High Content Screening for evaluation of nanoparticles interaction with cells

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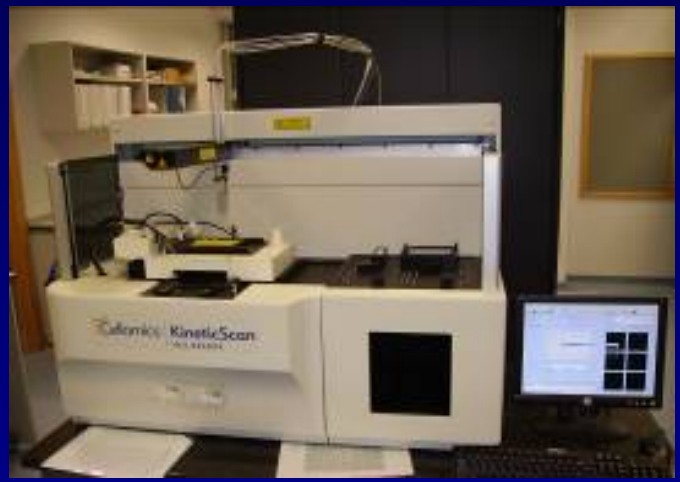
Introduction

- 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

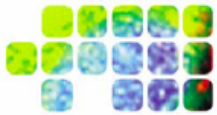
GE InCell Analyser 1000

- **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.**

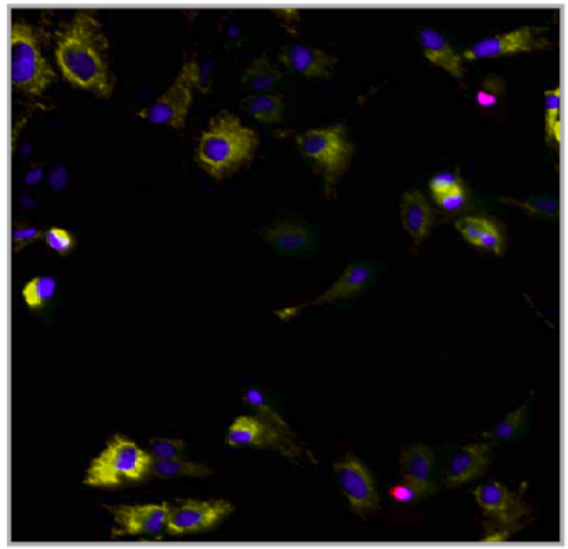
The Kinetic Scan HCS Reader

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



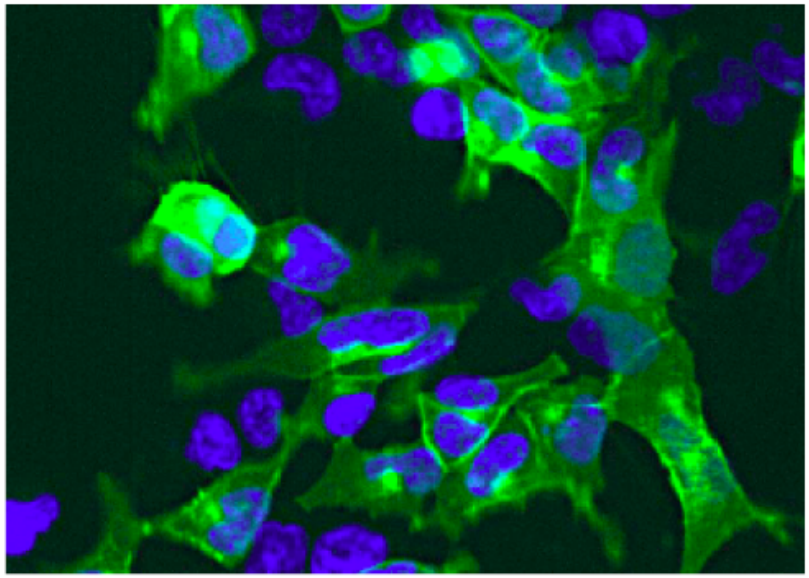
- Ability to use a wide variety of fluorescent indicators
- Excitation from 350-700 nm
- Decreased photodamage
- Decreased phototoxicity
- Low cost
- Low maintenance
- No daily alignment required



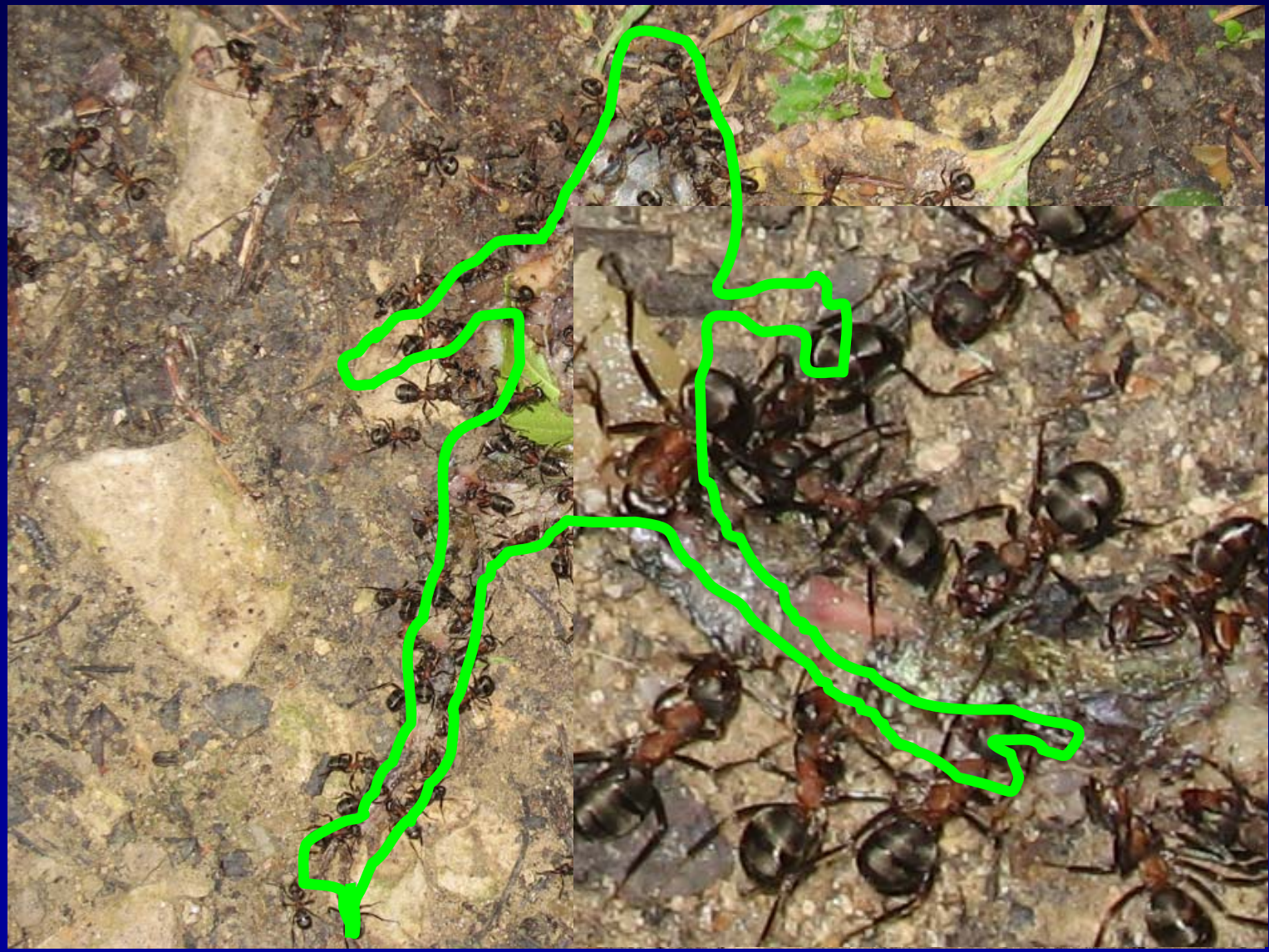
Blue: Nuclear Morphology
Green: Calcium Homeostasis
Yellow: Mitochondrial Transmembrane Potential
Red: Membrane Permeability

