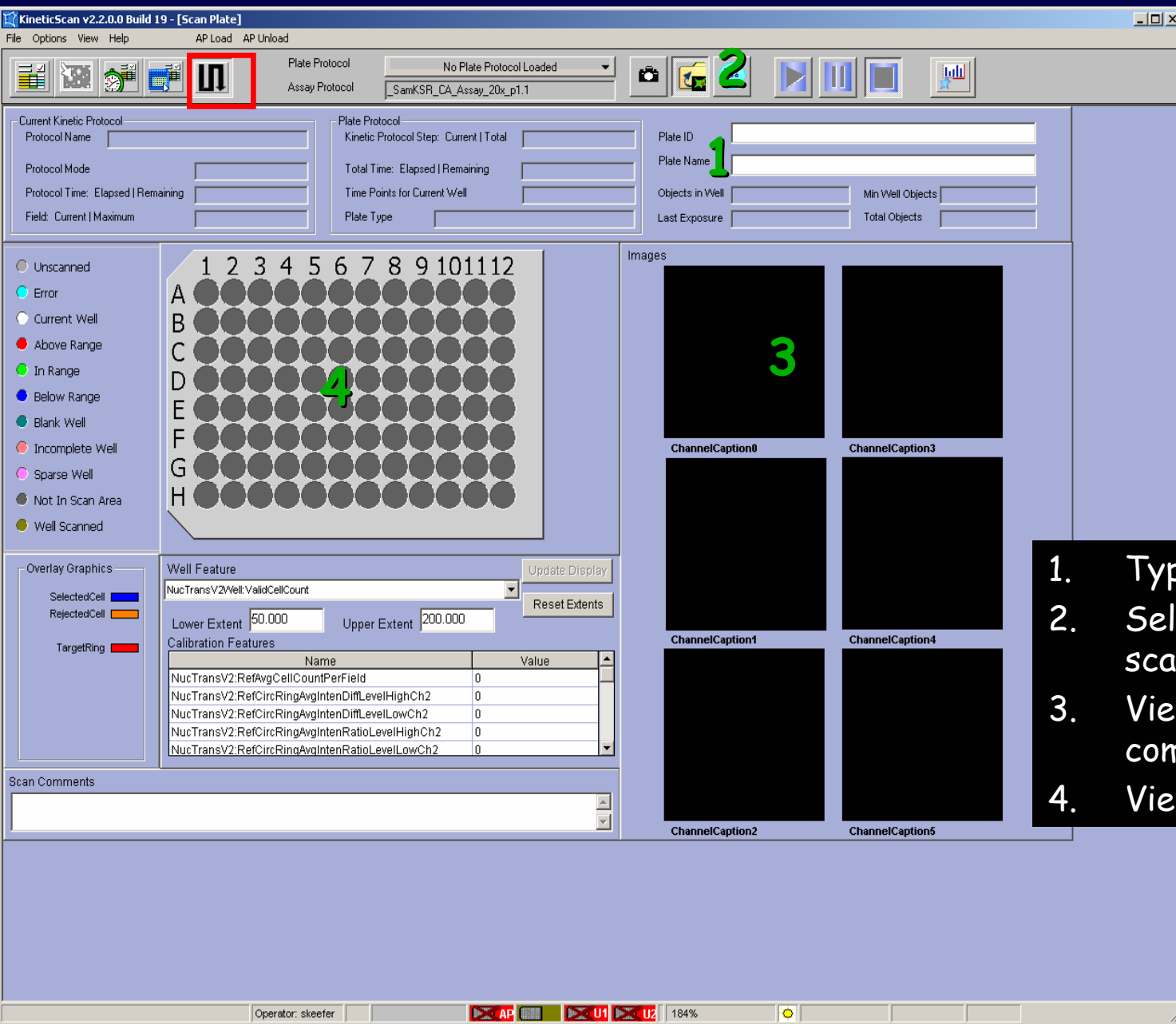
Step	Kinetics Protocol	Scan Area
1	anthony09.05.05kinetic	B3:F5
<<Add New Step>>		

Operator: skeefer

AP U1 U2 184%

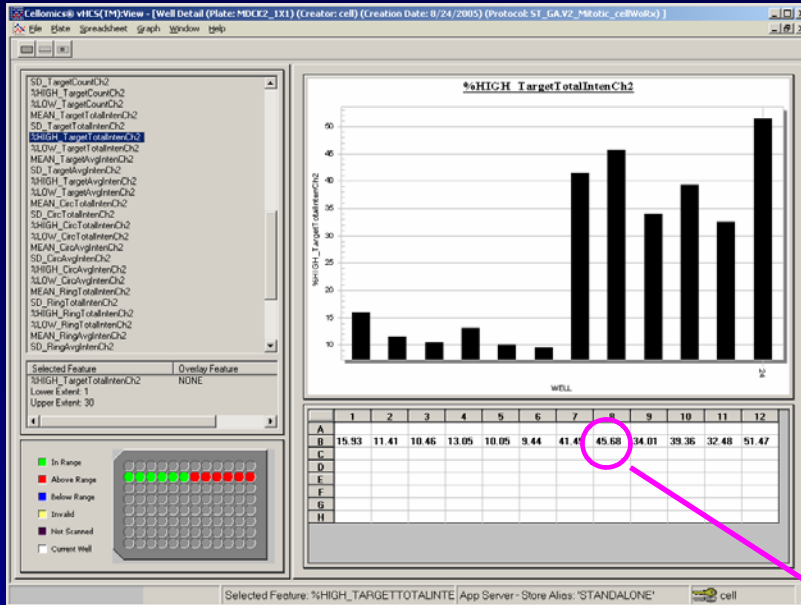
1. Plate type
2. Assay Protocol
3. Kinetic protocol
4. No. & pos. of wells

Scan View - Starts the plate imaging and processing...



1. Type in plate Id
2. Select field to start scan
3. View all channels + composite
4. View plate or values

vHCS™:View



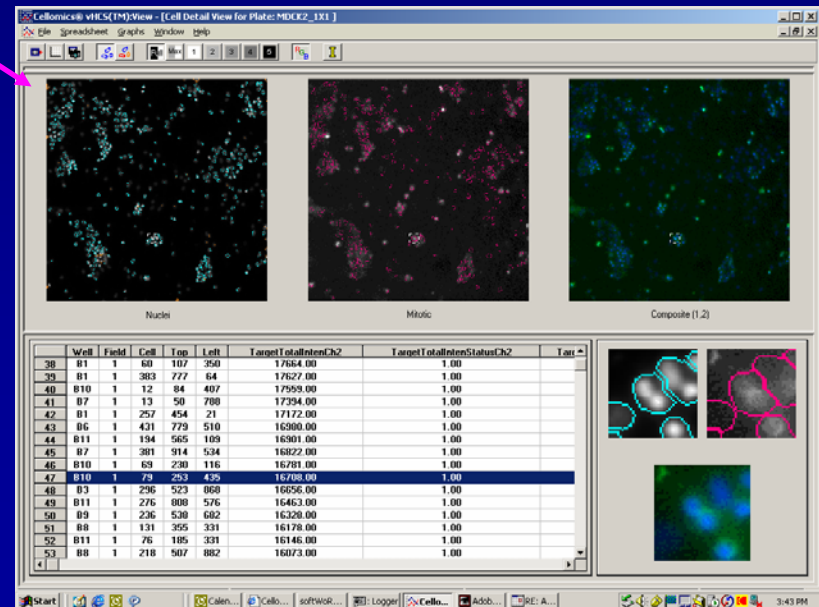
Well Level:

