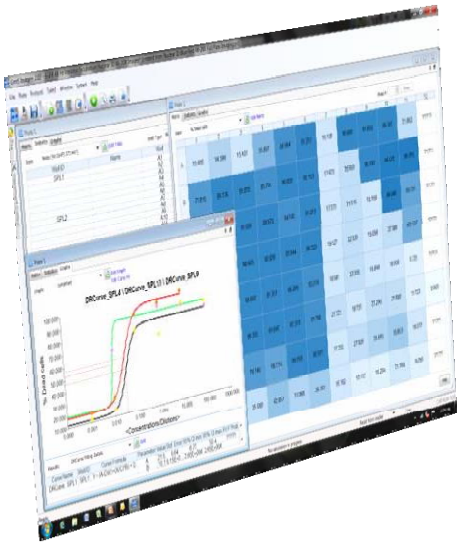
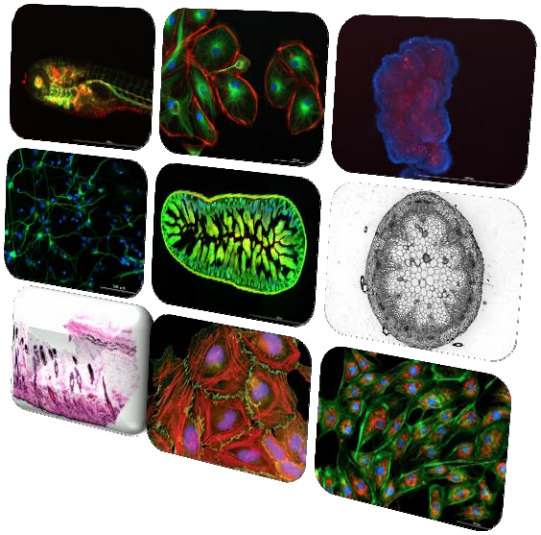
Think Possible

BioTek

The Multi-faceted Assays Enabled with Cytation 5 Technology

An Advanced Tool for Smarter Target-Based, Phenotypic Research & Screening





試機時間 11 月 2 日~11 月 13 日

臺中榮民總醫院 5F 醫學研究部精密儀器中心

Think Possible

BioTek®

Target vs Phenotypic Research & Development

Target-based Research

- Initial Target ID and Validation studies are critical for efficacy
- Assay development for screening focuses on drug interaction with target
- Screening hundreds of thousands of compounds is relatively easy

Phenotypic Research

- Use a phenotype model for the disease – no specific drug target
- Assay development for screening focuses on phenotype suppression
- Screening hundreds of thousands of compounds is relatively difficult

Example: Angiogenesis in Cancer: Tumor growth requires blood vessel development (angiogenesis).

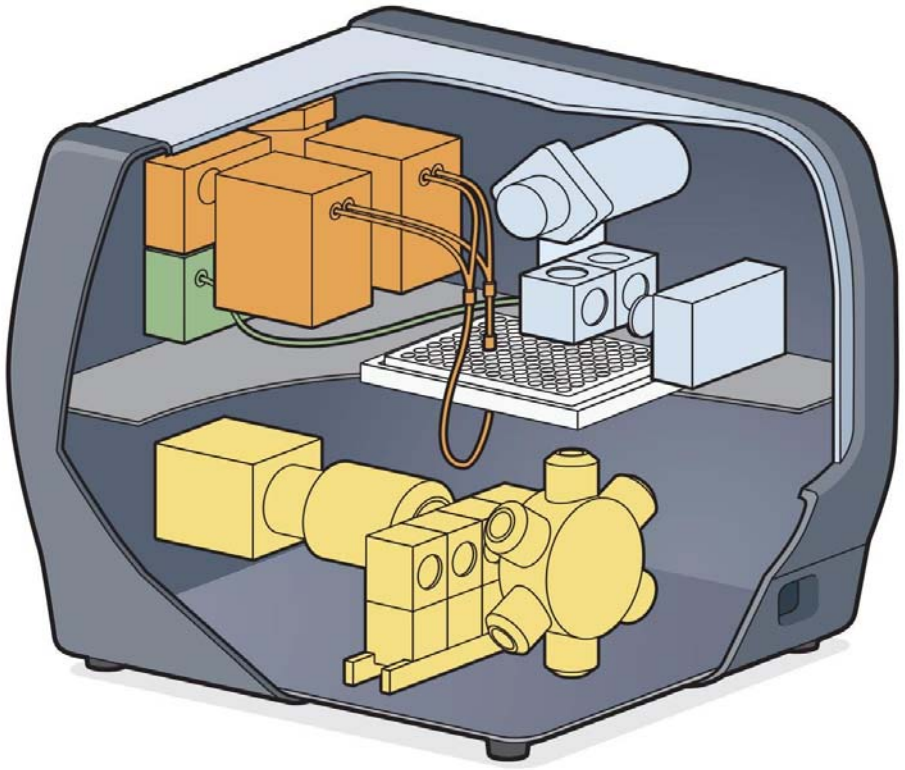
- Establish that VEGF binding to VEGFR induces blood vessel growth
- Develop assay that monitors binding to VEGF receptors
- Develop assay that monitors blood vessel growth

… need/desire to perform both types of screens to generate accurate in-vivo-like results

Think Possible

BioTek®

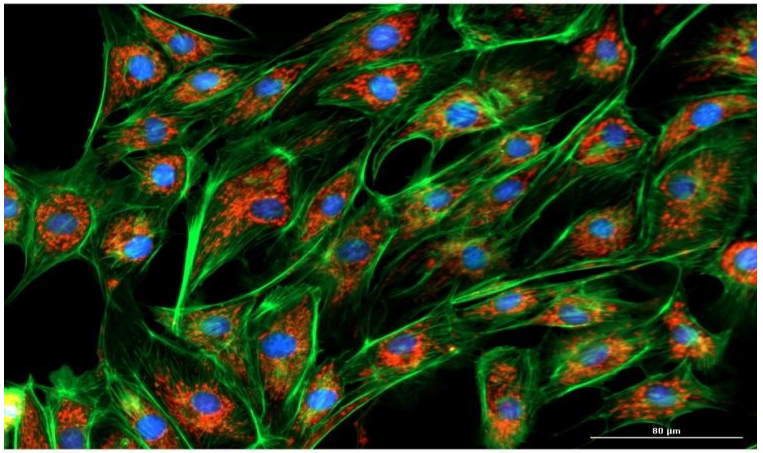
Microplate Reader with Imaging



- Fuller picture for cell biology research

	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	0.282	0.264	0.278	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	0.297	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	0.280	0.260	0.277	0.276	0.268	0.272	0.274	0.270
H	0.243	0.258	0.279	0.274	0.278	0.268	0.269	0.276	0.263	0.268	0.248	0.248

- Quantitative univariate data

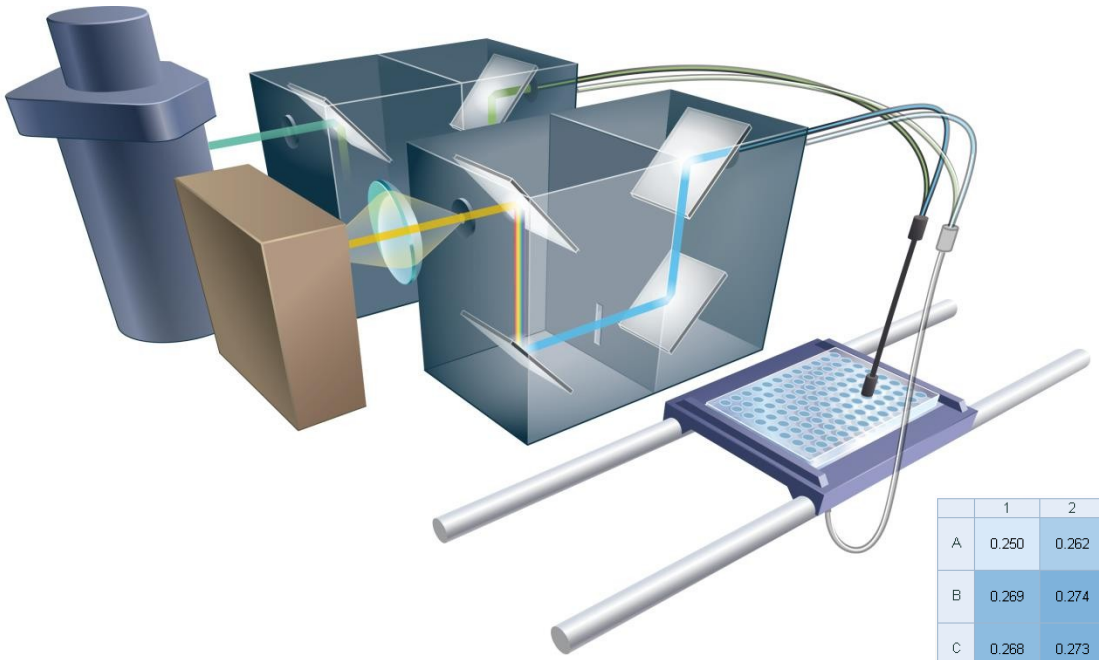


- Multivariate data

Think Possible

BioTek®

Traditional Plate Reader - Monochromator



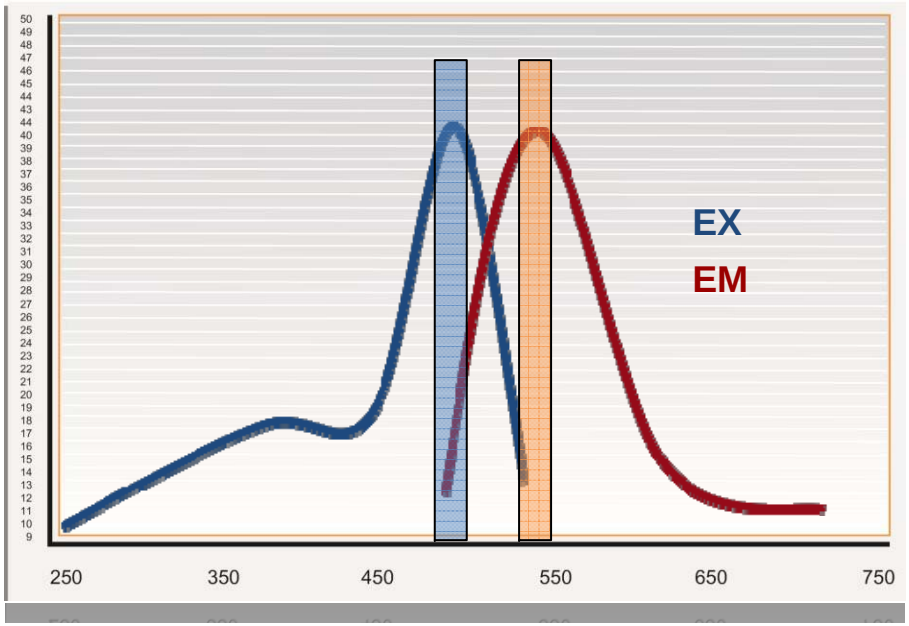
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.250	0.262	0.281	0.258	0.257	0.243	0.266	0.248	0.259	0.262	0.261	0.262
B	0.269	0.274	0.282	0.264	0.278	0.274	0.271	0.273	0.272	0.271	0.270	0.267
C	0.268	0.273	0.274	0.272	0.268	0.269	0.297	0.269	0.272	0.273	0.273	0.265
D	0.268	0.271	0.282	0.266	0.267	0.276	0.274	0.272	0.273	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	0.280	0.260	0.277	0.276	0.268	0.272	0.274	0.270
H	0.243	0.258	0.279	0.274	0.278	0.268	0.269	0.276	0.263	0.268	0.248	0.248

- Quantitative data and flexibility
- Precisely measure cellular activity

Think Possible

BioTek®

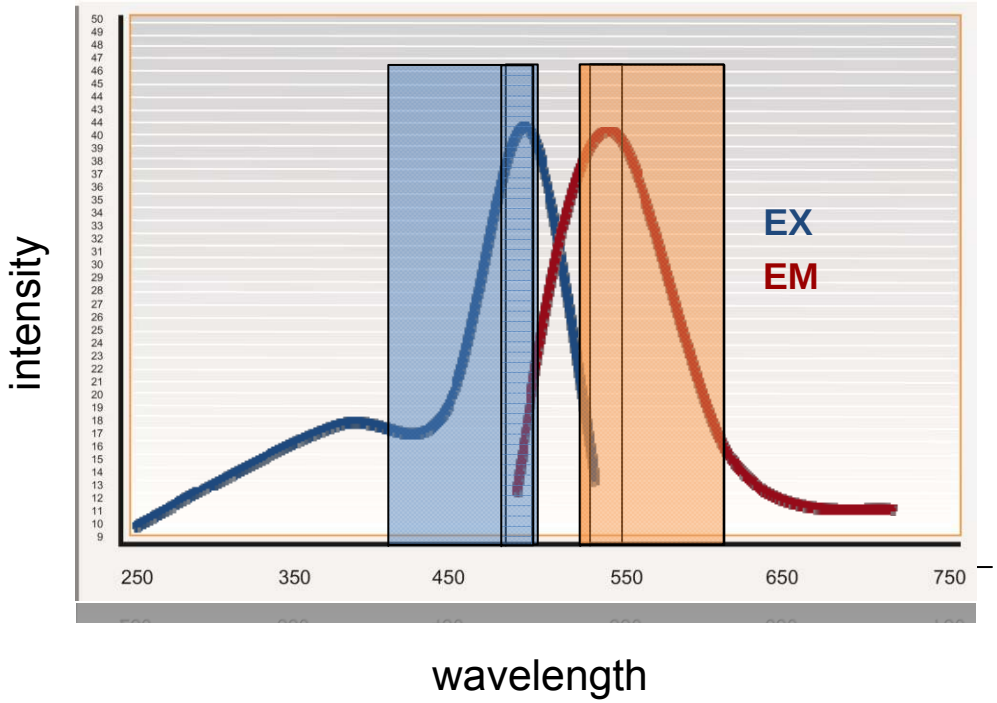
Variable Bandwidth Monochromators: Fluorescence



Wavelength Selection

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

Variable Bandwidth Monochromators: Fluorescence



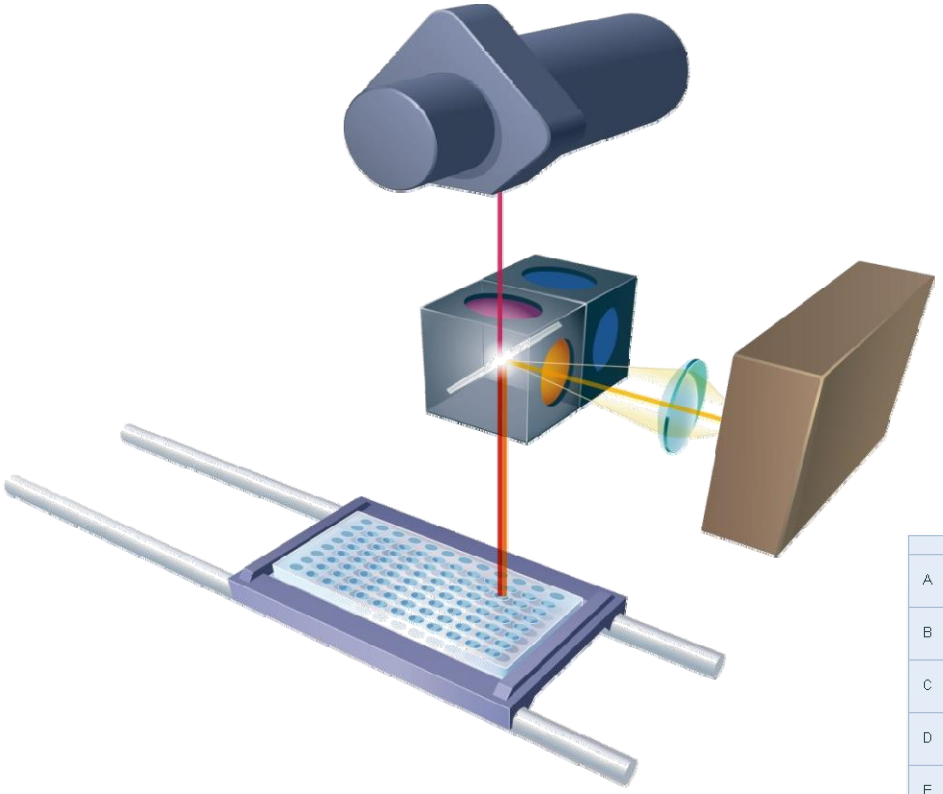
Selectable Bandwidth Benefits

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

Think Possible

BioTek®

Traditional Plate Reader - Filters

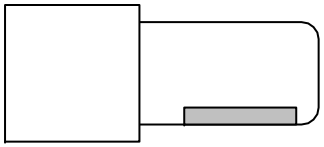


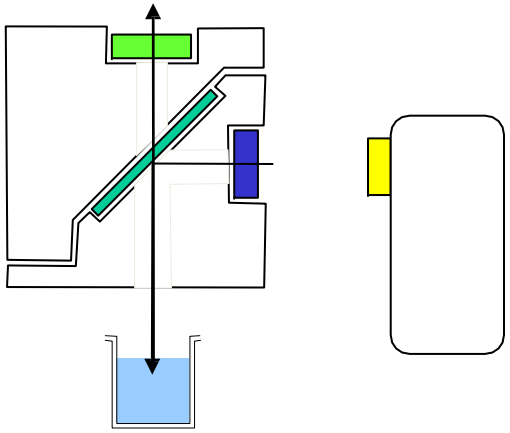
- Quantitative data and sensitivity
- Precisely measure cellular activity

	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	0.282	0.264	0.278	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	0.297	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	0.280	0.260	0.277	0.276	0.268	0.272	0.274	0.270
H	0.243	0.258	0.279	0.274	0.278	0.268	0.269	0.276	0.263	0.268	0.248	0.248

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

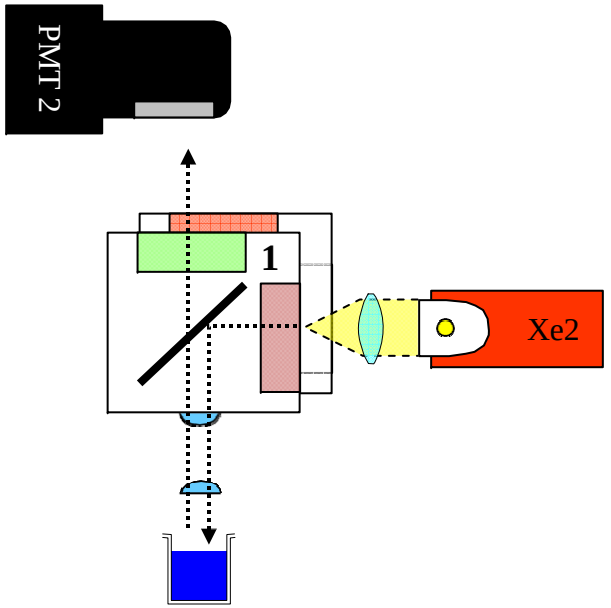




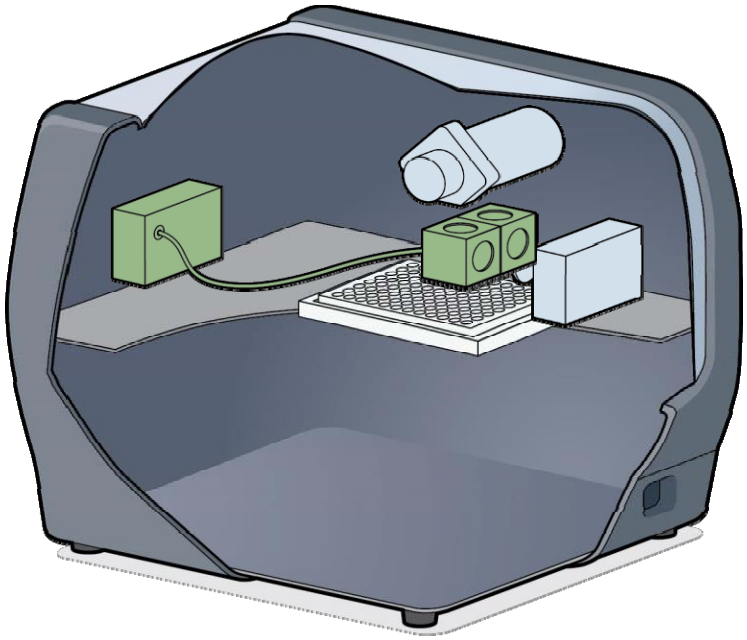
Filter Module: 2 Filter Sets per Cube

One filter cube ships with the F module:

- Default is 360/460 and 485/528 filter sets
- High-performance dichroic-based optics
- Can be customized per customer requirements
- Additional cubes can be ordered

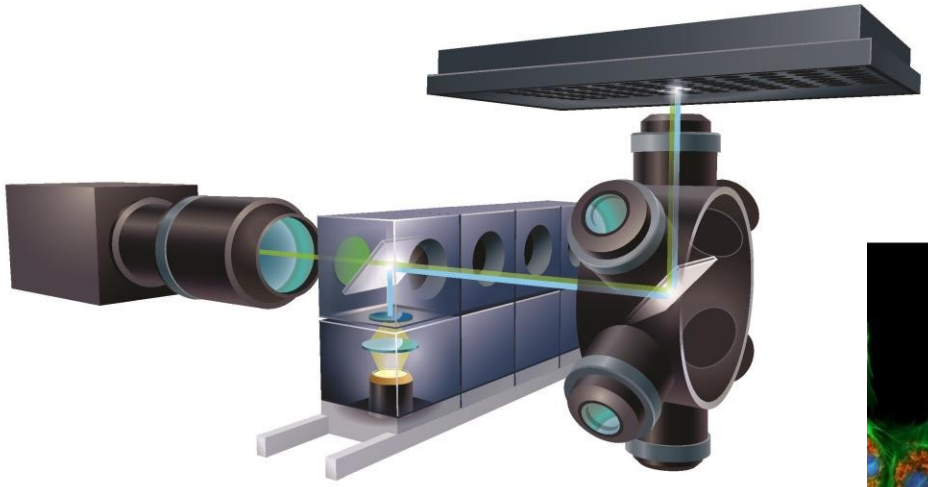


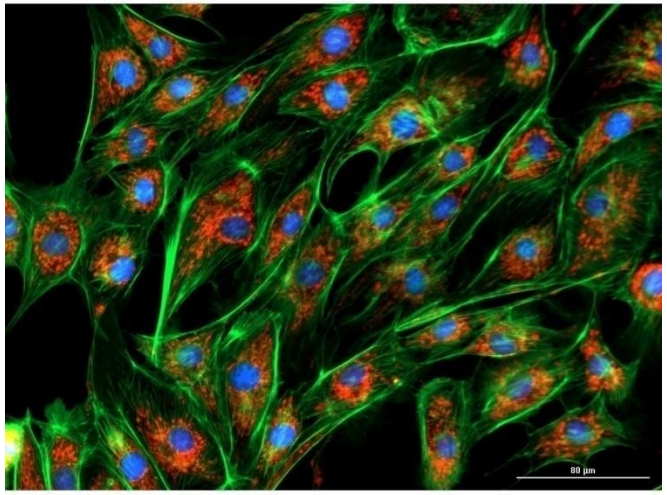
Laser-based Excitation: Alpha Assays



- Powerful 100 mW laser provides best sensitivity and high read speeds

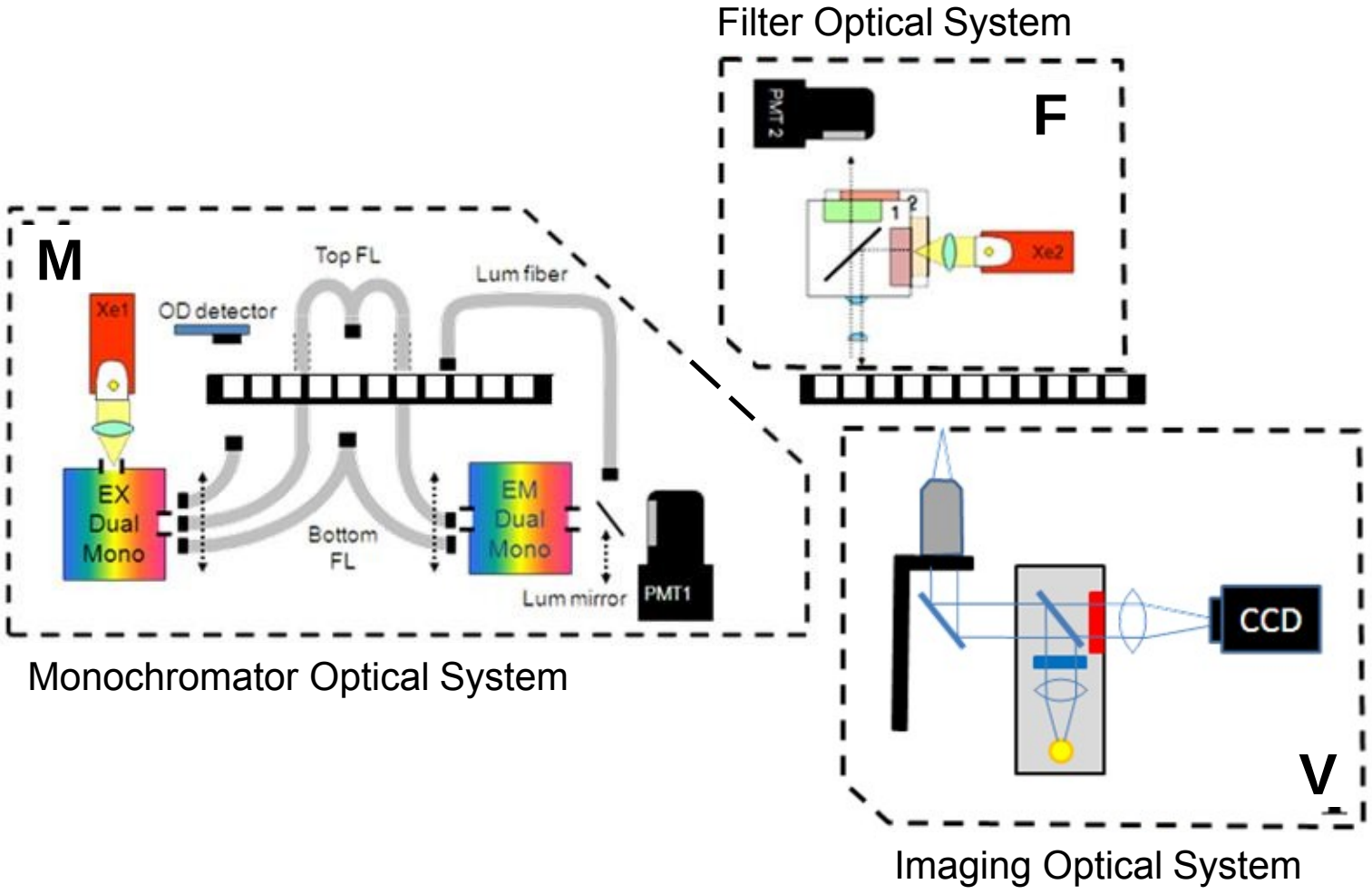
Imaging Optical Path





- Qualitative and Quantitative data
- Characterize change in phenotype

Microplate Reader Optics with Imaging



Think Possible

BioTek®

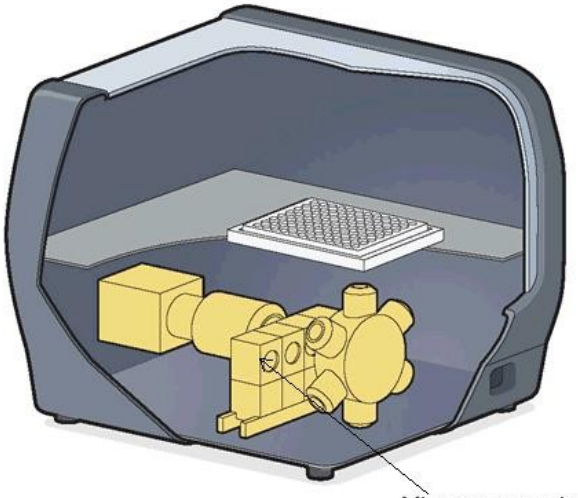
Cytation 5 Modularity & Upgradeability

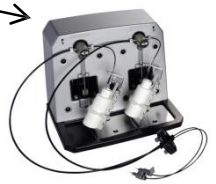
- CYT5M
- CYT5F
- CYT5FA
- CYT5V
- CYT5PV
- CYT5MF
- CYT5MFA
- CYT5MFV
- CYT5MV
- CYT5MPV
- CYT5FV
- CYT5FAV
- CYT5MFAV

- M - Quad Monochromator Optics
- F - Filter-based Optics
- V - Imaging Optics
- A - AlphaScreen Laser Optics
- P - Phase Contrast Optics

Additional Options:

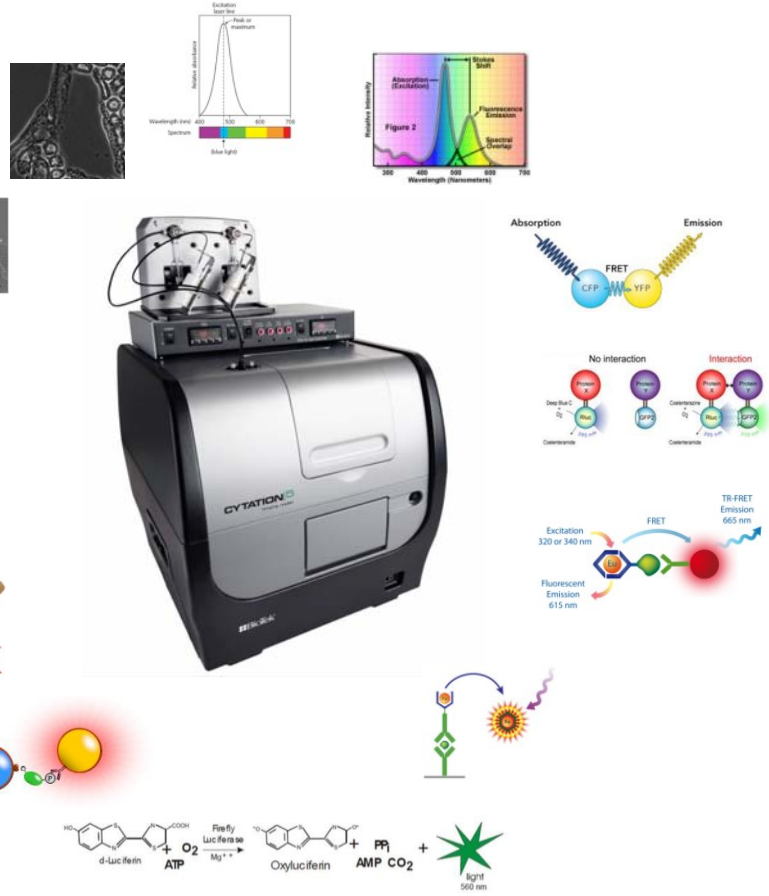
- *CO_2 Gas Controller*
- *O_2 / CO_2 Gas Controller*
- *Dual Reagent Injectors*
- *Joystick Controller*
- *BioStack4*





Cytation 5 Detection Modes

- UV/Vis Absorbance
- Fluorescence Intensity (top & bottom)
- Luminescence (glow & flash)
- Filtered Luminescence, e.g. BRET, BRET^2
- Heterogeneous TRF
- Homogenous TRF (TR-FRET)
- Fluorescence Polarization
- AlphaScreen® & AlphaLISA®
- Fluorescence Imaging
- Color Brightfield Imaging (H&E)
- Brightfield Imaging
- Phase Contrast Imaging

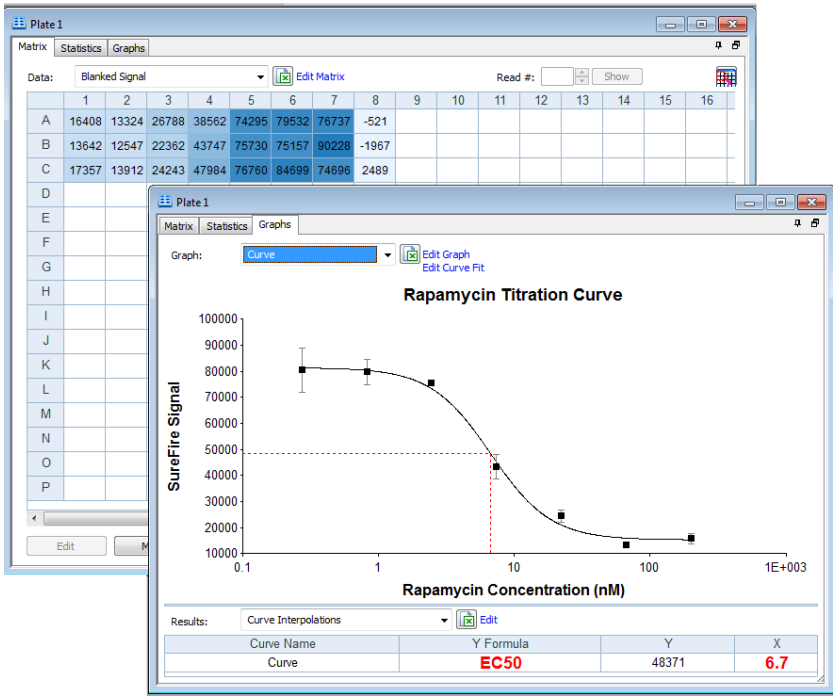


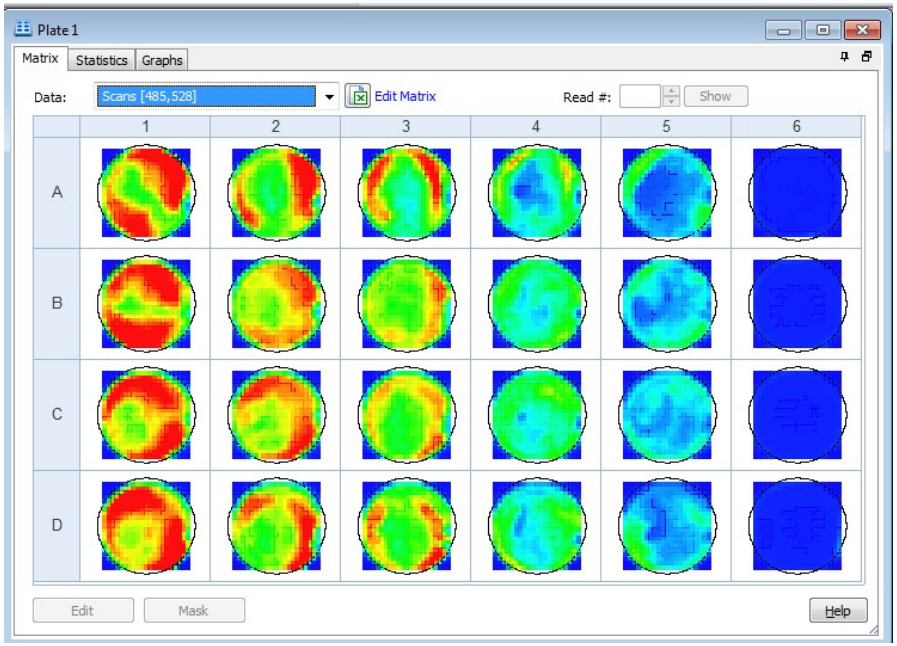
Think Possible

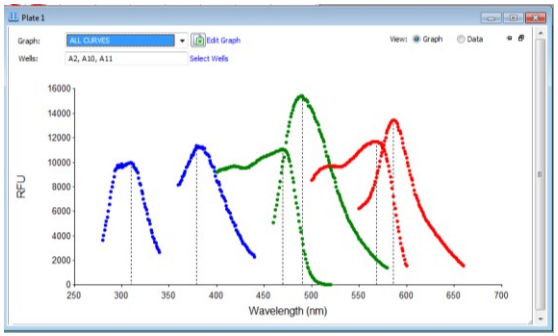
BioTek®

Gen5 Data Analysis Software: Powerful Functionality

- Results gradient
- Curves
- Area scan
- Spectral scan





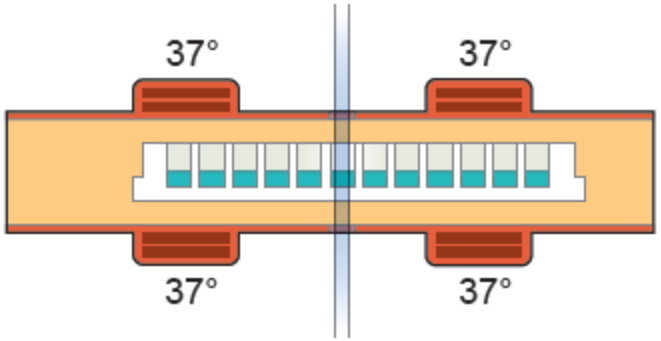


Think Possible

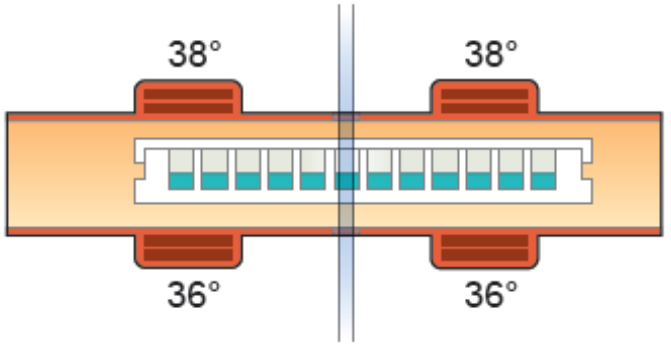
BioTek®

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 ºC @ 37 ºC



Uniform temperature set point in all four zones



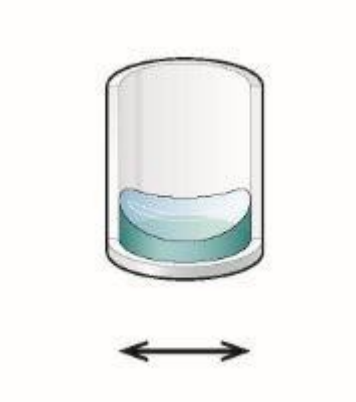
Top zones set higher for condensation control