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



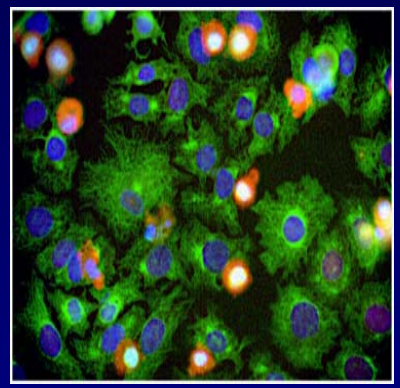
Assay Preparation

- 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

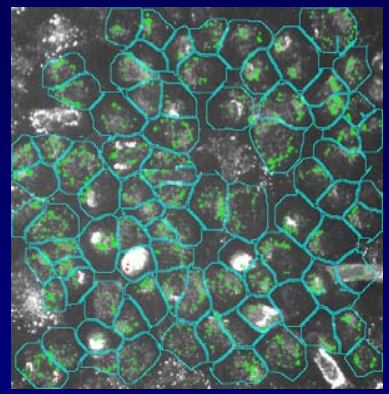
Cellomics Platform Workflow

Automated Plate Delivery

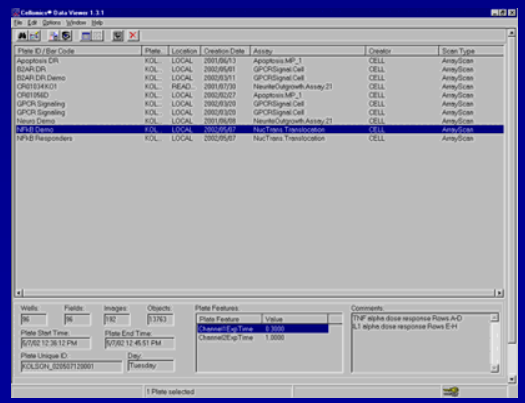
Auto-focus, Expose & Acquire



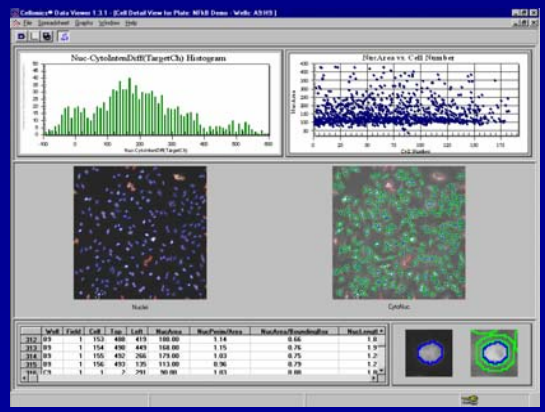
Automated Image Analysis



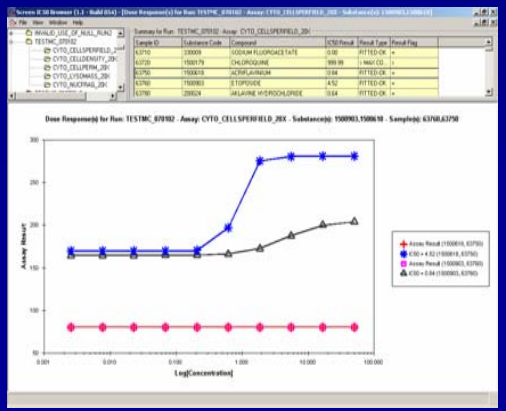
Automatic Data Archival



Instantaneous Data Display

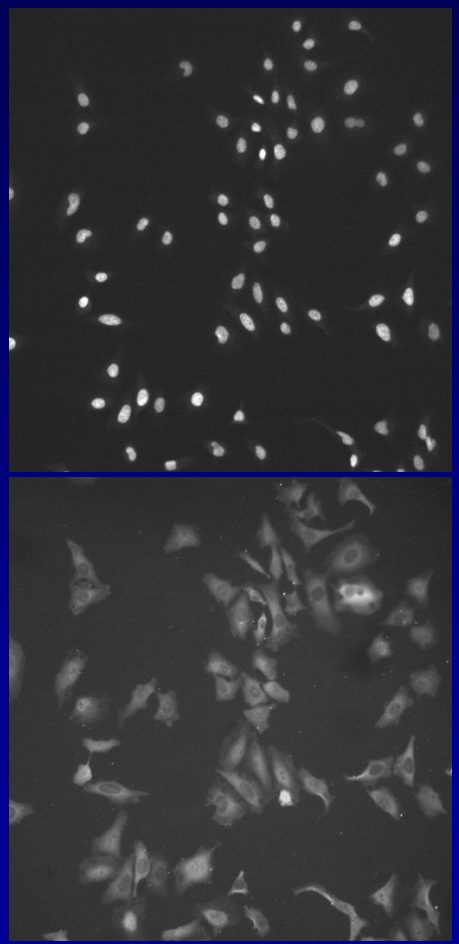


Analysis of Results

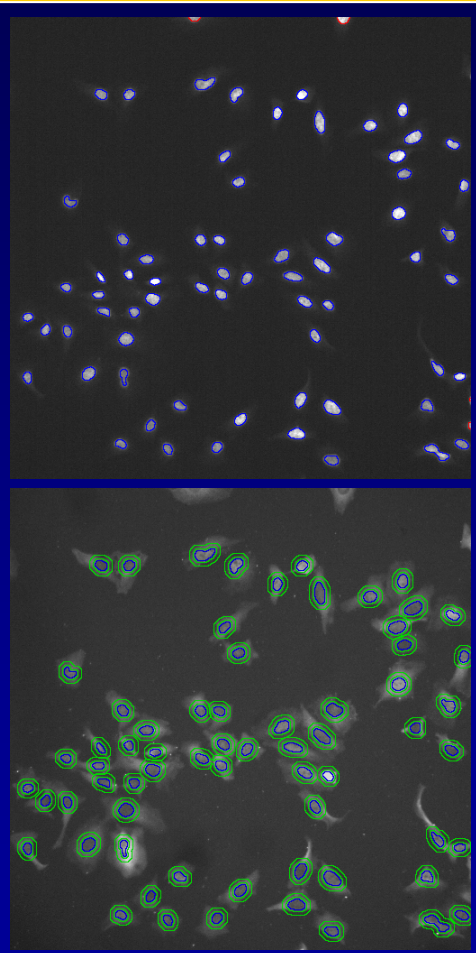


Processing of Images on the Cellomics® HCS Systems

Images Acquired



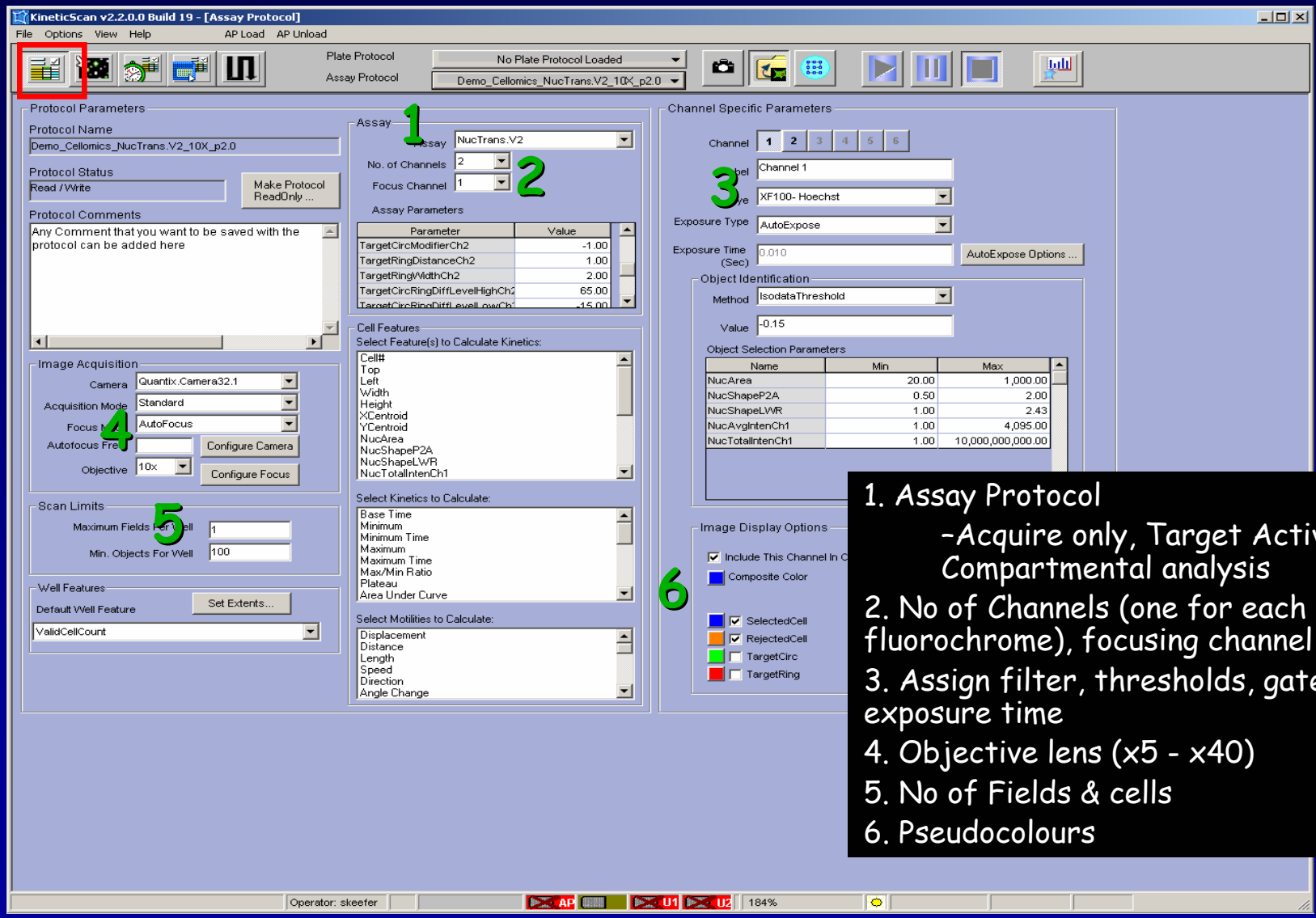
Auto-Threshold & Algorithm Applied



Data Extracted

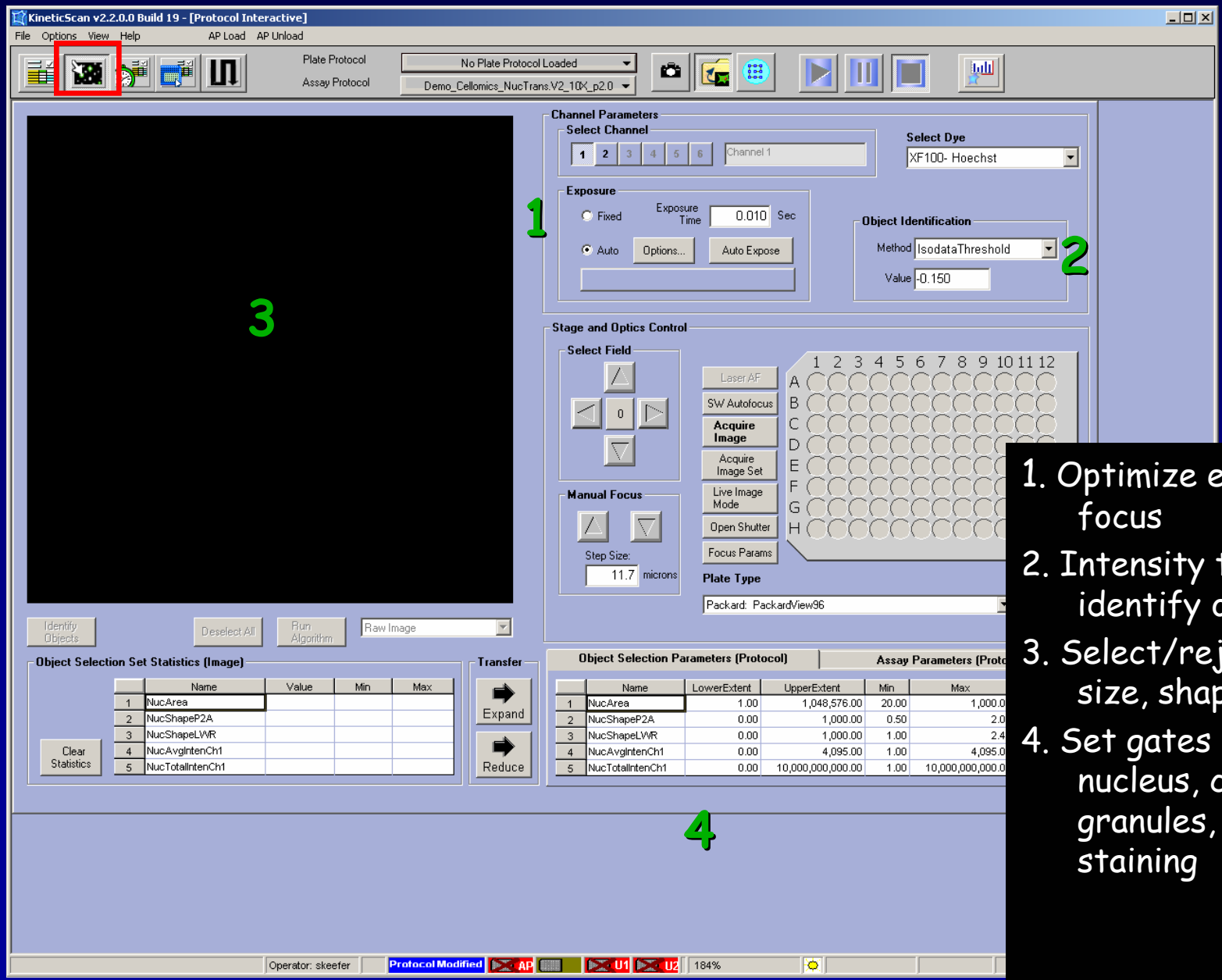
Avg CytoNuc Diff	66.0
Cell Count	148.5
Avg Nuclear Intensity	495.0
Avg Nuclear Area	154.2
Std Dev Nuclear Area	43.6
Std Dev Nuclear Stain Intensity	65.5
Std Dev CytoNuc Diff	205.7
Std Err Nuclear Area	5.4
Std Err Nuclear Intensity	8.1
Std Err CytoNuc Diff	25.3
COV Nuclear Area	0.3
COV Nuclear Intensity	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 …