- Graphs and spreadsheets on all parameters
 - Eg area, intensity, shape, texture
 - Basic statistics; mean, std dev
- Export data to Excel
- Access to images
- Kinetic time charts

Cell Level:

Information on individual cells

- Images
- Animated images of kinetic assays



ScanTM Toolbox

- Virtual Scan
- Choose another protocol or bioapplication
- Change parameters and gates
- Rescan previously acquired images

Procedure Summary

- Load 96 well plate
- Switch on lamp
- Assay protocol
- Kinetic protocol
- Plate protocol
- Interactive window
- Scan plate
- View™ (analysis)
- Scan™ (Toolbox)

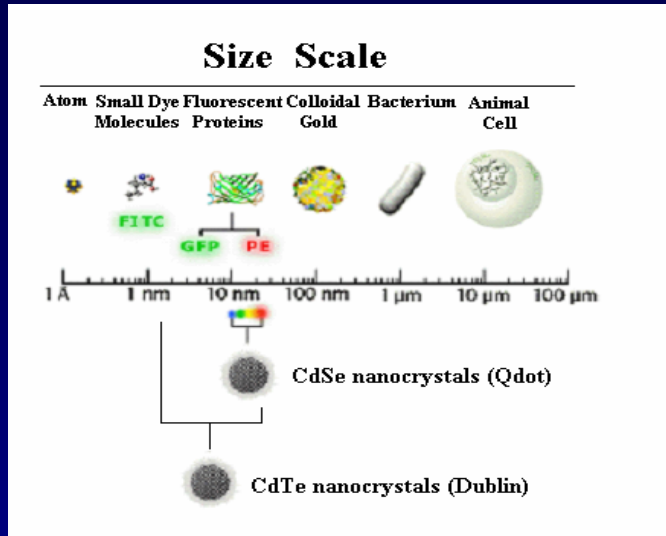
HIGH CONTENT ASSAYS CURRENTLY ESTABLISHED

- Apoptosis
- Multi-parameter cytotoxicity
- Cell Health
- Cell Cycle
- Cell Morphology
- Golgi Fragmentation
- Cell fibrillar organisation
- Neurite outgrowth
- Molecular translocation (various targets)
- Cell Migration
- Access to full-genome siRNA library (Dharmacon) and HCS evaluation of signalling-targeted effects

- **Case study:**

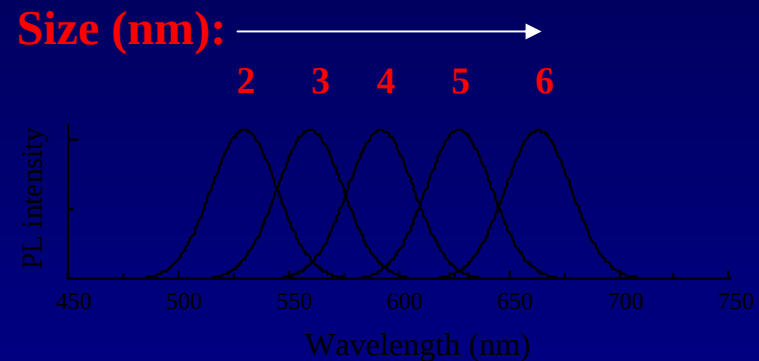
HCA in multi-parametric evaluation of nanoparticles uptake, subcellular distribution and cytotoxicity

Fluorescent semiconductor nanoparticles (Quantum Dots): tiny objects with a mega potential



- Based on metals from groups II-VI or III-V of the periodic table
- Significant advantages over conventional dyes in terms of brightness and stability
- Wide choice of colours
- Multiple targets can be detected in a single cell

Quantum-confinement in CdTe nanocrystals: emission spectrum shifts to the red with size



Samples of CdTe nanocrystals in water. Different colours correspond to different average size of nanoparticles (from 2 to 5 nm).

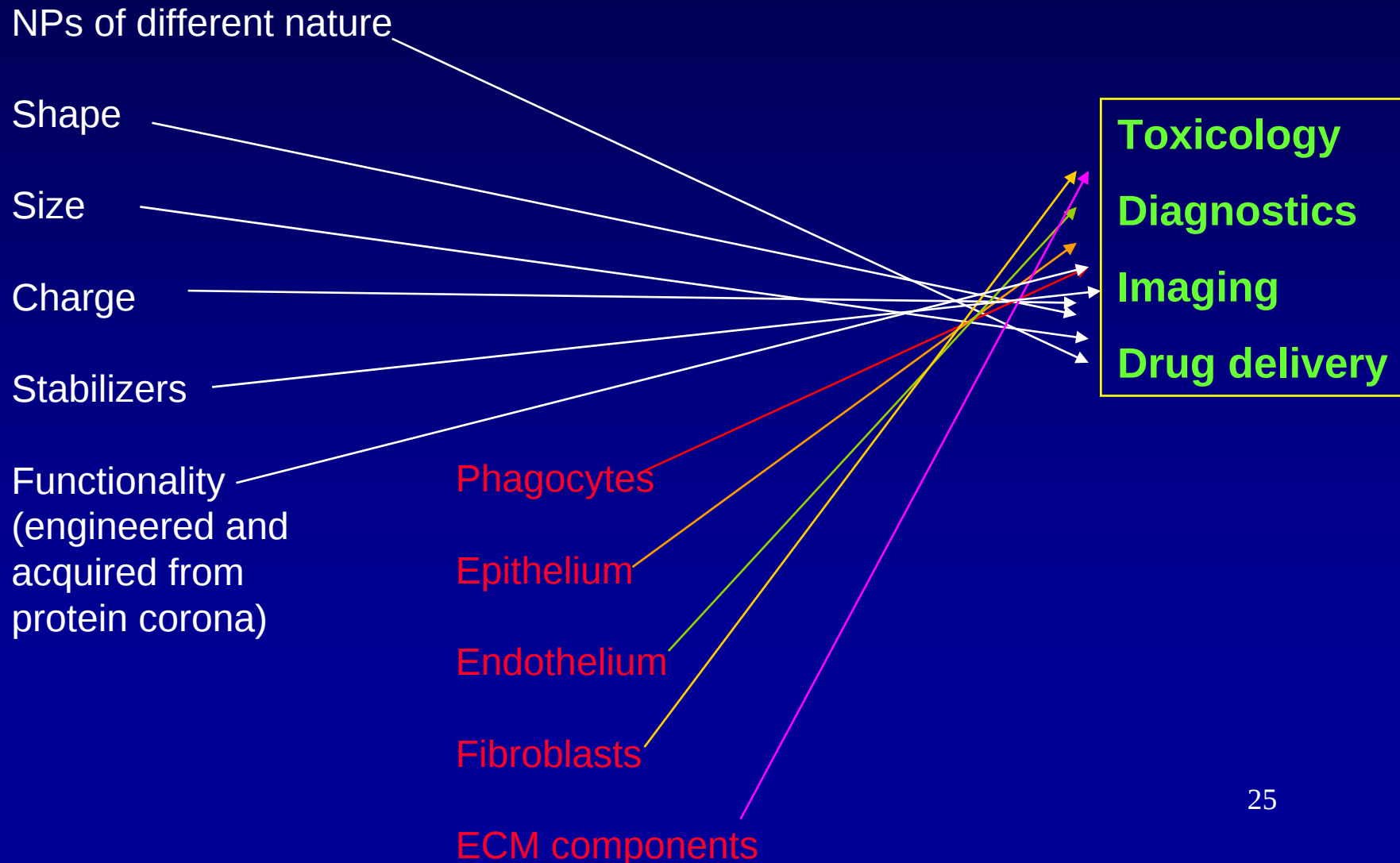
Traditional Fluorophores

- Overlapping absorption/emission profiles
- Prone to bleaching
- Limited use in long term imaging
- Difficult to look at multiple signals at the same time

