gibthai
A 3N HOLDING COMPANY

Basic Knowledge of Inverted Microscopy and Introduction of EVOS™ M7000 Fluorescent Imaging System

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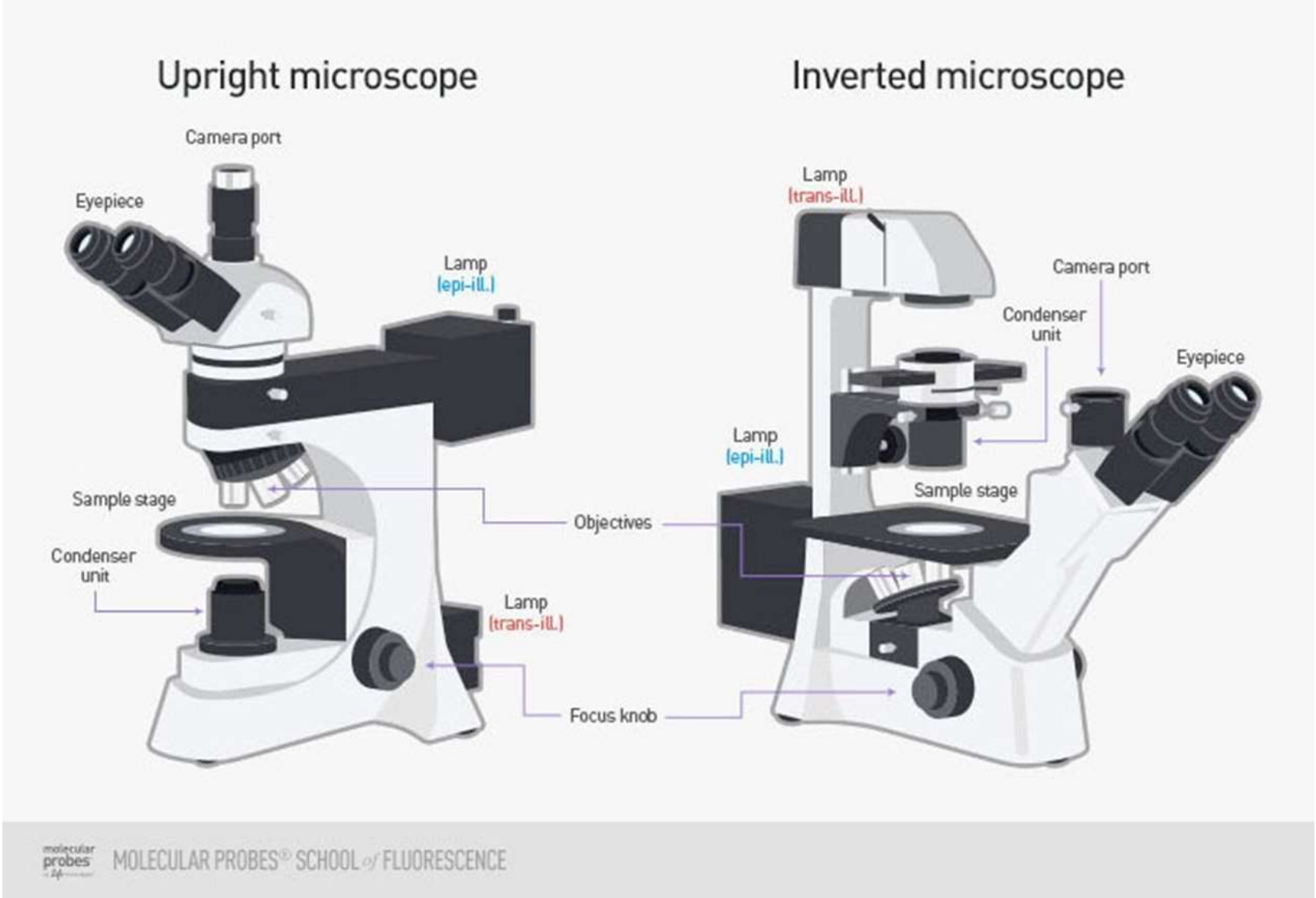
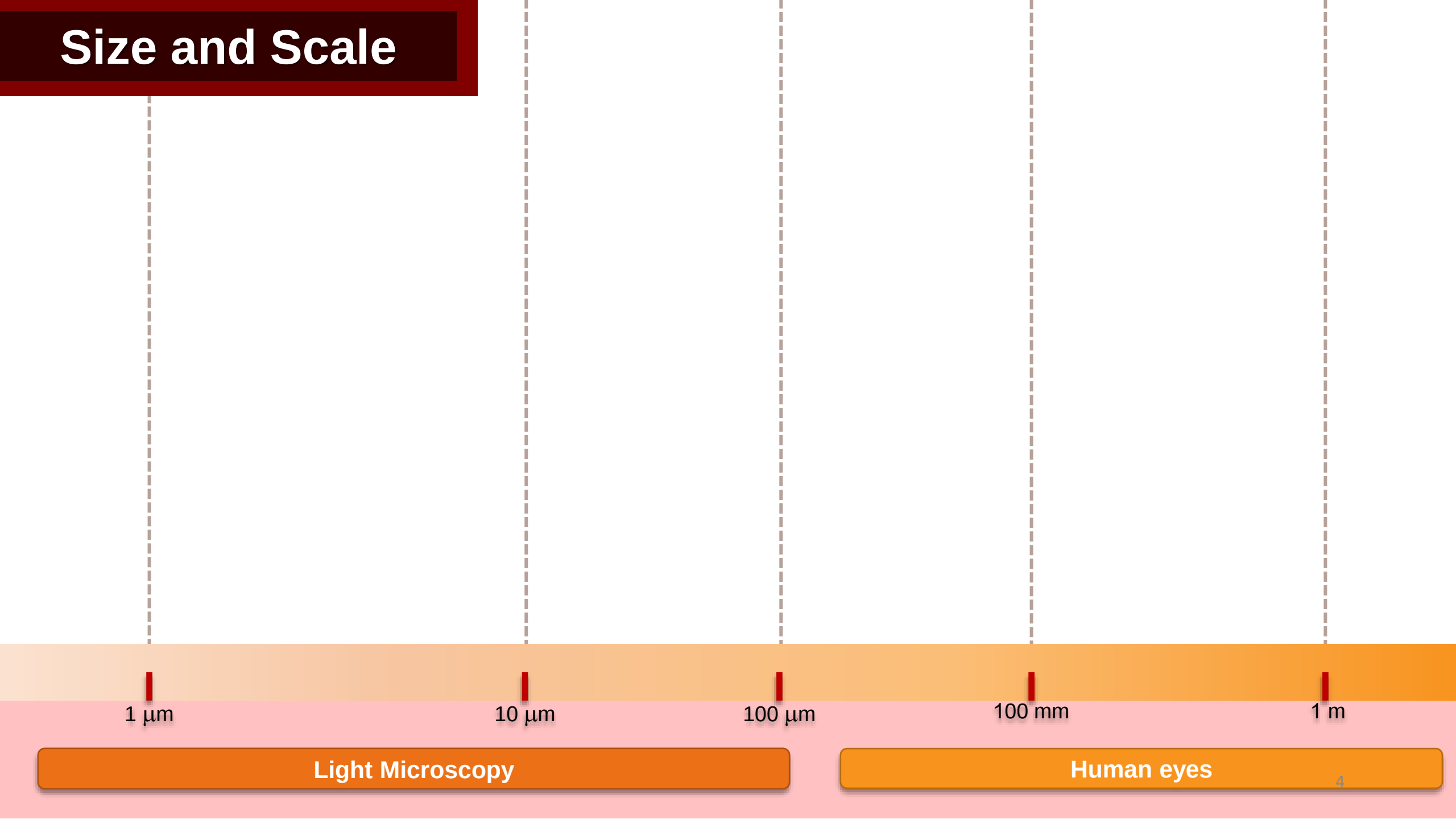
Topics

1. Type of Microscopy
2. Principle of Fluorescent Microscopy
3. EVOS M7000 Imaging System

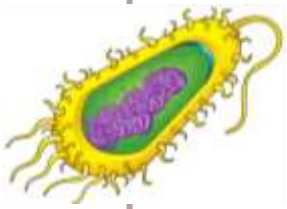
Why we use microscope ?



Size and Scale



Size and Scale



Bacteria cell

White blood cells

mitochonrion

Red blood cells

Animal cell

Chicken egg

Stomach

Heart

Lung

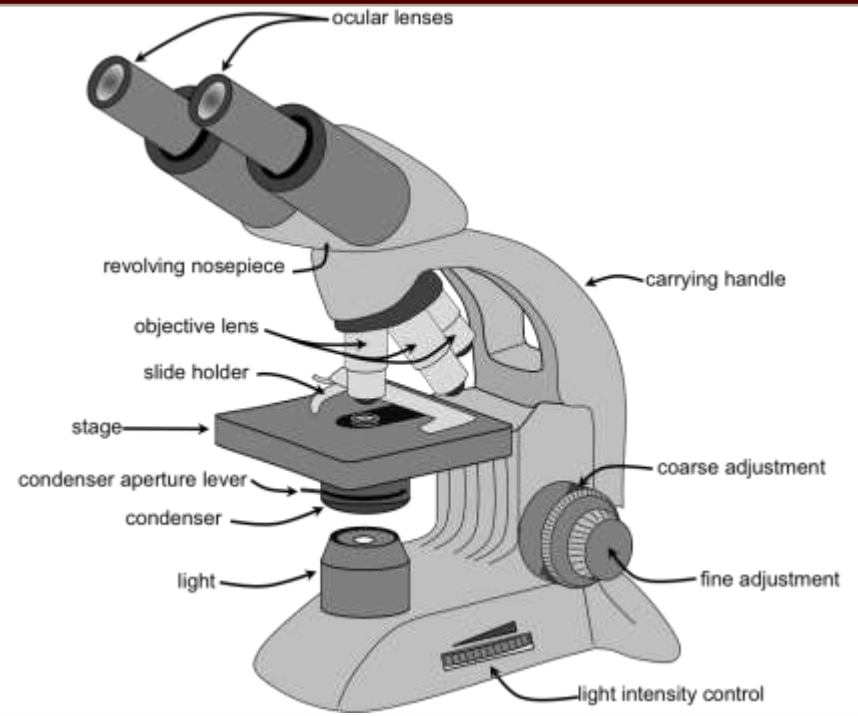
1 µm | 10 µm | 100 µm | 100 mm | 1 m

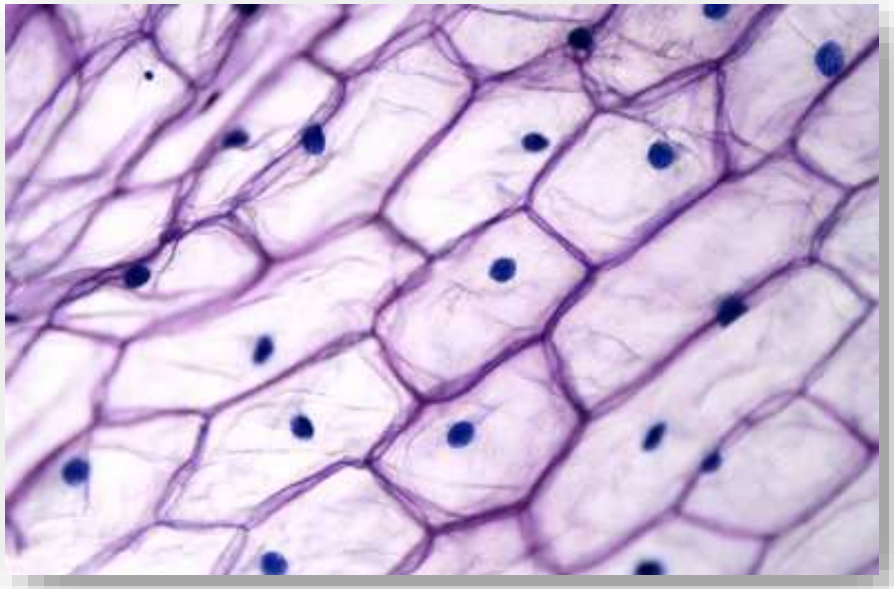
Light Microscopy

Human eyes

Microscope

- **Compound Microscope**



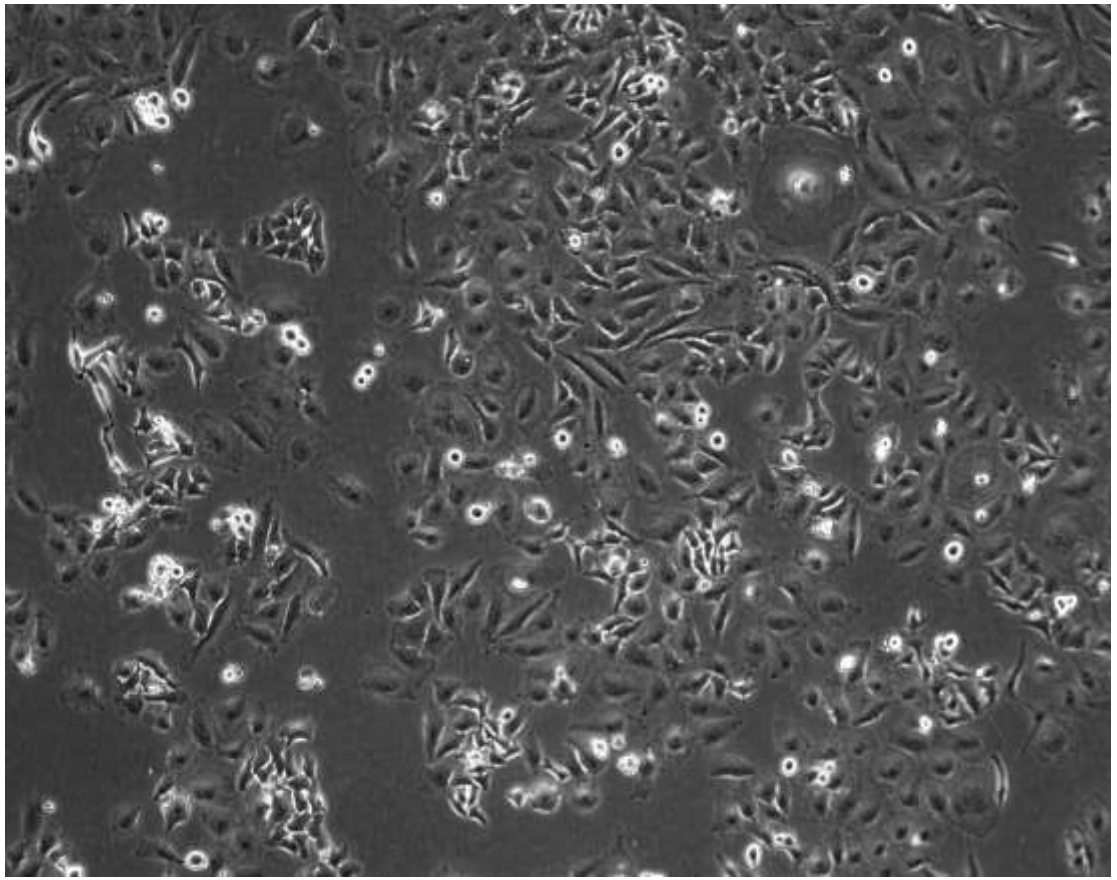


Microscope

- Compound Microscope
- **Inverted Microscope**

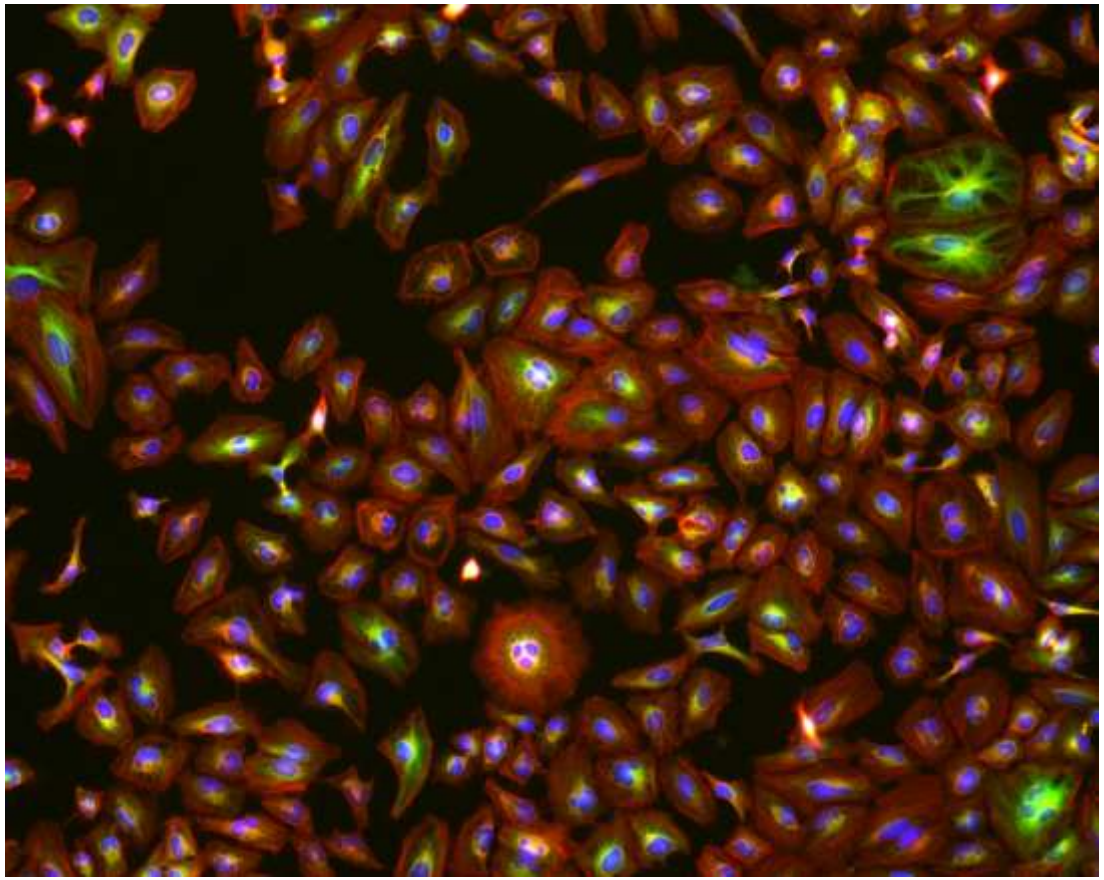
Why Fluorescence in Microscopy?

Brightfield



VS.

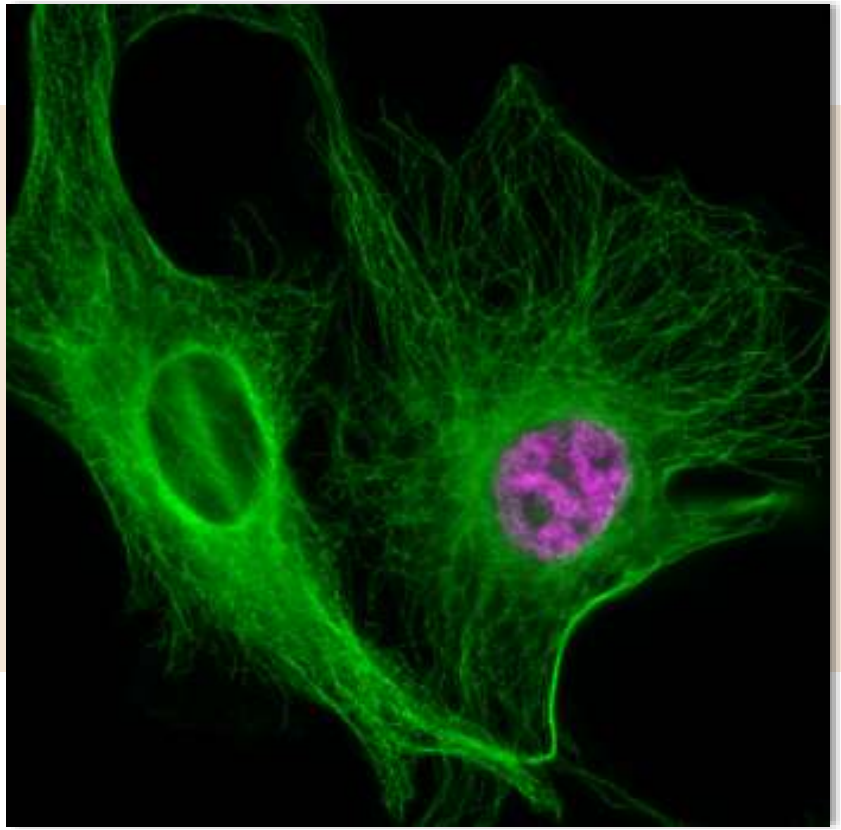
Fluorescence

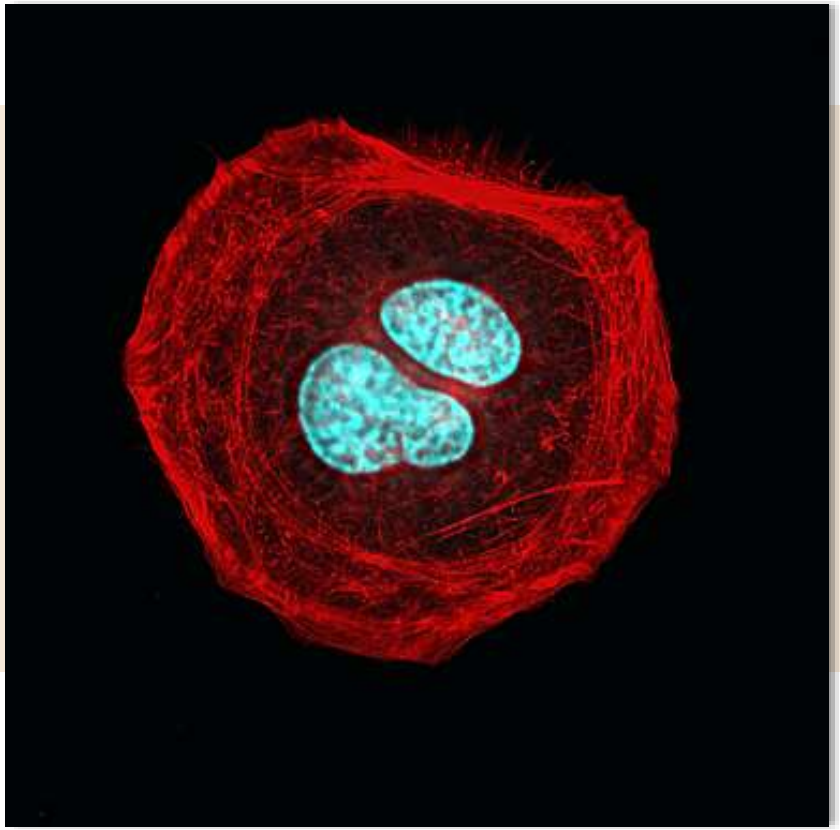


Why Fluorescence in Microscopy?

Beautiful images

tell a story





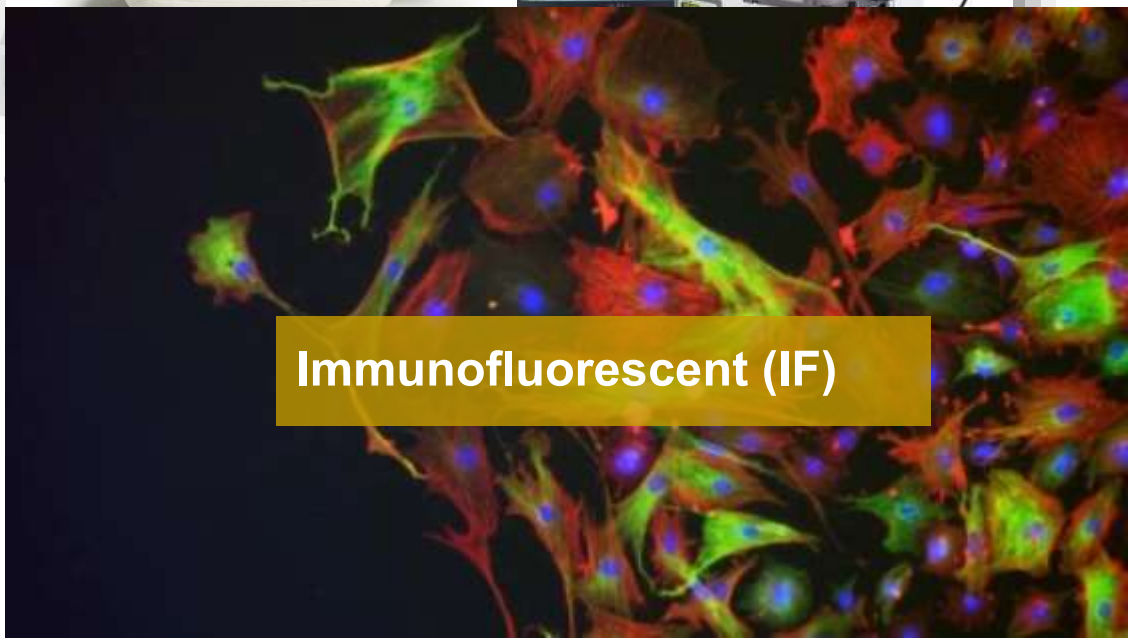
Microscope

- Compound Microscope
- Inverted Microscope
- **Fluorescent Microscope**

Epifluorescence microscope

Microscope

- Compound Microscope
- Inverted Microscope
- **Fluorescent Microscope**

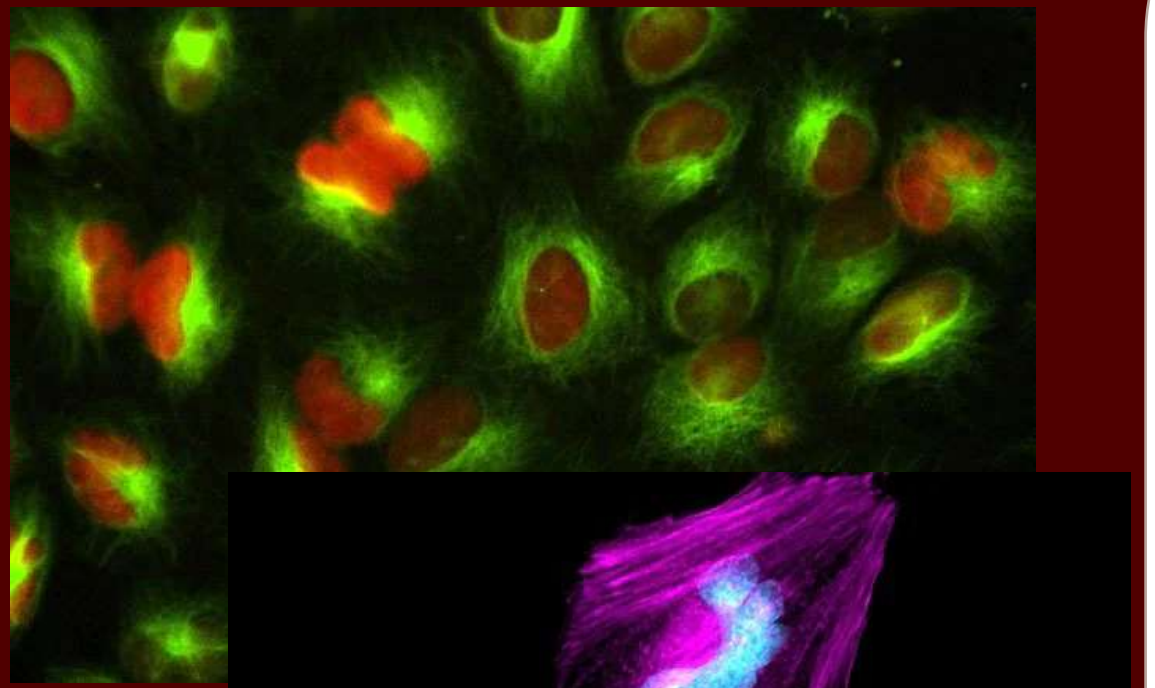


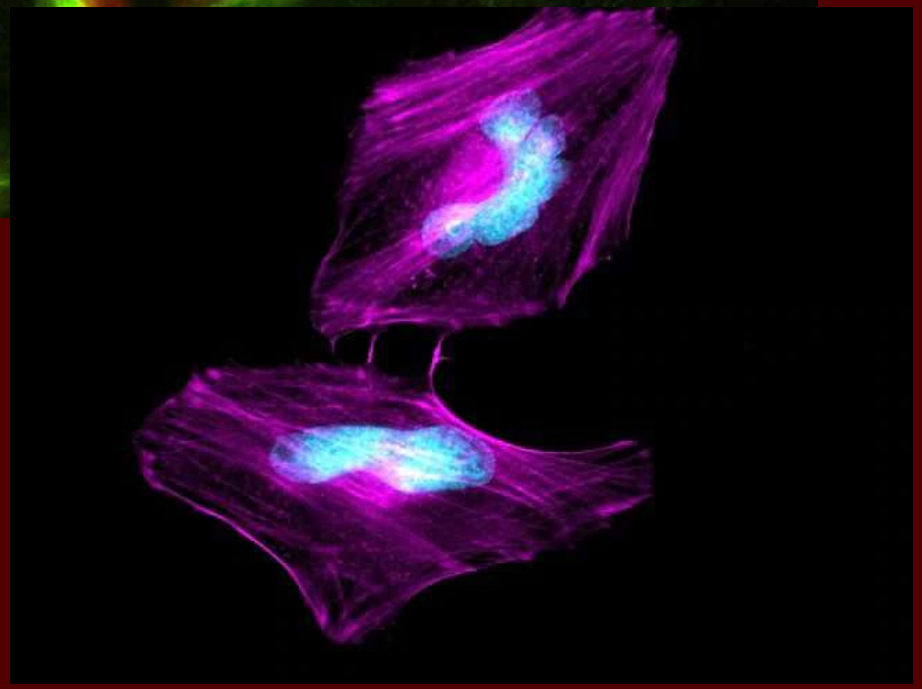
Epifluorescence microscope

Microscope

- Compound Microscope
- Inverted Microscope
- Fluorescent Microscope
- **Confocal Microscope**

