High Content Screening for evaluation of nanoparticles interaction with cells

Yuri Volkov Department of Clinical Medicine Institute of Molecular Medicine Trinity College Dublin

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





GE InCell Analyser 1000

- Thermo Fisher (Cellomics) KineticScan HCS Reader
- 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.

1. Live-cell Environmental Sample Chamber

The Kinetic Scan HCS Reader

- Pipettor Access Port
- 2. Pipetting Capability with 1-to-8 Channel Liquid Handling

Cellomics KineticScan

- 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



- Market Multiple States Sta
- 🔈 Green: Calcium Homeostasis
- Yellow: Mitochondrial Transmembrane Potential
- ked: 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 10⁴ 10⁵/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

Piese C. (For Costs) Press. Locates Costs Costs Costs Costs Amy/Costs Statute D. (Soc.) Statute D. (S	Prime D: (File: Cole) Pres. Locales Cole Tenne Cole Tenne Cole Amy/Strain Statution D: Statution						
Appelan (Ph. YCL. LOCAL 2018/H21 Appelan (M*) CUL. Amples Amples (M*) BORTRA KCL. LOCAL 2018/H21 Appelan (M*) CUL. Amples Amples (M*) CHELSACID KCL. HACAL 2018/H21 Amples M*) CUL. Amples Amples (M*) CHELSACID KCL. HACAL 2018/H21 M*) CUL. Amples Amples (M*) CHELSACID KCL. HACAL 2018/H21 M*) CUL. Amples (M*) CHELSACID KCL. HACAL 2018/H21 M*) CUL. Amples (M*) CHELSACID KCL. HACAL 2018/H21 M*) CUL. Amples (M*) Varia Davis KCL. LOCAL	Angebra (PL) ICL LOCAL STRIPL'A Angebra (PL) CLL Angebra (PL) StRIPL'A StRIPL'A StRIPL'A StRIPL'A StRIPL'A Angebra (PL) Angebra	Plate ID / Bar Code	Plate. Location	Creation Date	Assty	Creator	Scen Typ
Biol/LD Kr.D. LOCAL 2000/DB Of Of Digension Cells CELL American Biol/LD Kr.D. LOCAL 2000/DB Of Of Digension Cells CELL American OVERSIANCI Kr.D. LOCAL 2000/DB FGL CEL	BitAld Discover Kin, LOCAL DOUBT Of Of Object and B Chill Amplian BitAld Discover Kin, LOCAL DOUBT Of Of Object and B Chill Amplian BitAld Discover Kin, LOCAL DOUBT Of Of Object and B Chill Amplian Direl Statut Kin, LOCAL DOUBT Of Of Object and B Chill Amplian Oriel Statut Kin, LOCAL DOUBT Amplian Chill Amplian Oriel Statut Kin, LOCAL DOUBT Amplian Chill Amplian Value Chill LOCAL DOUBT Ma	Apoptosis DR	KOL. LOCAL	2001/06/13	Apoptosis.MP_1	CELL	AnayScar
Disk (ED) Kin, LUCA Disk (ED) Child Amylon Disk (ED) Kin, LUCA 2001/011 CP/CHSpace (AD) CL Amylon CPUID Kin, LUCA 2001/011 CP/CHSpace (AD) CL Amylon CPUID Kin, LUCA 2001/011 CP/CHSpace (AD) CL Amylon CPUID Kin, LUCA 2001/012 CP/CHSpace (AD) CL Amylon CP/CHSpace (AD) Kin, LUCA 2001/012 CP/CHSpace (AD) CL Amylon CP/CHSpace (AD) Kin, LUCA 2001/012 CP/CHSpace (AD) CL Amylon CP/CHSpace (AD) Kin, LUCA 2001/012 CP/CHSpace (AD) CL Amylon CP/CHSpace (AD) Kin, LUCA 2001/012 Amylon CL Amylon CP/CHSpace (AD) 2001/012 Amylon Amylon Amylon Amylon CP/CHSpace (AD) 2001/012 Amylon Amylon Amylon Amylon CP/CHSpace (AD) 2001/012 Amylon Amylon Amylon <td>Disk (E. Durse) Kin. LOGA Other State Constraint Constraint Constraint Constraint Amy Constraint Constraint Constraint Constraint Constraint Amy Constraint Constraint Constraint Constraint Amy Constraint Constraint Constraint Amy Constraint Constraint</td> <td>B2AR DR</td> <td>KOL. LOCAL</td> <td>2002/05/01</td> <td>GPCRSignal.Cell</td> <td>CELL.