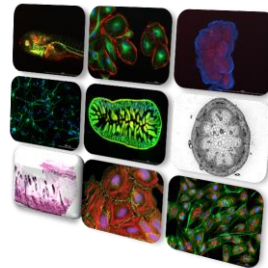
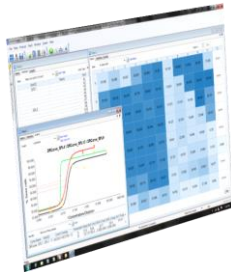


Think Possible




Cytation™ 5 Cell Imaging Multi-Mode Reader



翰新國際有限公司

Think Possible



Cytation 5 Cell Imaging Multi-Mode Reader

Cytation™ 5 is a fully integrated multi-mode reader combining digital fluorescence, brightfield, H&E stain and phase contrast imaging with BioTek's patented Hybrid Technology™ for optimal detection flexibility and performance with both filter and monochromator-based optics.

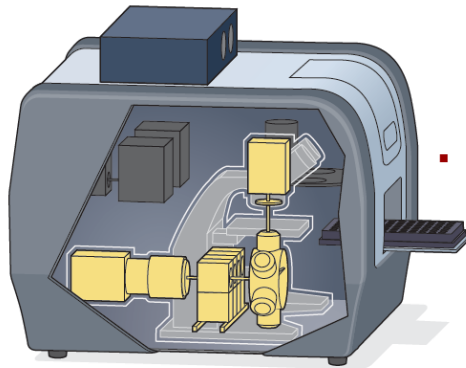
Cytation 5 features:

- Combined digital microscopy and multi-mode detection
- Hybrid Technology™: filter and monochromator-based optics
- Variable bandwidth monochromators for fluorescence
- Laser-based excitation for Alpha assays
- Cell assay optimized with CO₂/O₂ control, incubation and shaking

Think Possible



Digital Microscopy *and* Multi-Mode Detection



Microscopy

- Digital Fluorescence
- Brightfield
- H&E Stain (color brightfield)
- Phase Contrast

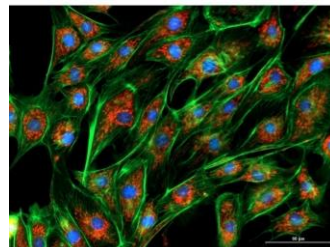
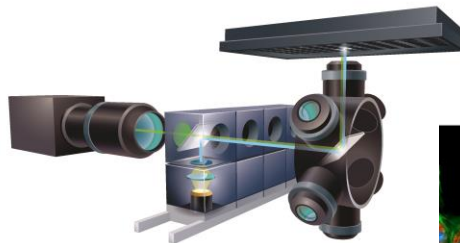
Multi-Mode Detection

- Monochromator Based
 - UV-Vis Absorbance
 - Fluorescence Intensity
 - Time Resolved Fluorescence
 - Luminescence
- Filter Based
 - Fluorescence Intensity
 - Time Resolved Fluorescence
 - Fluorescence Polarization
 - Luminescence
 - Alpha

Think Possible



Imaging Optical Path



- Qualitative and Quantitative data
- Characterize change in phenotype

Think Possible



High Quality Optical Components

- Semrock filters
- High power LEDs
- Zeiss & Olympus objectives
- 16-Bit CCD camera with Sony chip

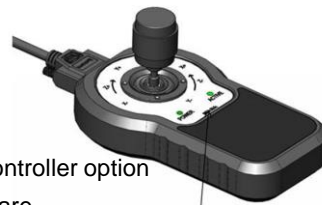


Think Possible



Imaging Basic Specifications
















- Matched LED light source and filter cube assemblies
- 4 filter cube onboard capacity: RGB, plus several other colors
- Six objectives onboard capacity: 1.25x, 2.5x, 4x, 10x, 20x, 40x and 60x
: 4x, 10x, 20x and 40x phase
- Read modes: End point / Time lapse / Montage
- Labware: 6- to 1536-well microplates
Microscope slides / chamber slides
Cell Counting Chamber
T25 flasks
Petri dishes
- AutoFocus, AutoIntensity, AutoExposure
- Software controlled microscopy & Joystick controller option
- Gen5 reader control and data reduction software



Think Possible



Imaging Filter Cube Options

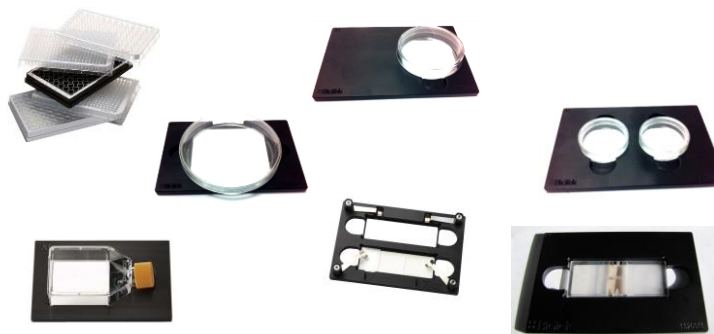
	Description	P/N	Excitation	Emission	Mirror	P/N	LED
	DAPI filter cube	1225100	377/50	447/60	409	1225000	365 nm
	TagBFP filter cube	1225115	390/18	447/60	409	1225009	390 nm
	CFP filter cube	1225107	445/45	510/42	482	1225001	465 nm
	GFP filter cube	1225101	469/35	525/39	497	1225001	465 nm
	YFP filter cube	1225104	500/24	542/27	520	1225004	505 nm
	CFP-YFP FRET filter cube	1225110	445/45	542/27	482	1225001	465 nm
	RFP filter cube	1225103	531/40	593/40	568	1225003	523 nm
	Phycoerythrin filter cube	1225113	469/35	593/40	568	1225001	465 nm
	Texas Red filter cube	1225102	586/15	647/57	605	1225002	590 nm
	Propidium Iodide filter cube	1225111	531/40	647/57	605	1225003	523 nm
	Acridine Orange filter cube	1225109	469/35	647/57	605	1225001	465 nm
	CY5 filter cube	1225105	628/40	684/40	660	1225005	623 nm
	Chlorophyll filter cube	1225112	445/45	685/40	482	1225001	465 nm
	CY5.5 filter cube	1225114	647/57	794/160	695	1225008	655 nm
	CY7 filter cube	1225106	716/40	809/81	757	1225006	740 nm

Think Possible



Labware: Multi-Vessel Compatibility

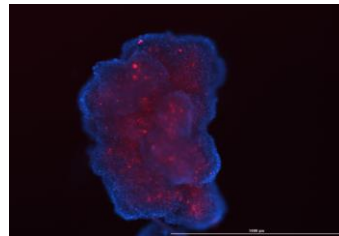
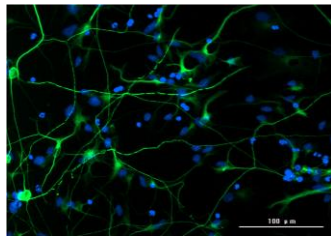
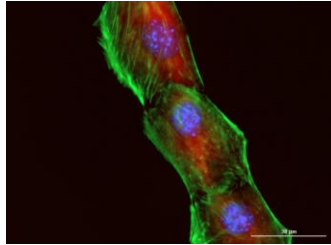
- 6- to 1536-well microplates
- Microscope slides / chamber slides
- T25 flasks
- 100, 60 and 35 mm Petri dishes
- Cell Counting Chamber



Think Possible

 BioTek®

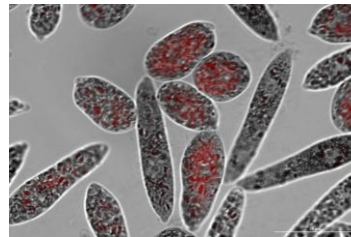
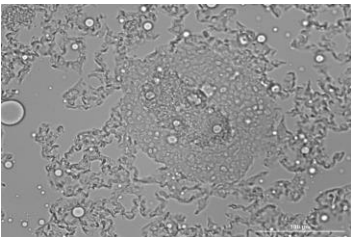
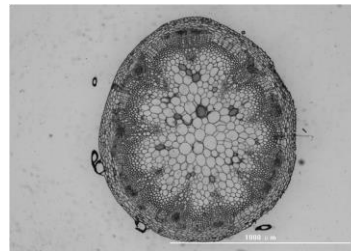
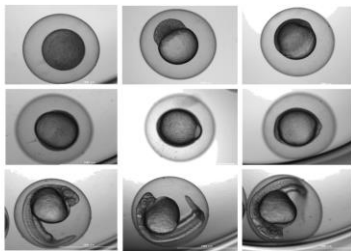
Digital Imaging Modes: Fluorescence



Think Possible

 BioTek®

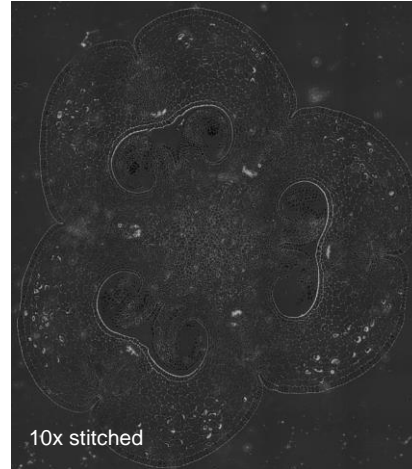
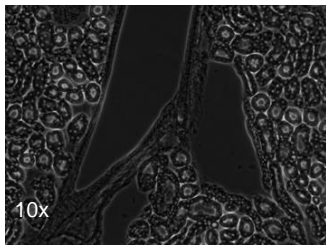
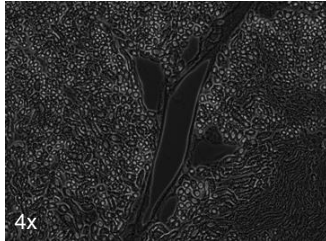
Digital Imaging Modes: Brightfield