Fluorescent Microscope



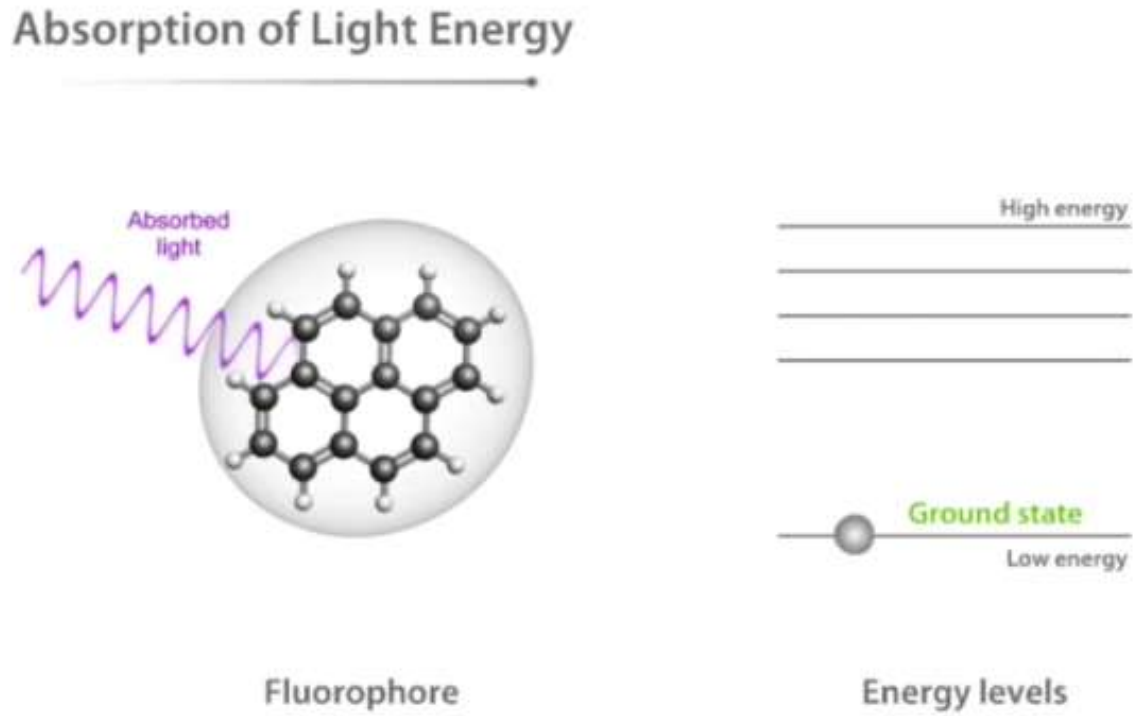


Epifluorescence microscope

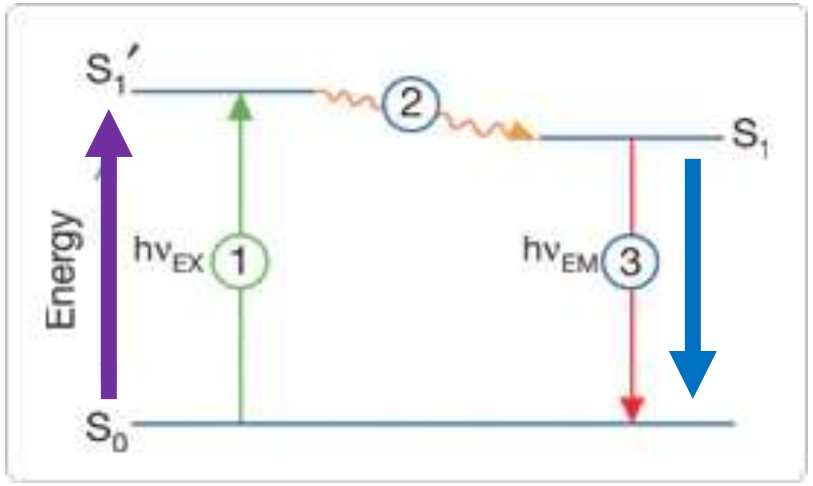
Topics

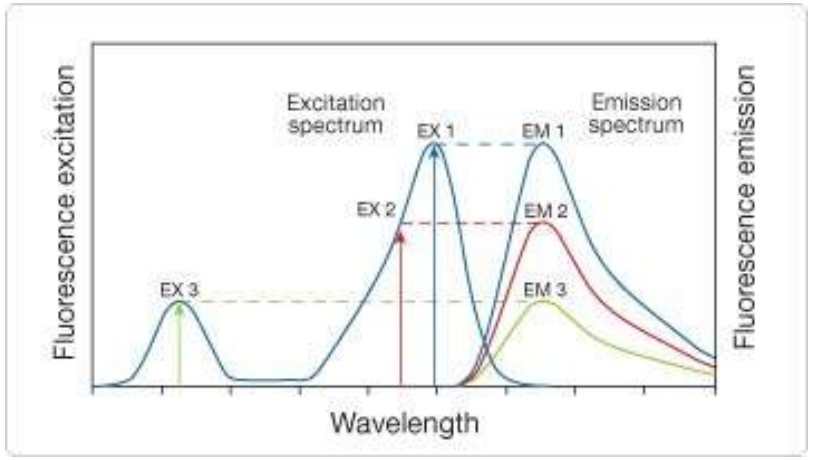
1. Type of Microscopy
2. Principle of Fluorescent Microscopy
3. EVOS M7000 Imaging System

The Principle of Fluorescence

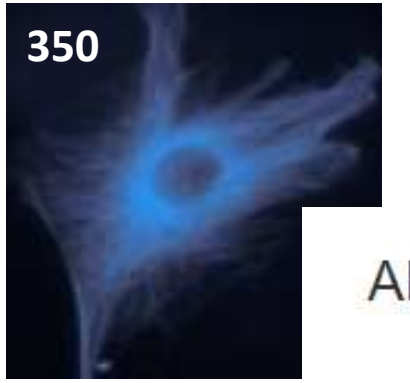


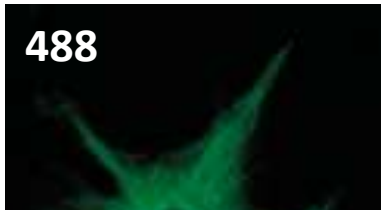
molecular probes

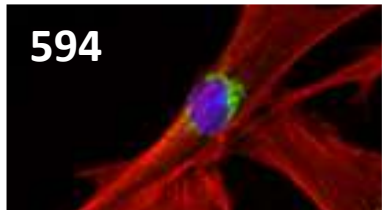


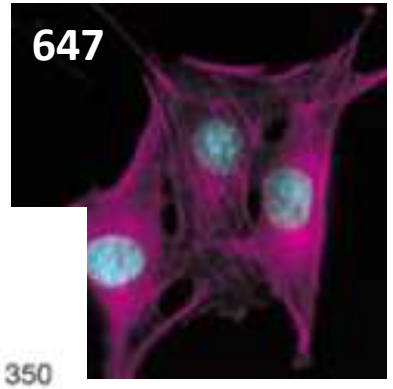


Fluorescence: The Main Colors









Blue

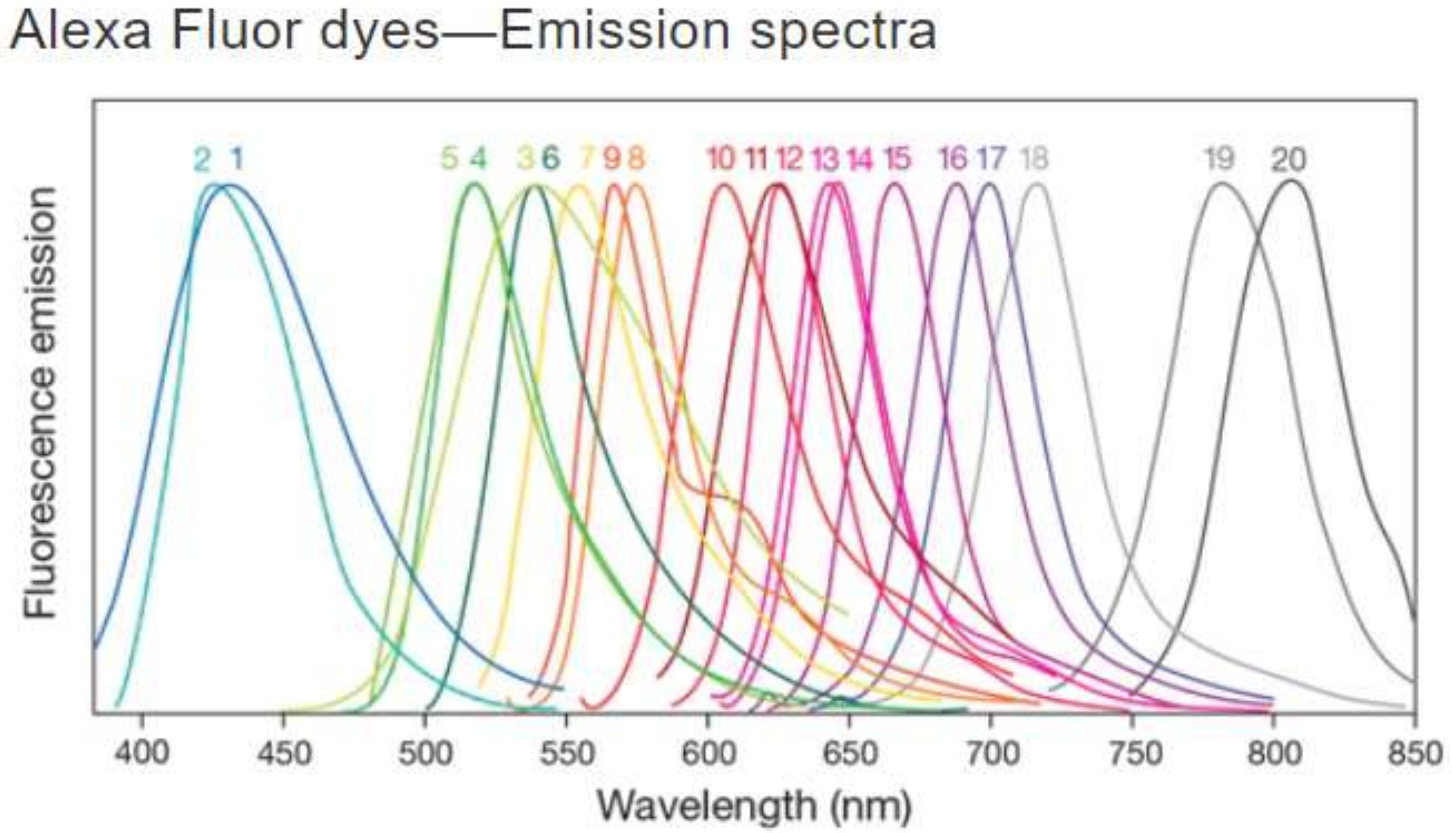
350

Alexa Fluor 405

DAPI

Hoechst

CFP



1. Alexa Fluor 350
2. Alexa Fluor 405
3. Alexa Fluor 430
4. Alexa Fluor 488
5. Alexa Fluor 500
6. Alexa Fluor 514
7. Alexa Fluor 532
8. Alexa Fluor 546
9. Alexa Fluor 555
10. Alexa Fluor 568
11. Alexa Fluor 594
12. Alexa Fluor 610
13. Alexa Fluor 633
14. Alexa Fluor 635
15. Alexa Fluor 647
16. Alexa Fluor 660
17. Alexa Fluor 680
18. Alexa Fluor 700
19. Alexa Fluor 750
20. Alexa Fluor 790

Figure 1. Emission spectra for Alexa Fluor dyes.

Far red

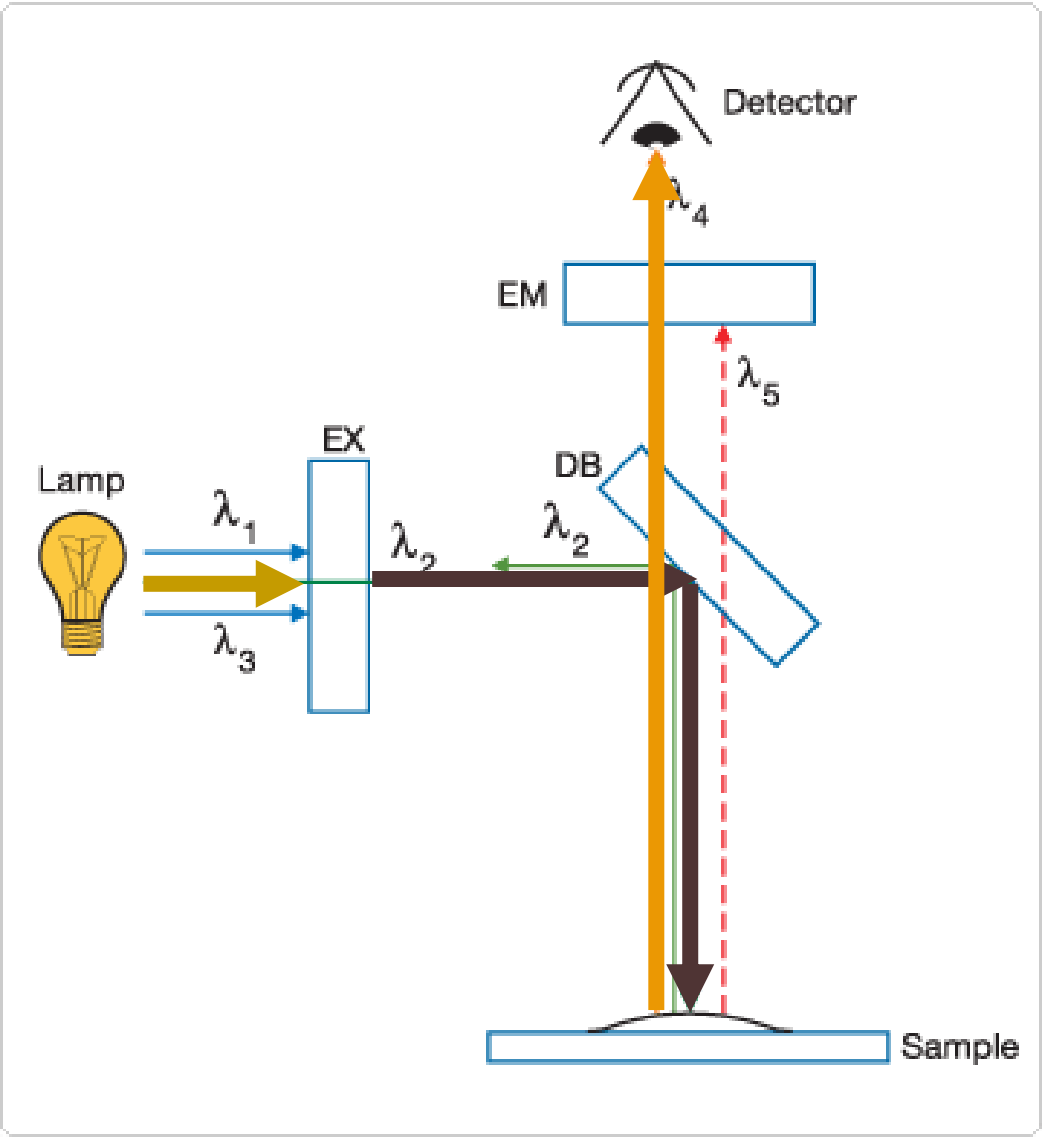
650

Alexa Fluor 647

Cy5

mPlum

Fluorescence Microscope Filters



- The Stokes shift of excitation and emission mean the two can be separated
- Excitation and emission filters and a dichroic mirror are used
- These can be mounted in a cube or separately in filter wheels

1. Excitation Filter
2. Dichroic Mirror
3. Emission Filter

invitrogen

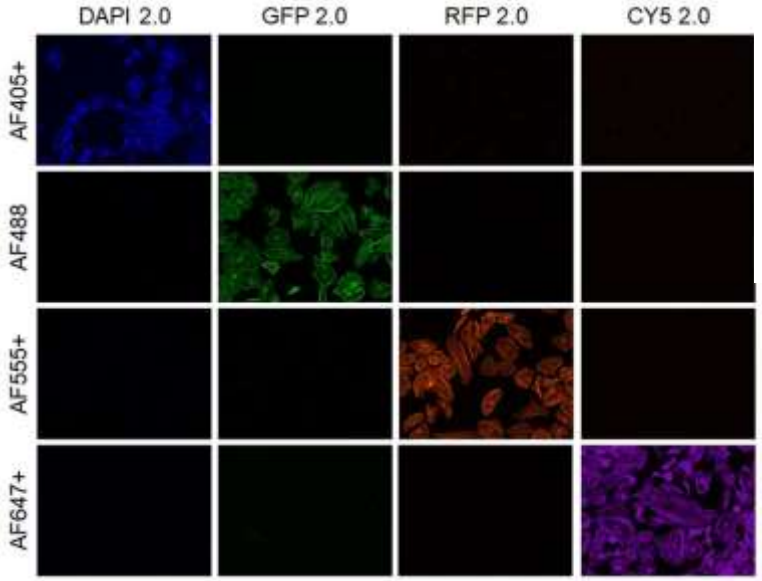
EVOS Light Cube

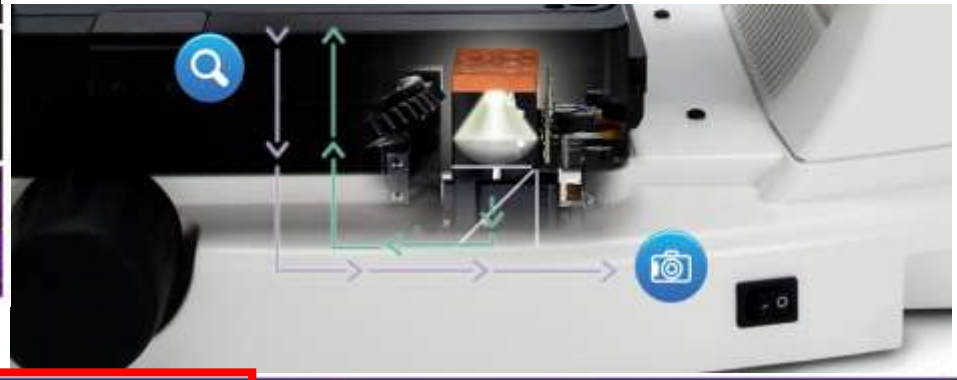
Specialty light cubes

- CFP-YFP
- AO
- AOred
- Qdot 525
- Qdot 545
- Qdot 565
- Qdot 585
- Qdot 605
- Qdot 625
- Qdot 655
- Qdot 705
- Qdot 800
- Qdot 525-800

Commonly used light cubes

- DAPI
- TagBFP
- CFP
- GFP
- YFP
- RFP
- Texas Red
- Cy5
- Cy5.5
- Cy7

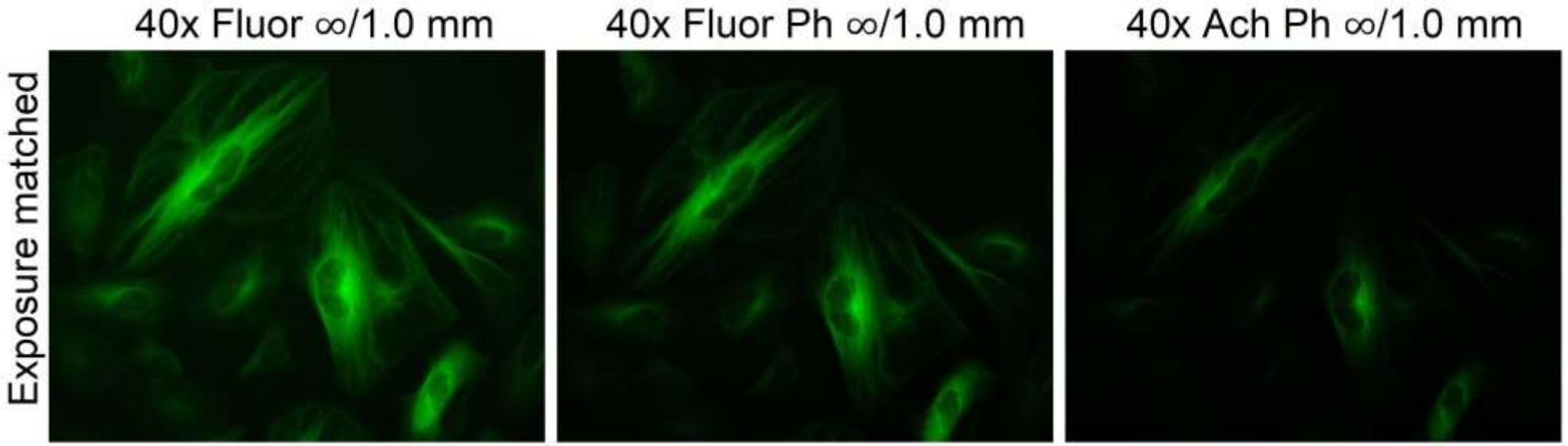




Light cube	Excitation (nm)	Emission (nm)	Common compatible dyes/fluorescent proteins
DAPI	357/44	447/60	DAPI, Hoechst, BFP
CFP	445/45	510/42	ECFP, Lucifer Yellow
GFP	470/22	525/50	GFP, Alexa Fluor 488, SYBR Green, FITC
YFP	500/24	542/27	EYFP, acridine orange (+DNA)
RFP	531/40	593/40	RFP, Alexa Fluor 546, Alexa Fluor 555, Cy3, DsRed, Rhodamine Red, dTomato
Texas Red	585/29	628/32	Texas Red, Alexa Fluor 568, Alexa Fluor 594, MitoTracker Red, mCherry
Cy5	628/40	692/40	Cy5, Alexa Fluor 647, Alexa Fluor 660, DRAQ5

The Microscope Objective Lens

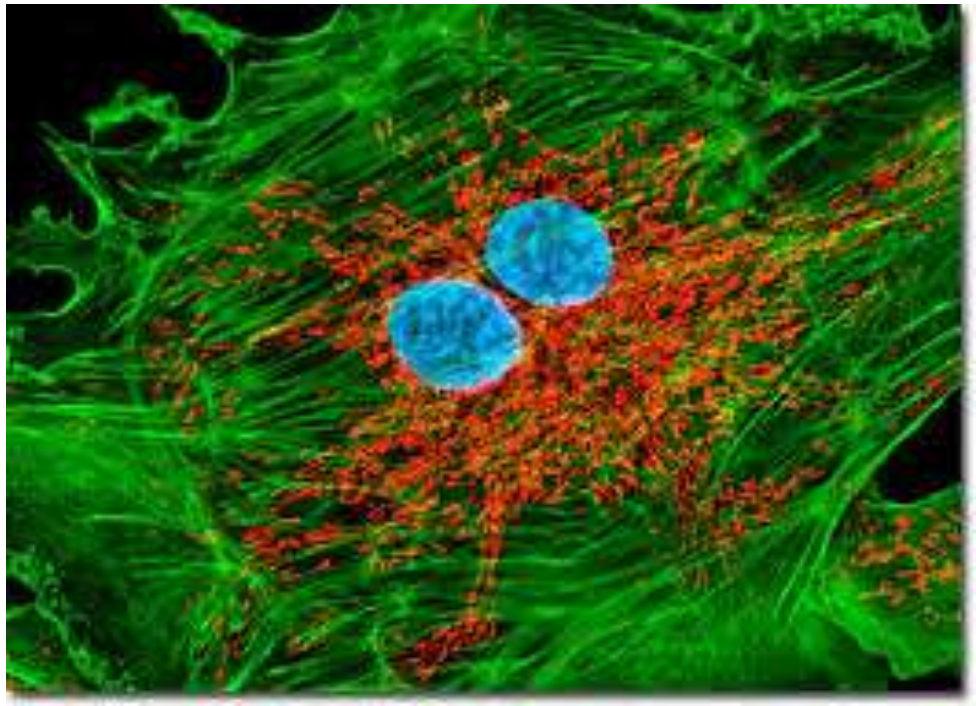
- Most important part of the microscope because it forms the image
- Nomenclature indicates things like field flatness and color correction
- Resolution is defined by numerical aperture (NA)
- Working distance generally is shorter for high resolution lenses
- It is important to match refractive indexes to avoid aberration



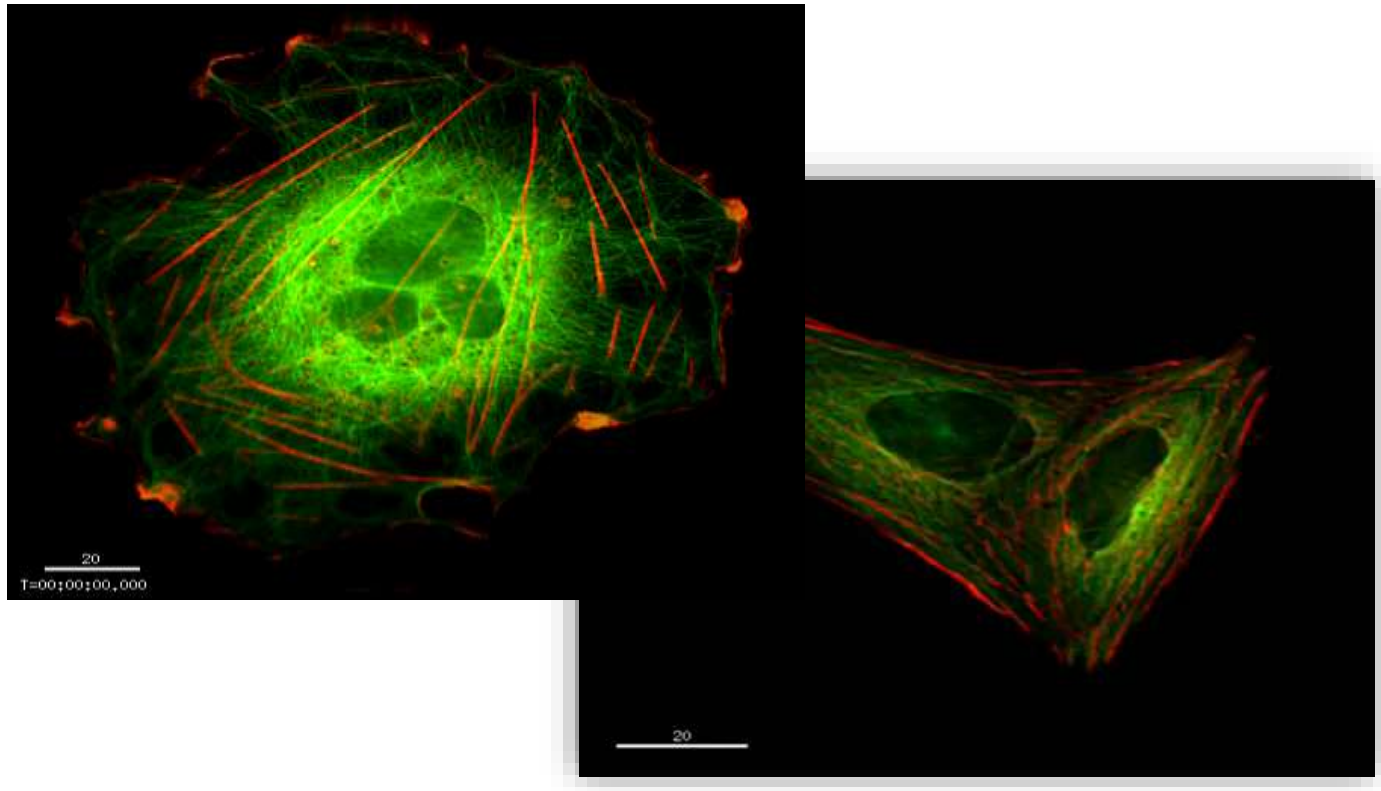
Impact of image quality comparing three types of LWD objectives with the same sample and exposure settings.

Fluorescence Microscopy: Fixed-cell vs Live-cell analysis

Fixed cell



Live cell



Usually higher resolution microscopy

Achievable, more Multiplexable

Dynamic measurement

Cellular events in real time

Topics

1. Type of Microscopy
2. Principle of Fluorescent Microscopy
3. EVOS M7000 Imaging System

Why EVOS®?

Go from all this……

‘Typical’ epifluorescence system

…to this

EVOS M7000

EVOS™ M7000 Imaging System

FAST scan speed

Powerful analysis software option

Choice of cameras – dual mono/color or high-res mono models

Four changeable light cubes PLUS transmitted light

Large bright monitor

Outstanding image quality

Robust and fast autofocus

Powerful image analysis options

Automated acquisition routines

Instrument installation AND training included

Why EVOS®?

Ergonomic layout and control

Large, bright monitor
Multiple users can view simultaneously

Simple, yet powerful user interface
Minimize training – anyone can operate

Accessories
Ease of use, image requirements, sample/vessel types

Designed for use on the bench
get out of the darkroom

Quality Optics
Wide range of objectives (2x to 100x)

Range of Vessel Holders
Microscope slides to multiwell plates

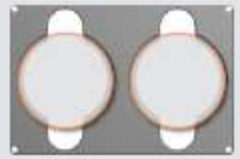
Proprietary Light Cubes
50,000 hour LED lifetime
Easy to change, exceptionally bright

EVOS vessel holders and stage plates

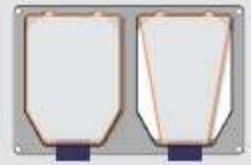
All models

AMEPVH009
Universal stage insert

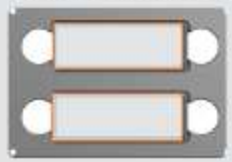
AMEPVH003
Holds two 60 mm Petri dishes



AMEPVH005
Holds two 25 cm² flasks (rectangular or triangular)

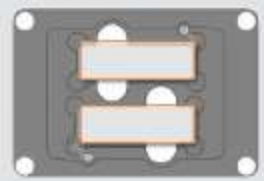


AMEPVH001
Holds two 25 mm x 75 mm standard microscope slides, chamber slides, etc.