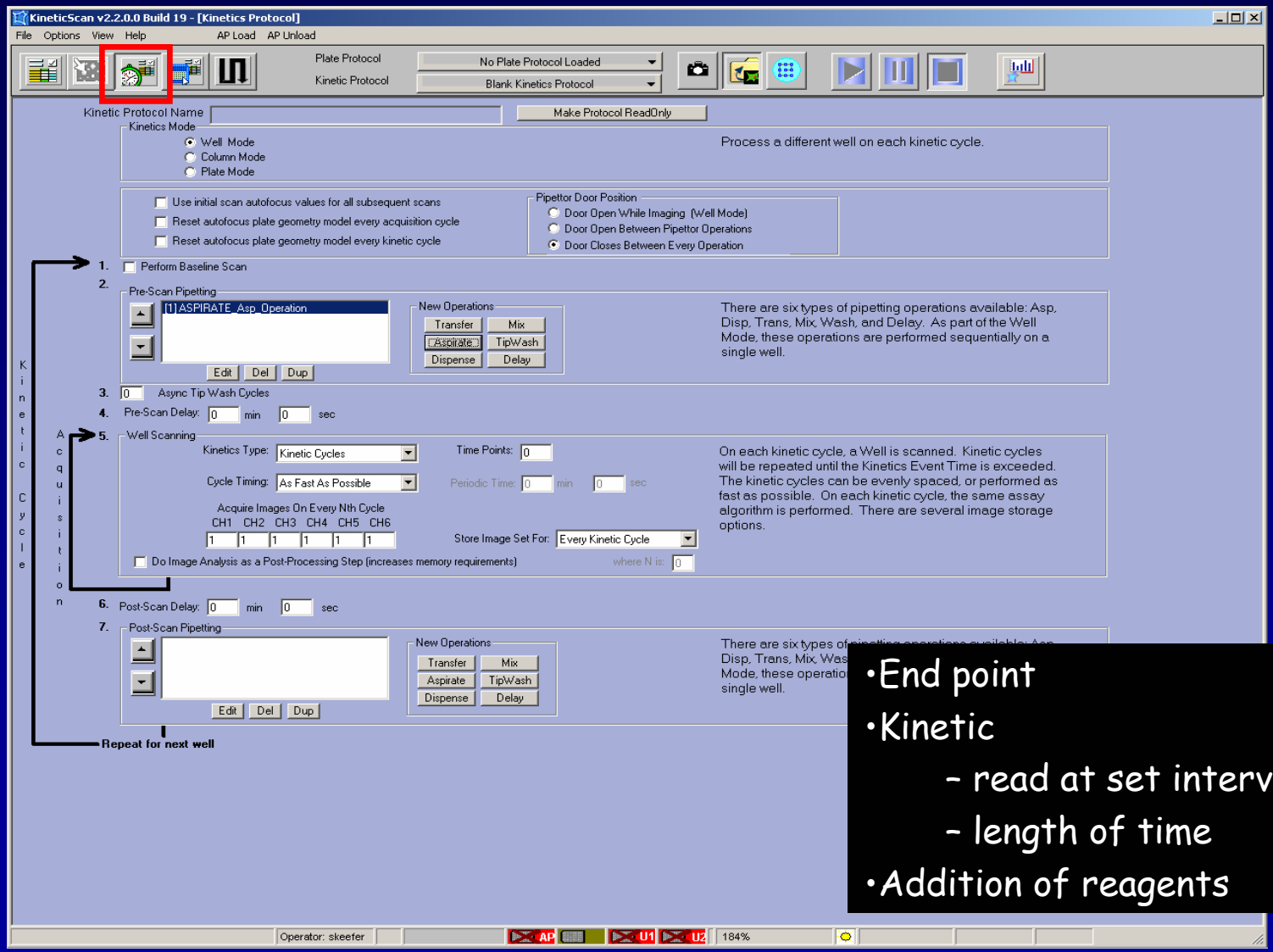
1. Assay Protocol
   - -Acquire only, Target Activation, Compartmental analysis
2. No of Channels (one for each fluorochrome), focusing channel
3. Assign filter, thresholds, gates, exposure time
4. Objective lens (x5 - x40)
5. No of Fields & cells
6. Pseudocolours

Next… interacting with your sample. Interactive Protocol



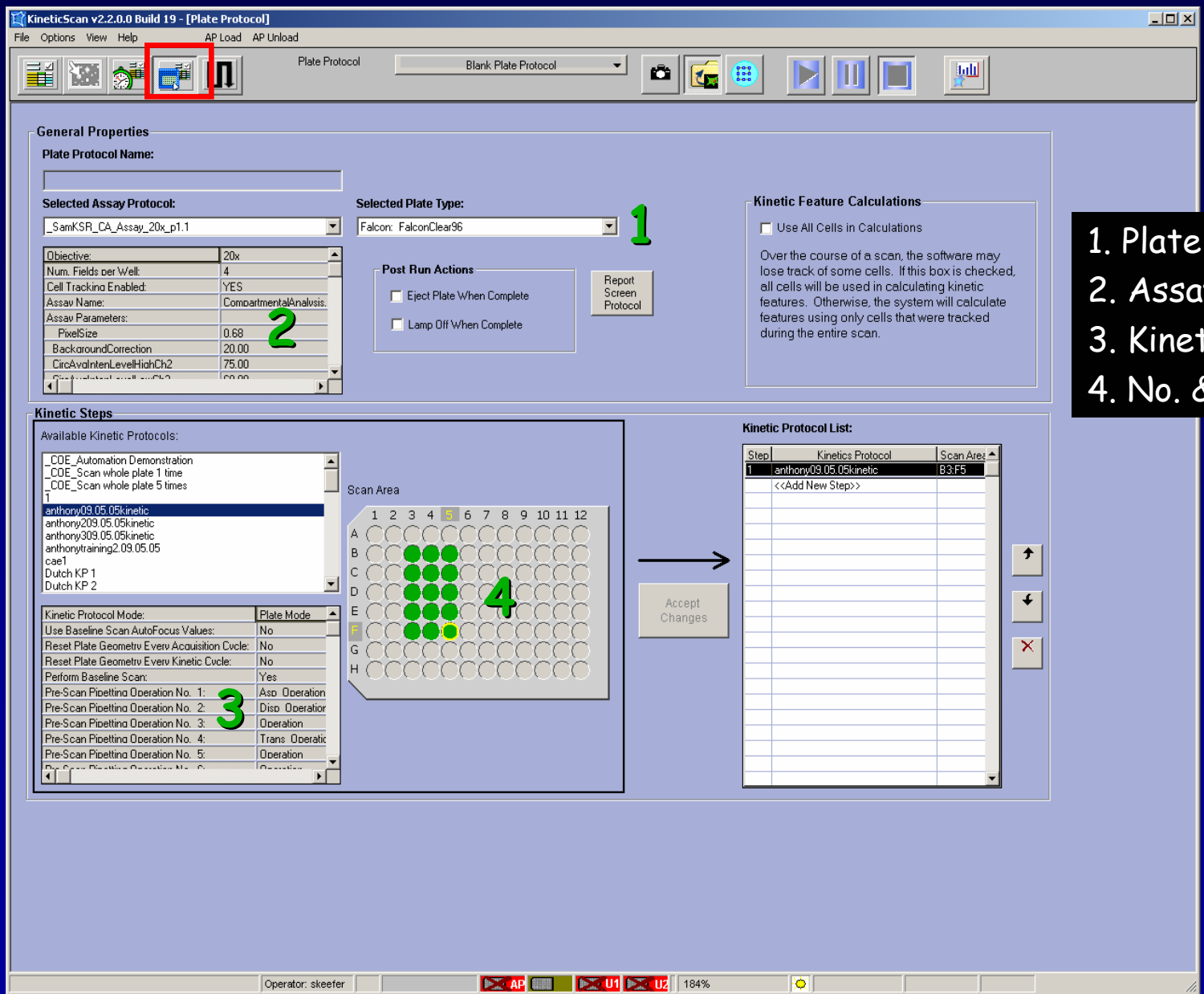
1. Optimize exposure and focus
2. Intensity threshold to identify objects
3. Select/reject cells by size, shape, intensity
4. Set gates to pick out nucleus, cytoplasm, granules, extracellular staining