</td> <td>Anny/Som</td>	Disk (E. Durse) Kin. LOGA Other State Constraint Constraint Constraint Constraint Amy Constraint Constraint Constraint Constraint Constraint Amy Constraint Constraint Constraint Constraint Amy Constraint Constraint Constraint Amy Constraint	B2AR DR	KOL. LOCAL	2002/05/01	GPCRSignal.Cell	CELL.	Anny/Som
Offention Kin. H&LCL. Minute Minute Minute Ammy Care OPEN Signing Kin. LACAL 2010/17/20 NewHorksgewing News/21 Clit. Ammy Care OPEN Signing Kin. LACAL 2010/17/20 NewHorksgewing News/21 Clit. Ammy Care OPEN Signing Kin. LACAL 2010/17/20 Minute Clit. Ammy Care OPEN Signing Kin. LACAL 2010/18/20 Clit. Ammy Care OPEN Signing Kin. LACAL 2010/18/20 Clit. Ammy Care OPEN Signing Kin. LOCAL 2010/18/20 Clit. Ammy Care OPEN Signing Kin. LOCAL 2010/18/20 Clit. Ammy Care OPEN Sig	Oriell Statution Kit. HB/LCL. 2010/17/20 Newberdbagewares/21 Cli.1 American Control Statution OPCR Statution Kit.L. KB/LCL. 2010/17/20 Newberdbagewares/21 Cli.1 American Control Statution OPCR Statution Kit.L. KB/LCL. 2010/17/20 Newberdbagewares/21 Cli.1 American Control Statution OPCR Statution Kit.L. Kit.L. Kit.L. Kit.L. Kit.L. American Control Statution Cli.1 American American American American OPCR Statution Kit.L. Kit.L. Kit.L. Kit.L. American American American American OPCR Statution Kit.L. Kit.L. Kit.L. Kit.L. American American American American OPCR Statution Kit.L. L. L. American American American Cli.L. American OPCR Statution Kit.L. L. L. American American Cli.L. American OPCR Statution Kit.L. L. L. L. American L. <	B2AR DR Demo	KOL. LOCAL	2002/03/11	GPORSignal Cell	CELL	Ana/Scar
Offer 1960 1975 Spanning K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen (1975 Spanning K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1974 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1974 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1974 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1974 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1974 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1976 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1977 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1978 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1977 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1978 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1978 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1978 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1978 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1979 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1970 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1970 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1970 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1970 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1970 