Think Possible

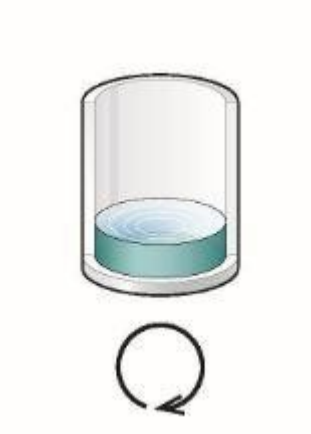
BioTek®

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

BioTek

Automation

- Compatible with automated systems
- Cytation 5 presents microplates in portrait orientation
- Compatible with BioStack 4
  - De-lidding and re-lidding capability for 96-, 384- and 1536-well plates
  - Stacking of 24- to 1536-well plates

Think Possible

BioTek®

More and More Assays are Run on Live Cells

Cytation 5 live cell features:

- Temperature control up to 65 ºC
- Gas controller (CO_2 / O_2)
- Automatic reagent dispenser

Example Applications:

- Calcium signaling
- Enzymatic activity
- Metabolic activity
- Mitochondrial membrane potential
- Oxidative burst
- Receptor binding
- Live Cell assays
- Cell migration
- Hypoxia & Oxidative stress
- Cell cycle analysis

Think Possible

BioTek®

Imaging Basic Specifications

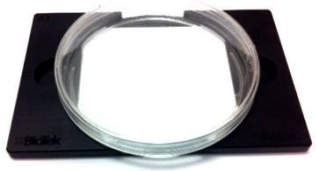
- Matched LED light source and filter cube assemblies
- 4 filter cube onboard capacity: RGB, plus several other colors
- Six objectives onboard capacity: 1.25x, 2.5x, 4x, 10x, 20x, 40x and 60x
  : 4x, 10x, 20x and 40x phase
- Read modes: End point / Time lapse / Montage
- Labware: 6- to 1536-well microplates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

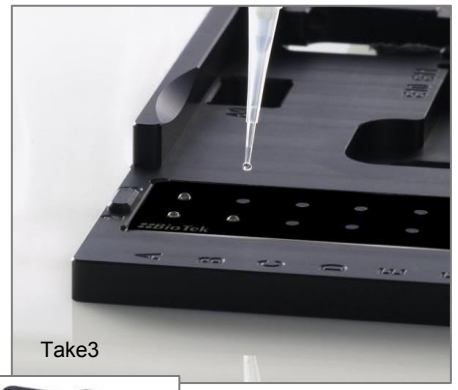
Think Possible

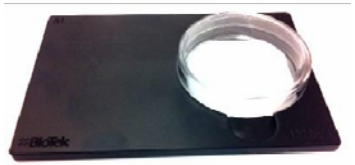
BioTek®

Labware: Multi-Vessel Compatibility

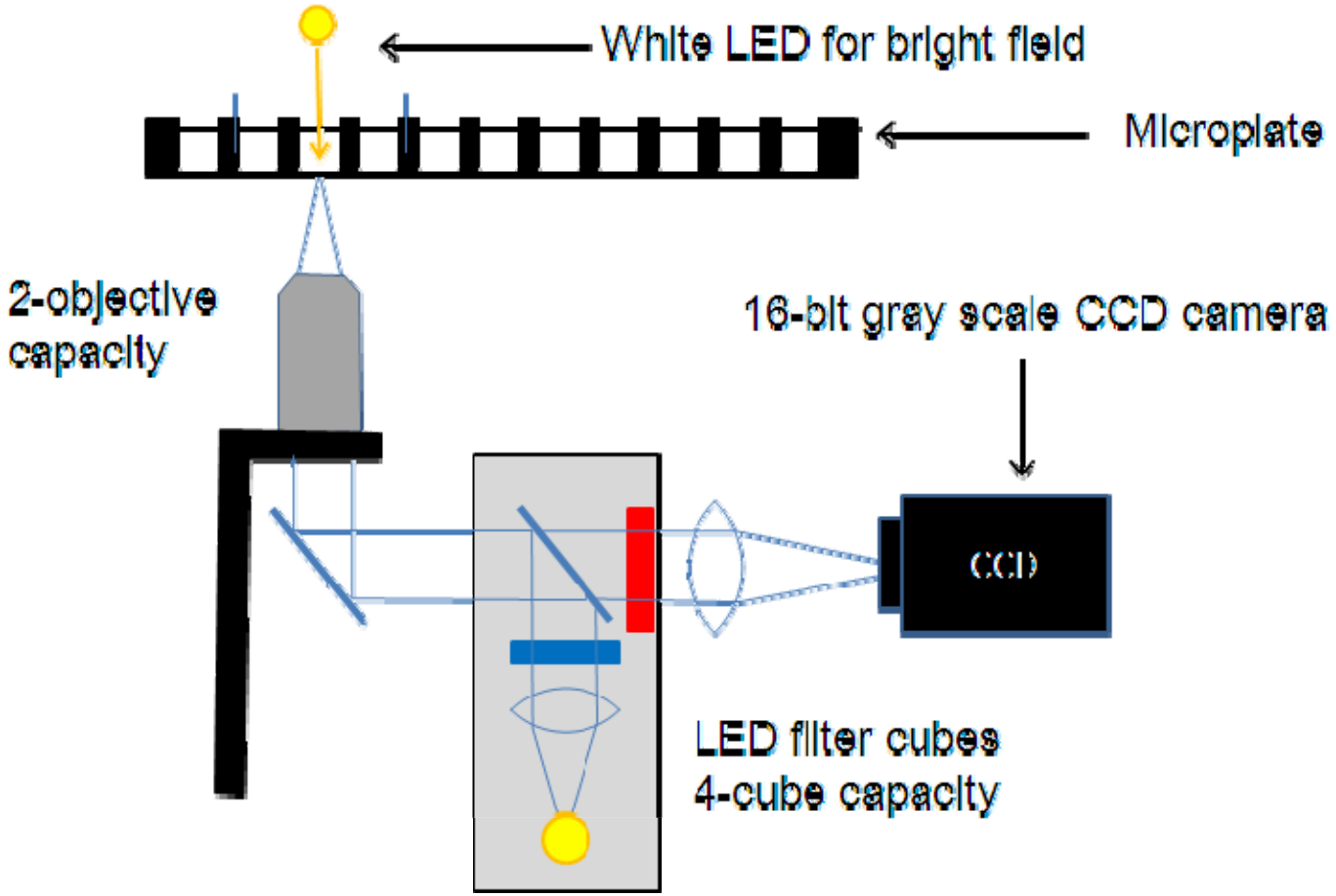
- 6- to 384-well microplates (up to 1536-well for imaging)
- Microscope slides / chamber slides
- T25 flasks
- 100, 60 and 35 mm Petri dishes
- Cell Counting Chamber
- Take3 & Take3 Trio







Imaging Optical Path



High Quality Optical Components

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

Think Possible

BioTek®

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

BioTek®

Objective Specifications

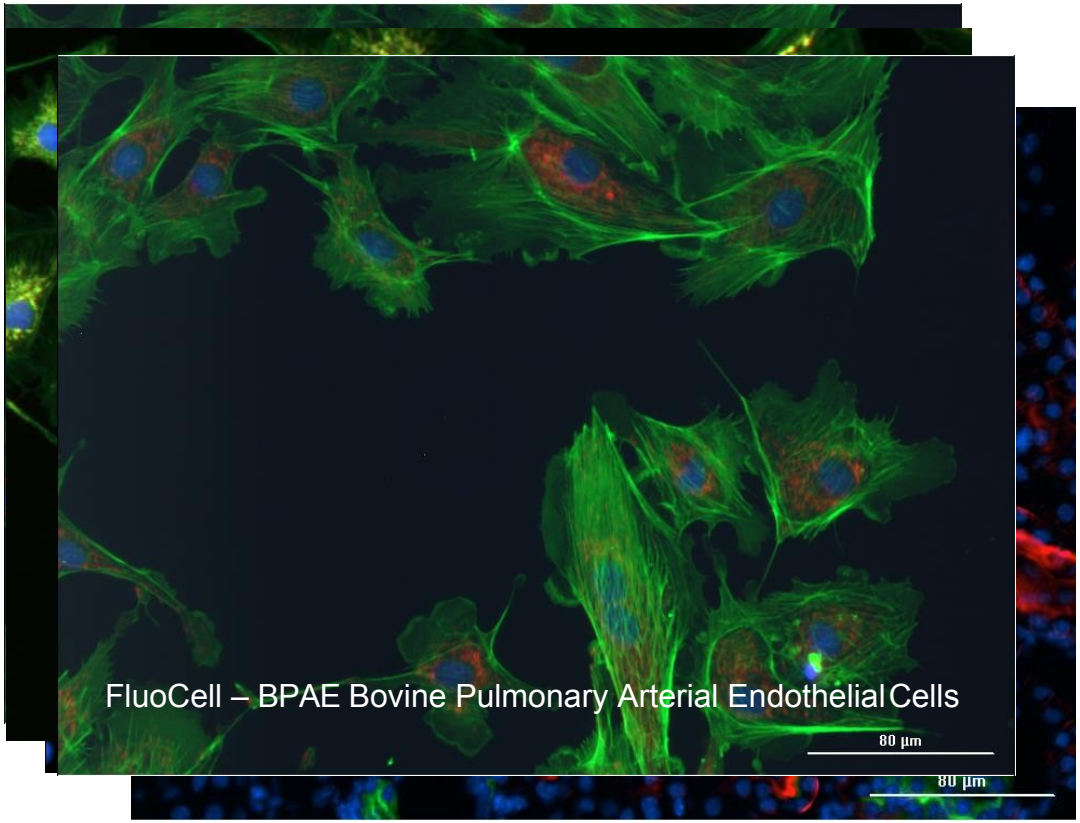
	Numerical Aperture	Resolution (nm)*	Depth of Field (µm)*	Working Distance (mm)
1.25x	0.04	4400	300	5
2.5x (2.25 x eff)	0.07	2500	132.2	5.7
2.5x (2.75x eff)	0.12	1458	50.7	6.3
4x	0.13	1346	38.9	17
Phase 4x	0.13	1346	38.9	17
10x	0.3	583	7.1	10
Phase 10x	0.3	583	7.1	10
20x	0.45	388	2.9	6.4 - 7.6
Phase 20x	0.45	388	2.9	6.4 - 7.6
40x	0.6	291	1.5	2.7 - 4.0
Phase 40x	0.6	291	1.5	2.7 - 4.0
60x	0.7	250	1.1	1.5 - 2.2

* Example is for blue (DAPI) at 350 nm

Think Possible

BioTek®

Fluorescence Imaging

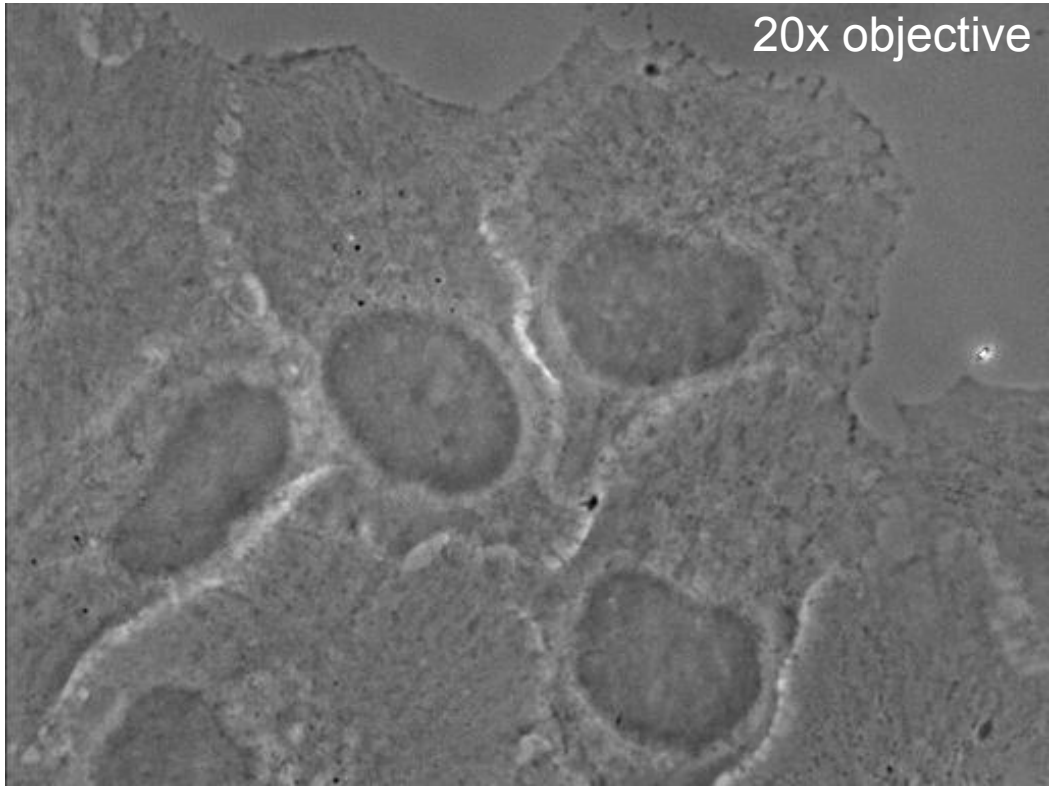


- 3-color image (blue, green red)
- 20x objective
- Images are acquired in sequence in gray scale
- Artificial color scale is applied to each color channel
- Finally, the 3 images are overlapped
- Used for quantitative measurements

FluoCells® is a registered trademark of Molecular Probes, Inc.

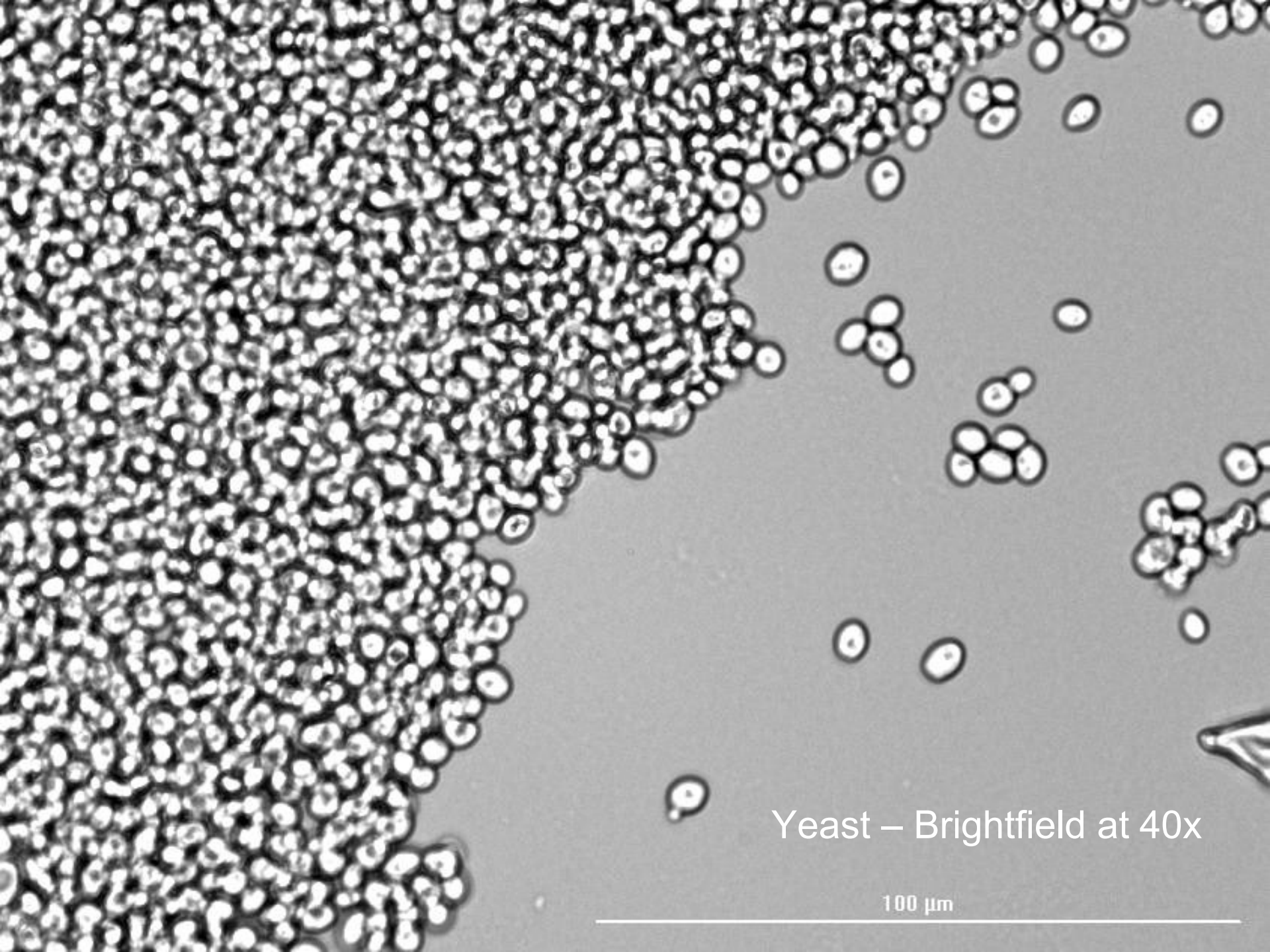
Brightfield Imaging

- Simple, gray scale image shows OD image
- Can be used for quantitative analysis, e.g. cell counting

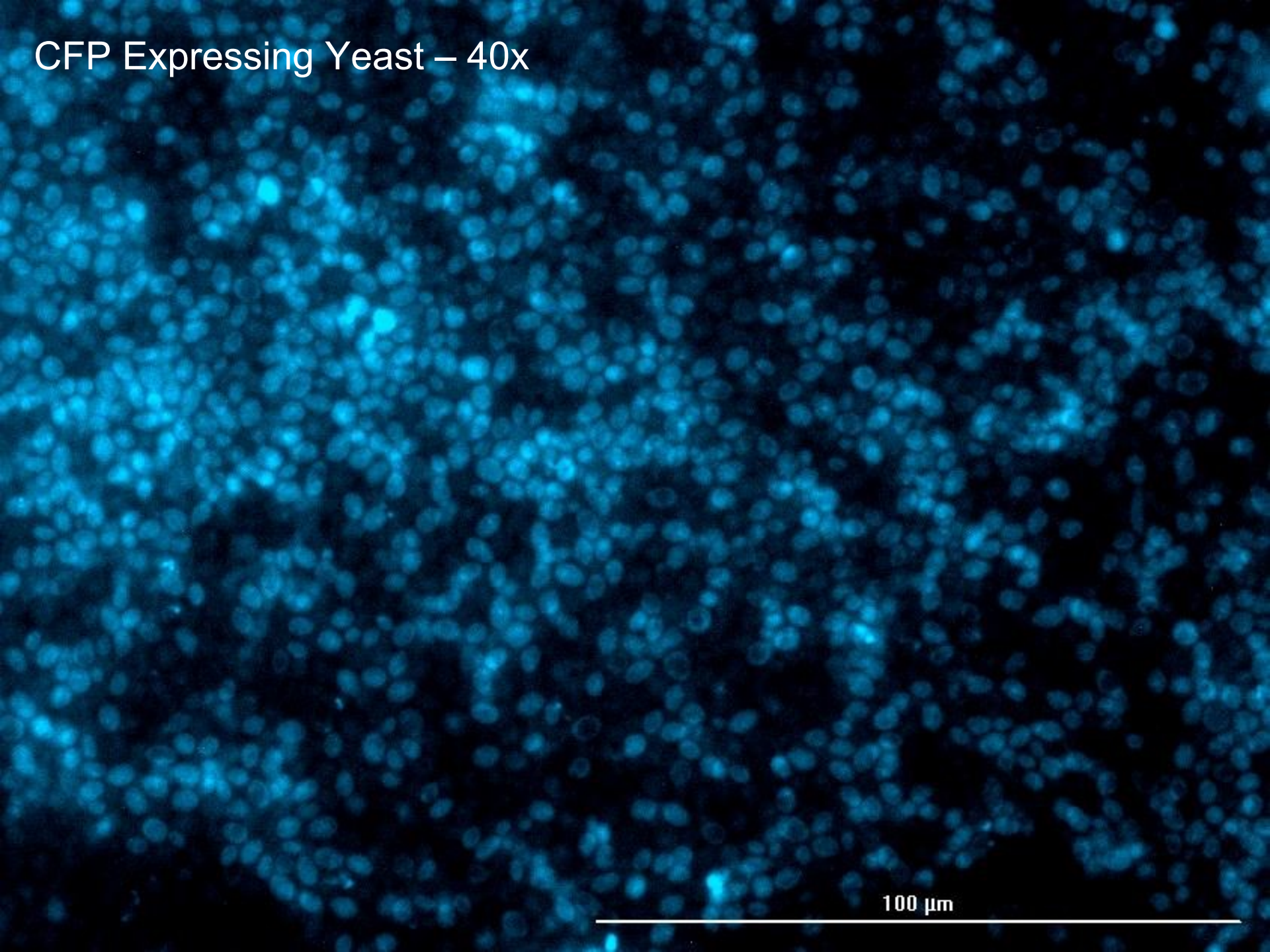


40x & 60x Example Applications

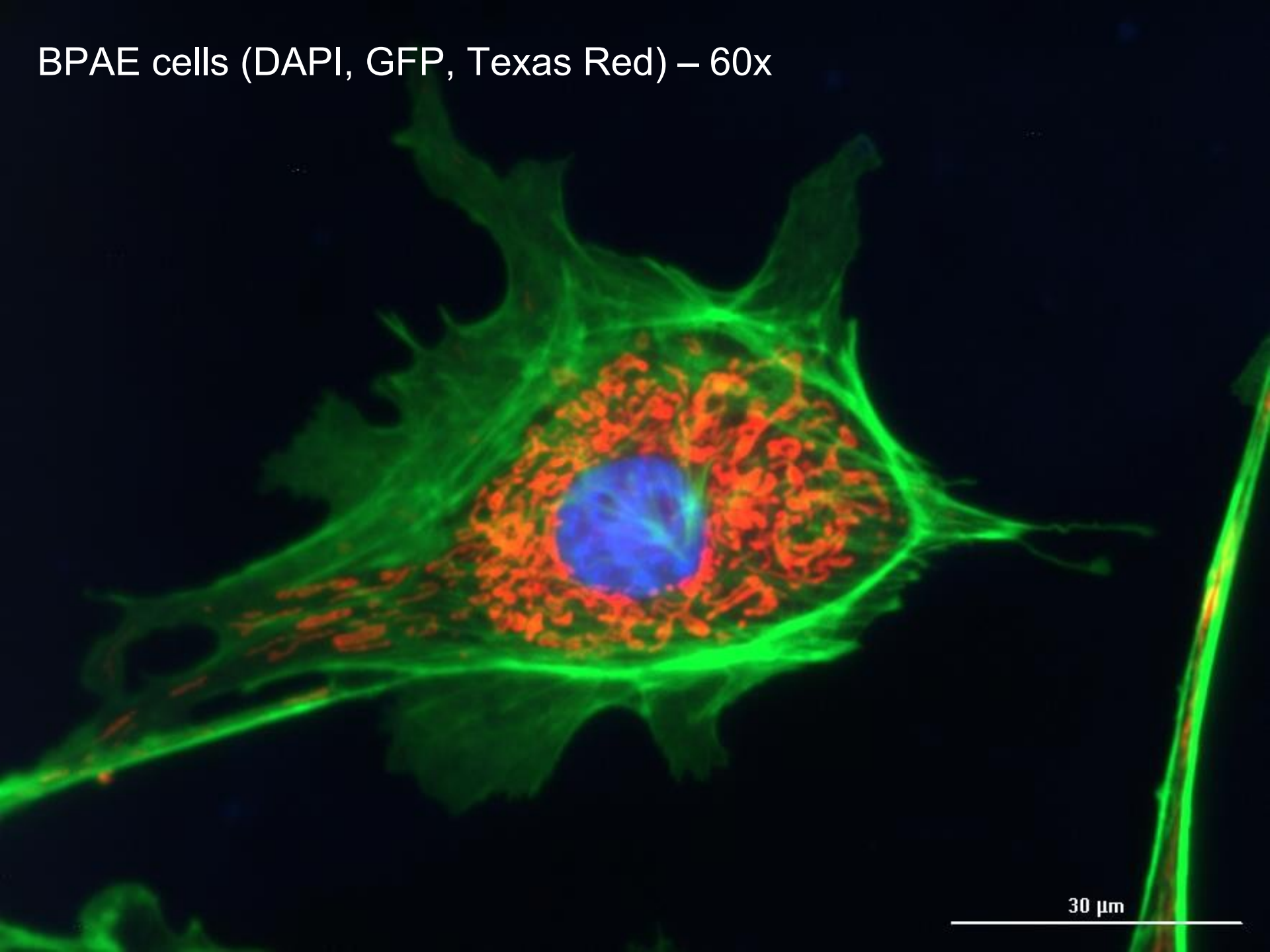
- 40x: Olympus, 2.7 mm working distance, 0.6 NA
- 60x: Olympus, 1.8 mm working distance, 0.7 NA
- Imaging yeast and bacteria
- Imaging smaller cellular structures, e.g. mitochondria
- Resolution at 60x and 500 nm is about 350 nm (0.35 µm)
  - 500 / 2 x 0.7 (wavelength / 2 x NA)