Image sequencing and pipetting… The Kinetic Protocol



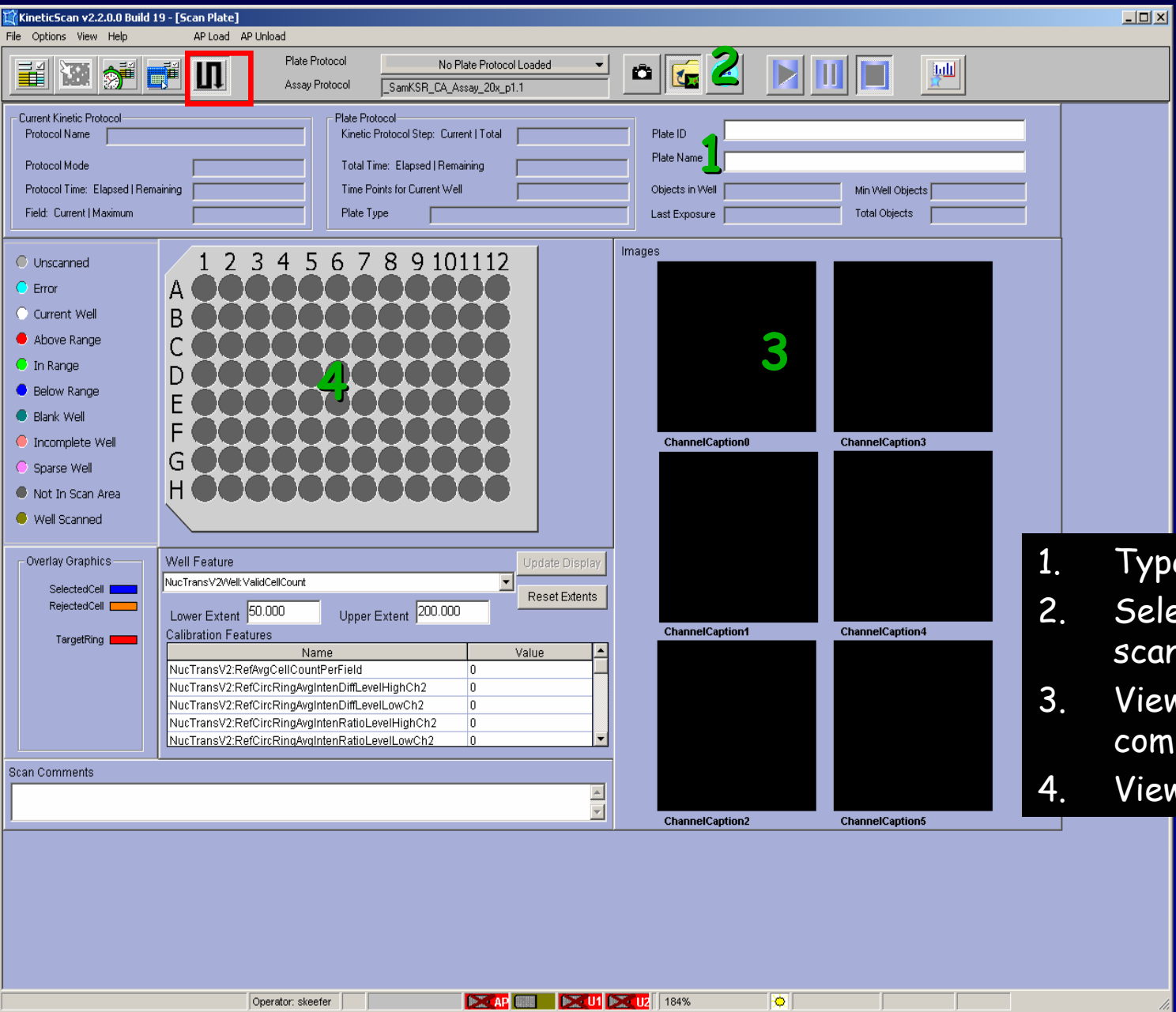
- End point
- Kinetic
  - read at set intervals
  - length of time
- Addition of reagents

Plate Protocol puts everything together…



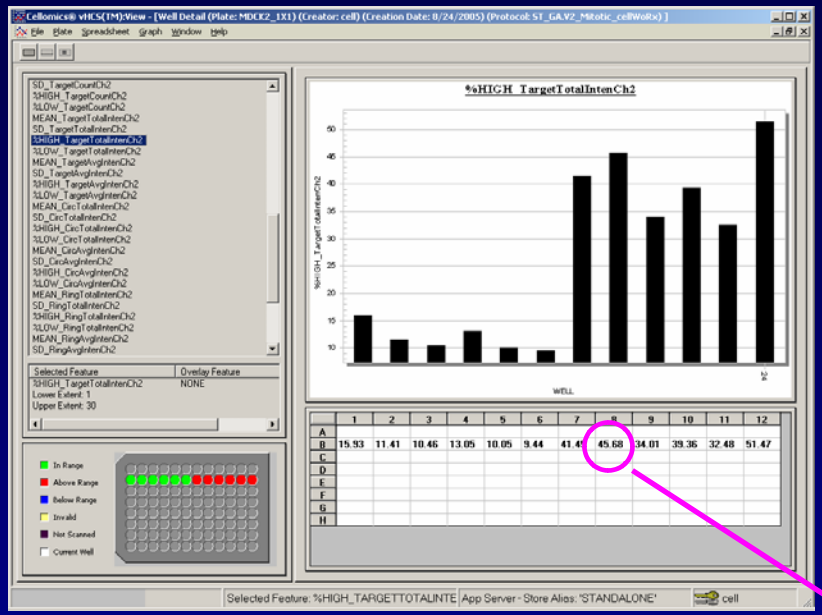
1. Plate type
2. Assay Protocol
3. Kinetic protocol
4. No. & pos. of wells

Scan View – Starts the plate imaging and processing…



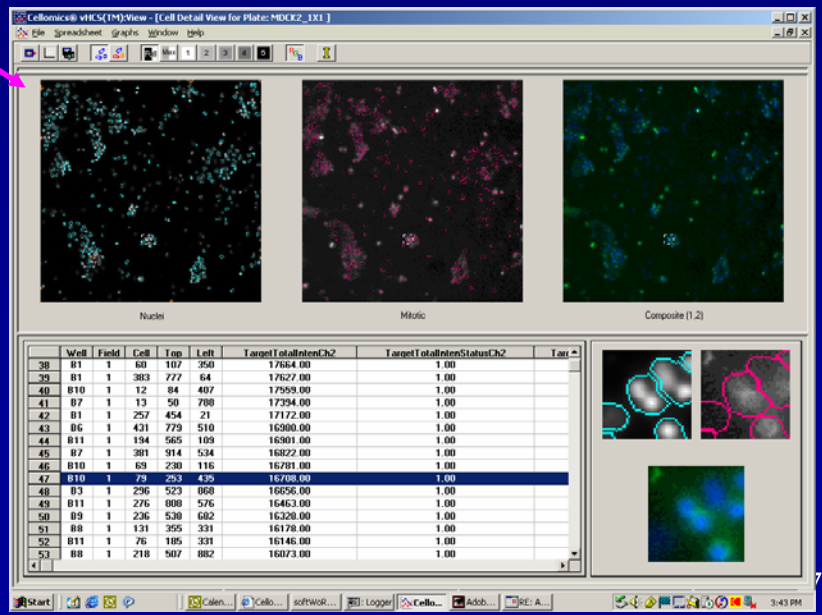
1. Type in plate Id
2. Select field to start scan
3. View all channels + composite
4. View plate or values

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

Scan™ 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)

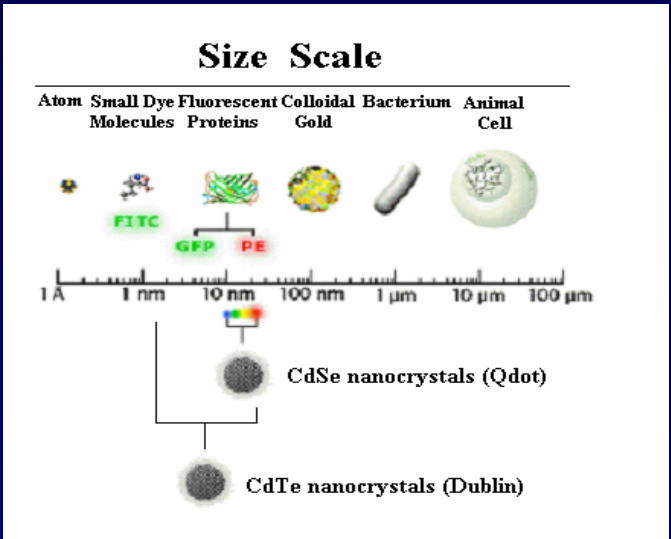
HIGH CONTENT ASSAYS CURRENTLY ESTABLISHED