AMEPVH006
Holds one Thermo Scientific™ Nunc™ T-75 flask (75 cm²)

AMEPVH021
Holds two microscope slides or chamber slides with retention clip



AMEPVH004
Holds one 100 mm Petri dish

AMEPVH007
Holds one hemocytometer

AMEPVH022
Holds one multiwell plate with retention clip for AMEPVH001 through AMEPVH018

AMEPVH002
Holds four 35 mm Petri dishes

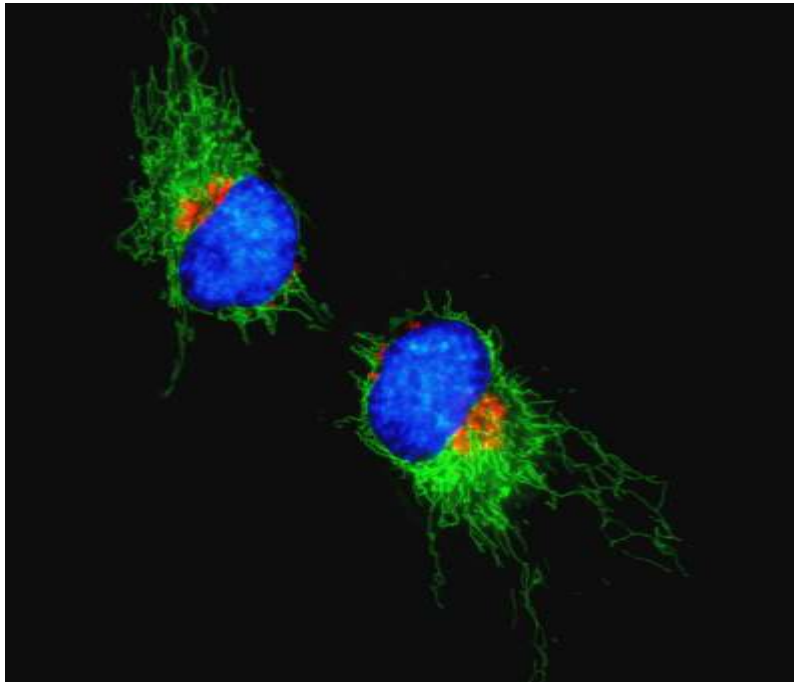
AMEPVH028
Holds one multiwell plate with retention clip

AMEPVH030
Holds two 35 mm Petri dishes

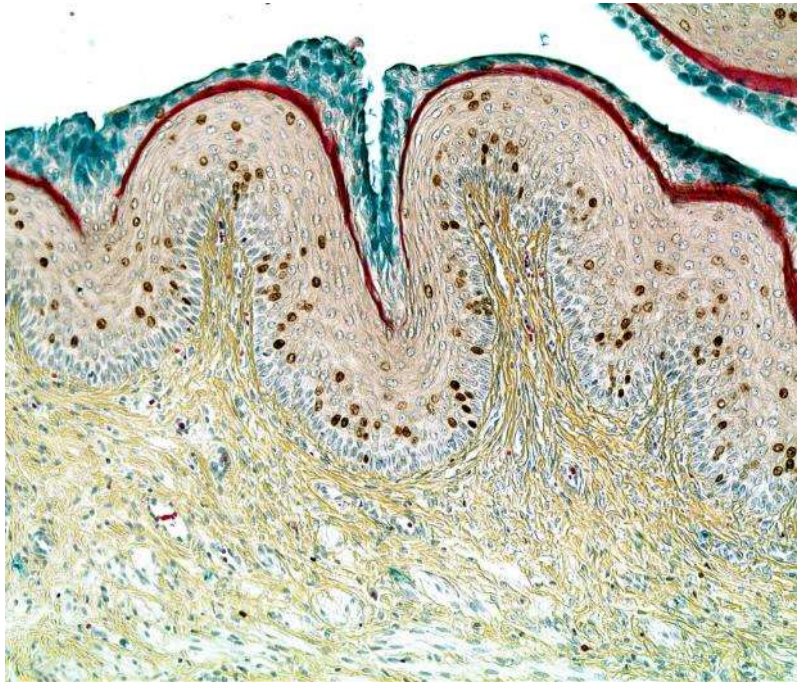
EVOS M7000 System—One Microscope, Two Cameras

Dual cameras:

Seamlessly switch between monochrome and color camera as needed for fluorescent or colorimetric samples



Monochrome camera



Color camera

Revolutionary Light Path

- The LED is placed as close as possible to the objective
- Minimizes the number of optical elements in the light path
- Increases efficiency of fluorophore excitation

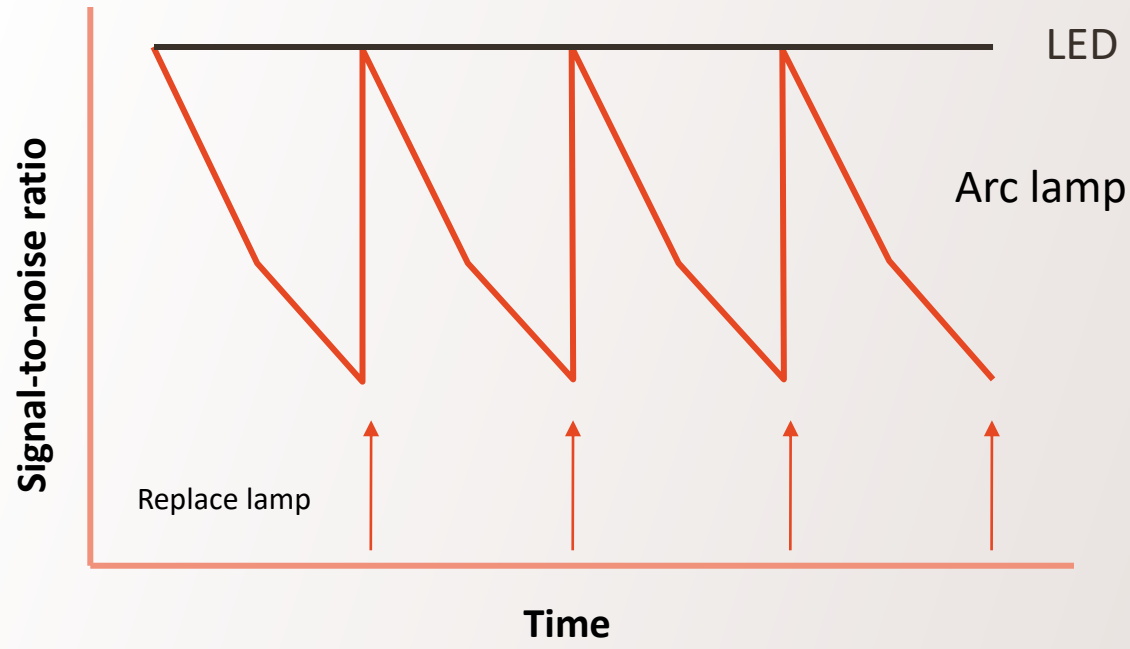
Better detection of
weak fluorescence signals

Illumination Stability

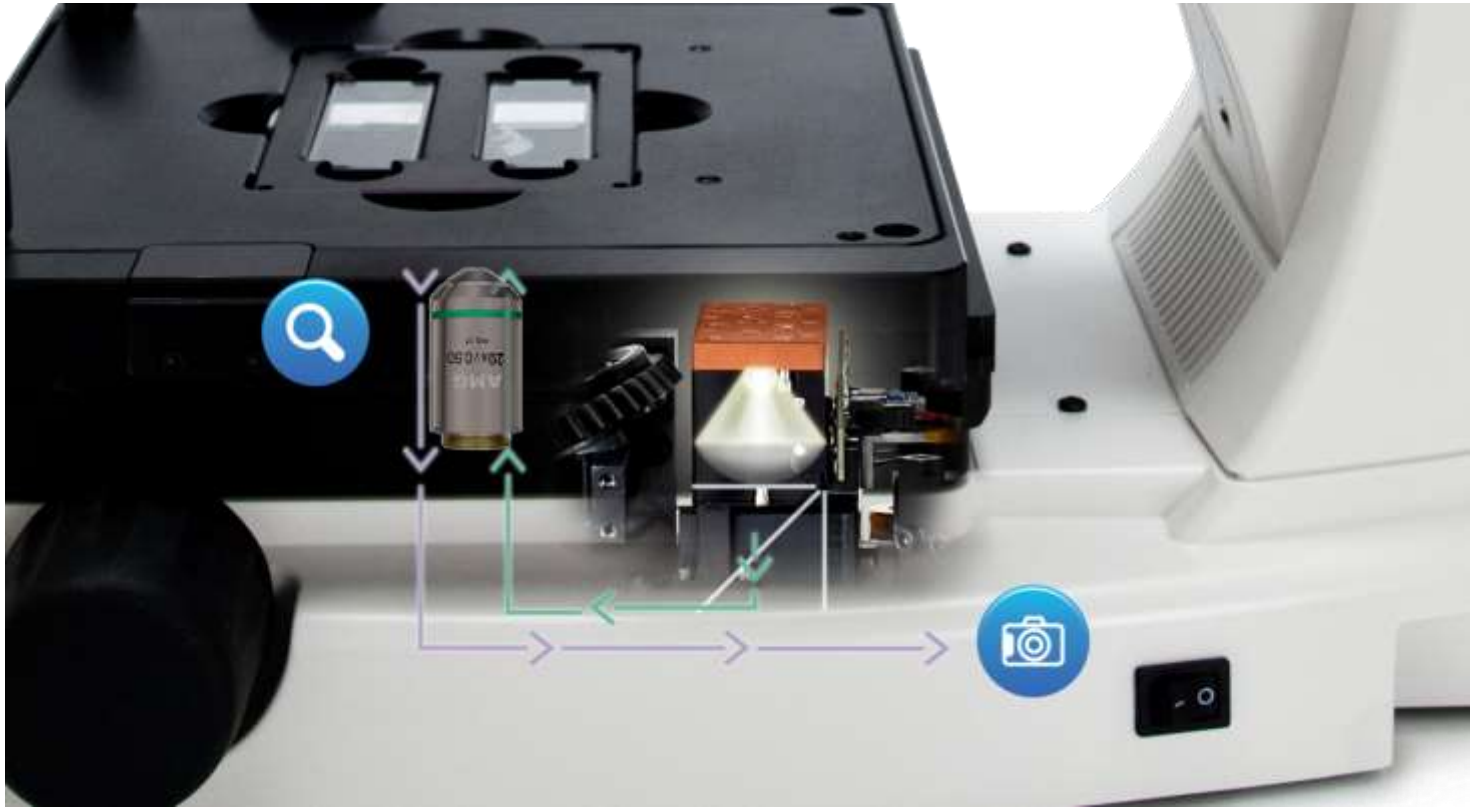
Mercury arc lamps lose 0.5% intensity per hour of use, resulting in 50% reduction in only 100 hours of use

Images acquired in different sessions suffer from quantitative variability when using mercury illumination without complicated calibrations

Mercury metal halide illumination vs. LED



LED Integral Design



Long life-time

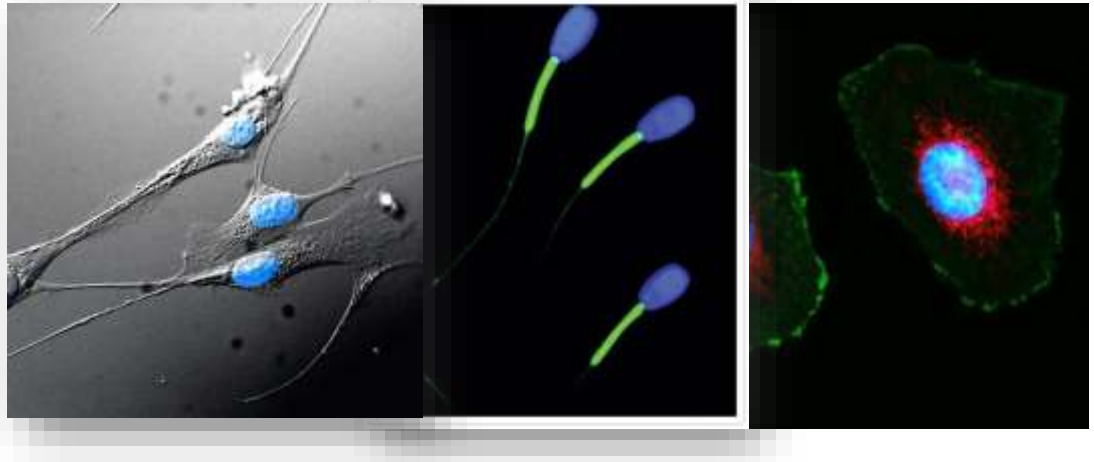
Stable

No photobleaching

No photo toxicity

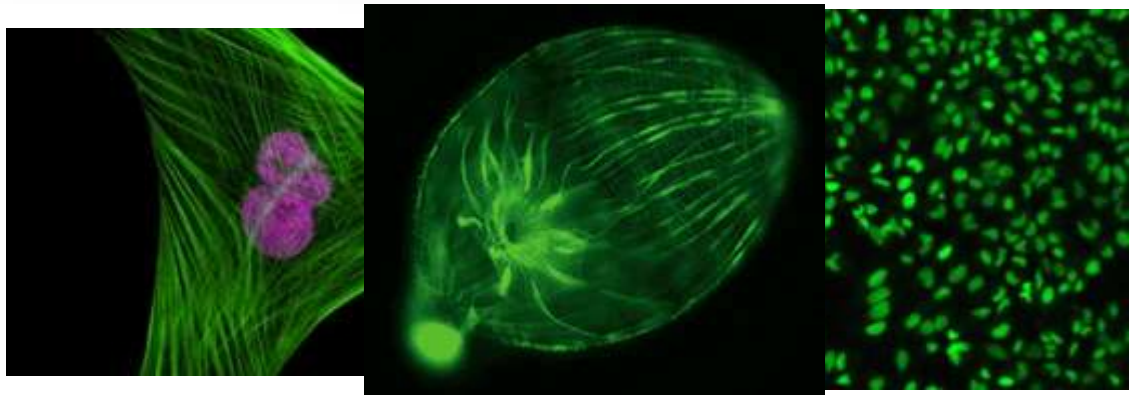
No warm up/cool down

EVOS™ Light Cube, DAPI 2.0 (357/44 nm Excitation; 447/60 nm Emission)



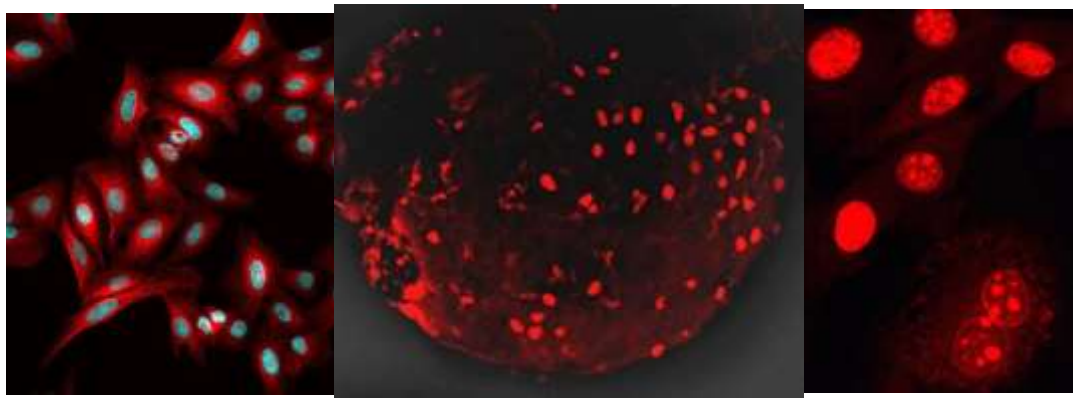
Alexa Fluor 350
DAPI
Hoechst 33342
LysoTracker Blue
NucBlue
Etc.

EVOS™ Light Cube, GFP 2.0 (482/25 nm Excitation; 524/24 nm Emission)



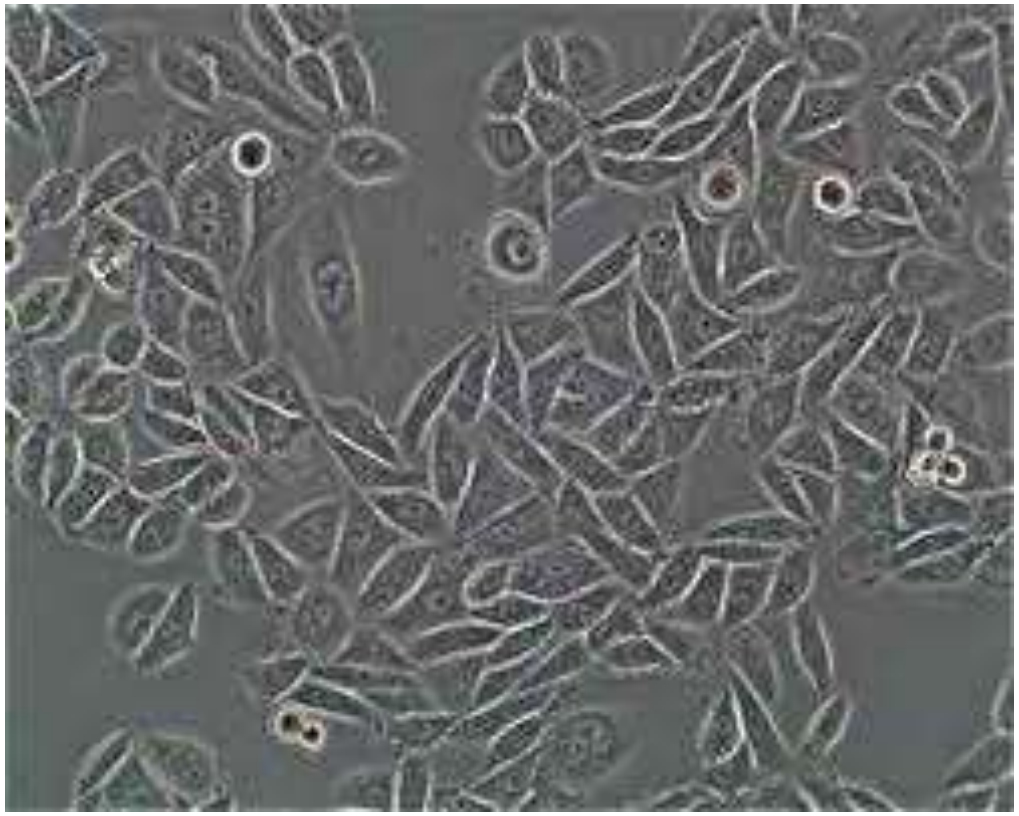
Alexa Fluor 488
CellROX Green
FITC
SYTO-9
YOYO-1
Etc.

EVOS™ Light Cube, Texas Red 2.0 (585/29 nm Excitation; 628/32 nm Emission)



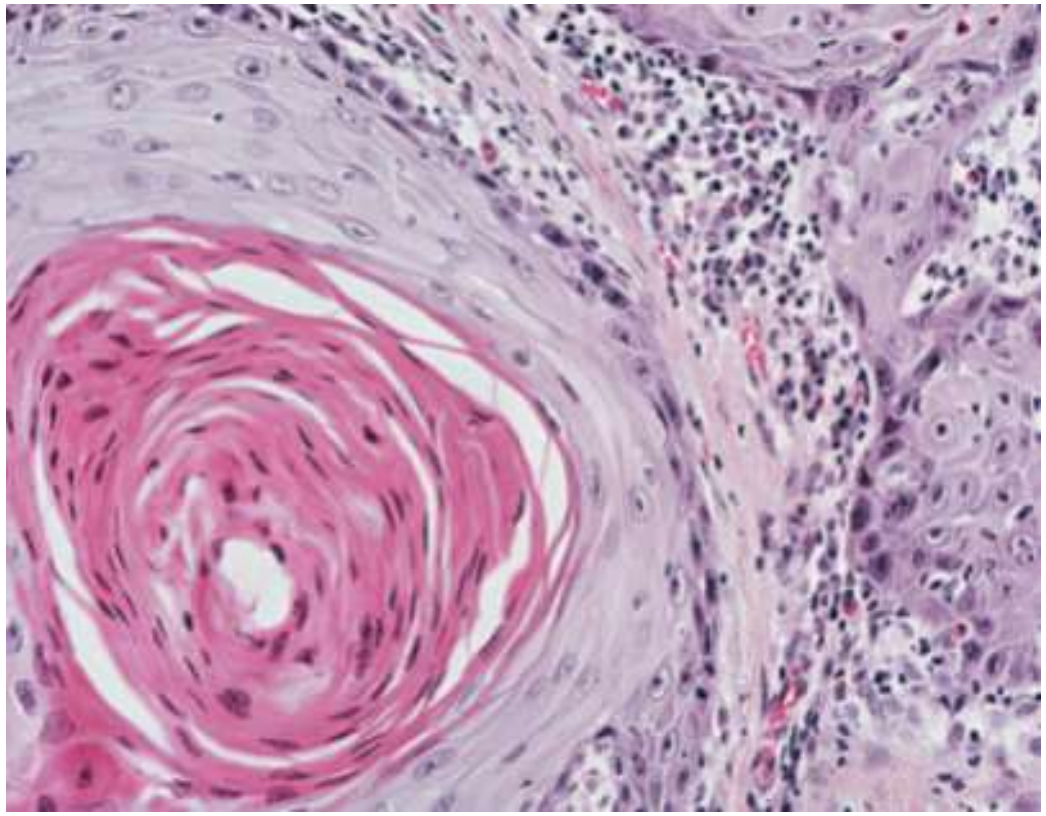
Texas Red
Alexa Fluor 594
mCherry
pHrodo Red
Cy3.5
Etc.

EVOS® M7000: Transmitted Light Applications



HeLa cells in culture

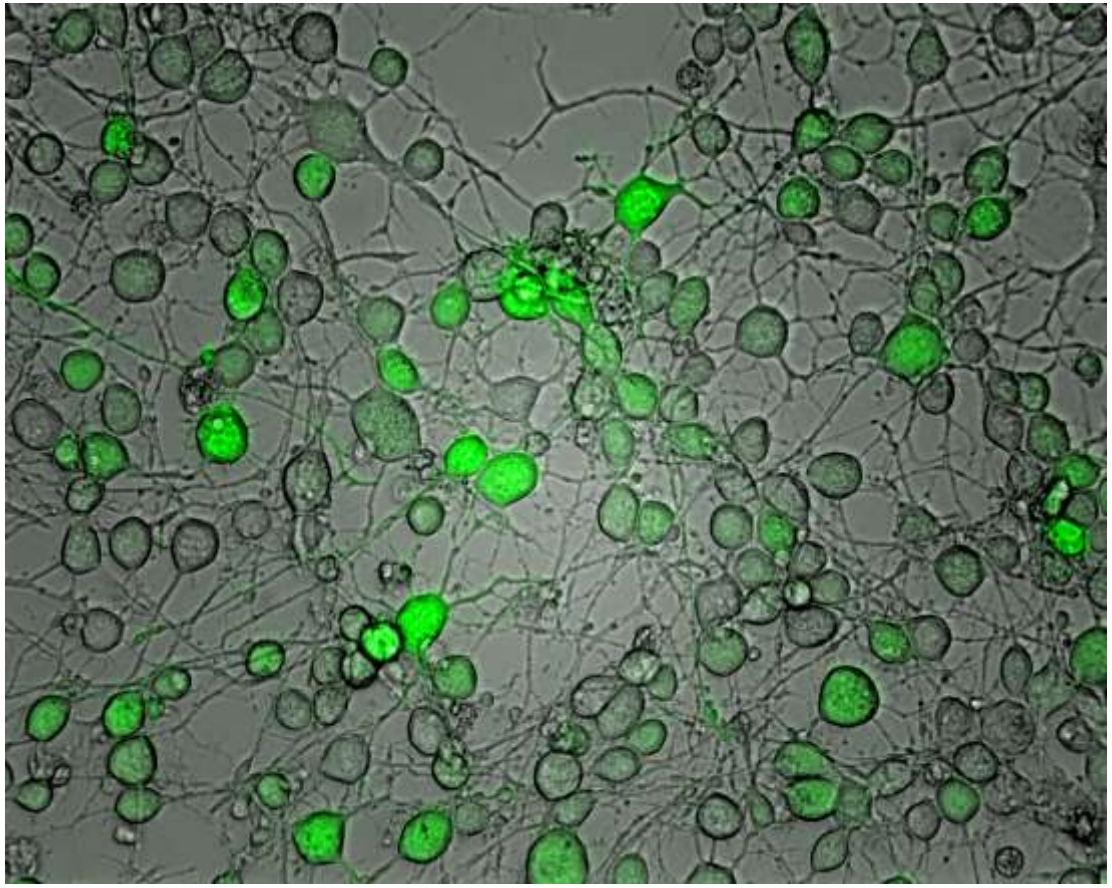
Cell culture: *Are my cells confluent and healthy?*



Immunohistochemical (IHC) staining of lung tissue with squamous cell carcinoma

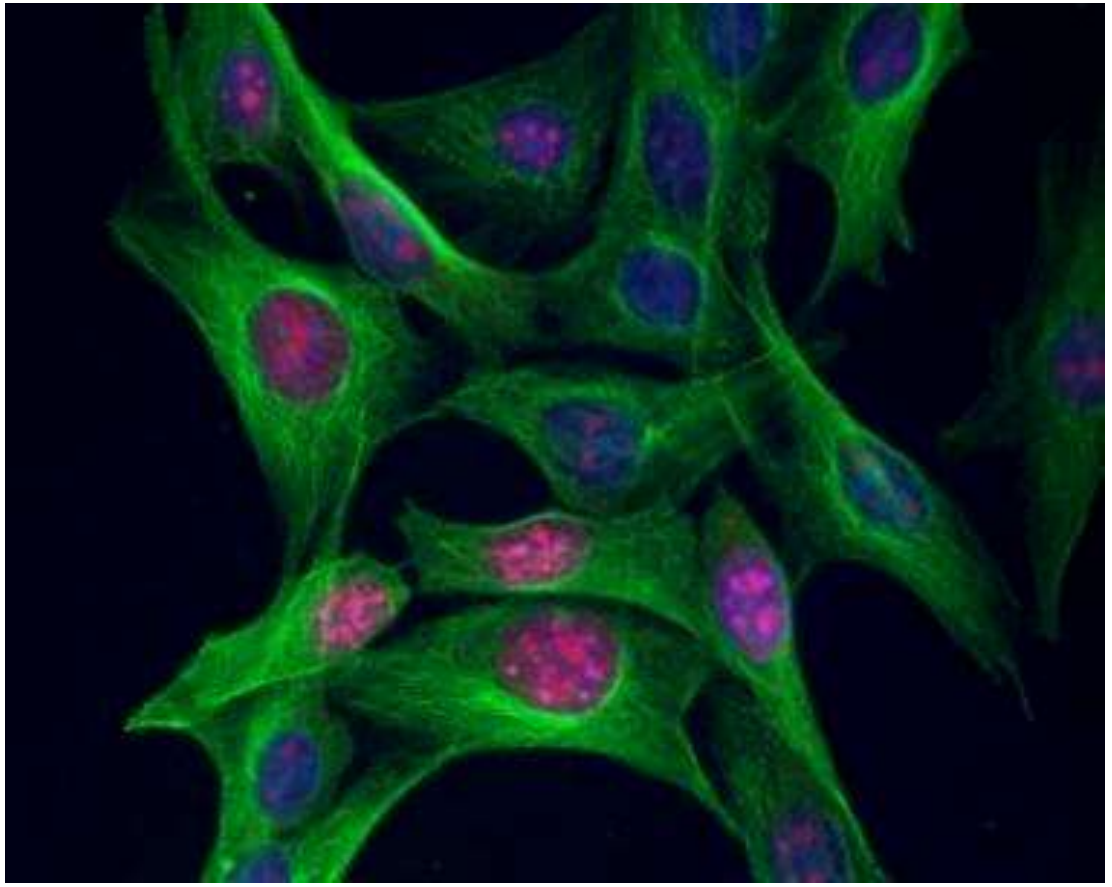
IHC: *Visual markers of disease*

EVOS® M7000: Fluorescent Applications



Neuronal stem cells expressing GFP

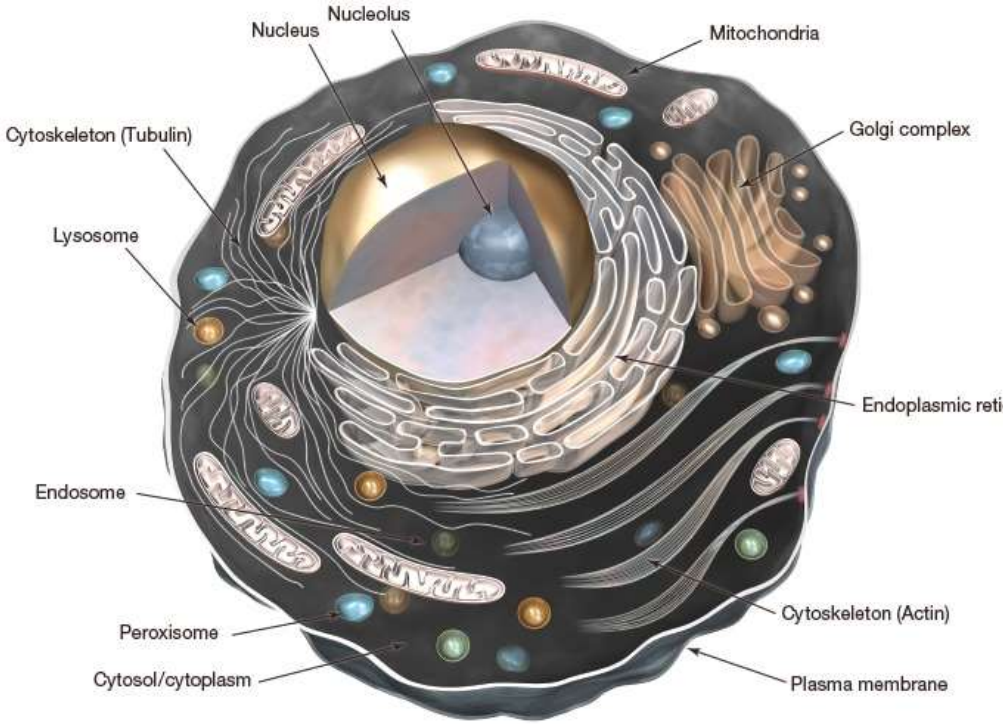
Cell culture: *Are my cells expressing GFP?*



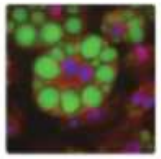
NIH 3T3 cells: mRNA (red), tubulin (green), and nucleus (blue) are clearly visualized

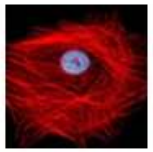
Cell health: *Do my cells look normal?*

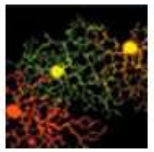
EVOS® M7000: Fluorescent Applications

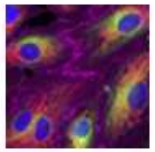


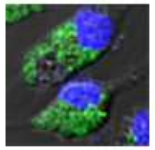
Find organelle-specific antibodies and organelle stains

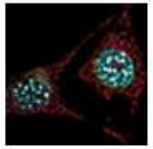

Adiposomes

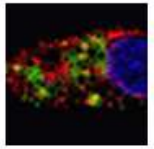

Cytoskeleton


Cytosol/cytoplasm

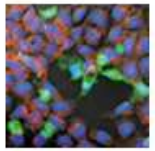

Endoplasmic reticulum

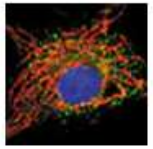

Endosomes

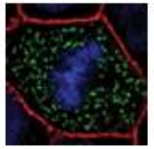

Golgi complex


Lysosomes


Mitochondria


Nucleus/nucleolus


Peroxisomes


Plasma membrane

https://www.thermofisher.com/th/en/home/life-science/cell-analysis/cell-structure.html