Think Possible

 BioTek®

Digital Imaging Modes: Phase Contrast



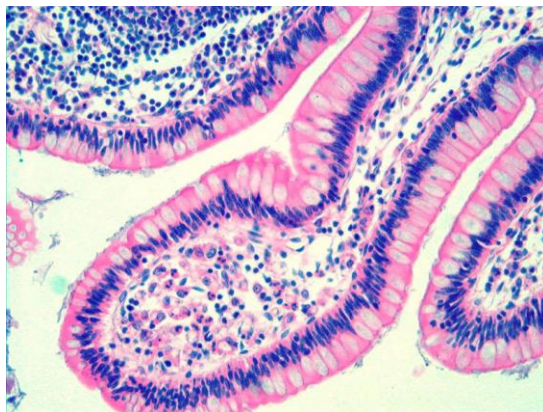
Think Possible

 BioTek®

Digital Imaging Modes: H&E Stain Imaging

Hematoxylin stain
(blue)

Eosin stain
(red)



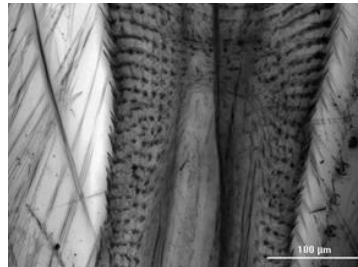
Think Possible



Imaging & Microscopy

- Imaging Methods
 - Single color
 - Multi-color
 - Montage
 - Time lapse
 - Z-Stacking
- Image Processing
 - Z-Projection
 - Digital Phase Contrast
 - Stitching

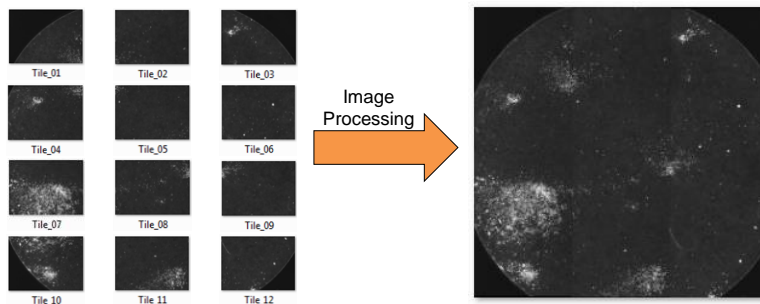
Z-projection in Gen5



Think Possible



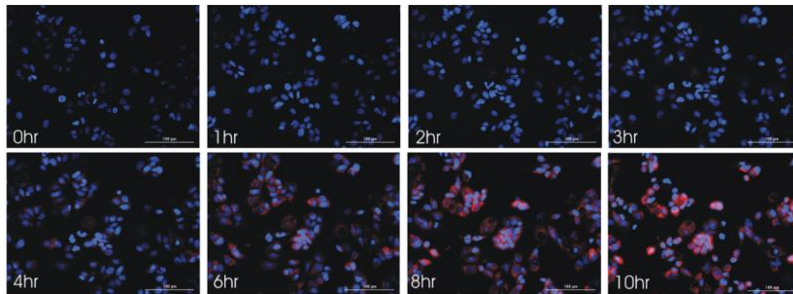
Imaging & Microscopy: Montage and Stitching



Think Possible

BioTek®

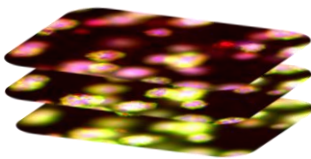
Imaging & Microscopy: Time Lapse



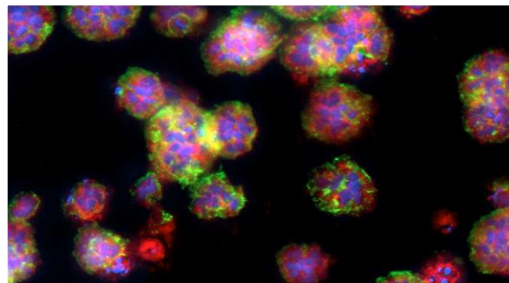
Think Possible

BioTek®

Imaging & Microscopy: Z-Stacking, Z-Projection



Z-Stacking

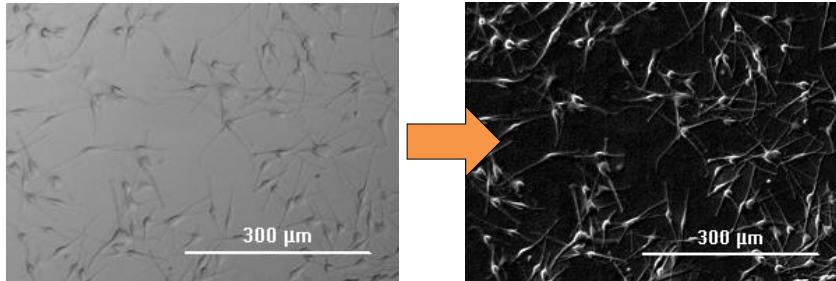


Z-Projection

Think Possible

BioTek®

Imaging & Microscopy: Digital Phase Contrast

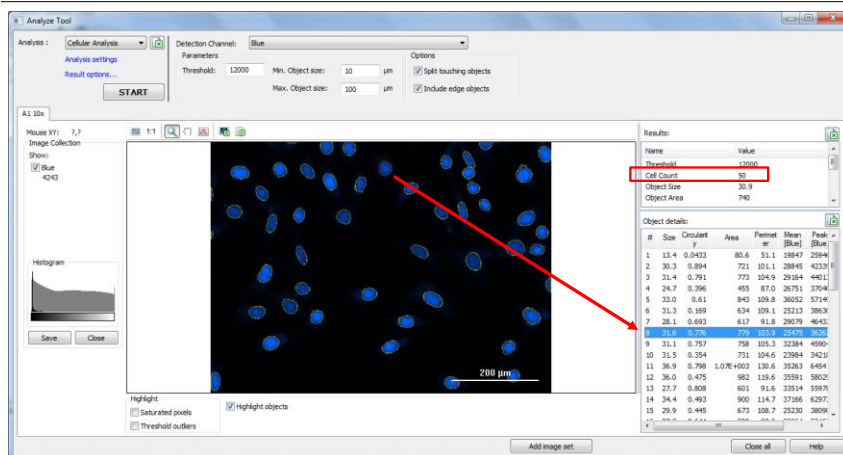


Digital Phase Contrast

Think Possible

BioTek®

Gen5 Interface

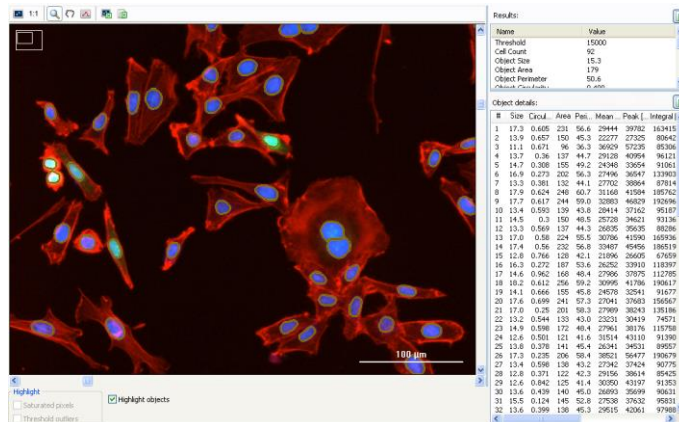


Gen5 automates cell counting and sub-population analysis in an uncluttered, easy to use interface.

Think Possible

BioTek

Image Analysis Can Be Very Complex

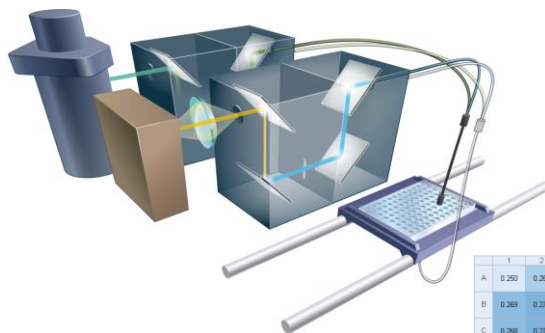


- One-click cell counting tool
- Designed for users with no image analysis experience

Think Possible

BioTek

Traditional Plate Reader - Monochromator



- Quantitative data and flexibility
- Precisely measure cellular activity

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.250	0.262	0.281	0.259	0.257	0.243	0.266	0.248	0.259	0.262	0.261	0.262
B	0.269	0.274	0.262	0.264	0.276	0.274	0.271	0.273	0.272	0.271	0.270	0.267
C	0.269	0.273	0.274	0.272	0.268	0.269	0.267	0.269	0.272	0.273	0.273	0.266
D	0.269	0.271	0.282	0.266	0.267	0.276	0.274	0.272	0.273	0.272	0.274	0.277
E	0.268	0.272	0.277	0.273	0.274	0.271	0.276	0.274	0.269	0.272	0.270	0.273
F	0.267	0.274	0.279	0.273	0.274	0.268	0.276	0.272	0.270	0.273	0.274	0.277
G	0.269	0.280	0.284	0.270	0.260	0.260	0.277	0.276	0.268	0.272	0.274	0.270
H	0.243	0.255	0.279	0.274	0.278	0.268	0.269	0.276	0.263	0.269	0.245	0.245

Think Possible



Detection mode :

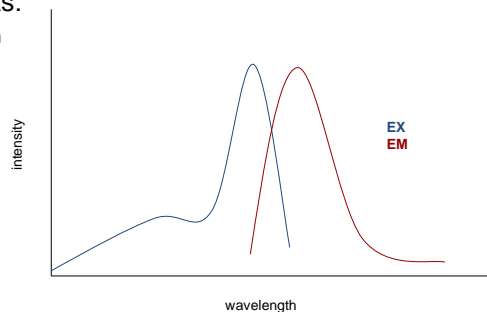
- Fluorescence Intensity
- Time-Resolved Fluorescence
- Luminescence
- UV-Vis Absorbance