- 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

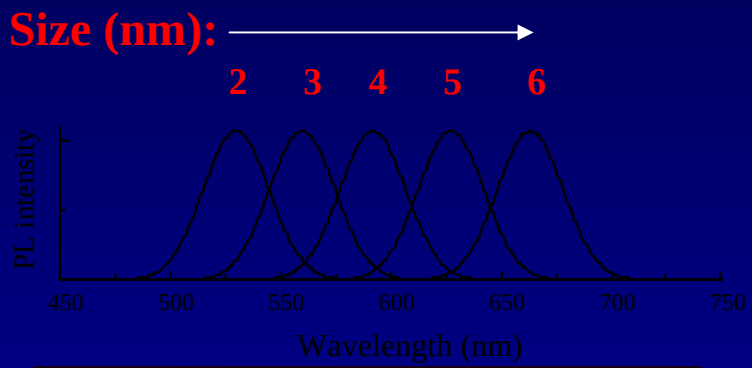
- **Case study:**

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

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



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



- 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

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

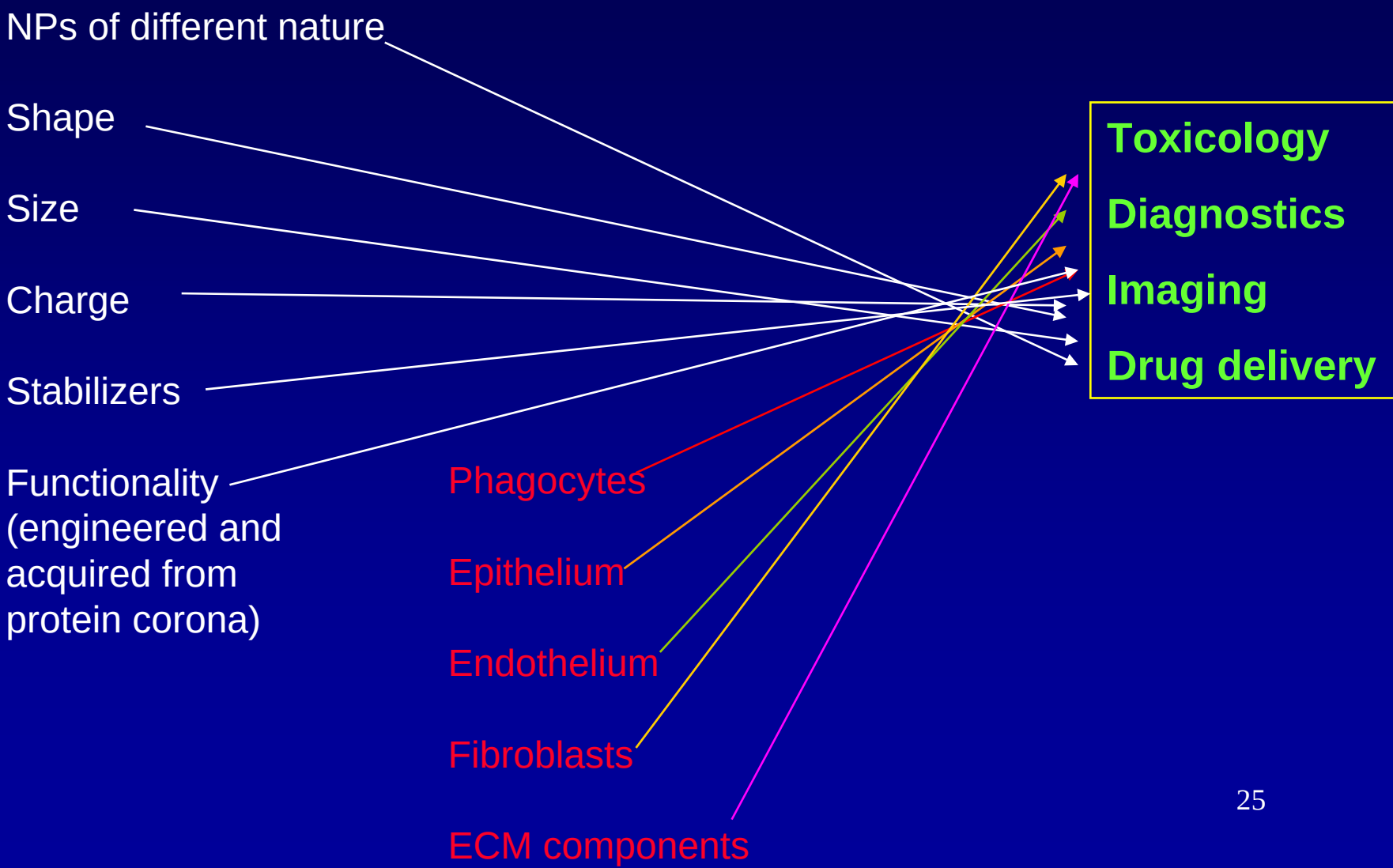
Traditional Fluorophores

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

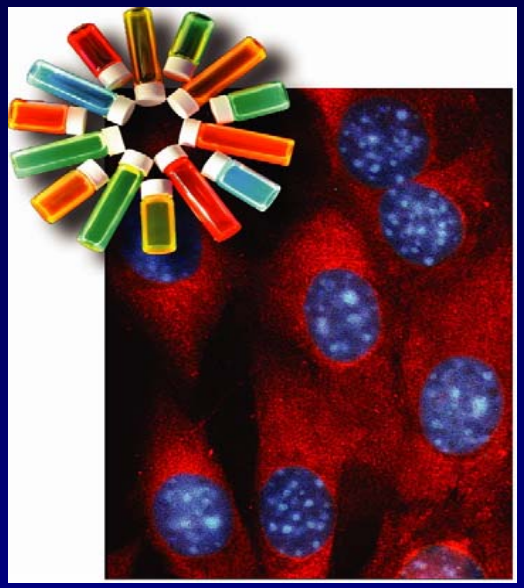
QDots-specific "issues"

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

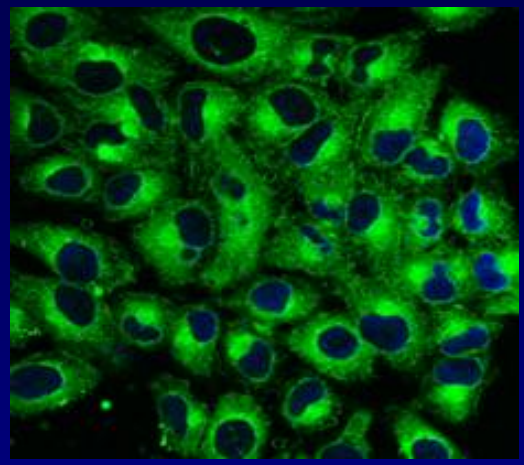
NP/CELL INTERACTION CHALLENGES: THE MULTIFACTORIAL ENVIRONMENT



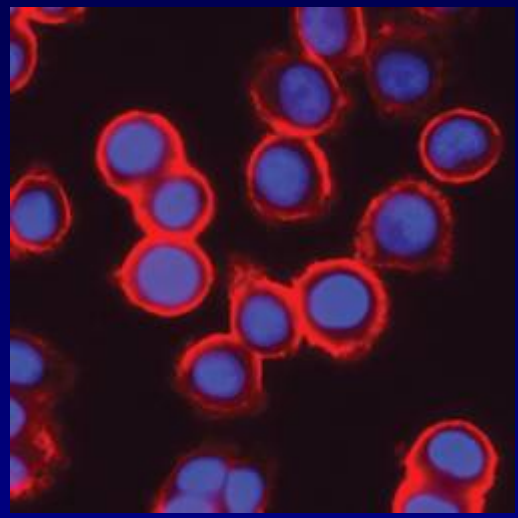
Nanoparticles and cells – clear patterns?



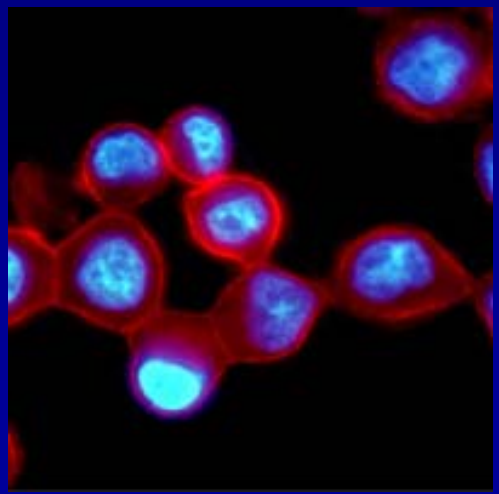
www.mdpi.org/ijms/specialissues/quantumdots.htm



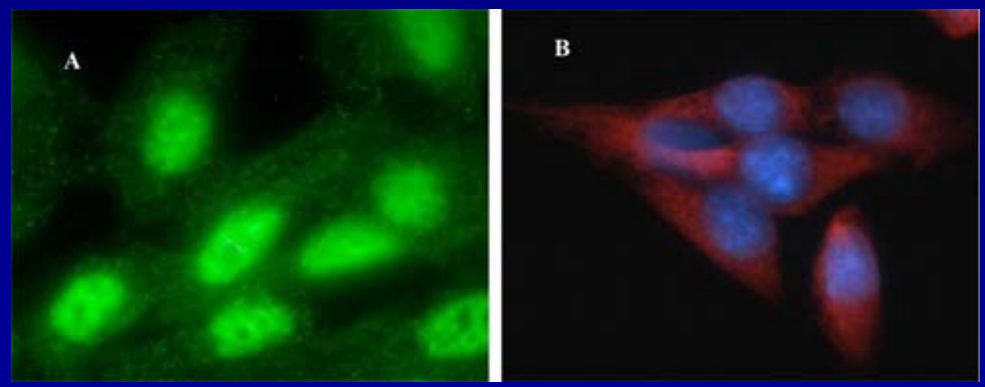
Dawson et al., UCD, -ESF Conference,Sant Feliu-2007



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



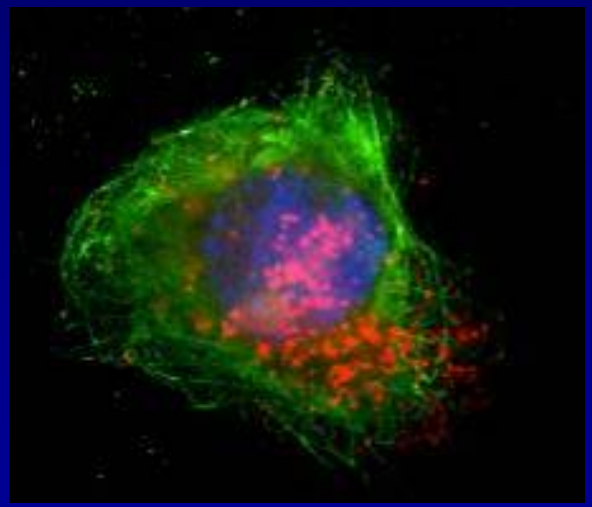
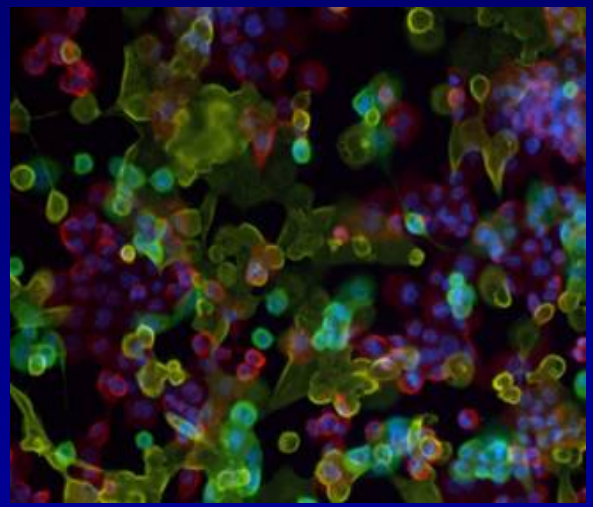
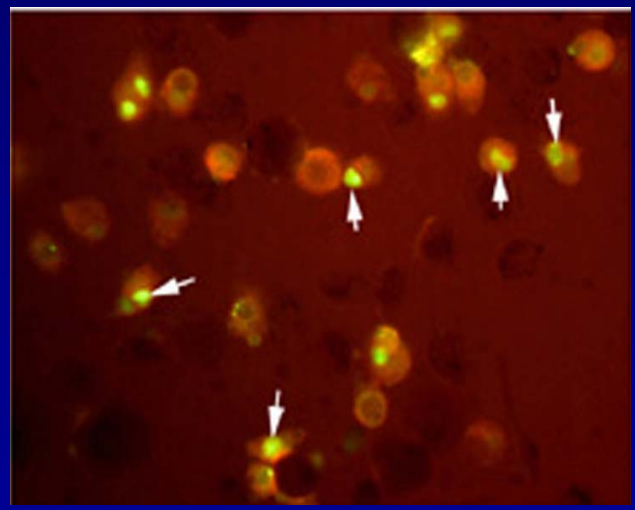
xnet.rrc.mb.ca/davidb/nanotools.htm