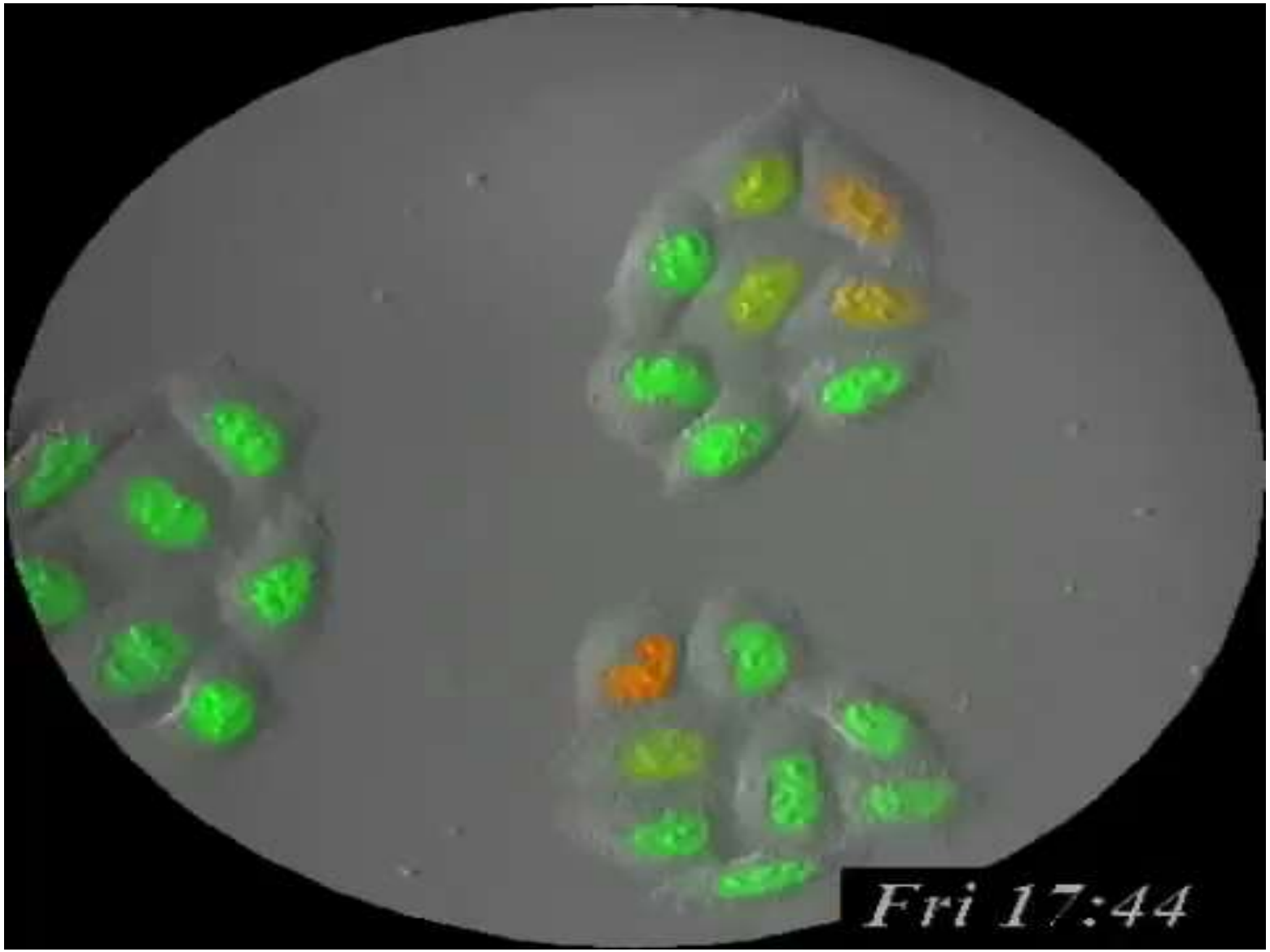
Live Cell Imaging Solution

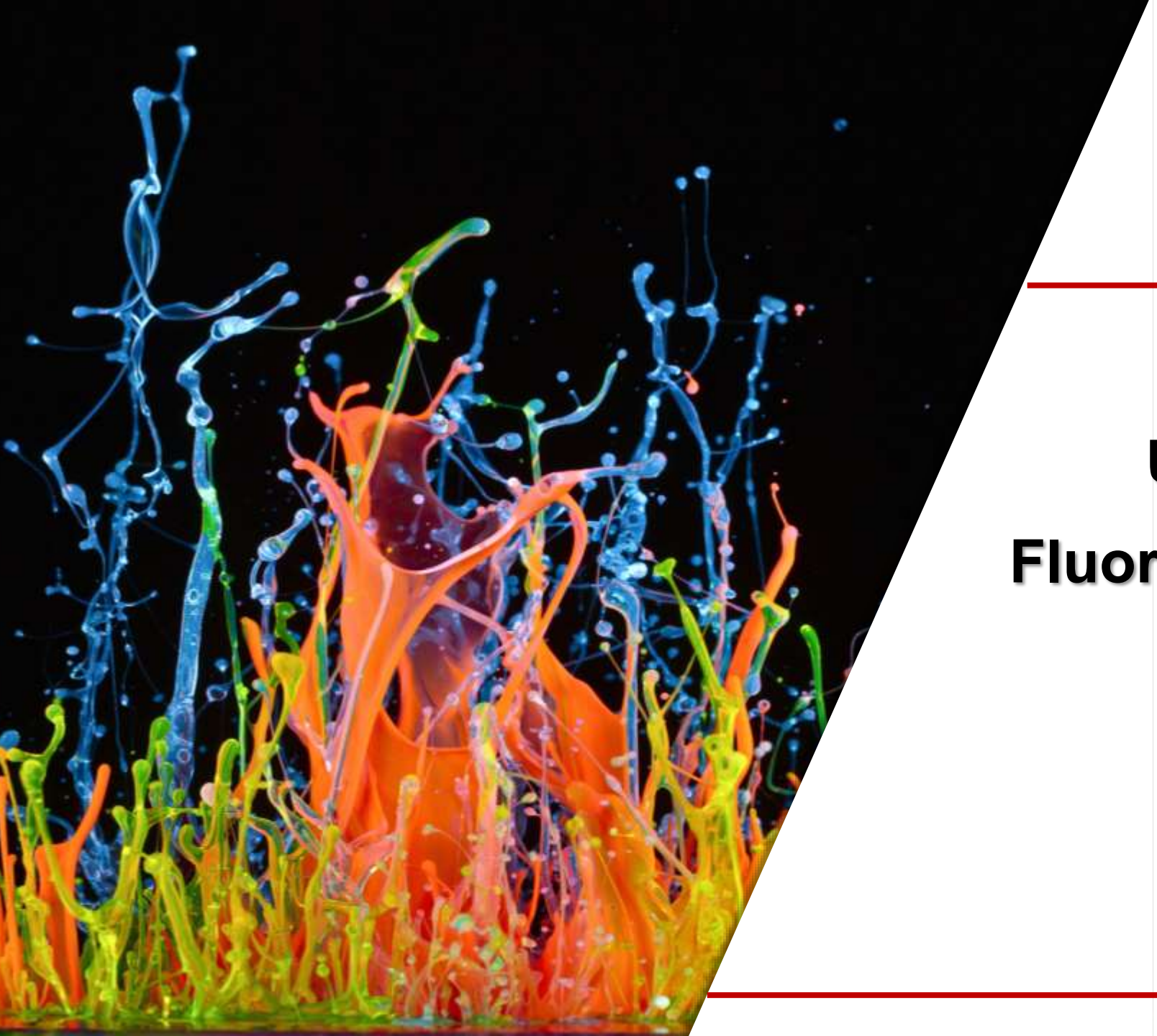
Complete solution to environmental control during live cell and time-lapse imaging studies

- Fully integrated environmental chamber for live cell time-lapse imaging
- Precisely maintain physiological or hypoxic conditions
- Intuitively set all acquisition parameters from the EVOS M5000 or M7000 interface
- Small footprint, robust design elements
- Use with a range of validated reagents

The EVOS M7000 and M5000 microscopes and the EVOS Onstage Incubator (OSI) operate as one fully integrated unit, for seamless live cell experiments, imaging, and analysis

EVOS® M7000: Time-lapse imaging





gibthai
A 3N HOLDING COMPANY

Using of EVOS™ M7000 Fluorescent Imaging System

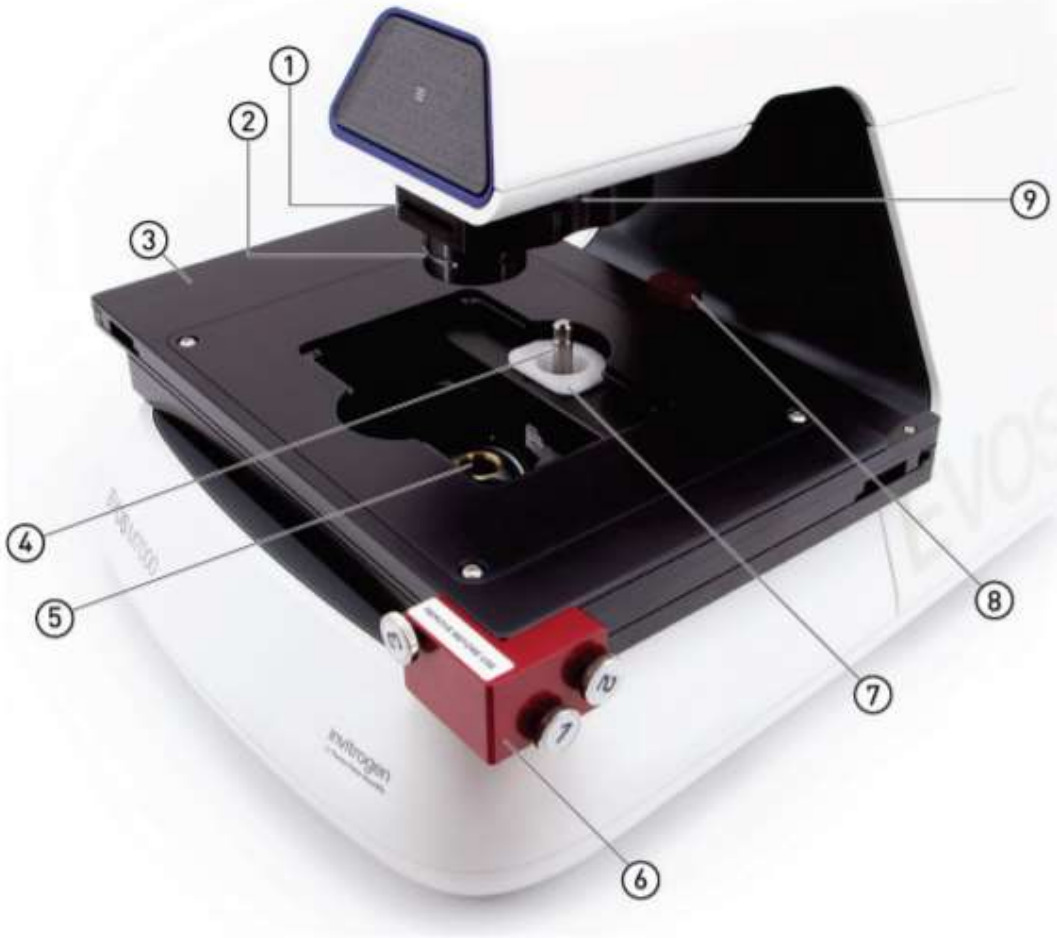
Chayaporn Subkamkaew
Technical Application Specialist
for Imaging and Cellular Analysis Product
Gibthai Co., Ltd.

EVOS™ M7000 Fluorescent Imaging System

Overview and Function

Instrument exterior components

Top view



1. Condenser slider slot
2. Condenser
3. Automatic X-Y axis stage
4. Light cube tool
5. Objective turret (accomodates up to 5 objectives)
6. X-Y stage shipping restraint
7. Light cube shipping restraint
8. Camera shipping restraint
9. Phase annuli selector

Instrument exterior components

Side view

1. Condenser
2. Automatic X-Y axis stage
3. Handholds

Instrument exterior components

Rear view

1. Power switch
2. 4-pin power input port (24 VDC, 5 A)
3. USB 3.1 Type B port

Basic operation

Turn on the instrument power switch

↓

Turn on the computer and monitor

↓

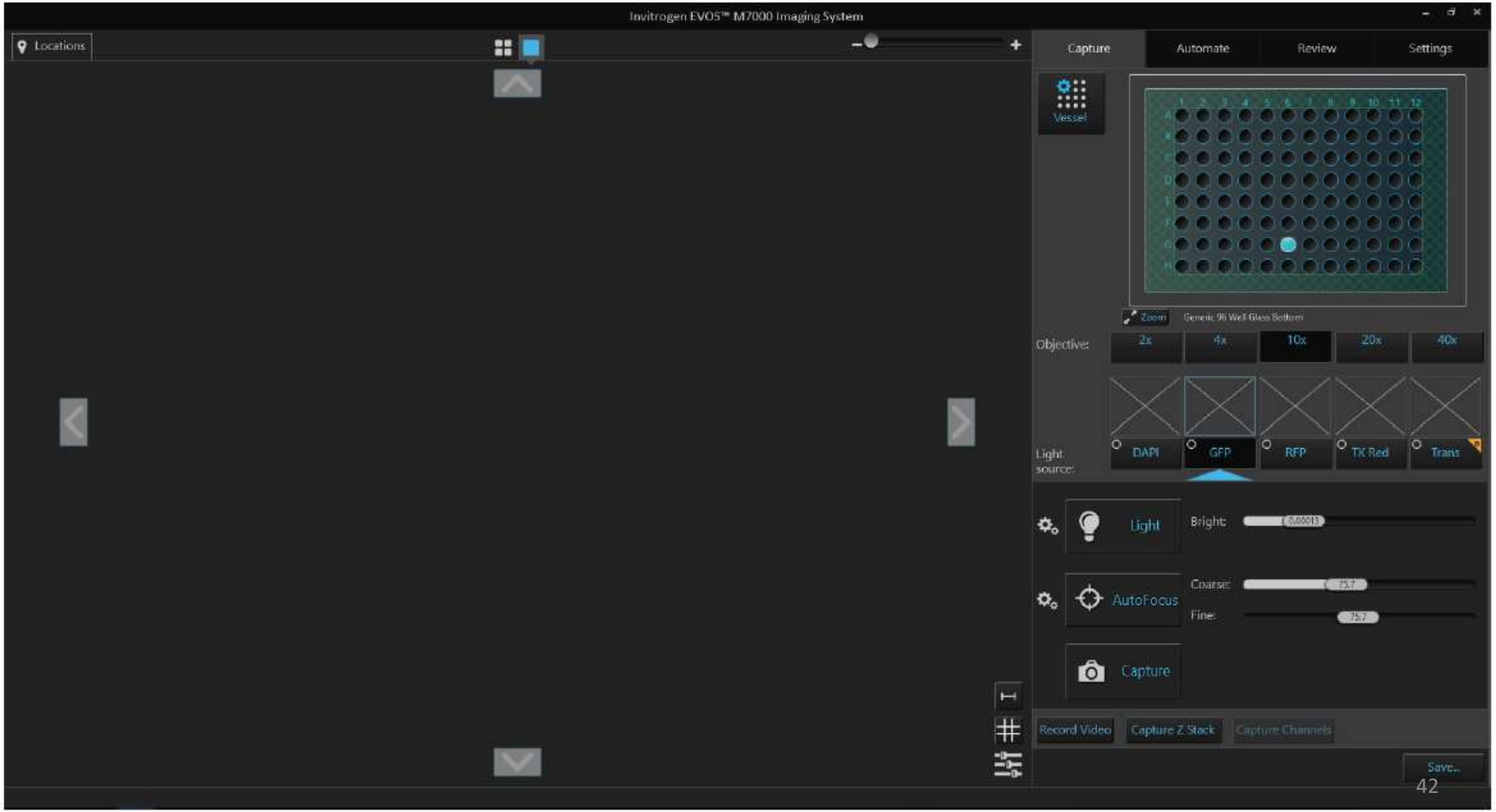
Click the M7000 icon on the desktop to start the EVOS™ M7000 software

↓

The Capture tab is displayed, the EVOS™ M7000 Imaging System is ready to use

Capture tab

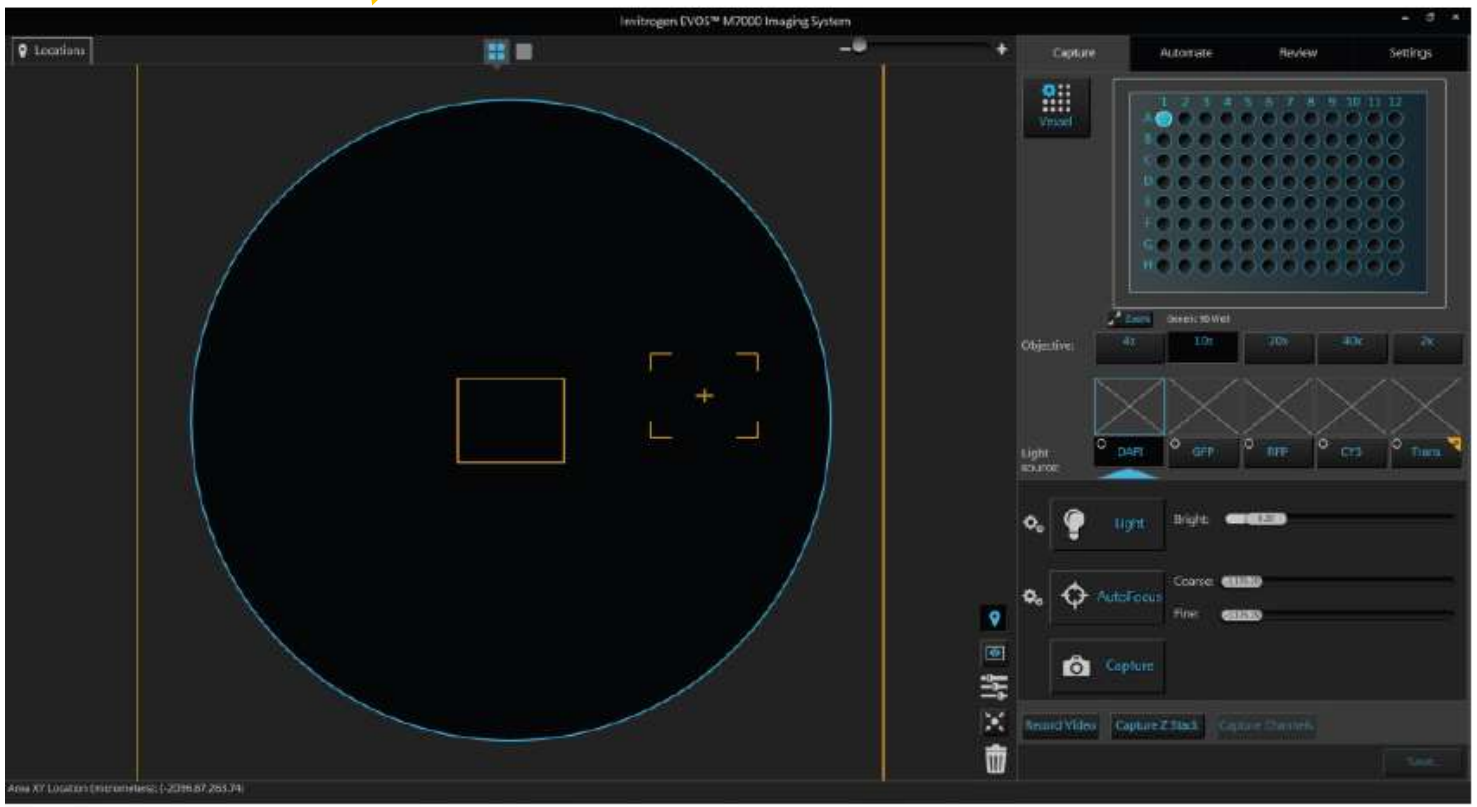

M7000


Invitrogen EVOS™ M7000 Imaging System
Locations
Capture
Automate
Review
Settings
Vessel
Zoom
Generic 96 Well Glass Bottom
Objective:
2x
4x
10x
20x
40x
Light source:
DAPI
GFP
RFP
TX Red
Trans
Light
Bright:
AutoFocus
Coarse:
Fine:
Capture
Record Video
Capture Z Stack
Capture Channels
Save...

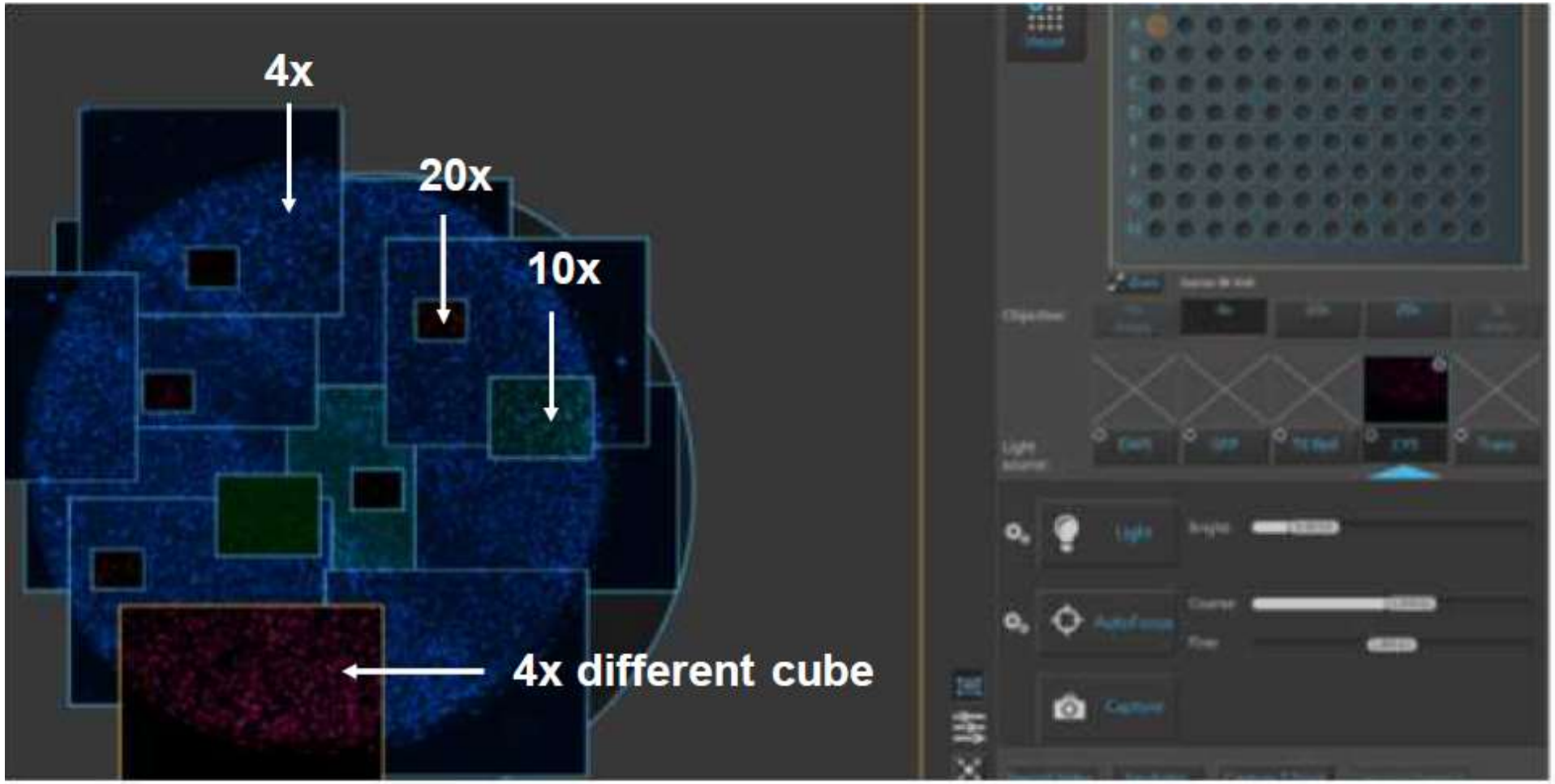
Capture tab


Vessel


Vessel Selection
Well Plates
Flasks
Dishes
Slides
Holder:
Well Plate | Generic Plate
Plate:
96 Well | NUNC | 167311
Cancel
Done

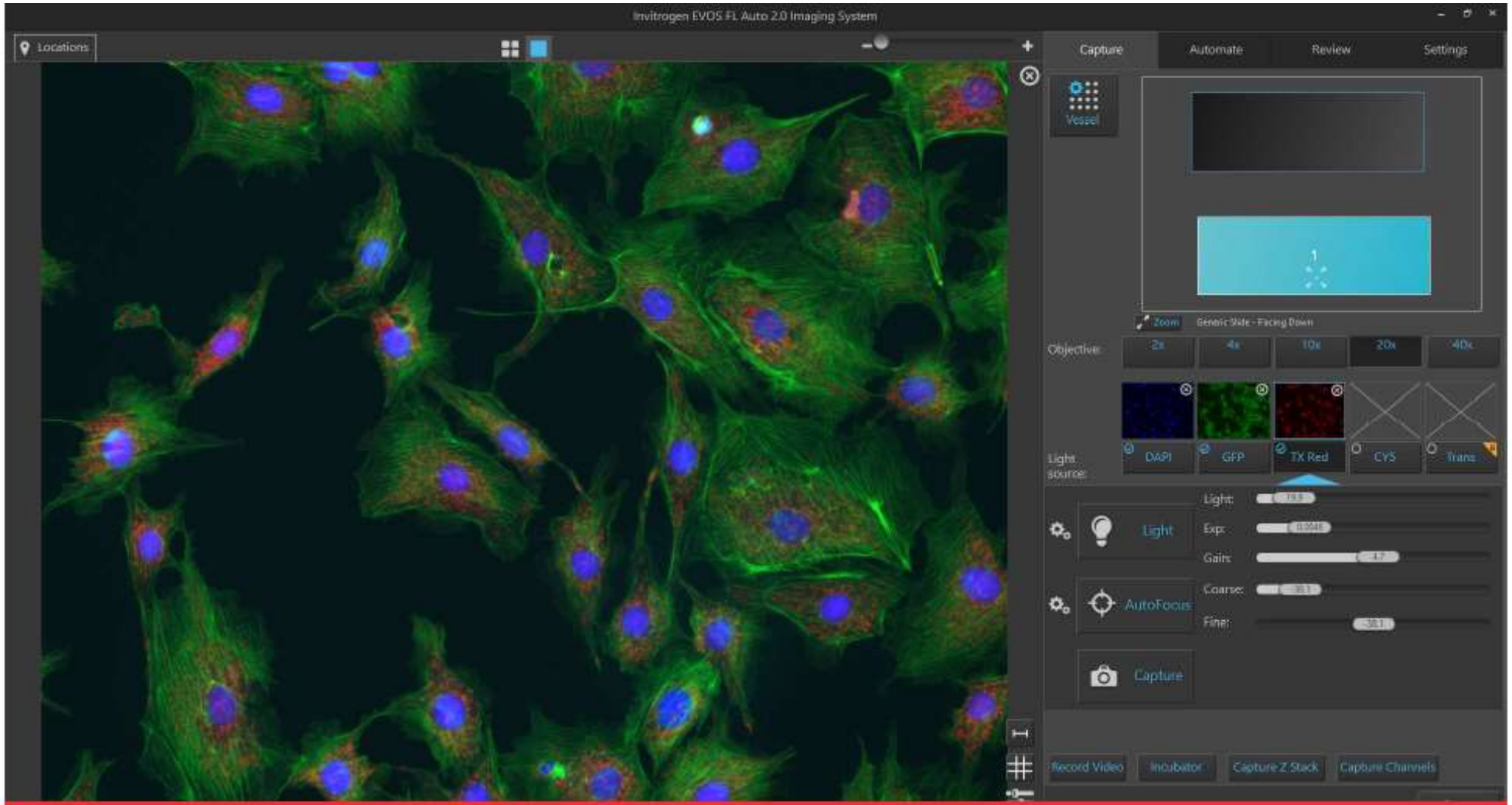

Capture
Automate
Review
Settings
Objective:
DAPI
GFP
RFP
CY5
Light
AutoFocus
Capture

Capture tab



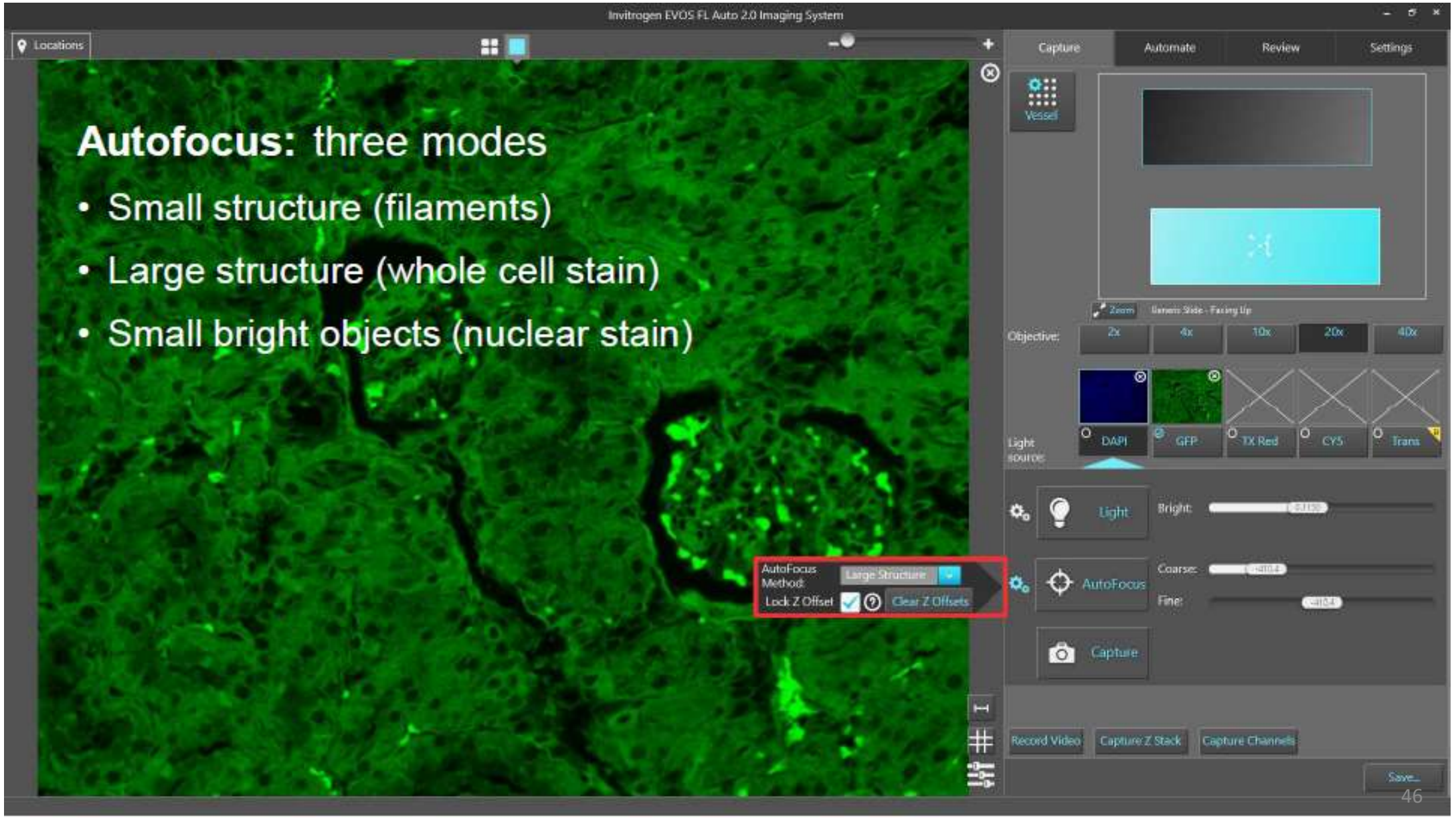
Area view mode

Capture tab

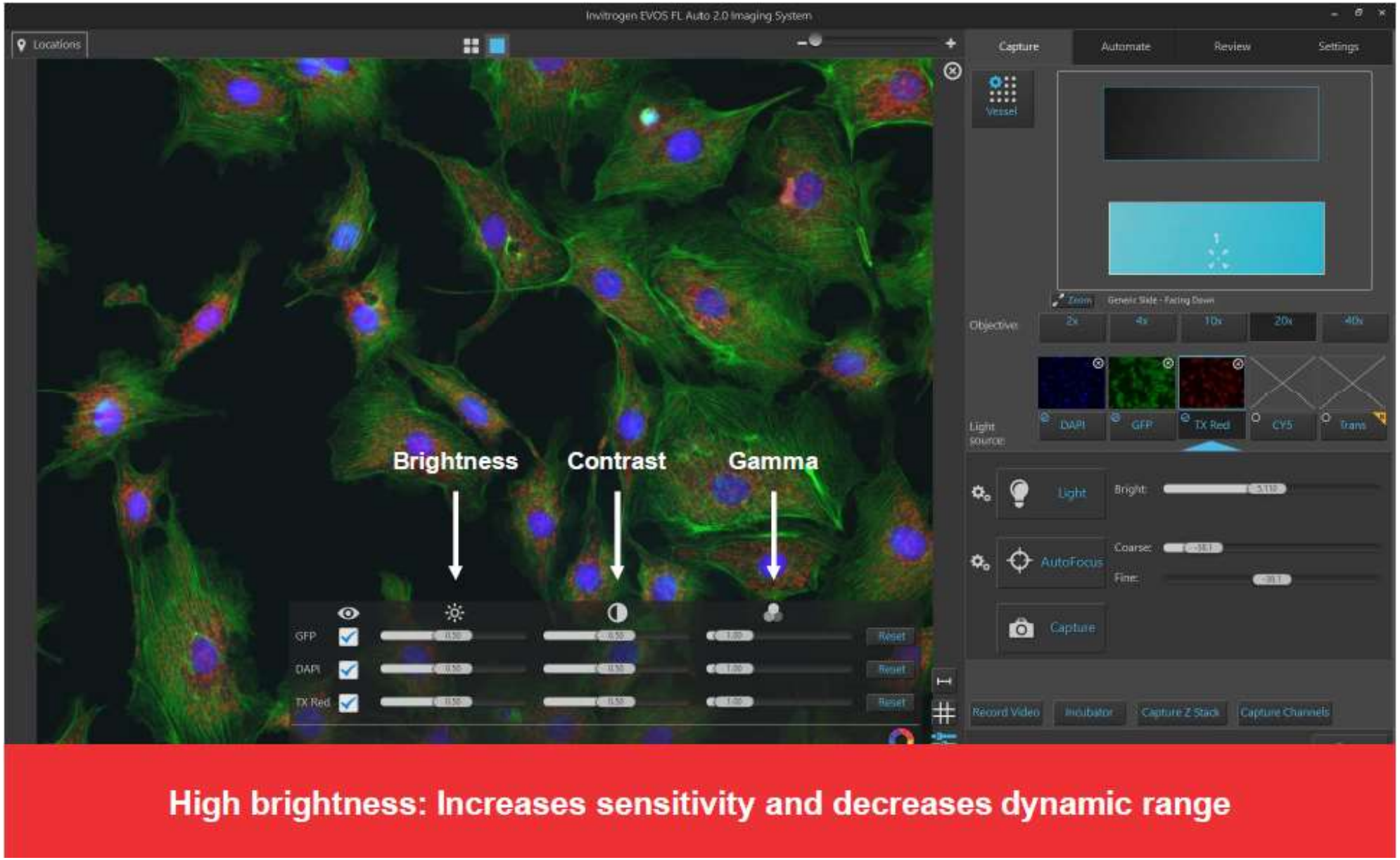


Field view mode

Capture tab

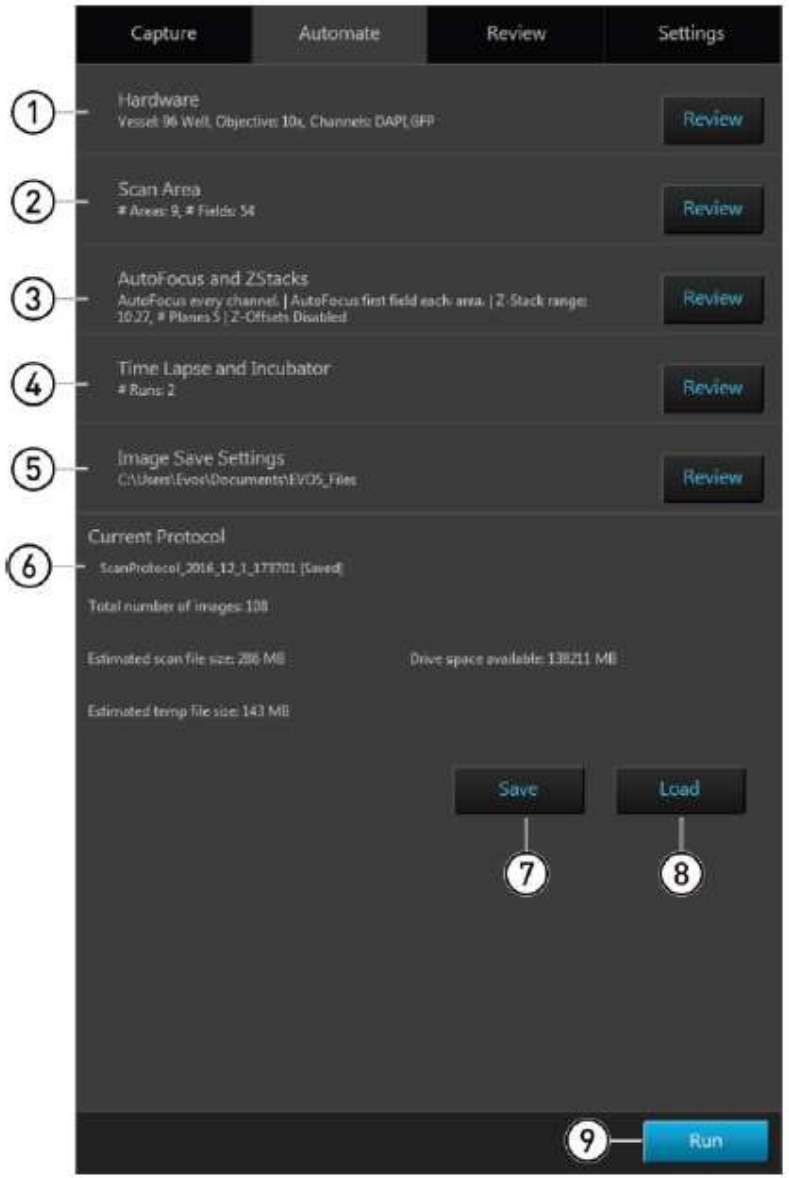

Invitrogen EVOS FL Auto 2.0 Imaging System
Locations
Capture
Automate
Review
Settings
Vessel
Objective:
2x
4x
10x
20x
40x
DAPI
GFP
TX Red
CY5
Trans
Light source:
Light
Bright:
AutoFocus
Coarse:
Fine:
Capture
AutoFocus Method:
Large Structure
Lock Z Offset
Clear Z Offsets
Record Video
Capture Z Stack
Capture Channels
Save...
Autofocus: three modes
• Small structure (filaments)
• Large structure (whole cell stain)
• Small bright objects (nuclear stain)