Yeast – Brightfield at 40x
100 µm

CFP Expressing Yeast – 40x



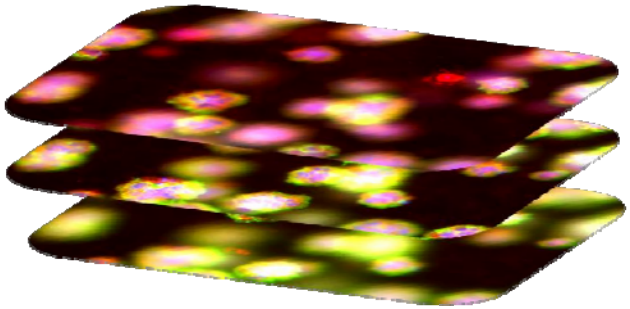
BPAE cells (DAPI, GFP, Texas Red) – 60x



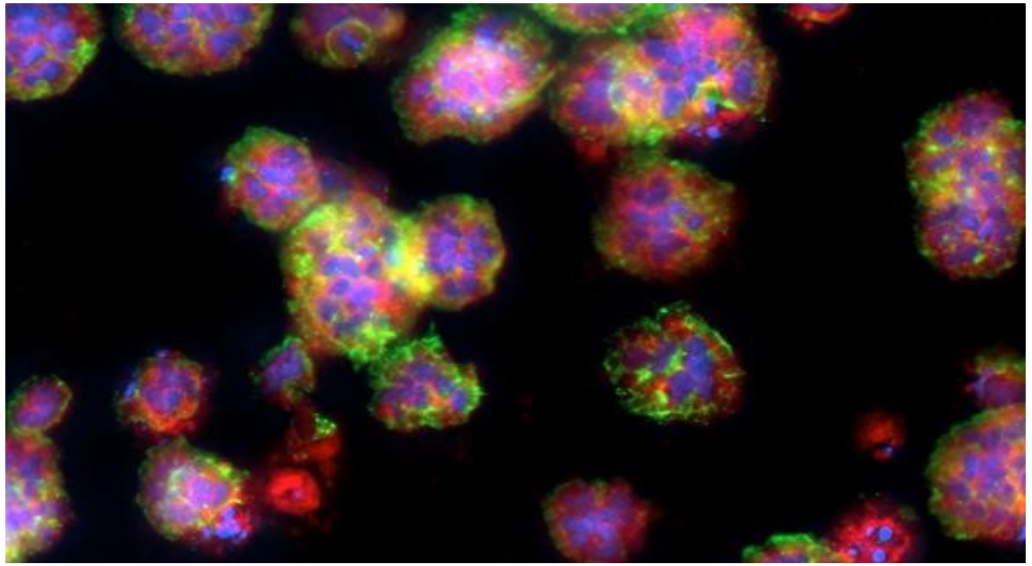
Think Possible

BioTek®

Imaging & Microscopy:Z-Stacking, Z-Projection



Z-Stacking

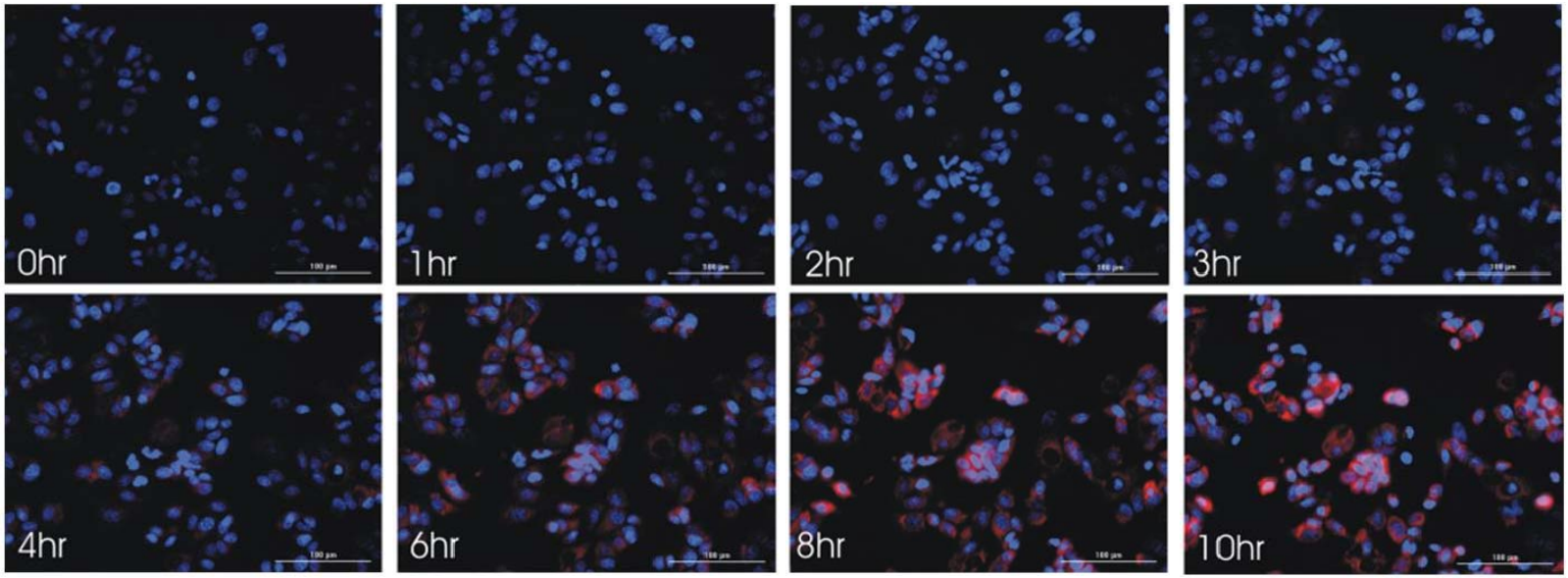


Z-Projection

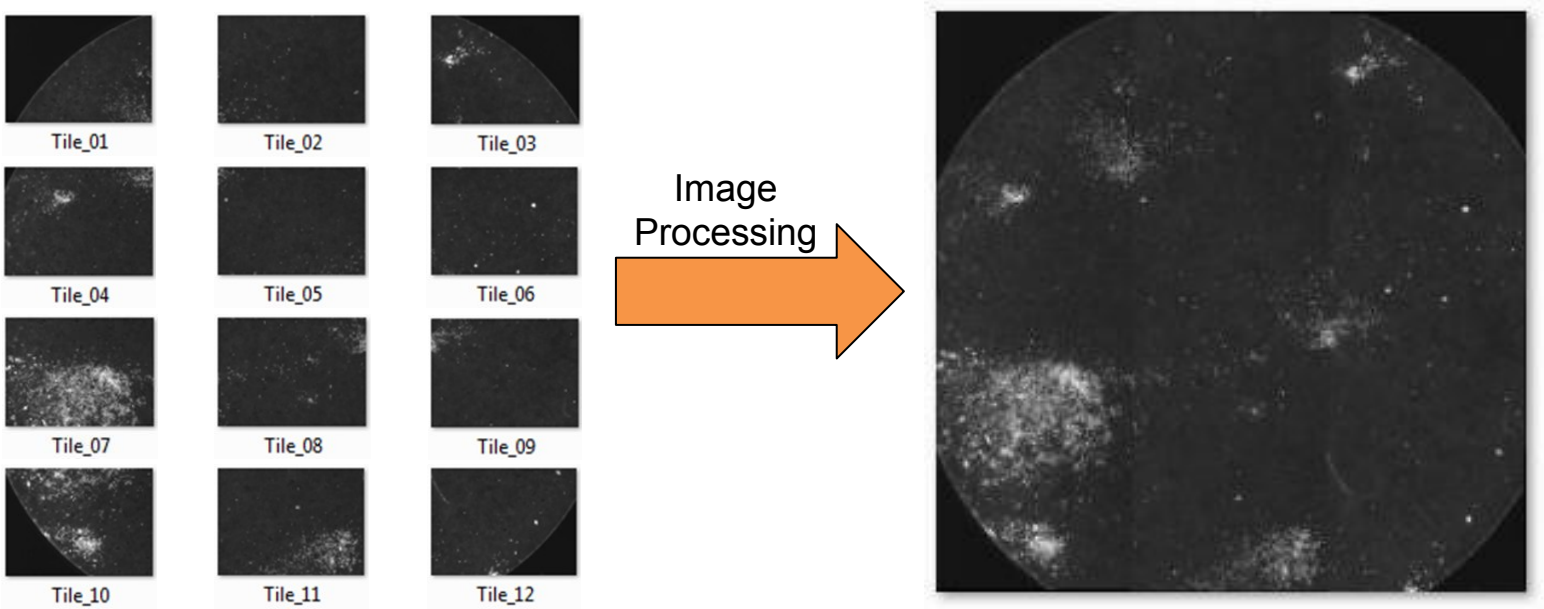
Think Possible

BioTek®

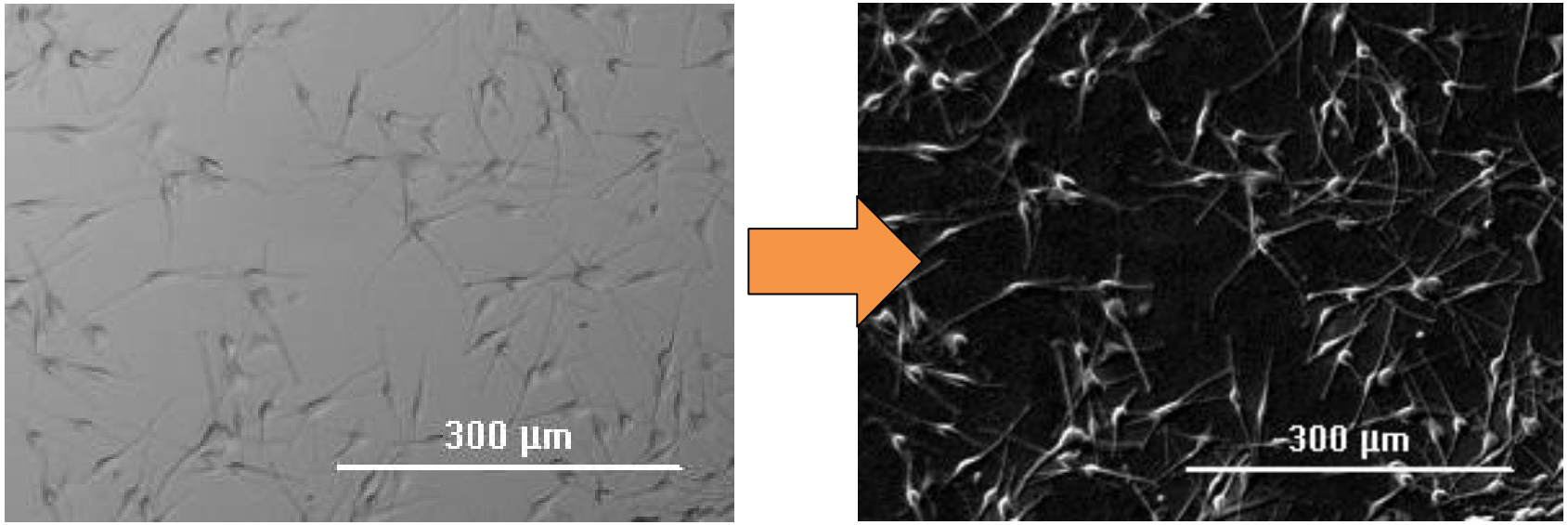
Imaging & Microscopy: Time Lapse



Imaging & Microscopy: Montage and Stitching

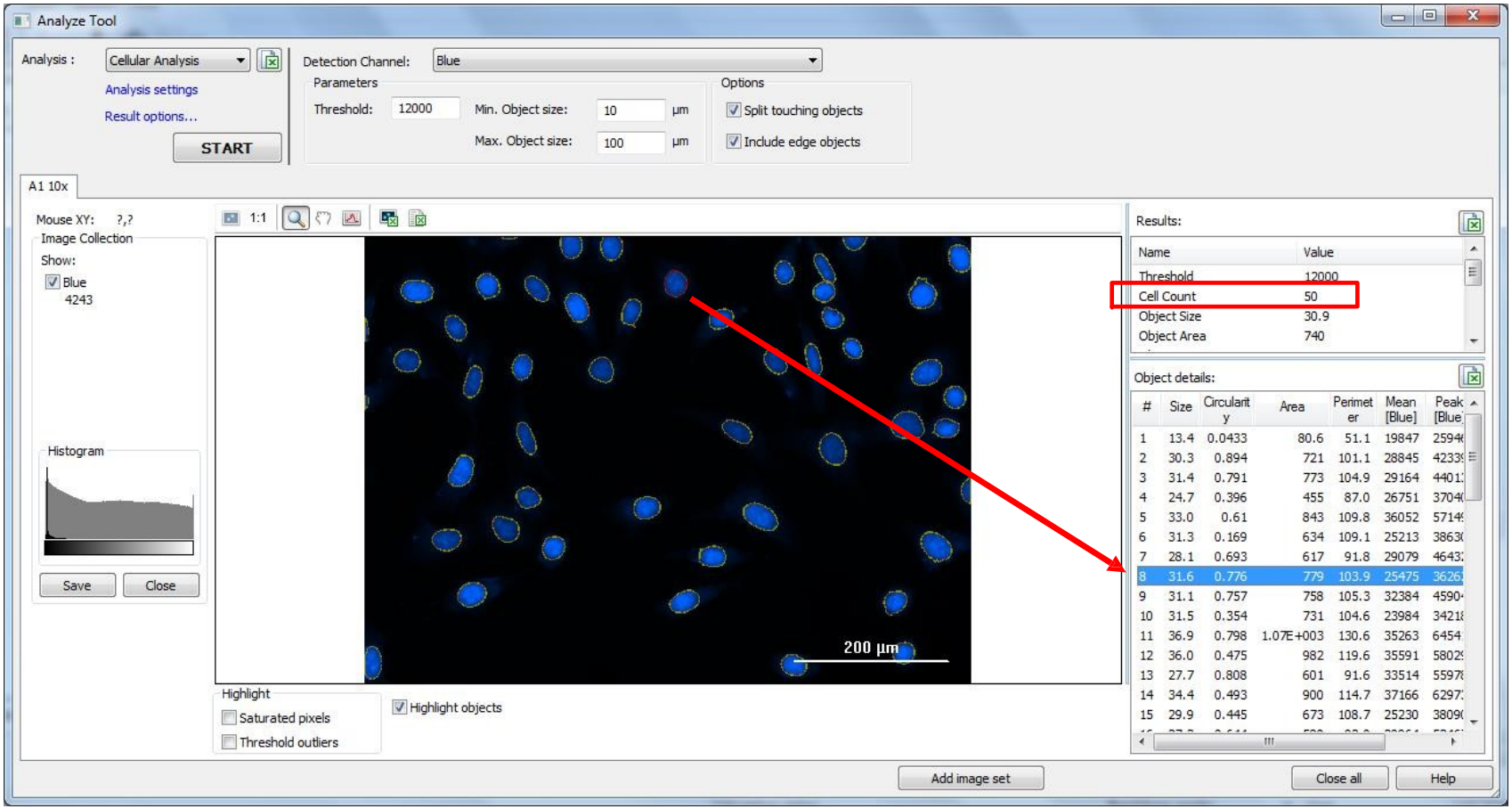


Imaging & Microscopy:Digital Phase Contrast



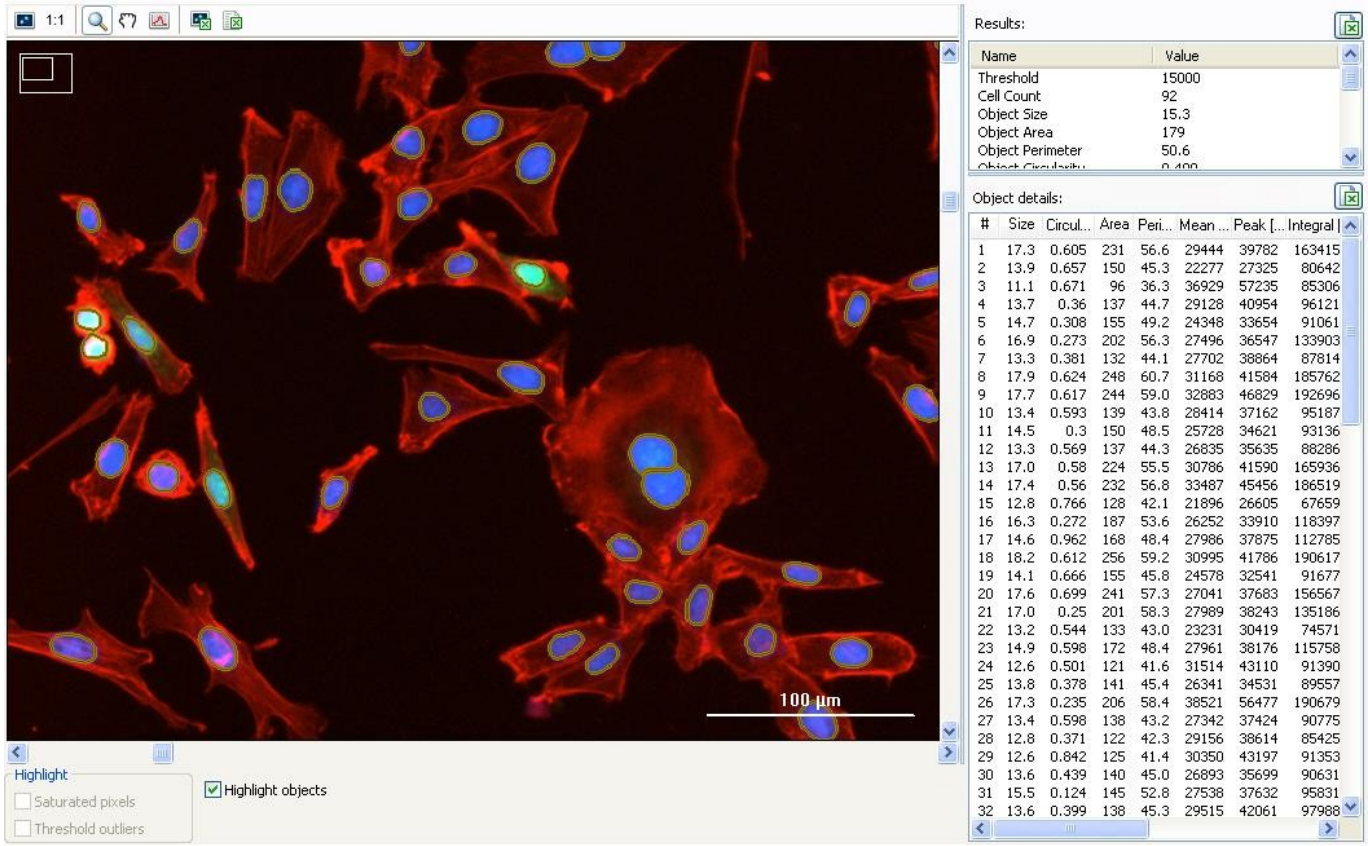
Digital Phase Contrast

Gen5 Interface



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

Image Analysis Can Be Very Complex



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

Software Interface

- Gen5 is BioTek's interface for plate readers
- Our new imager takes advantage of 30 years of plate reader software development.
- Interface allows to:
  - Control the device to automate simple or complex processes
    - End point, kinetic…
  - Multiplex imaging and plate reading steps
  - Analyze the data
  - Automatically export raw and calculated data to Excel or text file if required.

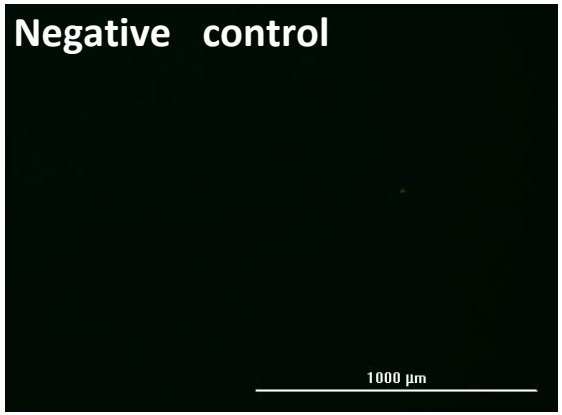
Image Analysis

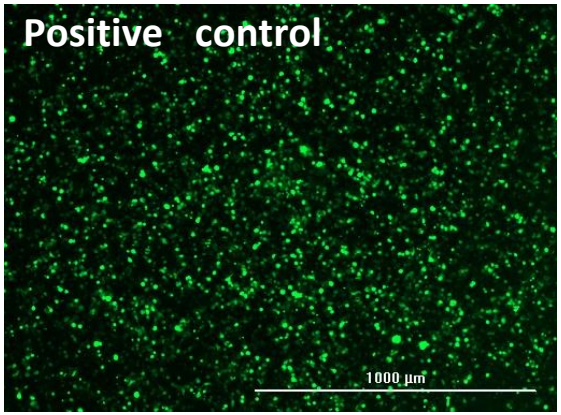
- Simple image analysis is included in the software
  - Image statistics
  - Cell counting using cell nuclei
  - Sub-population analysis using cell-count mask
- Output can then be processed as standard microplate data for more complex data reduction (e.g. EC_{50}, potency…)
- Images are saved in universal format for further analysis using other software packages if needed (Image J, Cell Profiler, MetaMorph, CombineZP…)

Think Possible

BioTek®

Improve Sensitivity with Imaging: Plate Reader vs. Microscope





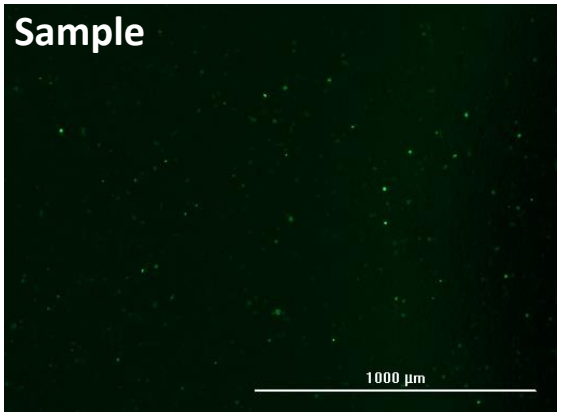
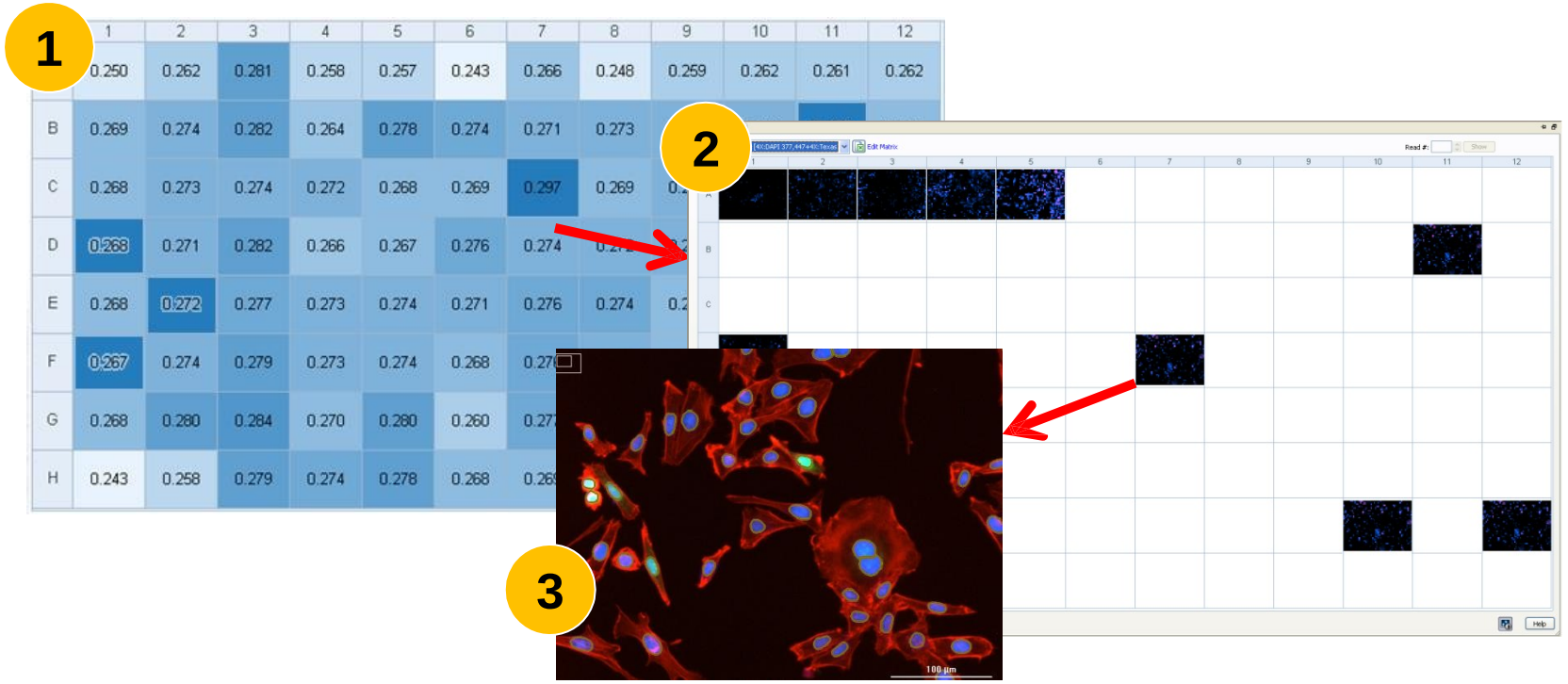


	Plate Reader PMT (RFU)		Microscope CCD Camera (Cell count)	
	Well value	**S/B**	Well value	**S/B**
Negative control	19556		1	
Positive control	45029	**2.3**	2668	**2668**
Sample	24118	**1.2**	104	**104**

Think Possible

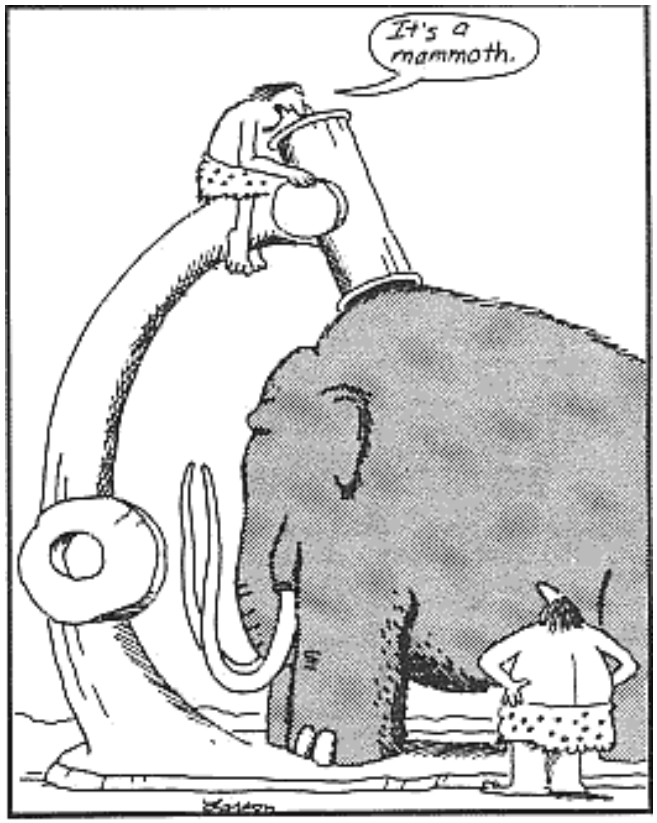
BioTek

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)

Applications



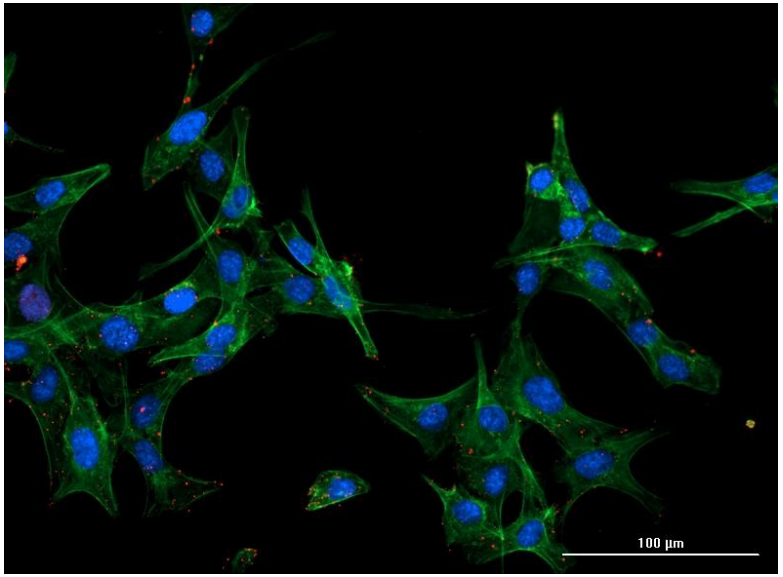
Early microscope

Think Possible

BioTek®

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

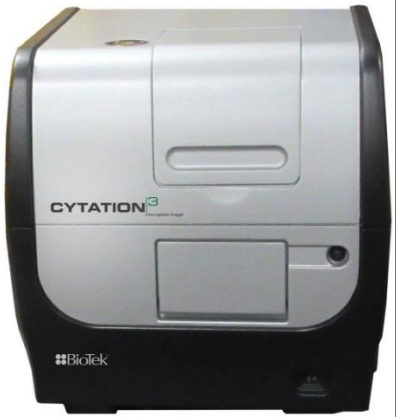


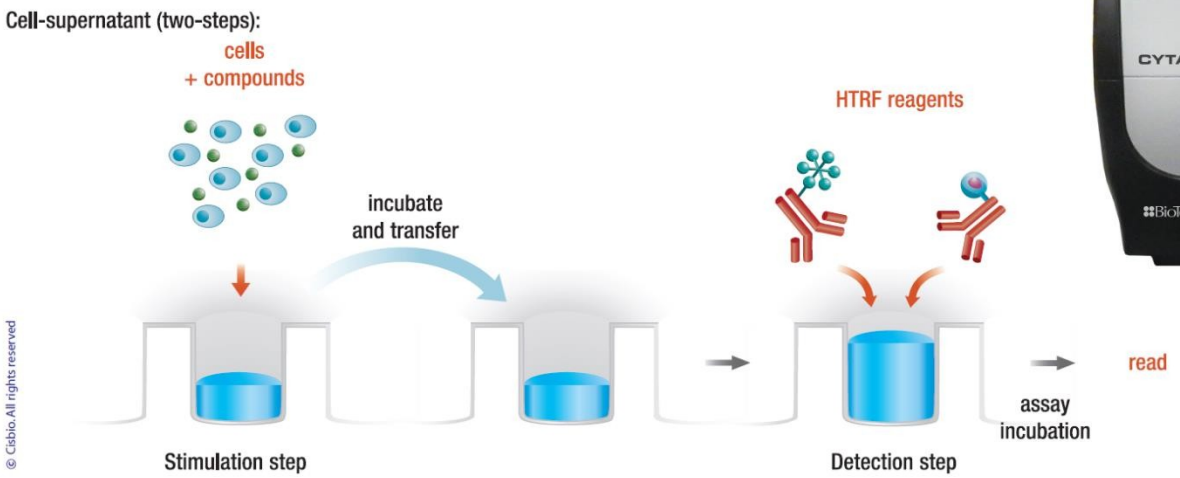
Think Possible

BioTek®

Multiplexed Assay for IL-6 secretion and Cell Viability

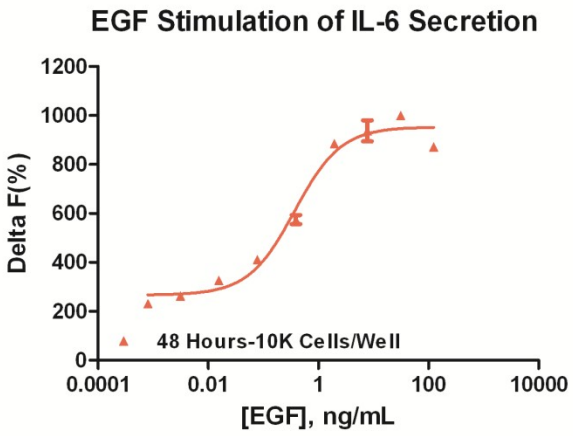
- Endothelial Ovarian Cancer Cell Line: SKOV-3
- IL-6 secretion induced by EGFR activation of NFkB pathway
- IL-6 quantified by 2-step HTRF assay through plate transfer
  - Top read, spectral filters / dichroic
- LIVE/DEAD assay performed on cells post-transfer step
  - Bottom read, digital fluorescence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



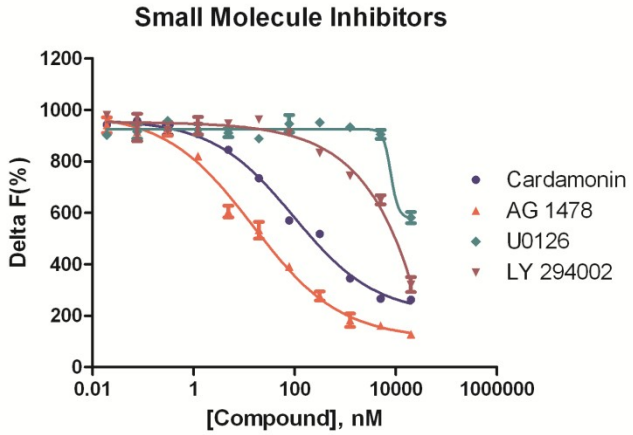


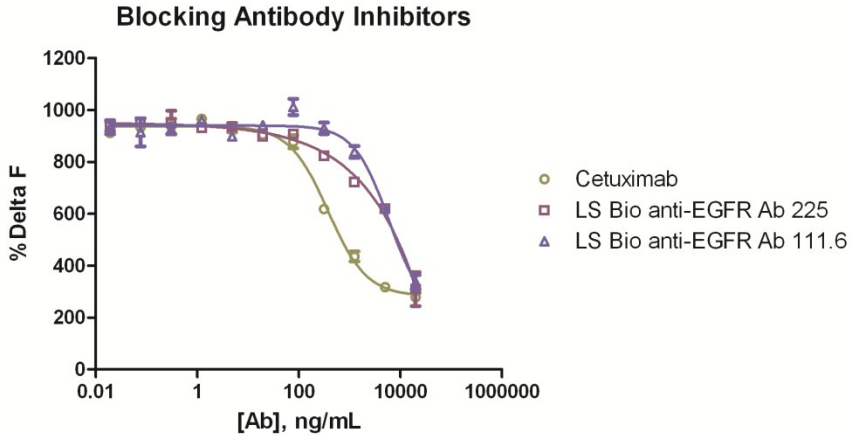
IL-6 secretion – 48 hr incubation

Activation

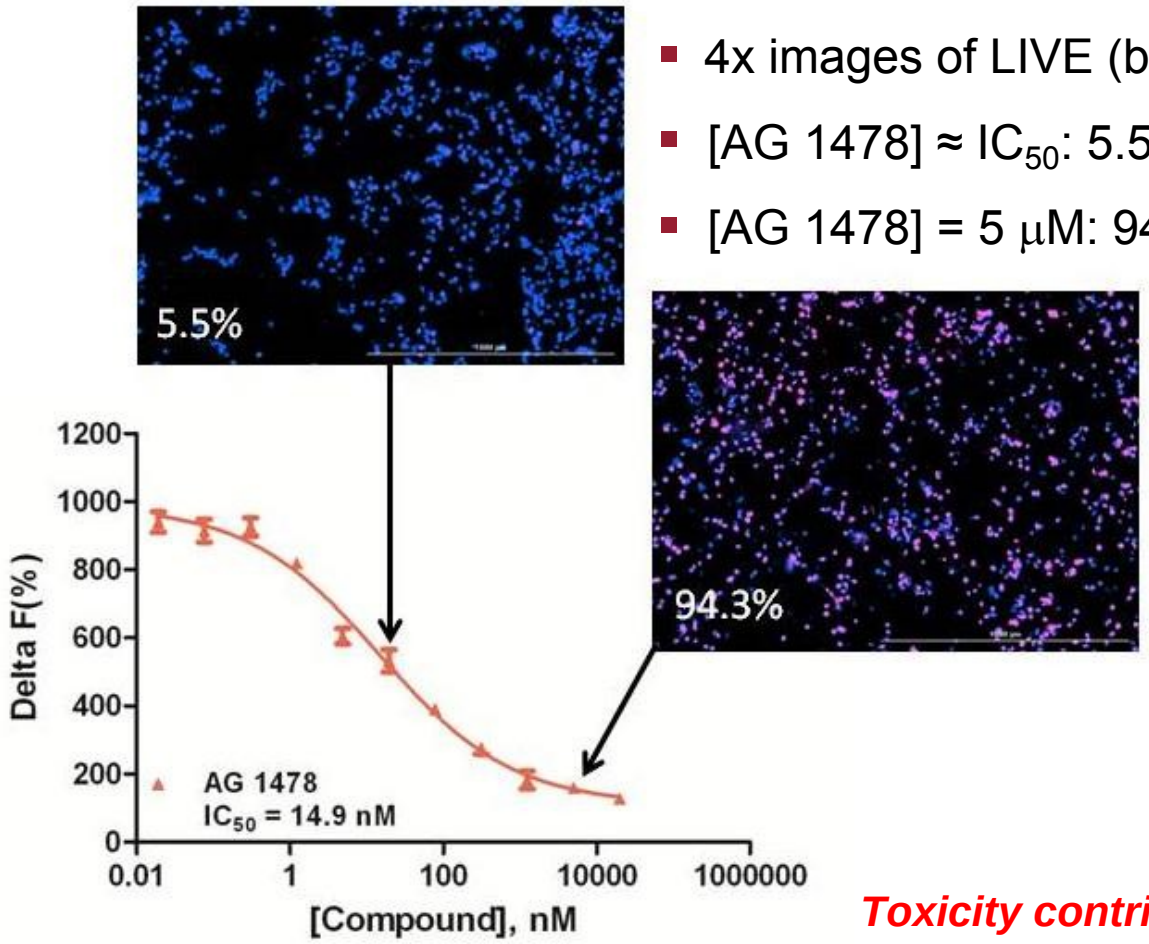


Inhibition





Toxicity Assessment of AG 1478 using LIVE/DEAD Probes

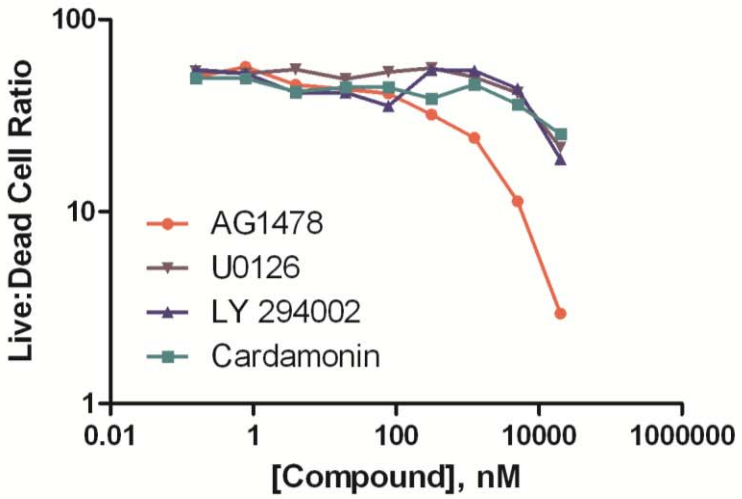


- 4x images of LIVE (blue) and DEAD (red)
- [AG 1478] ≈ IC_{50}: 5.5% DEAD cells
- [AG 1478] = 5 μM: 94.3% DEAD cells

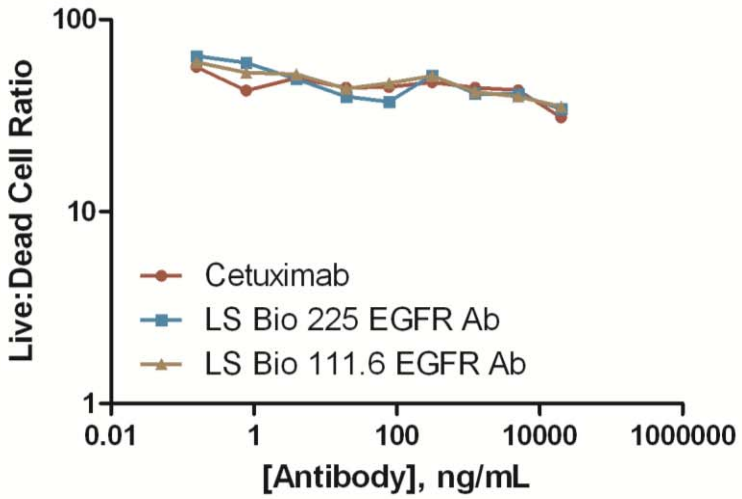
Toxicity contributing to AG 1478 potency