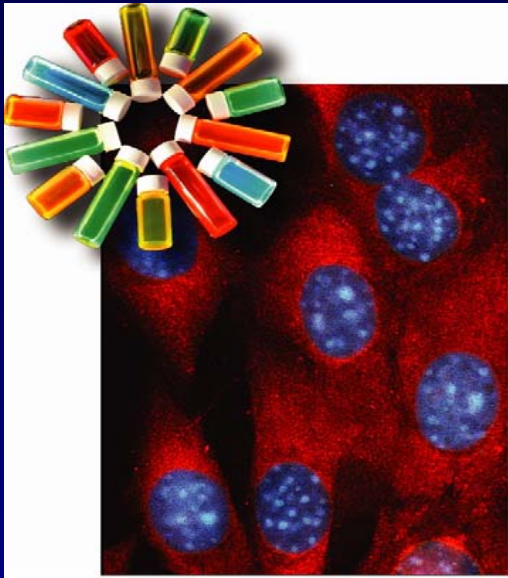
QDots-specific "issues"

- Toxicity
- Solubility in water
- Aggregation
- Stability in physiological environment
- Size specificity

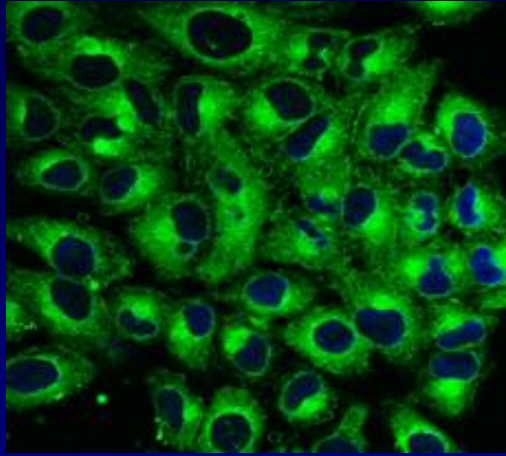
NP/CELL INTERACTION CHALLENGES: THE MULTIFACTORIAL ENVIRONMENT



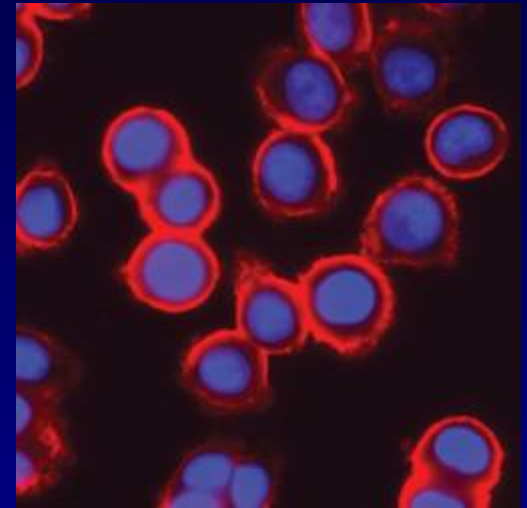
Nanoparticles and cells - clear patterns?



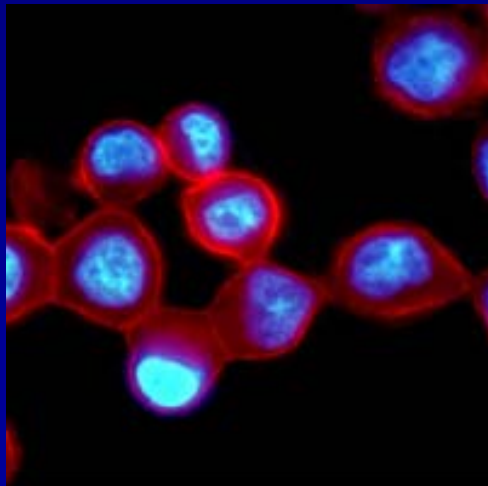
www.mdpi.org/ijms/specialissues/quantumdots.htm



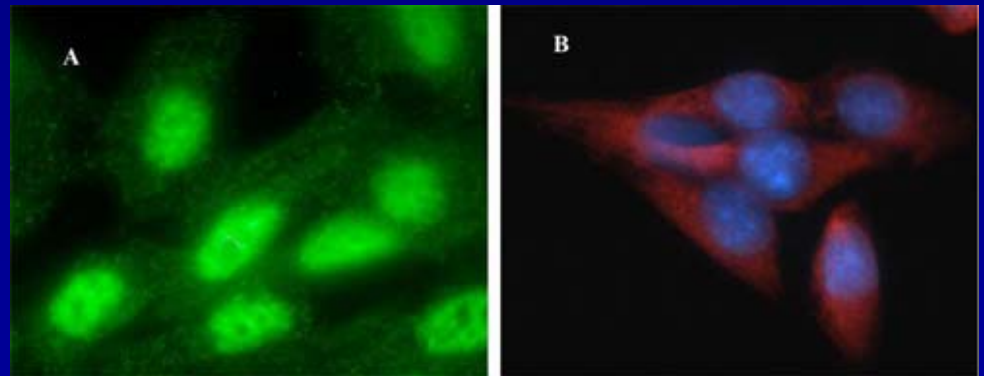
Dawson et al., UCD, -ESF Conference, Sant Feliu-2007



http://www.sciencenews.org/articles/20030215/a3060_2710.jpg



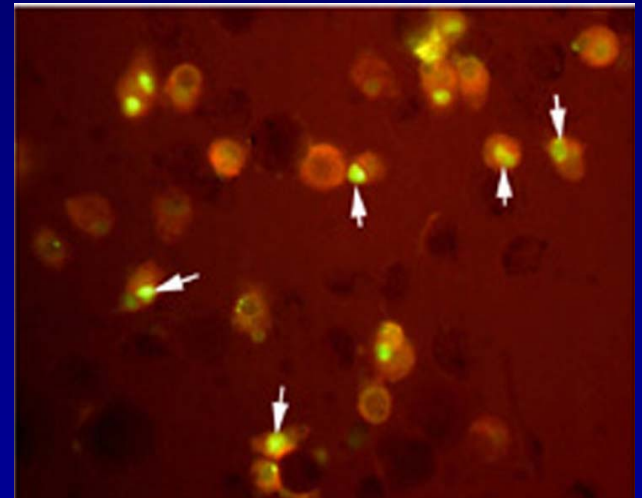
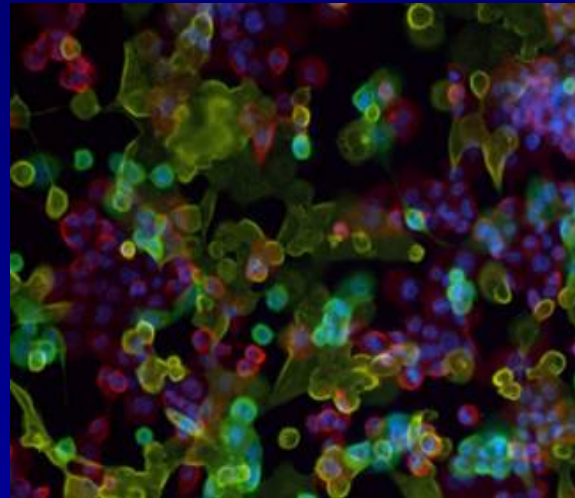
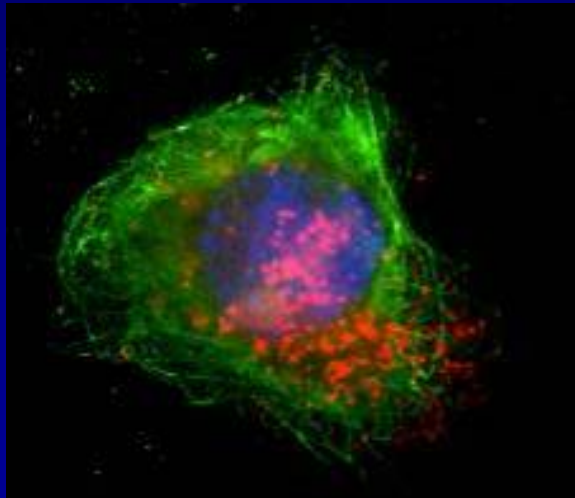
xnet.rrc.mb.ca/davidb/nanotools.htm