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. LOCA, 2002 SP. Appress MF.J. C.L. Anny Gen 1970 December K.L. LOCA, 2002 SP. Appress MF.J. C.L. Appress MF.J. C.L. Appress MF.J. LOCA, 2002 SP. Appress MF.J. C.L. Appress MF.J. LOCA, 2002 SP. Appress MF.J. LOCA, 200	Odd 1980 Kin. LOA Modelson & Kin. Chin. Amydian Marking Odd 1980 Kin. LOA 2002/02 Angelass Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mark Domony Kin. LOA 2002/02 Amydian Marking Chin. Amydian Marking Mar	CR01034K01	KOL. READ.	2001/07/30	NeuriteOutgrowth.Assay.21	CELL.	Anny/Sch
Verbillingendigen Werbillingendigen Hans Dams K.C., LOOA, 2005/000 (CHORAGINAL CHI, CHI, American United States), Control (CHI, China), China States), China States, China (China), China States), LOOA, 2005/007 (King States), China States), China (China), China States), LOOA, 2005/007 (King States), China States), China States), China (China), China States), LOOA, 2005/007 (King States), China States), Chi	Units Park None Dist Park Park <t< td=""><td>CR01056D</td><td>KOL. LOCAL</td><td>2002/02/27</td><td>Apoptosis.MP_1</td><td>CELL</td><td>Ana/Sca</td></t<>	CR01056D	KOL. LOCAL	2002/02/27	Apoptosis.MP_1	CELL	Ana/Sca
CHAT Expension CHAT Expension 2014 El Expension	Oct-No Description Coling Co	GPCR Signaling	KOL. LOCAL	2002/03/20	GPCRSignal Cell	CELL	Ana/Sca
and Execution of the Local policies relations executions CEL Analysis MPLB Responders KOL LOCAL 2002/92/97 Nucl'hers Translocation CELL Analysis	Angle Secondary KOL LOCA, 2002/927 NacTressTowellocates, Clinicates,	GPCR Signaling	KOL. LOCAL	2002/03/20	GPCRSignal Cell	CELL	Ana/Sca
Ale Brandware ED, UDA, OBERGE BLANDERS CONTRACTOR CONTRACTOR AND AN	Contracting and a set of the	Neuro Demo	KOL. LOCAL	2001/06/08	NeurleOutgrowth.Assey.21	CELL	Ana/Sca
enne megendent KUL LUGAL deutstelt met hens i nemendekkol GELL Annycom	View Parks Integration View Parks View	Periodi Cento	10L 10CM	couchean	Nucliness Literatoceton	uu	ANNYOCH
	Image Description Page Factores Connects Image File Fi						
ROVE 12:34:12:PM ROVE 12:34:15:PM Channel/Dup Time 1.0000 Prove Lingue Do David Prove Lingue Do David ROULDING LINGTON Prove Lingue Do David Prove Lingue Do		Vieta Pietote Imag pie pieto Ben Time Pieto Rock 12:581:2794 Pieto Rock 12:581:2794 Pieto Rock 12:581:2795 Rock 12:585:071:55607	per Objects 1 [3703] a End Tree R2 12 451 PM Day Truesday	Plate Postures. Plate Posture Dame (20) Channel 20) T	Value = 3000 re 1.000	Contradents. The mighter doore respondence respon	once Rows A/O nee Rows EH
Fride:12.117.mc Fride:12.117.mc Fride:12.117.mc Channel/Cup Tree 1.000 Frid:12.12.016 Data Fride:12.000 Tree 1.000 Frid:12.12.016 Data Fride:12.000 Tree 1.000	1 Plate selected	Volta Patta Inno Pat Dat Ten Pat Dat Ten Pat Dat Ten Pat Dat Den Pat Dat Den Pat Den Pat Dent Dent Pat Dent	en Oracite 13363 12453 104 047 1946 1946 104 1 Pline selected	Rate Features. Plate Features Plate Feature Channel Charlow Charlow Channel Charlow	Videa # 3300 me 12000	Commentes. [The apples dose range 1.1 bytes dose range	onse Pows A-O nse Rows E-H