Toxicity Assessment of All Inhibitors using LIVE/DEAD Probes

Small Molecule Inhibitors



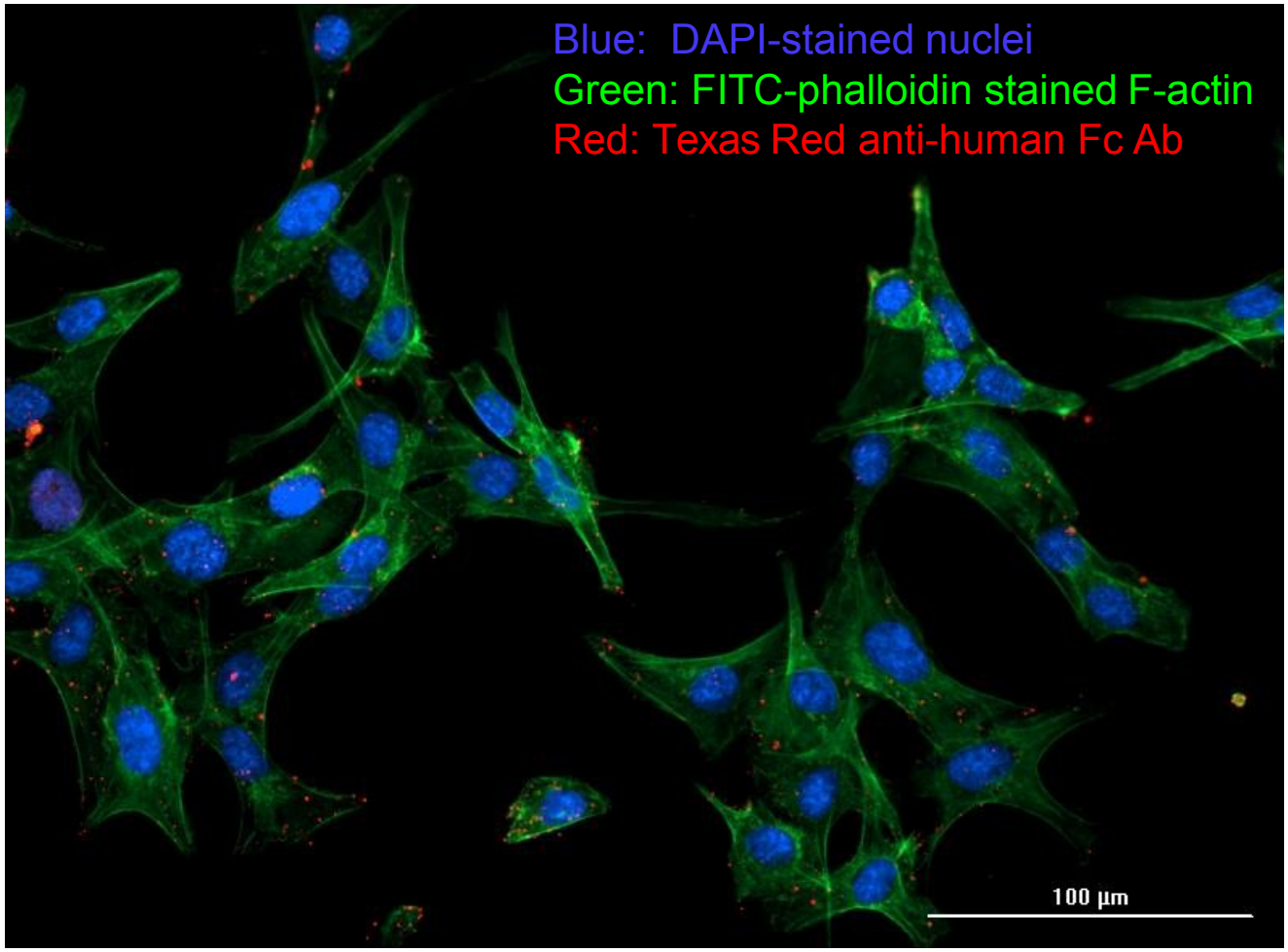
Blocking Antibody Inhibitors



Think Possible

BioTek®

Validation of Cetuximab Binding by 20x Imaging

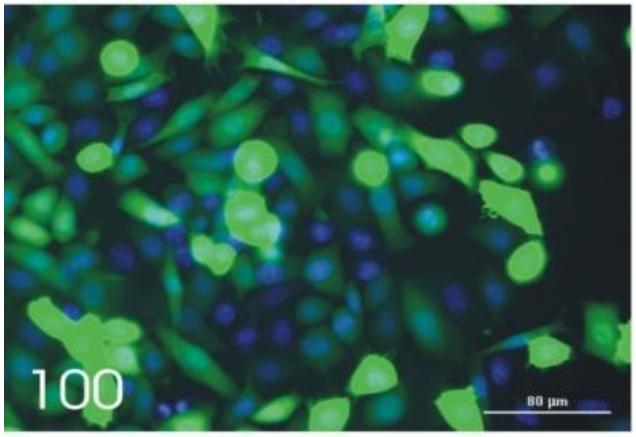


Think Possible

BioTek®

Green Fluorescent Protein (GFP) Transfection

Assessing GFP Gene Delivery using Cellular Imaging and Microplate Reader Technologies



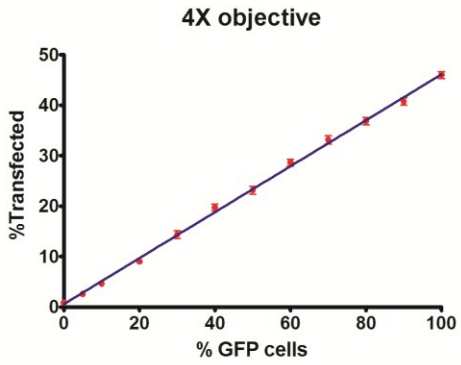
Transfection Efficiency Assessment Procedure

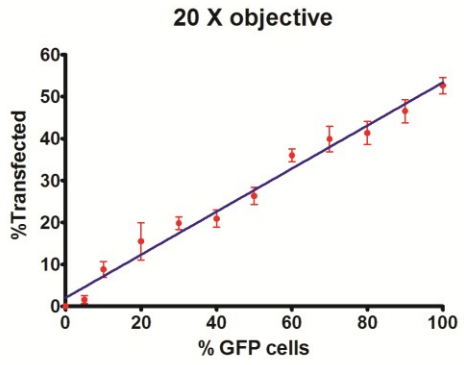
- NIH3T3 (mouse embryo fibroblast) cell model
- 20,000 cells in total added per well
  - Combination of NIH3T3-GFP and NIH3T3 cells
  - Different combinations added to mimic multiple transfection efficiencies
- Cell-permeable Hoechst 33342 nuclear stain added to identify complete cell population
- 4x and 20x fluorescence microscopy images captured to assess theoretical transfection efficiency
- Top read, whole well fluorescence measurements made for validation purposes

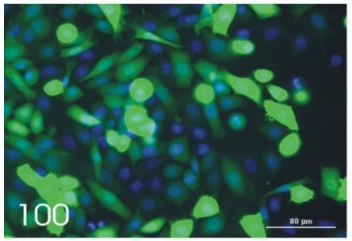
Think Possible

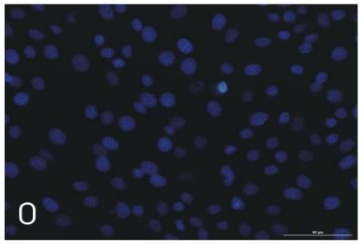
BioTek®

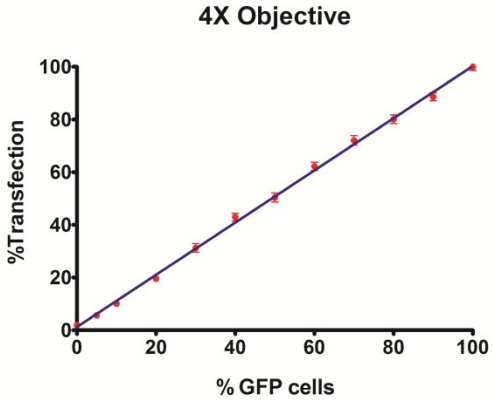
Analysis of Transfection Efficiency using Gen5 Software











Cell count feature in Gen5 software used to determine cell number transfected and total cell count

4x images taken every 2 hours

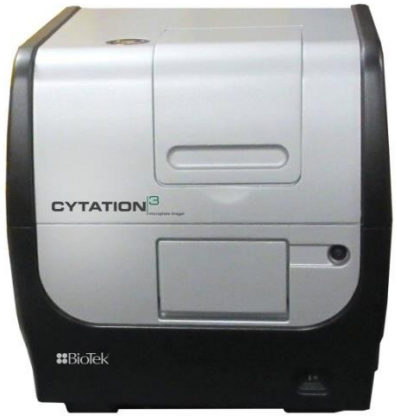
- Cellular expression of GFP fusion protein visible via fluorescence as soon as 6 hours
- Maximum expression reached between 16 and 20 hours

Think Possible

BioTek®

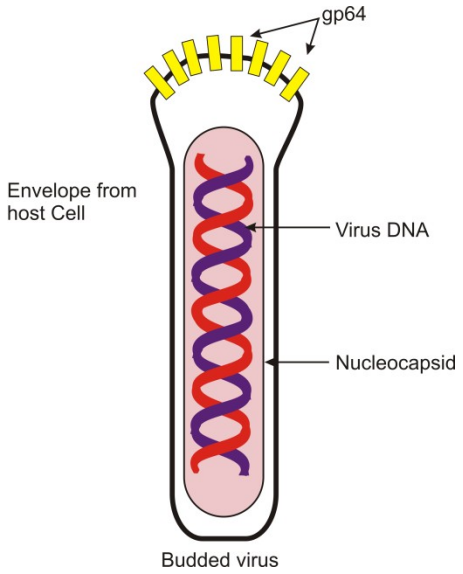
BacMam Transfection

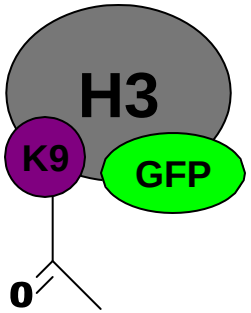
Assessment of Transient Histone H3-GFP Gene Delivery using Cellular Imaging and Microplate Reader Technologies



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 ^{o}C/5% CO_2 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

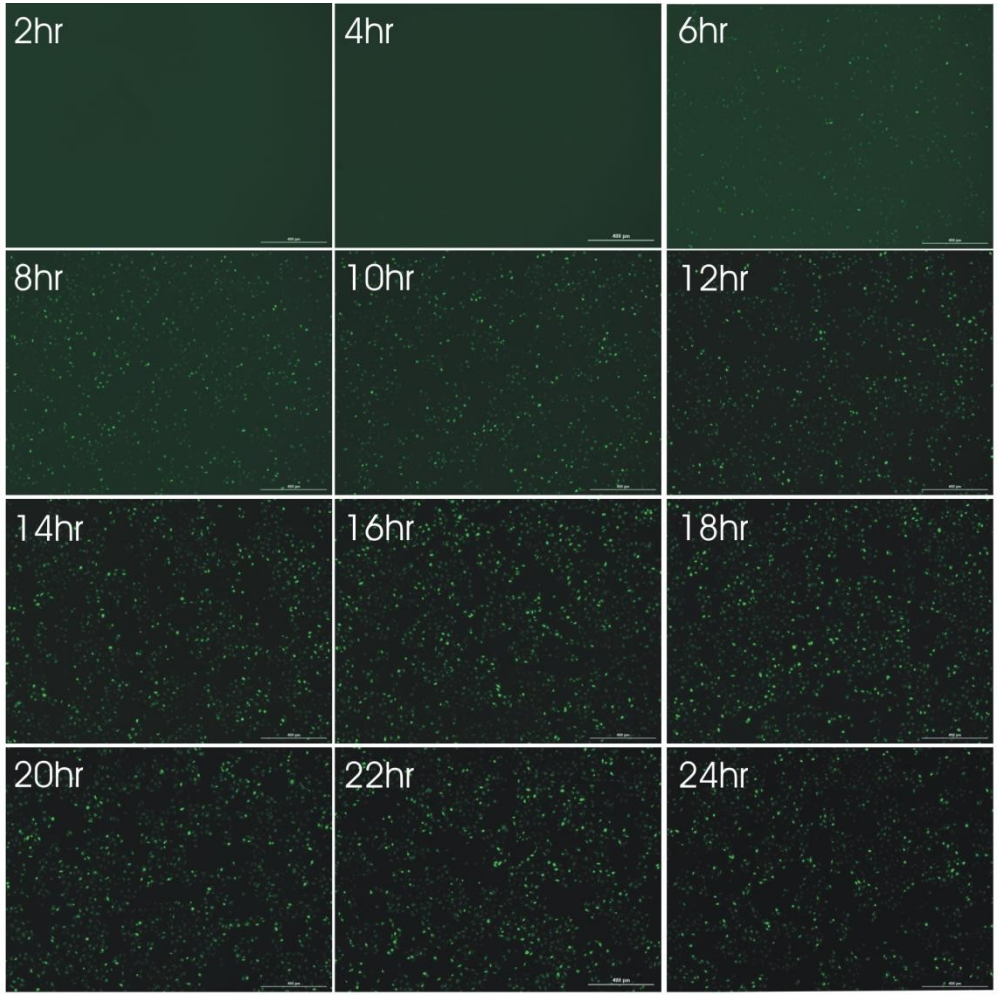




Think Possible

BioTek®

BacMam Transfection Time Course Study

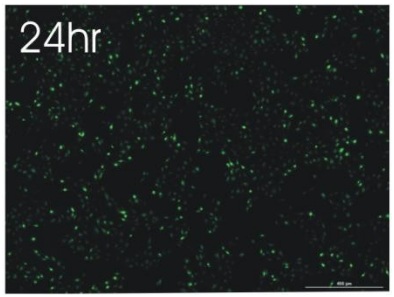


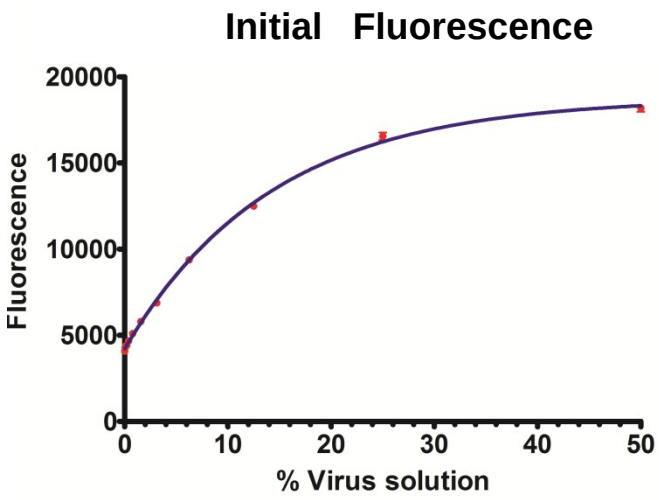
- U-2 OS cells infected with 7.5% virus concentration
- 4x images taken every 2 hours
- Cellular expression of GFP fusion protein visible via fluorescence as soon as 6 hours
- Maximum expression reached between 16 and 20 hours

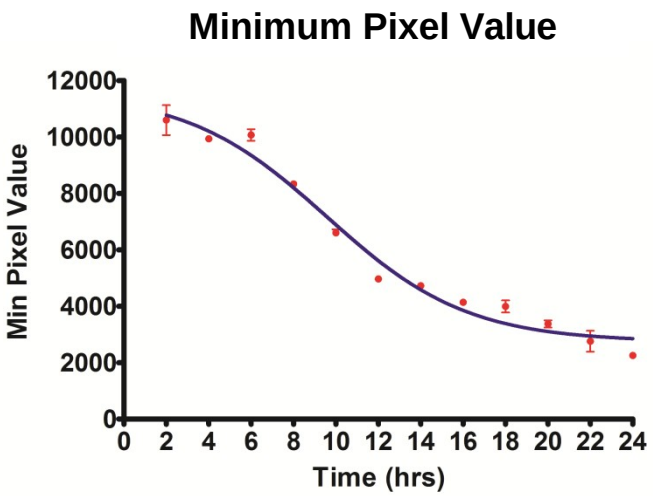
Think Possible

BioTek®

Assessment of BacMam Viral System Fluorescence

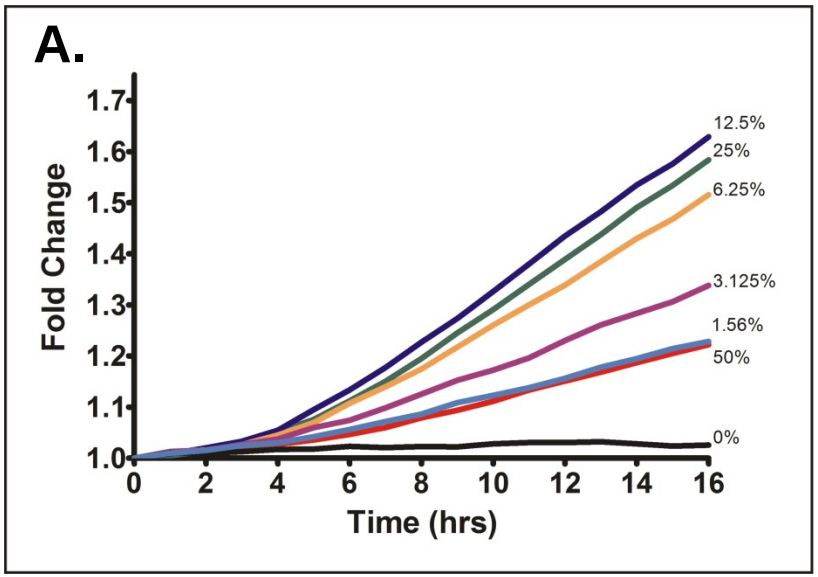


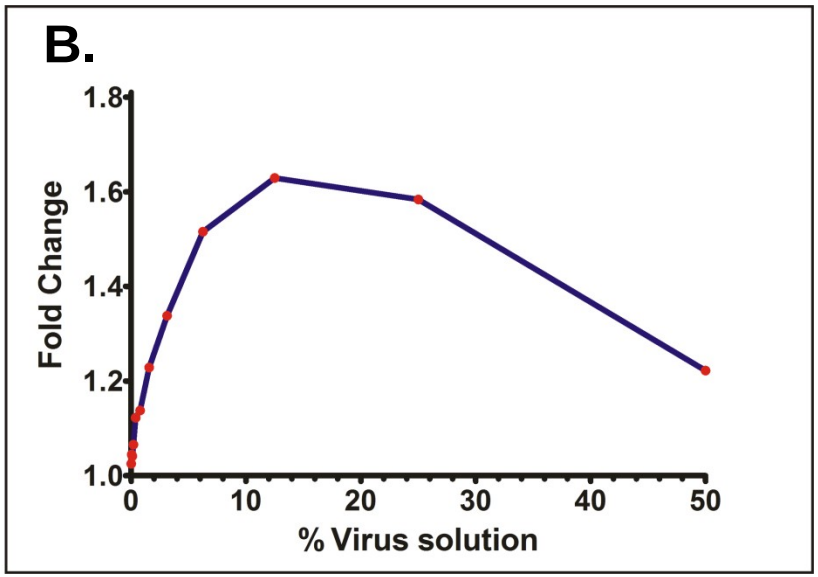




- High green fluorescent background signal seen at 2 hour time point
- Time zero whole well fluorescent reads confirm signal linked to viral introduction
- Background fluorescence drops over time, as seen from 4x images and subsequent image analysis
  - Indicative of viral uptake into cells and nuclear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

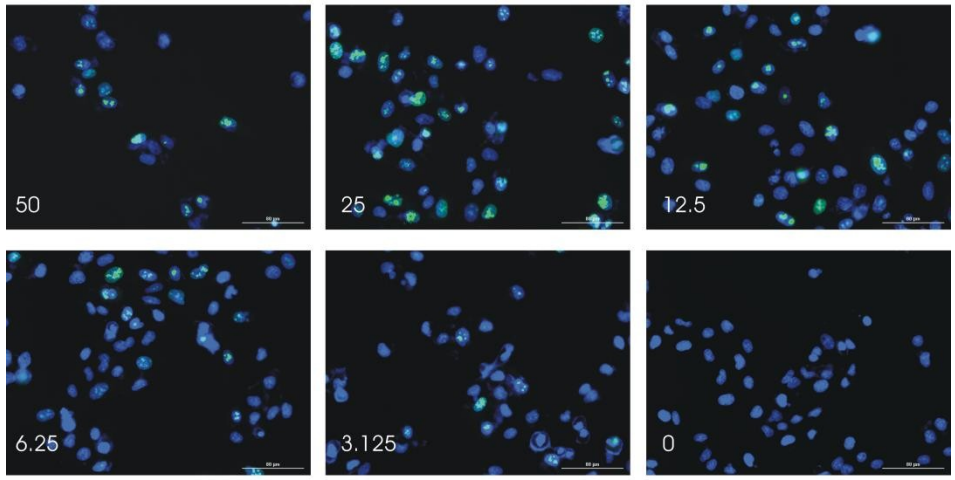
Transfection Optimization-Top Read



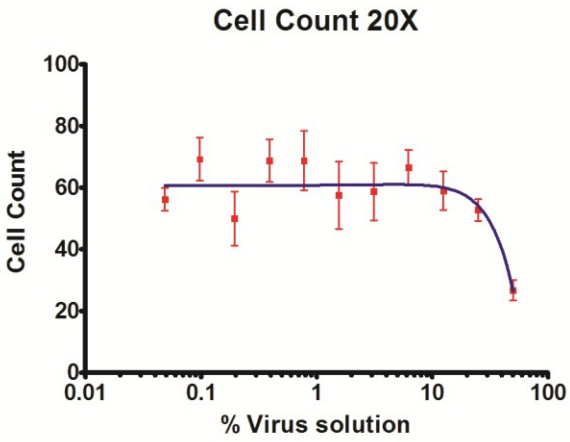


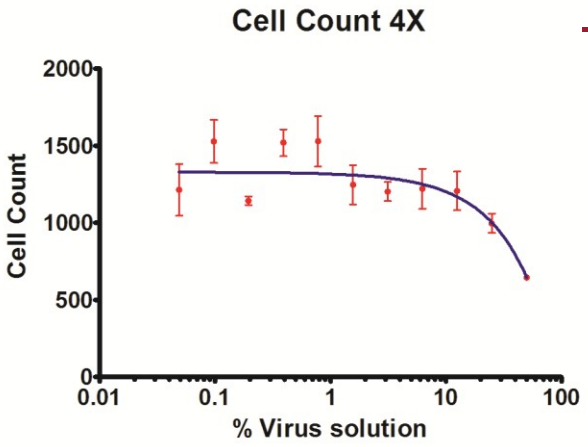
- Fold change generated by comparing top fluorescence reads of GFP signal
- Optimal incubation time seen to be 16 hours with all viral concentrations tested
- 12.5% concentration seen to give largest fold change
  - Higher viral concentrations may be toxic to cell model

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





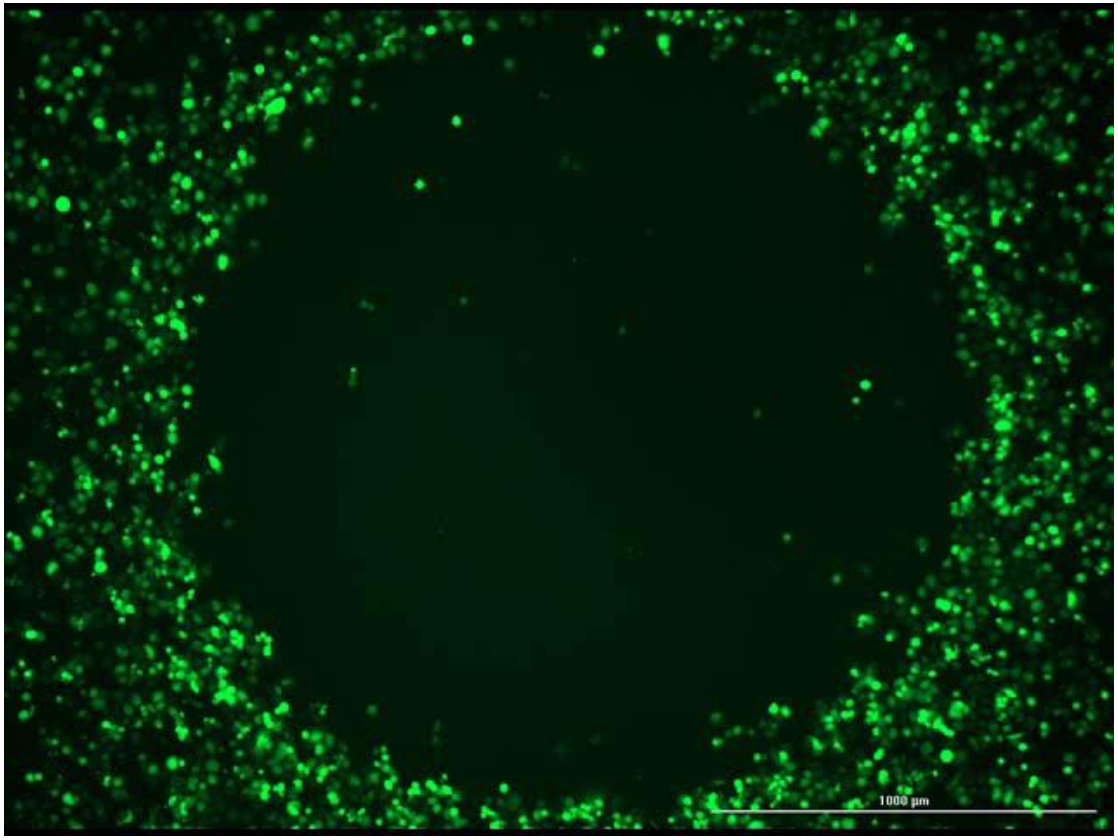
Think Possible

BioTek®

PLATYPUS TECHNOLOGIES

Cell Migration

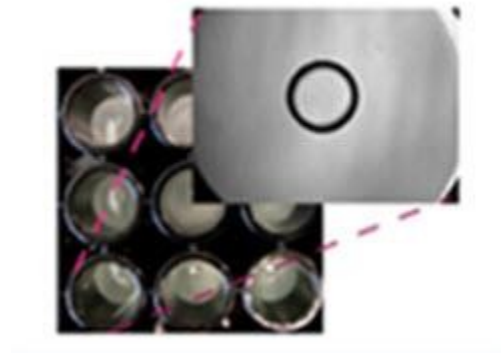
Exploring Cell Movement

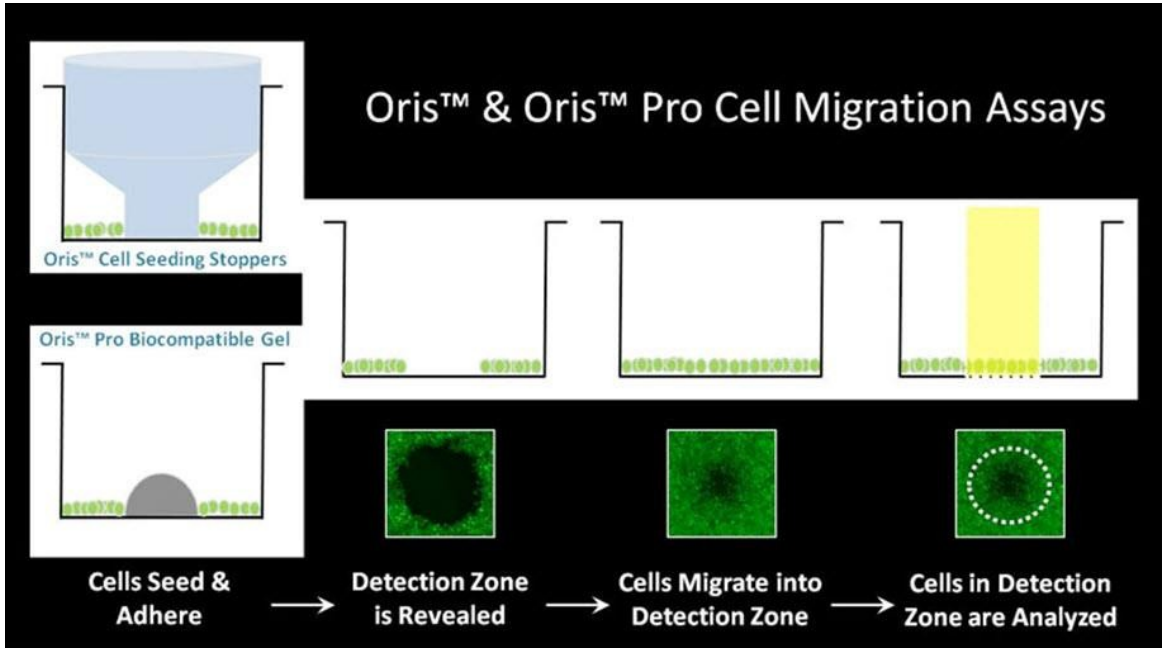


Think Possible

BioTek®

Platypus Oris™ &Oris™ Pro Cell Migration Assay





Think Possible

BioTek®

Cell Migration Assay Workflow

Cell Preparation

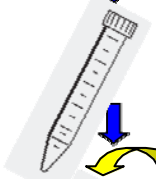
HT1080 cells

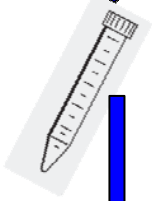
Harvest cells

MDA-MB-231 GFP

Resuspend in phenol/serum free media

Resuspend in phenol free media + FBS



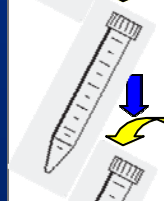


Add Cell Tracker Green CMFDA dye

Incubate 37 °C with stirring 3X10 min.

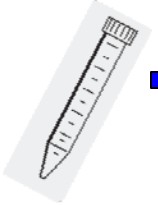
Plate 20 or 90 µL cells (96- or 384-well, respectively) and briefly centrifuge

Incubate 37 °C, 5% CO_2 for 1-4 hours*



Wash cells with phenol/serum free medium

Incubate 37 °C with stirring 3X10 min.



*

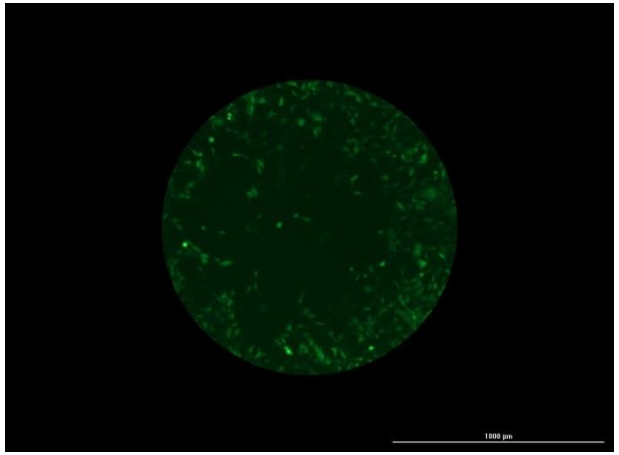
Add 20 or 10 uL compound or control (96- or 384-well, respectively

Image wells w/ Cytation 3 in kinetic mode w/5% CO2 @ 37 °C

Resuspend cells in phenol free medium + FBS

Methods of Image Analysis

- Gen5 – utilizes mask to define cell free zone for GFP intensity analysis
  - Reader control and data analysis



- Area of coverage can be measured in Gen5
  - Plug and threshold

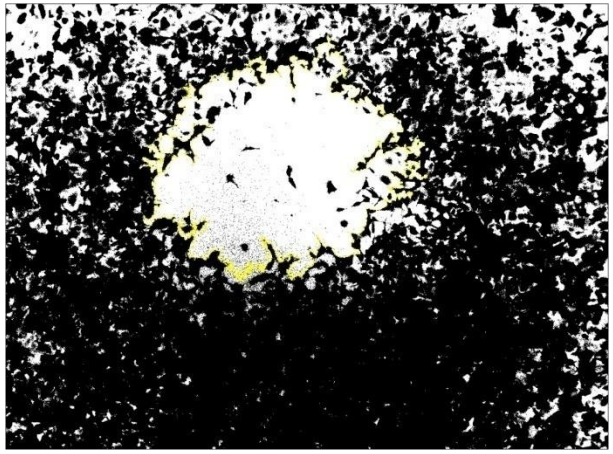
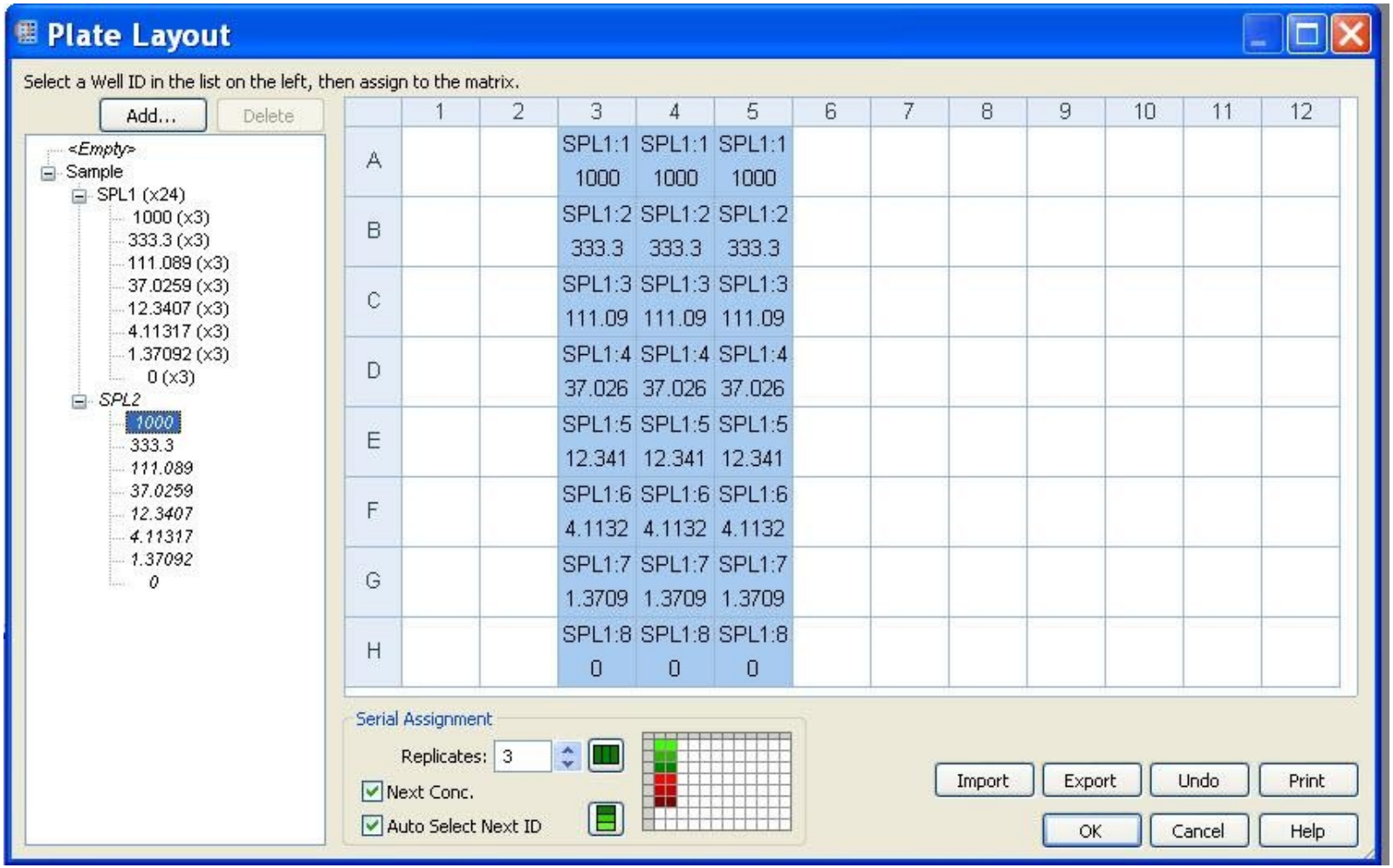