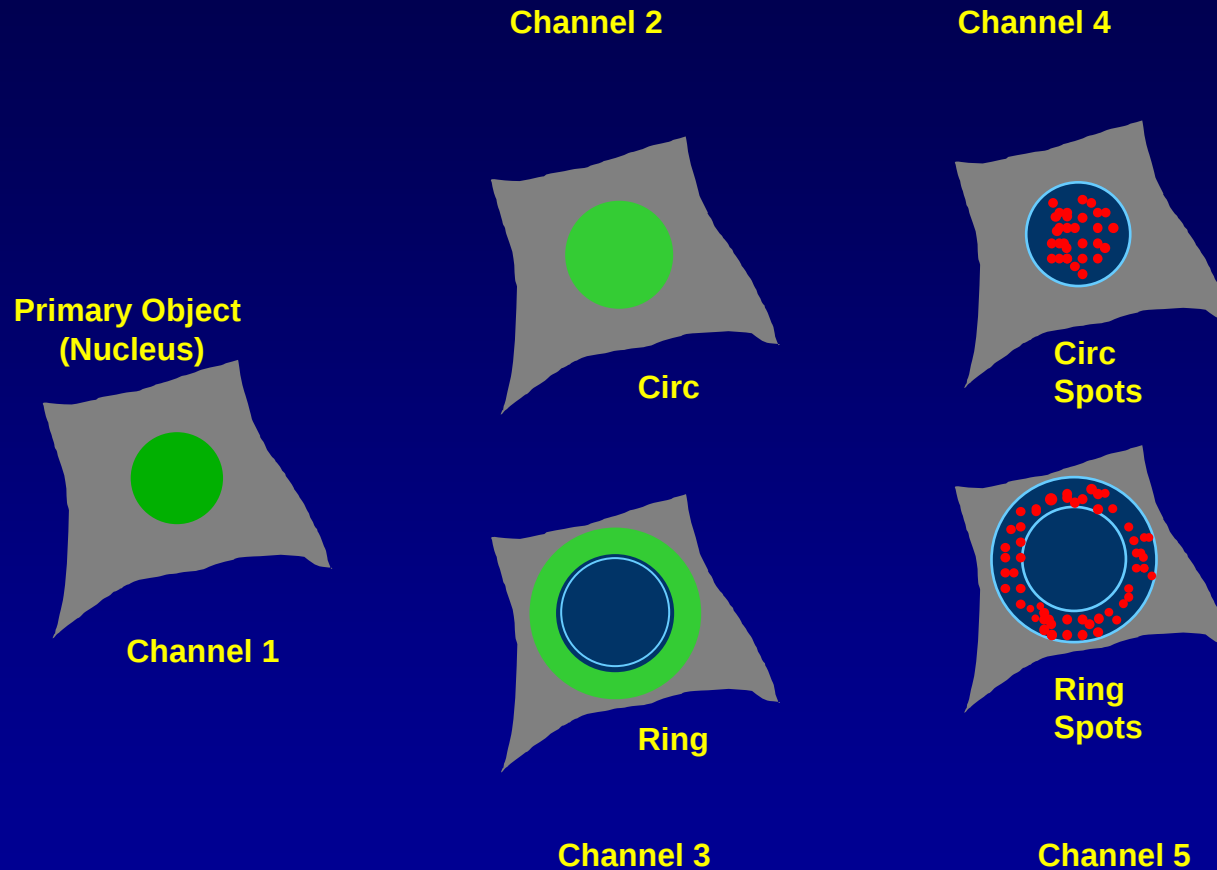
<http://www.esi-topics.com/fbp/2005/february05-GabrielASilva.html>

Nanoparticles and cells - clear patterns?

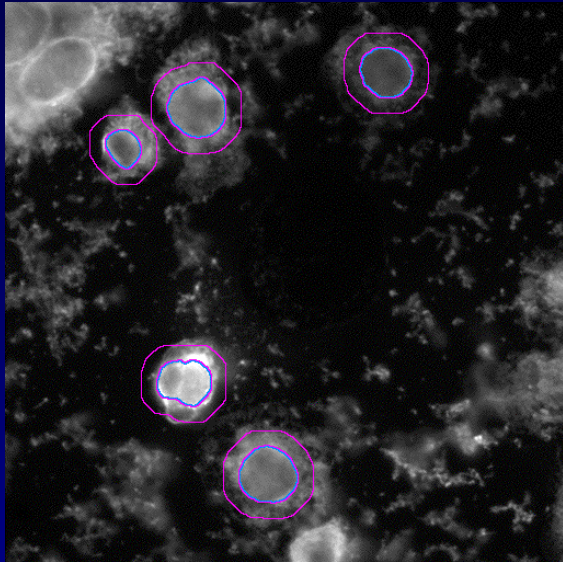
- Partially matching patterns
- Multi-parametric (multiplexed) readout is required
- Not everything fits within the conventional (convenient...?) 95%