Think Possible



Variable Bandwidth Monochromators: Fluorescence

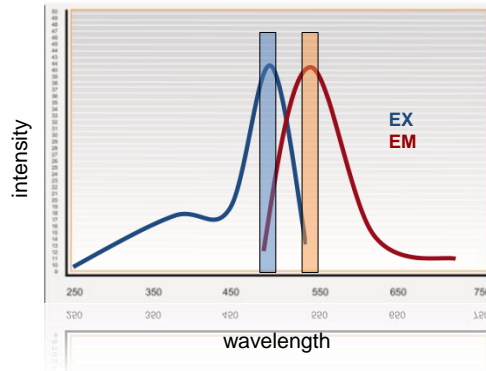
- Fluorescence wavelength selection
 - Range 250 nm to 750 nm
 - Select bandwidth from 9 nm to 50 nm, in 1 nm increments
- Selectable bandwidth benefits:
 - Allows optimization of detection conditions for fluorophores, (for example fluorophores with small Stokes shift)
 - Better performance compared to conventional monochromators



Think Possible



Variable Bandwidth Monochromators: Fluorescence



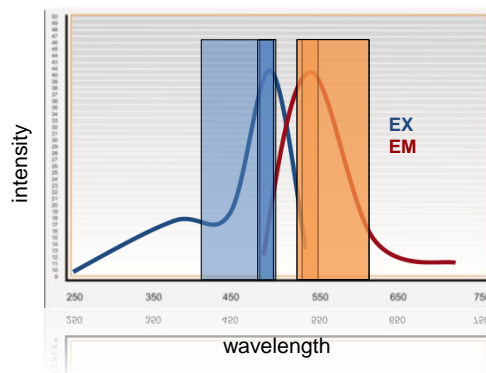
Wavelength Selection

- Range 250 nm to 750 nm
- Select bandwidth from 9 nm to 50 nm, in 1 nm increments

Think Possible



Variable Bandwidth Monochromators: Fluorescence



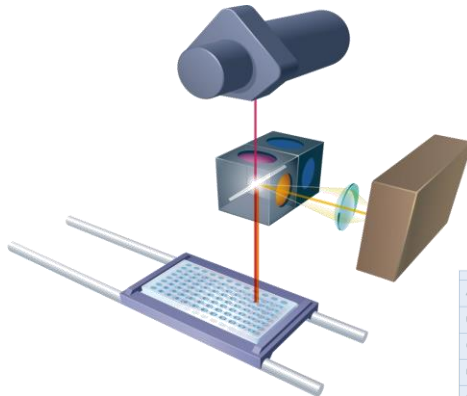
Selectable Bandwidth Benefits

- Allows optimization of detection conditions for fluorophores, e.g. narrow stokes shift fluorophores
- Better performance compared to conventional monochromators

Think Possible



Traditional Plate Reader - Filters



- Quantitative data and sensitivity
- Precisely measure cellular activity

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.250	0.262	0.281	0.258	0.257	0.243	0.266	0.248	0.259	0.262	0.261	0.262
B	0.269	0.274	0.262	0.264	0.276	0.274	0.271	0.273	0.272	0.271	0.270	0.267
C	0.268	0.273	0.274	0.272	0.268	0.269	0.267	0.269	0.272	0.273	0.273	0.265
D	0.268	0.271	0.282	0.266	0.267	0.276	0.274	0.272	0.273	0.272	0.274	0.277
E	0.268	0.272	0.277	0.273	0.274	0.271	0.276	0.274	0.268	0.272	0.270	0.273
F	0.267	0.274	0.279	0.273	0.274	0.268	0.276	0.272	0.270	0.273	0.274	0.277
G	0.268	0.280	0.284	0.270	0.260	0.260	0.277	0.276	0.268	0.272	0.274	0.270
H	0.243	0.259	0.279	0.274	0.279	0.268	0.269	0.276	0.263	0.269	0.248	0.248

Think Possible



Detection mode :

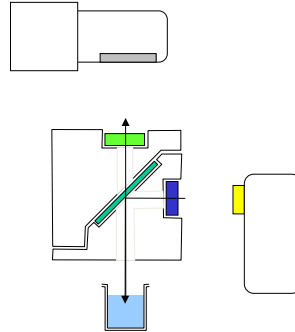
- Fluorescence Intensity
- Time-Resolved Fluorescence
- Fluorescence Polarization
- Luminescence
- Alpha Detection

Think Possible



Filter Module: Very High Optical Efficiency

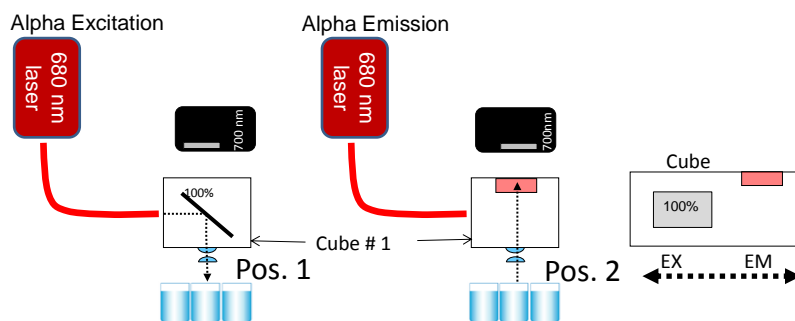
- Light source and PMT are directly coupled with a cube that contains filters and dichroic mirrors
- No-fiber system allows using a fairly small light source with very high efficiency: low-cost, high-performance system
- Filter cube arrangement is similar to system found in fluorescence microscopy: extremely easy-to-use



Think Possible



Laser-based Excitation: Alpha Assays



- Powerful 100 mW laser
- 15 year life time running one hundred 384-well plates per day
- Low noise PMT for best sensitivity, light path improvement reduces cross-talk

Think Possible



Cell Assay Optimization: CO₂/O₂ Control, Incubation and Shaking

- Gas Controller for
 - CO₂/O₂
 - CO₂ only
 - Controls and monitors
- 4-Zone™ incubation to 65 °C
 - Condensation Control™ prevents buildup on plate lids during incubated run
- Linear, orbital and double orbital shaking
 - Flexibility for all assay conditions

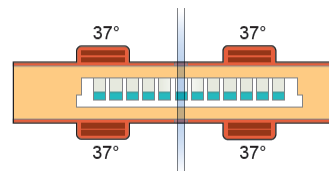


Think Possible

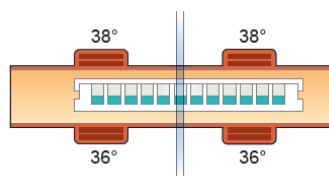


4-Zone™ Temperature Control: With Condensation Control Mode

- Proven natural convection incubator design:
 - Provides precise temperature control in the entire reading chamber
 - “Fan-free” design helps prevent edge effect common in other systems
- Condensation control mode option
 - Set the top heaters slightly higher than bottom heaters when using lidded plates
 - Even during long-term incubated kinetics, there is no condensation, so measurements aren't affected
- Excellent uniformity +/- 0.5 °C @ 37 °C



Uniform temperature set point in all four zones



Top zones set higher for condensation control

Think Possible



Advanced Shaking

- Three shake movement profiles to suit multiple applications:



Linear shaking provides strong agitation in the well, ideal for maximum mixing for non-cell based assays



Orbital shaking provides gentle yet thorough mixing, ideal for cell-based assays where cell disruption should be minimized



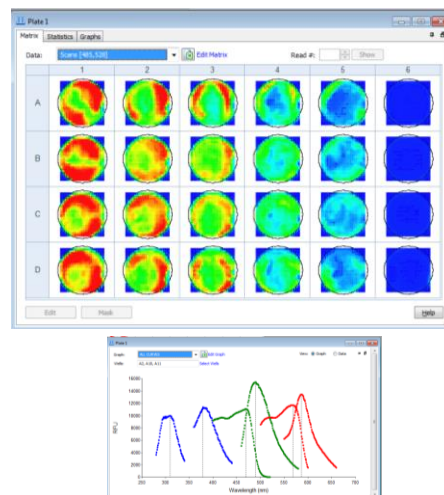
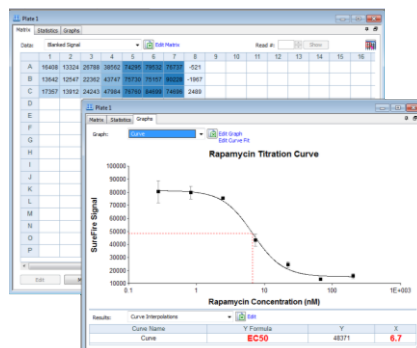
Double-orbital shaking provides strong agitation multi-directional shaking, good for mixing to prevent cell clumping

Think Possible



Gen5 Data Analysis Software: Powerful Functionality

- Results gradient
- Curves
- Area scan
- Spectral scan

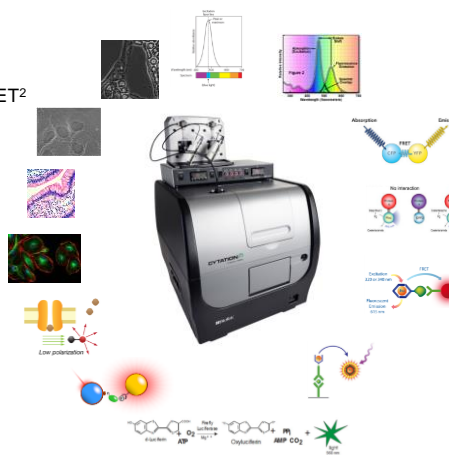


Think Possible



Cytation 5 Detection Modes

- UV/Vis Absorbance
- Fluorescence Intensity (top & bottom)
- Luminescence (glow & flash)
- Filtered Luminescence, e.g. BRET, BRET²
- Heterogeneous TRF
- Homogenous TRF (TR-FRET)
- Fluorescence Polarization
- AlphaScreen® & AlphaLISA®
- Fluorescence Imaging
- Color Brightfield Imaging (H&E)
- Brightfield Imaging
- Phase Contrast Imaging