Plate Layout

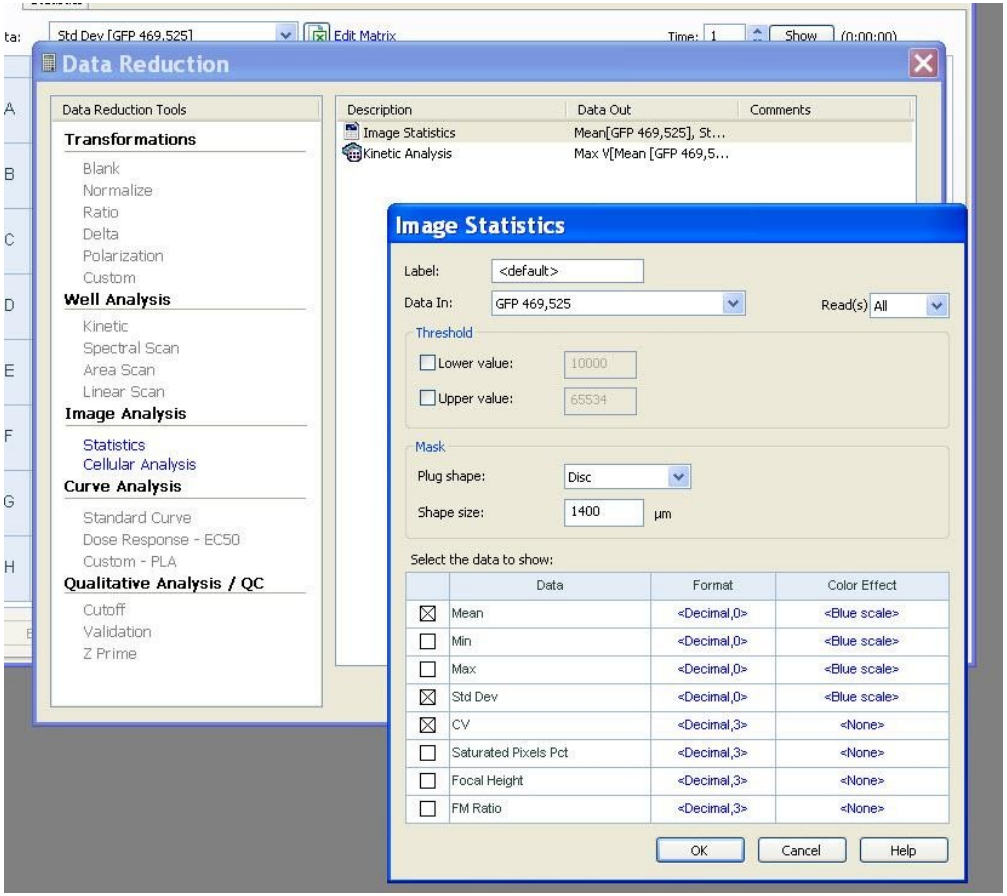


Think Possible

BioTek®

Gen5 Data Analysis: 2 Ways to Set up Statistical Analysis

Gen5 data reduction step

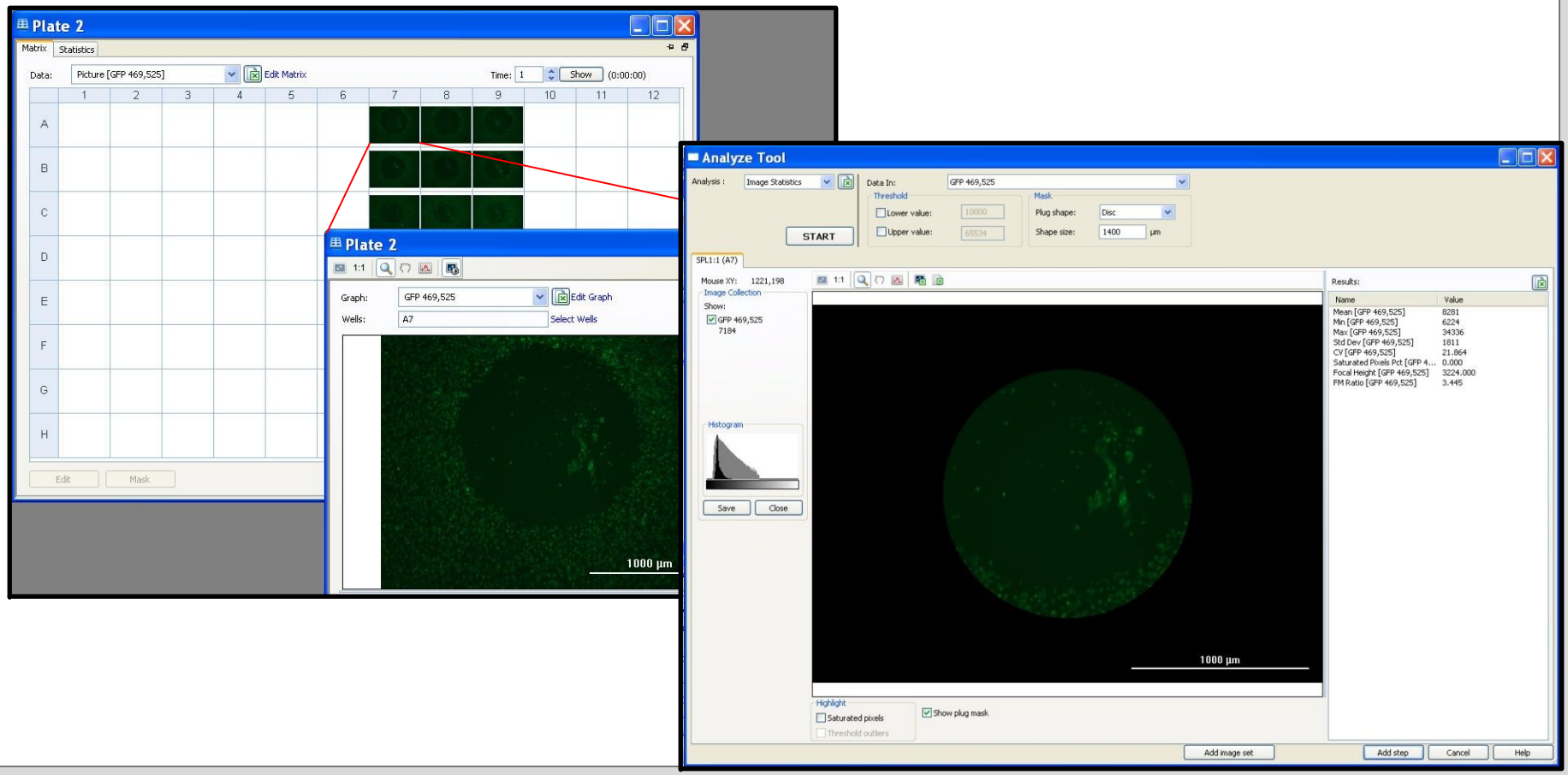


Think Possible

BioTek®

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

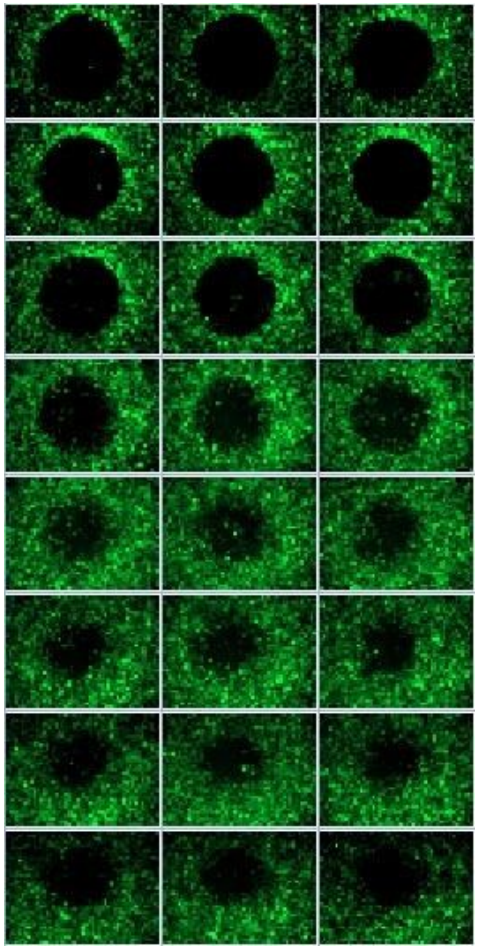
Use of **analyze tool** from picture view



Think Possible

BioTek®

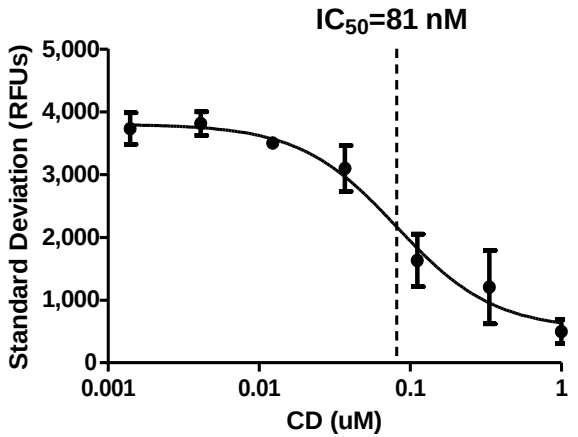
Statistical Analysis of MDA-MB-231 Cells: Standard Deviation



569	281	649
1122	1827	672
1156	1797	1944
2732	3463	3097
3426	3476	3610
3611	3984	3845
3651	3531	4015
3386	3721	3343

~7-fold assay window

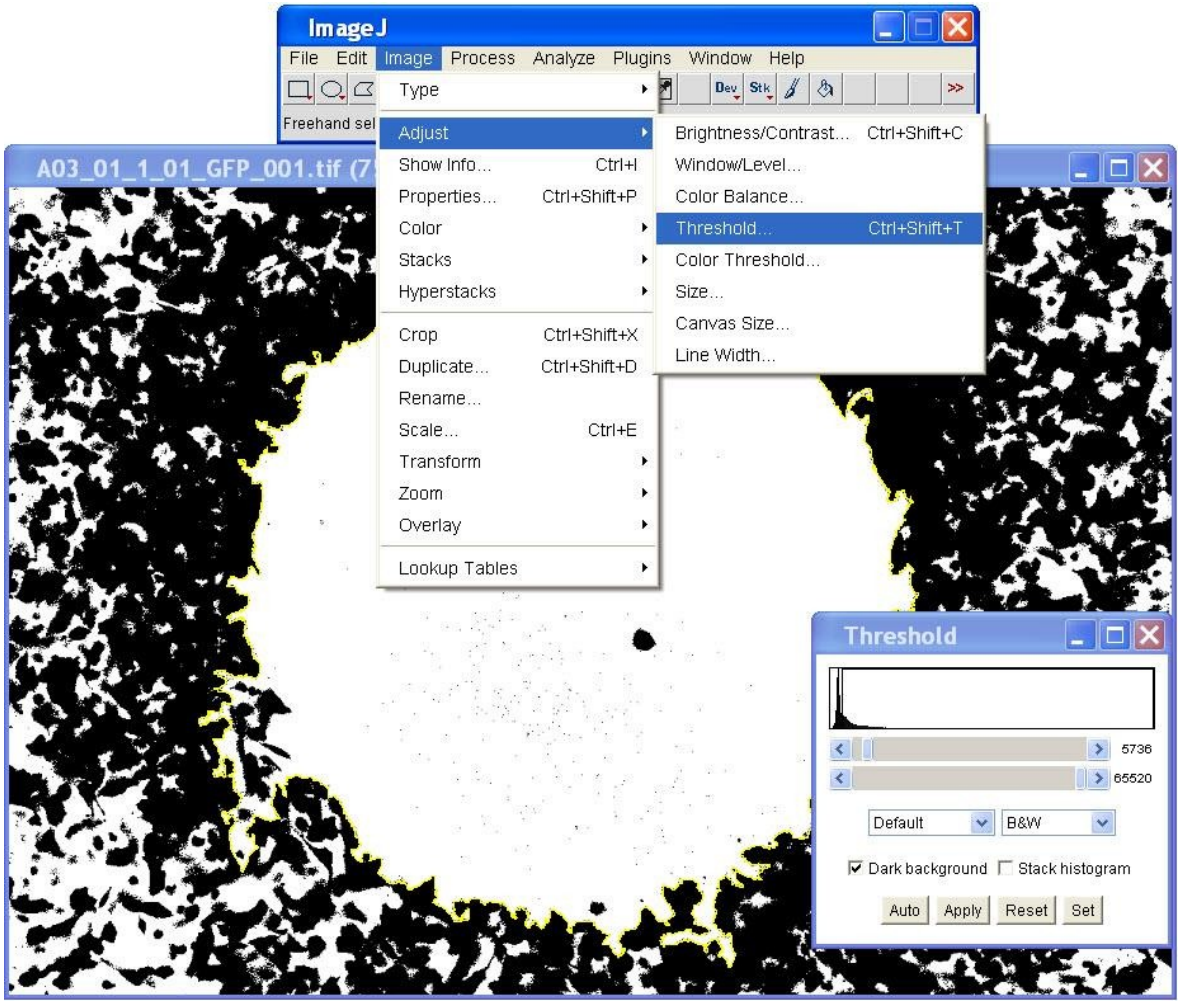
Standard Deviation Analysis of MDA MB 231 GFP cells



ImageJ Data Analysis: Portability of Cytation 3 Imaging Data

- Several software packages are available for image analysis
- ImageJ – open source software: http://rsbweb.nih.gov/ij/
  - ImageJ is written in Java, which allows it to run on Linux, Mac OS X and Windows, in both 32-bit and 64-bit modes

ImageJ Analysis



1) Open image

1) Adjust threshold to increase contrast

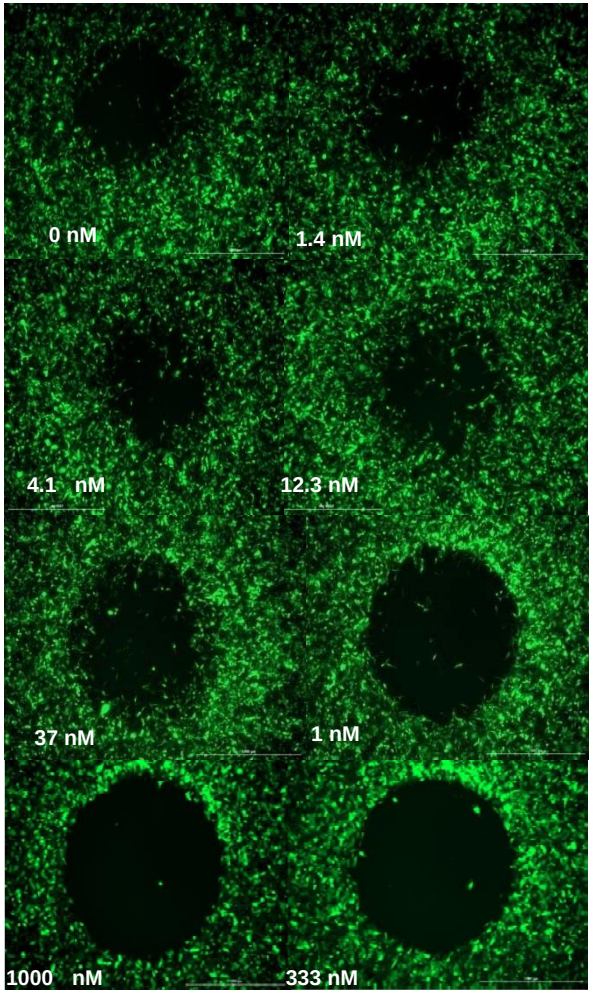
3) Use magic wand tool to select area

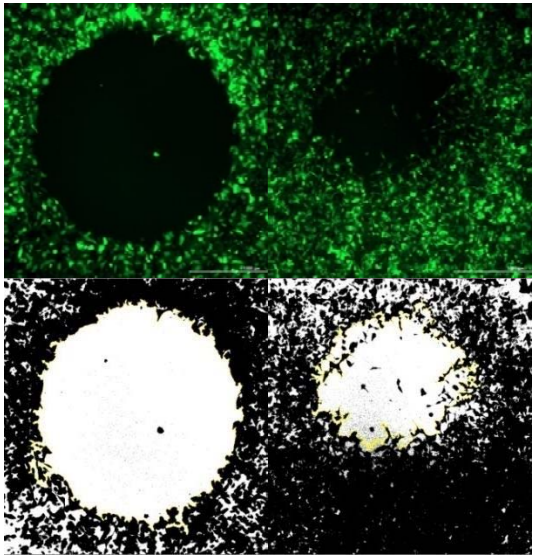
3) Select measure to calculate area

Think Possible

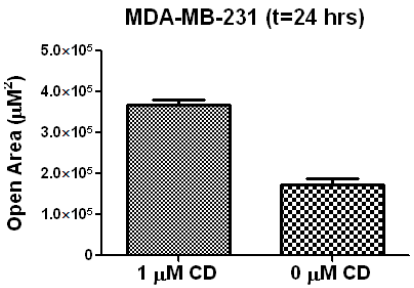
BioTek®

ImageJ Analysis



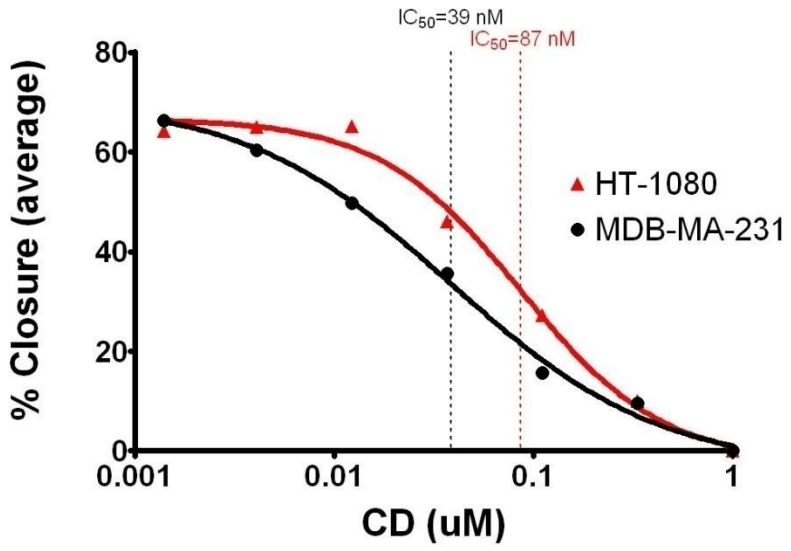


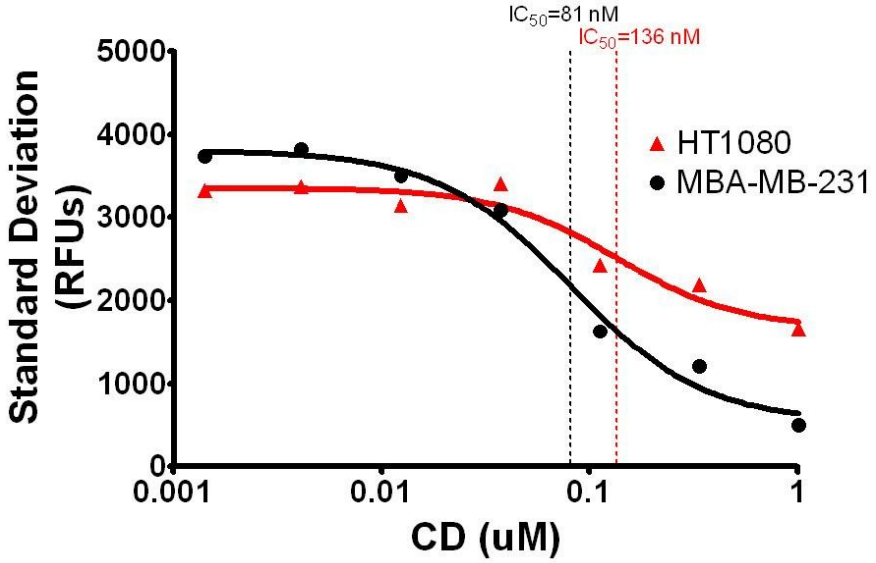
1 µM CD 0 µM CD



~3-fold assay window

Comparative Analysis



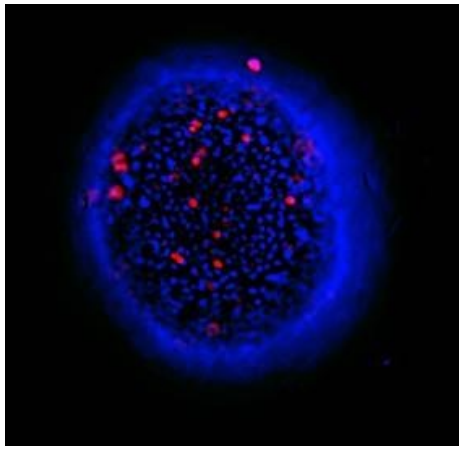


Think Possible

BioTek®

3D Cell-Based Imaging

Analysis of Single-plane and Z-stacked Tumoroid and Spheroid Images



Think Possible

BioTek®

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®

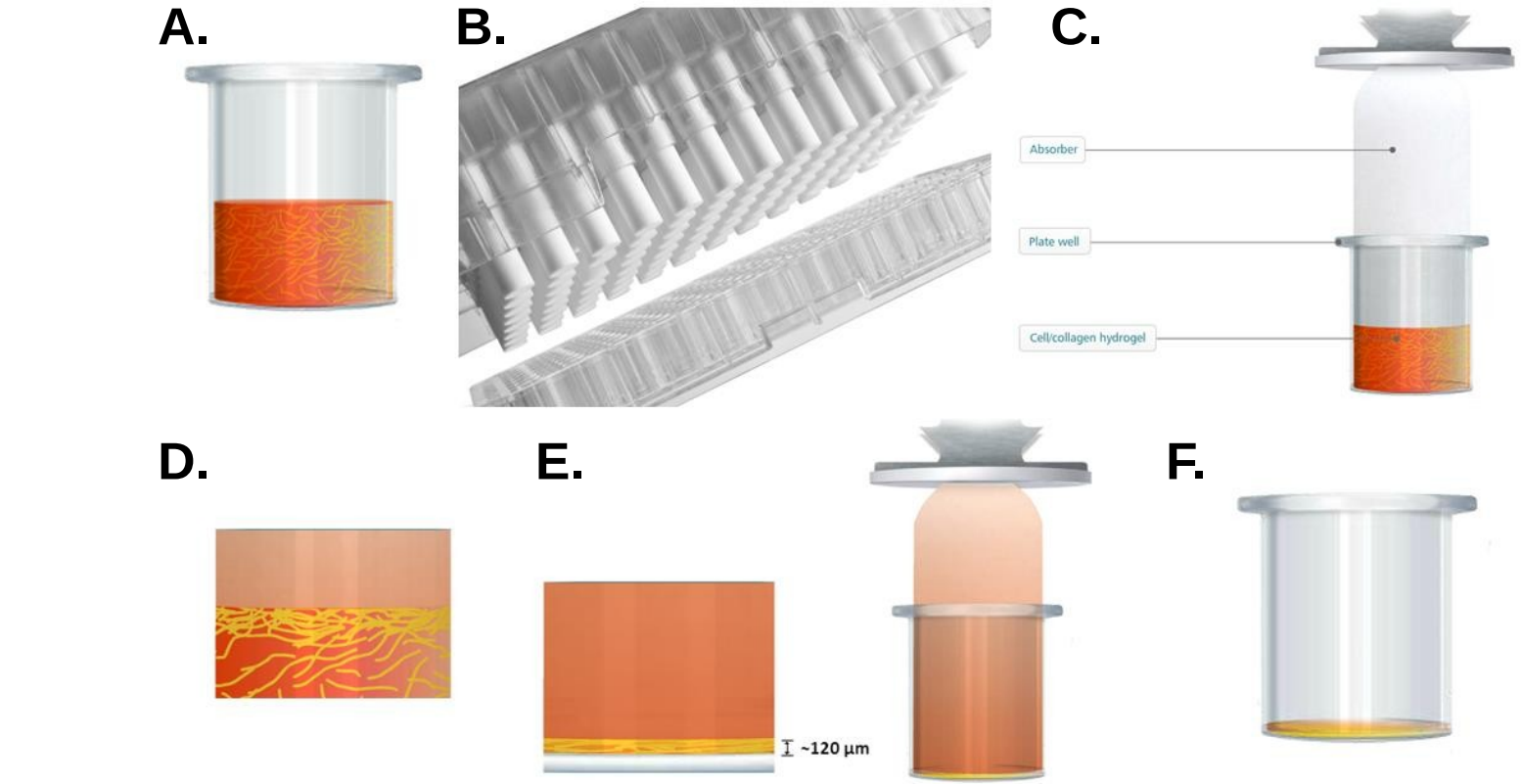
TAP Biosystems RAFT System HCT116 Tumoroid Analysis

- HCT116 cells cultured in T75 flasks
- Cells mixed with collagen mixture, plated, and incubated at 37 ^{o}C for 15 mins
- Excess medium removed by proprietary absorbers for 15 minutes
  - Creates a 100 um thick layer of cells and collagen
- Plates incubated for three days to allow “tumoroids” to form within collagen
- Cells stained with nuclear, actin, and cell membrane fluorescent probes
- Pipetting performed manually, however MultiFlo FX also used to automate assay procedures using RAFT system
- Imaging, and cellular analysis performed using the Cytation 3 and Gen5 software
  - Manual imaging performed to capture images
  - Z-stacking also performed using external software

Think Possible

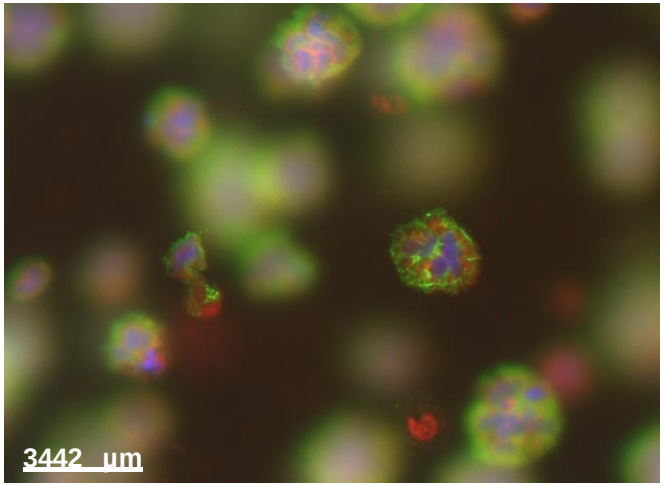
BioTek®

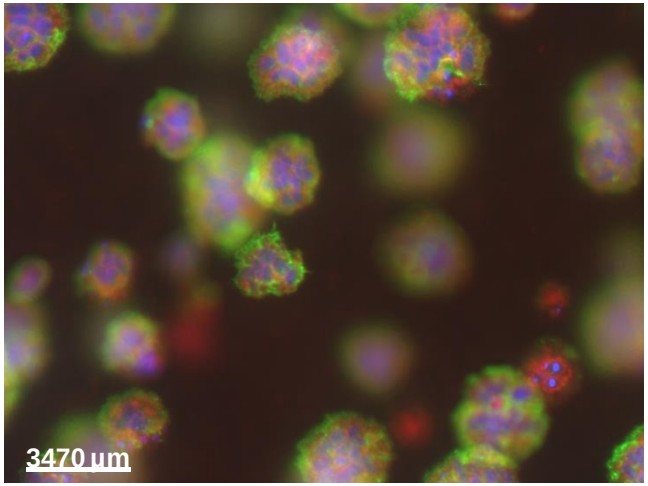
RAFT 3D Cell Culture Creation Procedure

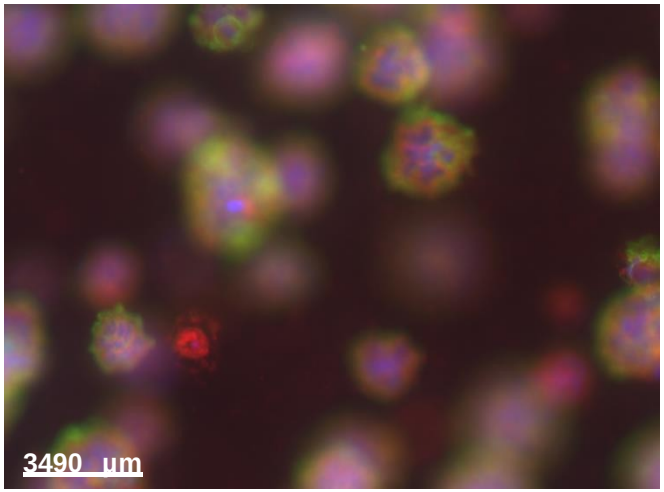


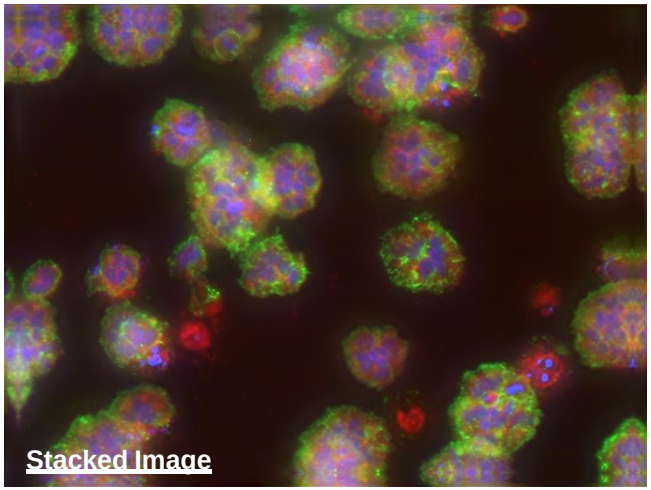
The RAFT 3D Cell Culture System. **(A)** Cell/collagen mix within a microplate well. **(B)** Specialized RAFT plate showing sterile absorbers. **(C)** Absorbers remove medium to concentrate the cell/collagen mix. **(D)** Concentrated cell/ collagen mix. **(E)** Cell/collagen layer showing absorbed medium.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

Tumoroid Image









Objective: 20x

Cell Stains:

Blue – DAPI

Green – Alexa Fluor 488 Phalloidin

Red – CellMask Orange Plasma Membrane Stain

Think Possible

BioTek®

Z-Stacking Procedure

- Manual images are captured with the Cytation 3 using the same exposure settings and X and Y position
- Individual color images saved as 16-bit .tif files
- Z-stack captures images on multiple planes, Gen5 processes based on user-specified selections
- Gen5's z-projection processes stacked images into a compiled 3D image
- Image and cellular analysis performed on individual 'slices" or on the z-projection
- Compatible with both endpoint and kinetic (time lapse) read modes

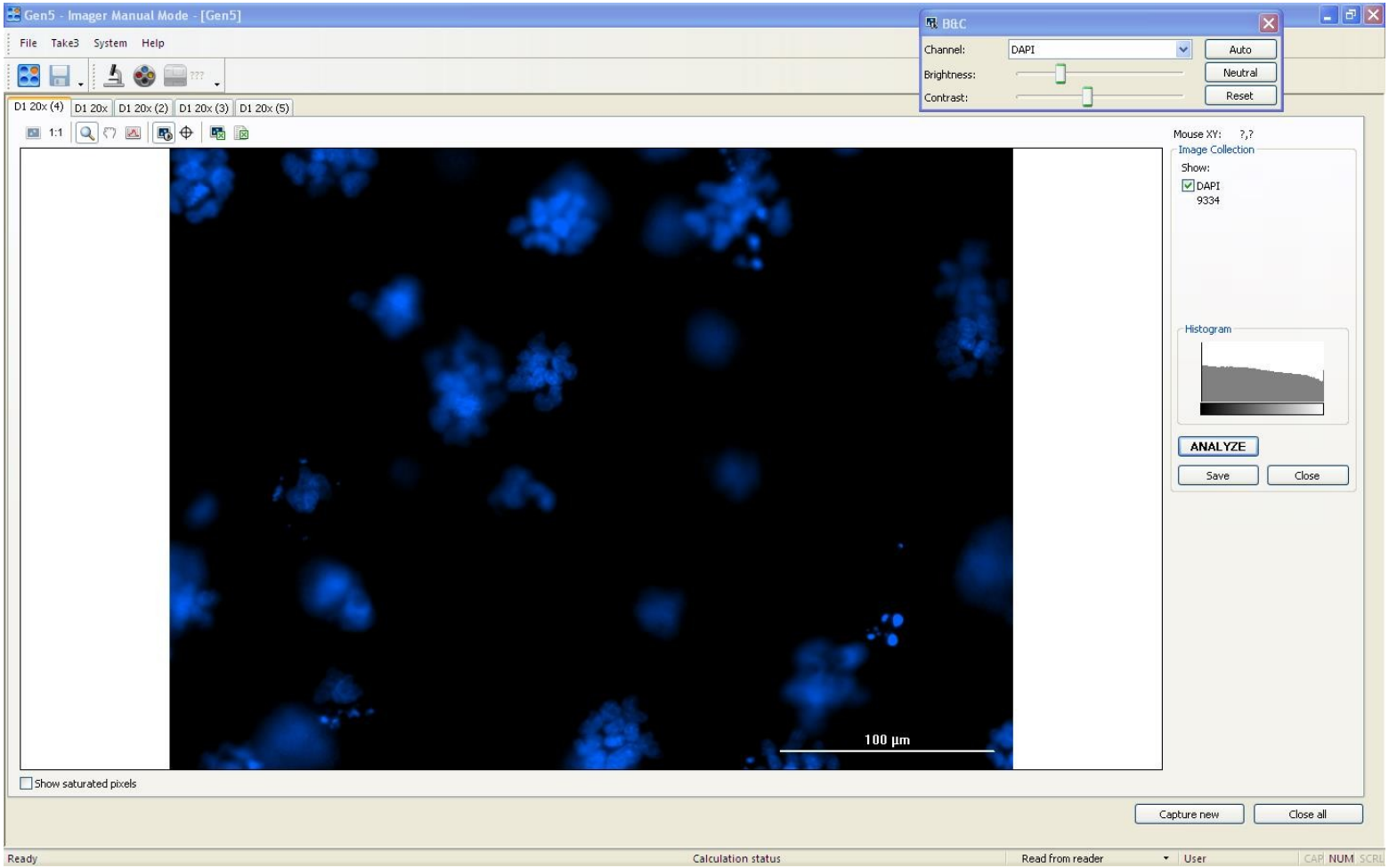
Other Software with Z-Stacking Capability