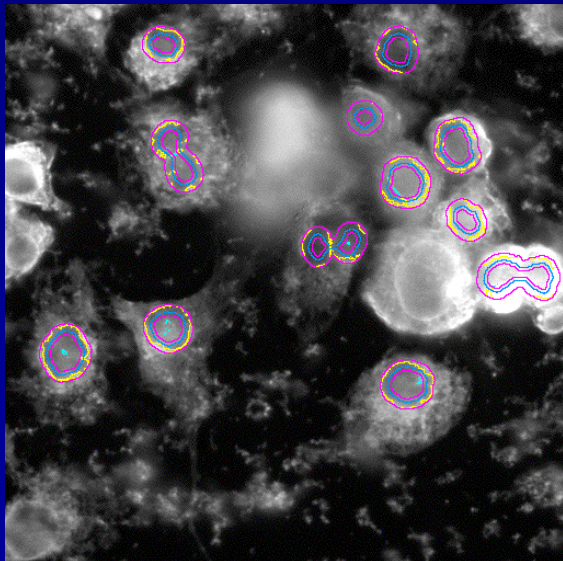
Compartmental Analysis BioApplication



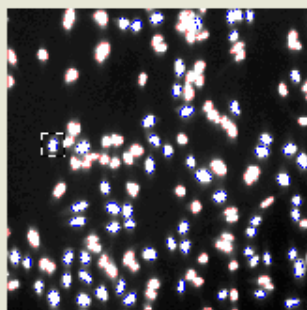
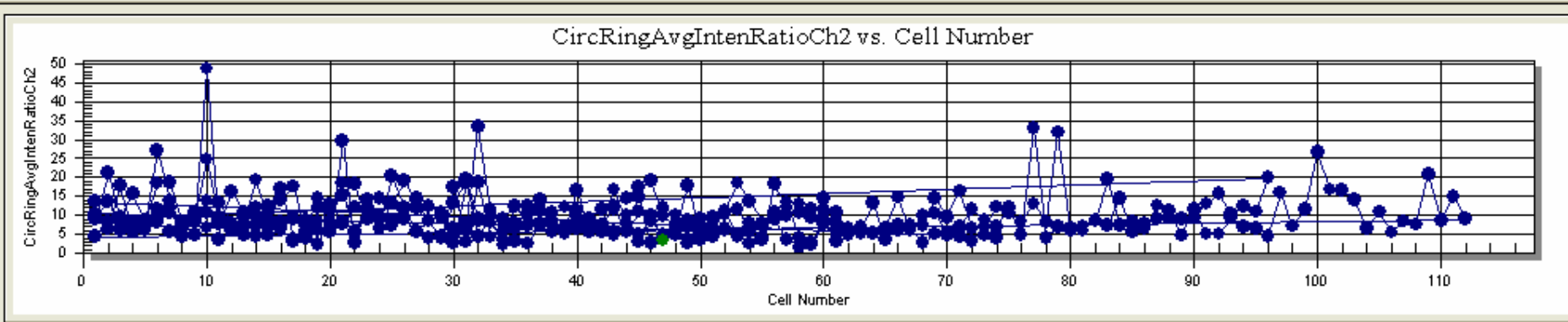
Compartmental Analysis©



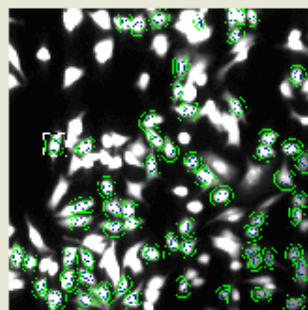
- Blue circle = nucleus
- Pink circle = cytoplasm



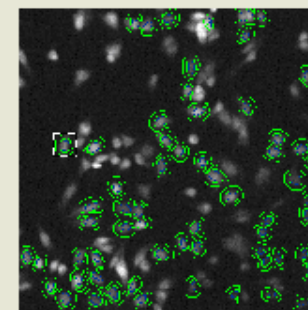
- Turquoise dots = nucleoli
- Yellow dots = nuclear rim



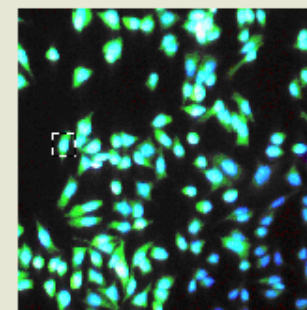
Channel1



Channel2

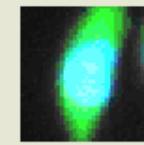
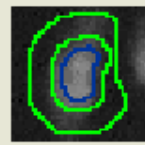
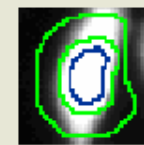
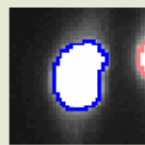


Channel3

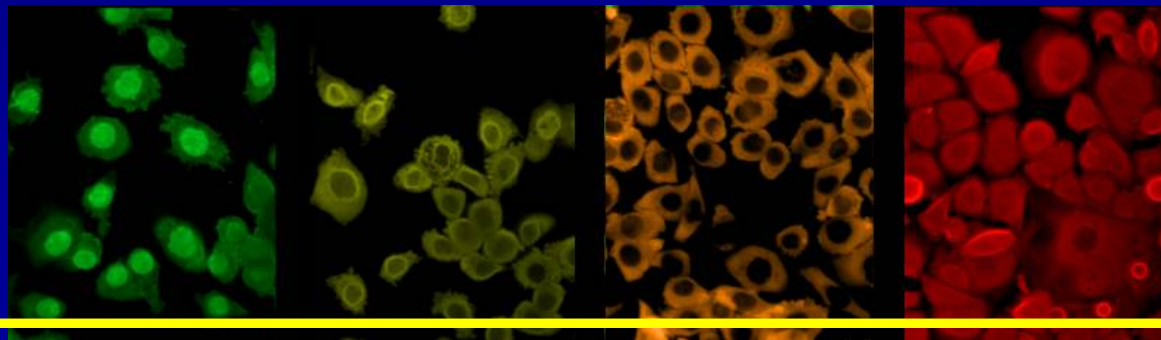
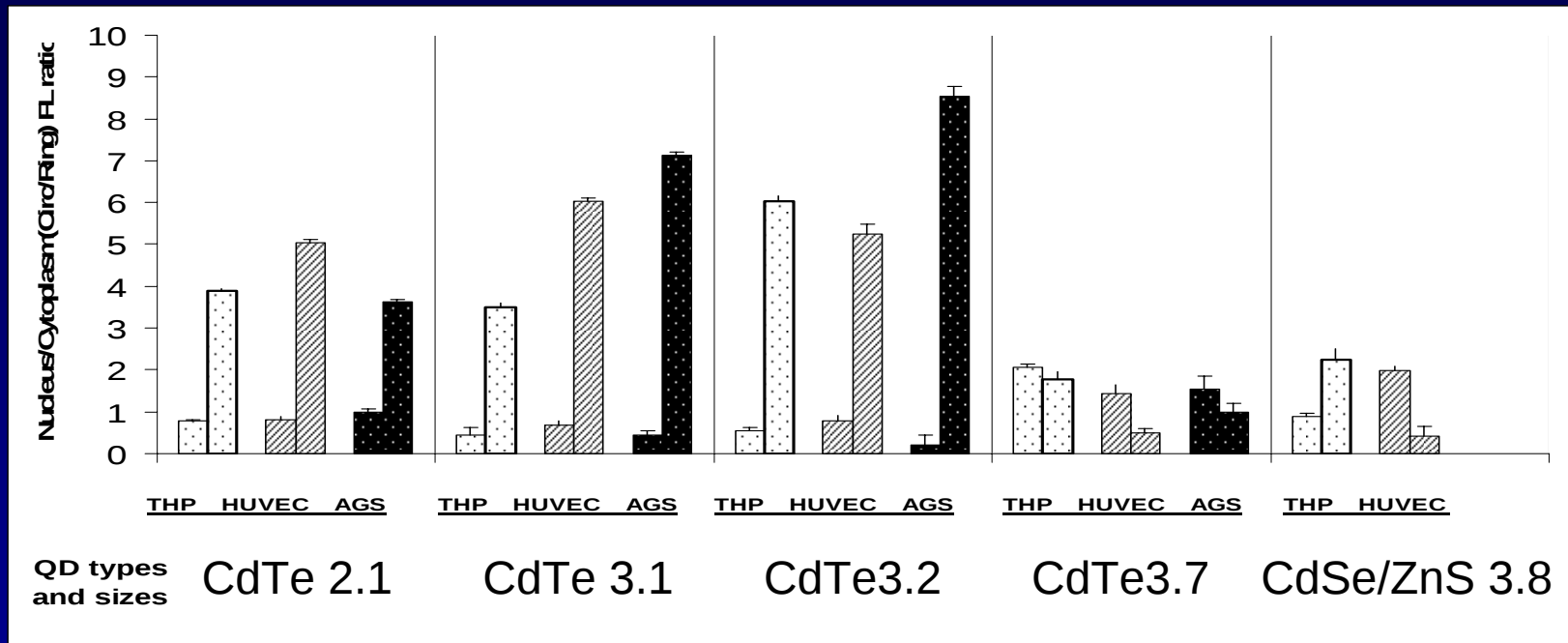