Think Possible



Examples of Absorbance Applications

Assay	Targets
AlamarBlue®	Cell Proliferation, Viability & Counting
Amplex® Red	Enzyme Activity
phosphoELISA	Kinases
Vybrant assay C12 Rezarurin	Metabolic Labeling
Vybrant MTT Cell Proliferation Assay Kit	Cell Counting
Lipid Peroxidation Assay Kit (using MDA)	Oxidative Stress
CellTiter 96® Cell Proliferation Assay (MTT)	Cell Proliferation
DC Protein, Bradford, Lowry & BCA Protein Assays	Protein Quantification
OxiSelect™ Superoxide Dismutase Activity Assay	Oxidative Stress
Glycoprotein Carbohydrate Estimation Kit	Glycoprotein Quantification
IDEXX FlockChek™ Avian Influenza Multi-species ELISA Assay	Avian Influenza
Melamine ELISA Assay	Food Safety
CaspACE™ Assay	Apoptosis
Growth Assay (OD at 600nm)	Cell Growth
Haemoglobin denaturation Assay	Haemoglobin

Think Possible



Examples of Fluorescence Intensity Applications

Assay	Targets
Adapta® Universal Kinase Assay	Kinases
AlamarBlue®	Cell Proliferation, Viability & Counting
Amplex® Red	Enzyme Activity
EnzCheck	Proteases
Fluo-4 AM	GPCR
LIVE/DEAD® Viability/Cytotoxicity Kit	Apoptosis
PIPer™ Phosphate Assay Kit	Phosphatases
SYTOX® stains	Viability
Vivid® CYP450	Cytochrome P450
Quant-iT™ PicoGreen® dsDNA Assay Kit	Nucleic Acid Quantification
Transcreener® GDP FI Assay	GTPase
FluxOR™ Potassium Ion Channel Assay Kits	Ion Channels
OxiSelect™ ROS Assay Kit	Oxidative Stress
CellTiter-Fluor™ Cell Viability Assay	Cell Viability
FluoroBlok Cell Invasion Assays (using Calcein AM)	Cell Migration

Think Possible



Examples of Luminescence Applications

Assay	Targets
CellTiter-Glo® Luminescent Cell	Cell Proliferation
Dual-Luciferase® Reporter (DLR™) Assay	Gene Expression
P450-Glo™ Assay	CYP Activities
ADP-Glo™ Assay	Kinase
Caspase-Glo® 3/7 Assay	Apoptosis
cAMP-Glo™ Assay	GPCR
GloSensor™ cAMP Assay	GPCR
Aequorin	GPCR
ATPlite™ Luminescence Assay	Cell Proliferation/Kinases
aCella -TOX™ Bioluminescence Cytotoxicity Assay	Cell Death
ToxiLight® Non-destructive Cytotoxicity BioAssay Kit	Cell Death/Cell Proliferation
ATP quantification assay (luciferine-luciferase)	ATP Quantification
Britelite™ plus Reporter Gene Assay	Gene Expression
Steady-lite plus™ Reporter Gene Assay	Gene Expression
Neolite Reporter Gene Assay	Gene Expression

Think Possible



Spectrum of Targets Covered by Time-Resolved Fluorescence & AlphaScreen

	AlphaScreen	TRF	
		LANCE/HTRF	DELFI
GPCR Functional Assays: cAMP IP ₃ GTP Binding	✓ ✓ ✓		✓ ✓
Receptor Binding Assays: GPCRs Nuclear	✓ ✓	✓ ✓	✓ ✓
Enzyme Assays: Tyrosine Kinase Serine/Threonine Kinase Phosphatase Protease Transferase Polymerase Phospholipase Helicase Phosphodiesterase	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓
Protein Interactions: Protein:Protein Protein:Peptide Protein:DNA	✓ ✓ ✓	✓ ✓ ✓	✓ ✓ ✓
Other Functional: Cell Proliferation/Viability			✓

Think Possible



AlphaLISA Immunoassay Targets

Angiogenesis	Biologics & Bioprocess	Cancer	Cardiovascular
<ul style="list-style-type: none"> EPO TNFr TNFr (mouse) VEGF VEGFA (mouse) VEGFB VEGFC VEGFD 	<ul style="list-style-type: none"> CHO-P E. coli HCP IgA IgE IgG IgM ISO-P Residual Protein A Albumin 	<ul style="list-style-type: none"> AFP CA125 Caspase-3 (active) CXCL11/I-TAC EGF-R EPO ERBB2 / HER2 HGF/Sc-MET IFN-β MMP1 MMP2 MMP3 MMP9 MMP9 (mouse) PSA TFF3 TMPI TNFr β-NGF 	<ul style="list-style-type: none"> Alpha-2 macroglobulin Cardiac Troponin 1 D-dimer EPO ICAM-1 Myeloperoxidase Myoglobin NT-proBNP PAI-1 PCSK9 Plasminogen Renin/Prorenin sFPA
Inflammation		Metabolic	Central Nervous System
<ul style="list-style-type: none"> CCL2 / MCP1 CCL2/MCP-1 (mouse/rat) CCL3/MIP-1α CCL4/MIP-1β CCL5 / RANTES CCL5 / RANTES (mouse) COMP CRP CXCL1/GRO-α CXCL10/IP-10 CXCL9/MIG G-CSF GM-CSF GM-CSF (mouse) IFN-α IFN-γ IFN-γ (mouse) IL10 IL10 (mouse) IL11 IL12 (p70) IL13 IL15 IL15 (mouse) IL17 IL17A CXCL9/MIG (mouse/rat) IL18 IL1α IL1β IL1β (mouse) IL2 IL2 (mouse) IL3 IL4 IL5 IL6 (mouse) IL6 IL7 IL7 (mouse) IL8 IL1β (rat) TNFr TNFr (mouse) 		<ul style="list-style-type: none"> Adiponectin Adiponectin (mouse) Albumin Albumin (mouse) C-peptide (mouse/rat) GH GLP-1 IGF1 IGF2 Insulin Leptin Prolactin 	<ul style="list-style-type: none"> Amyloid β 1-15 /16 Amyloid β 1-40 Amyloid β 1-40 (High Specificity) Amyloid β 1-40 (mouse/rat) Amyloid β 1-42 Amyloid β 1-42 (High Specificity) Amyloid β 1-42 (mouse/rat) Amyloid β 1-x Amyloid β oligomers sAPPα (C-term specific) sAPPβ sAPPβ (High Sensitivity) Tau
			Virology
			<ul style="list-style-type: none"> HIV p24 HIV p24 (High Sensitivity)

Think Possible



Examples of Fluorescence Polarization Applications

Assay	Targets
PolarScreen™	Kinases
PolarScreen™ Competitive Binding Assays	Nuclear Receptors
Transcreener® AMP/GMP Assay	PDE's
Transcreener® GDP FP Assay	GTPase
Transcreener® UDP Assay	UDP-glycosyltransferases
Transcreener® ADP2 FP Assay	Kinases
[FP] ²	cAMP

Think Possible



Improve Sensitivity with Imaging: Plate Reader vs. Microscope

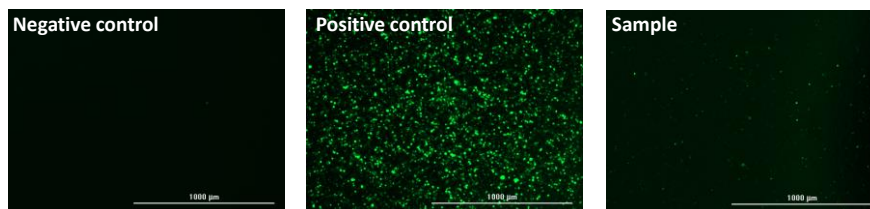
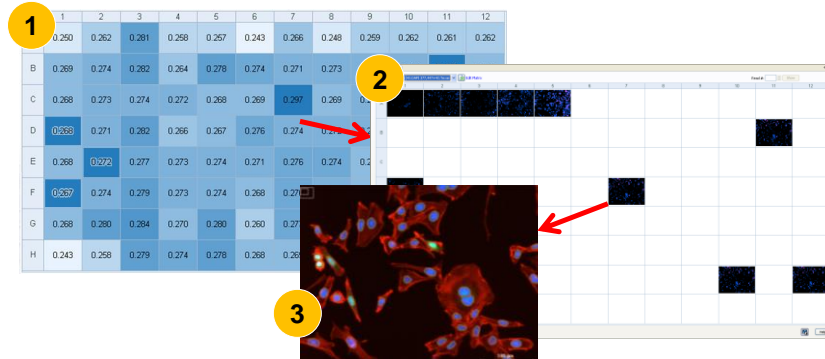


	Plate Reader PMT (RFU)		Microscope CCD Camera (Cell count)	
	Well value	S/B	Well value	S/B
Negative control	19556		1	
Positive control	45029	2.3	2668	2668
Sample	24118	1.2	104	104

Think Possible



Cytation 5 Unique Feature: Hit Picking

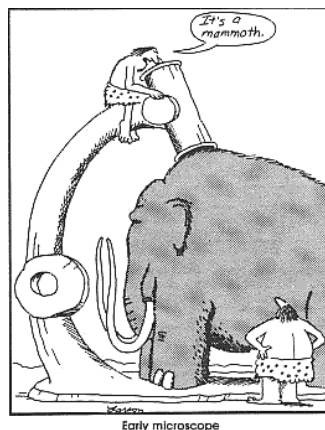


1. Plate is pre-screened with plate reader optics (<1 minute)
2. Only hit wells are imaged
3. Significantly faster, with fewer images to process and store
 - (one 96-well plate with one image per well = 200 MB)