- If further z-stack tools are needed, other options are available:
  - Adobe Photoshop (proprietary)
  - ALE (GPL/open source)
  - Enfuse (combined with align_image_stack or similar. GPL/open source)
  - Stack Focuser (GPL/open source)
  - Tufuse (“Freeware”)
  - ImageJ (GPL/open source)
  - Combine ZP

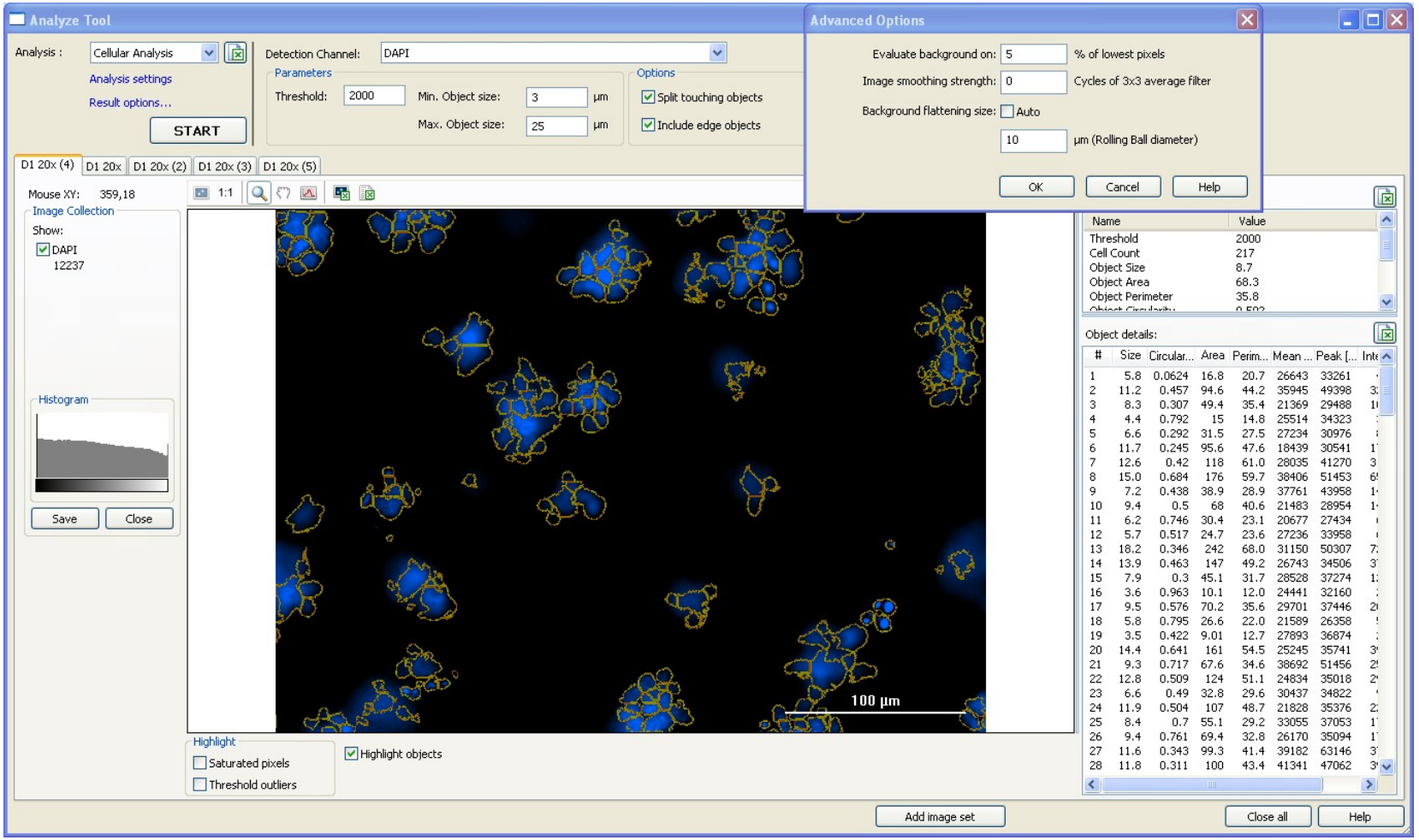
Think Possible

BioTek®

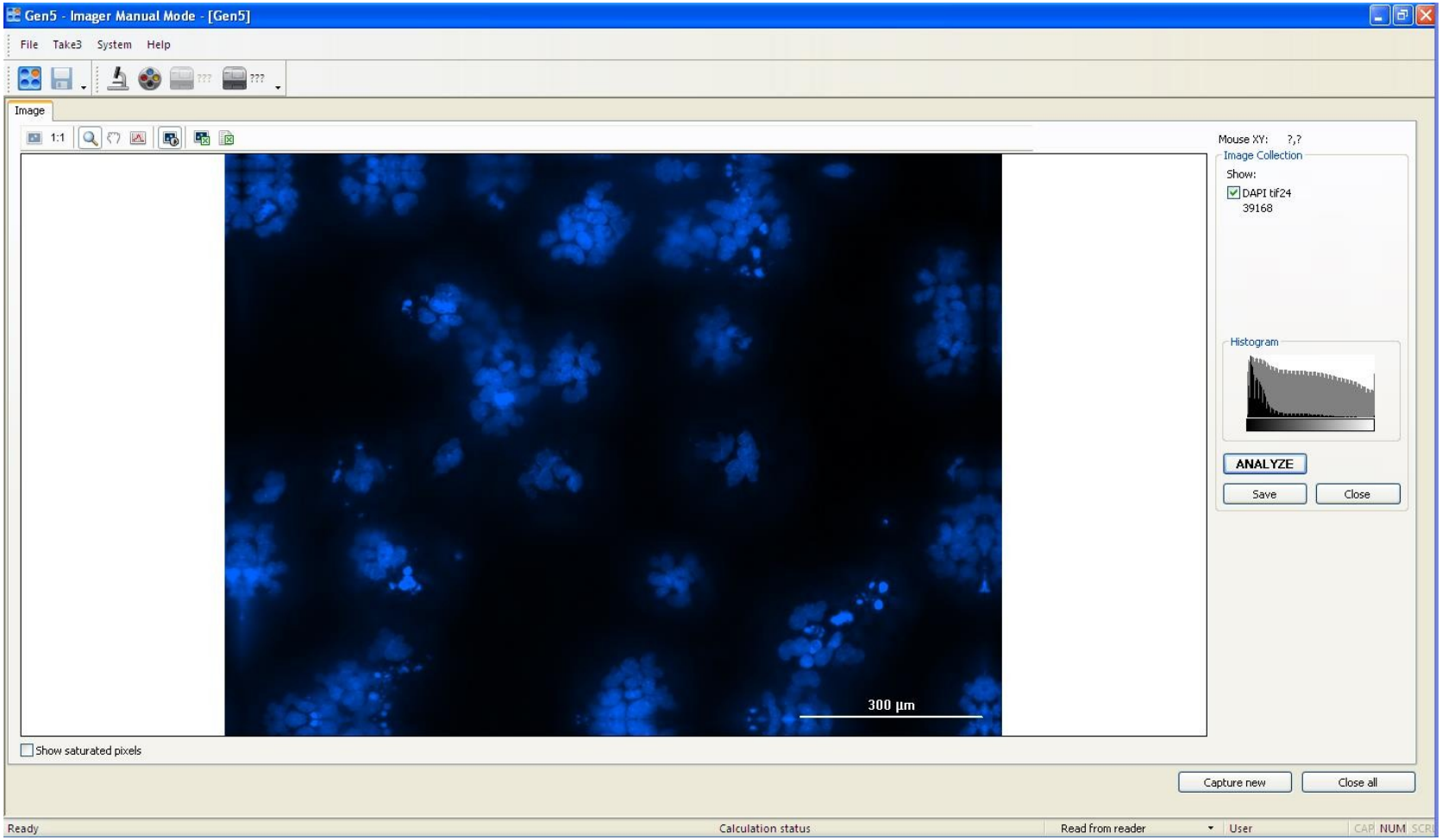
Single Plane Tumoroid Image (20x Objective/DAPI Channel)



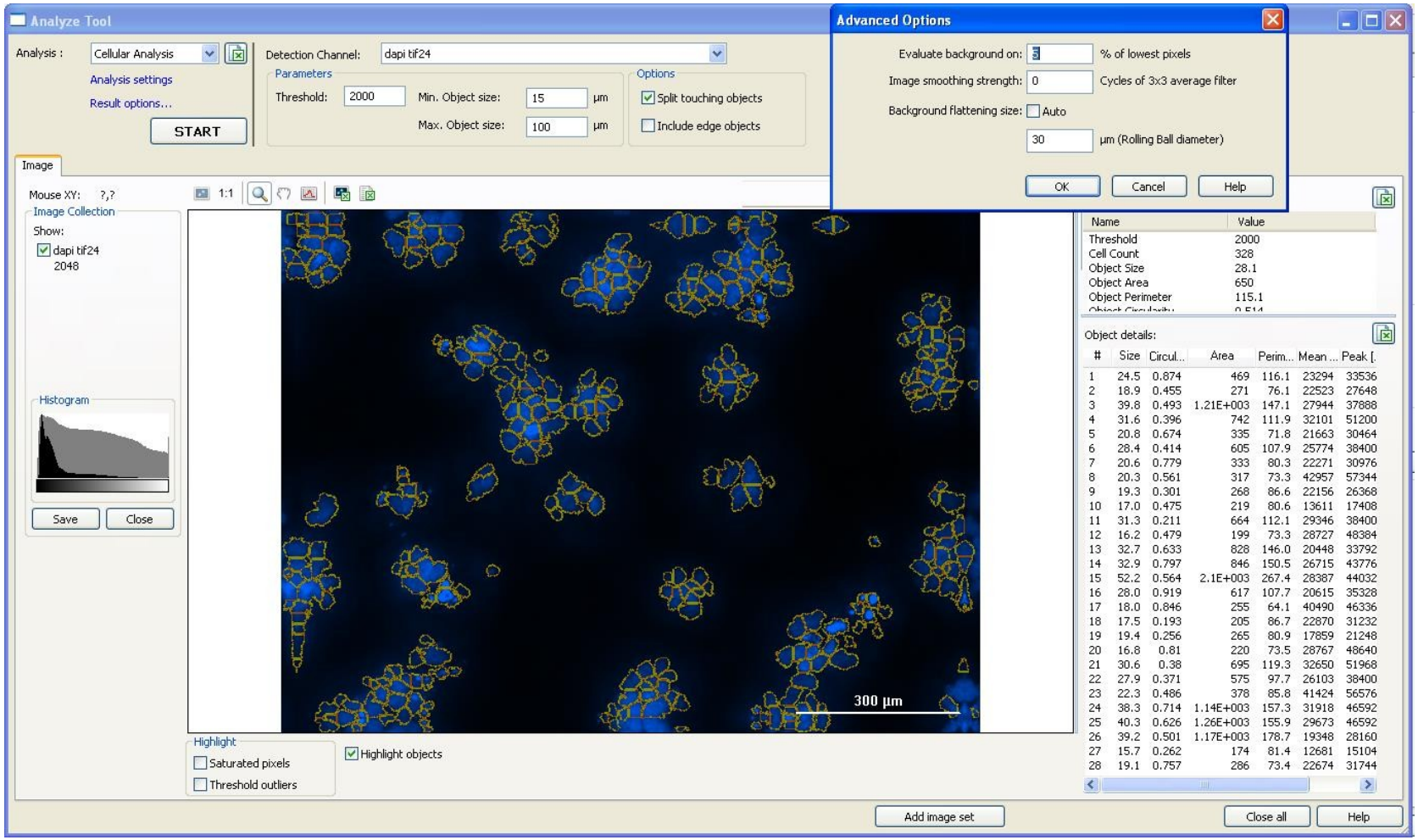
Single Plane Tumoroid Cell Analysis Parameters



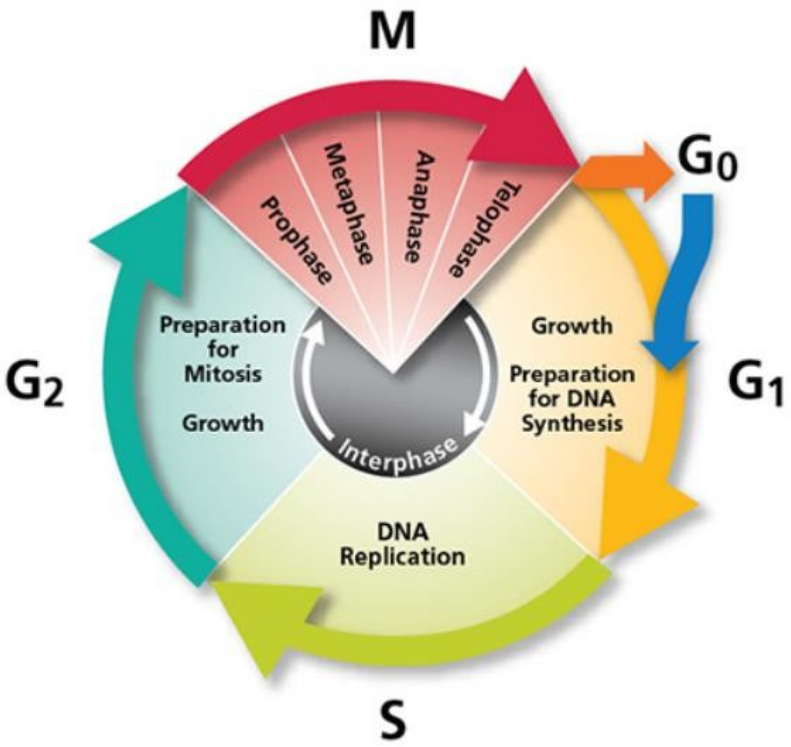
Z-stacked Tumoroid Image (20x Objective/DAPI Channel)



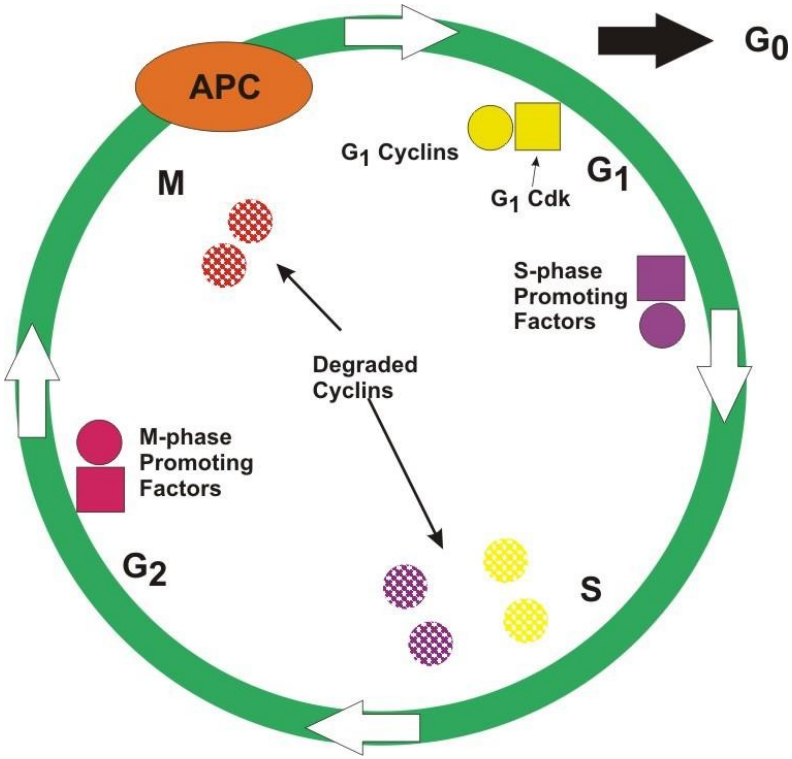
Z-stacked Tumoroid Cell Analysis Parameters



Cell Cycle Analysis: Mitosis



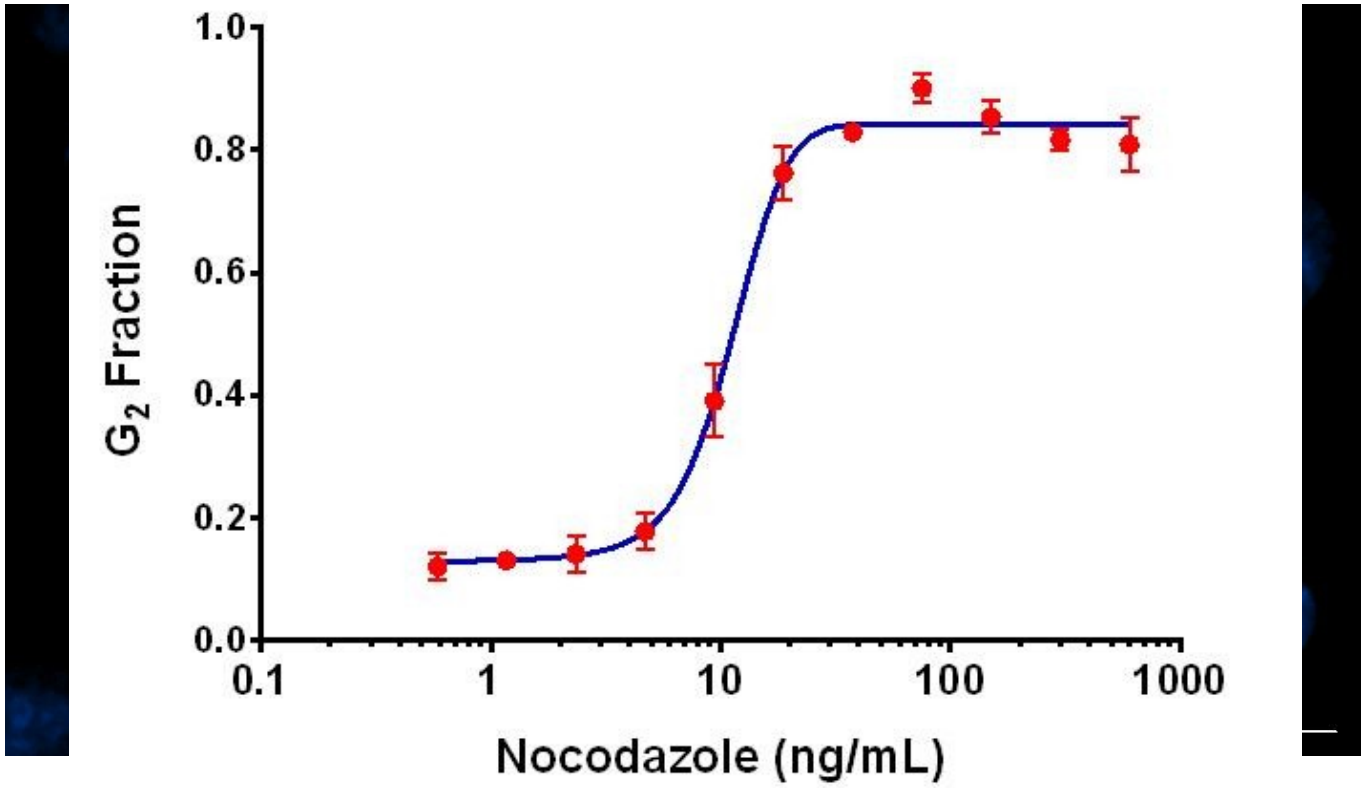
Cell Cycle Analysis



Think Possible

BioTek®

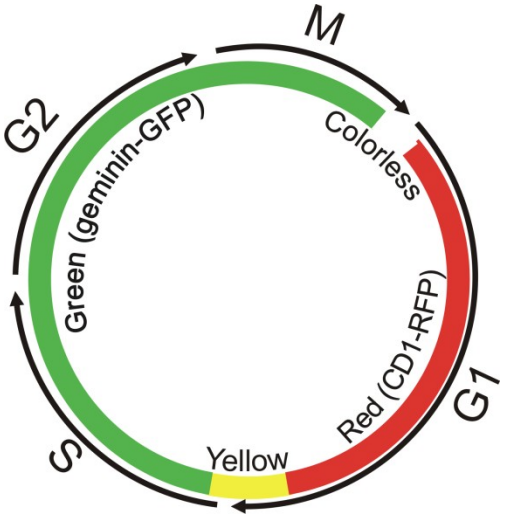
Cell Cycle Analysis: DNA content

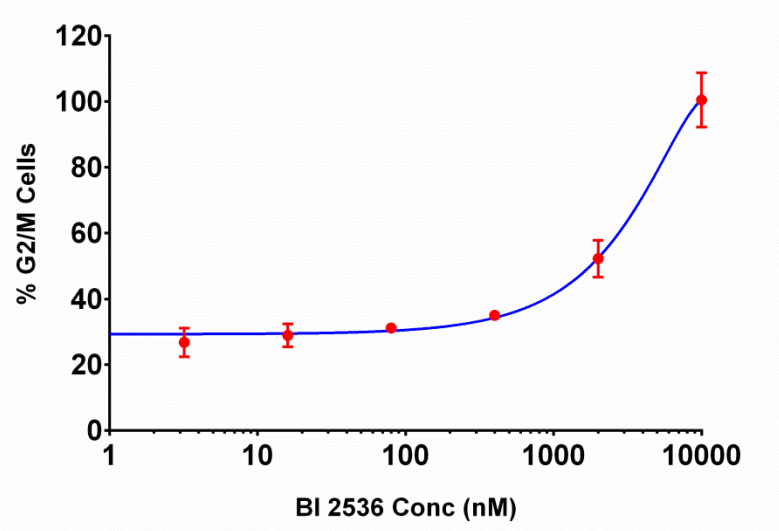


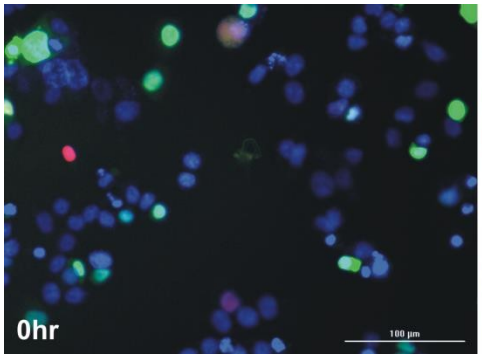
Think Possible

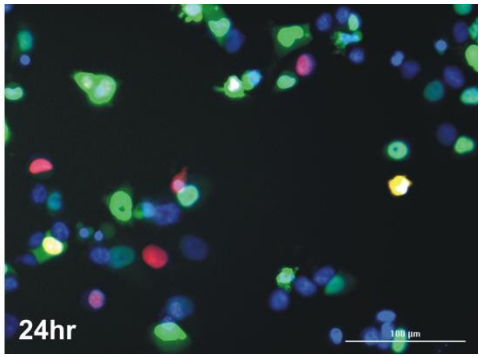
BioTek®

Cell Cycle Analysis: FUCCI dyes







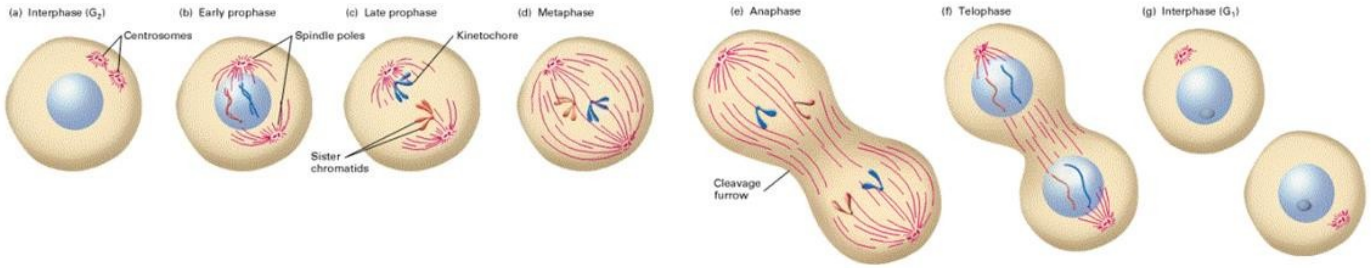


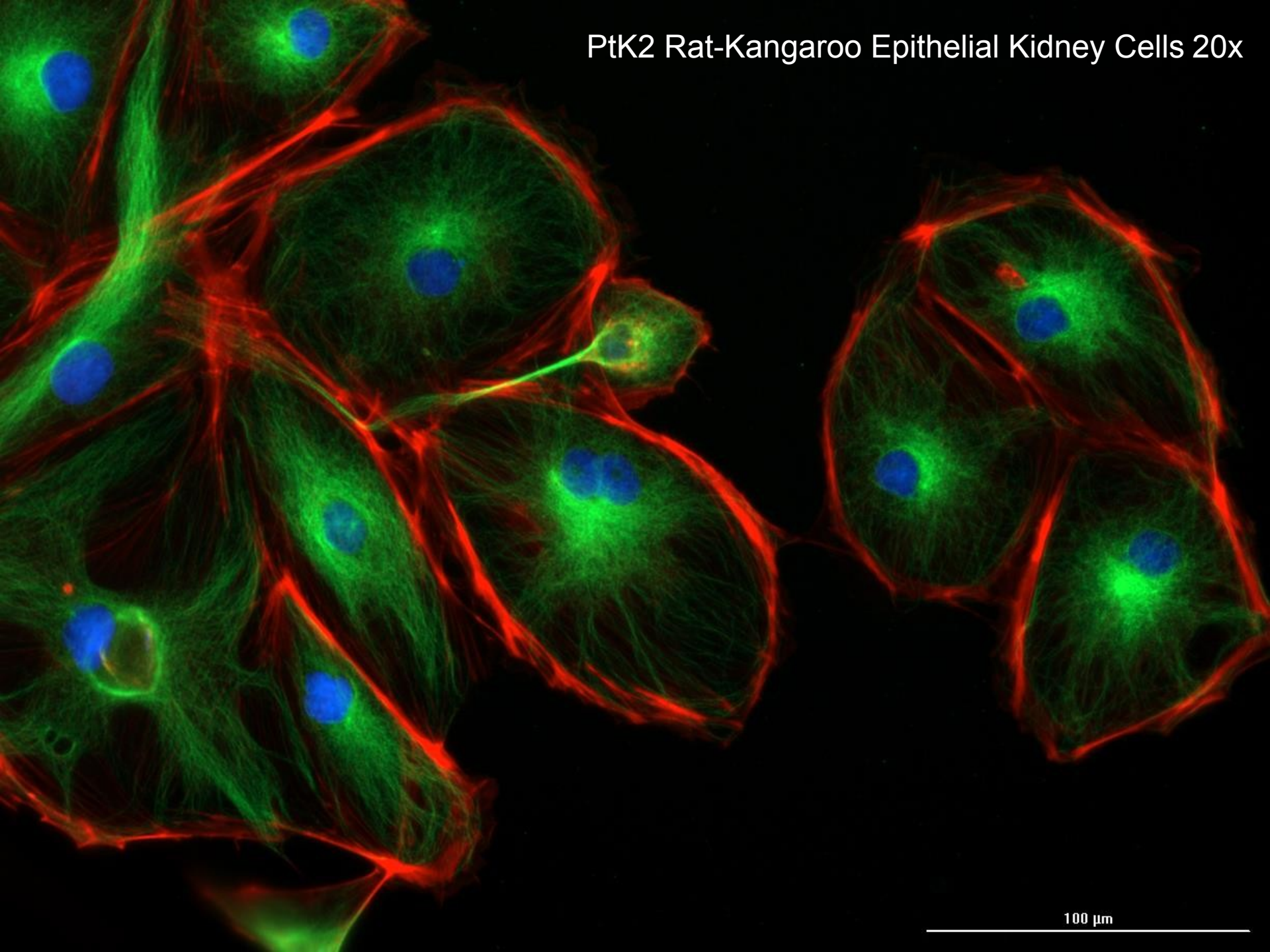
Think Possible

BioTek®

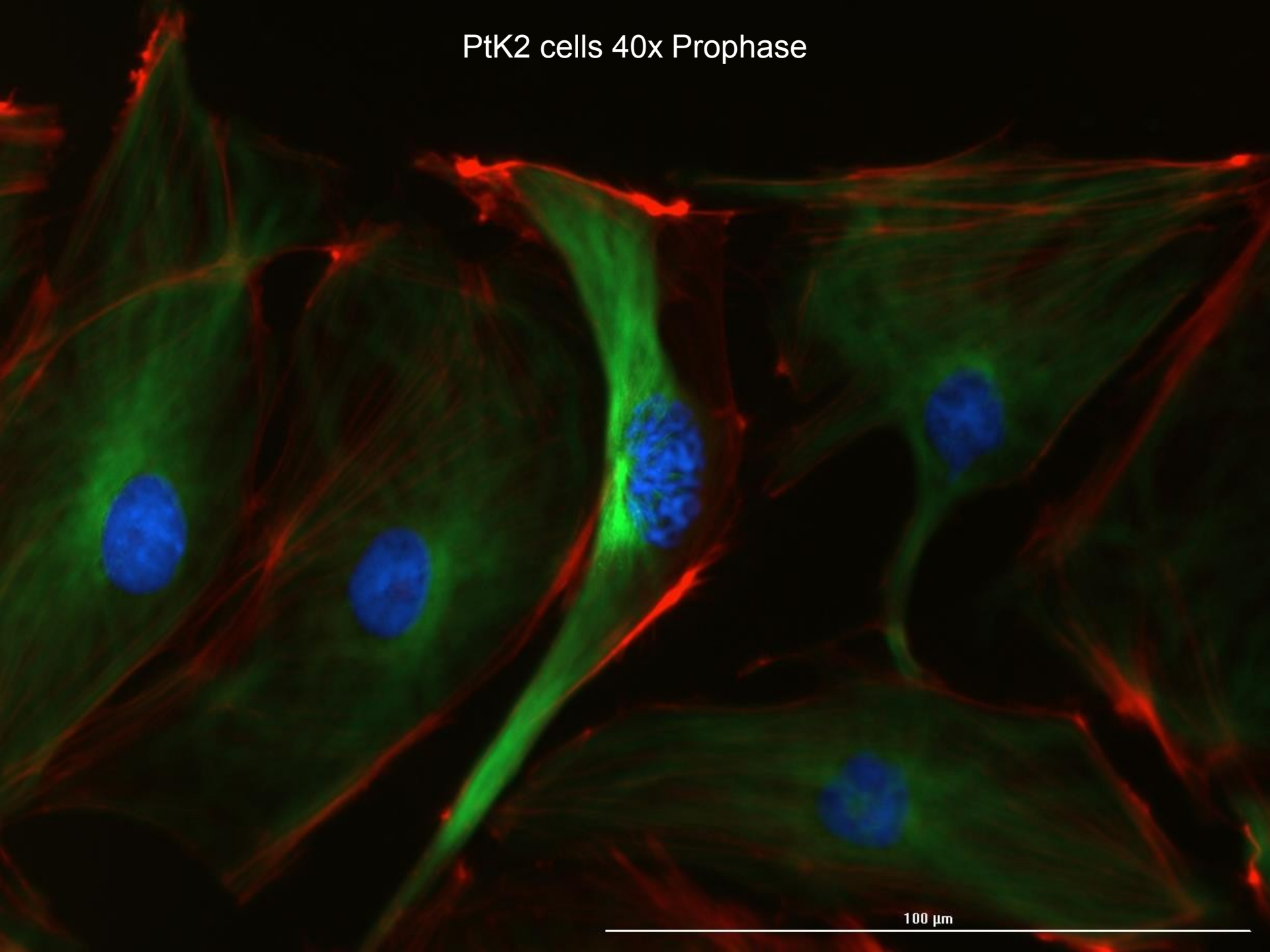
Cell Cycle Imaging to Study Mitosis

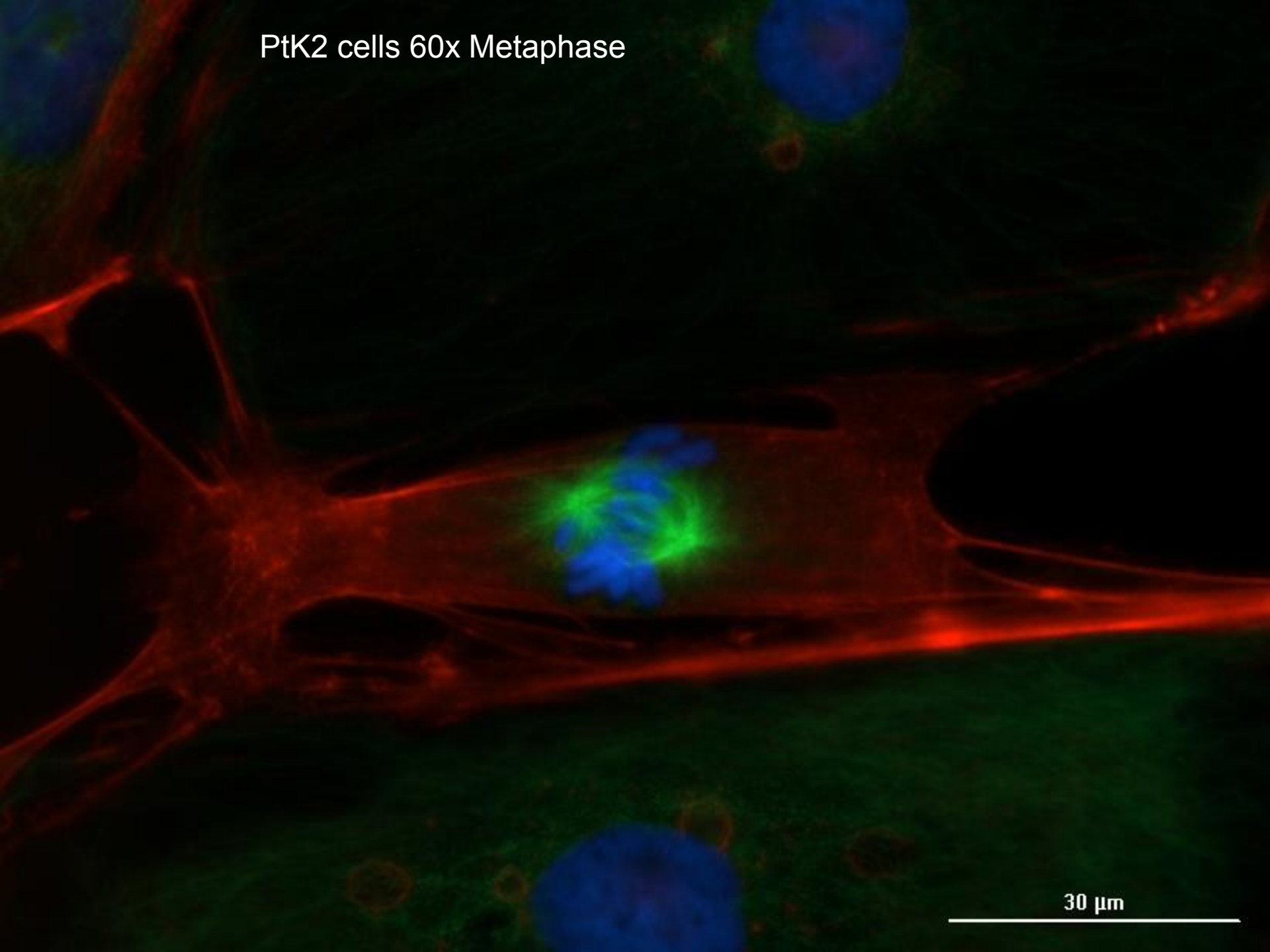
- PtK2 cell line derived from male rat-kangaroo epithelial kidney cells
  - Relatively large cells
  - Small number of chromosomes
  - Cells remain flat during mitosis
    - Most adherent cell types tend to round up making mitosis hard to visualize
- Labeling:
  - Anti-tubulin mouse monoclonal primary
  - Alexa 488 secondary goat anti-mouse
  - DAPI
  - Texas Red phalloidin
- Cells were imaged at varied time points at 20, 40 or 60x to visualize different stages of cell cycle