Composite [1,2,3]

	Well	Field	Cell	Top	Left
379	A2	5	43	258	419
380	A2	5	44	259	263
381	A2	5	45	263	467
382	A2	5	46	265	115
383	A2	5	47	268	68
384	A2	5	48	277	239
385	A2	5	49	279	428
386	A2	5	50	310	227
387	A2	5	51	327	292

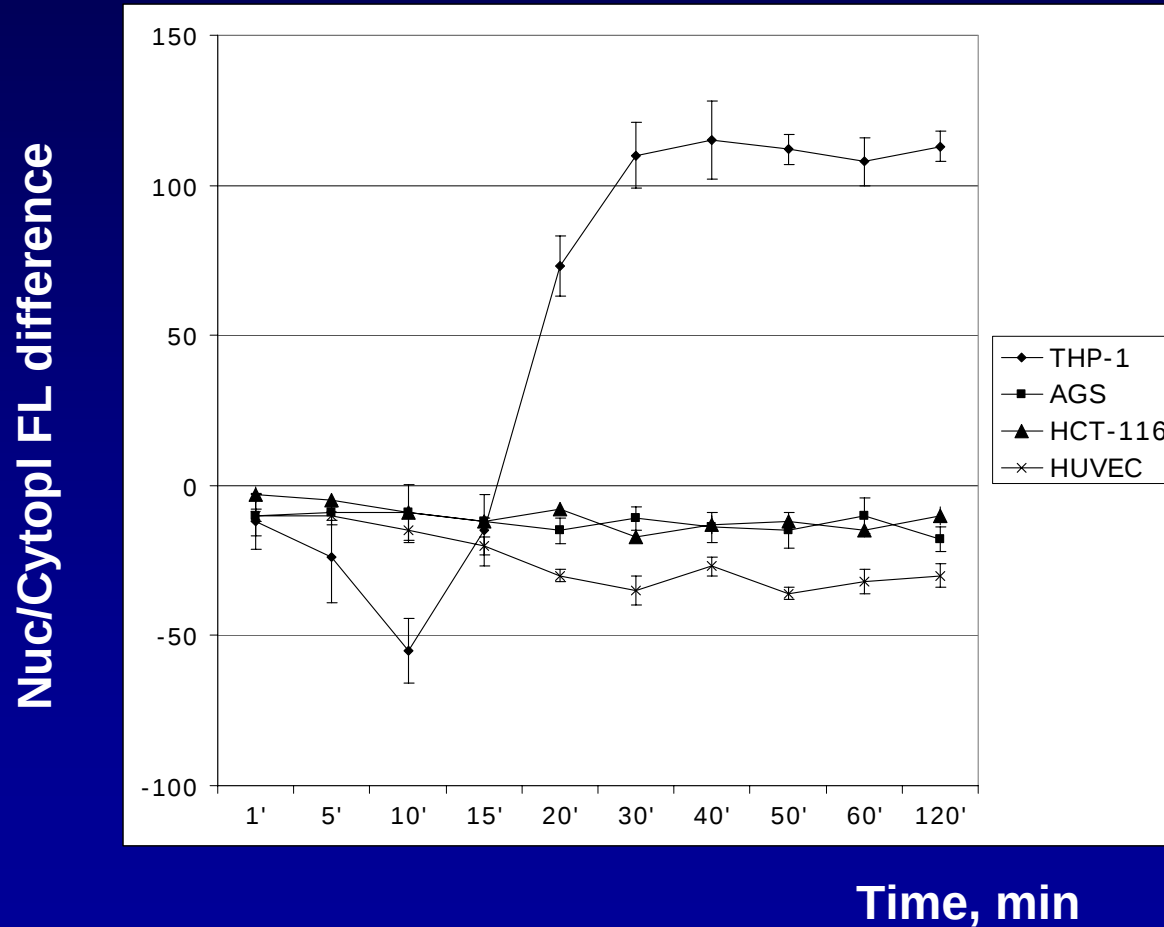


NP and cell-specific cellular response diversity: new models for targeted drug delivery