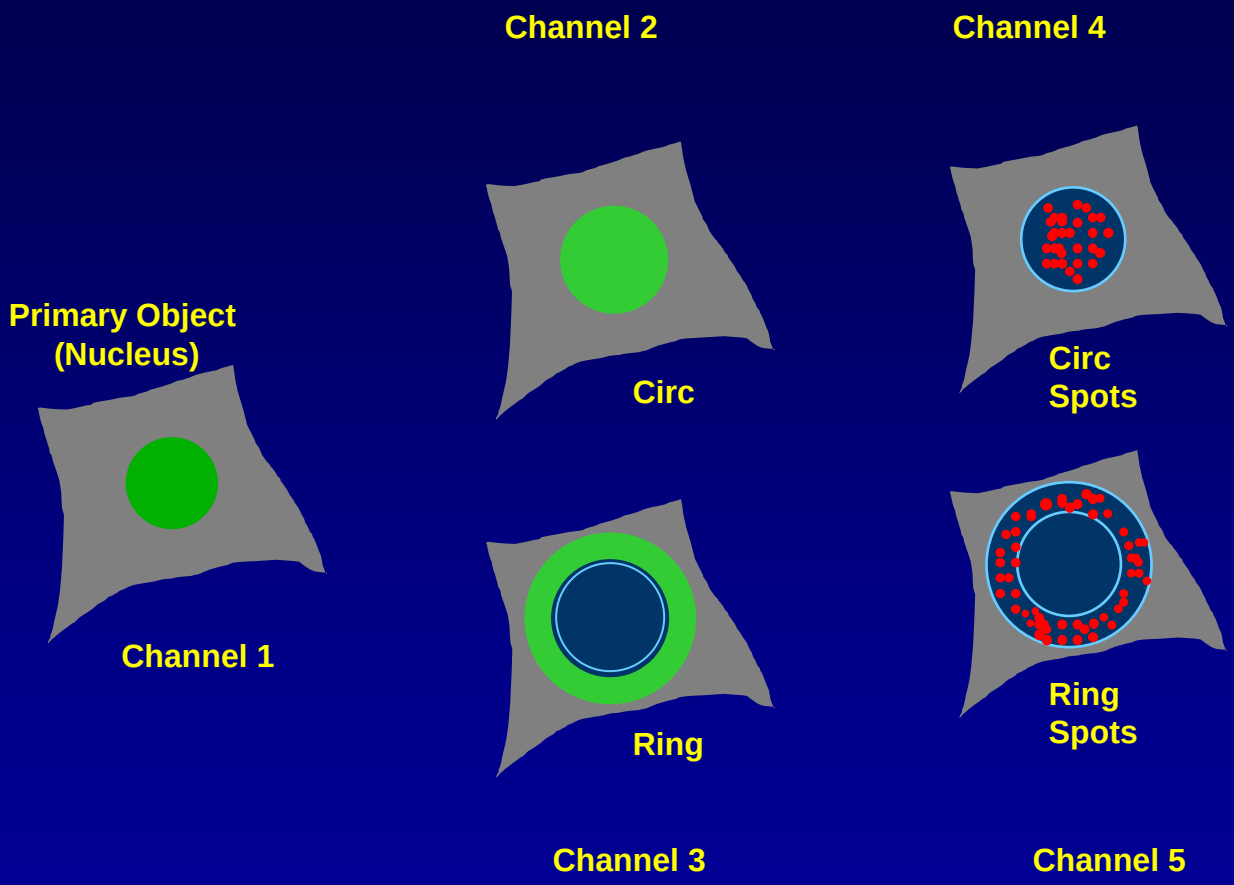
http://www.esi-topics.com/fbp/2005/february05-GabrielASilva.html

Nanoparticles and cells – clear patterns?

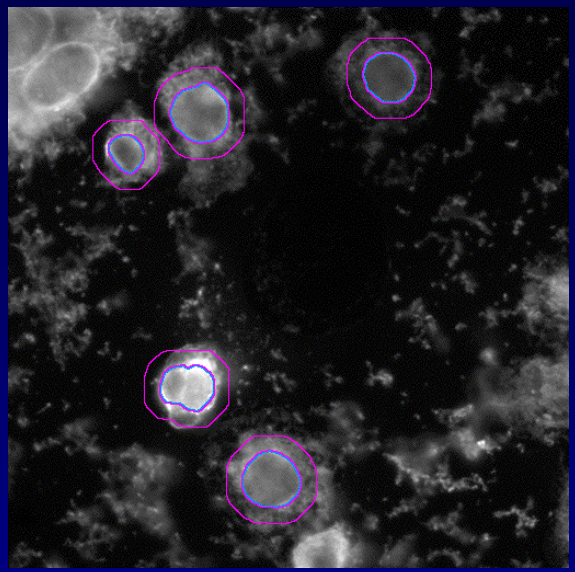
- Partially matching patterns
- Multi-parametric (multiplexed) readout is required
- Not everything fits within the conventional (convenient…?) 95%

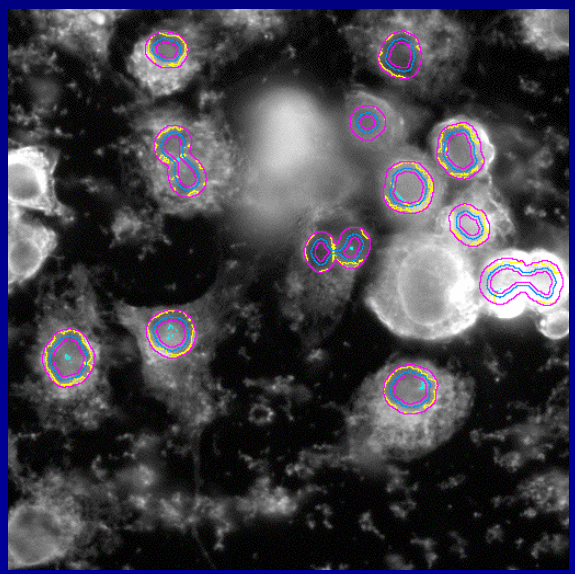




Compartmental Analysis BioApplication



Compartmental Analysis©



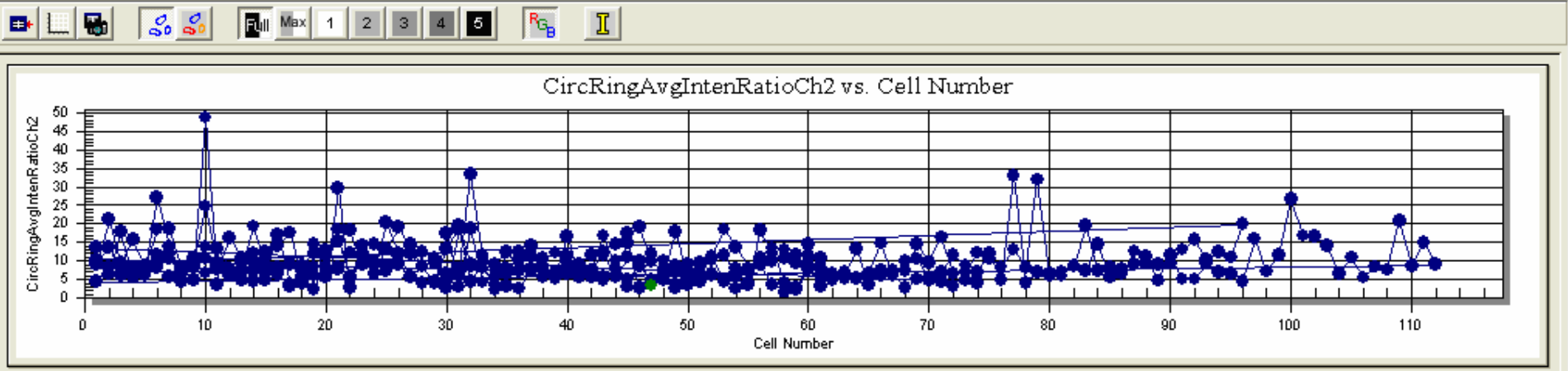


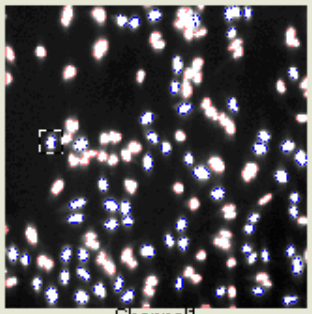
- Blue circle = nucleus
- Pink circle = cytoplasm

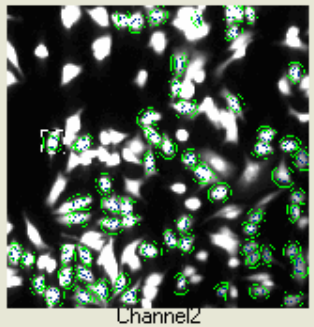
- Turquoise dots = nucleoli
- Yellow dots = nuclear rim

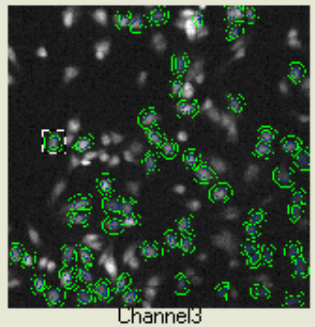
Cellomics® vHCS(TM):View - [Cell Detail View for Plate: med178158_12:41:01]

