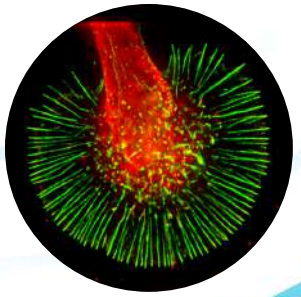
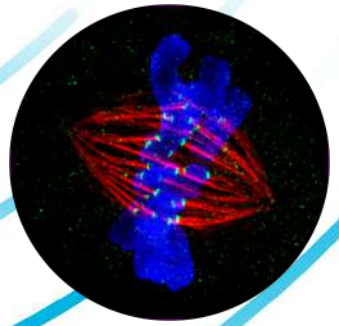
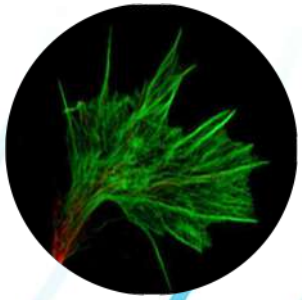
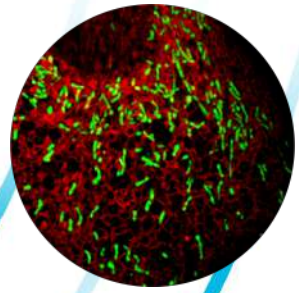
均泰生物科技有限公司
Genetech Biotech Co., Ltd

Advanced Imaging Solution: DeltaVision Imaging System

蔣明涵 Michelle Chiang
techsupport@gtbiotech.com.tw

Cell Analysis Portfolio

Increasing content/number of cells

Smart Cell imager

inCellis

bertin INSTRUMENTS

High Content

IN Cell Analyzer 2500HS

IN Cell Analyzer 6500HS

High Resolution

DeltaVision™ Ultra

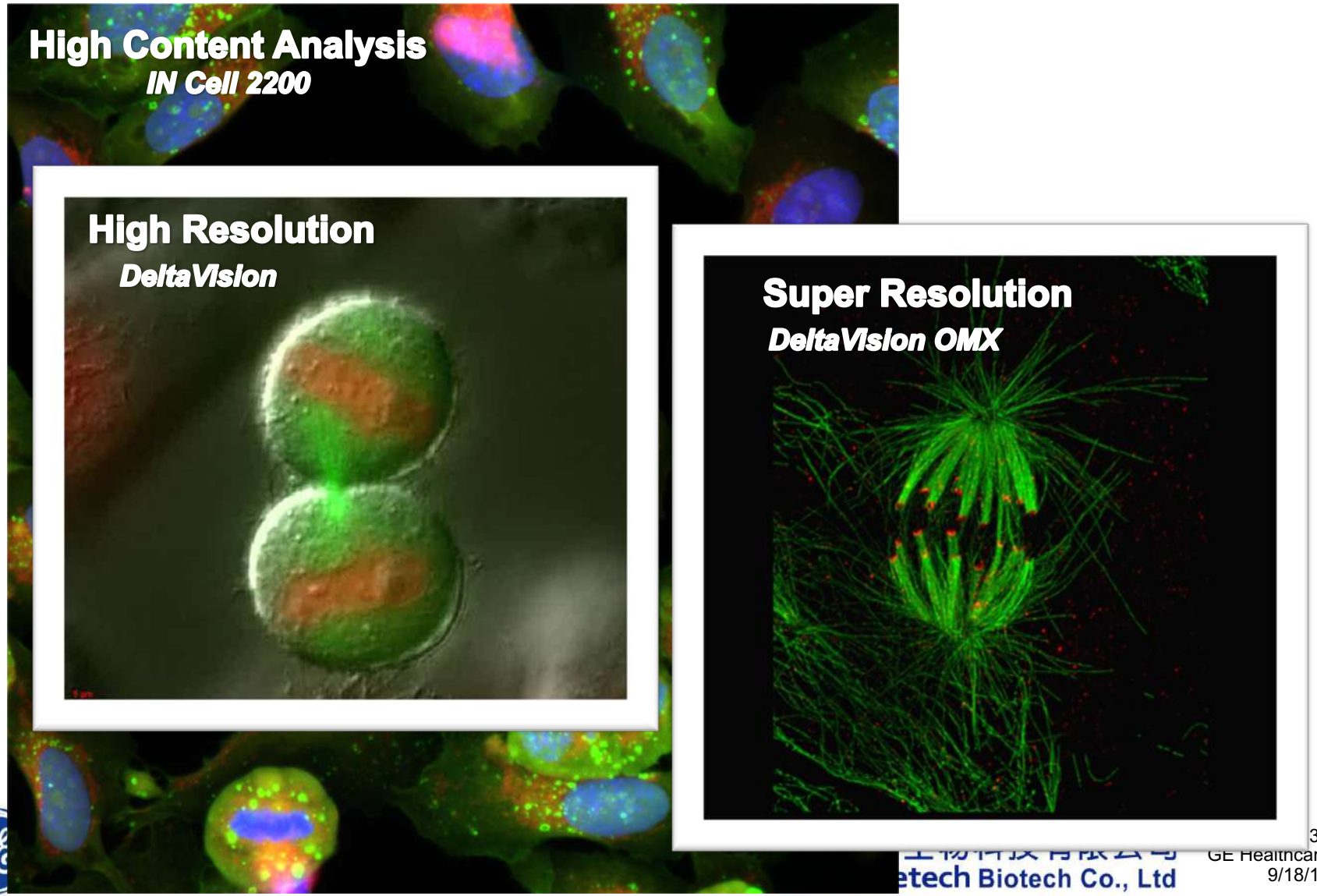
Super Resolution

DeltaVision™ OMX Flex

Increasing resolution/detail

Cellular Imaging – Cell Cycle

Embracing complexity in breadth, depth & detail



Introduction to DeltaVision

Widefield Restoration Deconvolution Imaging Systems

Everyone can get robust data fast!

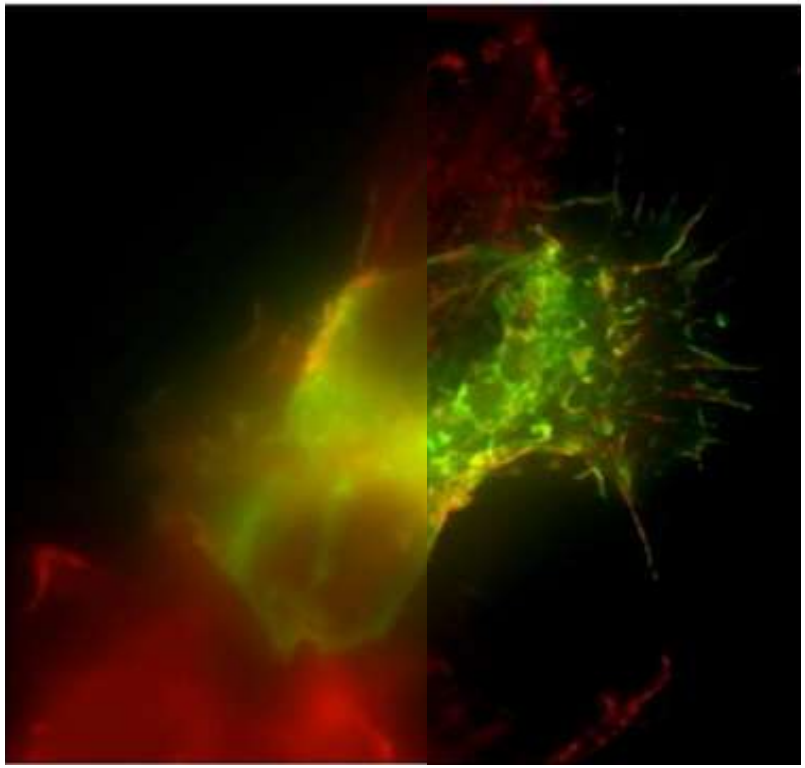
Easy-to-use interface

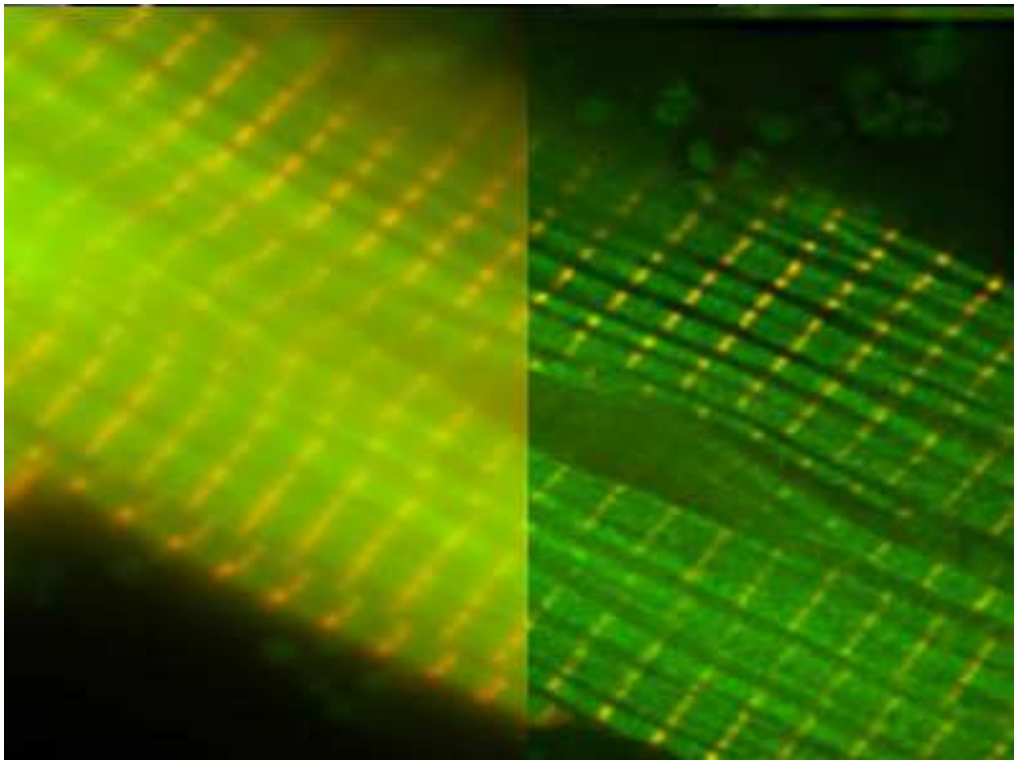
Simple Workflows

Minimal training burden

Get to results FASTER!