Think Possible



Applications



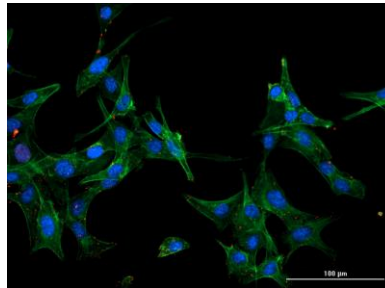
Early microscope

Think Possible



HTRF™ IL-6 and Nuclear-ID™ Cell Viability Assays

Assessment of IL-6 Secretion and Cell Viability using Microplate Reader and Cell-Based Imaging Technologies

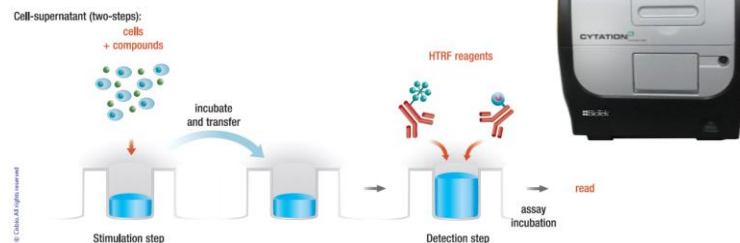


Think Possible



Multiplexed Assay for IL-6 secretion and Cell Viability

- Endothelial Ovarian Cancer Cell Line: SKOV-3
- IL-6 secretion induced by EGFR activation of NFκB pathway
- IL-6 quantified by 2-step HTRF assay through plate transfer
 - Top read, spectral filters / dichroic
- LIVE/DEAD assay performed on cells post-transfer step
 - Bottom read, digital fluorescence microscopy

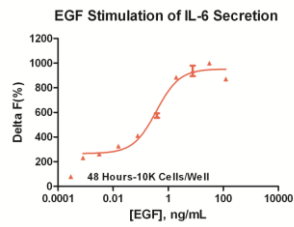


Think Possible



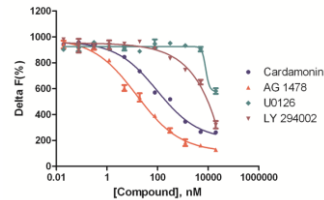
IL-6 secretion – 48 hr incubation

Activation

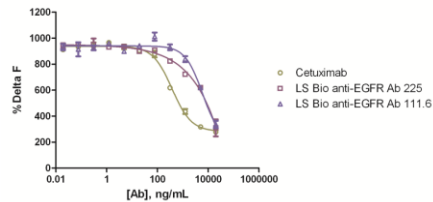


Inhibition

Small Molecule Inhibitors



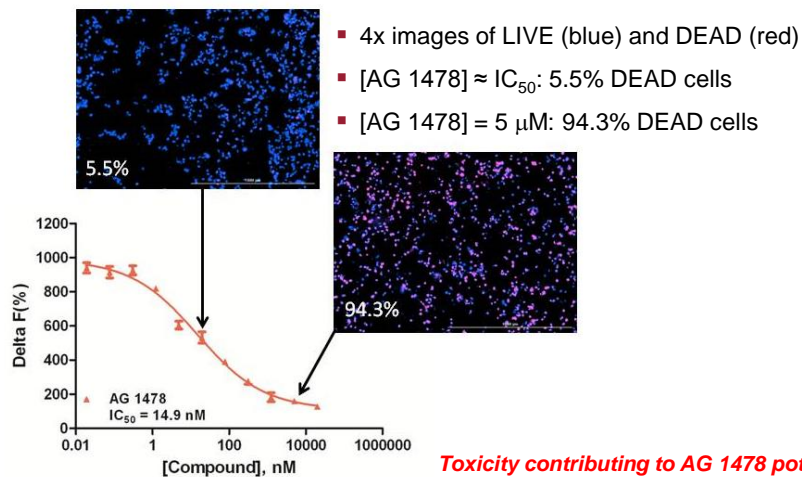
Blocking Antibody Inhibitors



Think Possible



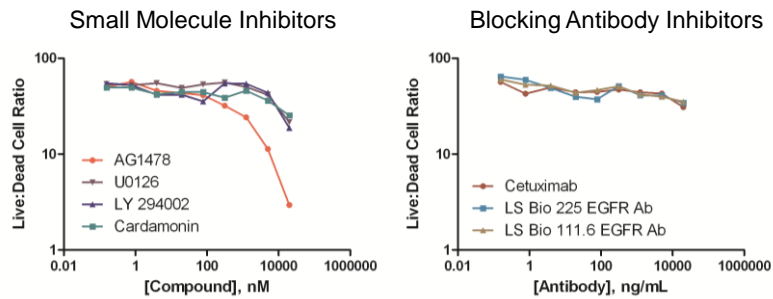
Toxicity Assessment of AG 1478 using LIVE/DEAD Probes



Think Possible



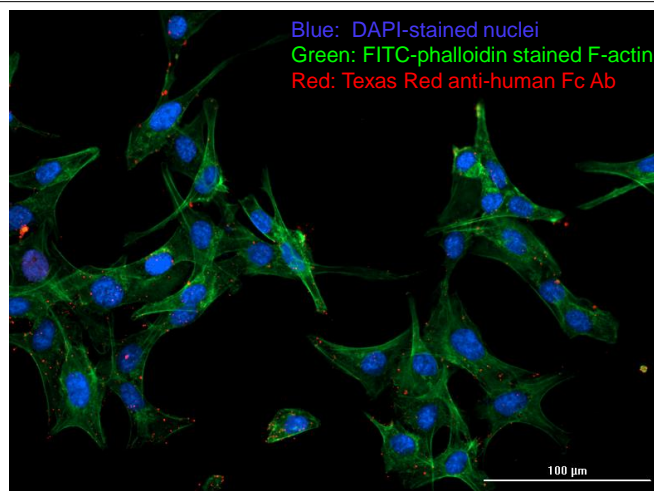
Toxicity Assessment of All Inhibitors using LIVE/DEAD Probes



Think Possible



Validation of Cetuximab Binding by 20x Imaging

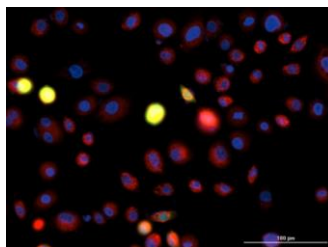


Think Possible



Hypoxia/Oxidative Stress and CellTiter-Glo® Cell Viability Assays

Phenotypic Assessment of Hypoxia and Oxidative Stress plus Bioluminescent Cell Viability Determination



Think Possible



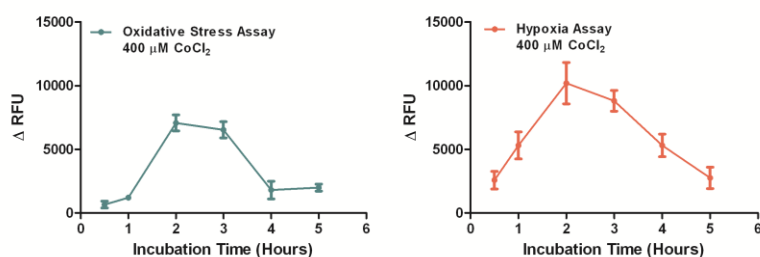
Phenotypic Assay for Hypoxia / Oxidative Stress

- Immortalized Keratinocytes as cell model
- Individual fluorogenic cell permeable probes for oxidative stress and hypoxia
 - Oxidative stress a measure of ROS production
 - Hypoxia a measure of nitroreductase activity
- Oxidative stress / Hypoxia chemically induced by CoCl_2
 - Whole well fluorescence intensity measurements used for assay optimization and hypoxia inhibitor screening
 - Digital fluorescence microscopy for assay & hit validation
- Bioluminescent reagent for cell viability
 - Whole well luminescence measurements used to confirm true inhibition of antioxidant compounds
 - Reagent added following microplate detection and imaging using hypoxia and oxidative stress probes

Think Possible



Incubation Time Studies – 400 μM CoCl_2

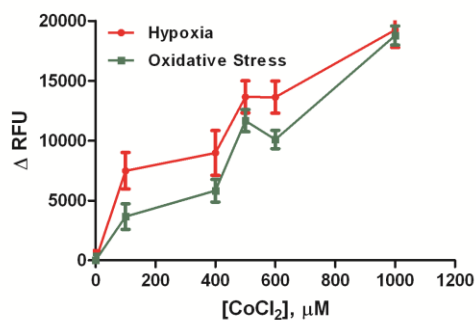


- CoCl_2 incubation time optimal at 2 hours
 - Oxidative stress (green) measured with spectral filters / dichroic
 - Hypoxia (red) with monochromators

Think Possible



CoCl_2 Dose Response – Whole Well Intensities

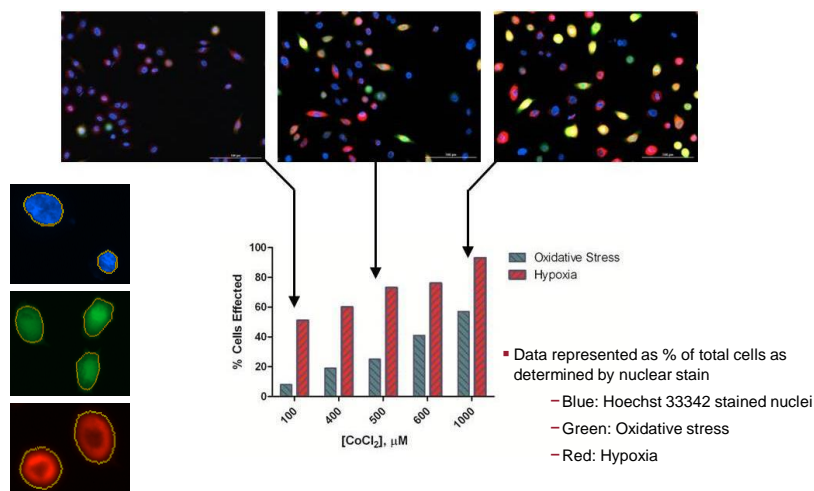


- Rapid measurement of oxidative stress and hypoxia
 - Oxidative stress (green) measured with spectral filters / dichroic
 - Hypoxia (red) with monochromators