Capture tab



High brightness: Increases sensitivity and decreases dynamic range

Automate tab



① **Hardware:** Allows you to configure hardware options (such as sample vessel, objective, light source etc.) for the scan protocol

② **Scan Area:** Allows you to specify the scan areas and fields to capture for the scan protocol

③ **AutoFocus and Z Stacks:** Allows you to configure AutoFocus options and Z-Stack settings for the scan protocol

④ **Time Lapse and Incubator:** Allows you to specify time lapse options (duration, capture frequency etc.) and incubator settings (temperature, oxygen etc.) for the scan protocol

⑤ **Image Save Settings:** Allows you to select a save location for captured images and to set image save options

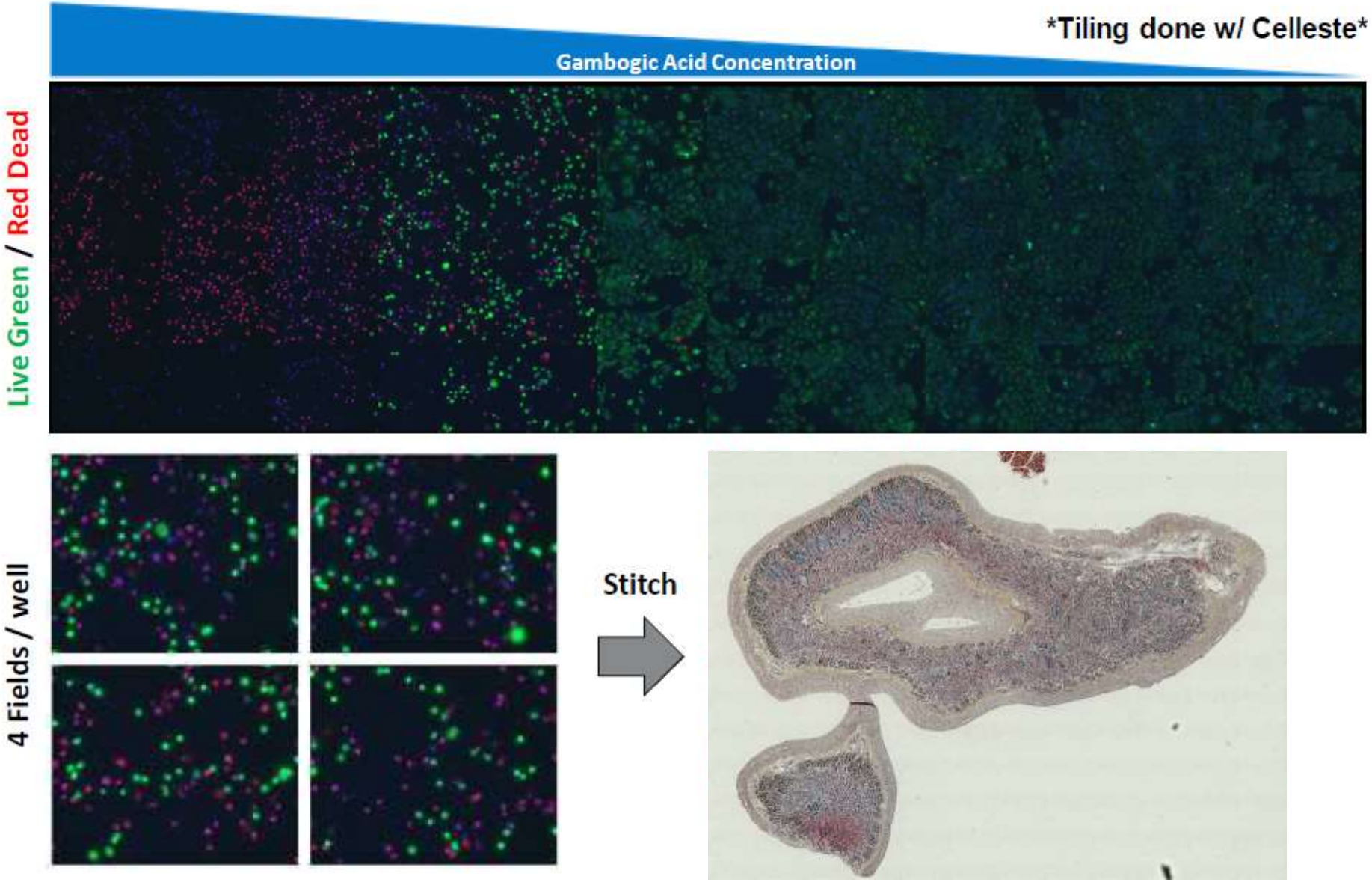
⑥ **Current Protocol:** Displays the name of the currently selected scan protocol and provides additional information (total number of images, estimated scan file size, estimated temporary file size, and drive space available).

⑦ **Save:** Saves the automated scan protocol for future experiments.

⑧ **Load:** Opens the Load dialog, which allows you to recall a previously saved scan protocol to run with new samples.

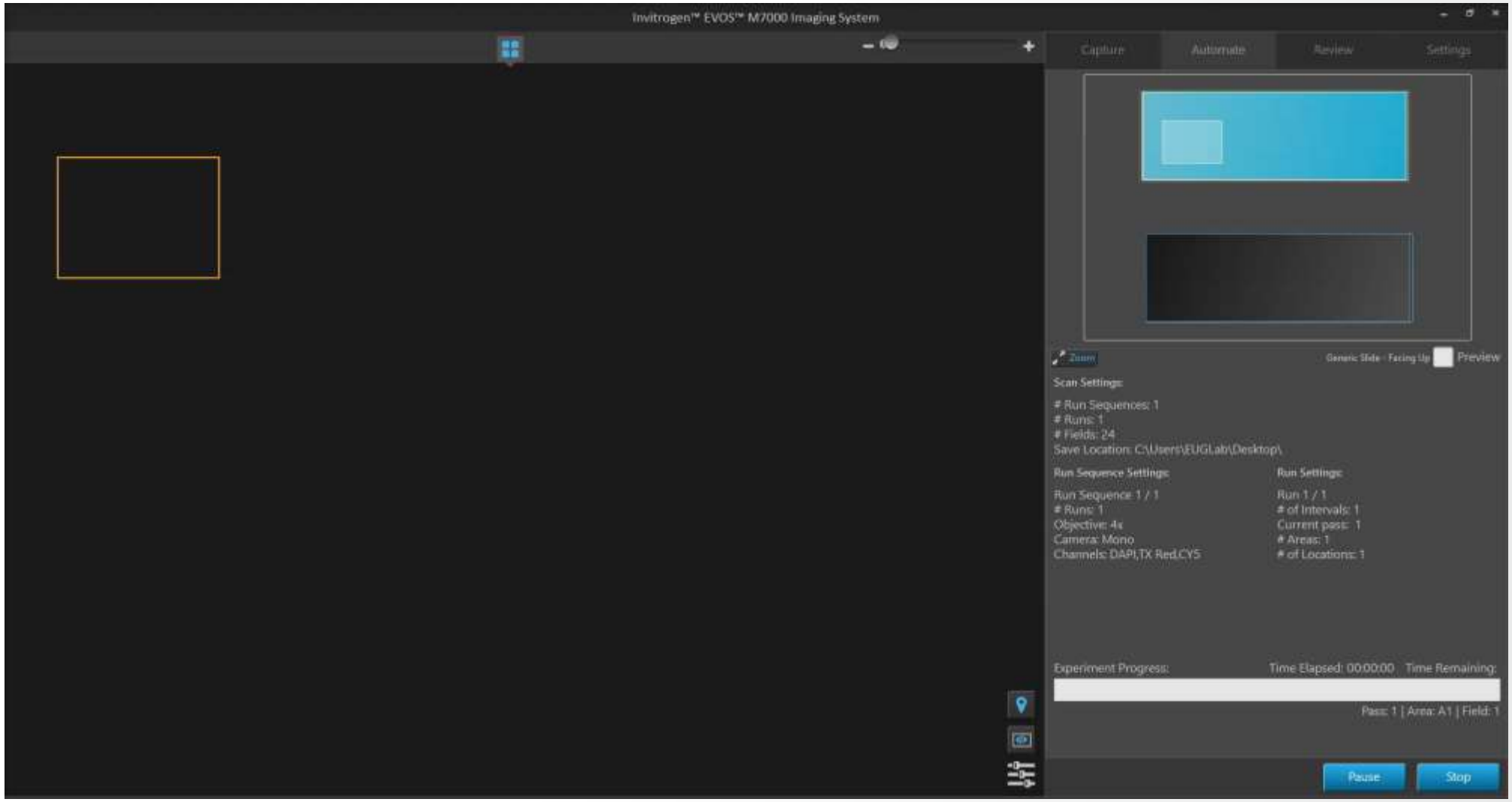
⑨ **Run:** Runs the automated scan protocol (newly created or recalled).

Results…Images – Presented is the first image acquired from each well, for channel 1 (NucBlue), channel 2 (Live Green) and channel 3 (Dead Red)



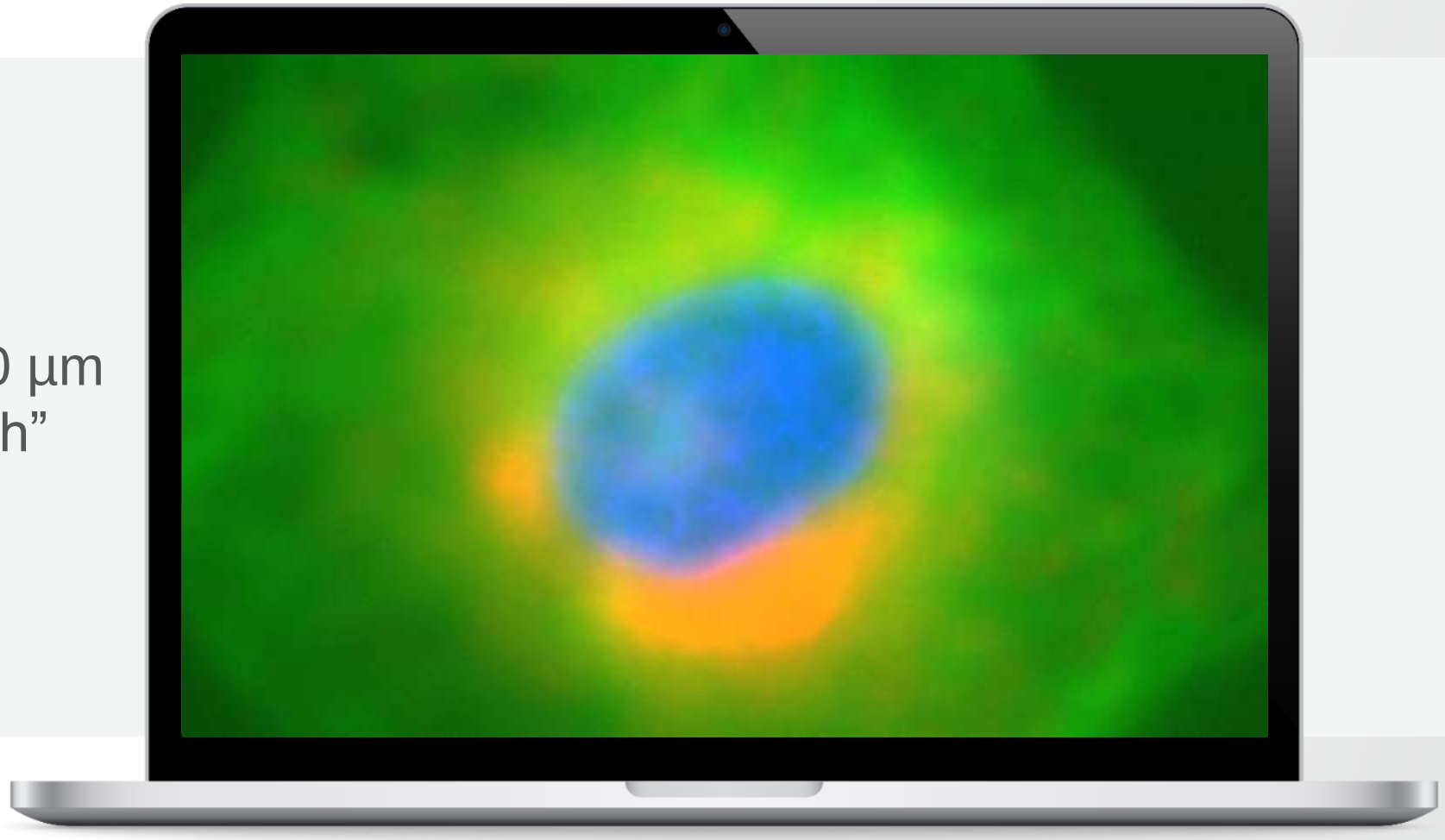
Imaging Over Space: X and Y Scanning

Blazingly fast acquisition and tile-stitching speed

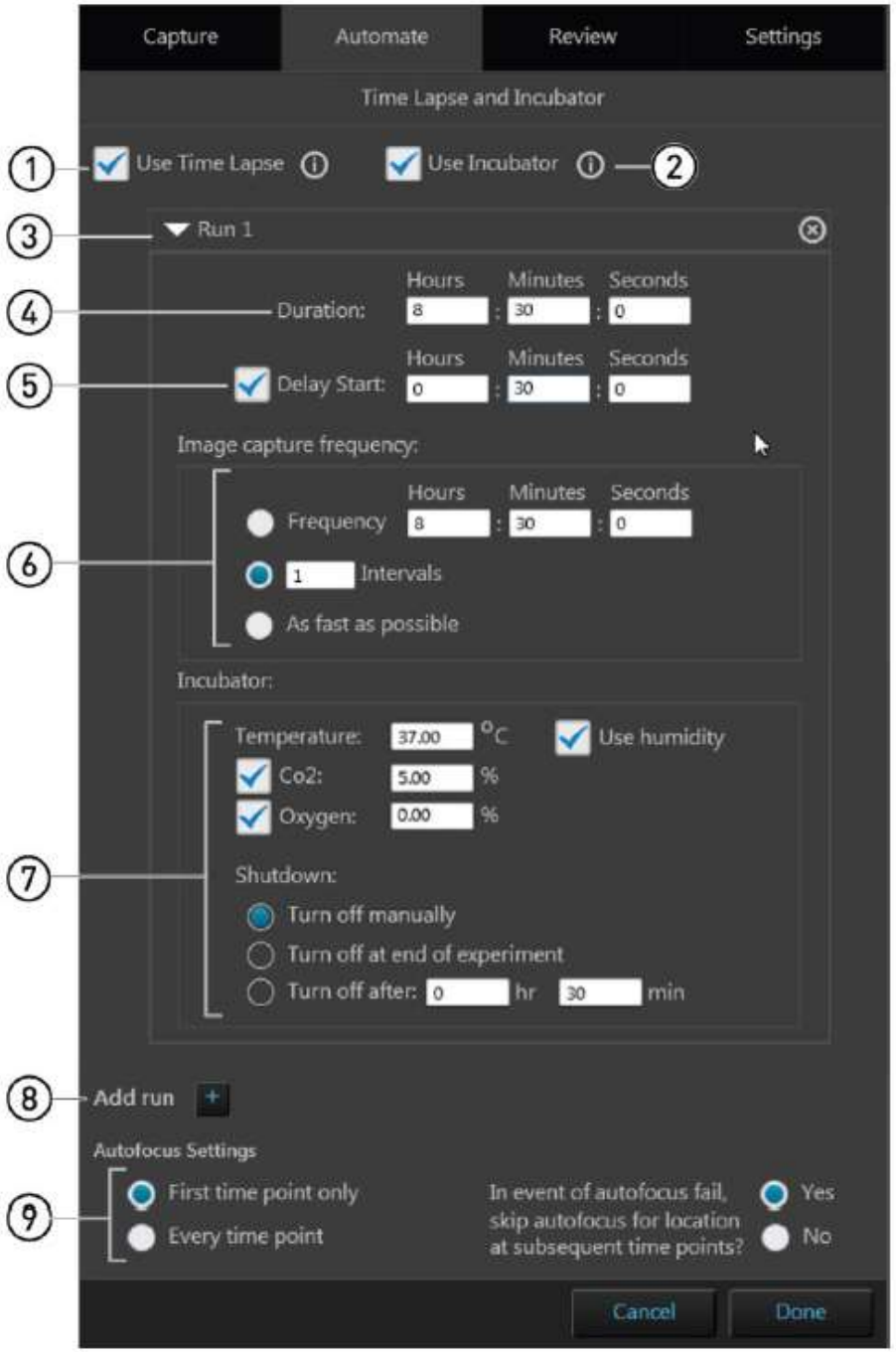


Imaging Through Space: Z-Stack

Z-stacks: Collect layers in step sizes down to 0.150 µm thickness and “walk through” an object

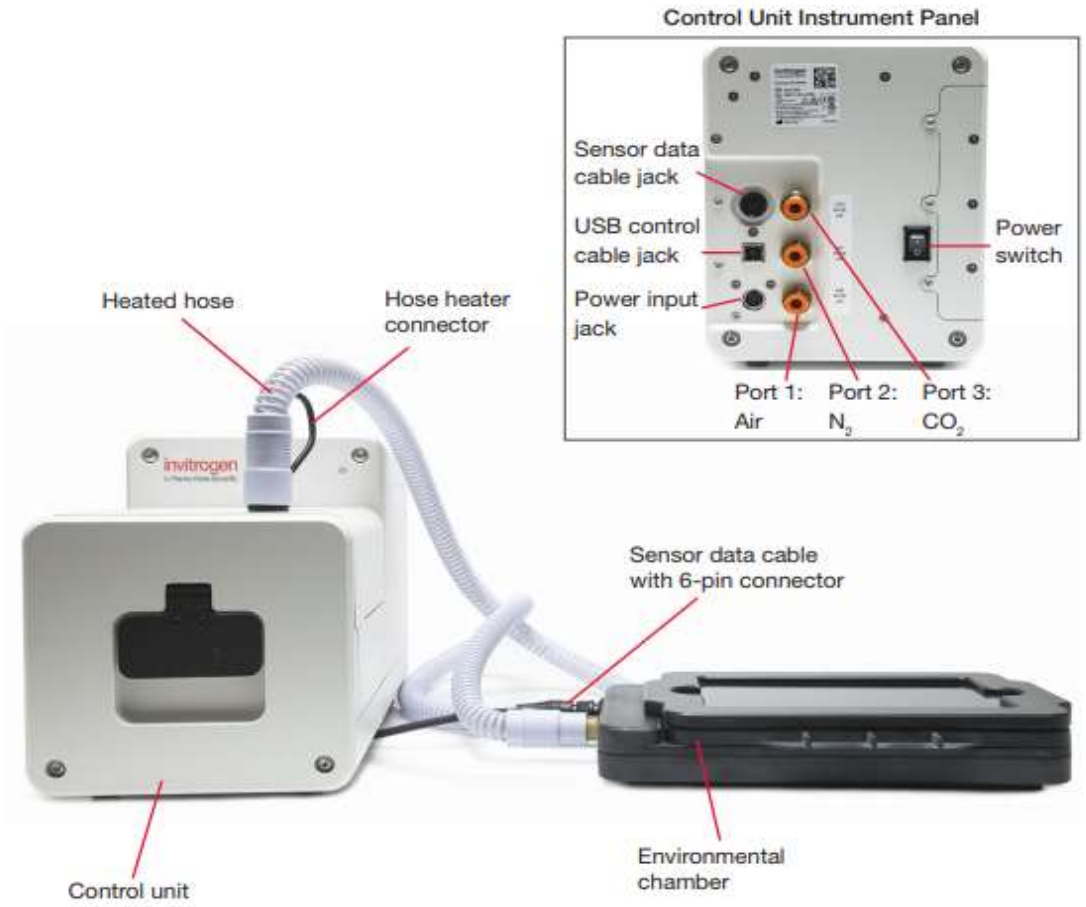


Automate tab : Time Lapse and Incubator



① Use Time Lapse
② Use Incubator
③ Run
④ Duration
⑤ Delay Start
⑥ Image capture frequency
⑦ Incubator
⑧ Add run
⑨ Autofocus Settings

EVOS Onstage Incubator



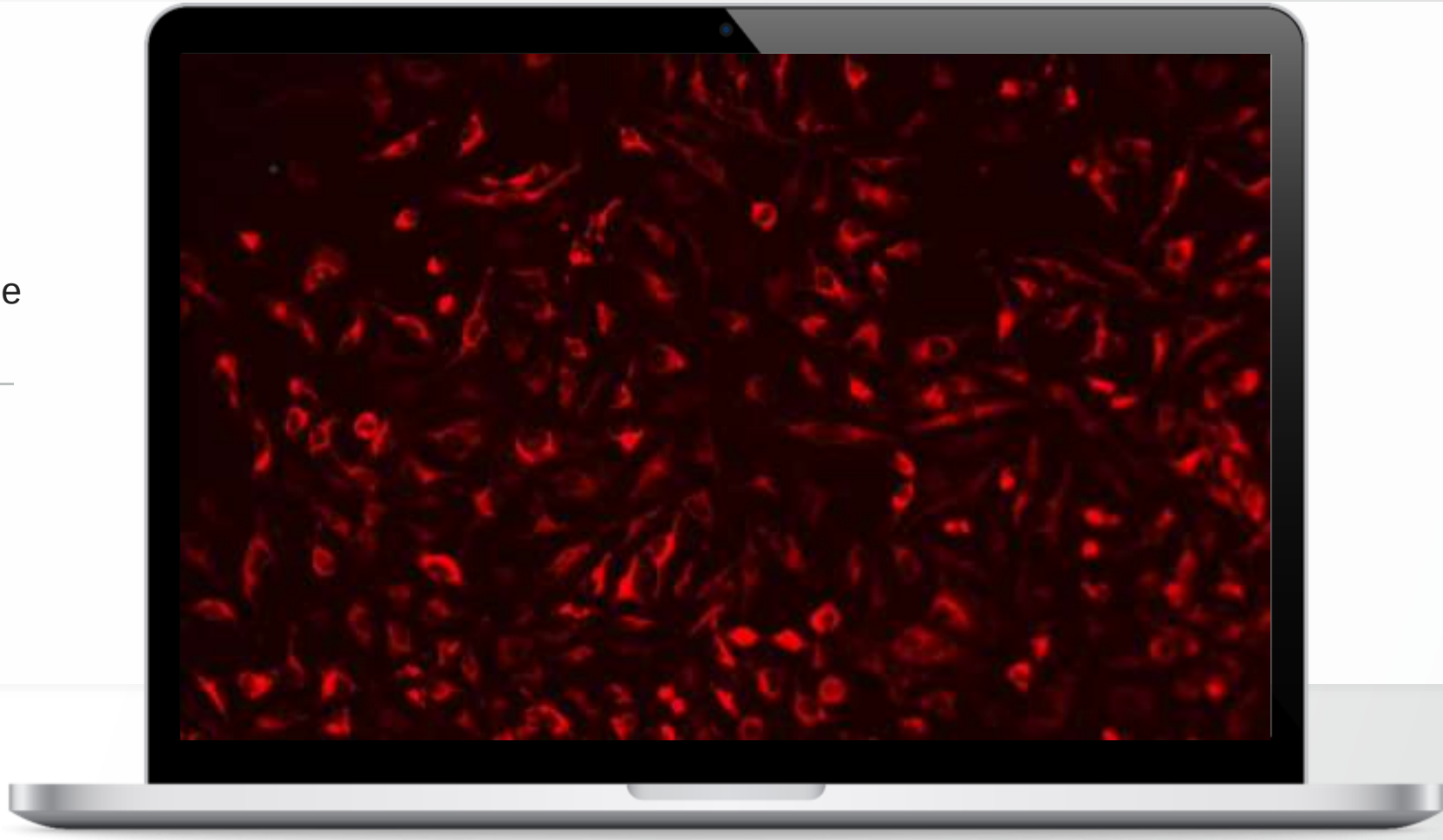
Time-lapse imaging

Visualize and measure biological processes and dynamics over time

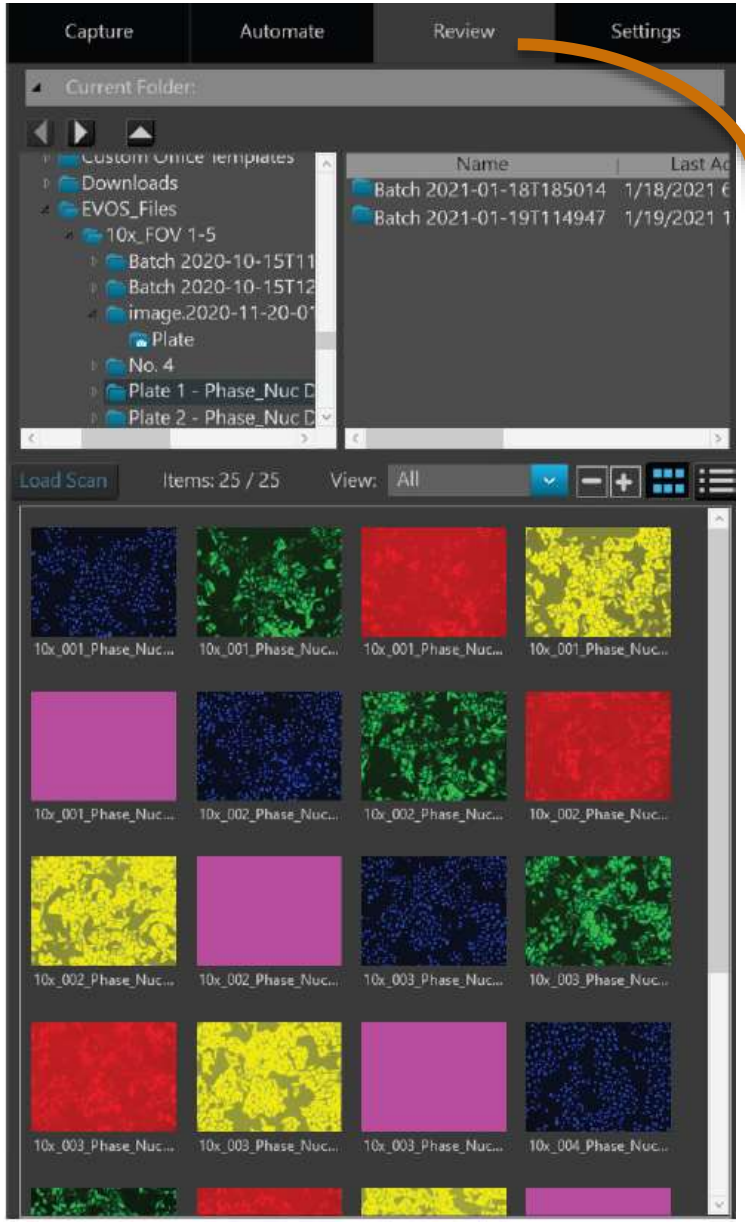
Apoptosis and Toxicity

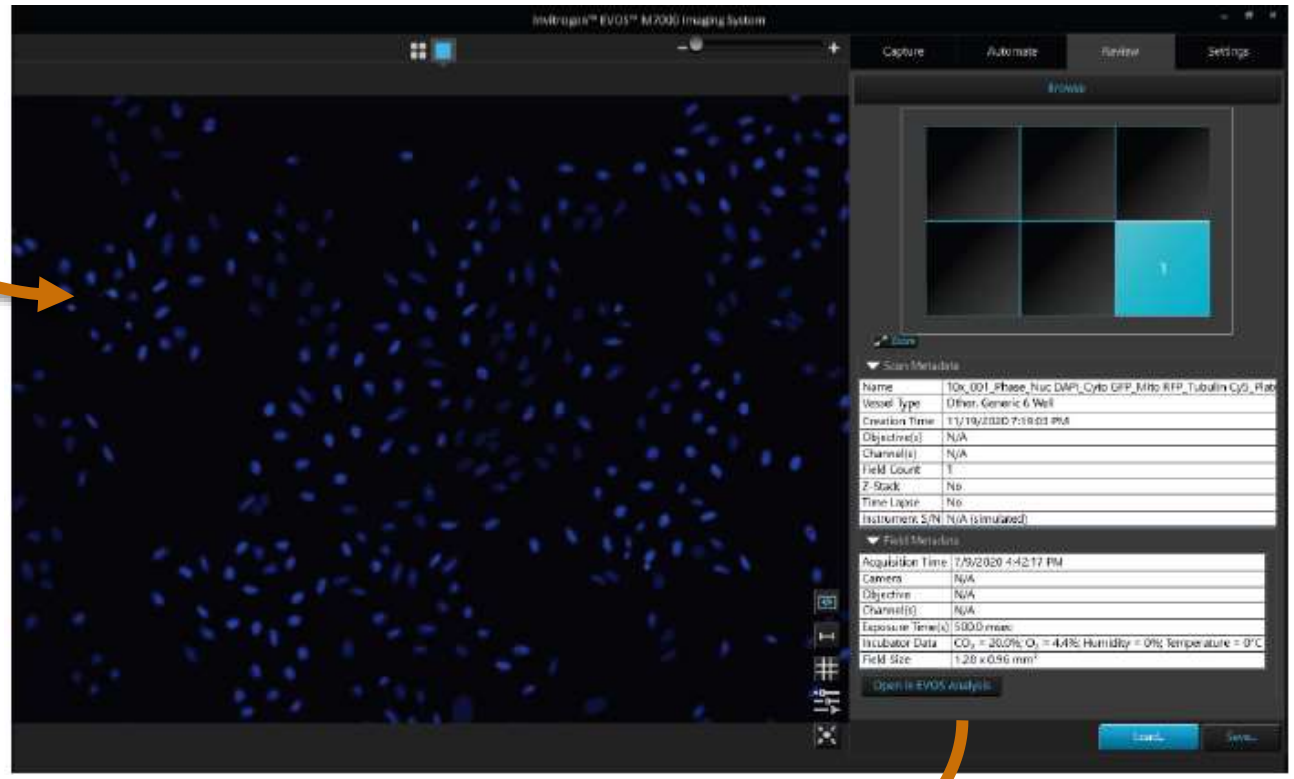
Red: TMRM (mitochondrial membrane potential indicator)
Fading red signal indicates loss of membrane potential and pre-lethal toxicity