Effect of restorative deconvolution

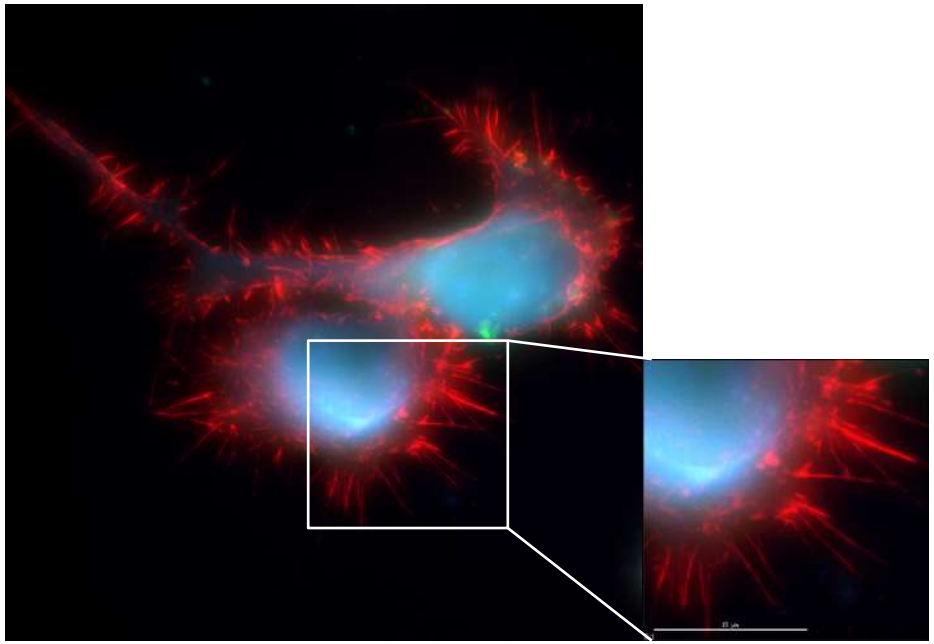




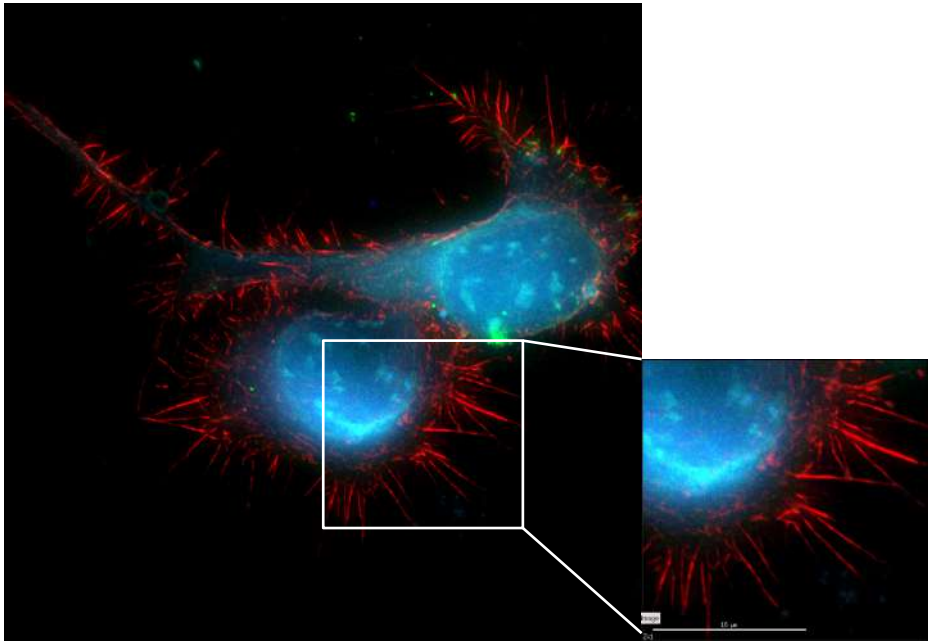
Effect of restorative deconvolution

Improvement in image quality

Widefield

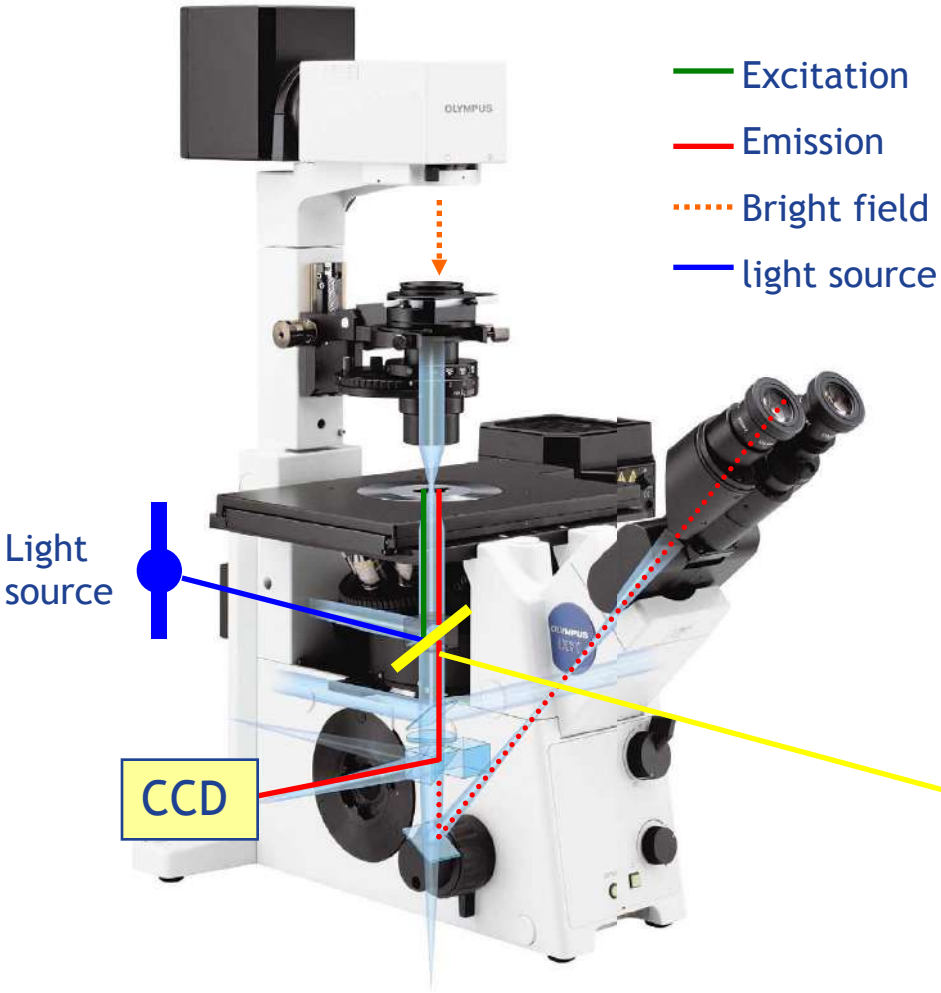


Widefield Deconvolution

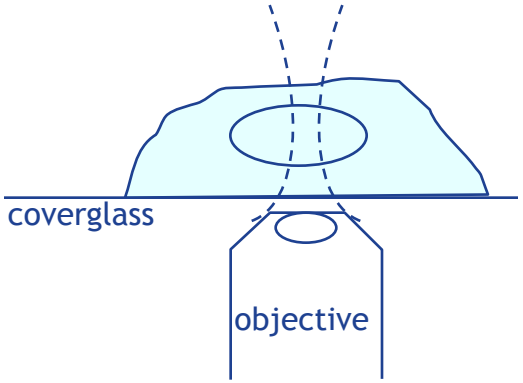


Restoration Deconvolution

Widefield microscope



Inverted microscope



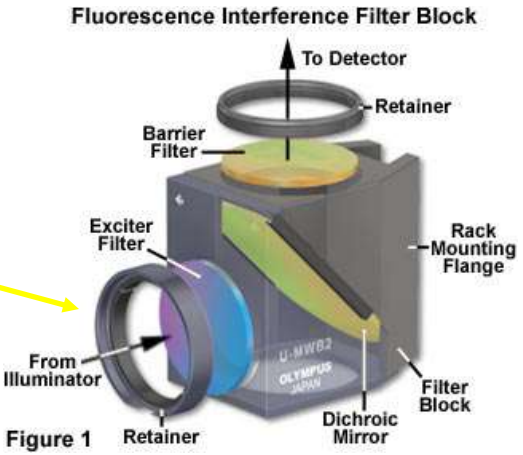
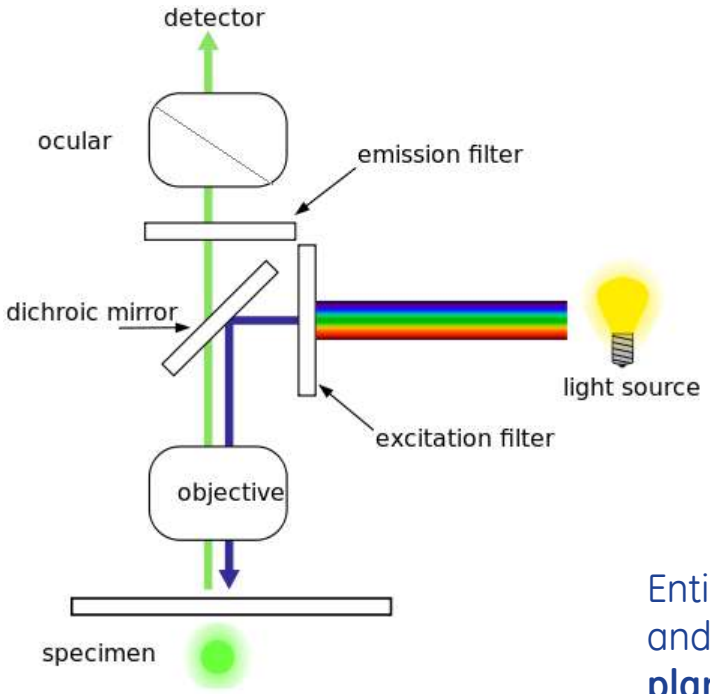
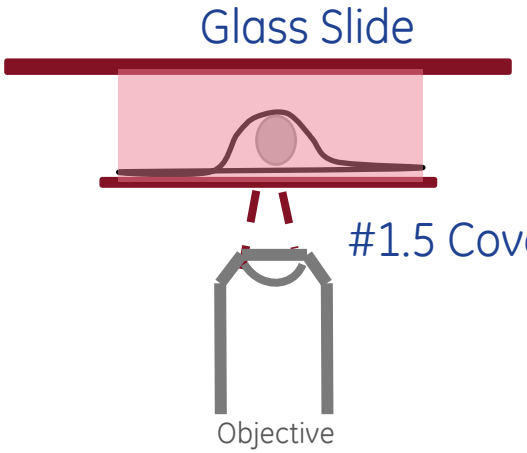
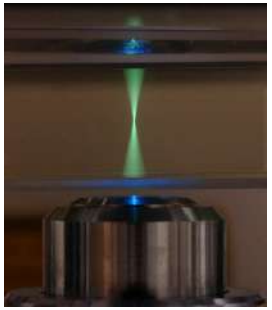


Figure 1

What is widefield or epi-Illumination







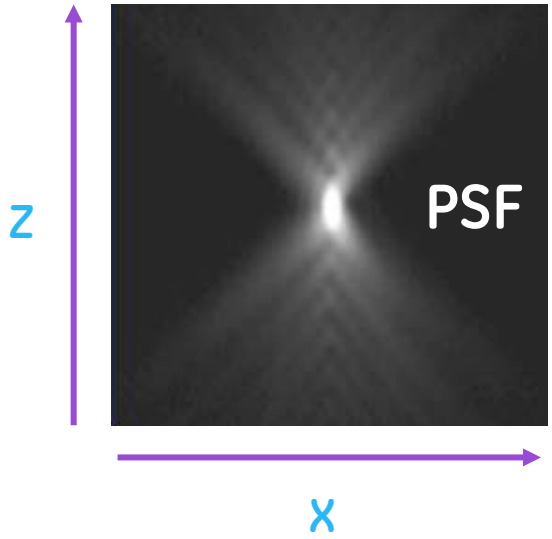
Side-view

Entire specimen is illuminated under a WF microscope and therefore **regions above and below the focal plane will also fluoresce** and be captured

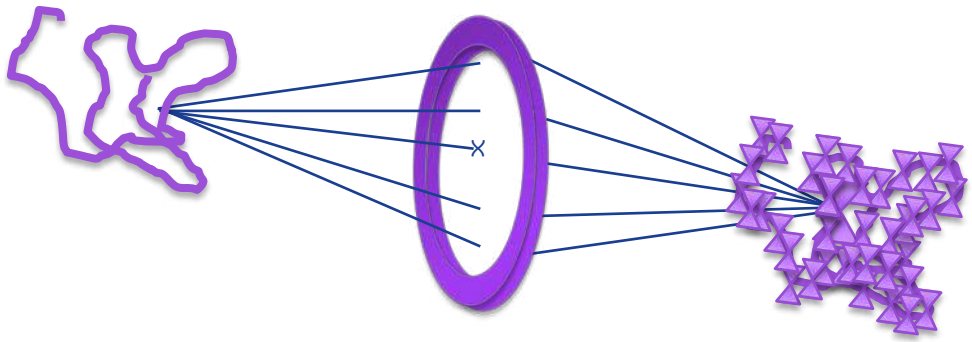
What is widefield or epi-Illumination

How does a point or a bead look like with all the out-of-focus light?

Side view of a single bead

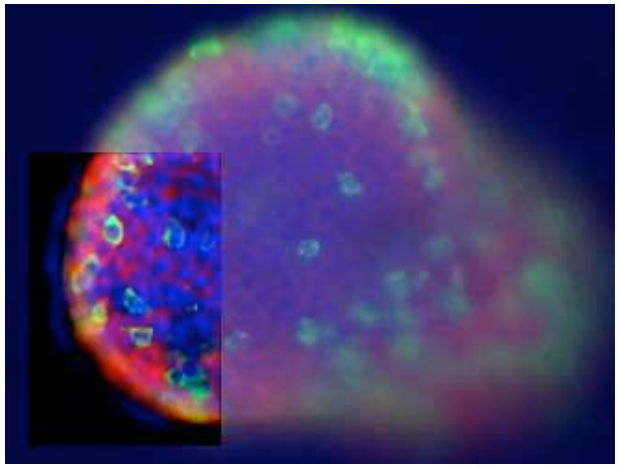


Many PSFs in a sample → ?

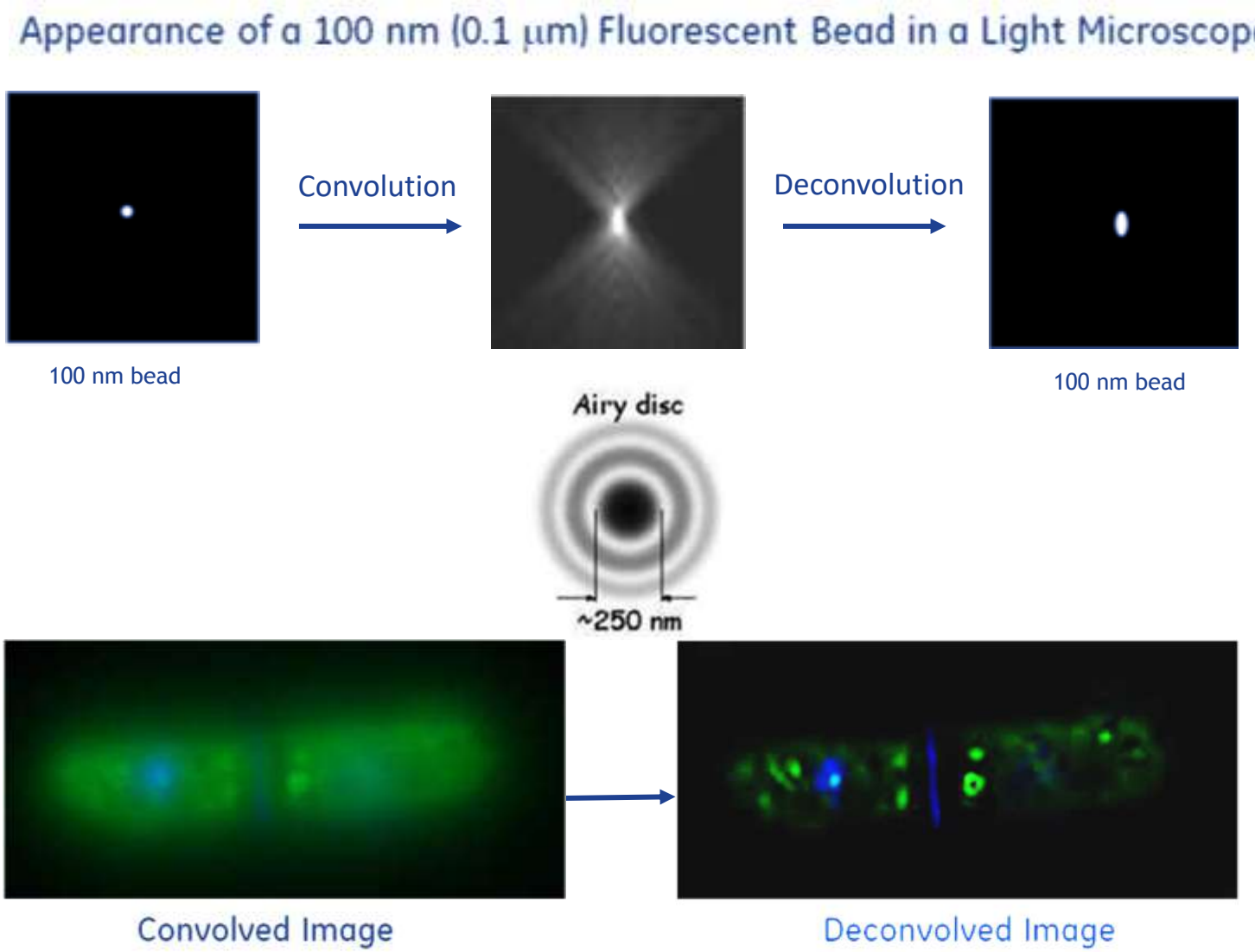


Principal of Deconvolution microscopy

An intrinsic problem exists in 3D wide-field fluorescence microscopy: out-of-focus blur is detected.
The out-of-focus blur comes from throughout the specimen and interferes with the visualization of information in the focal plane.
Deconvolution is the computational process that reduces out-of-focus blur.



How Restorative Deconvolution Works

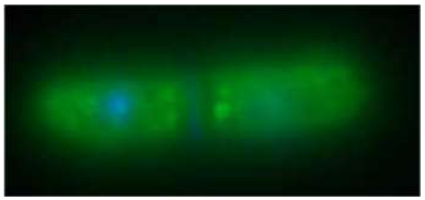


Restorative Deconvolution vs LSCM

Appearance of a 100 nm (0.1 mm) Fluorescent Bead in a Light Microscope

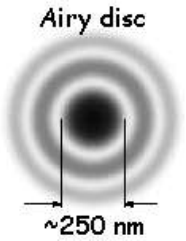
PSF is to the image what the brick is to the house

100 nm bead

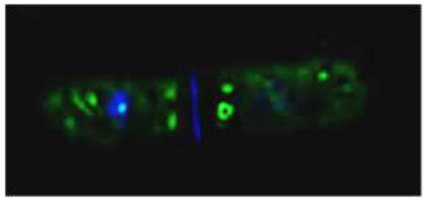


Convolved Image

Image from the
Bead is called
Point Spread Function (PSF)