Think Possible



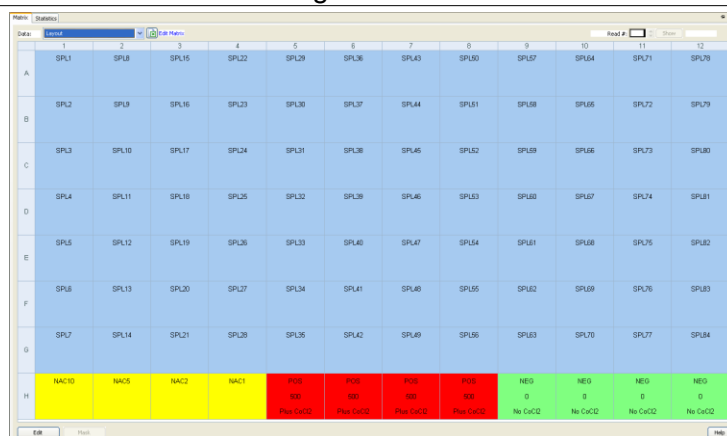
CoCl₂ Dose Response – 20x Imaging plus Cellular Analysis



Think Possible



Integration of Gen5 Hit Picking Protocol



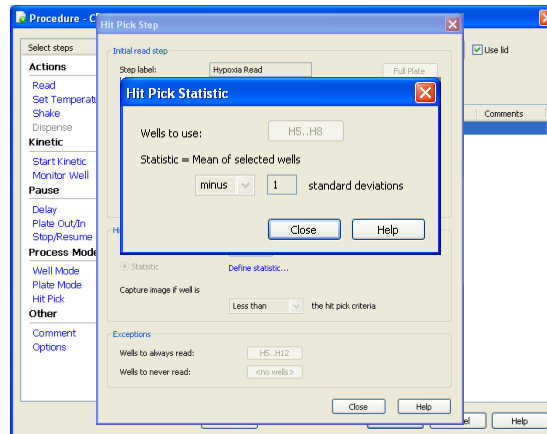
	1	2	3	4	5	6	7	8	9	10	11	12
A	SPL1	SPL8	SPL15	SPL22	SPL29	SPL36	SPL43	SPL50	SPL57	SPL64	SPL71	SPL78
B	SPL2	SPL9	SPL16	SPL23	SPL30	SPL37	SPL44	SPL51	SPL58	SPL65	SPL72	SPL79
C	SPL3	SPL10	SPL17	SPL24	SPL31	SPL38	SPL45	SPL52	SPL59	SPL66	SPL73	SPL80
D	SPL4	SPL11	SPL18	SPL25	SPL32	SPL39	SPL46	SPL53	SPL60	SPL67	SPL74	SPL81
E	SPL5	SPL12	SPL19	SPL26	SPL33	SPL40	SPL47	SPL54	SPL61	SPL68	SPL75	SPL82
F	SPL6	SPL13	SPL20	SPL27	SPL34	SPL41	SPL48	SPL55	SPL62	SPL69	SPL76	SPL83
G	SPL7	SPL14	SPL21	SPL28	SPL35	SPL42	SPL49	SPL56	SPL63	SPL70	SPL77	SPL84
H	NAC10	NAC5	NAC2	NAC1	DMSO	DMSO	DMSO	DMSO	NE10	NE10	NE10	NE10
					No CoCl ₂	No CoCl ₂	No CoCl ₂	No CoCl ₂	No CoCl ₂	No CoCl ₂	No CoCl ₂	No CoCl ₂

- Library and control compounds, uninhibited CoCl₂, and negative controls wells identified in Plate Layout

Think Possible



Hit Pick Criteria

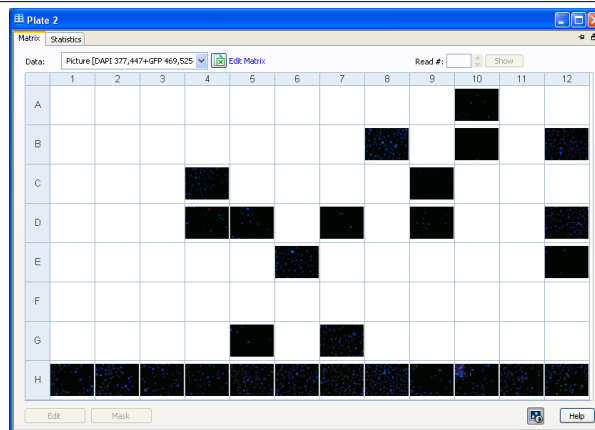


- Hit pick Gen5 feature allows for only wells which meet identified criteria to be immediately imaged

Think Possible



Hit Pick Imaging

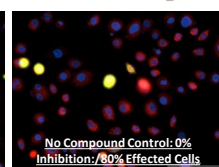
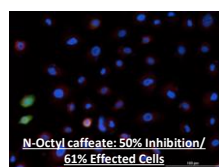
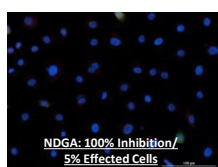
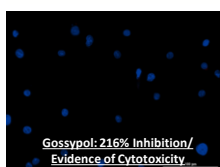
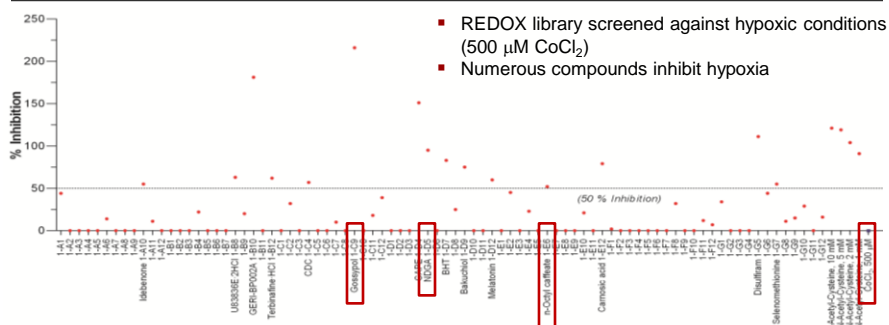


- 20x imaging of hit wells plus identified control wells

Think Possible



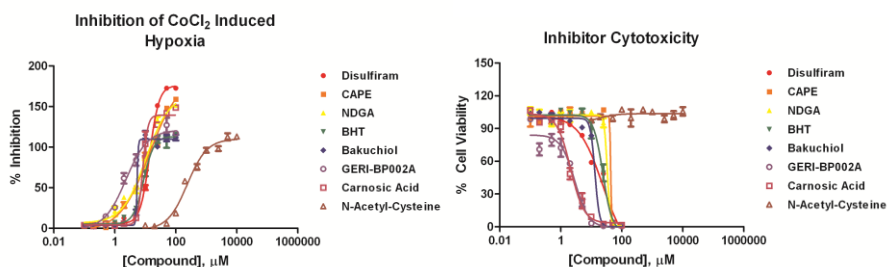
Screen for Hypoxia Inhibition (Monochromators)



Think Possible



Inhibition Studies (Monochromators and Luminescence)



- Full dose-response for select compounds from REDOX library
 - Numerous compounds demonstrate excessive inhibition beyond controls
- Cell viability assessed with CellTiter-Glo demonstrates compound toxicity at higher doses

Think Possible



BacMam Transfection

Assessment of Transient Histone H3-GFP Gene Delivery using Cellular Imaging and Microplate Reader Technologies

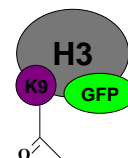
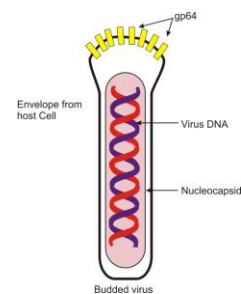


Think Possible



BacMam Transfection Optimization Procedure

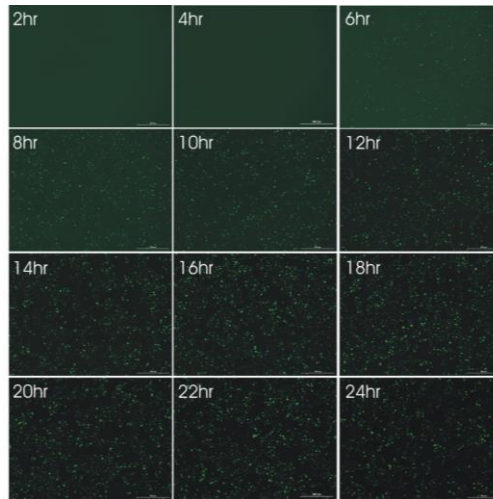
- U-2 OS (human osteosarcoma) cell model
- Viral concentration and incubation time optimization performed
 - Multiple concentrations of Histone H3 virus transfected into cells, ranging from 0-50%
 - Kinetic reads carried out using Cytation3 over 24 hours using stable 37 °C/5% CO₂ atmosphere
- Cell-permeable Hoechst 33342 nuclear stain added following BacMam incubation to identify complete cell population
- **4x and 20x fluorescence microscopy images** captured to assess appropriate viral incubation time, transfection efficiency, and cytotoxicity
- Top read, whole well **fluorescence measurements** made for validation purposes



Think Possible



BacMam Transfection Time Course Study

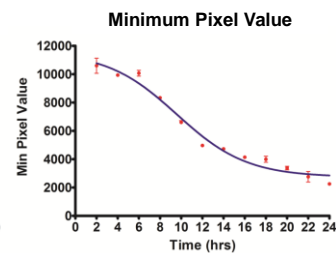
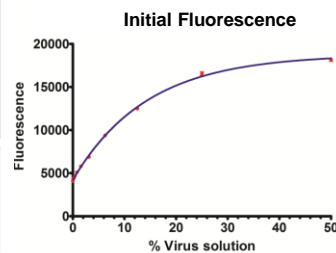
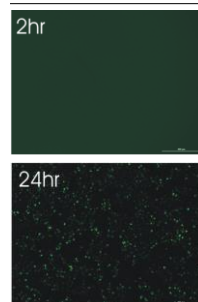