Analysis of Results



Instantaneous Data Display



Processing of Images on the Cellomics[®] HCS Systems





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 ...



Next... interacting with your sample. Interactive Protocol

🈰 KineticScan v2.2.0.0 Build 19 - [Protocol Interactive]	
File Options View Help AP Load AP Unload	
Plate Protocol No Plate Protocol Demo_Cellomics_NucTrar	Loaded
	Channel Parameters
3	Select Channel Select Dye 1 2 3 4 6 Channel 1 Image: Select Channel Image: Select Dye Image: Select Dye Image: Select Dye Image: Fixed Exposure 0.010 Select Identification Image: Fixed Method IsodataThreshold Image: Select Field Stage and Optics Control 1 2 3 4 5 7 8 9 10 11 2 Select Field 1 2 3 4 5 7 8 9 10 11 2 Image: Select Field Select AF A 2 4 5 7 8 9 10 11 2 Image: Select Field Select AF A 2 4 5 7 8 9 10 11 2
	Manual Focus Acquire image Set Manual Focus Image Set Mode Dpen Shutter Focus Parame Focus Parame 11.7 micrors Plate Type
	Packard: PackardView96 identify objects
Identify Deselect All Raw Image	
Object Selection Set Statistics (Image)	Object Selection Parameters (Protocol) Assay Parameters (Proto 3. Select/reject cells by
Name Value Min Max 1 NucArea Image: Statistics Image: Statistics Image: Statistics 2 NucShapeLVMR Image: Statistics Image: Statistics Image: Statistics 3 NucArgintenCh1 Image: Statistics Image: Statistics 5 NucTotalIntenCh1 Image: Statistics	Name LowerExtent UpperExtent Min Max size, shape, intensity 1 NucShapeP2A 0.00 1,000.00 0.50 2.0 3 NucShapeP2A 0.00 1,000.00 2.0 2.0 4 NucArea 0.00 1,000.00 0.00 2.0 5 NucTotalintenCh1 0.00 10,000,000.00.00 1.00 4.095.00 5 NucTotalintenCh1 0.00 10,000,000,000.00 1.00 10,000,000,000.00
	aranules extracellular
	staining
Operator: skeefer Protocol Modified	

Image sequencing and pipetting... The Kinetic Protocol

Kinet	ticScan v	v2.2.0).0 Build 19 -	[Kinetics Pro	tocol]								<u>_ ×</u>	
File Op	tions Vie	iew ł	Help	AP Load	AP Unload									
			st 🖬		Plate Protocol Kinetic Protocol	No Plate F Blank Kir	Protocol Loaded netics Protocol	· _ ↓	Č	a				
	Kin	netic F	Protocol Nam	e			Make Pro	tocol ReadOnly						
			Kinetics Mode © ©	Well Mode Column Mode Plate Mode					Pro	ocess a differ	rent well c	on each kinetic cycle.		
	_		🗖 Use i 🗖 Rese 🗖 Rese	nitial scan auto t autofocus pla t autofocus pla	focus values for all subsequent te geometry model every acqui te geometry model every kinetic	scans sition cycle c cycle	Pipettor Door Door 0 Door 0 Door 0	Position pen While Imagin pen Between Pip loses Between Ev	g (Well Mod ettor Operatio very Operatio	e) nns n				
	-> ;	1. [2	Perform Bas	eline Scan										
ĸ		2.	Pre-Scan Pipe	tting PIRATE_Asp_C Edit De	Iperation	New Operations Transfer Mix (Aspirate TipWa: Dispense Delay	sh j		Th Dis Mo sin	ere are six ty p, Trans, Mix de, these op gle well.	pes of pip < Wash, a erations a	petting operations available: Asp, and Delay. As part of the Well are performed sequentially on a		
n	3	3. 🔽) Async T	ip Wash Cycles	3									
е	4	4 . F	Pre-Scan Delay	0 min	0 sec									
t C y C I e	A P ! q i i t i	5.	Well Scanning	Kinetics Type Cycle Timing Acquire Im CH1 CH2 1 1 Analysis as a F	Kinetic Cycles As Fast As Possible ages On Every Nth Cycle CH3 CH4 CH3 CH4 1 1 1 1 Yost-Processing Step (increase	Time Points: 0	min () sec netic Cycle where N is:	On will The fas alg opt	each kinetic be repeated kinetic cyclo as possible orithm is perf ions.	cycle, a V I until the I es can be . On eacl ormed. T	Well is scanned. Kinetic cycles Kinetics Event Time is exceeded. e evenly spaced, or performed as ch kinetic cycle, the same assay There are several image storage		
	• – – į	6. p.	ost-Soon Delau		0									
	7	7. E	Post-Scan Pip	etting	lo sec									
						New Operations Transfer Mix Aspirate TipWash Dispersed Delay	1		Th Dis Mo sin	ere are six ty p, Trans, Mix de, these op gle well.	pes of ein < Was ieration	•End point		
L		Пере	eat for next v	Edit D	el Dup							 Kinetic 		
												- read at	set intervo	als
												- length o	ftime	
												•Addition of re	eagents	
					Operator: skeefer				U2 184	%				14
					, , ,									

Plate Protocol puts everything together...