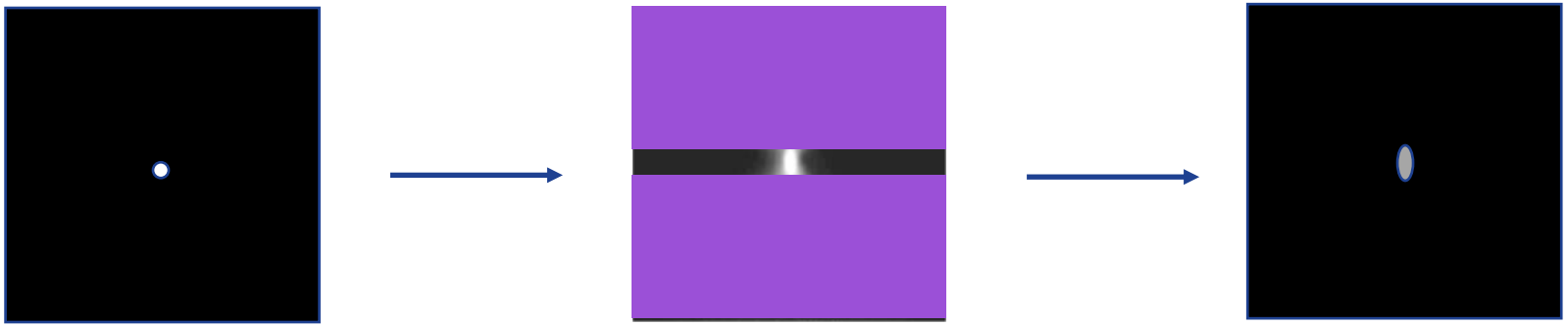
100 nm bead



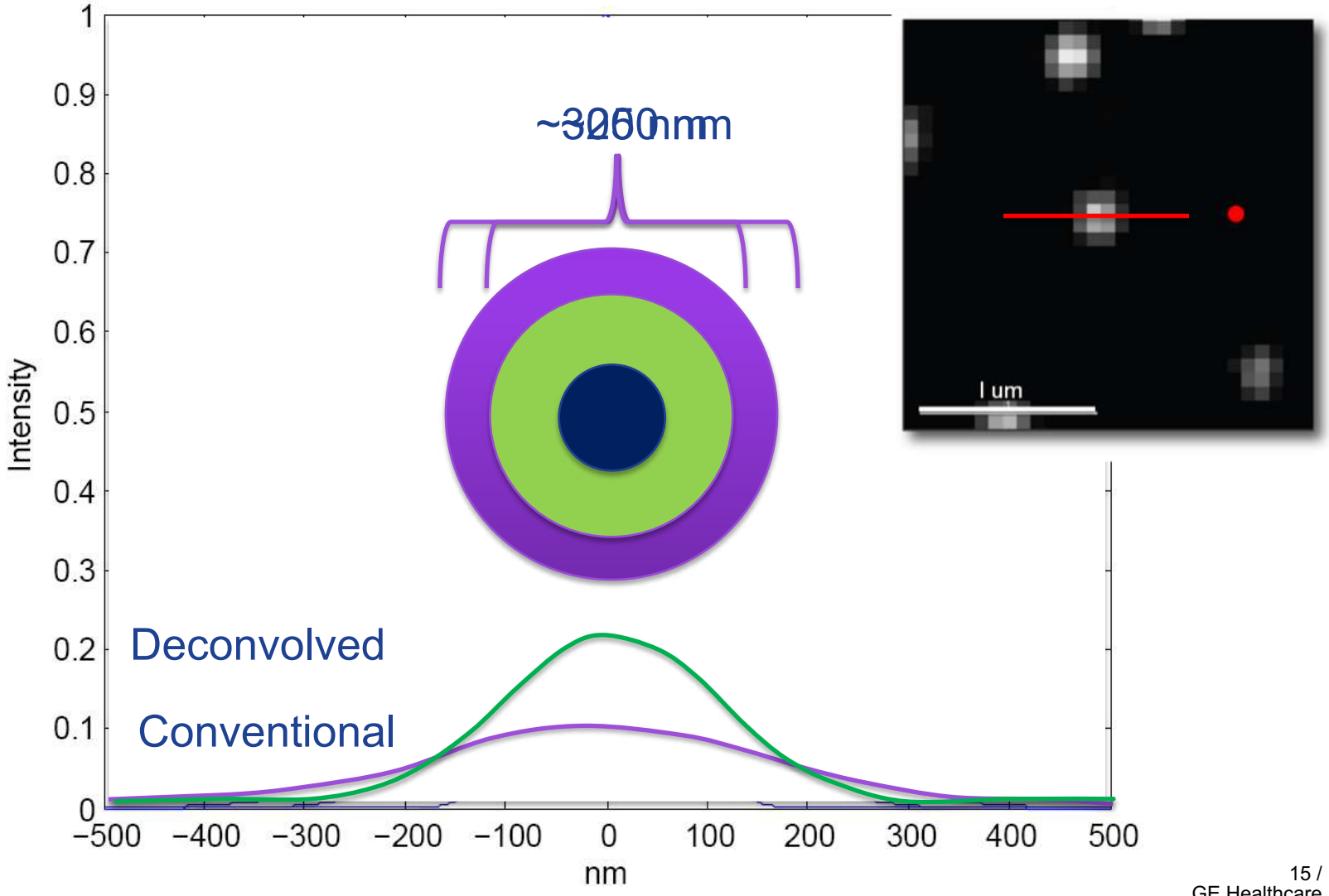
Deconvolved Image

Lifting the Fog

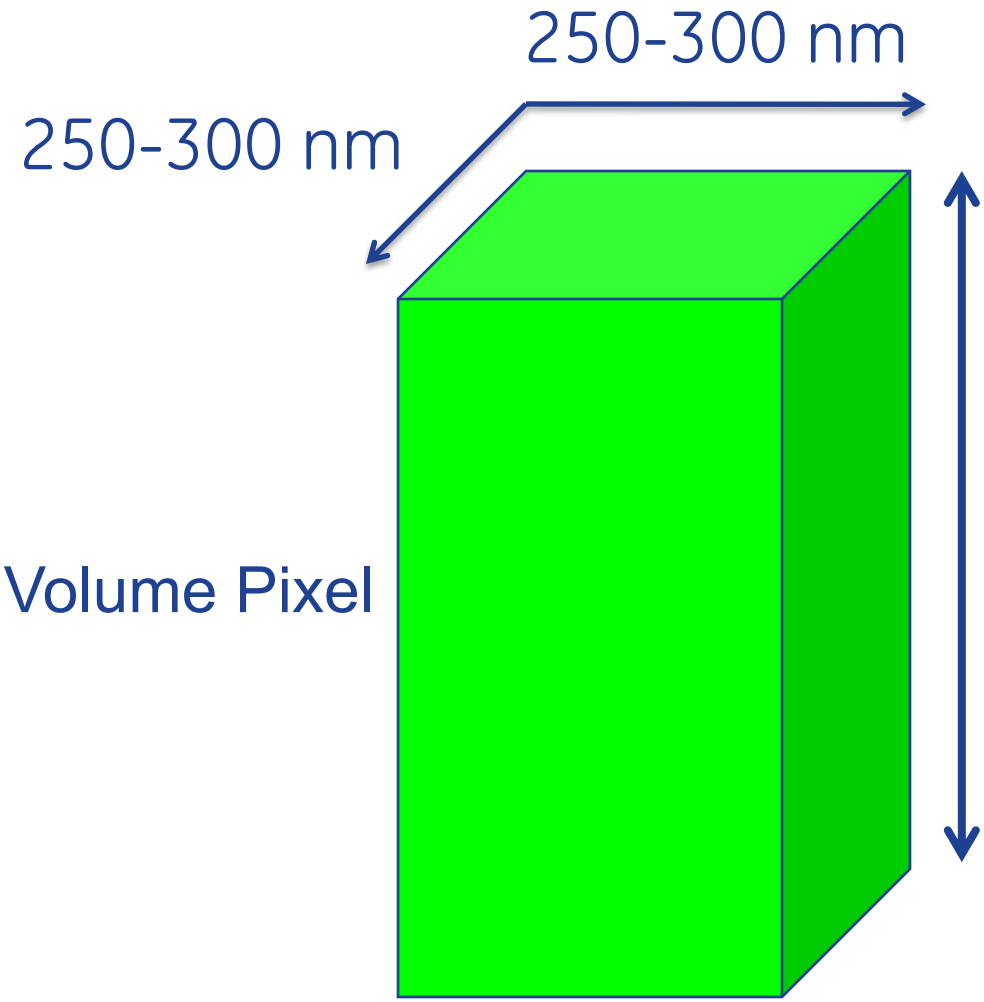
Confocal



Gaussian Fit of 100nm Beads



Diffraction limited volume



Voxel = Volume Pixel

Image VERY Small Particles

- HIV particles are approximately 120 nm in diameter and can be visualized within the cell.
- HIV (green) and microtubules (red).

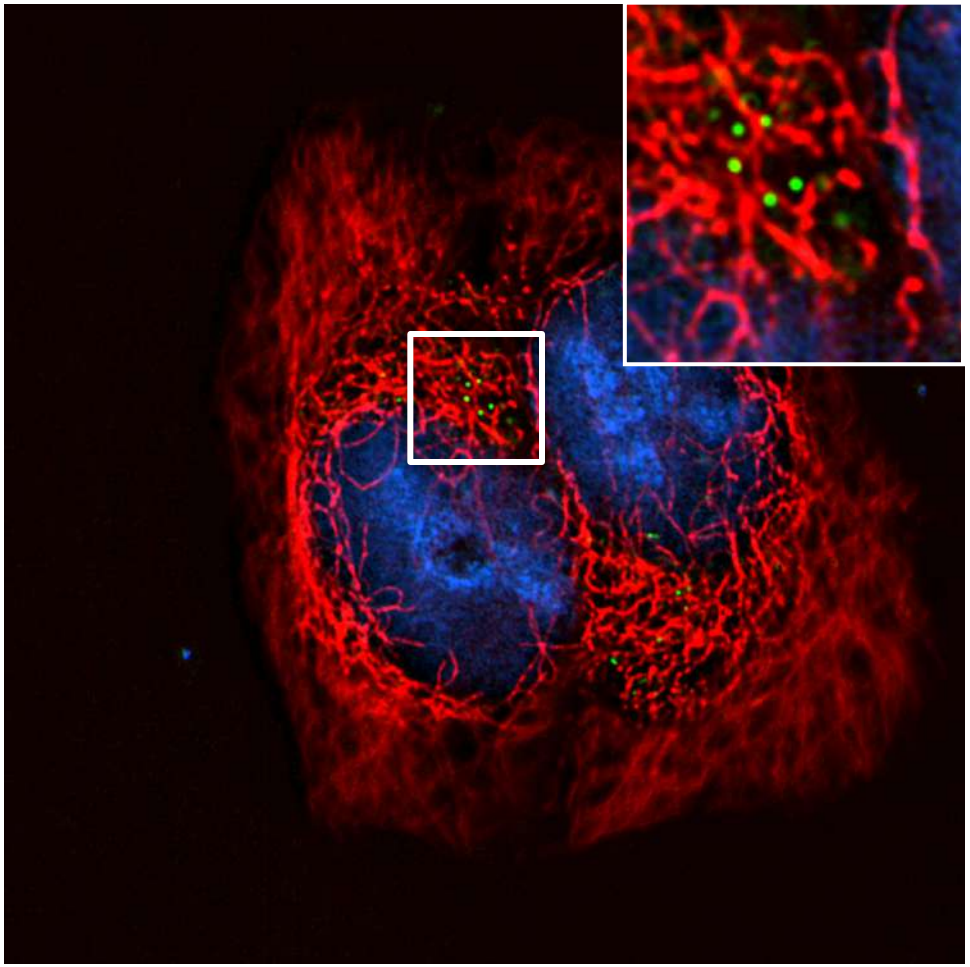
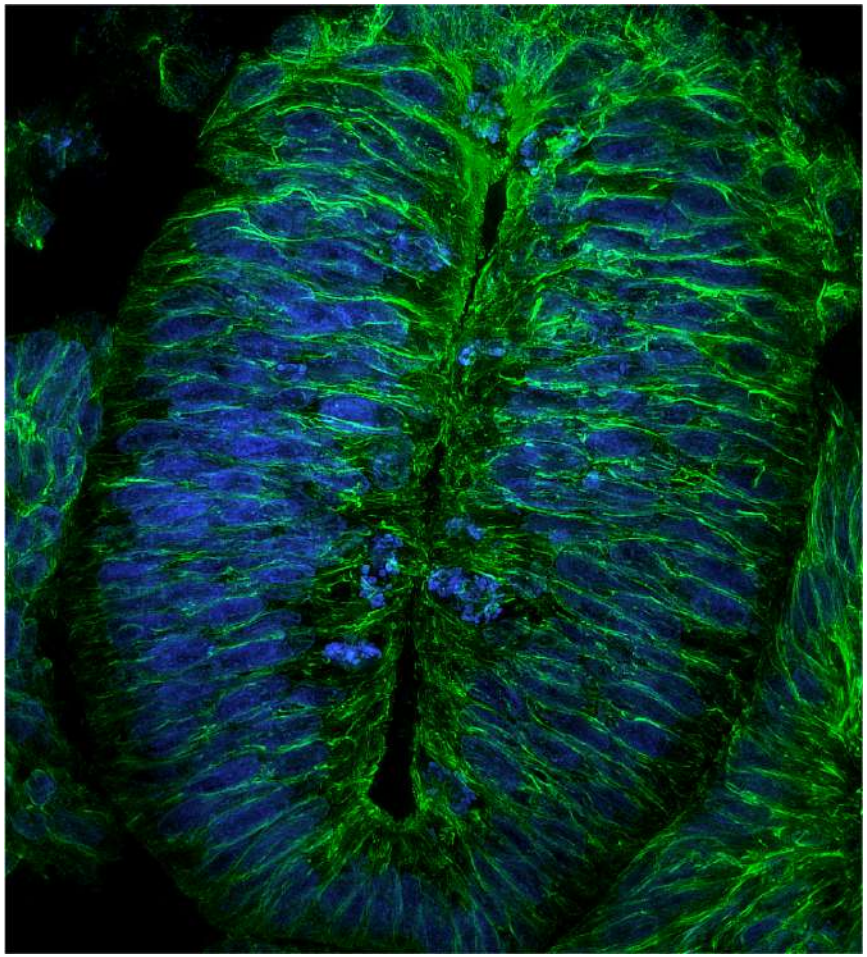
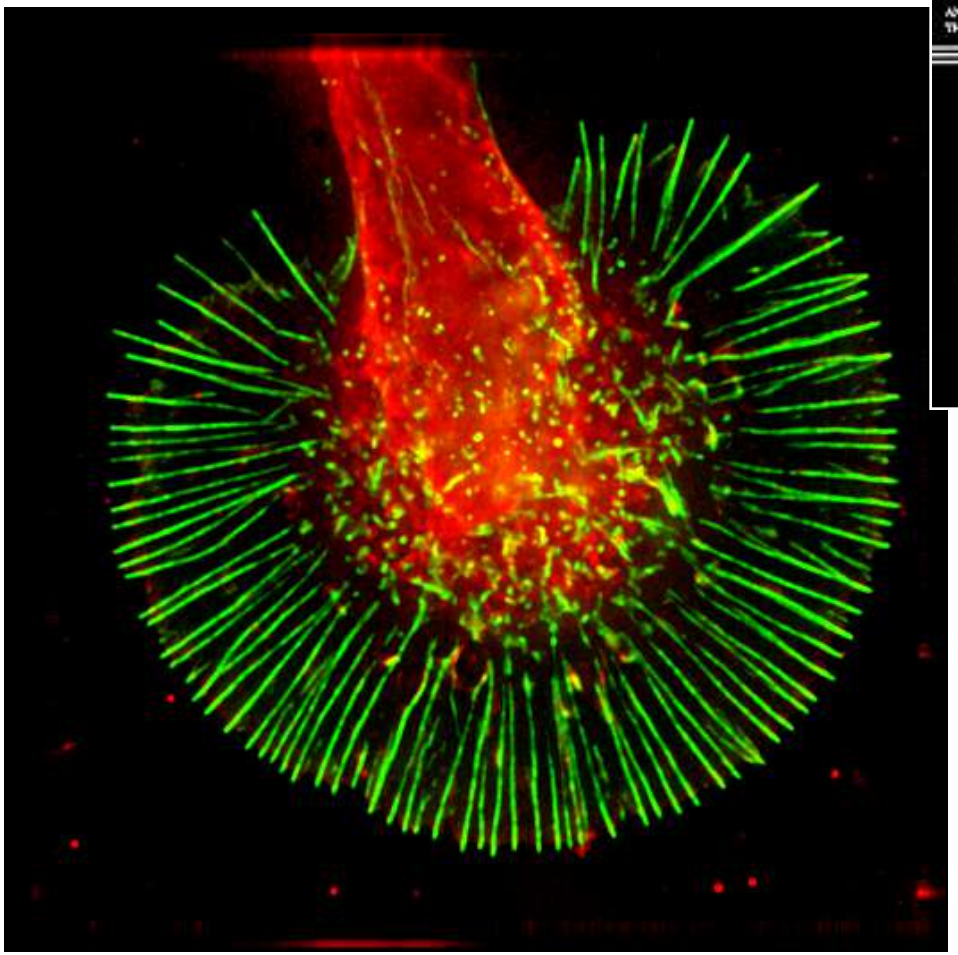


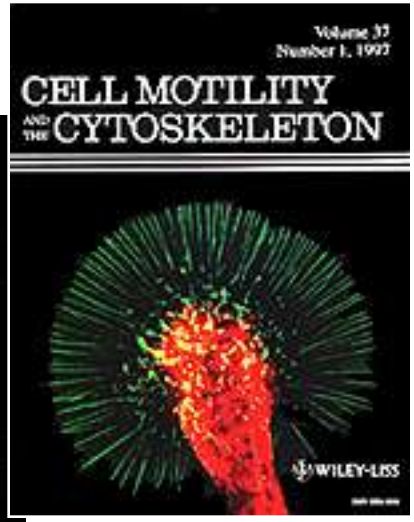
Image Thick Specimens

- Chick Embryo Neural Tube.
- Fixed tissues up to 70 - 100 microns thick.
- Labeled for microtubules (green) and DNA (blue).