- U-2 OS cells infected with 7.5% virus concentration
- 4x images taken every 2 hours
- Cellular expression of GFP fusion protein visible via fluorescence as soon as 6 hours
- Maximum expression reached between 16 and 20 hours

Think Possible



Assessment of BacMam Viral System Fluorescence

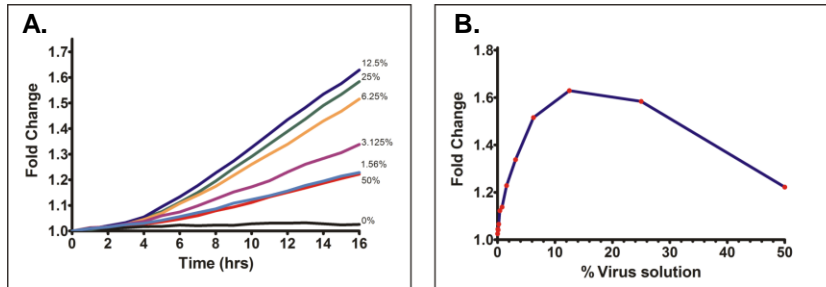


- High green fluorescent background signal seen at 2 hour time point
- Time zero whole well **fluorescent reads** confirm signal linked to viral introduction
- Background fluorescence drops over time, as seen from 4x images and subsequent image analysis
 - Indicative of viral uptake into cells and nuclear localization

Think Possible



Transfection Optimization- Top Read

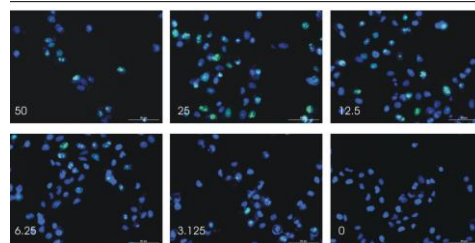


- Fold change generated by comparing top fluorescence reads of GFP signal
- Optimal incubation time seen to be 16 hours with all viral concentrations tested
- 12.5% concentration seen to give largest fold change
 - Higher viral concentrations may be toxic to cell model

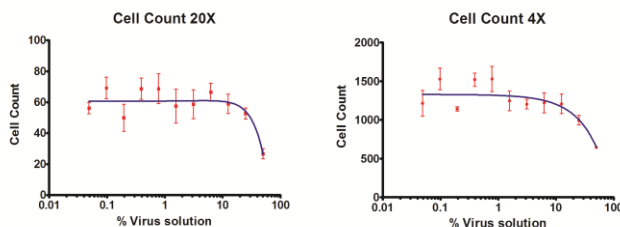
Think Possible



Optimal Transfection Efficiency Determination- Imaging



- Imaging plus automated cell count feature used to quantify number of cells transfected (green) as well as total cell number (blue)
- Graph of cell number ratio also confirms optimal viral concentration to be 12.5%



- Cytotoxicity at high viral concentrations also confirmed from total cell count

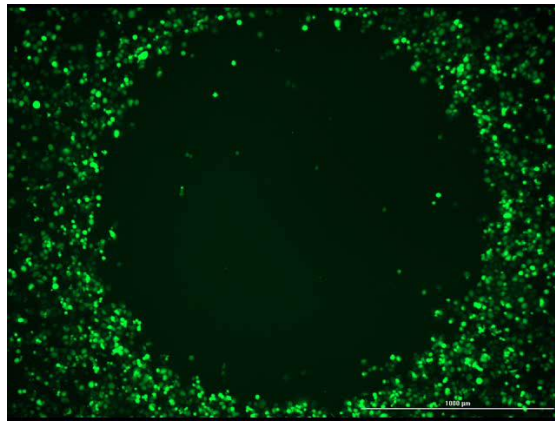
Think Possible

BioTek®

PLATYPUS
TECHNOLOGIES

Cell Migration

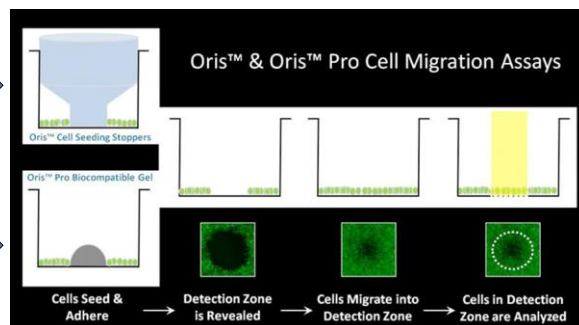
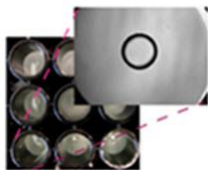
Exploring Cell Movement



Think Possible

BioTek®

Platypus Oris™ & Oris™ Pro Cell Migration Assay

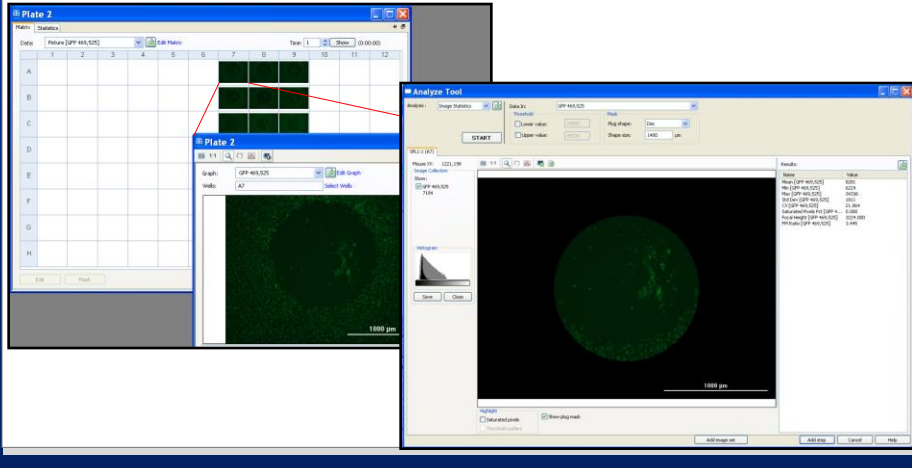


Think Possible



Gen5 Data Analysis: 2 Ways to Set up Statistical Analysis (Cont.)

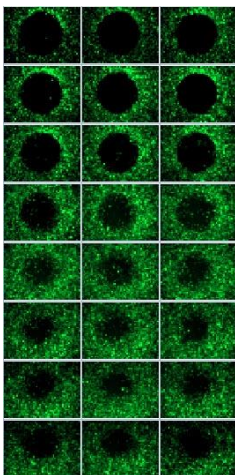
Use of **analyze tool** from picture view



Think Possible



Statistical Analysis of MDA-MB-231 Cells: Standard Deviation

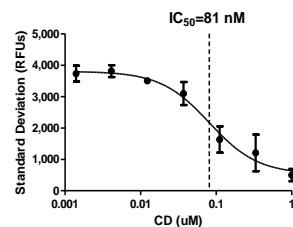


569	281	649
1122	1827	672
1156	1797	1944
2732	3463	3097
3426	3476	3610
3611	3984	3845
3651	3631	4015
3386	3721	3343

Std Dev [GFP 469,525]		
[CD] (uM)	mean	stdev
1.000	499.7	193.5
0.333	1207.0	582.2
0.111	1632.3	419.0
0.037	3097.3	365.5
0.012	3504.0	95.1
0.004	3813.3	188.5
0.001	3732.3	252.0
0.000	3483.3	206.9

~7-fold assay window

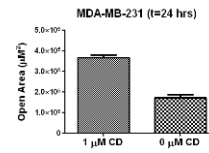
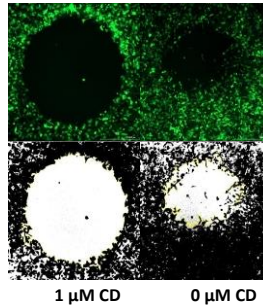
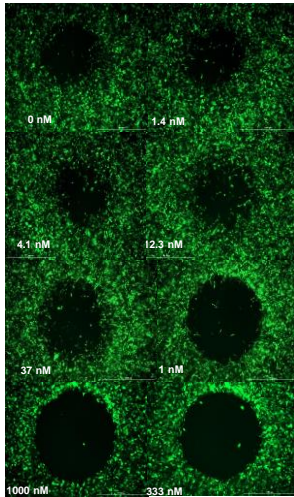
Standard Deviation Analysis of MDA MB 231 GFP cells



Think Possible



ImageJ Analysis

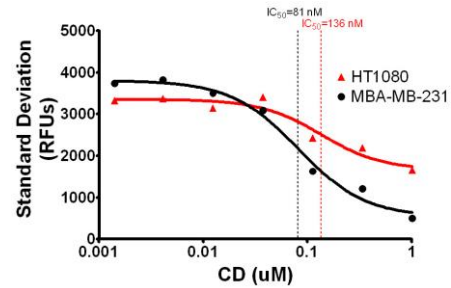
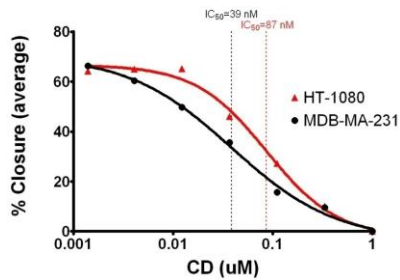


~3-fold assay window

Think Possible



Comparative Analysis

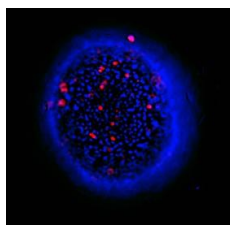


Think Possible



3D Cell-Based Imaging

*Analysis of Single-plane and Z-stacked
Tumorous and Spheroid Images*



Think Possible



3D Cell Culture...