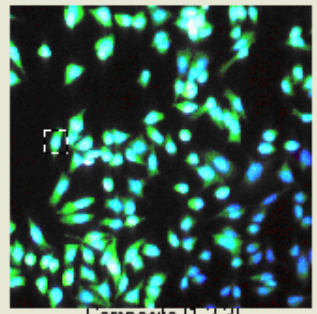
File Spreadsheet Graphs Window Help



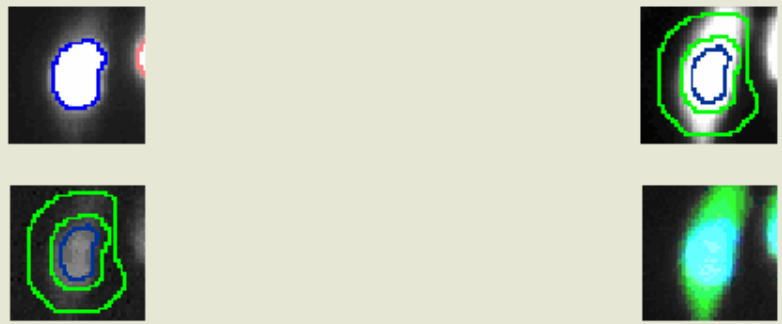

Channel1


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

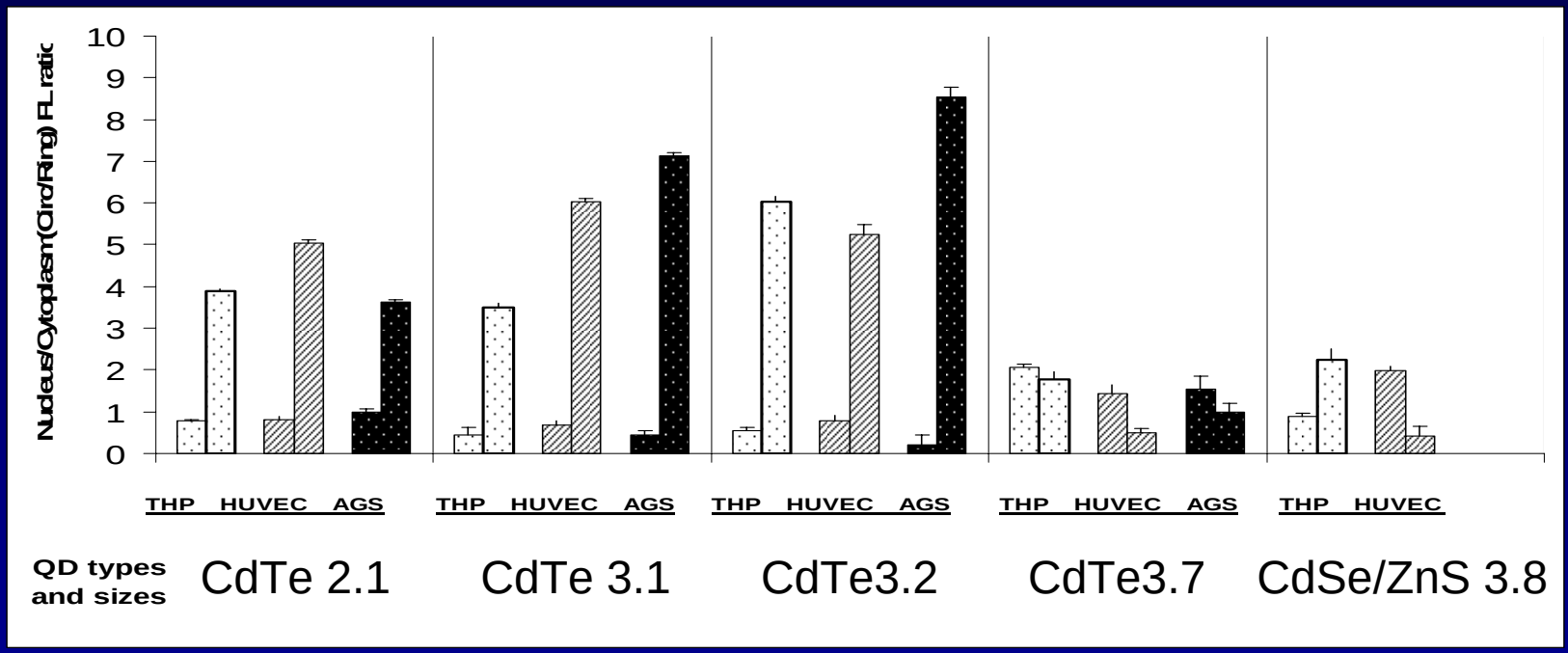


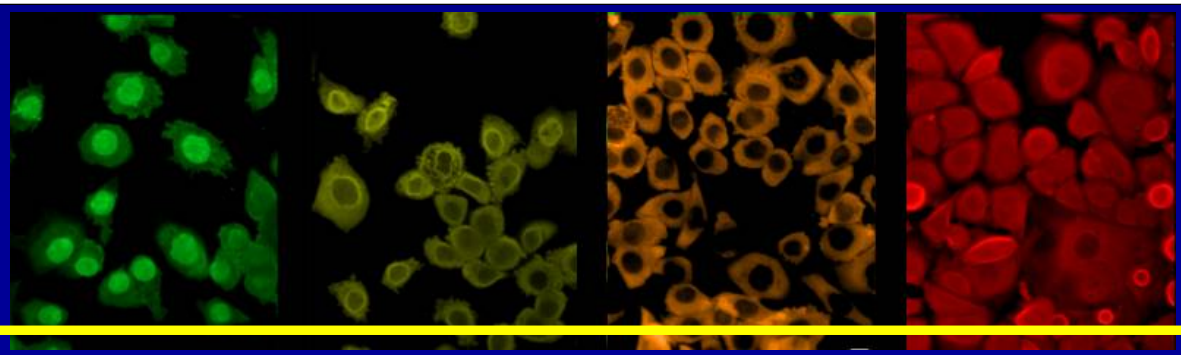
App Server - Store Alias: 'STORE'

cell

start Cellomics® vHCS(TM)... Doc1.doc - Microsoft ... 16:32

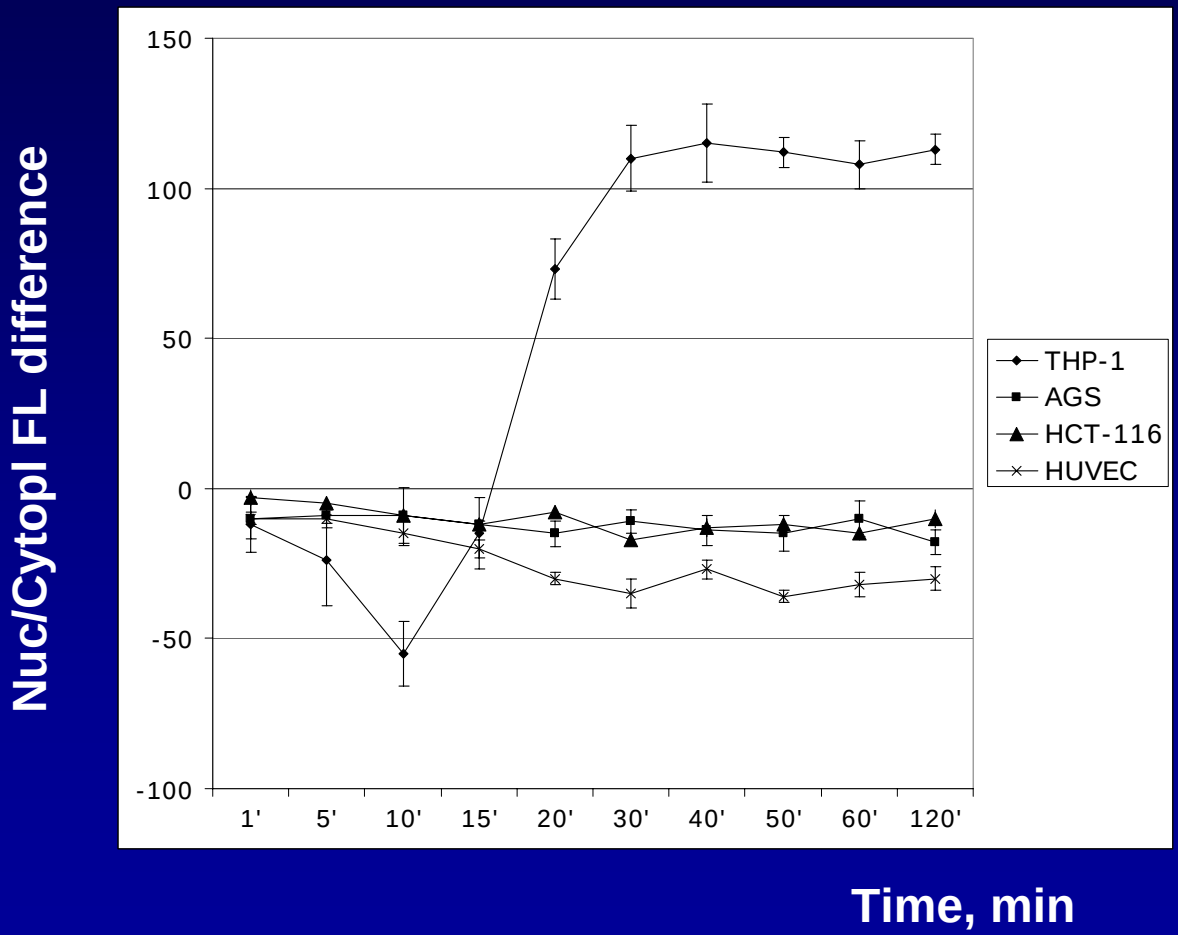
NP and cell-specific cellular response diversity: new models for targeted drug delivery