Growth Cone Morphogenesis in Helisoma Neurons

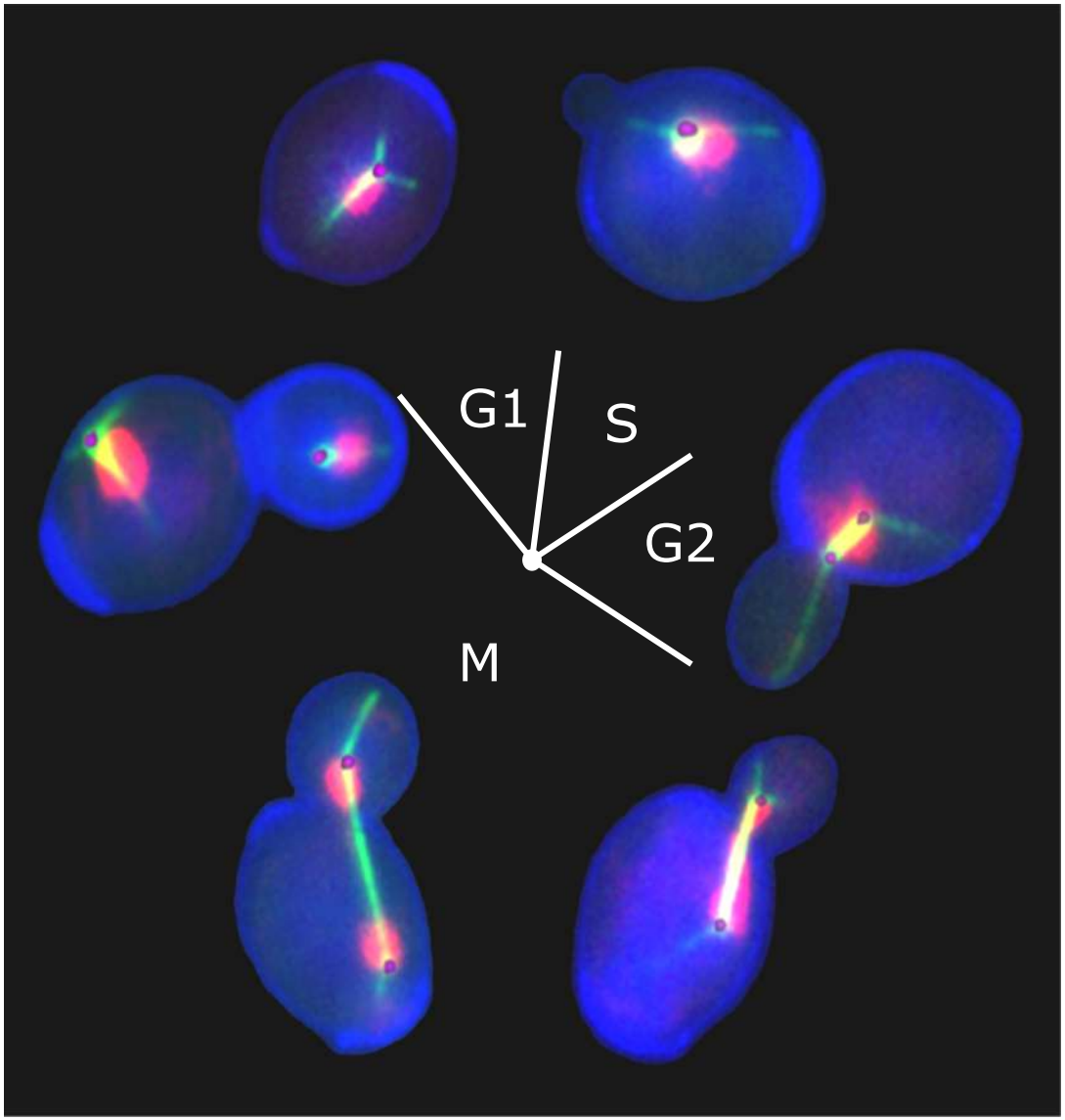




Actin is a prominent and dynamic cytoskeletal component in the lamellipodia and filopodia of growth cones. Actin filaments are arranged as radially-aligned bundles that project into the filopodia and also as a more loosely organized meshwork that extends throughout the lamellipodium. These actin filaments exhibit a retrograde flow that moves filaments from the peripheral edge of the growth cone, where actin is assembled, to the central domain of the growth cone, where actin is disassembled.

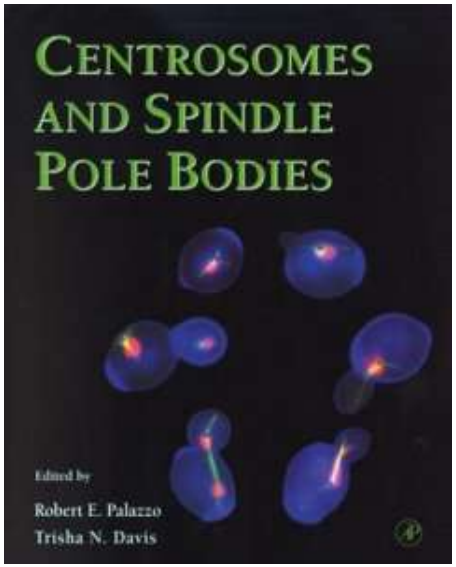
Welnhofer, E., Zhao, L., and Cohan, C.S. (1997). Actin dynamics and organization during growth cone morphogenesis in Helisoma neurons, Cell Motil Cytoskel. 37: 54-71

The Yeast Cell Cycle



Live Cell Labeling

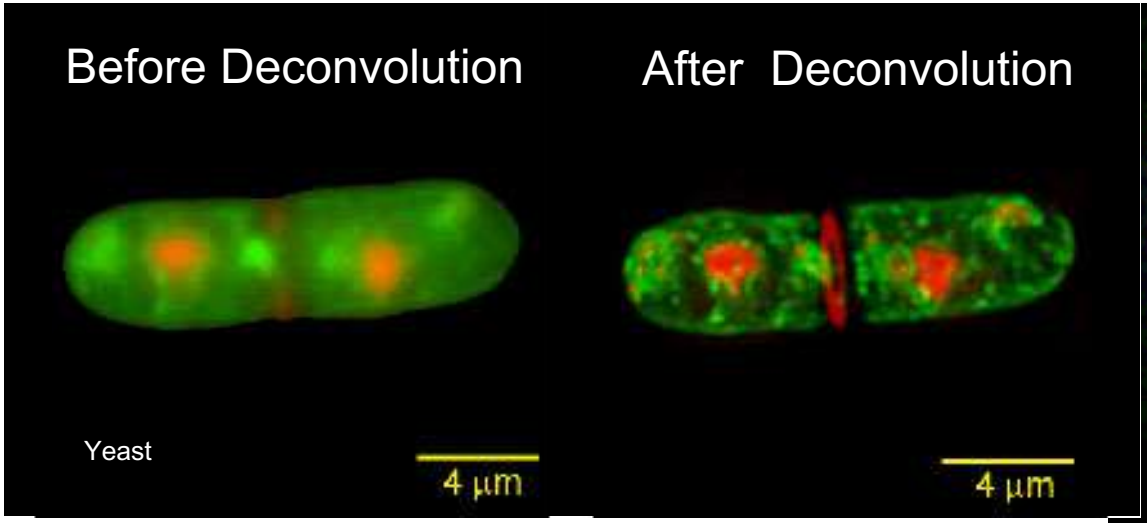
Cell Outline
Con A-Alexa633
Microtubules
YFP-Tub1
DNA
DAPI
Spindle Pole Body
Spc29-CFP



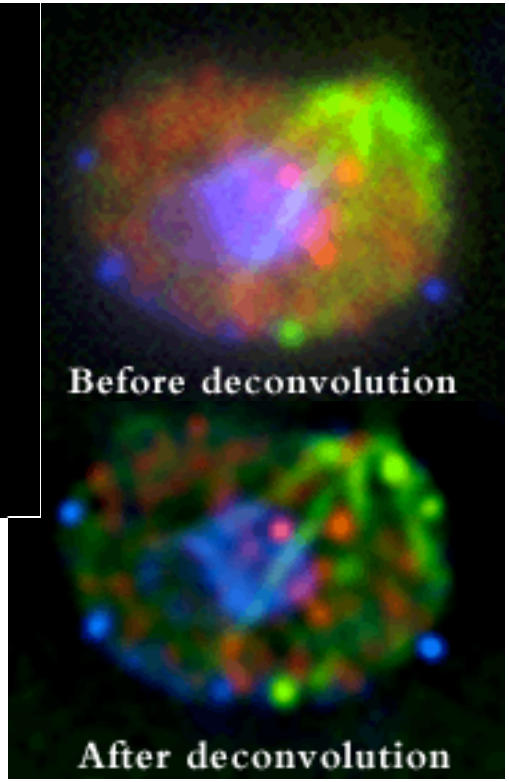
Centrosomes and Spindle Pole Bodies: Methods in Cell Biology, Volume 67 . Robert E. Palazzo, Trisha N. Davis. San Diego: Academic press, 2001. 375p

(Hailey, Muller & Davis, Yeast Resource Center, University of Washington)

The Best Imaging System for Small or Dim Cells

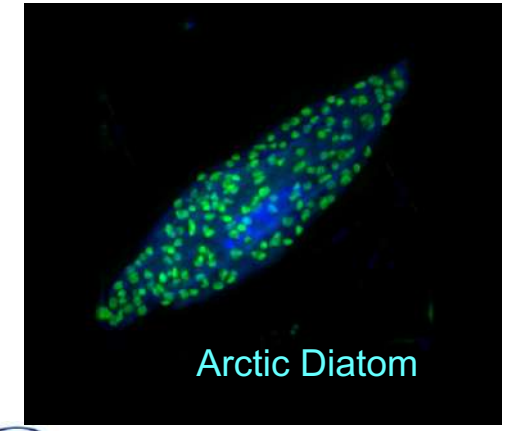


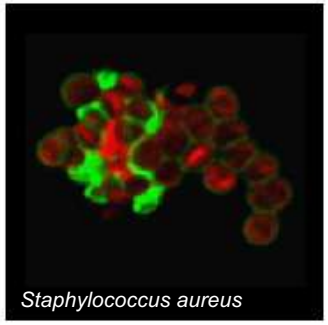
The ring of the developing medial septum (red) is forming between the two nuclei (red) that arose by nuclear division during the previous mitosis. Cellular membranes are also shown (green). Both the DNA and the medial septum are stained with Hoechst 33342 and membranes are visualized using the fluorescent lipophilic dye DiOC6. *Image courtesy of Janos Demeter and Shelley Sazer, Department of Biochemistry, Baylor College of Medicine, Houston, Texas*



Yeast cells labeled for actin (green), Cln2 (red) and nuclei (blue). Images were captured and deconvolved using the DeltaVision image restoration microscope, allowing fine structures to be revealed within these small cells.

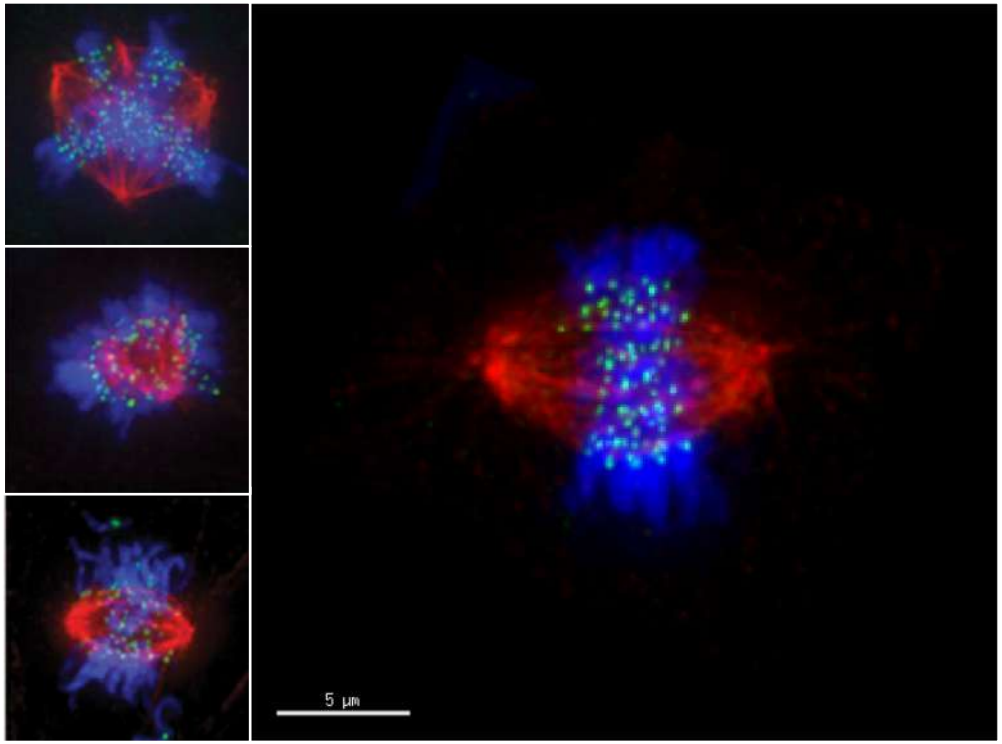
Mary Miller and Frederick Cross (Rockefeller University)





Staphylococcus aureus surface-labeled with green-fluorescent Alexa Fluor 488 wheat germ agglutinin (W11261) and stained with SYTO 59 red-fluorescent nucleic acid stain (S11341). A series of z-section images was acquired with a DeltaVision wide-field optical sectioning microscope (Applied Precision, Inc.). A three-dimensional projection movie was generated from a deconvolved z-image stack. *Courtesy. Molecular Probes*

Mitotic spindle in an induced pluripotent stem cell



The volume view shows a normal centrosomal structure in green of the iPSC. Organized microtubules in red provide structure for the cell, as well as work to pull chromatids in blue apart during cell division. Katanin-mediated severing serve to promoting microtubule disassembly and efficient movement.