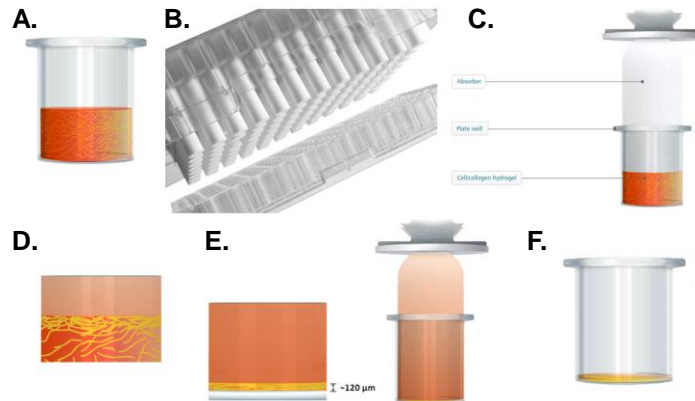
3D Cell Culture System	Vendor	Scaffold Material	Degradable	Format
Micro-tissues	InSphero AG	No	N/A	Microtissues in 96-well microplates
Spheroids	3-D Biomatrix	No	N/A	Hanging drop microplates, 96-and 384-well densities
AlgiMatrix	Life Technologies	Gelatin, PEG	Yes	Scalable
BD Matrigel	BD Biosciences	Proprietary mixture	Yes	Scalable
RAFT	TAP Biosystems	Collagen	Yes	96-well microplates
Alvetex	Reinnervate	Polystyrene	No	6- to 96-well microplates
MICA 3-D	CellASIC	No	N/A	Microfluidic plate

...driving for greater biological relevance in drug discovery

Think Possible

 BioTek®

RAFT 3D Cell Culture Creation Procedure

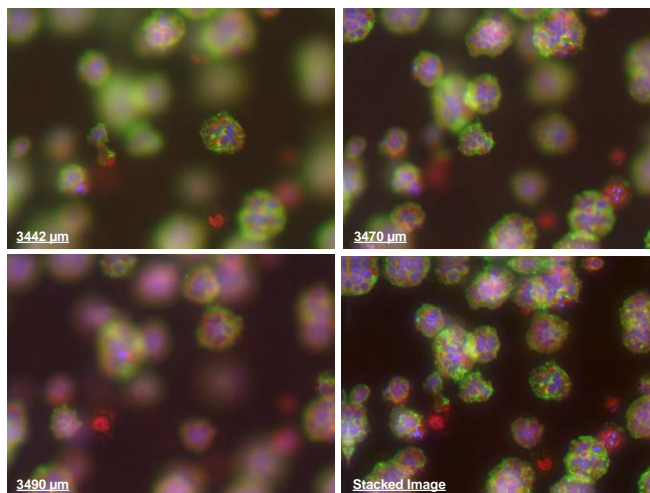


The RAFT 3D Cell Culture System. **(A)** Cell/collagen mix within a microplate well. **(B)** Specialized RAFT plate showing sterile absorbers. **(C)** Absorbers remove medium to concentrate the cell/collagen mix. **(D)** Concentrated cell/collagen mix. **(E)** Cell/collagen layer showing absorbed medium. **(F)** Final cell/collagen layer in the microplate well prior to dispensing fresh cell medium.

Think Possible

 BioTek®

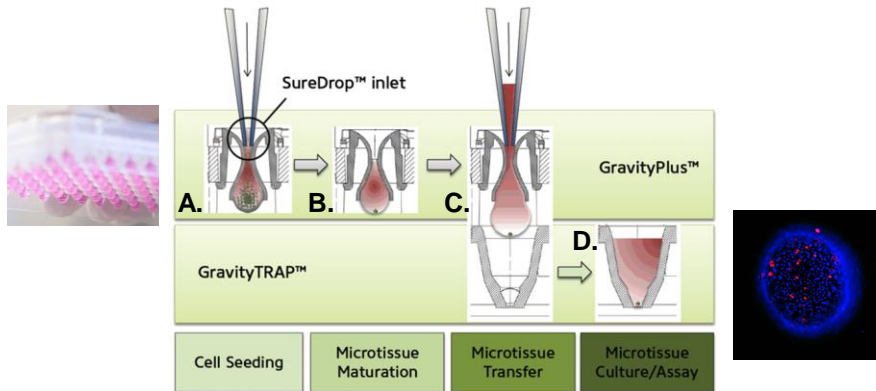
Tumoroid Image

**Objective:** 20x**Cell Stains:****Blue** – DAPI**Green** – Alexa Fluor 488
Phalloidin**Red** – CellMask Orange
Plasma Membrane Stain

Think Possible



InSphero 3D Spheroid Microtissue Creation Procedure

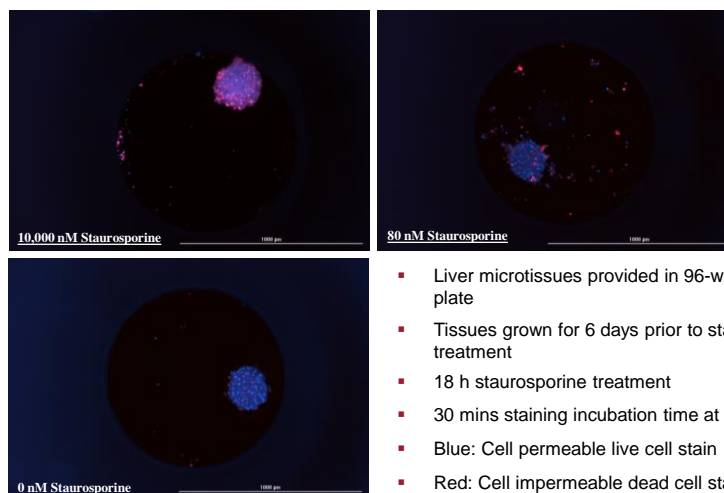


InSphero Spheroid Creation and Assay Process. **(A)** Load up to 50 μ L of cell suspension into a well of the GravityPlus plate. **(B)** Allow the microtissue spheroid to form (usually within 2-4 days). **(C)** Harvest the microtissue by adding additional media to the well, forcing the tissue to fall into the GravityTRAP plate. **(D)** Continue to culture microtissue or perform assay as usual.

Think Possible



Live/Dead Cell Analysis using Nuclear-ID Assay

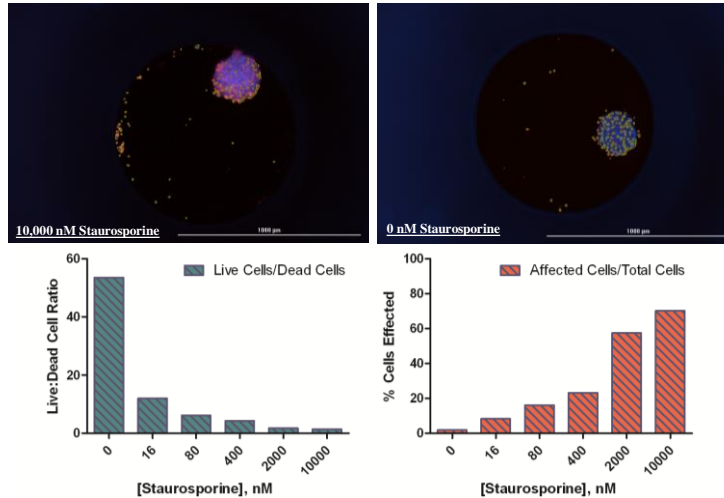


- Liver microtissues provided in 96-well GravityTRAP plate
- Tissues grown for 6 days prior to staurosporine treatment
- 18 h staurosporine treatment
- 30 mins staining incubation time at 37°C
- Blue: Cell permeable live cell stain
- Red: Cell impermeable dead cell stain

Think Possible



Live/Dead Cell Analysis using Nuclear-ID Assay



Think Possible



Some of our many users...



Dr. Tara Haas, York University, School of Kinesiology & Health Science

Dr. Hassan Zaidi, Ontario Institute for Cancer Research, Cancer Genomics Group

Dr. Kirk McManus, University of Manitoba, Manitoba Institute of Cell Biology

Dr. Donald Weaver, Toronto Western Research Institute, Toronto Western Hospital

Dr. Éric Déziel, Centre INRS – Institut Armand Frappier

Dr. Getulio Nogueira, University of Toronto, Department of Dentistry

Dr. Balakrishnan Prithiviraj, Dalhousie University, Department of Environmental Sciences

Dr. Marnie Taylor, Health Canada, Banting Research Centre

Dr. Raymond Thomas, Memorial University of Newfoundland, Department of Biochemistry

Dr. Patrick Gunning, University of Toronto, Department of Chemical & Physical Sciences

Dr. Maxim Berezovski, University of Ottawa, Department of Chemistry

Dr. Alex Wong, Carleton University, Department of Biology

Dr. Cory Harris, University of Ottawa, Department of Biology

Dr. Luc Berthiaume, University of Alberta, Department of Cell Biology

Dr. Ryan Pelis, Dalhousie University, Department of Pharmacology

Dr. Craig Moore, Memorial University of Newfoundland, Department of Neuroscience

Dr. Jean Legault, Université du Québec à Chicoutimi, Department of Basic Sciences

Dr. Géraldine Delbès, INRS-Institut Armand-Frappier, Department of Reproductive Biology

Dr. Jan-Eric Ahlfors, New World Laboratories

Dr. Jean-Yves Masson, Université Laval, Oncology

Dr. Turlough M. Finan, McMaster University, Department of Biology

Dr. Alessandro Datti, Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, HTS Core Facility

Memorial
University of Newfoundland

Inspiring Innovation and Discovery

Lunenfeld-Tanenbaum
Research Institute

MOUNT SINAI HOSPITAL

Just and Well. Making Health Count.

Think Possible

 BioTek®

Cytation 5

Do What You Never Thought Possible!