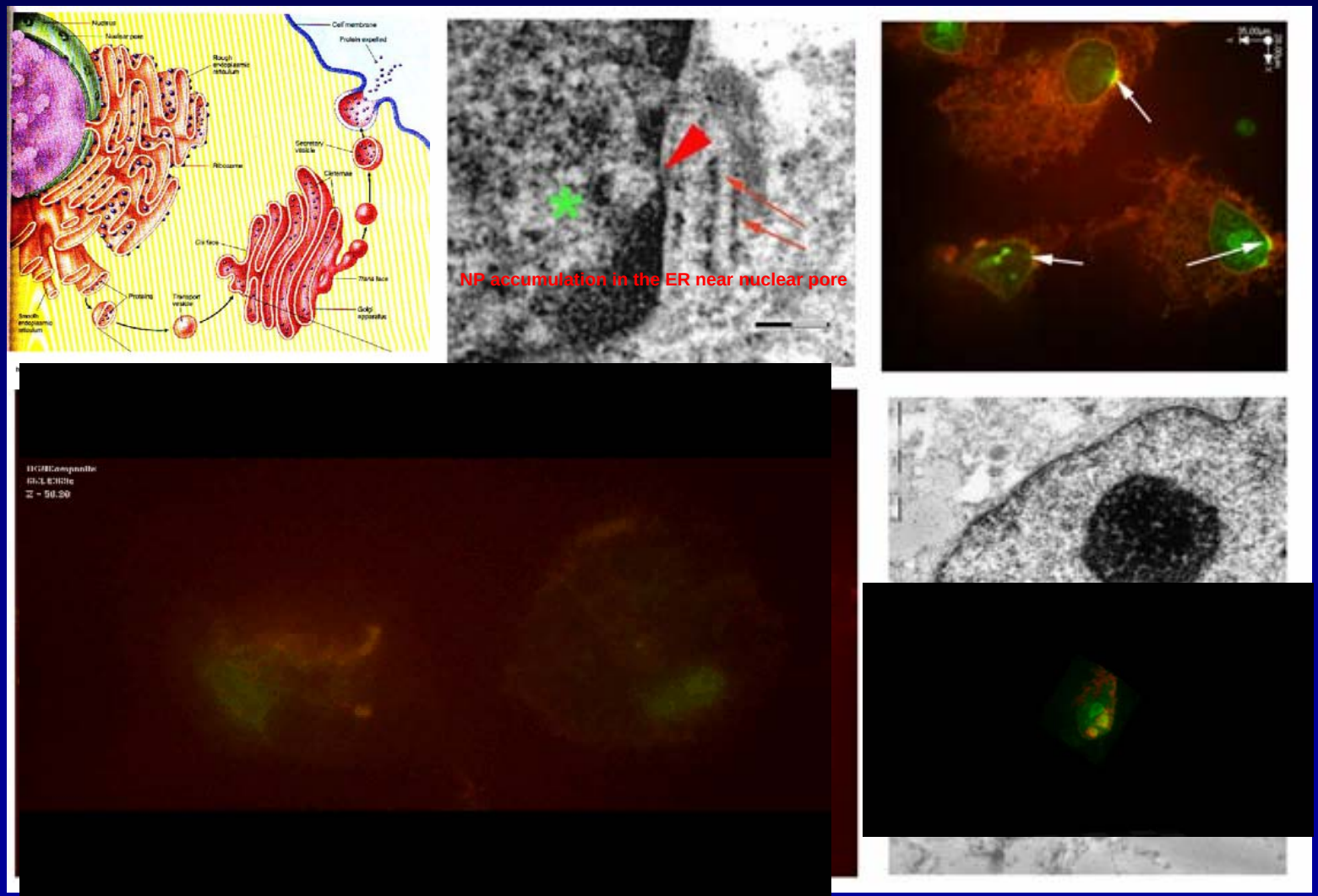


NP increasing size from 2 to 5 nm

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

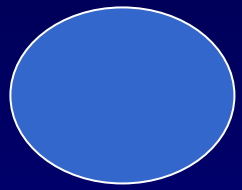
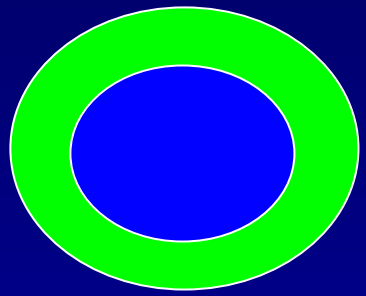
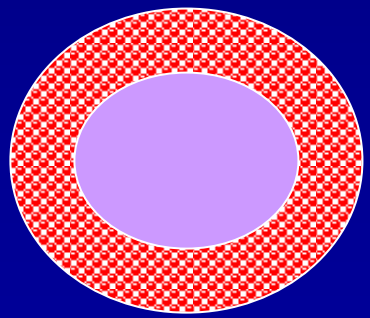


CdTe QDs: uptake and intracellular transport verified by "conventional methods"

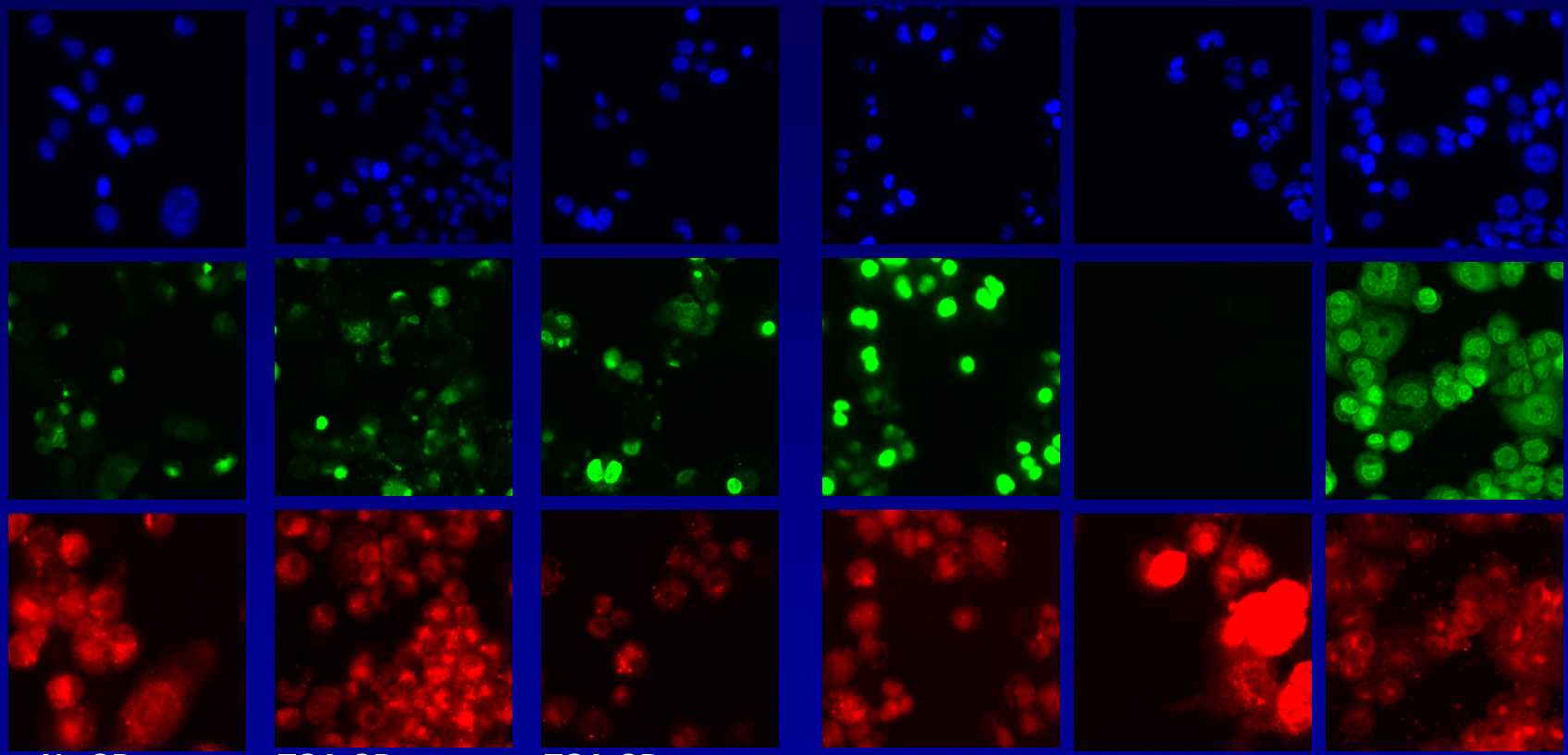


Nanoletters, November 2007

Cytotoxicity Assay

Channel 1		Nucleus	Nuclear count, size, shape, intensity,
Channel 2		Cytosol	Membrane Permeability
Channel 3		Inclusion in Cytoplasm	Changes in lysosomal mass/pH

Comparative cytotoxicity of unmodified and gelatine-coated QDs



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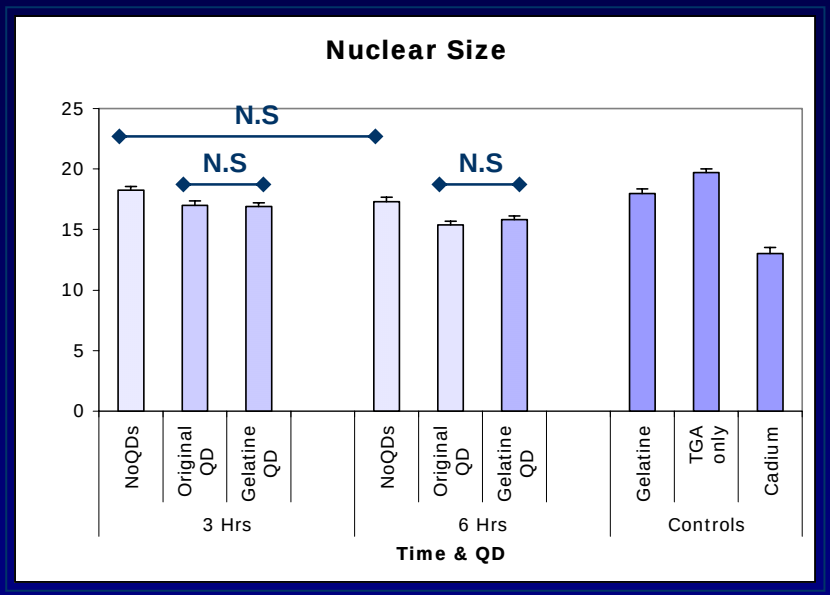
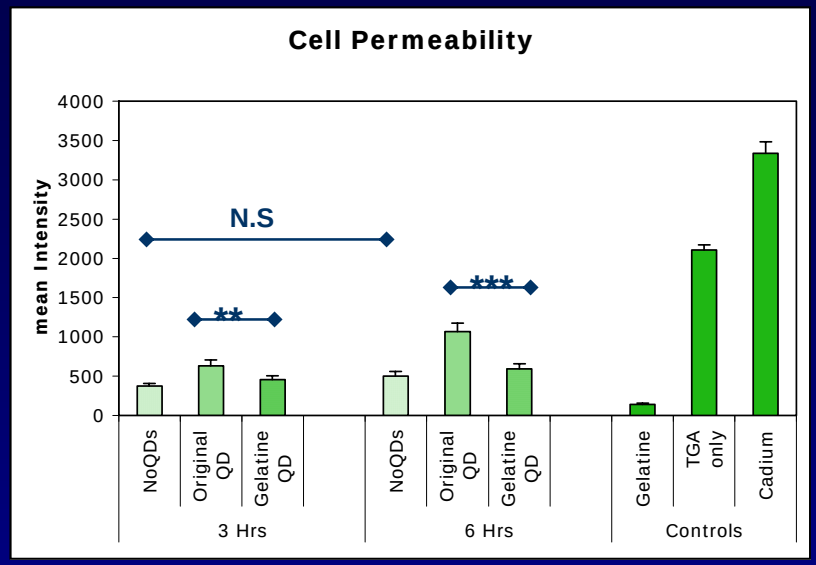
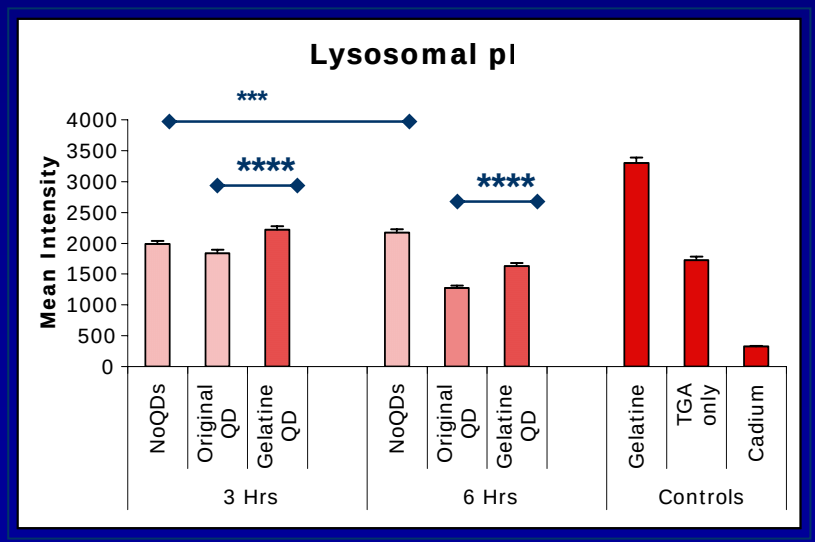
Comparative cytotoxicity of unmodified and gelatine-coated QDs







N.S not significant

**** P < 0.05**

*****P <0.005**

******P <0.0001**

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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 intracelluar 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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