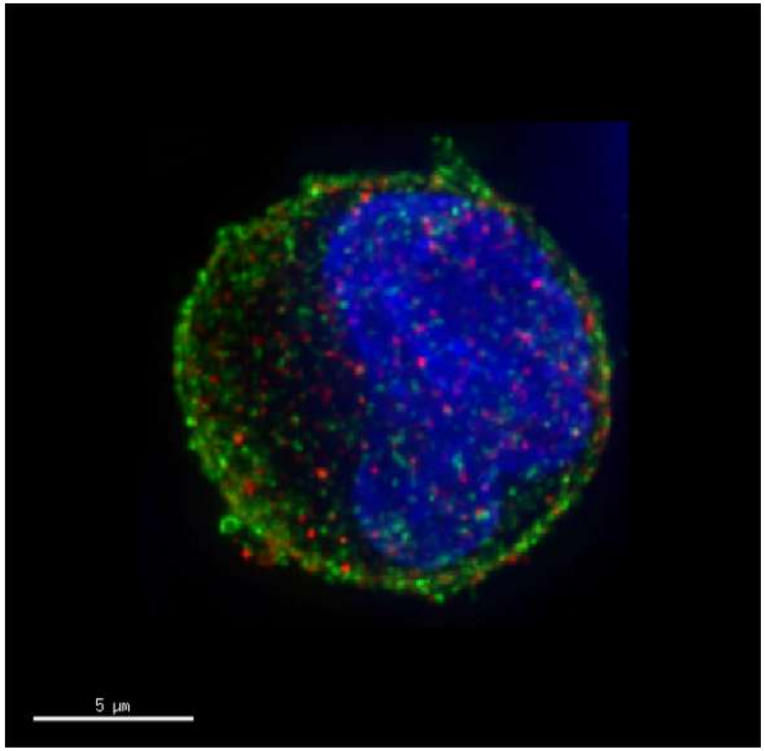
Loss of Katanin p80 disrupts spindle structure and mitosis - **10% of cells displayed multipolar** spindles, with supernumerary chromosomes and excess kinetochores, **22% of cells displayed abnormal monoastral** spindles, and an additional **19% contain misaligned chromosomes.**

Hu et al, Neuron. 2014
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4485387

DAPI, Centromere, β-Tubulin, scale bar 5 um

HIV virus-like particles localize on cell surface

3D volume & Z stack view shows localization of virus-like particles near cell surface marker



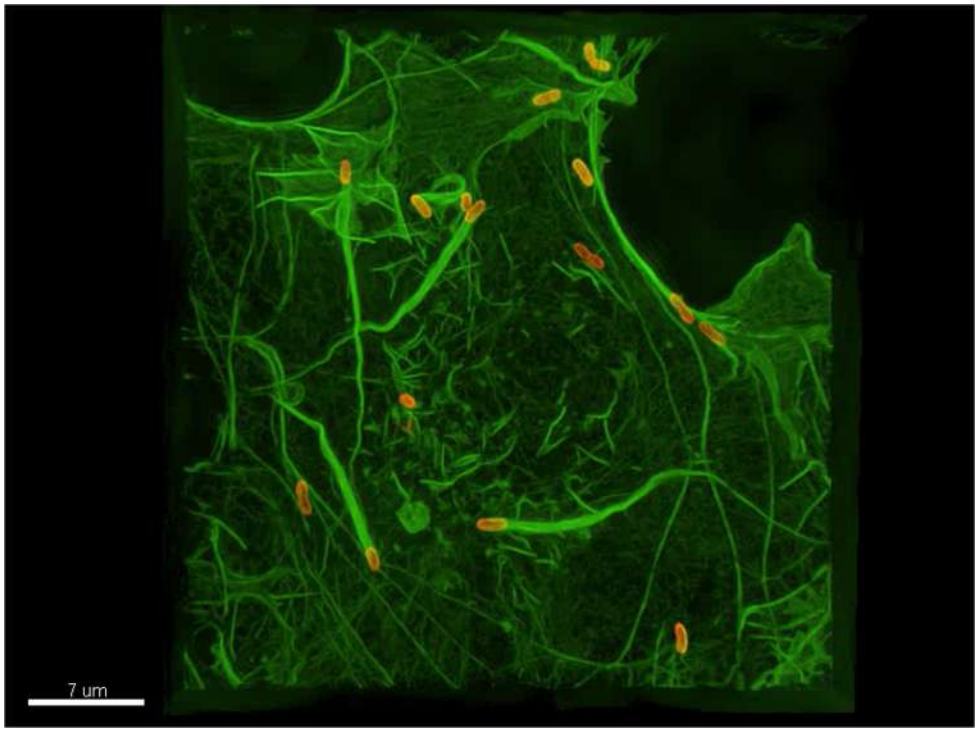
CD169+ virus-containing compartments (VCCs) in green is a mechanism of DC-mediated HIV-1 trans-infection, hence there is an interest to study the mechanism of VCC formation and its role in immune evasion mechanisms of HIV-1.

The researchers are looking at localization of HIV virus-like particles to the cell surface. The cell surface is marked with CD169 in green and the HIV virus like particles are in red. Notice how **as the cell rotates through Z you see that the red dots localize spatially near the green dots.**

Akiyama et al, PLoS Path 2015
http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004751

DAPI, CD169, Gag-mCherry VLP, scale bar 5 um

Widefield deconvolution gives great contrast and high-resolution images of bacteria also!



Protein requirements for Rickettsia motility differ from other pathogens use a distinct molecular mechanism of actin assembly and organization. This study use imaging to define a core set of actin cytoskeletal proteins critical for actin-based motility of rickettsia.

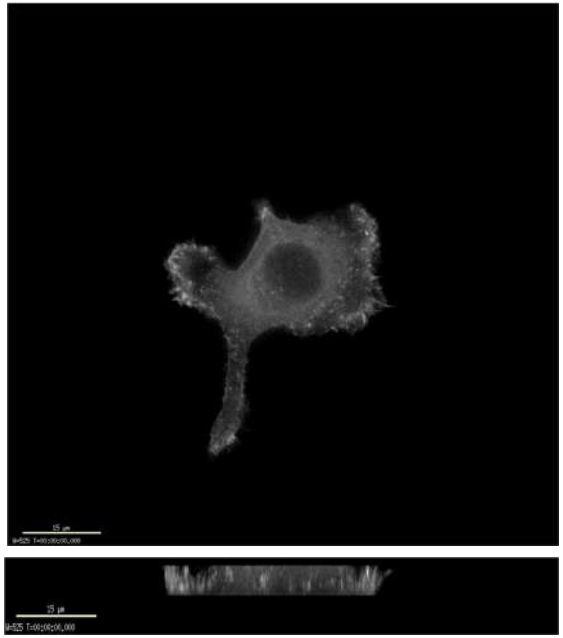
Alisa W. Serio, Robert L. Jeng, Cat M. Haglund, Shawna C. Reed, Matthew D. Welch May 2010

Actin, Bacteria, scale bar 7 um

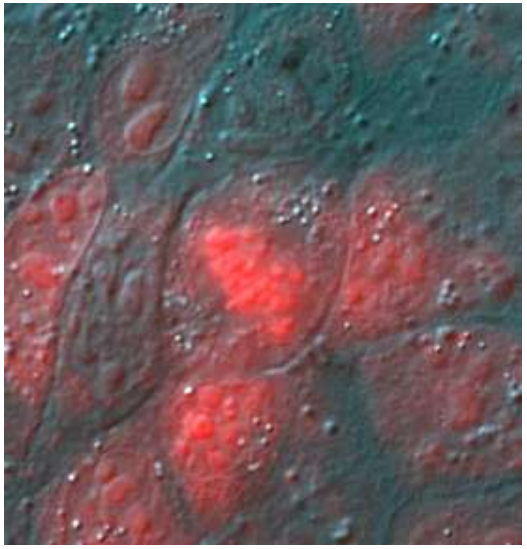
Effect of Restorative Deconvolution

Perfect for live-cell imaging

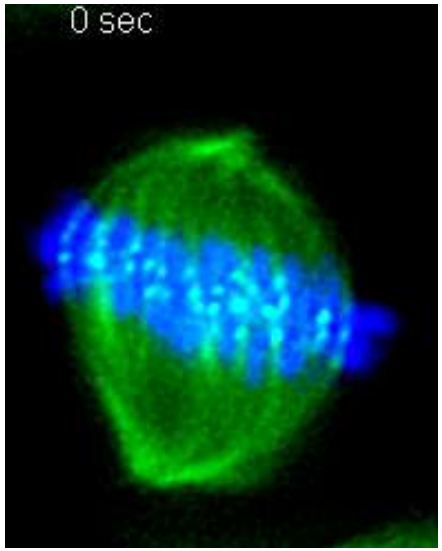
Fish keratocytes with LifeAct (Actin-GFP)