Green: Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent
Green signal increases with onset of apoptosis



EVOS Analysis

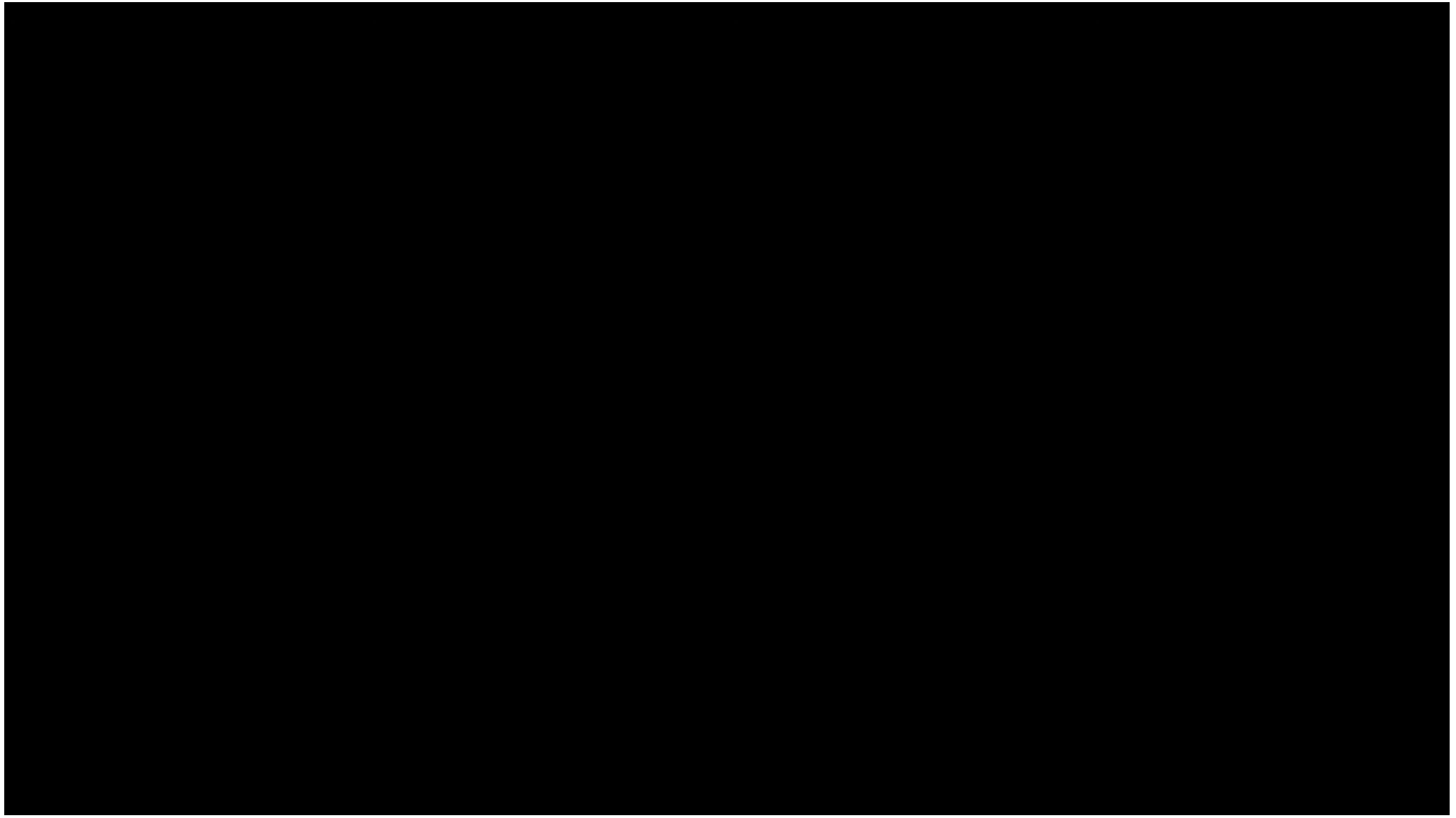




Open in EVOS Analysis

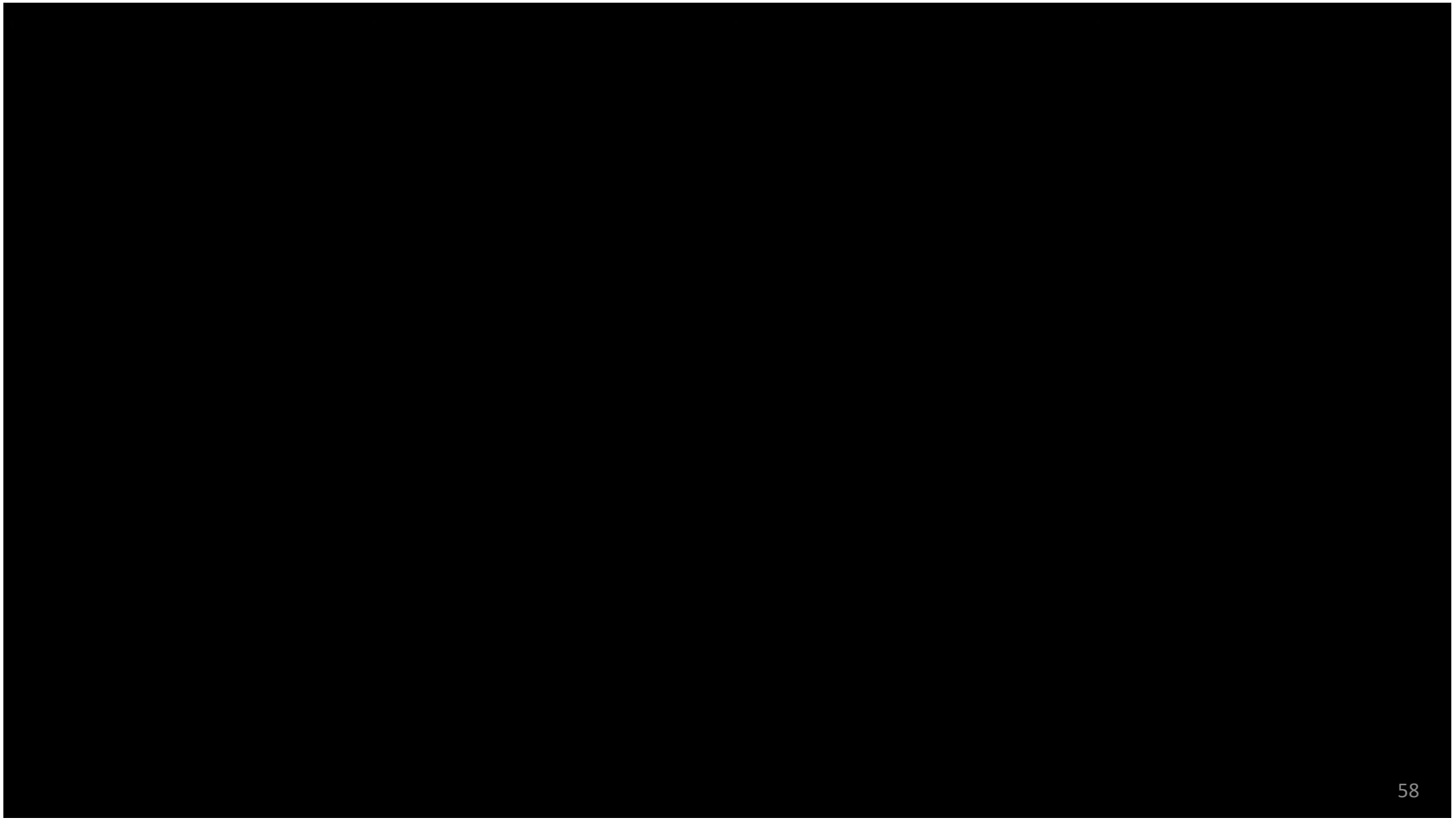
EVOS Analysis

Image enhancements, measurement, and annotations



EVOS Analysis

Cell Count



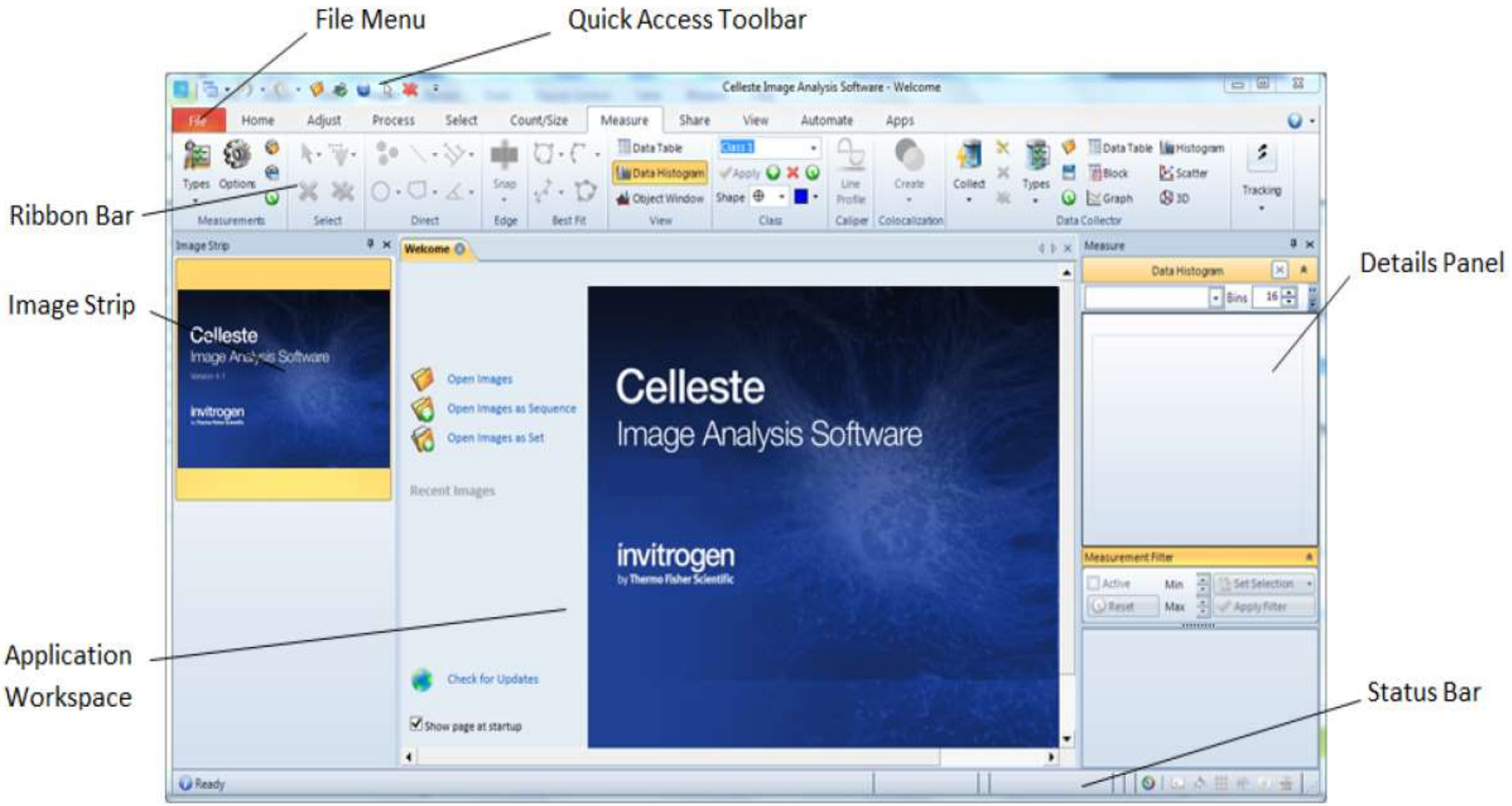
Celleste Image Analysis Software

What is Celleste software?

An image-centric analysis solution

- Easily generate data for commonly performed assays
- Use deep capabilities for advanced image analysis and quantitation
- Segment, count/size, classify, and analyze complex images
- Apply one-click macros, batch analysis and export functions to large image sets
- Adjust, visualize, and share

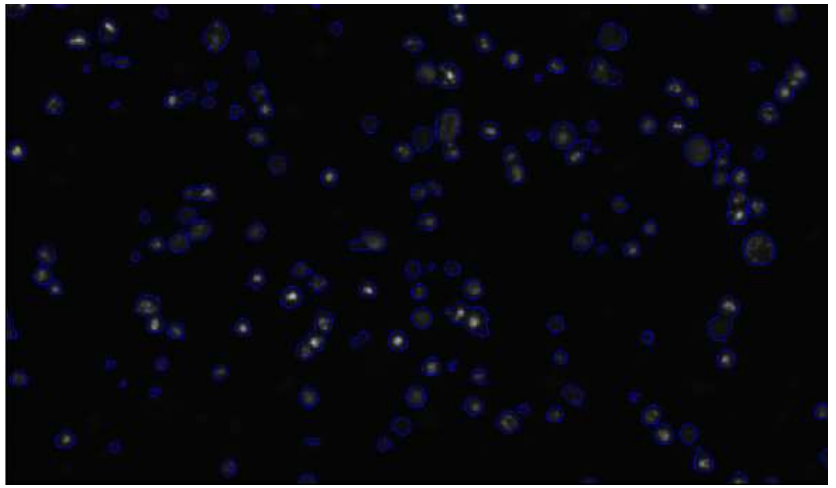
Celleste Image Analysis Software

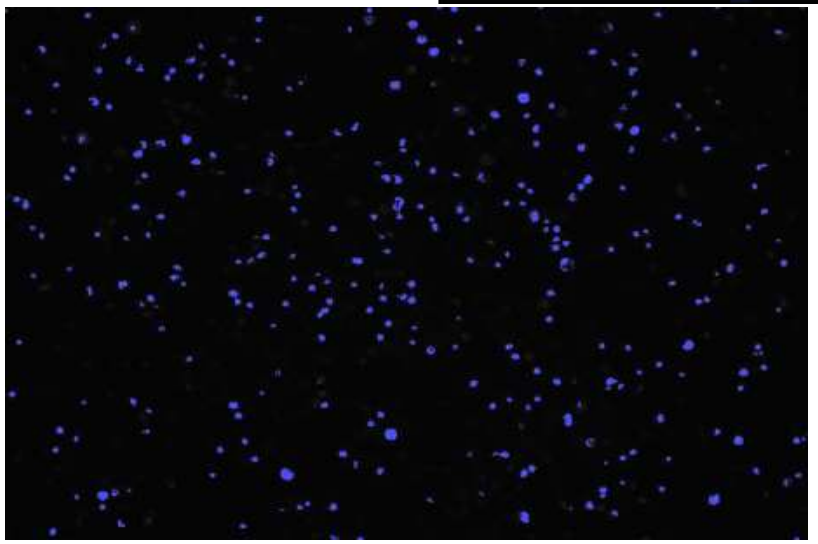


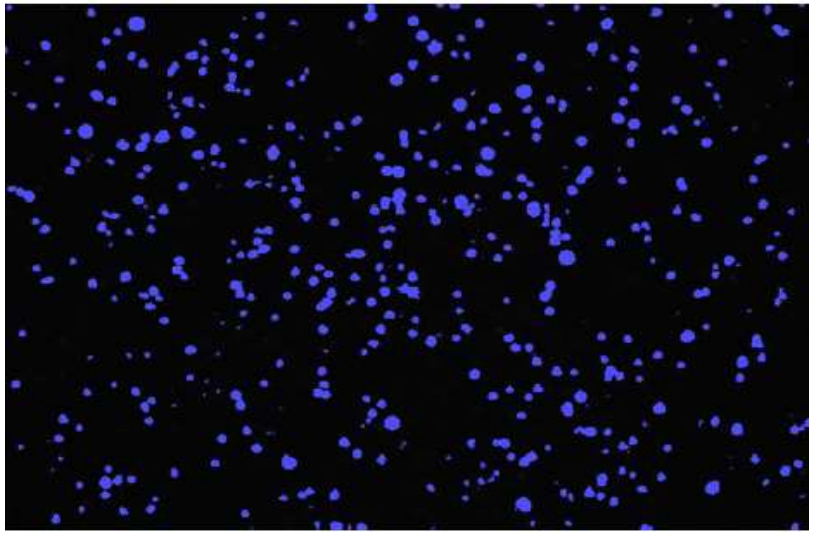
Celleste Image Analysis Software

Cell counting

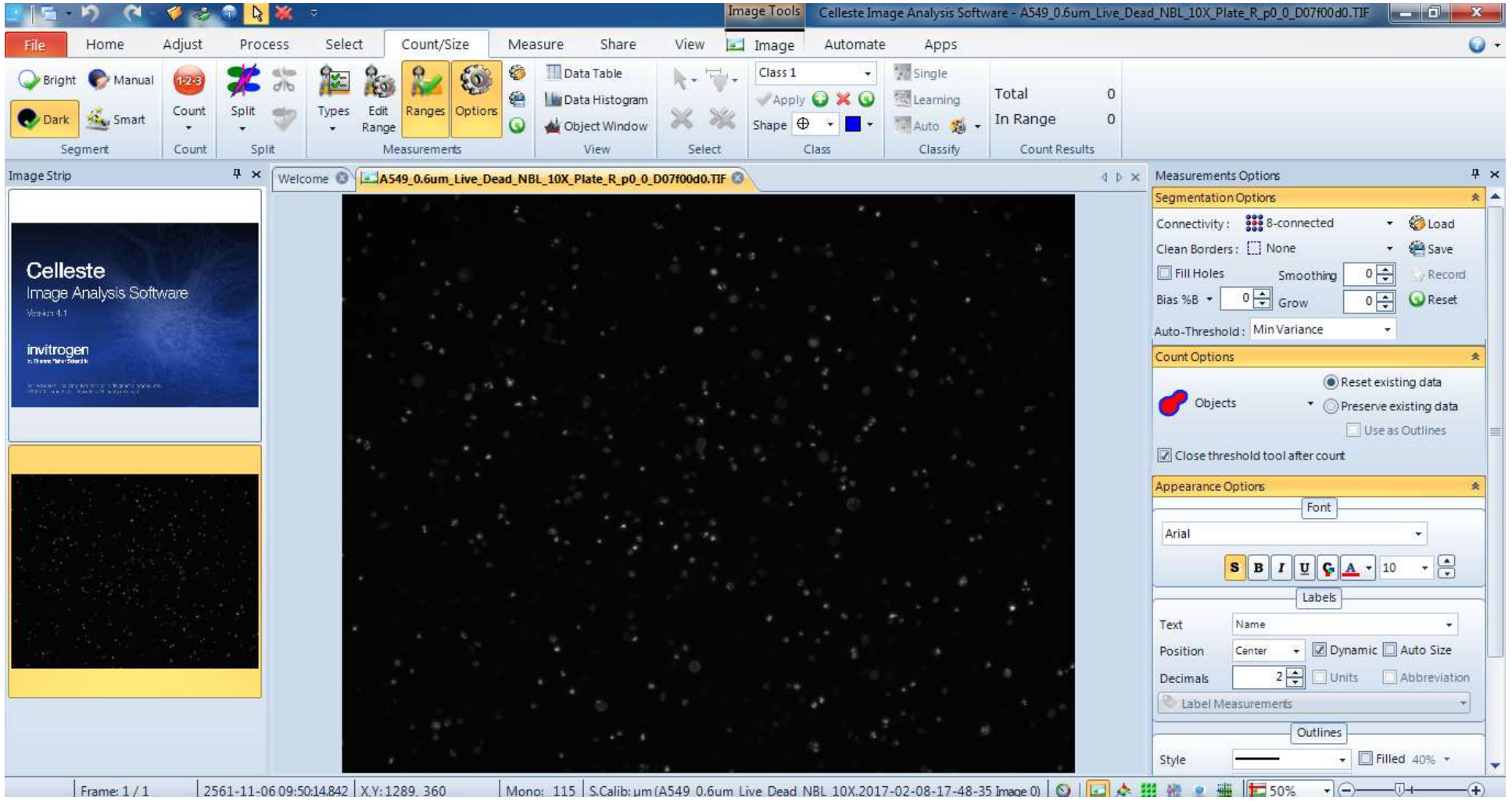
Cell counting has traditionally been known to be laborious and inaccurate. With Celleste Image Analysis Software, cells can be counted with the ability to capture cell counts over time, you can easily measure proliferation rates.