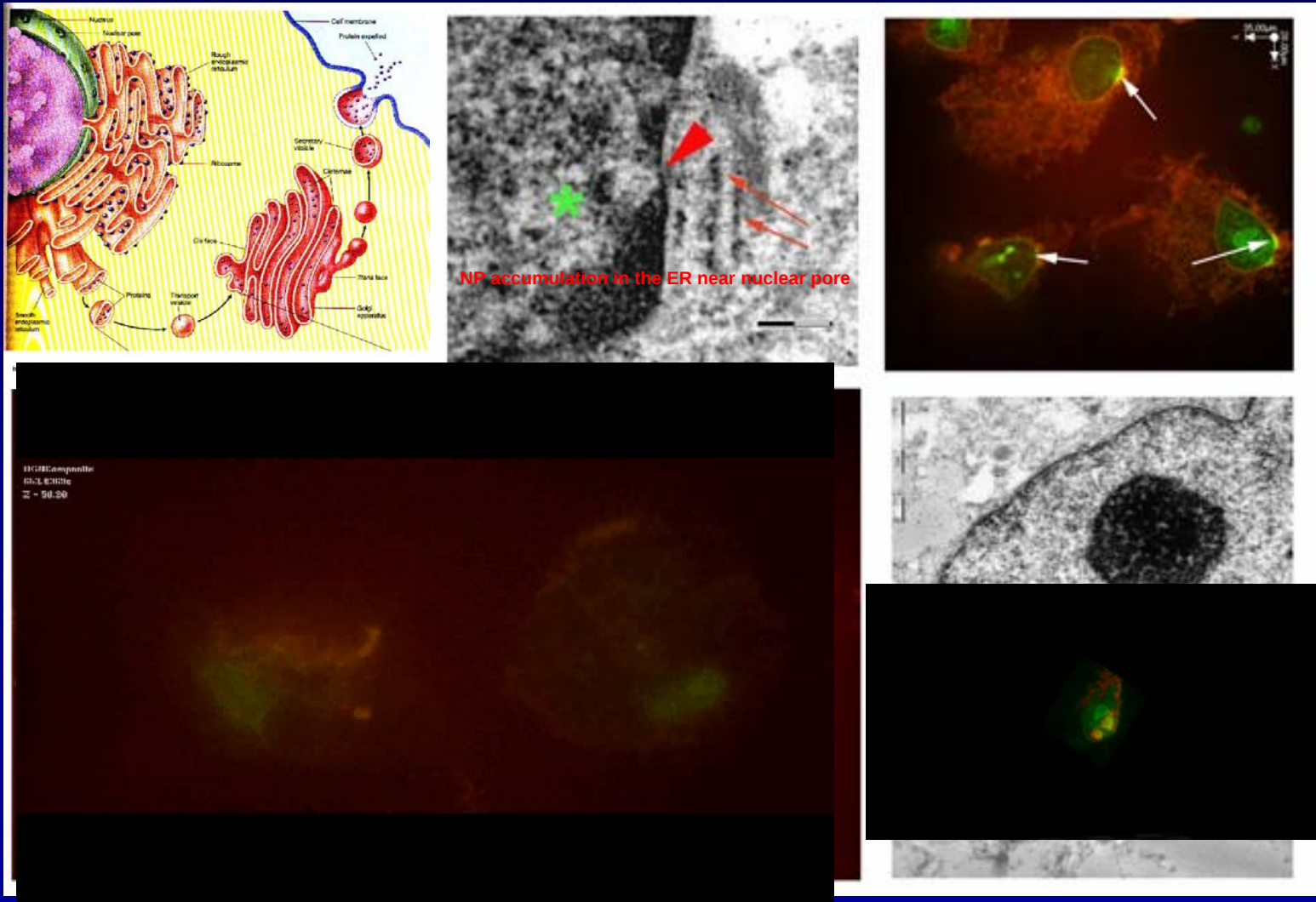


NP increasing size from 2 to 5 nm

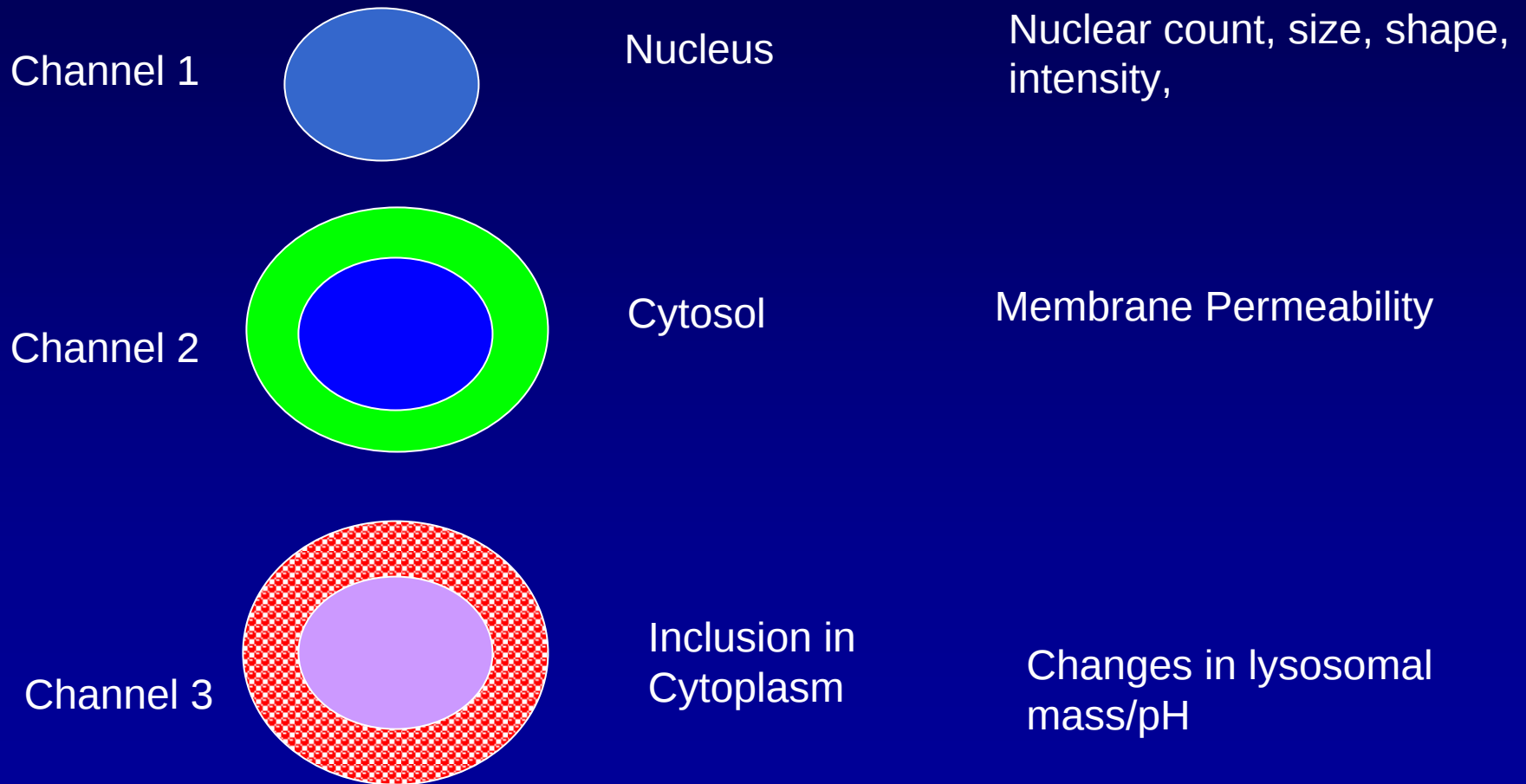
HCA-based quantitative dynamic assessment of CdTe QD uptake into the nuclei in cell lines



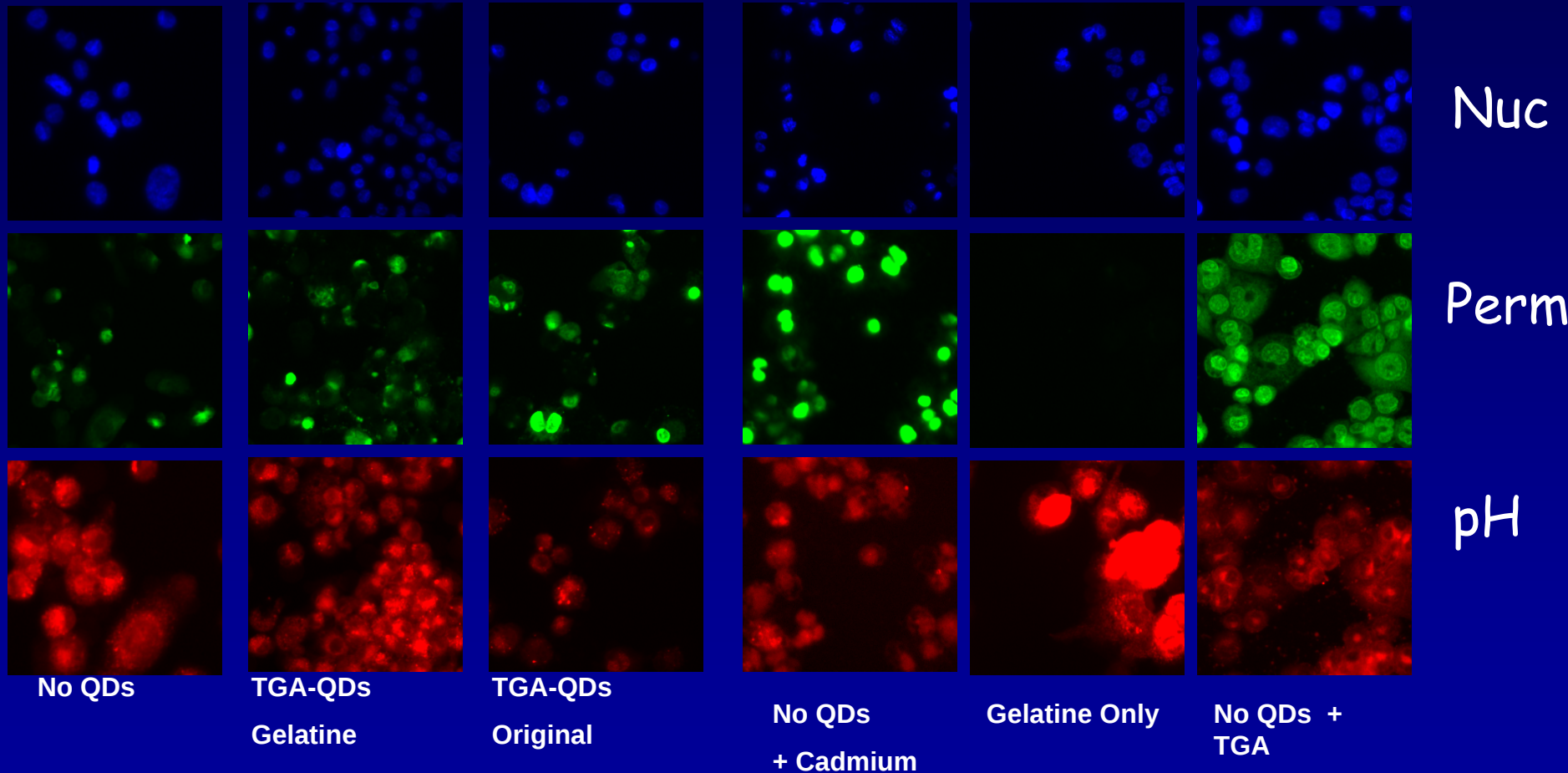
CdTe QDs: uptake and intracellular transport verified by "conventional methods"