Cell division and chromosome movement in live kidney epithelial cells



Cell division

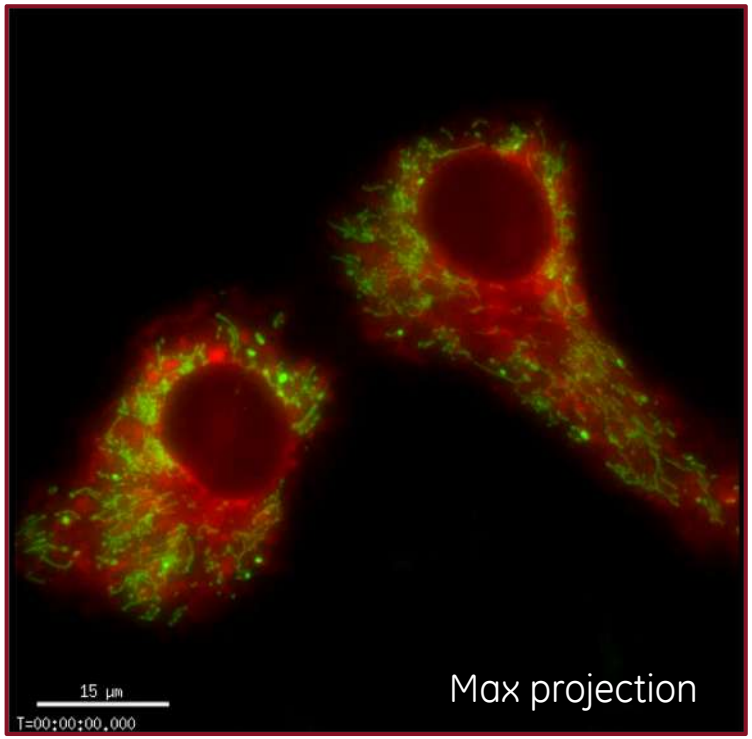


1. OMIBS, Woods Hole, MA
2. Alison North, Ph.D. Rockefeller University Bio-Imaging Facility

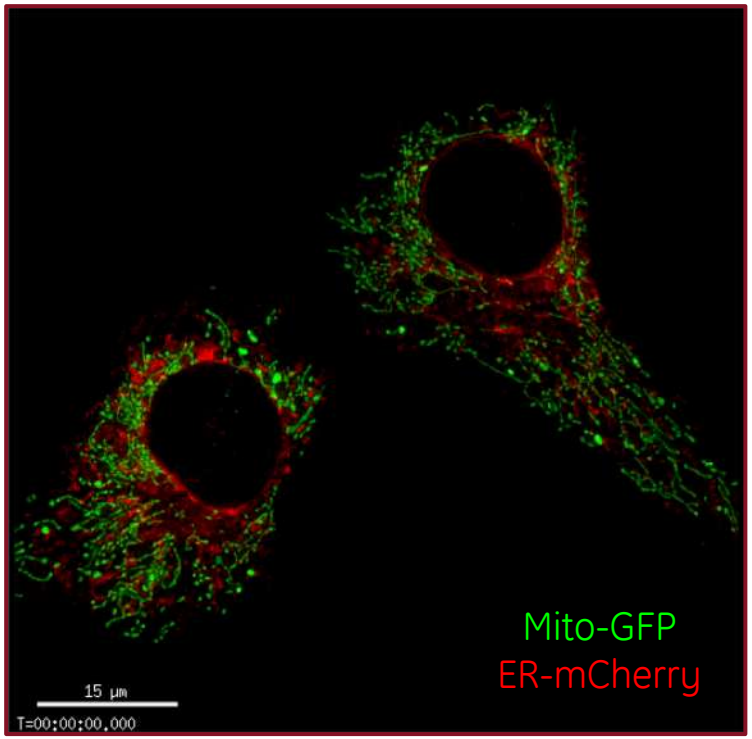
Effect of Restorative Deconvolution

Perfect for live-cell imaging

Widefield



Widefield Deconvolution



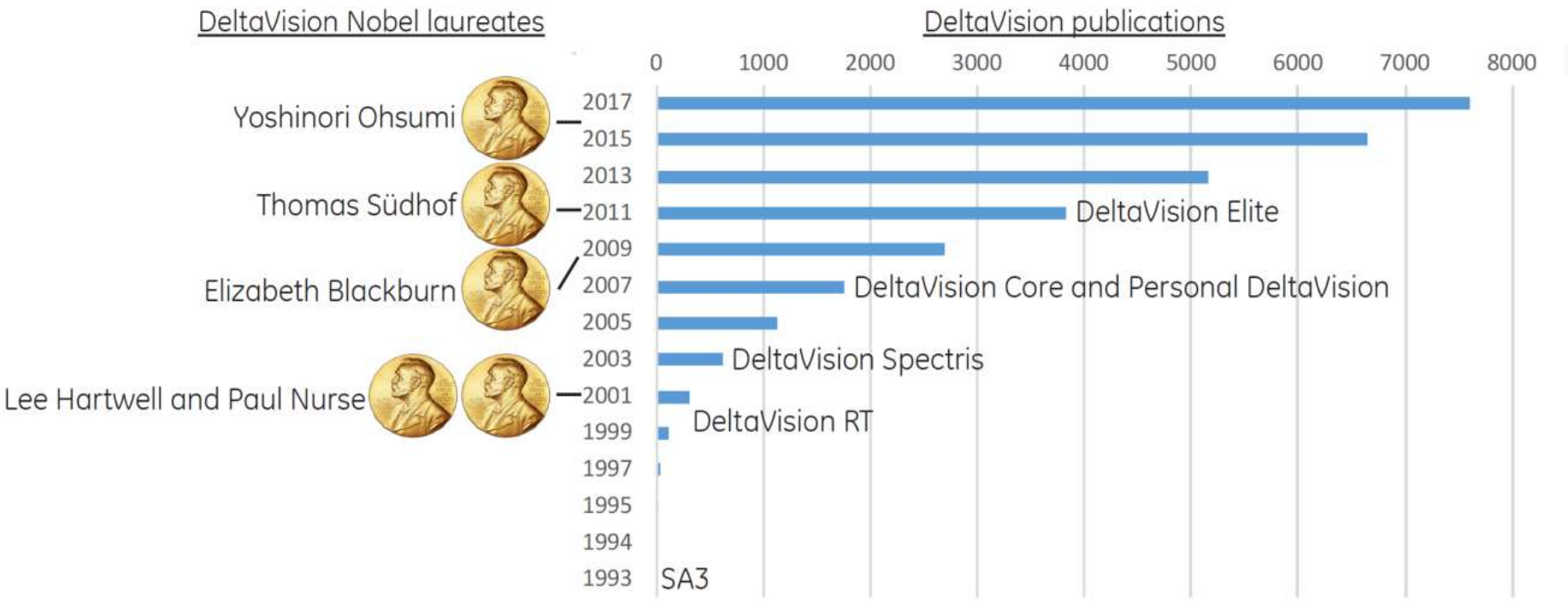
Vaughan Lab, UW

imagination at work

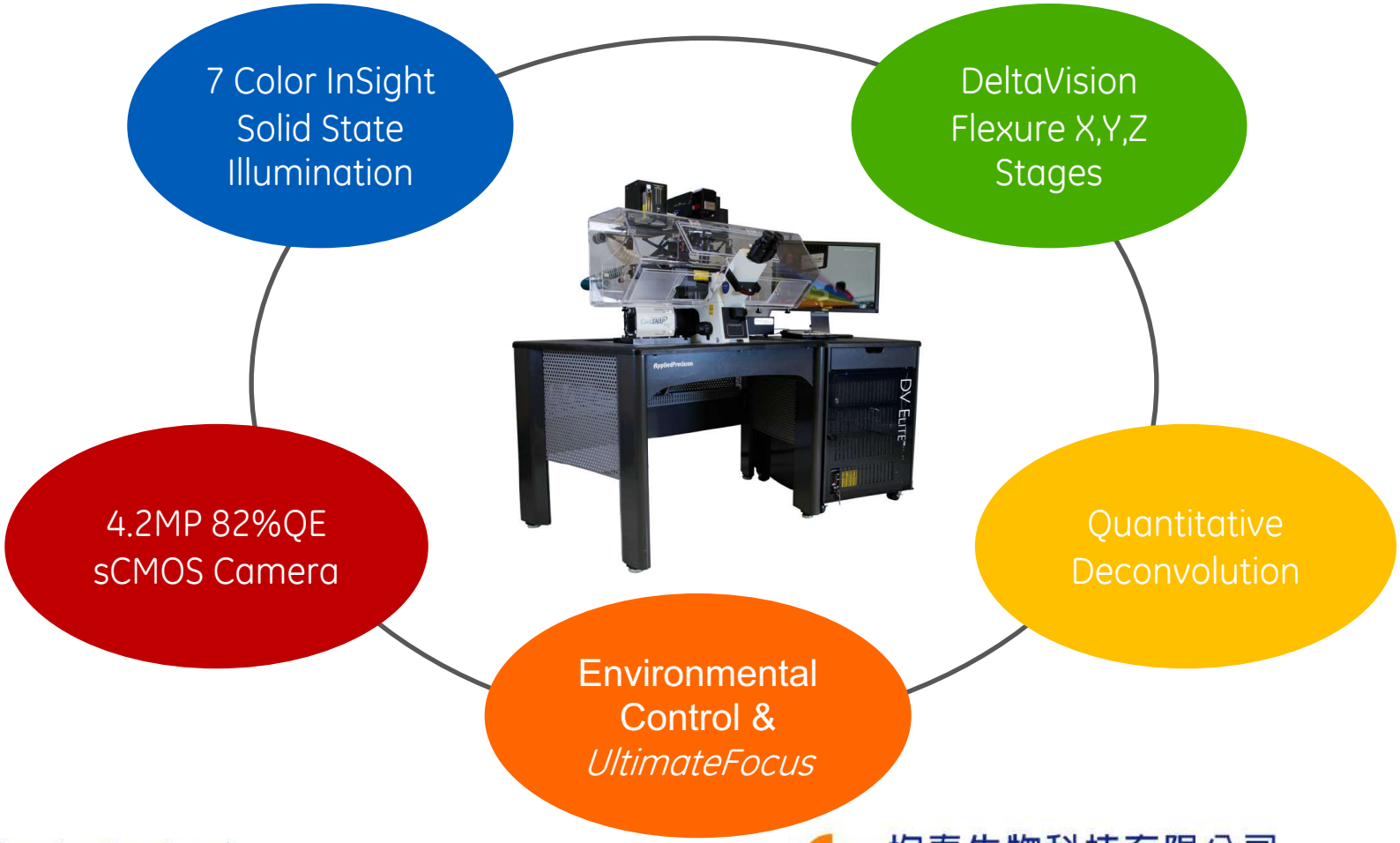
均泰生物科技有限公司
Genetech Biotech Co., Ltd

Publish robust scientific data

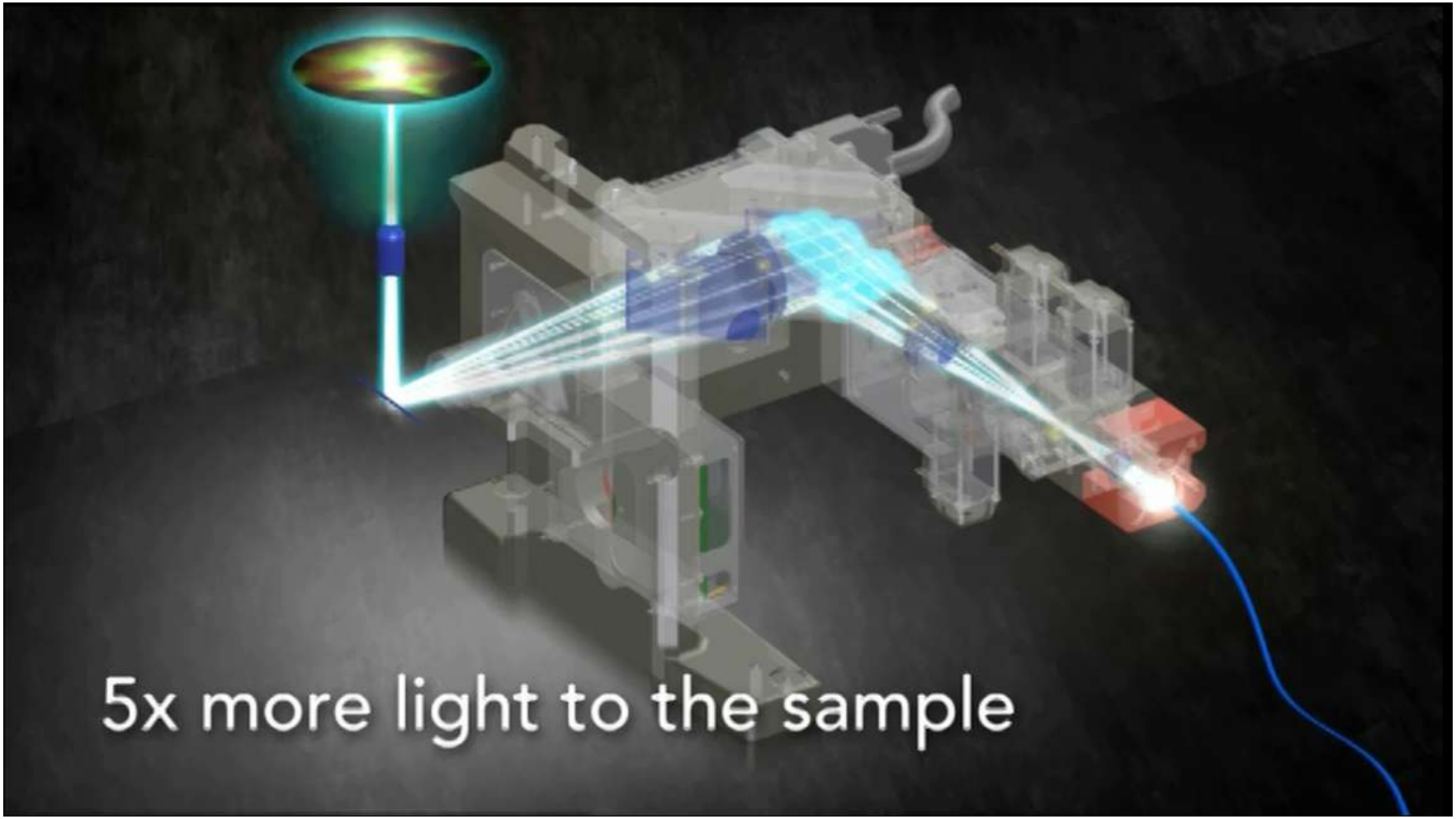
Historical DeltaVision Publications



DeltaVision- Attention to details



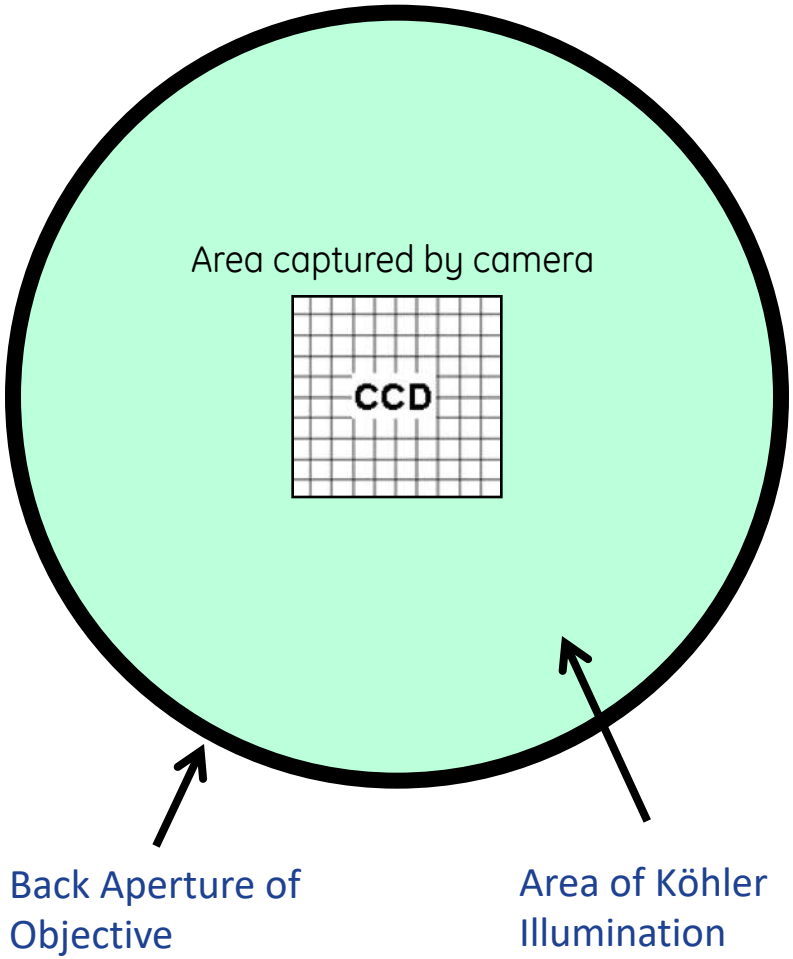
The TruLight Illumination System



Köhler vs. Critical Illumination

Köhler Illumination

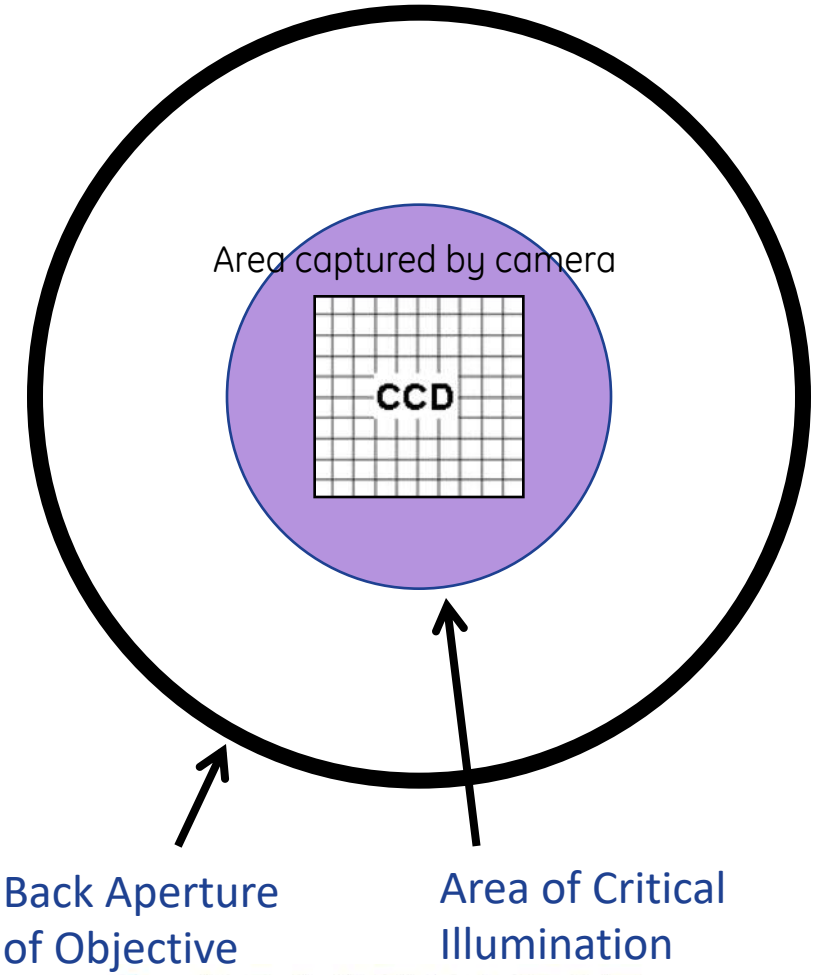
- Light is focused at the back aperture of the objective and is defocused at sample plane.
- Illuminates full field of view through oculars.
- Illuminates area greater than CCD chip.



Köhler vs. Critical Illumination

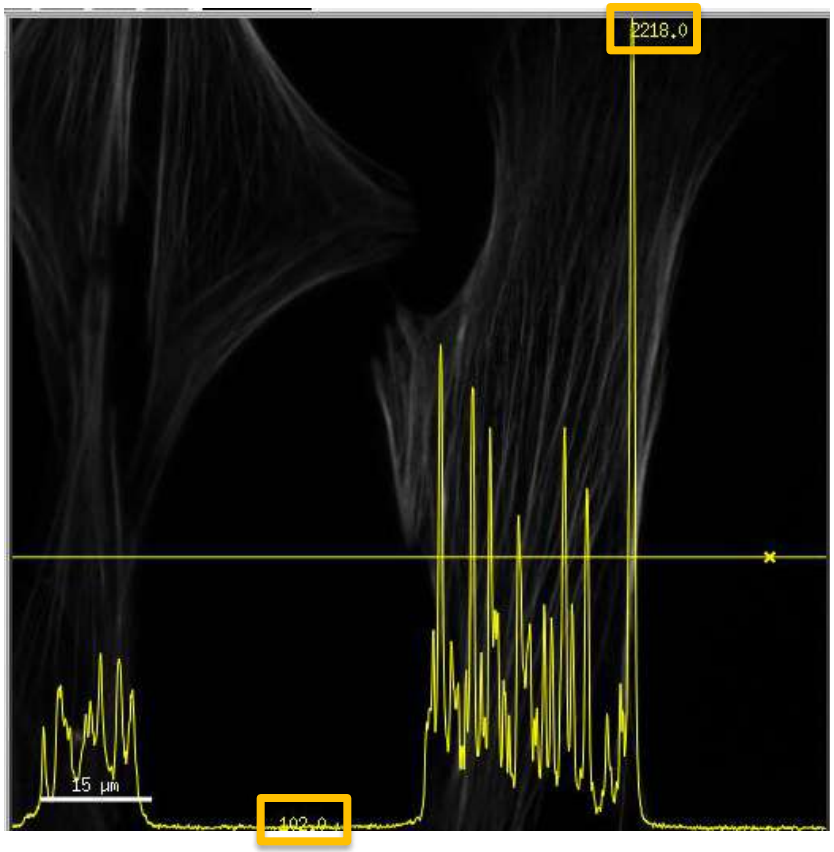
Critical Illumination

- Light is focused at the sample plane.
- Illuminates area surrounding CCD chip.
- Increases intensity of light over Köhler illumination.
- May decrease viewing area of the oculars.