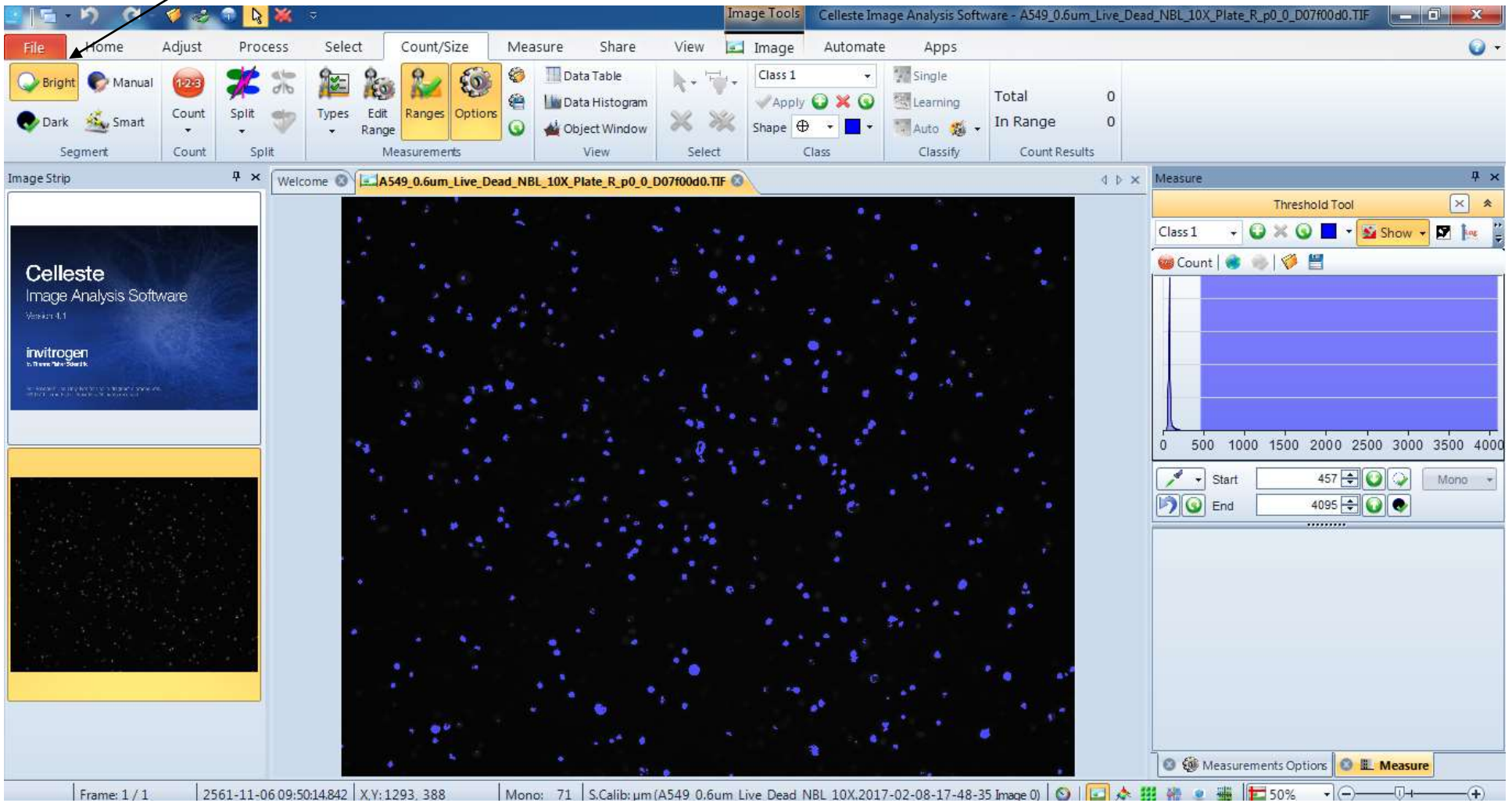


Celleste Image Analysis Software

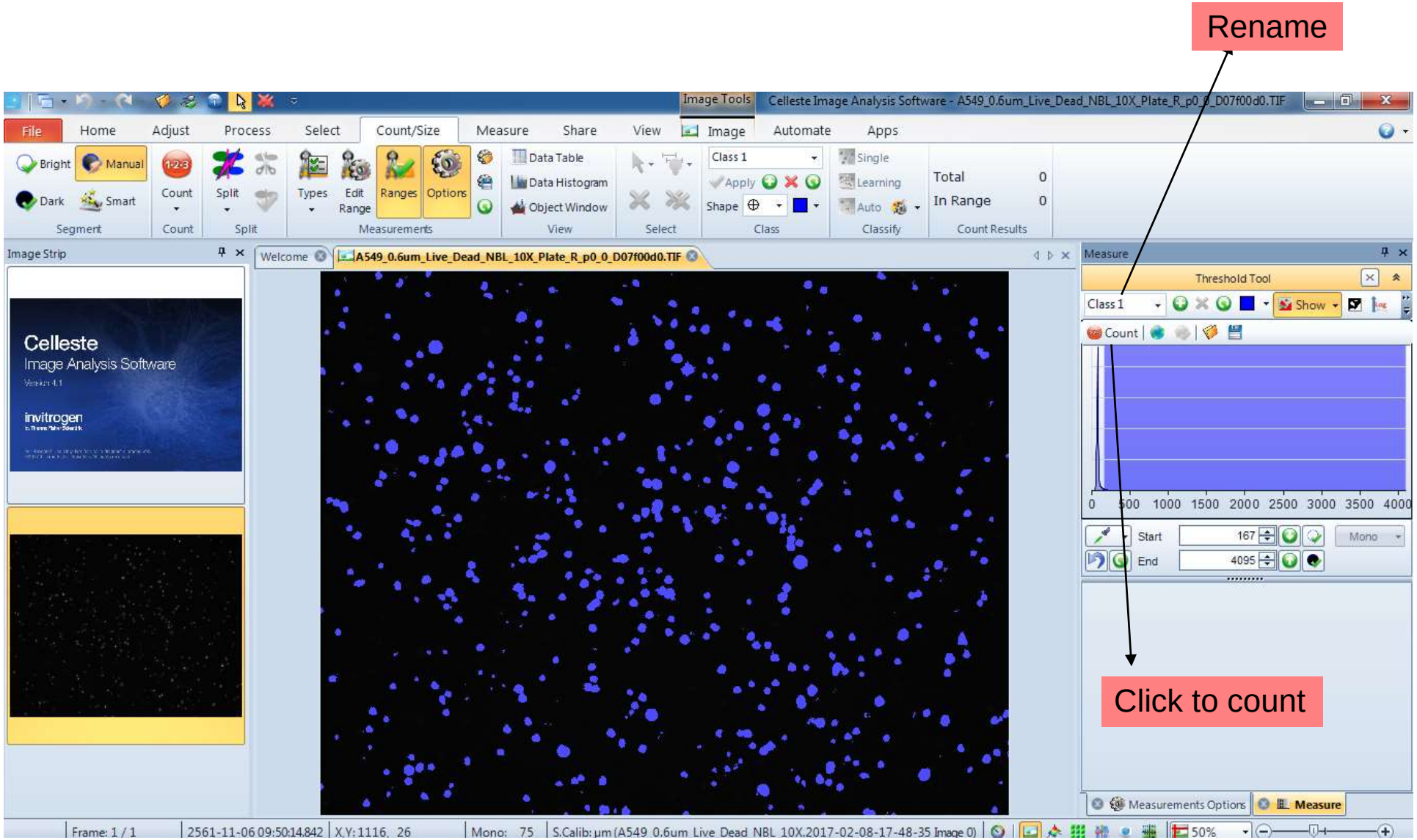


Celleste Image Analysis Software

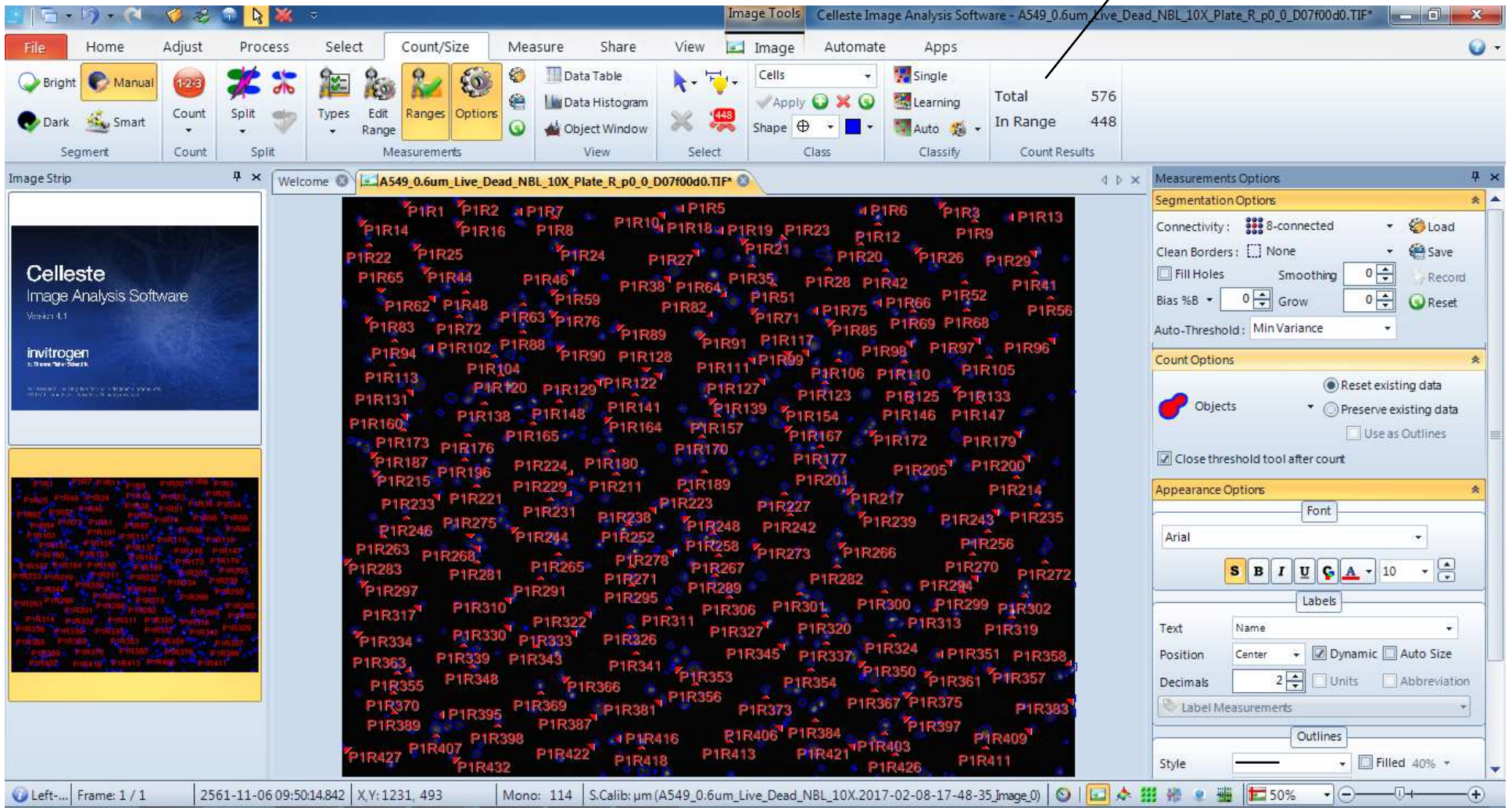
Auto-segment bright object



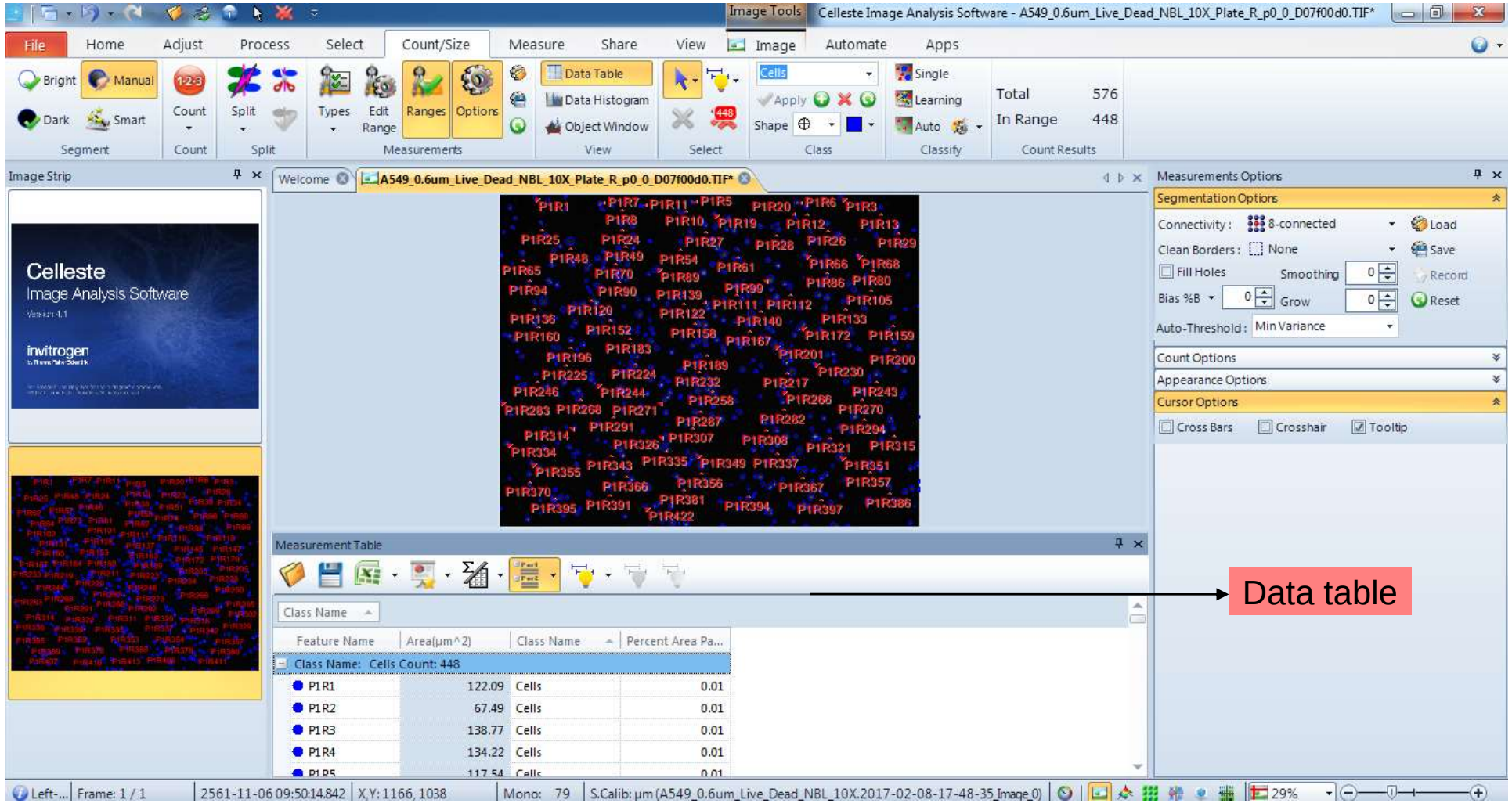
Celleste Image Analysis Software



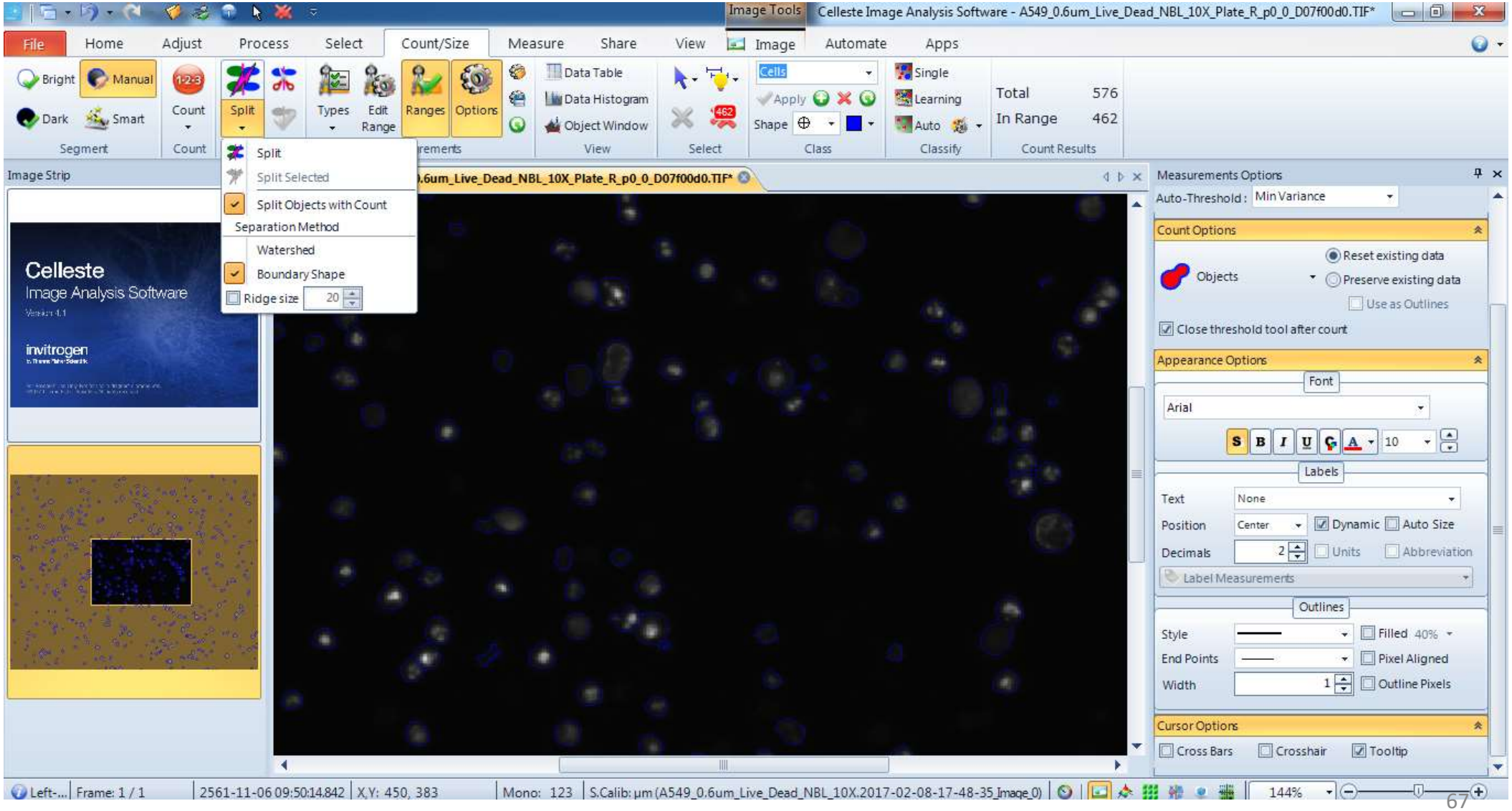
Celleste Image Analysis Software



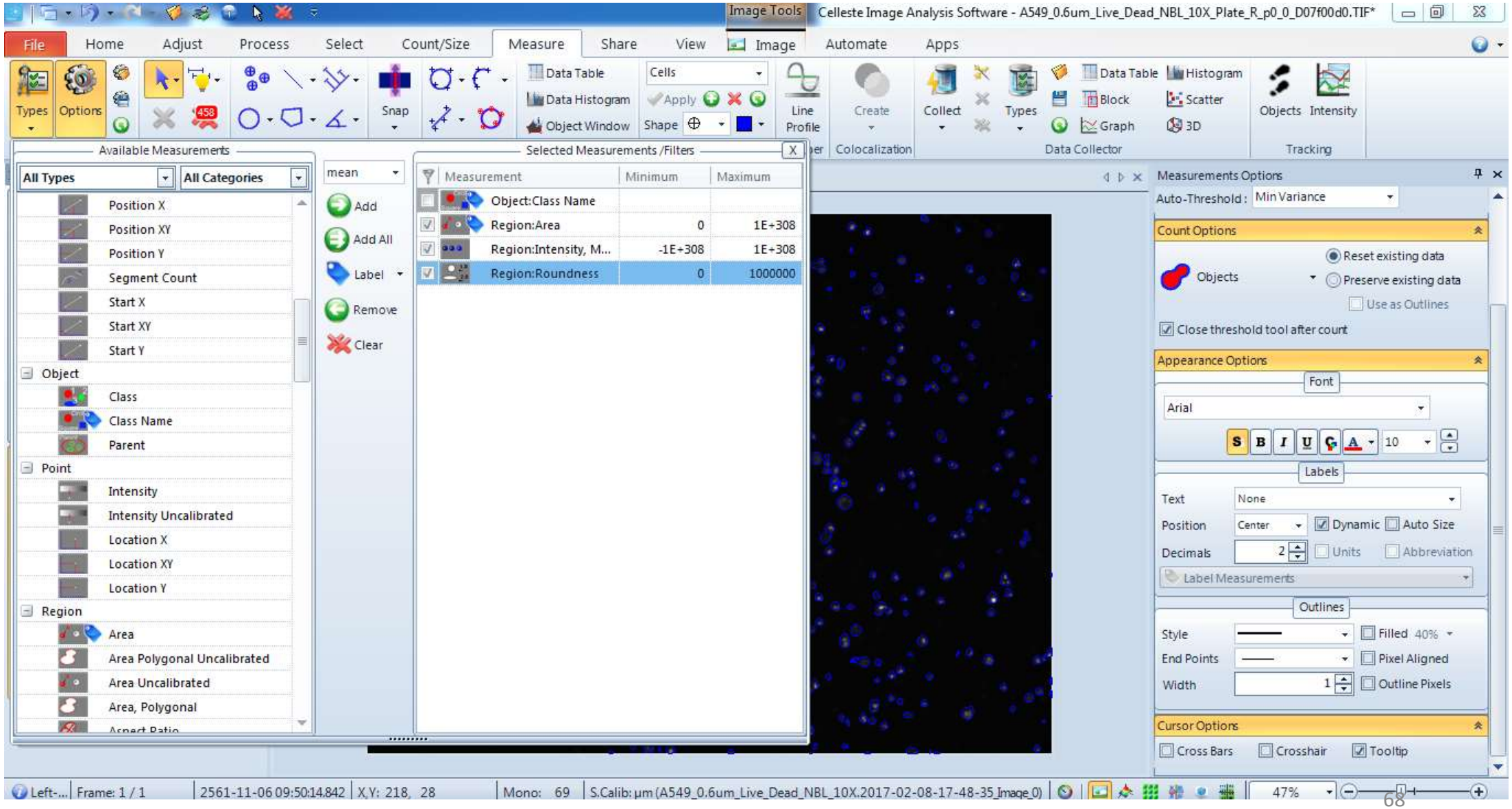
Celleste Image Analysis Software



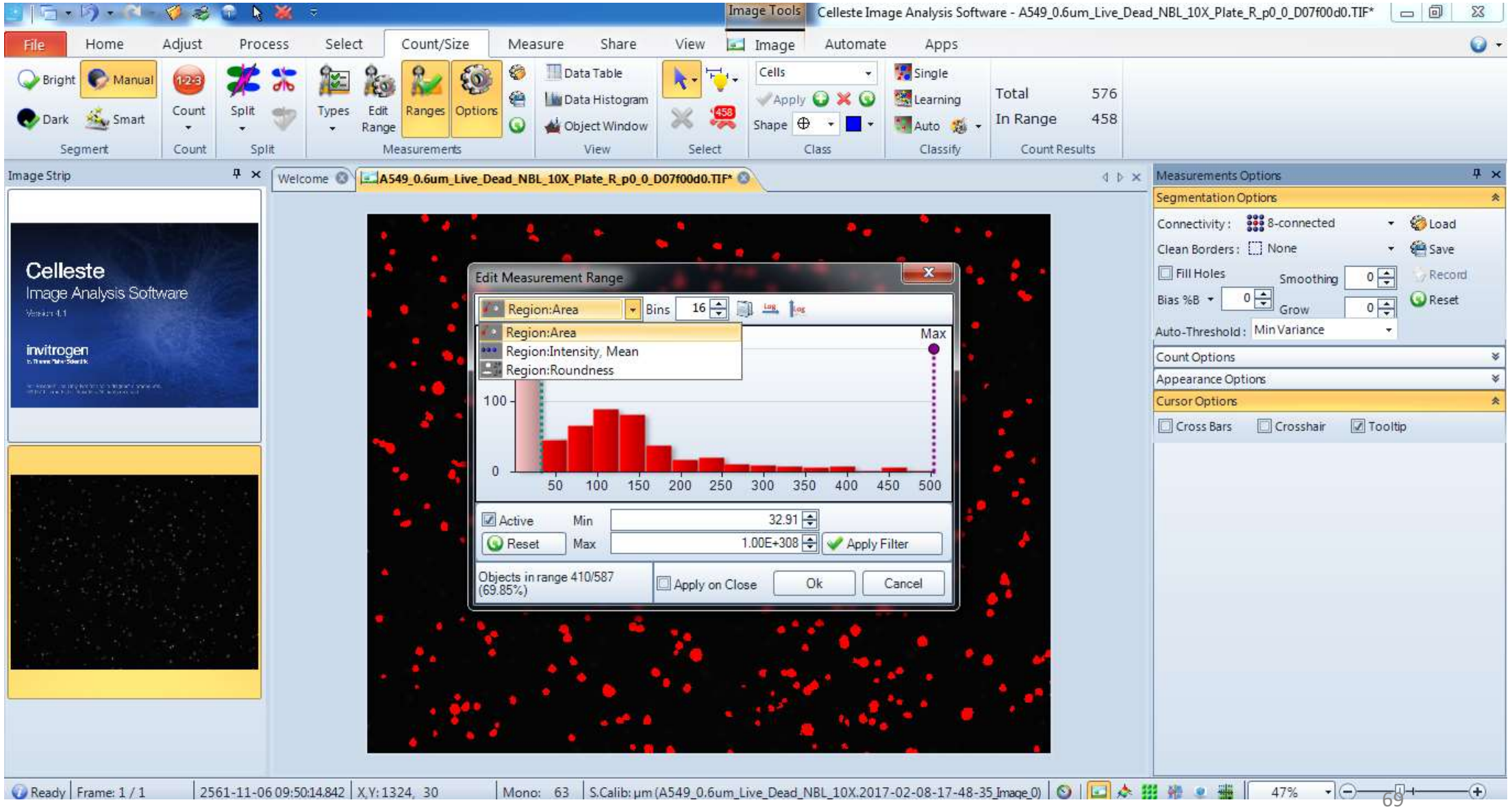
Celleste Image Analysis Software



Celleste Image Analysis Software

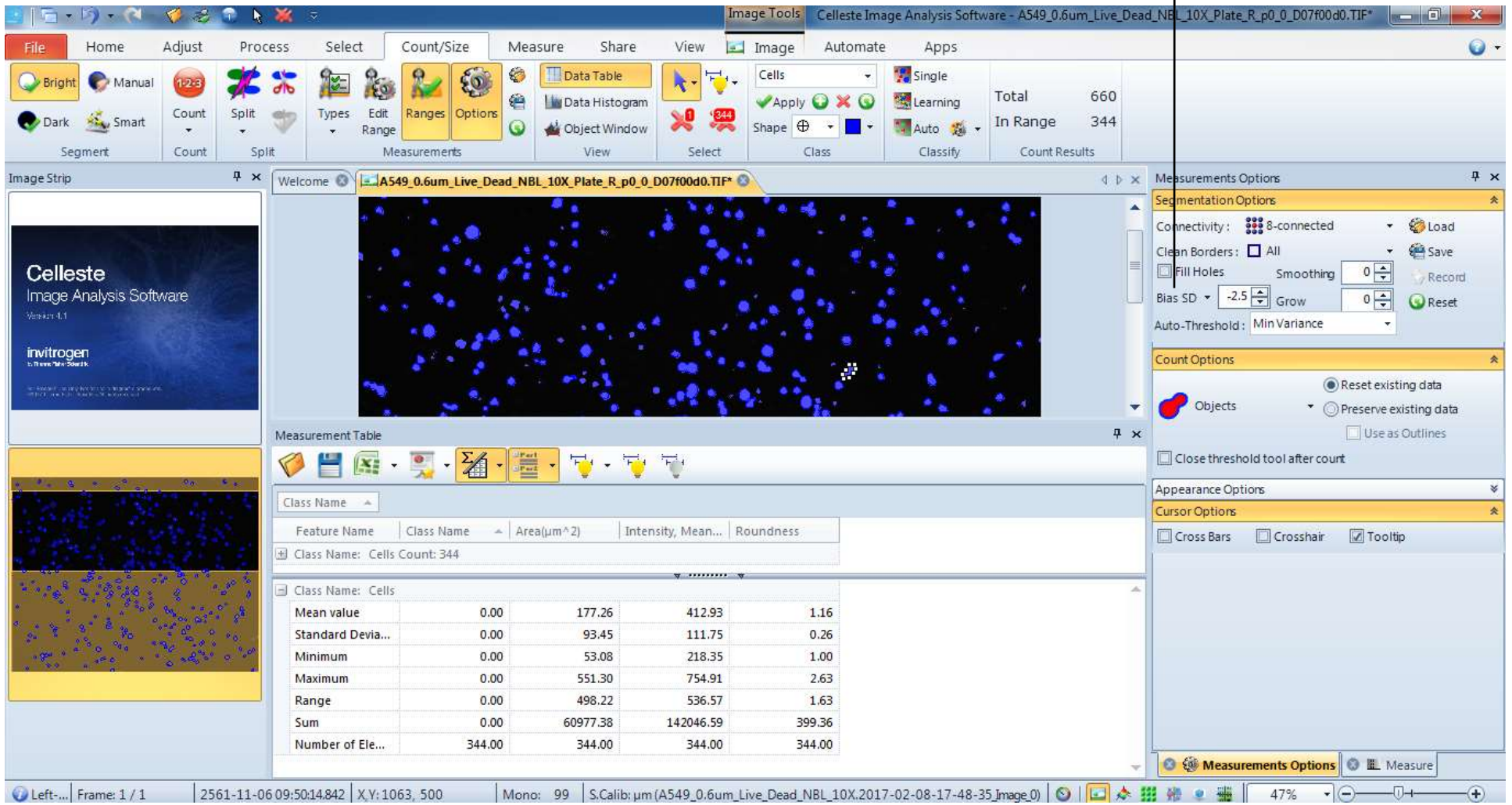


Celleste Image Analysis Software



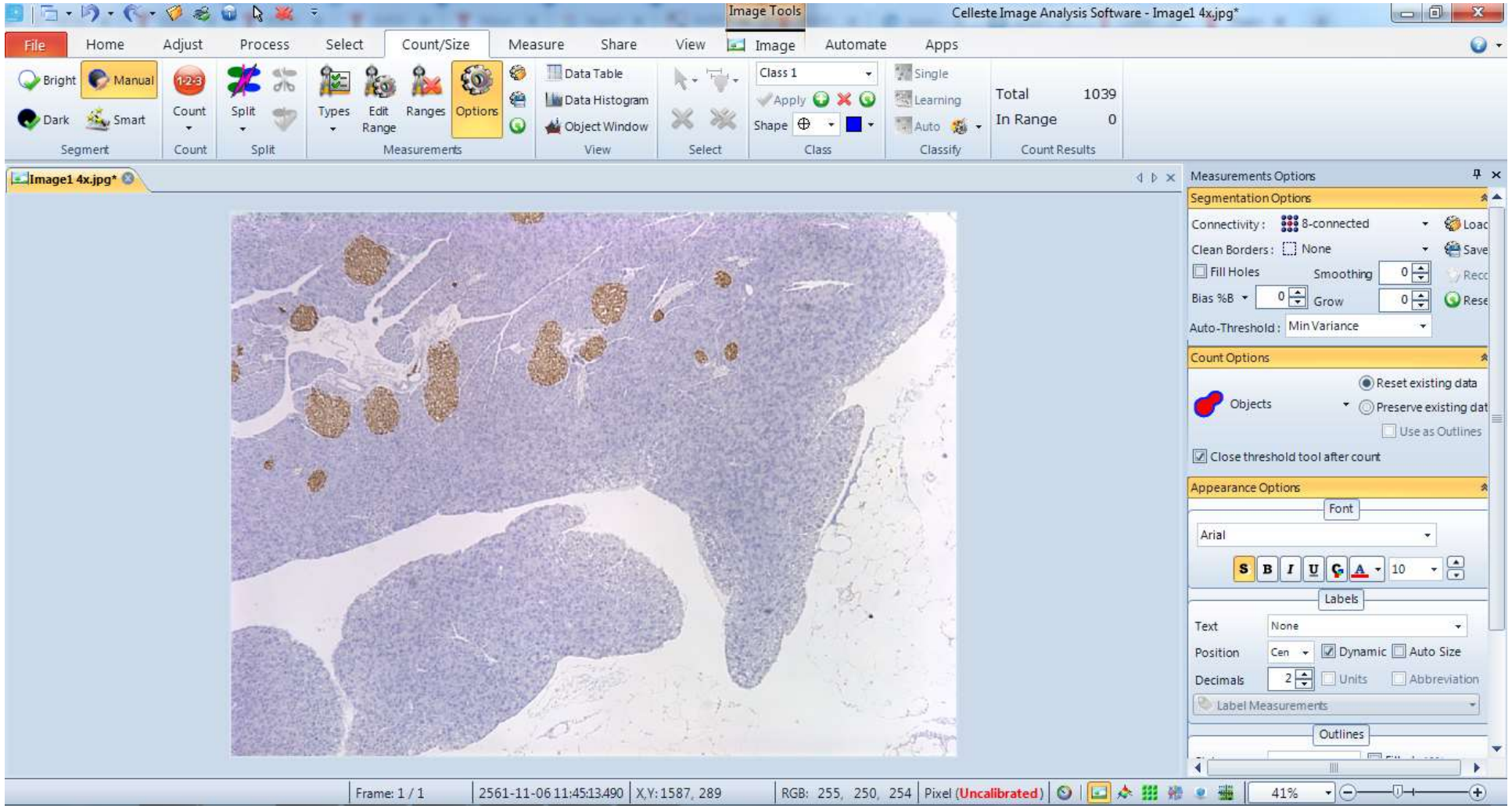
Celleste Image Analysis Software

auto-threshold

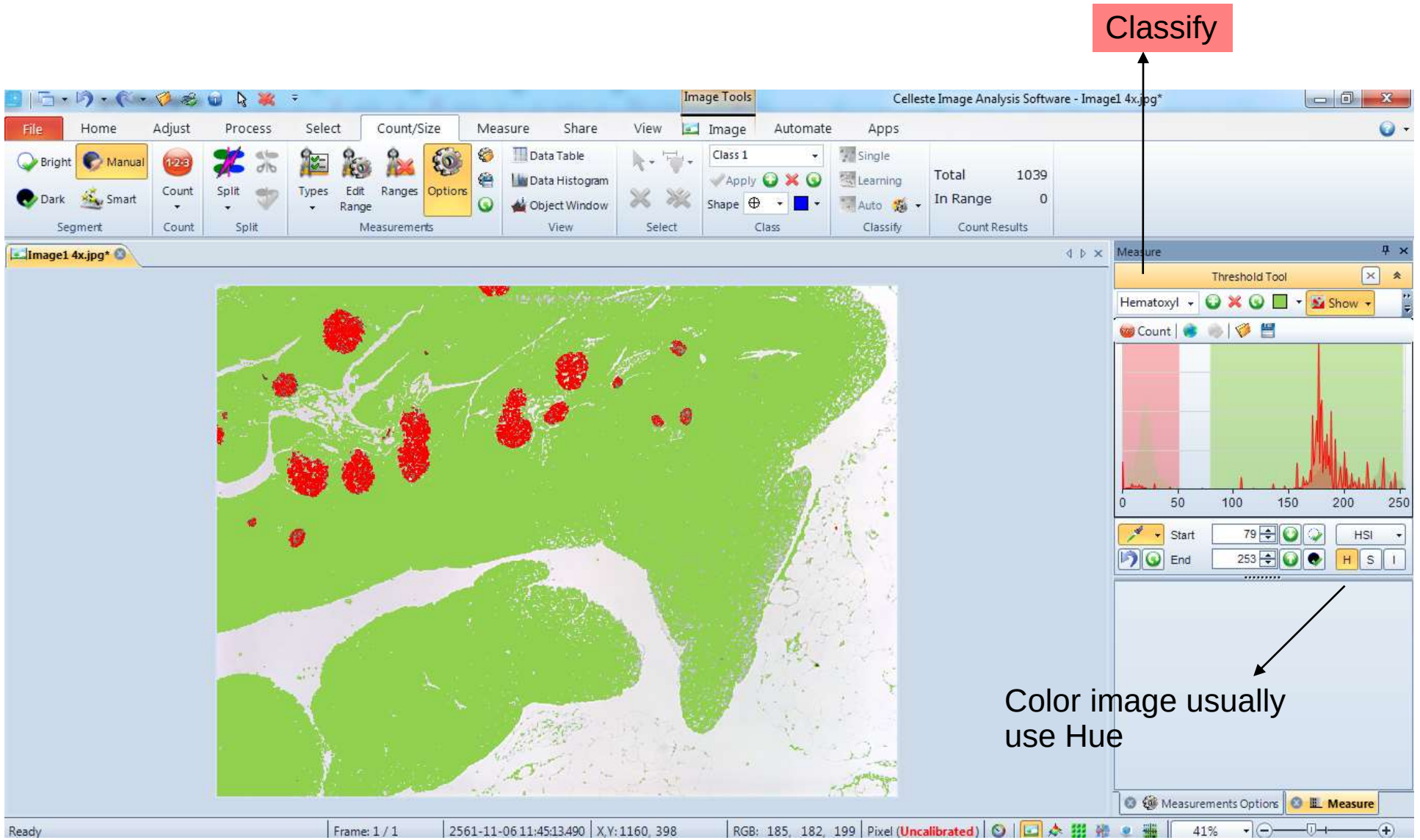


Celleste Image Analysis Software

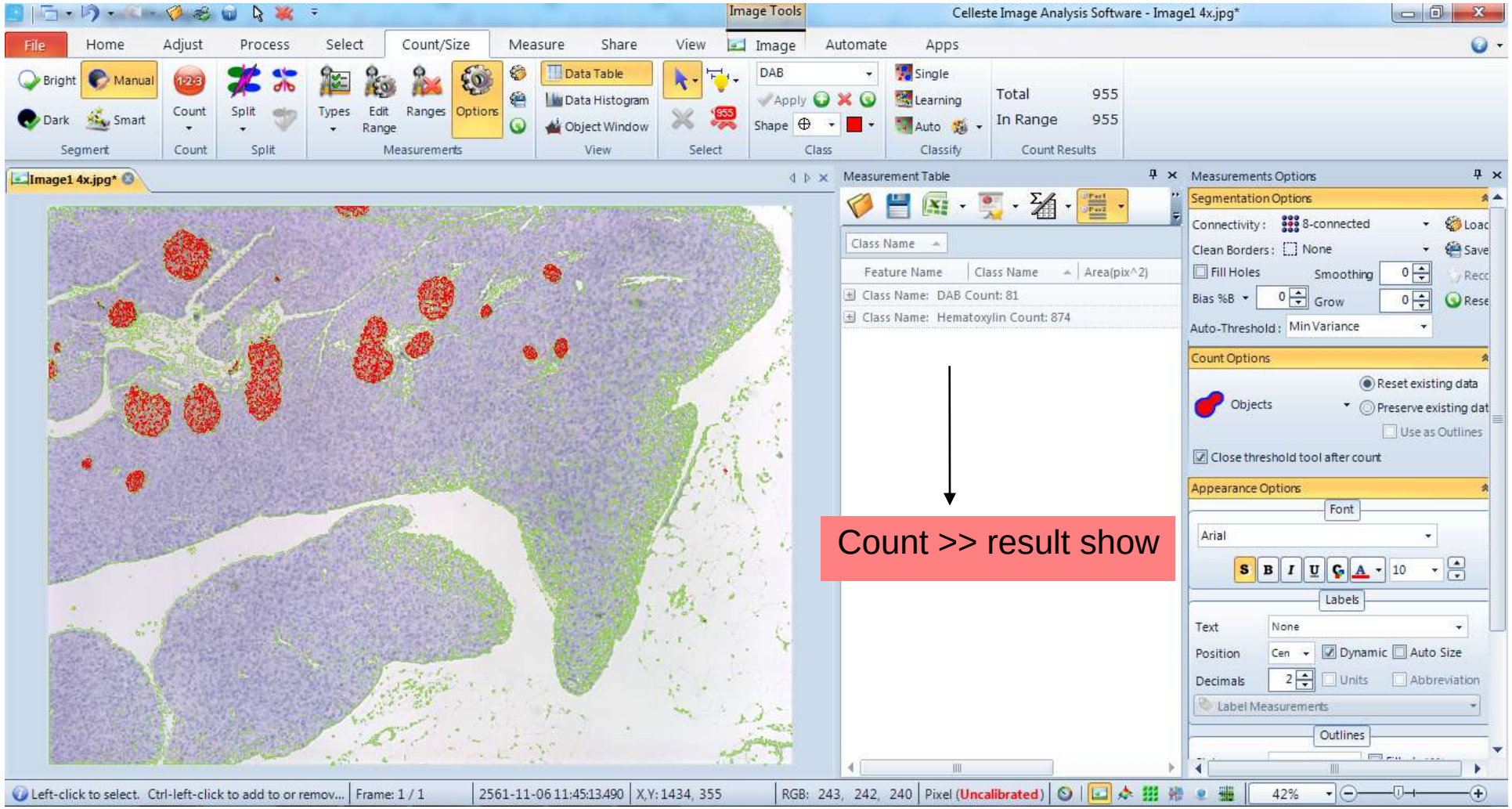
Classification and Counting Multiple Classes