	Operato	or: skeefer	AP	
--	---------	-------------	----	--

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

🏹 KineticScan v2.2.0.0 Build 1	19 - [Scan Plate]	
File Options View Help	AP Load AP Unload	
	Assay Protocol SamKSR_CA_Assay_20x_p1.1	
Current Kinetic Protocol	Plate ID Pla	
Protocol Mode Protocol Time: Elapsed I Rem	Total Time: Elapsed I Remaining Plate Name	
Field: Current Maximum	Immer of ins to current wear Copeus in vear mill vear objects Plate Type Last Exposure Total Objects	
C Unscanned		
C Error		
 Current Well Above Range 		
 In Range 		
 Below Range 		
 Blank Well Incomplete Well 	F ChannelCaption® ChannelCaption3	
Sparse Well		
Not In Scan Area		
Well Scanned		
Overlay Graphics	Well Feature Update Display	Type in plate Id
SelectedCell	Reset Extents	Select field to start
TargetRing	Calibration Features ChannelCaption1 ChannelCaption4	con
	NucTransV2:RefAvgCellCountPerField 0	scan
	NucTransV2:ReiClickingAvgintenDiffLevelLowCh2 0 NucTransV2:ReiClickingAvgintenDiffLevelLowCh2 0	View all channels +
	NucTransV2:RefCircRingAvgIntenRatioLevelHighCh2 0 NucTransV2:RefCircRingAvgIntenRatioLevelLowCh2 0	composite
Scan Comments		View plate on values
	ChannelCaption2 ChannelCaption5	view plate or values
		16
	Operator: skeefer	

vHCS[™]:View



Cell Level:

Information on individual cells

- •Images
- Animated images of kinetic assays

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



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



- 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 sifts 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





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] **_** 7 X 💢 File Spreadsheet Graphs Window Help _ 8 × Full Max 1 2 3 4 5 🗗 🛄 🖶 80 80 RGB Ι CircRingAvgIntenRatioCh2 vs. Cell Number 50 45 CircRingAvgIntenRatioCh2 40 35 30 25 20 15

60

Cell Number



Channel¹

20

10

n.

0

The second second

10

40

50

30

L'hannel/



70

1 1 1

80

- **T**I

90

Channel3



110

100

Composite [1,2,3]

16:32

379 380 381 382 383 384 385 386 386 387 ↓ ↓ ↓	Well A2 A2	Field 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Cell 43 44 45 46 47 48 49 50 51 51	Top 258 259 263 265 268 277 279 310 327 229	Left 419 263 467 115 68 239 428 227 292 292	
						 App Server - Store Alias: 'STORE'

Doc1.doc - Microsoft

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





NP increasing size from 2 to 5 nm

31

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



Time, min

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



Nanoletters, November 2007

Cytotoxicity Assay



Comparative cytotoxicity of unmodified and gelatine-coated QDs



Small, 2007

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 "nanoparticledriven" 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

TCD/Dept of Clinical Medicine:

- Bashir Mohamed
- Yvonne Williams
- Jennifer Conroy
- Anthony Davies
- Aine Whelan

TCD/CRANN:

School of Chemistry

- Stephen Byrne
- Yurii Gun'ko

School of Physics

- Yury Rakovich
- John Donegan

School of Pharmacy

Marek Radomski

UCD: Iseult Lynch Kenneth Dawson

Rheims

- Igor Nabiev
- Alyona Sukhanova

Munich

Andrey Rogach

Dresden

Nikolai Gaponik

FUNDING:

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