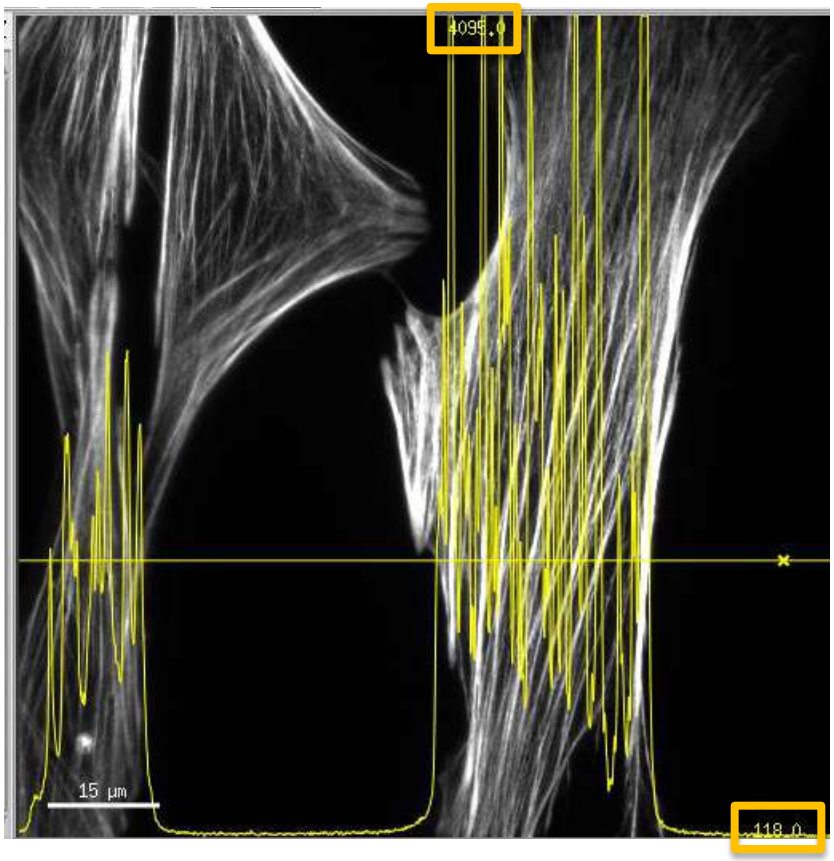


TruLight Illumination Systems

Viewing Mode

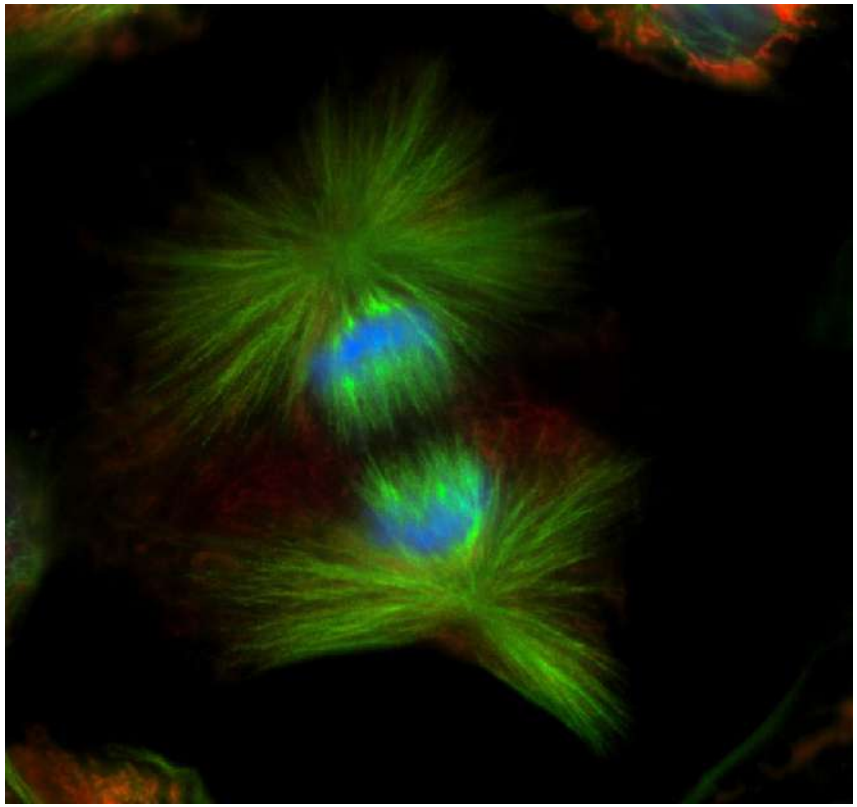


Imaging Mode

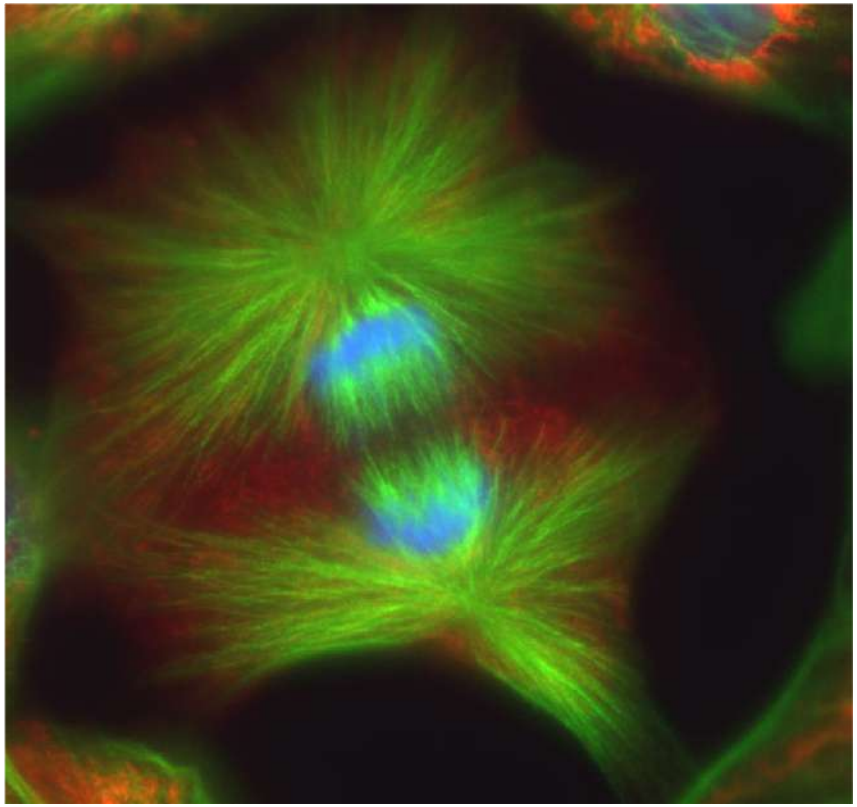


Same field of view, same exposure conditions

Delivers 5x More Light to the Sample



Standard Light Path



TruLight Illumination

The TruLight Illumination System

- Use lower exposure times to collect signal- improves viability
- Collect images more frequently to capture faster events
- Better signal to noise ratio
- Restrict illumination to the region of the camera chip-- protects cells in neighboring areas

InsightSSI Illumination Units

- High performance light technology
  - Increased power across ALL wavelengths
  - Uniform and consistent illumination across channels
  - Discrete channels of illumination eliminate UV leakage
- Faster performance
  - Instant on/off
  - Microsecond switching between wavelengths
  - Electronic shuttering eliminates moving parts for maximum speed
- Flexible options
  - 7 Color Combined Set for maximum flexibility

InsightSSI illumination module

Filter Set

Filter name	Probe	Ex (nm)	Em (nm)
1. Standard filter set (DAPI-FITC-TRITC-CY5)			
DAPI	DAPI, Hoechst, Alexa 350	381-412	420-456
FITC	Fluorescein, EGFP, Alexa 488	464-492	500-523
TRITC	Rhodamine, Texas Red, Cy3, Alexa 568	531-565	573-611
CY-5	Cy-5	619-646	654-700
2. mCherry filter set (DAPI-FITC-mCherry-CY5)			
DAPI	DAPI, Hoechst	381-412	420-456
FITC	Fluorescein, GFP, CY2, AL488	464-492	500-549
mCherry	Alexa 594, mCherry	557-590	598-617
CY-5	Cy-5, Alexa 647	625-646	654-700

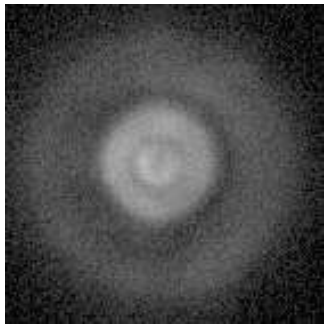
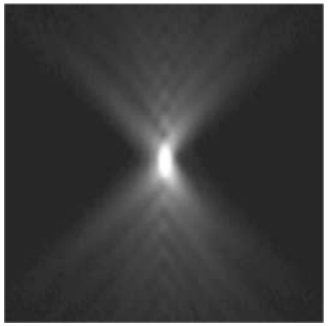
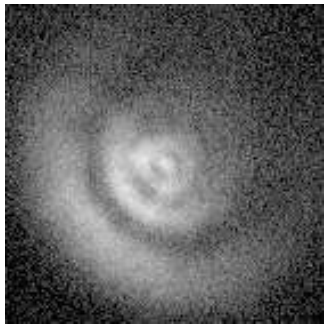
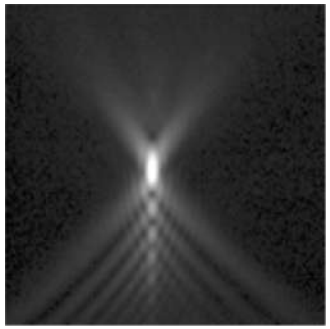
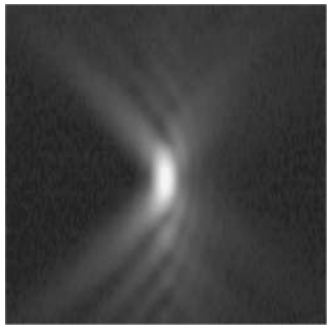
Filter Set

Filter name	Probe	Ex (nm)	Em (nm)
3. Live cell filter set (CFP-YFP-mCherry)			
CFP	CFP, Pacific blue	400-453	463-487
YFP	YFP	497-527	537-549
mCherry	mCherry, A594	557-592	602-662

- Dichroic 3 (CY-mCherry) is using for imaging CFP/YFP/mCherry.
- Using Dichroic 2 (mCherry) for imaging GFP/mCherry.

Hand-selected Objectives

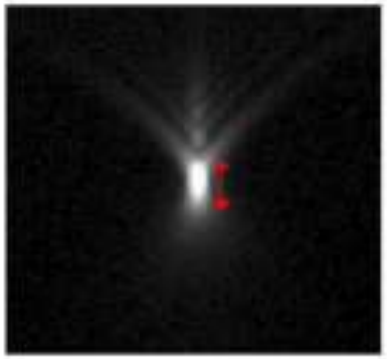
Objectives are hand selected in order to obtain the best PSF possible for every DeltaVision.

	Astigmatism & Coma	Spherical Aberration	Axial Skew
Accept			
Reject			

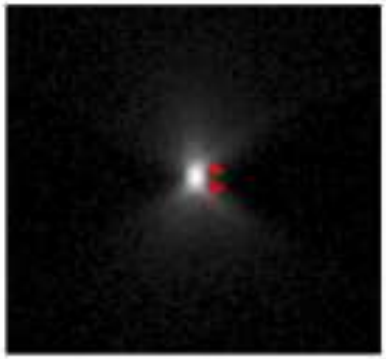
Importance of choosing the correct Oil

Oil Selection

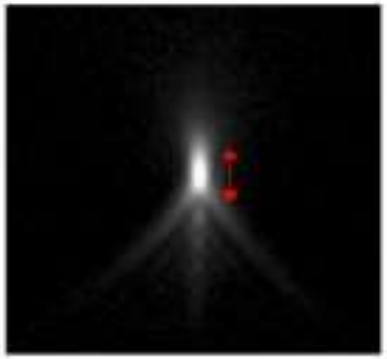
- RI of oil ~ RI of sample
- Crucial step to eliminate spherical aberration
  - Minimize blur due to out of focus light
  - Increase in focus signal level



1.508 RI
Max: 2031

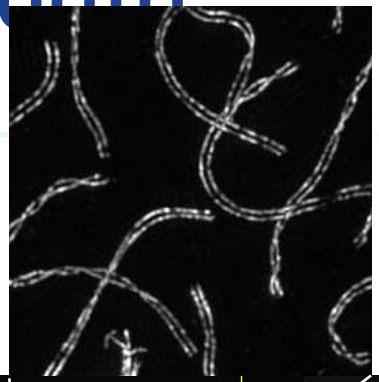


1.514 RI
Max: **3071**



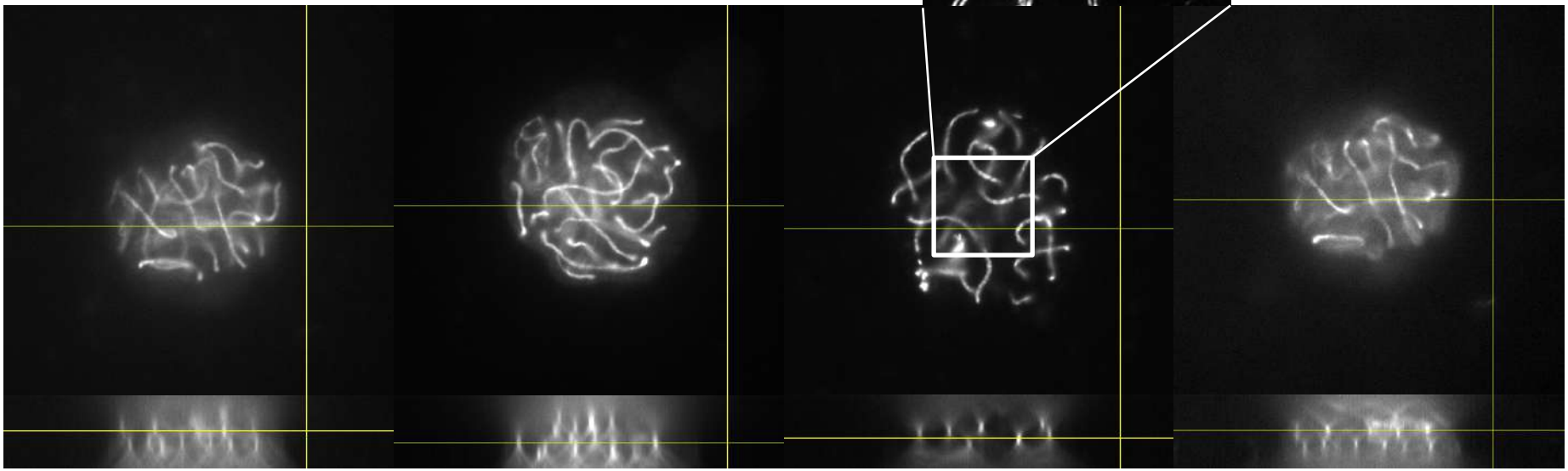
1.520 RI
Max: 1756

A real life situation optimized oil selection for better raw data



3D-SIM
Max
Projection

Better PSF = Better Contrast!!!



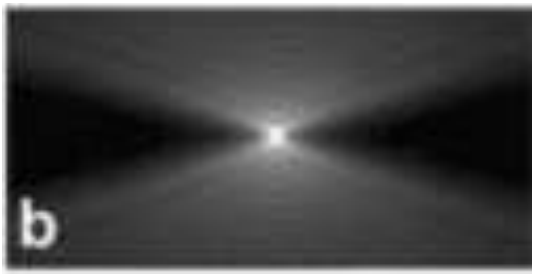
1.518 1.522 1.530 1.534

Oil kit

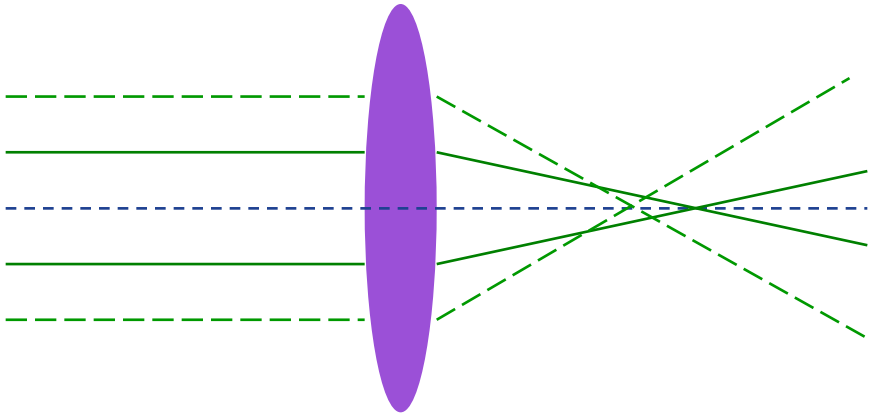
To get perfect PSF

Oil match calculator

- Temperature
- Refractive index of medium
- Distance to sample
- Coverslip thickness



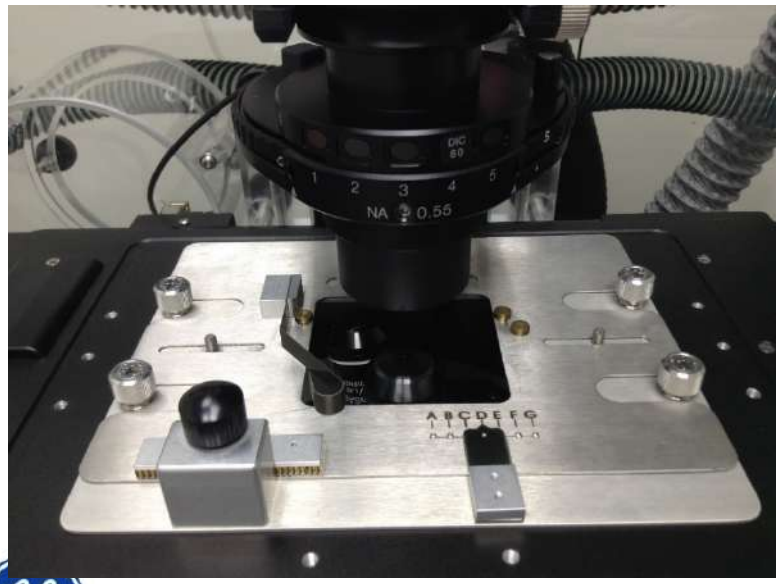
Perfect PSF



Spherical aberration

Stages

- 25 mm x 50 mm Flexure Stage
- Microtiter Stage
- Precision control over stage motion minimizes axial and lateral drift
- Minimal drift that occurs is localized to within optical resolution of the camera