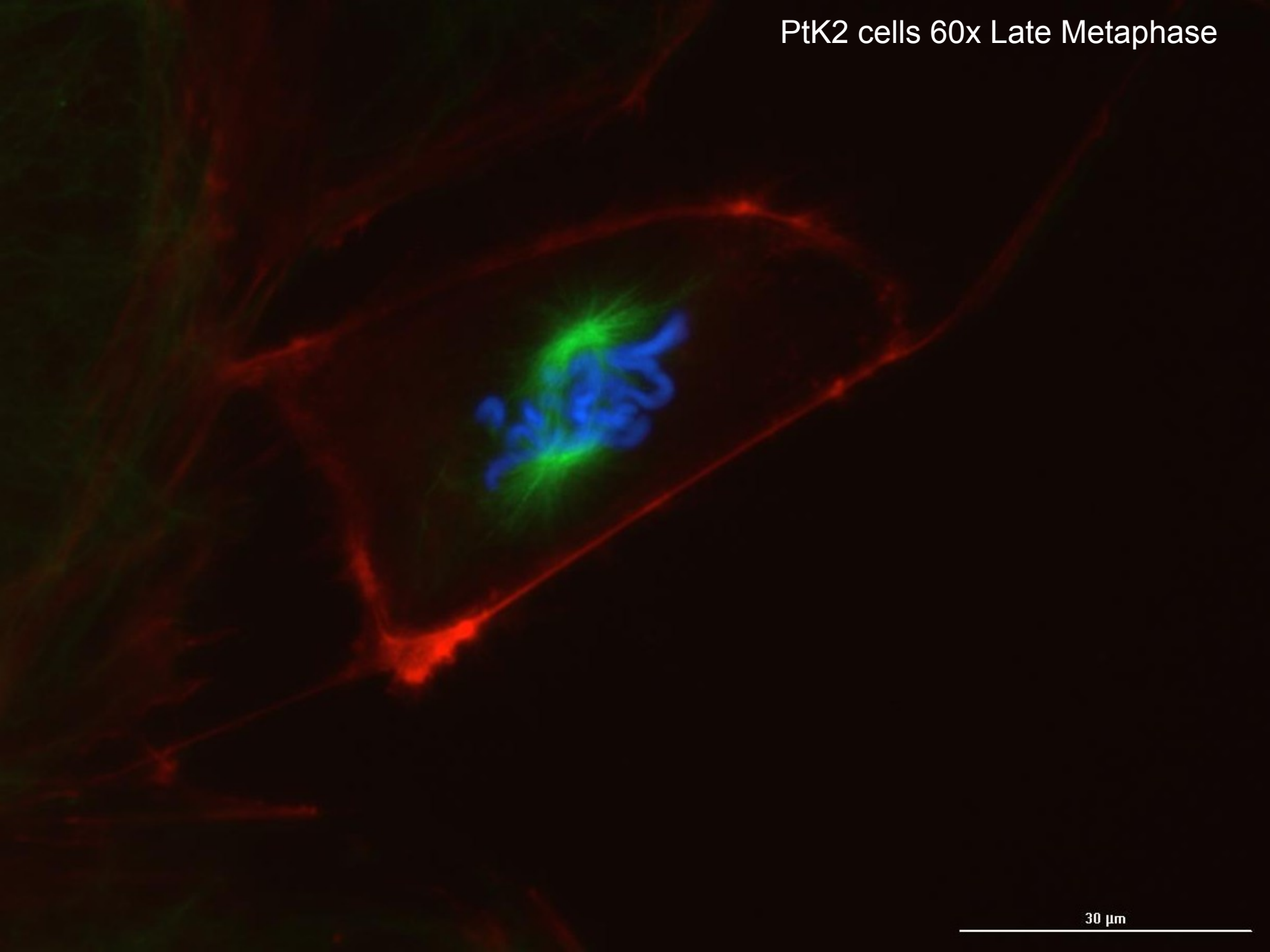


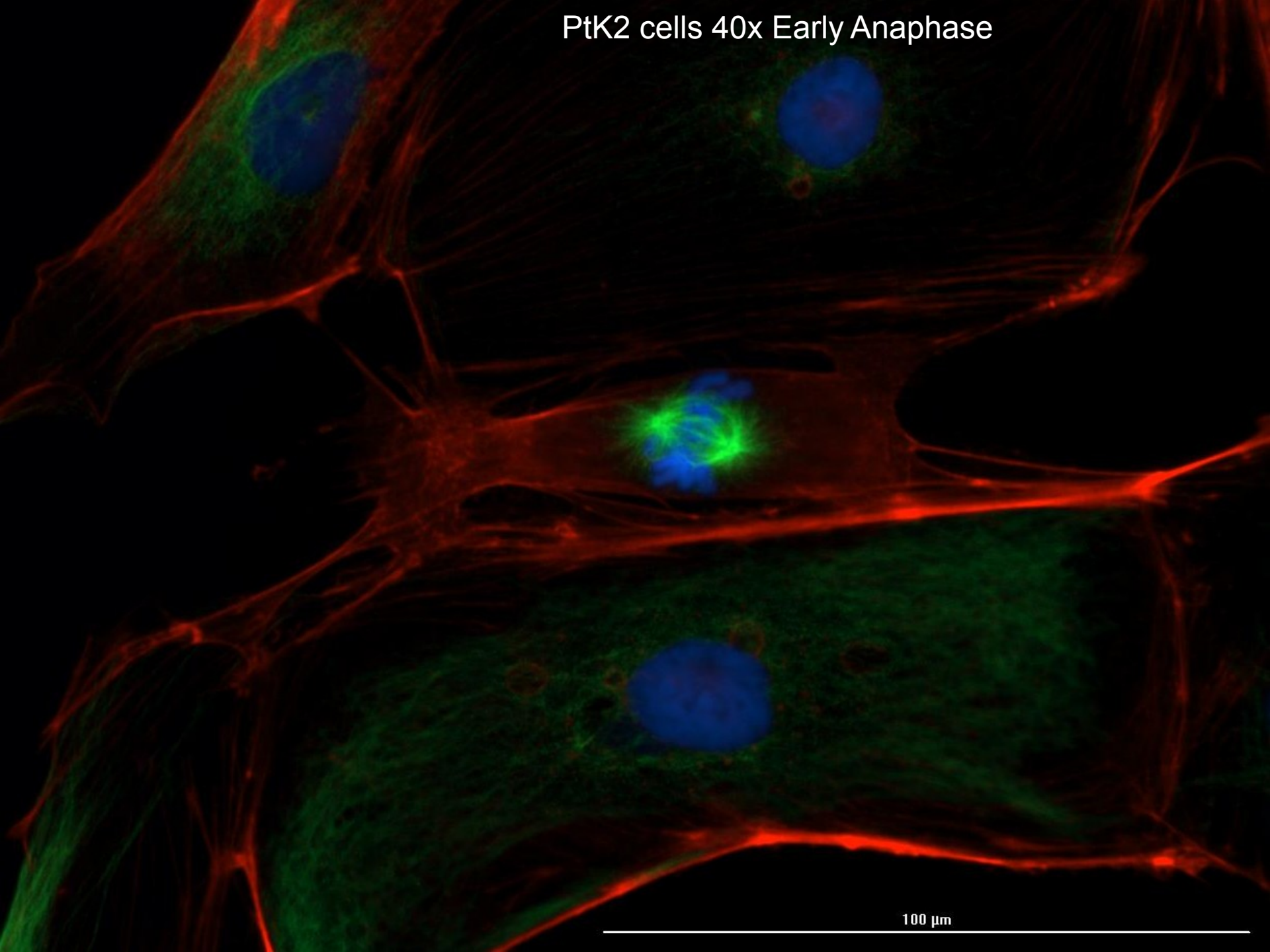

PtK2 Rat-Kangaroo Epithelial Kidney Cells 20x
100 µm

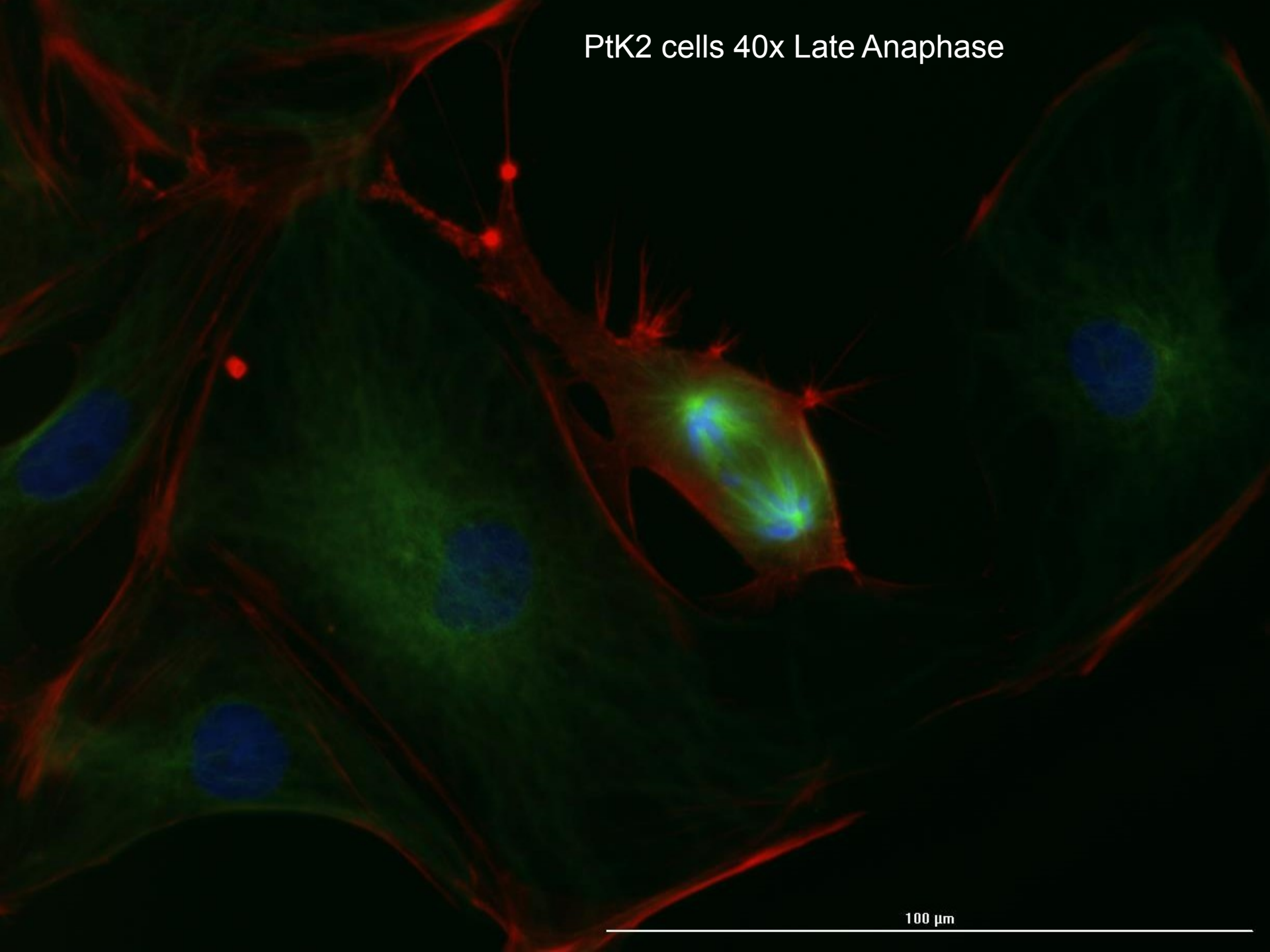
PtK2 cells 40x Prophase

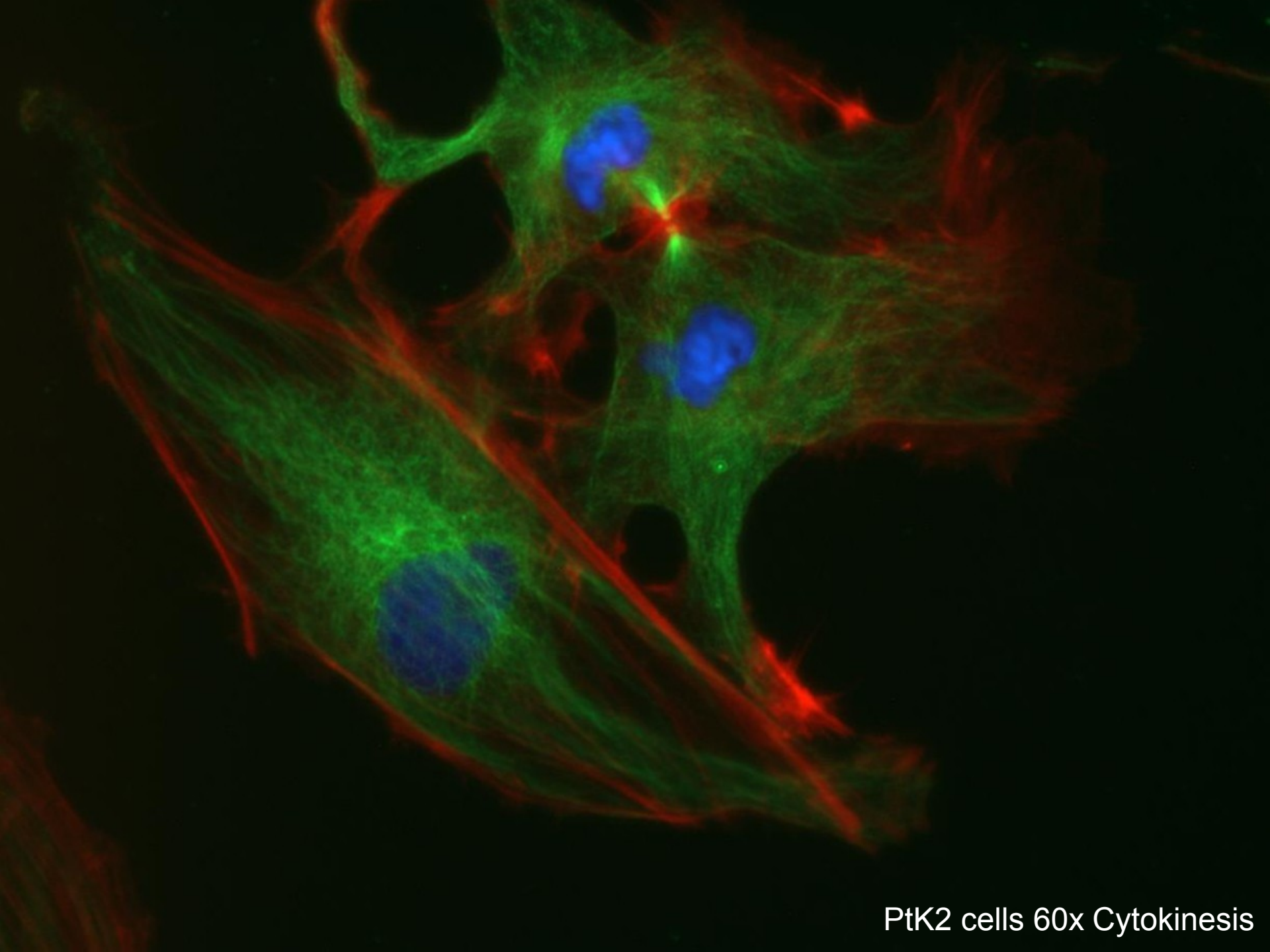



PtK2 cells 60x Metaphase
30 µm

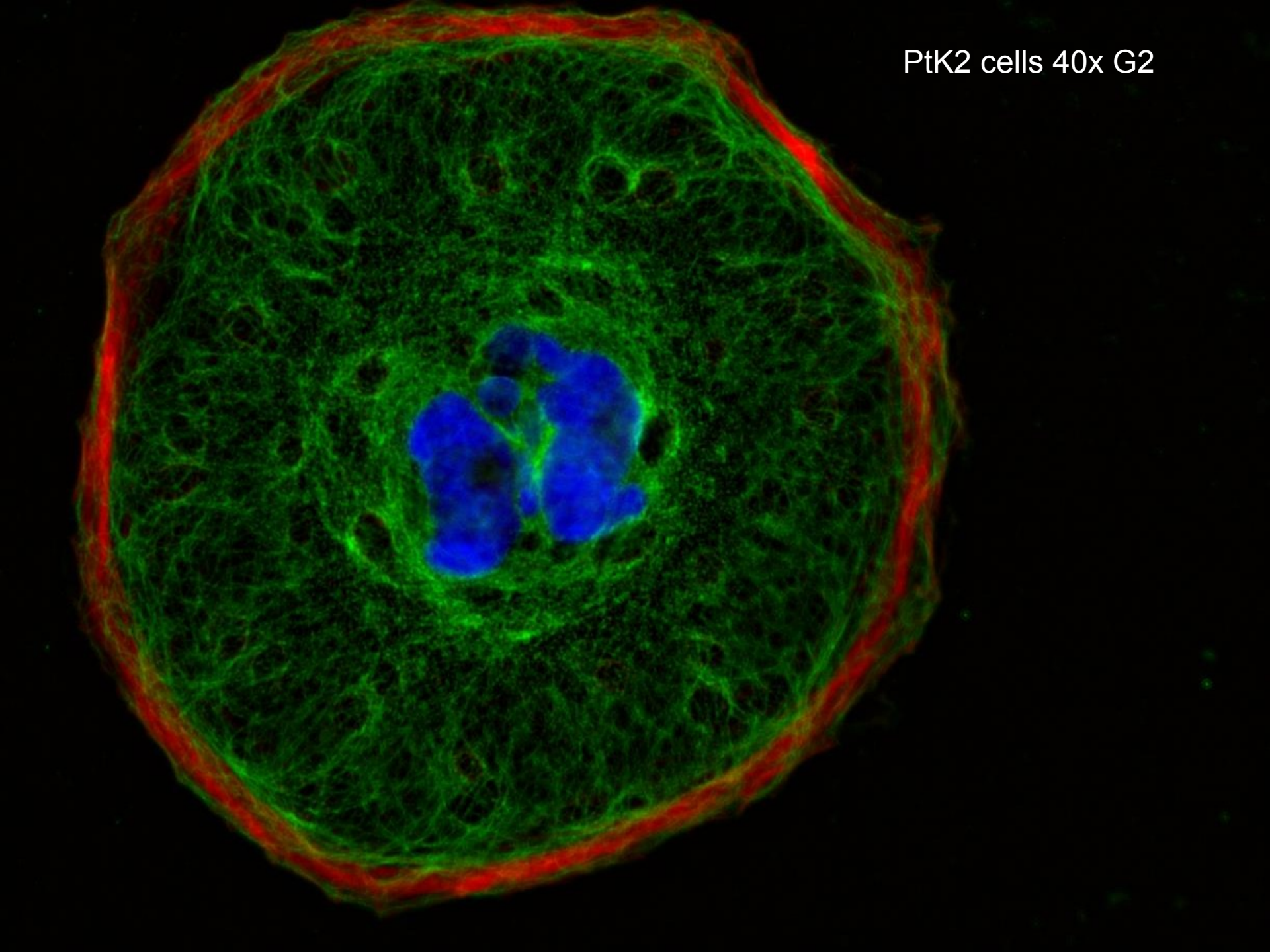

PtK2 cells 60x Late Metaphase
30 µm


PtK2 cells 40x Early Anaphase
100 µm


PtK2 cells 40x Late Anaphase
100 µm



PtK2 cells 60x Cytokinesis

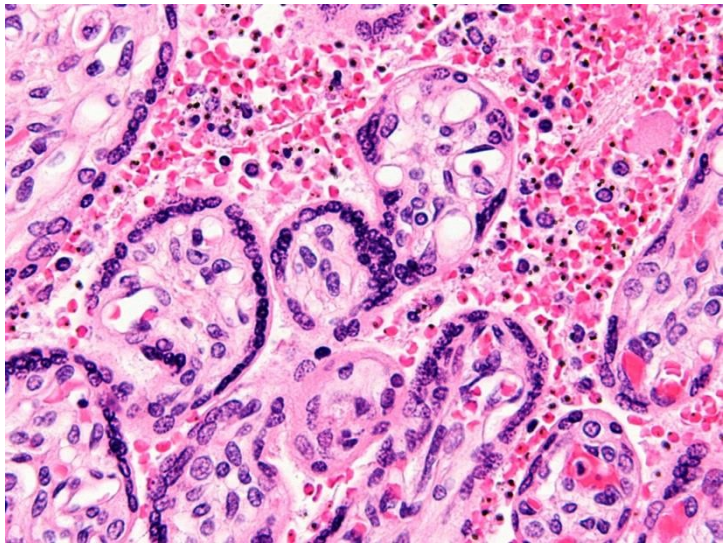

PtK2 cells 40x G2

Think Possible

BioTek®

Color Brightfield

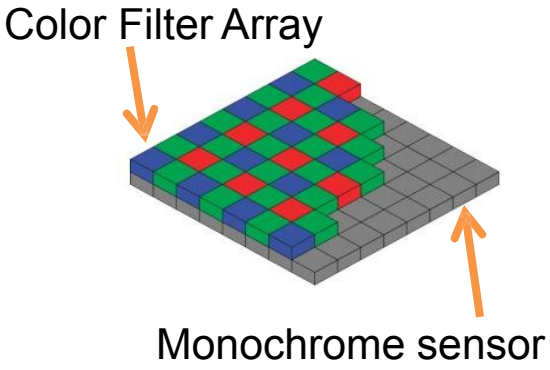
Histology



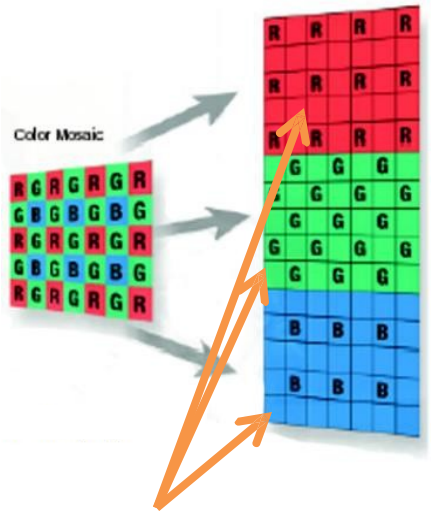
Tissue Staining

Color Camera

- Uses a monochrome (gray-scale) sensor
- Gets color information by applying a color filter array (CFA)
  - typically the CFA is the Bayer pattern
- CFA and Monochrome sensor makes a “Color Camera”



- To create a color image, a single exposure is taken
  - sampling of only one of the primary red, green or blue colors at each pixel location.
- Two un-sampled colors are then interpolated from adjacent pixels that have values for the color being calculated.
- To construct a color RGB image from this sampling method, 66% of the intensity values must be calculated.

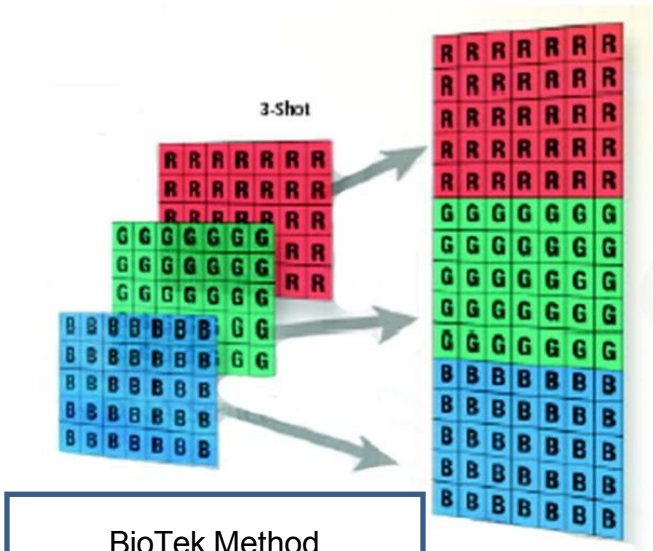


Graphics from spotimaging.com

BioTek Approach to Color

- Sequentially capture a red image, a green image and a blue image without filters by using sequential Red/Green/Blue LED illumination.

- Three sets of image data are then combined pixel by pixel to provide RGB sampled color at each pixel location.
  - Advantage: Each pixel samples all colors (Red/Green/Blue) so no interpolation



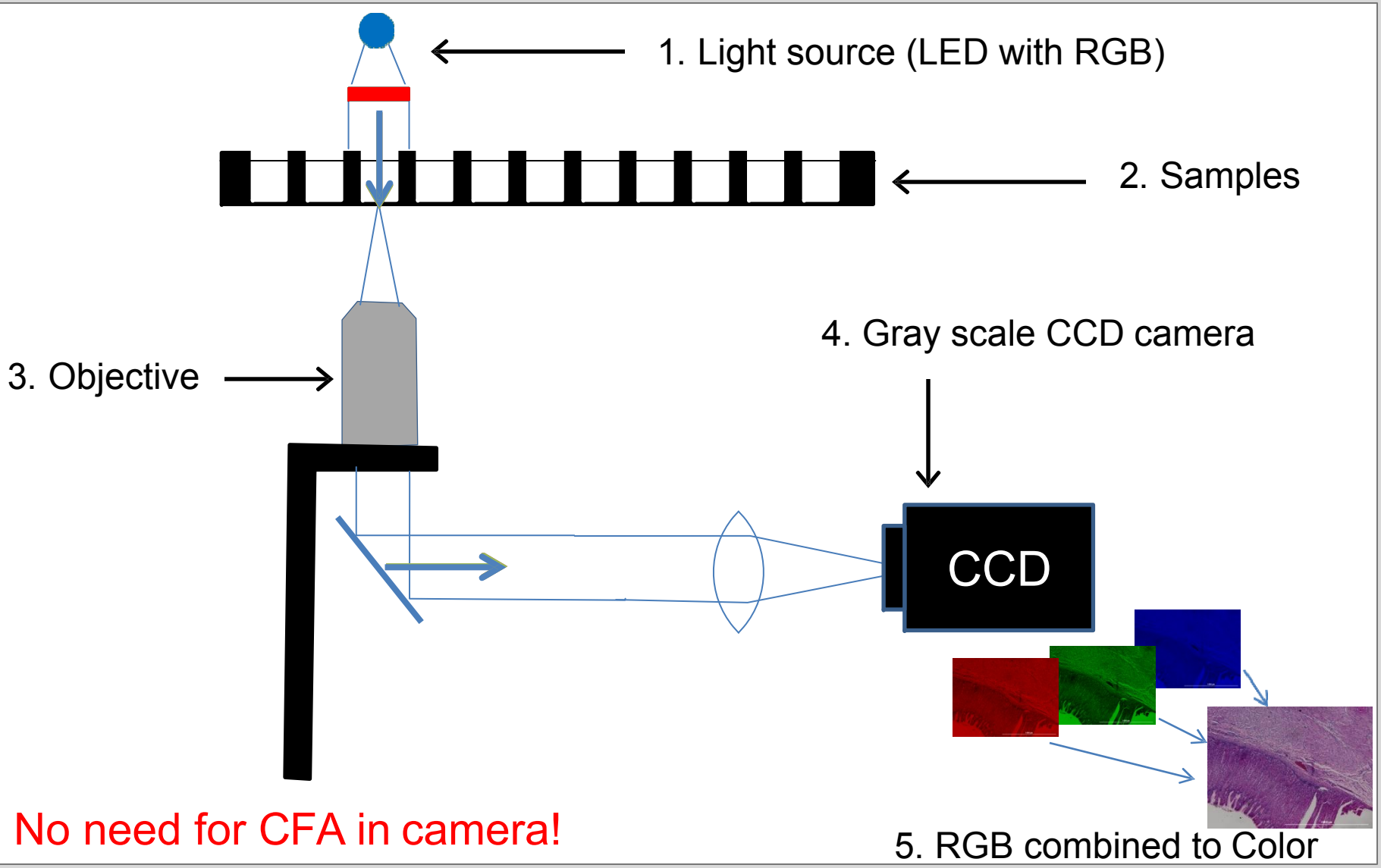
BioTek Method

Graphics from spotimaging.com

Think Possible

BioTek

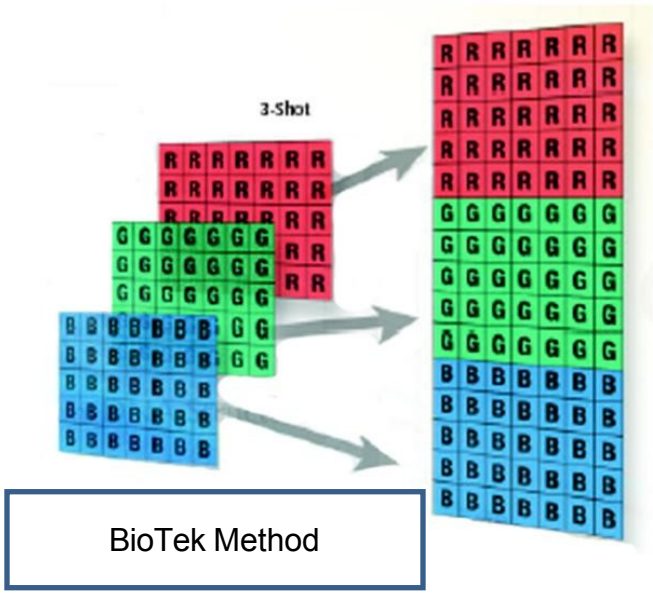
BioTek's Color Brightfield Optical Path



No need for CFA in camera!

BioTek Approach to Color

- Significant Benefits:
  - Monochrome camera supports fluorescence AND color
    - Less hardware, cost, maintenance
  - Increased resolution in final image
  - 3 separate color channels for image analysis



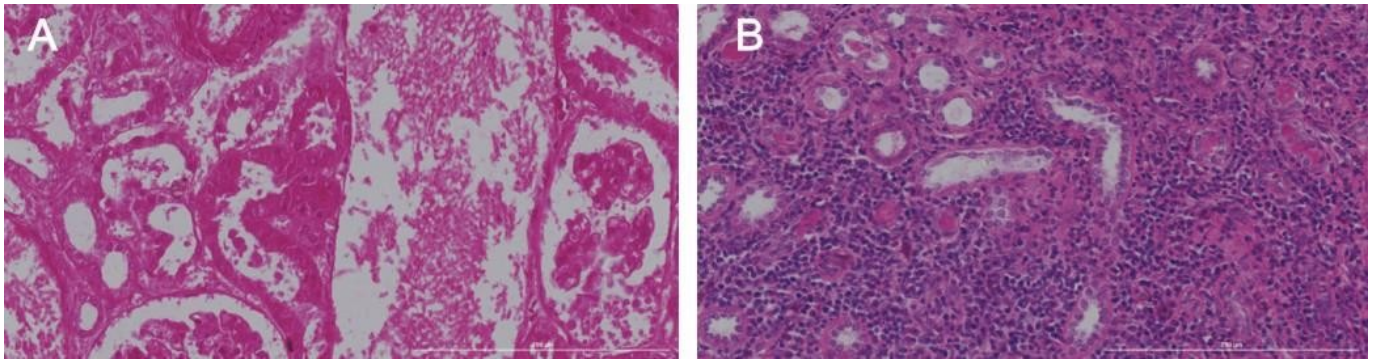
BioTek Method

Graphics from spotimaging.com

Think Possible

BioTek®

Hematoxylin and Eosin (H&E) Staining

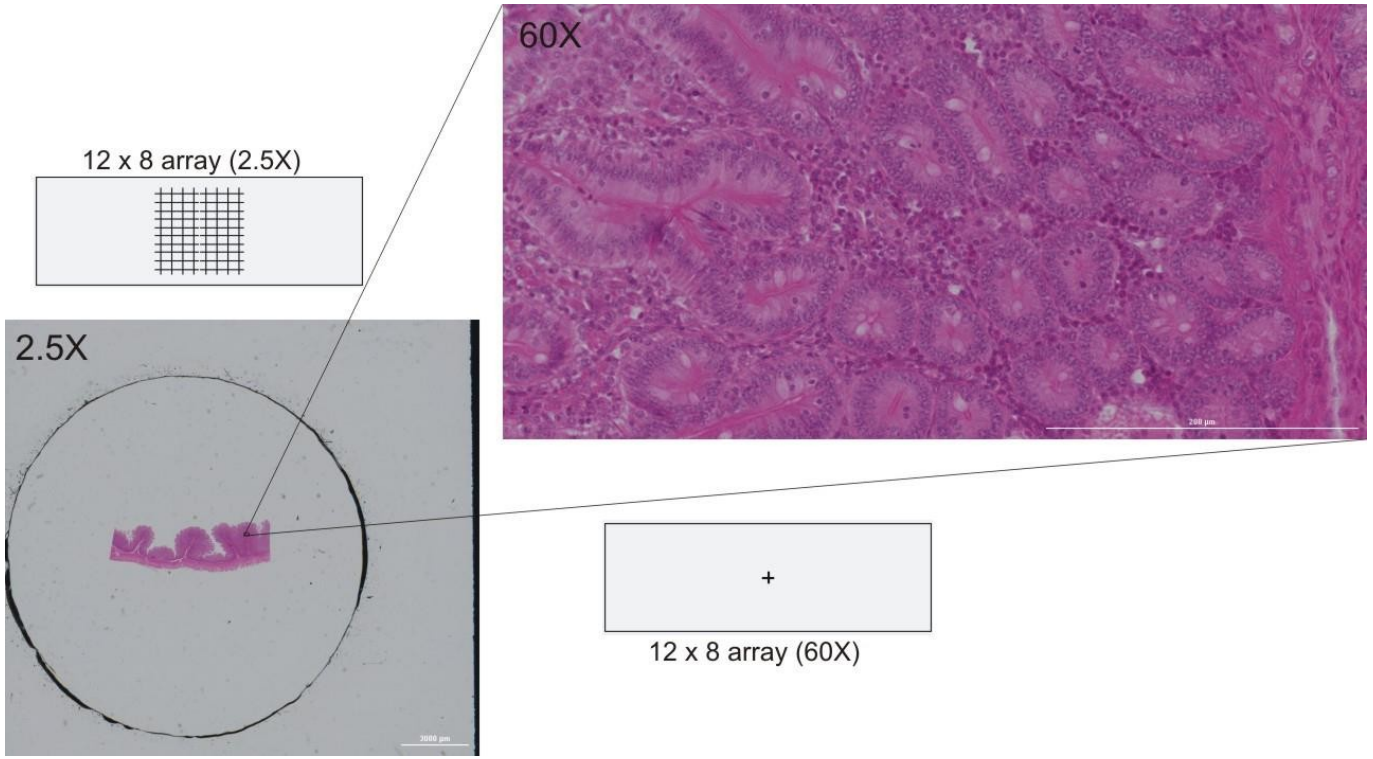


H&E stained tissue from (A) **normal kidney** and (B) **chronic nephritis kidney**.