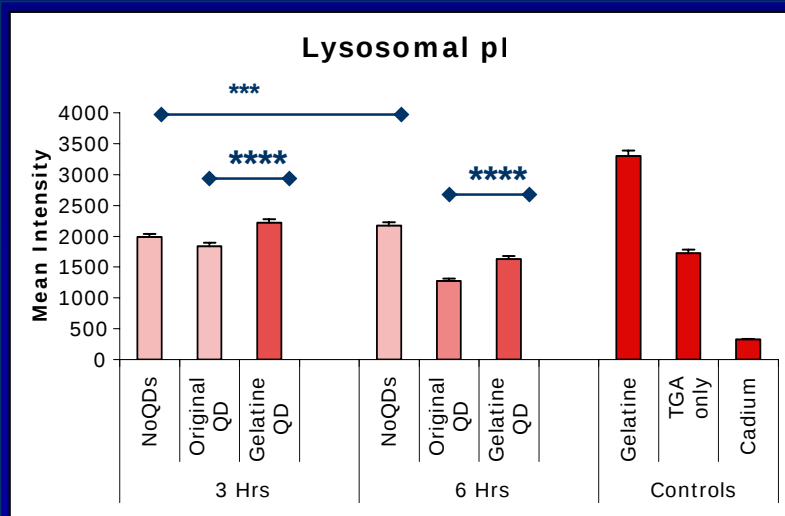
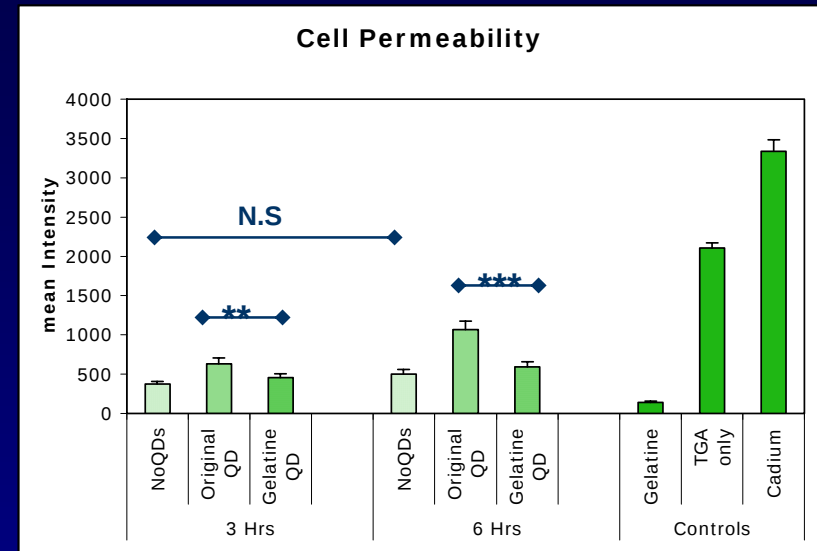
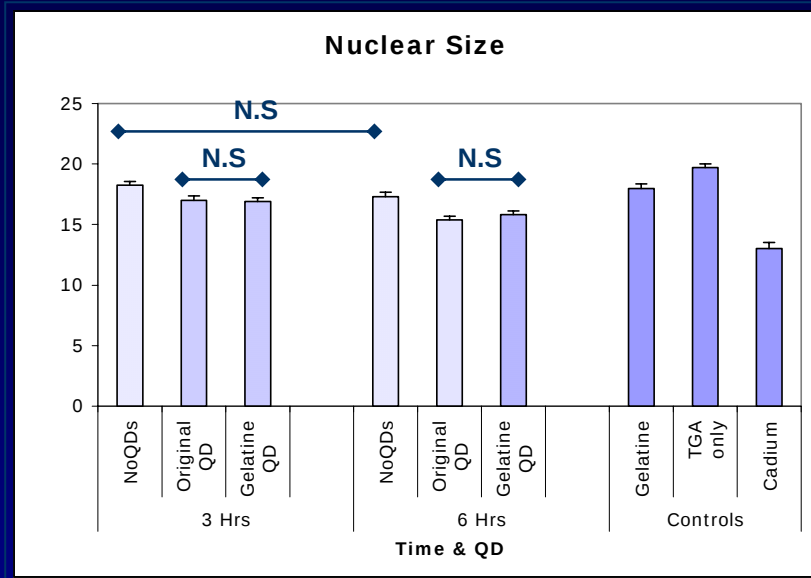
Cytotoxicity Assay



Comparative cytotoxicity of unmodified and gelatine-coated QDs



Comparative cytotoxicity of unmodified and gelatine-coated QDs



N.S not significant

**** P < 0.05**

*****P < 0.005**

******P < 0.0001**

Small, 2007

Conclusions:

a novel phenomenon of "nanoparticle-driven" science and R&D

- Need to evaluate and *quantify* far more complex patterns than encountered before
- Numerous parameters need to be read simultaneously, in real time
- Below average responses (rare events) are not uncommon – can we still be happy with a 95% effect in cell populations?
- *Solution: new technologies AND new mode of scientific thinking*

Conclusions:

a novel phenomenon of “nanoparticle-driven” science and R&D

- Most likely, there is no such thing as a biologically “inert” nanoparticle
- Medium to mild effects might be just overlooked at the cell population level
- Standards across the labs/centres (NP size, charge, geometry etc. are essential)
- Think how to handle the Terabytes!

HCS is a perfect match for the challenging tasks

Conclusions-contd

- HCA/HCS is an organically emerging integral technological platform for screening and investigation of the mechanisms of uptake, distribution and intracellular targeting of nanoparticles
- Nano-bio studies today closely resemble the drug discovery process
- *HCA does not solve all the problems* - high quality EM, spectroscopic methods etc. are still indispensable
- Key to success - is the multidisciplinary environment (biomedical sciences, physics, chemistry, IT...)

TCD research and education
High Content Screening and Analysis facility:

- Part of the biomedical imaging centre at the IMM
- Recently introduced MSc in Molecular Medicine with HCA and NanoMedicine modules
- Short practical course to be launched next year



The Team

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FUNDING:

Science Foundation of Ireland
Enterprise Ireland
Health Research Board of Ireland
EU - NanoInteract consortium