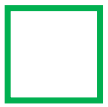
DeltaVision Flexure Stages

- Precision and accuracy are critical in motion control
- Enabled by patented NanoMotion motors and controls

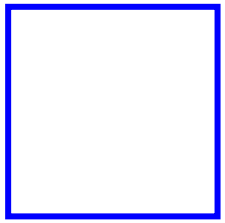
Absolute Accuracy	Repeatability	Step Resolution
< 10 µm per 25 mm (x,y)	< ± 0.2 µm (x,y)	20 nm (x,y)
< 0.6 µm per 13 µm (z)	< ± 0.1 µm (z)	5 nm (z)

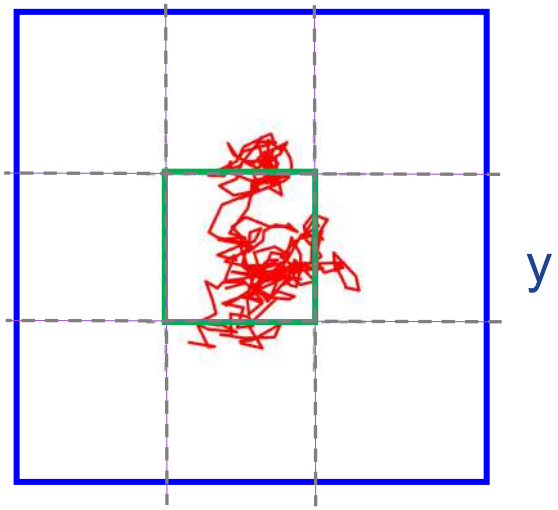
DeltaVision stages confine drift to levels undetectable by the camera for superior time lapse-imaging

Pixel= 64 nm in x, y



Optical Resolution= 200 nm in x, y





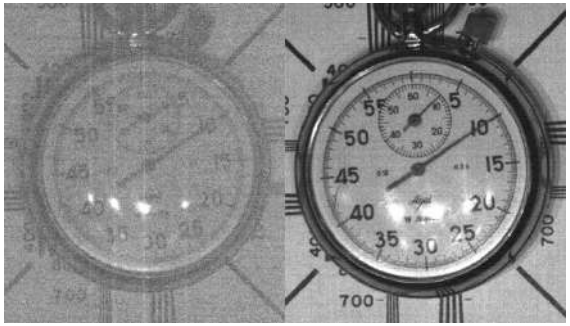
Camera Types

- CoolSnap HQ2: Standard camera
- Evolve EMCCD: High sensitivity (optional secondary camera)
- sCMOS: Fast speed, big area
- All camera software is integrated into softWoRx to optimize speed and performance

sCMOS Camera

- Scientific grade CMOS chip
- 2040 x 2040 pixels (4.2 Megapixel)
- >80% QE
- ~400 fps (512x512, single channel)

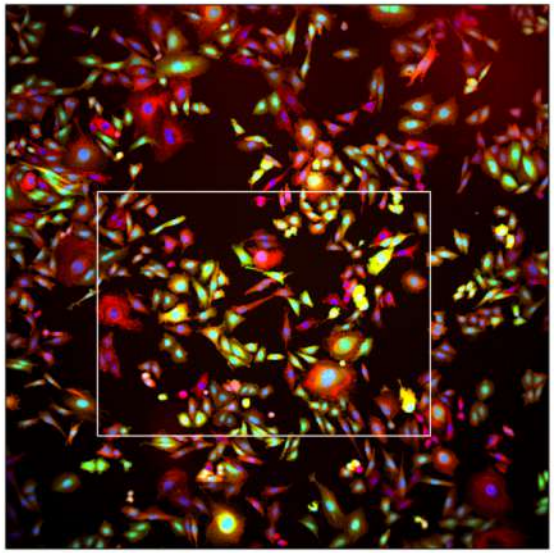
Lower image noise



CCD sCMOS

sCMOS data and images are taken from PCO.edge sCMOS

Large Field of View



Environmental Control

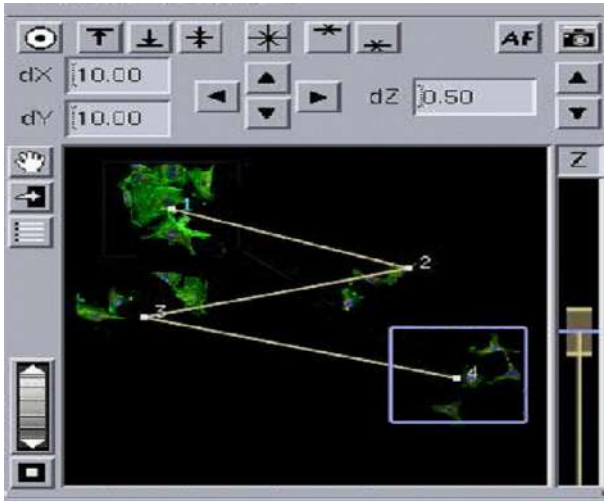
Control over the sample

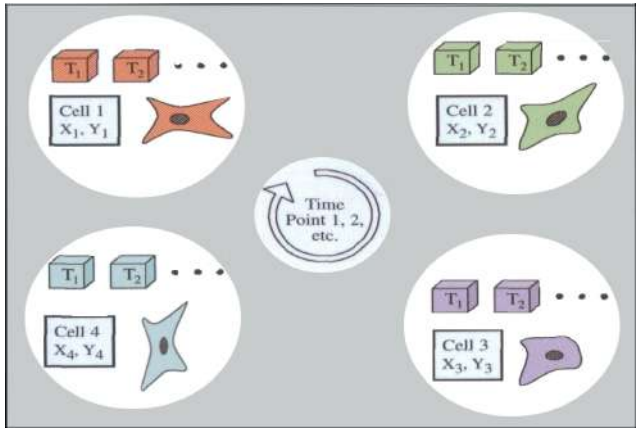
- Cell viability improves when the system mimics the incubator
  - Humidified CO_2
  - Dark environment

Control over the system

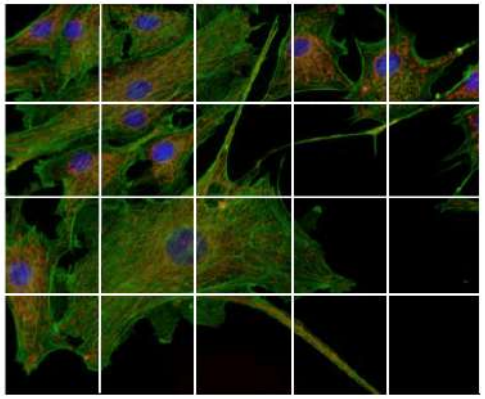
- Mechanical drift is decreased with thermal control
  - Eliminate drafts in the room
  - Enclose as much of the system as possible

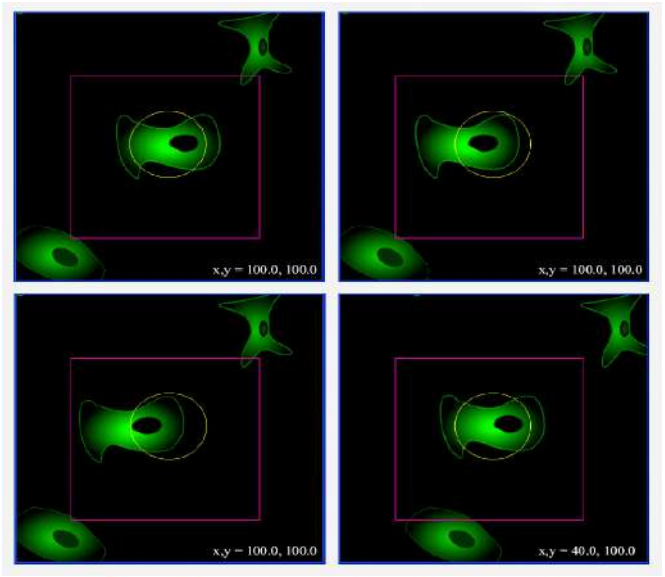
Nanometer Precision XYZ stage For Point-Visiting and Stitching





Example of stitching

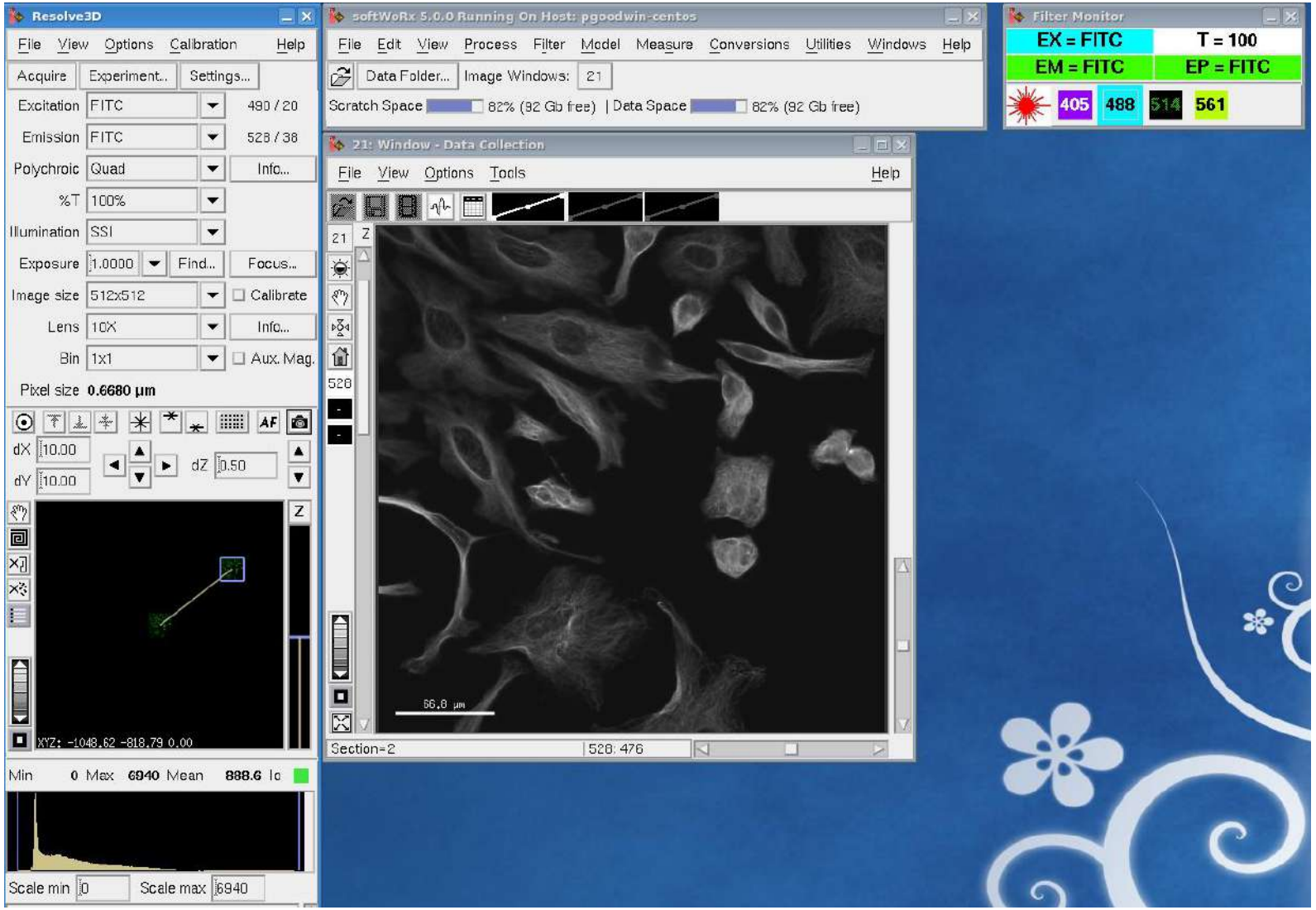




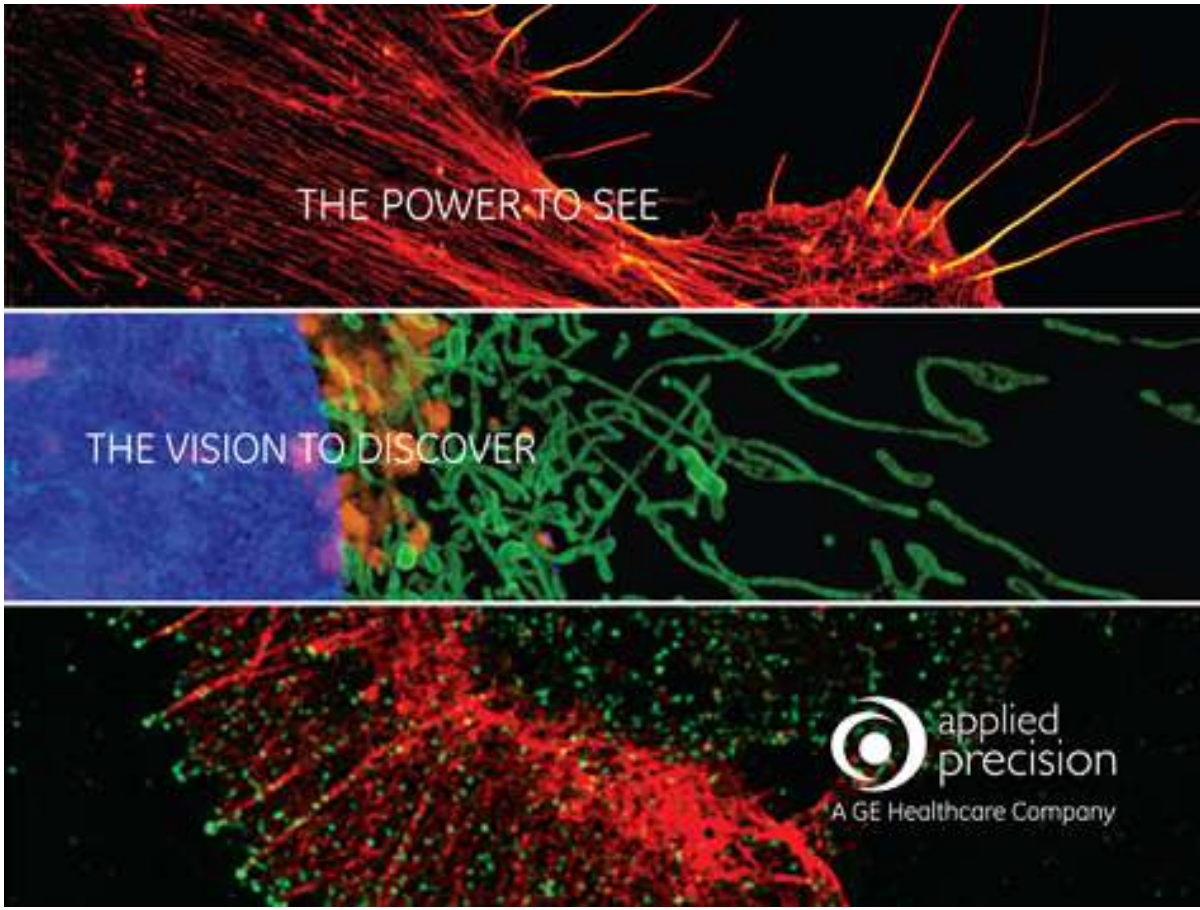
imagination at work

均泰生物科技有限公司
Genetech Biotech Co., Ltd

Software interface



Thanks and any question?



imagination at work

均泰生物科技有限公司
Genetech Biotech Co., Ltd