Images represent a stitched 12 x 8 montage made using a 60X objective.

Scale bar represents 200 µm.

Range of Magnification

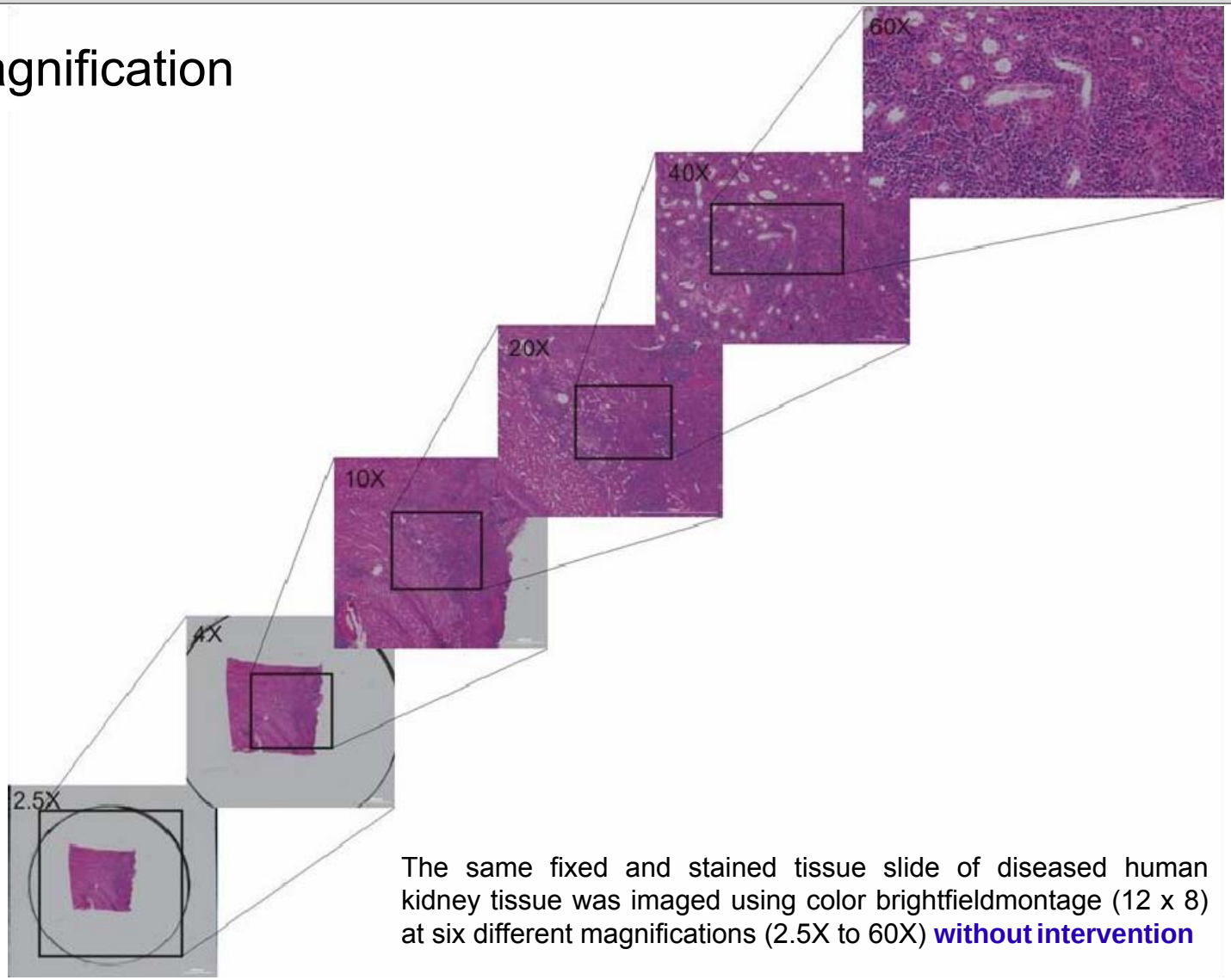


Fixed and stained tissue slice of human intestinal wall immobilized on a 1” x 3” slide was imaged with the 2.5X and the 60X objectives to generate a 12 x 8 montage.

Think Possible

BioTek®

Range of Magnification

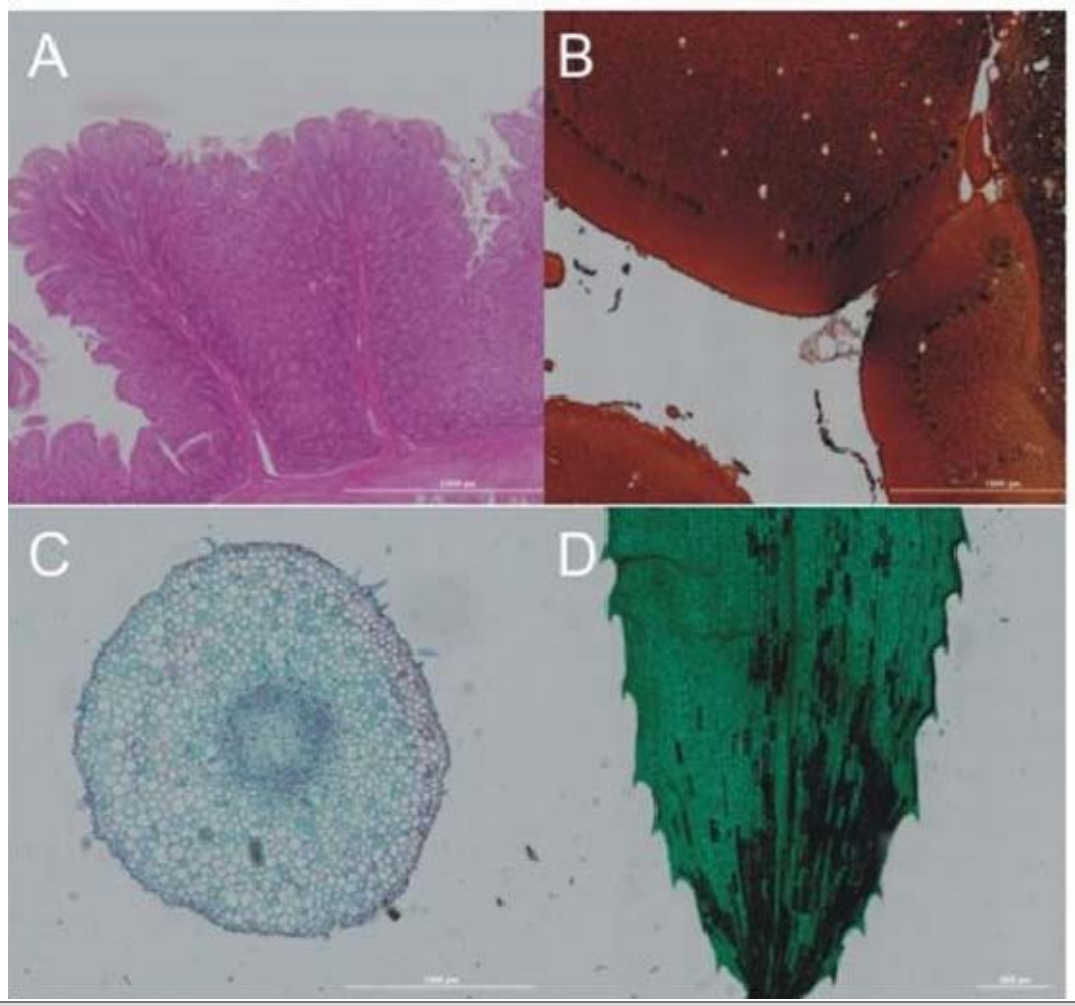


The same fixed and stained tissue slide of diseased human kidney tissue was imaged using color brightfieldmontage (12 x 8) at six different magnifications (2.5X to 60X) **without intervention**

Think Possible

BioTek®

Intrinsic color can be faithfully rendered …



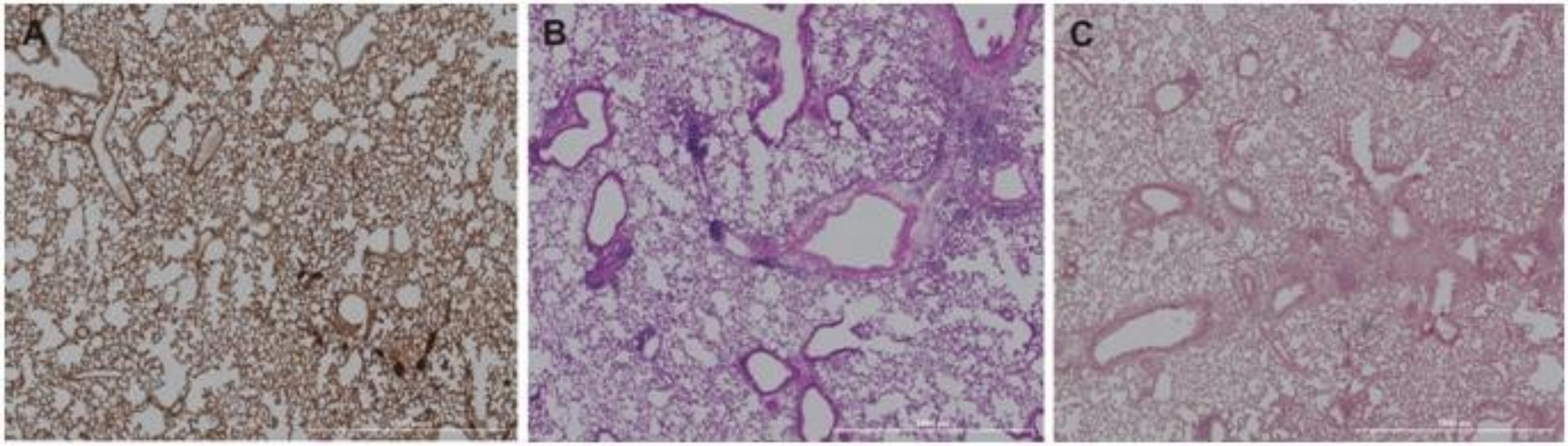
20x Imaging

A. H&E-stained tissue
B. Cerebellum tissue
C. Bean root
D. Hydrilla verticillate leaf

Think Possible

BioTek®

Use of other Colorimetric Stains



Mouse Lung tissue stained using various methods. Lung tissue was stained using:

A) HRP antibody conjugate reacted with DAB to form a brown reporter color

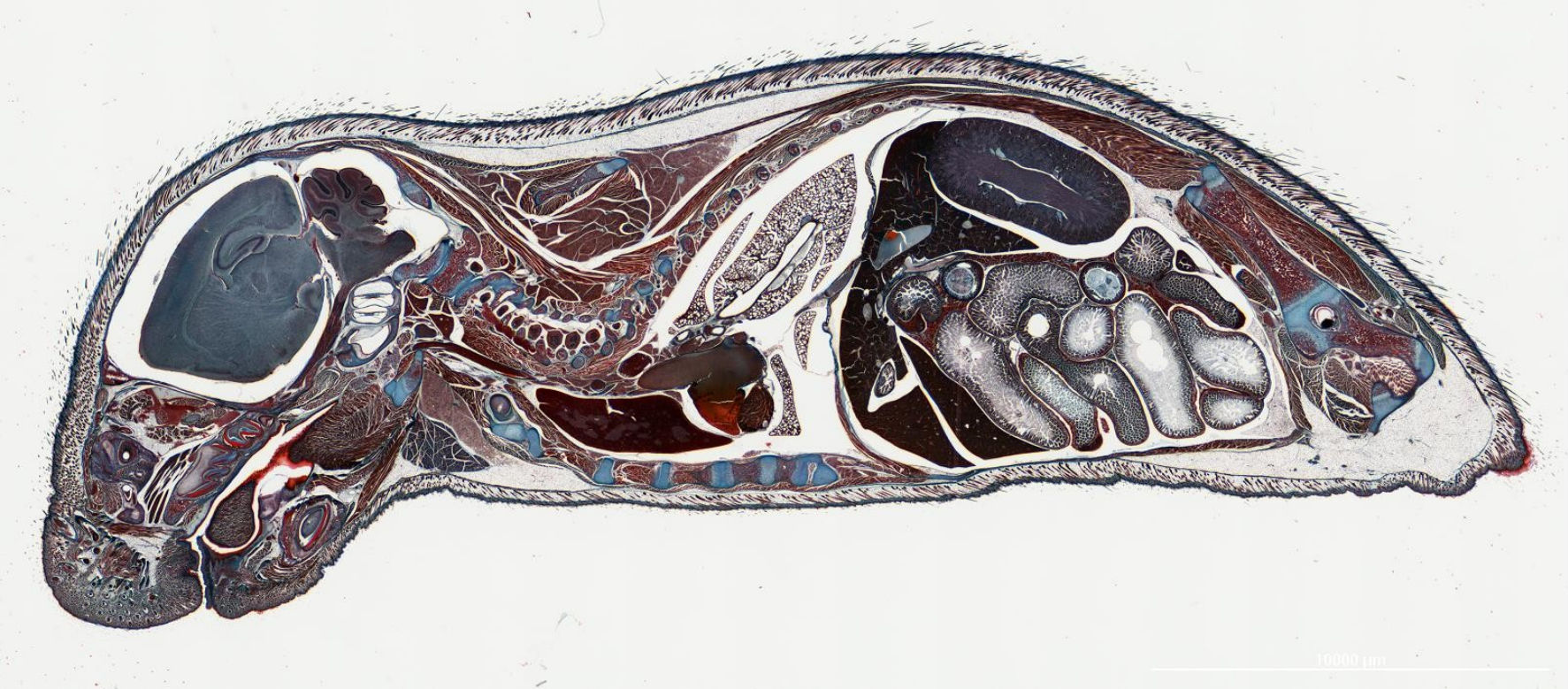
B) H&E dyes

C) Alkaline Phosphatase conjugate reacted with AEC to form pink products

Think Possible

BioTek®

Young Mouse with Internal Organs (2.5x Color Brightfield Montage)

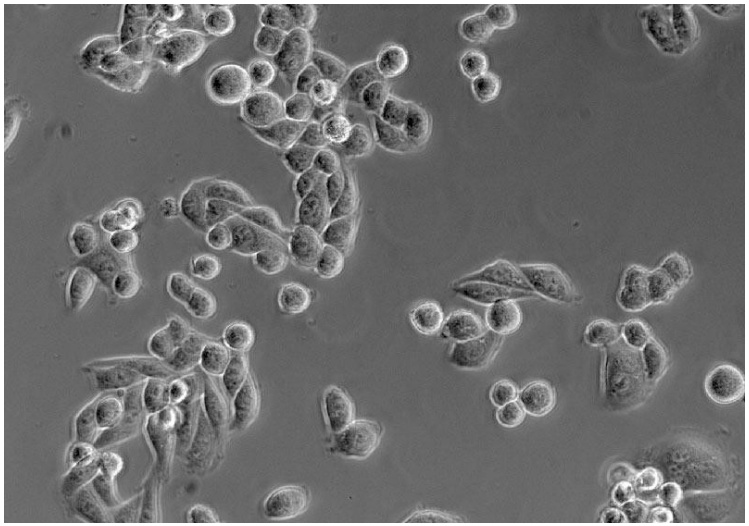


Think Possible

BioTek®

Apoptosis

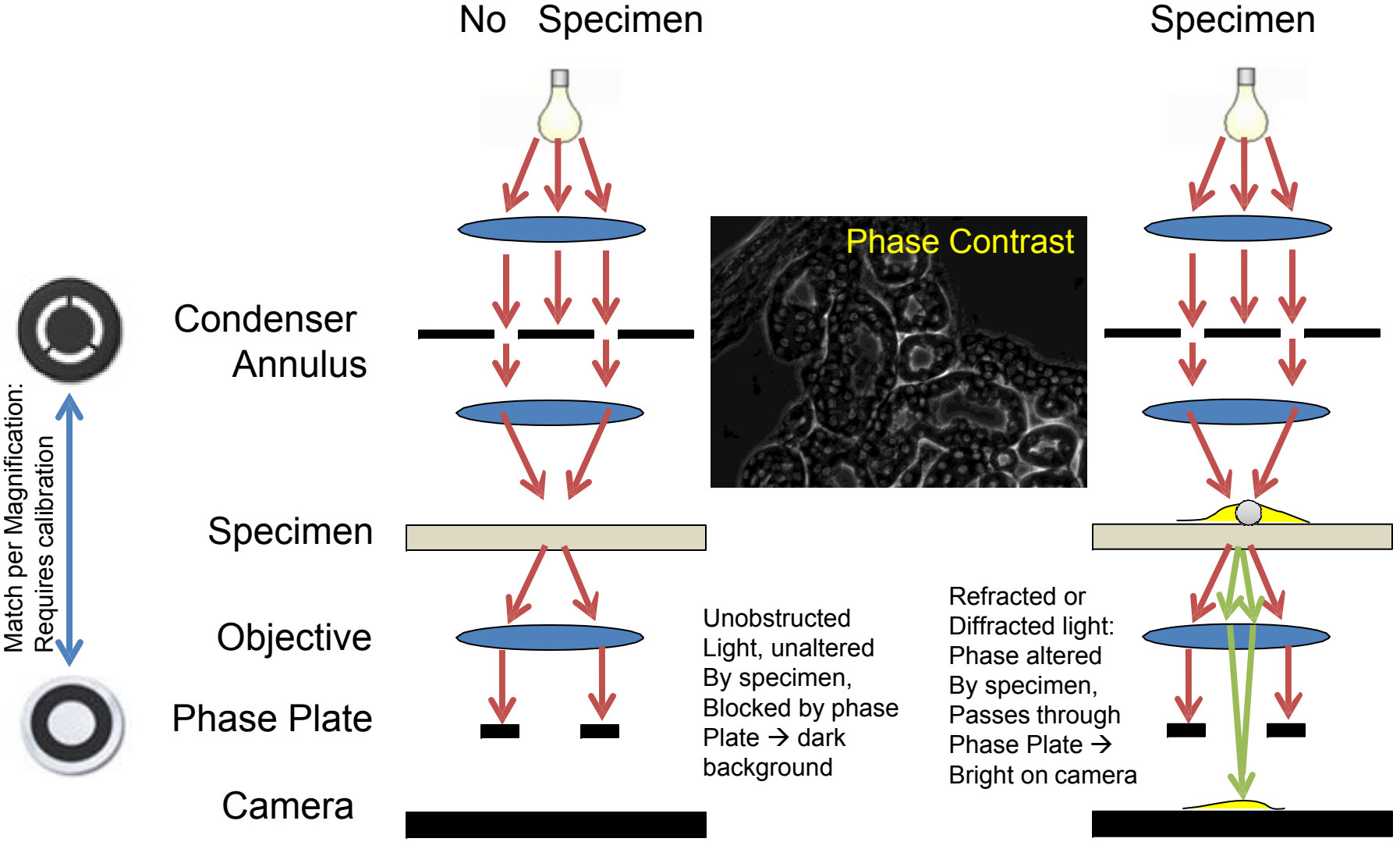
Phenotypic Assay



Phase Contrast

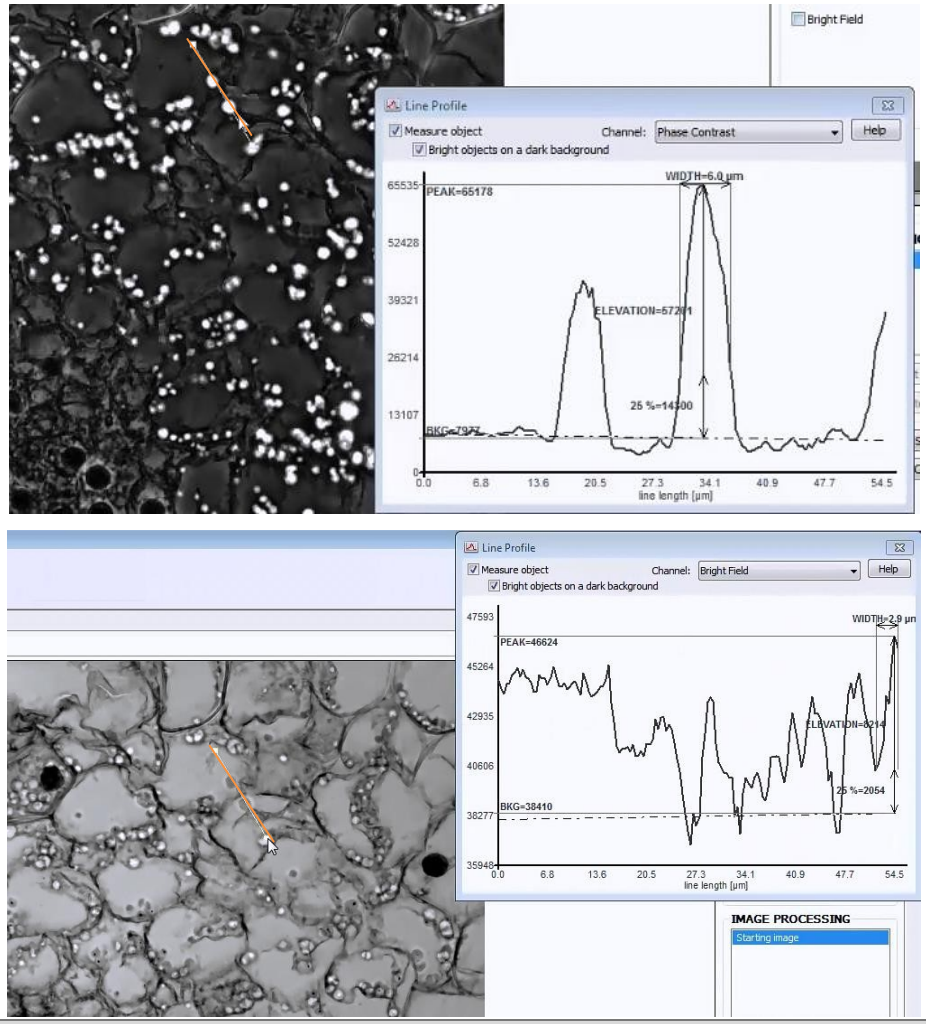
Think Possible

BioTek®



Phase Contrast: Image Analysis Benefit

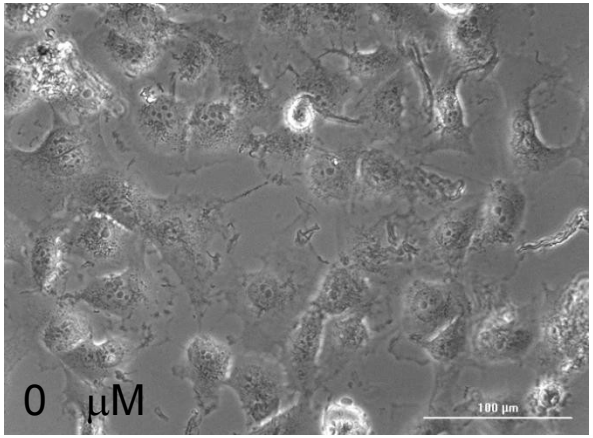
- Line profile tool comparison of the same spots visualized in Phase Contrast and Brightfield
- Much higher signal to noise in Phase Contrast allows for better Image Analysis of spots

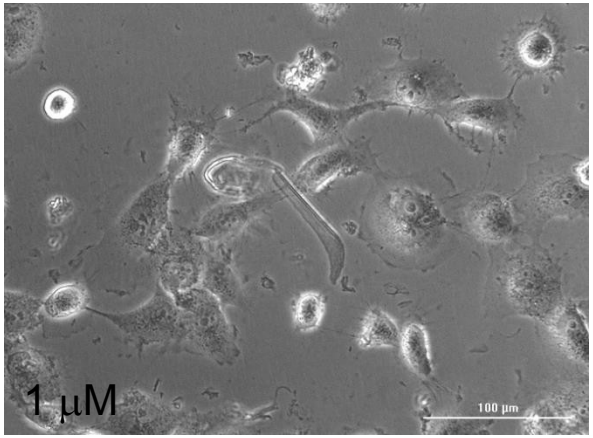


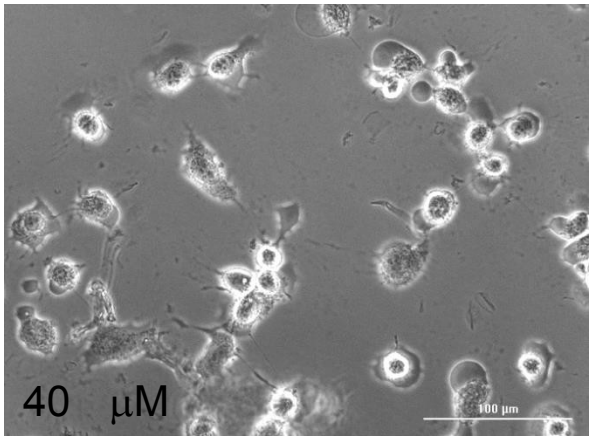
Think Possible

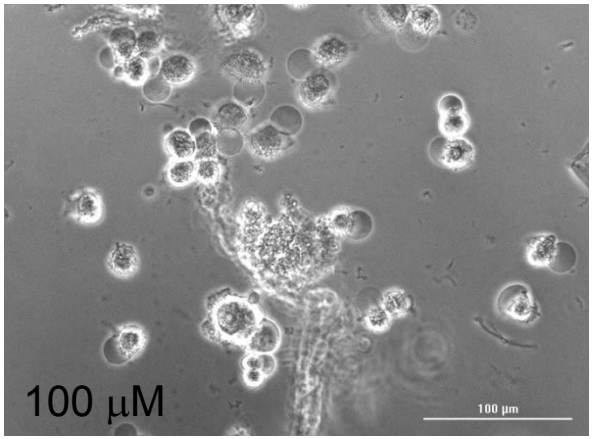
BioTek®

Phenotypic Assay: Oridonin-induced Apoptosis:





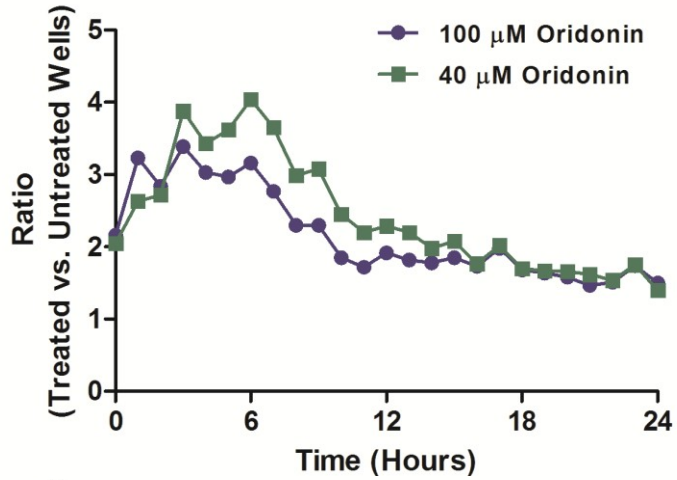


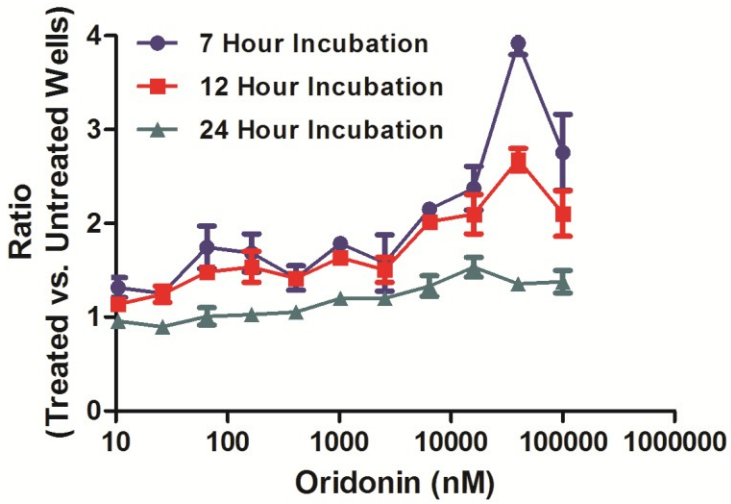


- MDA-MB-231 cells
- 6 hr incubation
- 20x Phase

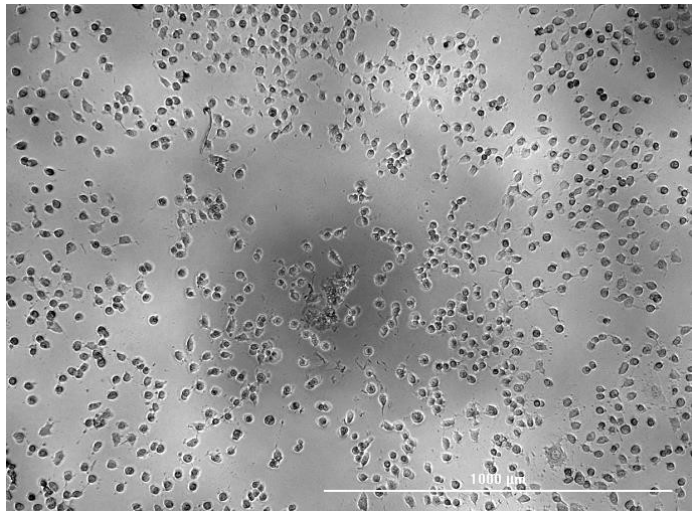
… phenotype of cell rounding easily visualized by phase contrast

Quantifying the Cell Rounding Phenotype



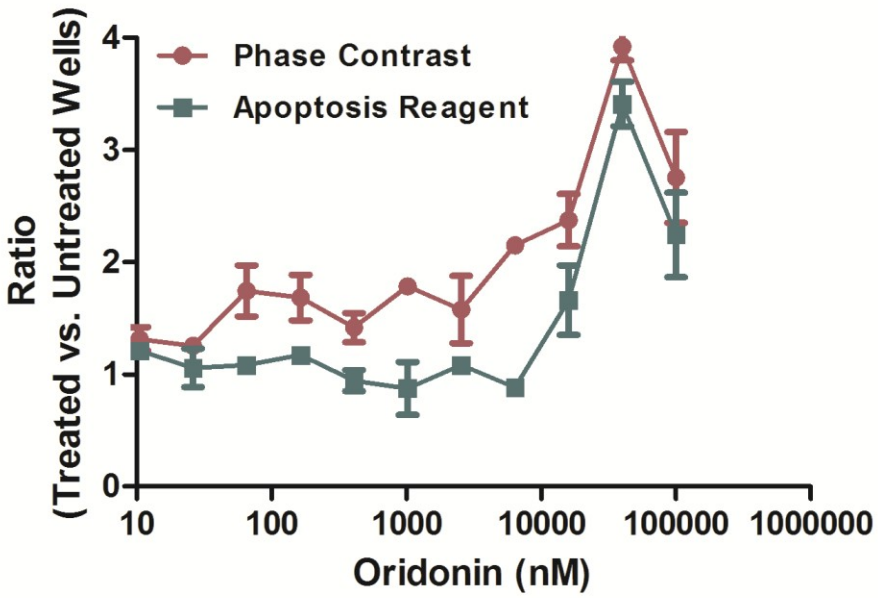


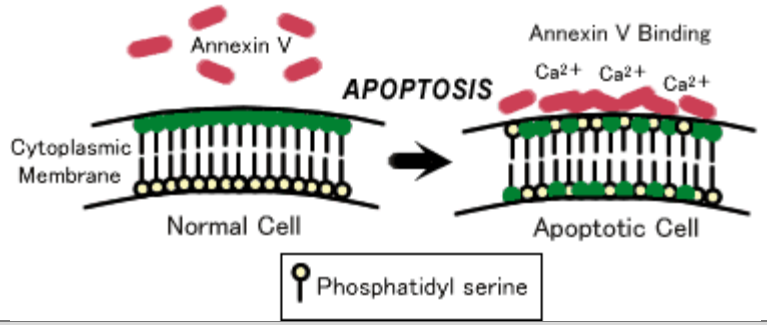
- 6 hr incubation
- 4x Phase
- Threshold 6,000 RBU
- Min. Object Size 10 µm
- Max. Object Size 40 µm
- Circularity > 0.4

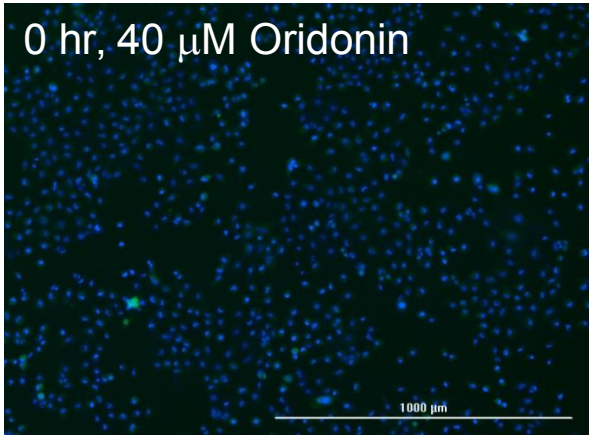


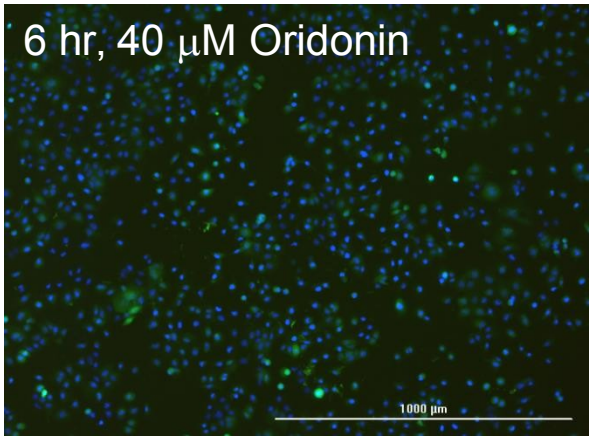
… phenotype peaks at ~ 6 hrs and 40 µM dose

Comparison to pS Green Fluorogenic Probe









… good correlation of phenotypes

Conclusions

- Cytation 5 – three separate optical paths
  - Digital widefield fluorescence microscopy (1.25x, 2.5x, 4x, 10x, 20x, 40x, 60x): **rich phenotypic data**
  - Excitation and Emission monochromators with quadruple gratings: **wavelength selection flexibility**
  - Excitation and emission spectral filters separated by dichroic mirror: **high sensitivity detection**
- Enables target-based and phenotypic assays
- HTRF, fluorescence intensity, luminescence, digital microscopy demonstrated in two case studies
  - Other detection modes available: absorbance, FRET, TRF, FP, filtered luminescence

Think Possible

BioTek®

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

Think Possible

BioTek®

Cytation 5

Do What You Never Thought Possible!

Think Possible

BioTek®

Questions?

"Mr. Osborne, may I be excused? My brain is full."