Celleste Image Analysis Software



Celleste Image Analysis Software

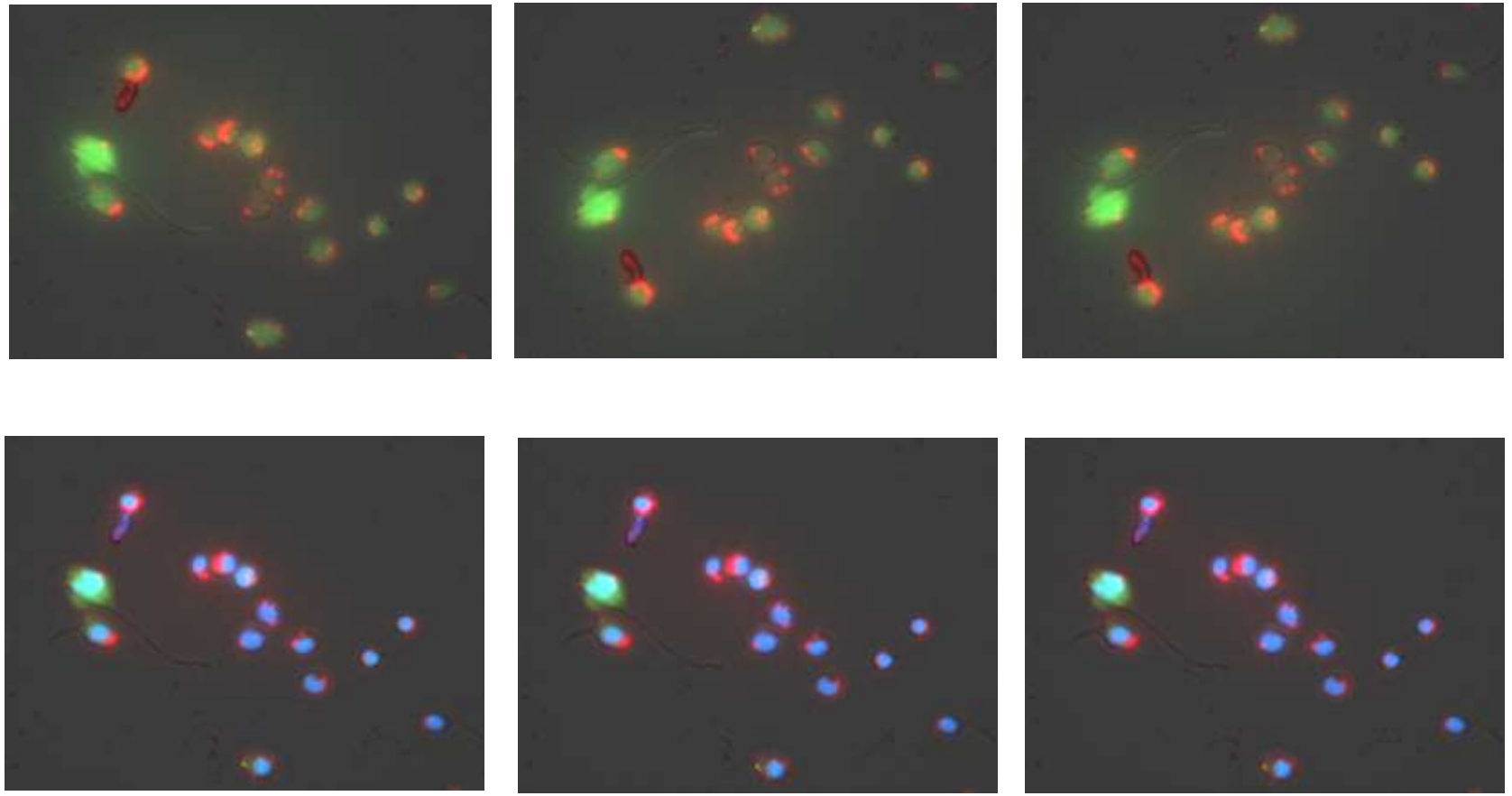


Celleste Image Analysis Software

Intensity Tracking

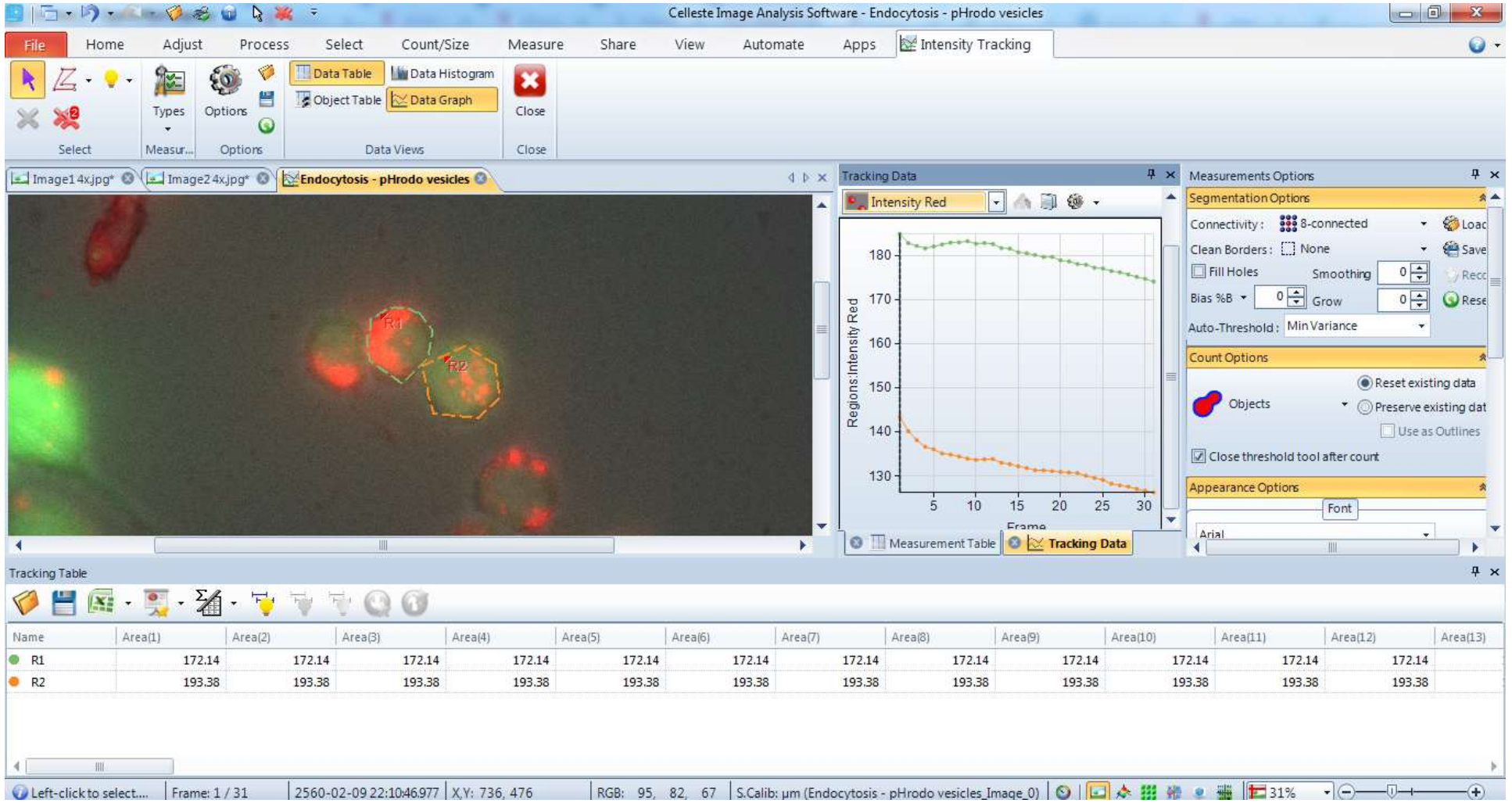
pHrodo Indicators for pH Determination

Proprietary, pH-sensitive Invitrogen pHrodo dyes are almost non-fluorescent at neutral pH and fluoresce brightly in acidic environments, making them ideal for use as pH indicators for a variety of applications.



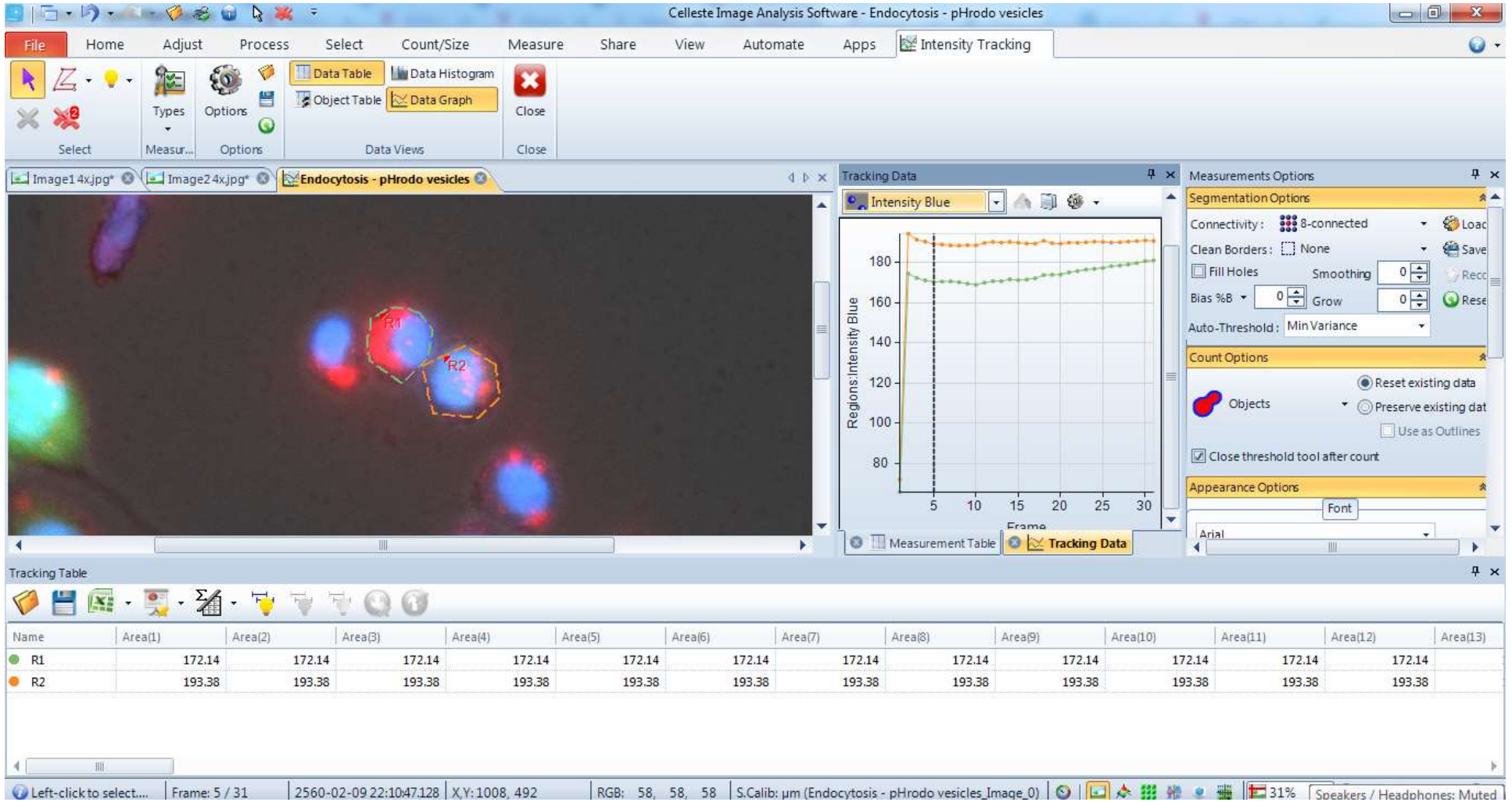
Celleste Image Analysis Software

Intensity Tracking



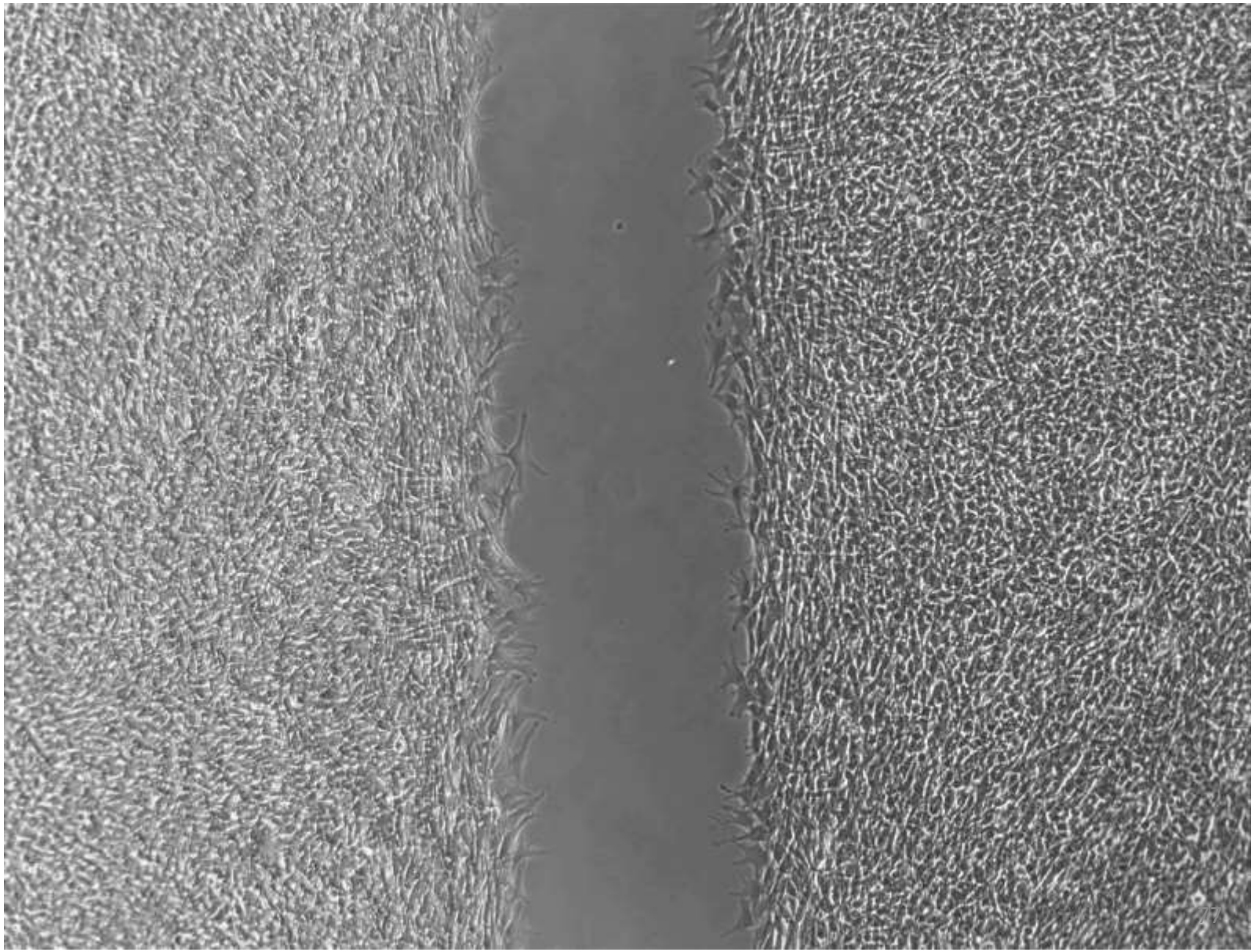
Celleste Image Analysis Software

Intensity Tracking



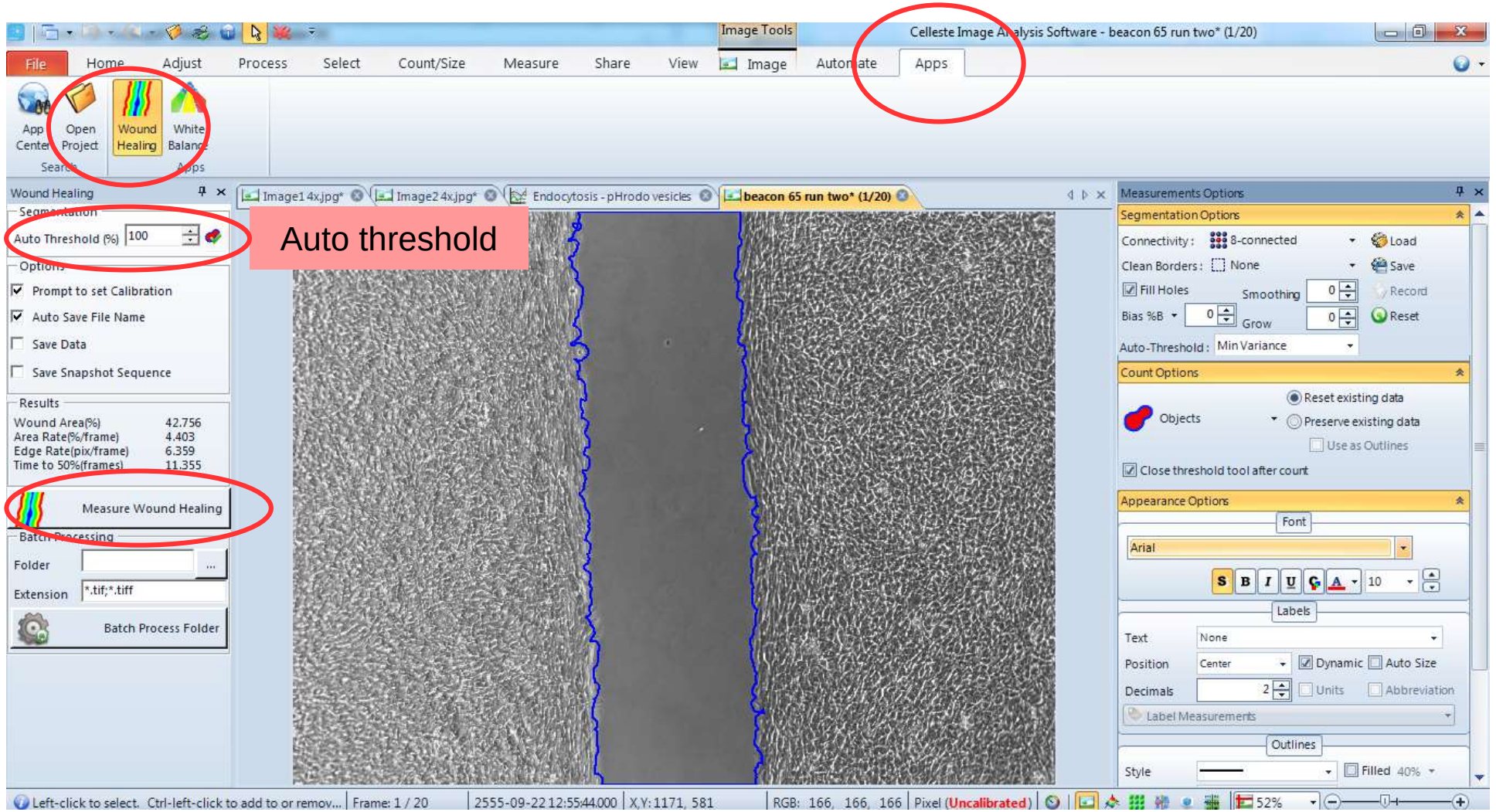
Celleste Image Analysis Software

Wound Healing



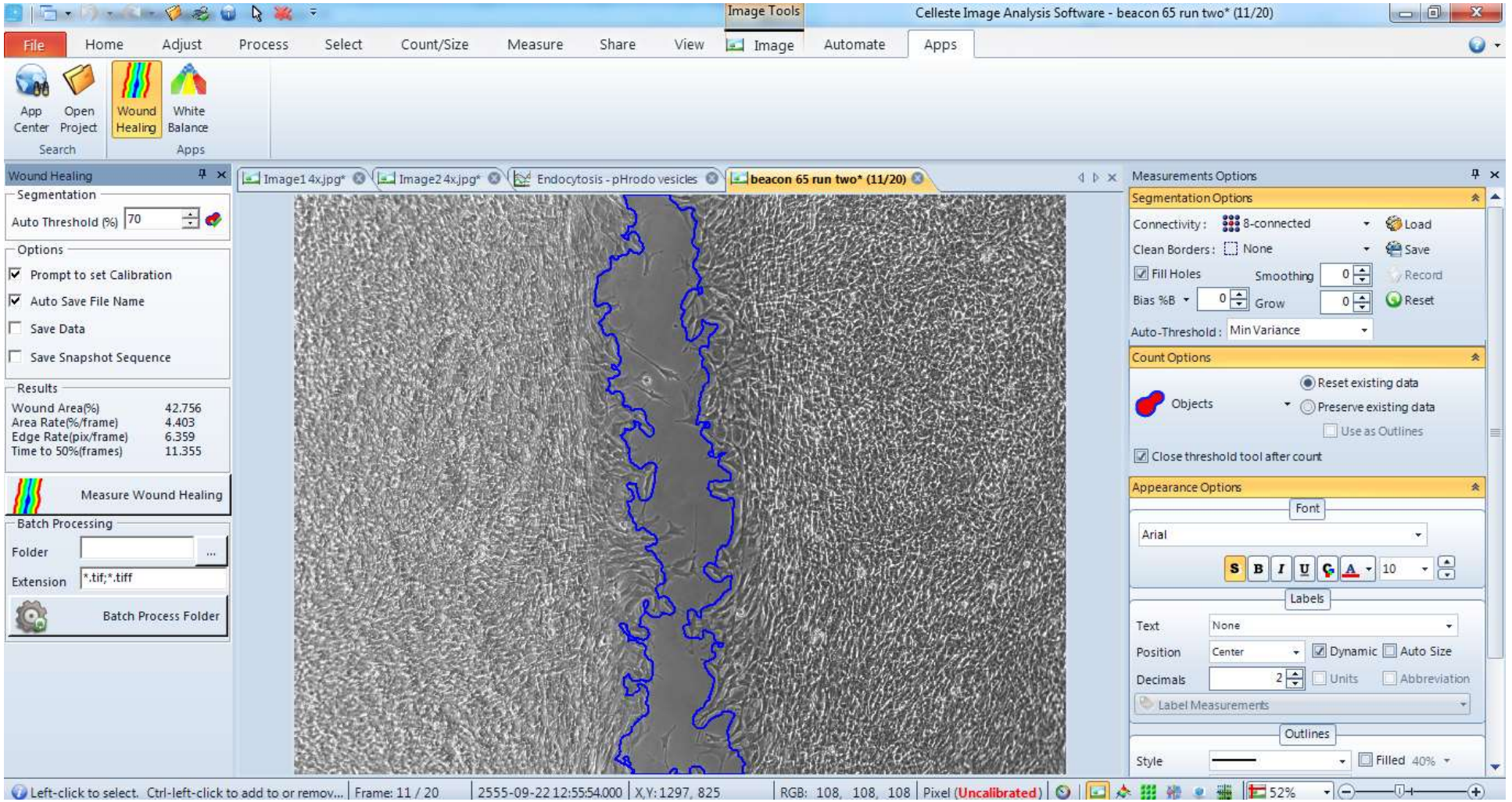
Celleste Image Analysis Software

Wound Healing



Celleste Image Analysis Software

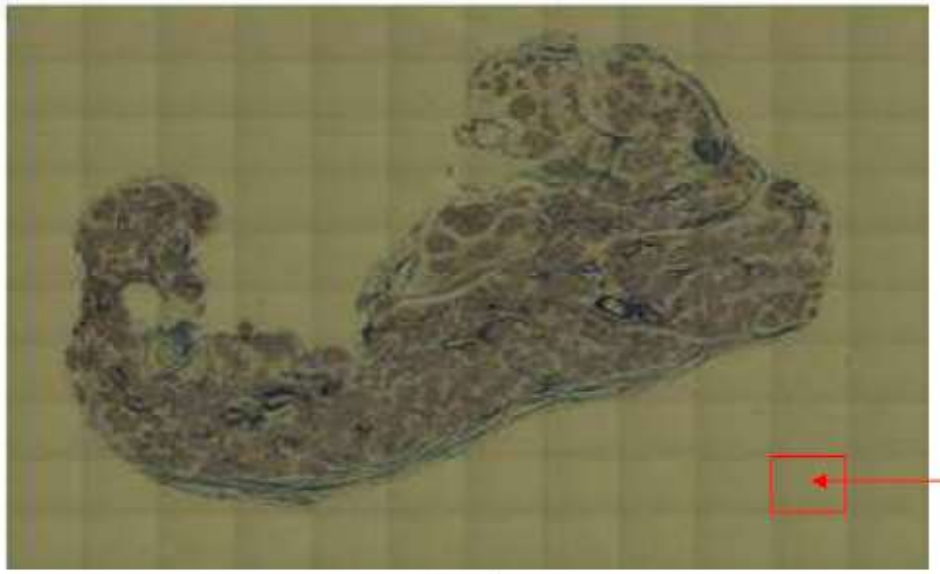
Wound Healing



Celleste Image Analysis Software

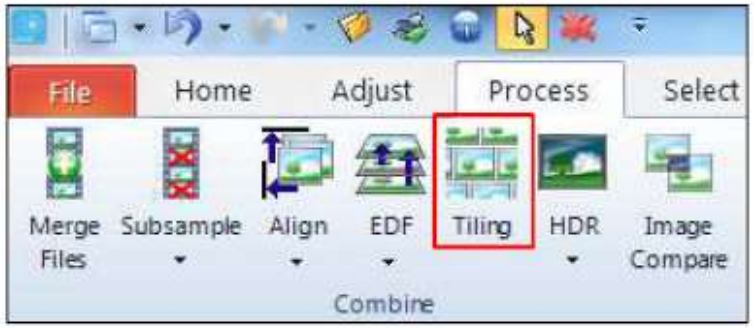
Tile Image

FL Auto 2 Stitch

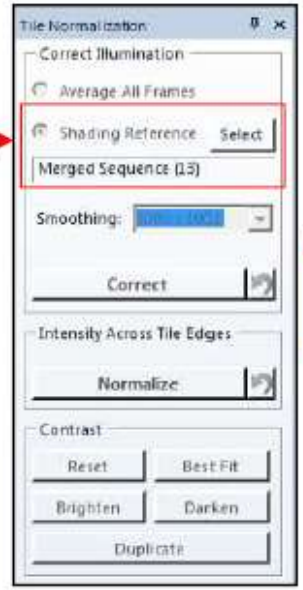


Celleste Corrected

1. Assemble overlapping images



2. Correct edges using Tile App

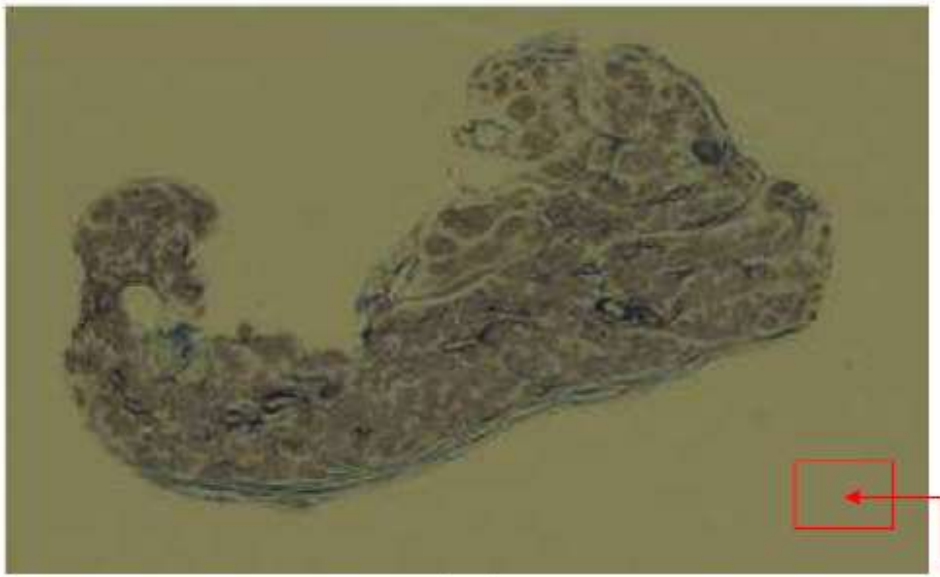


Set shading reference to background FOV

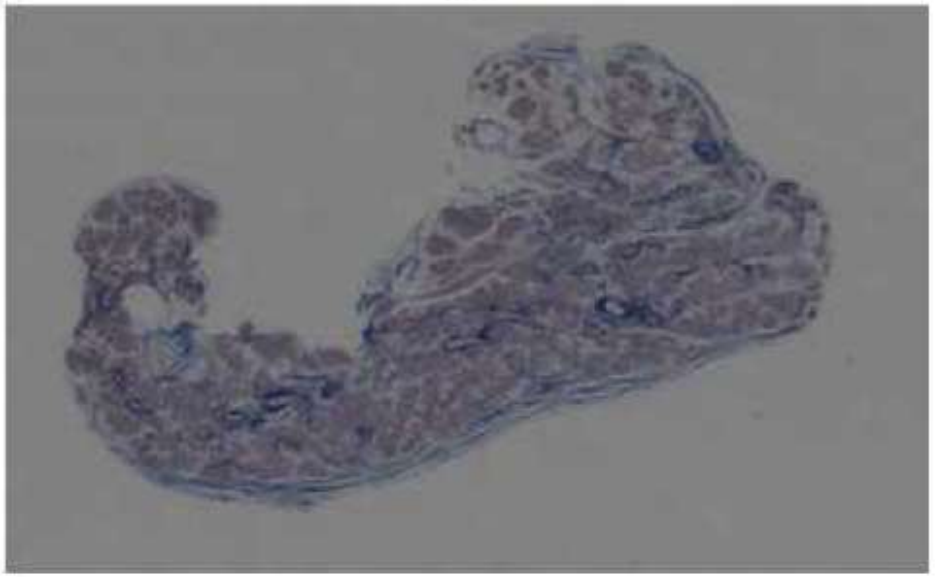
Data by: Trillium Blackmer
Celleste by: Oggie Golub

Celleste Image Analysis Software

Celleste Stitched

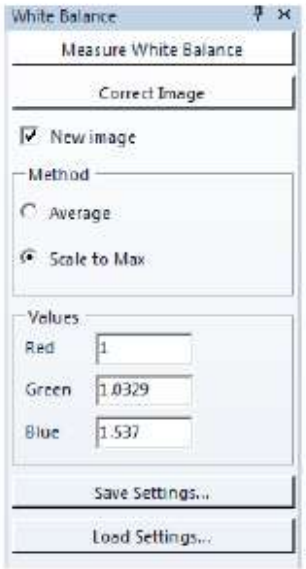


White Balance Corrected



1. Activate White Balance App

2. Measure White Balance & Correct

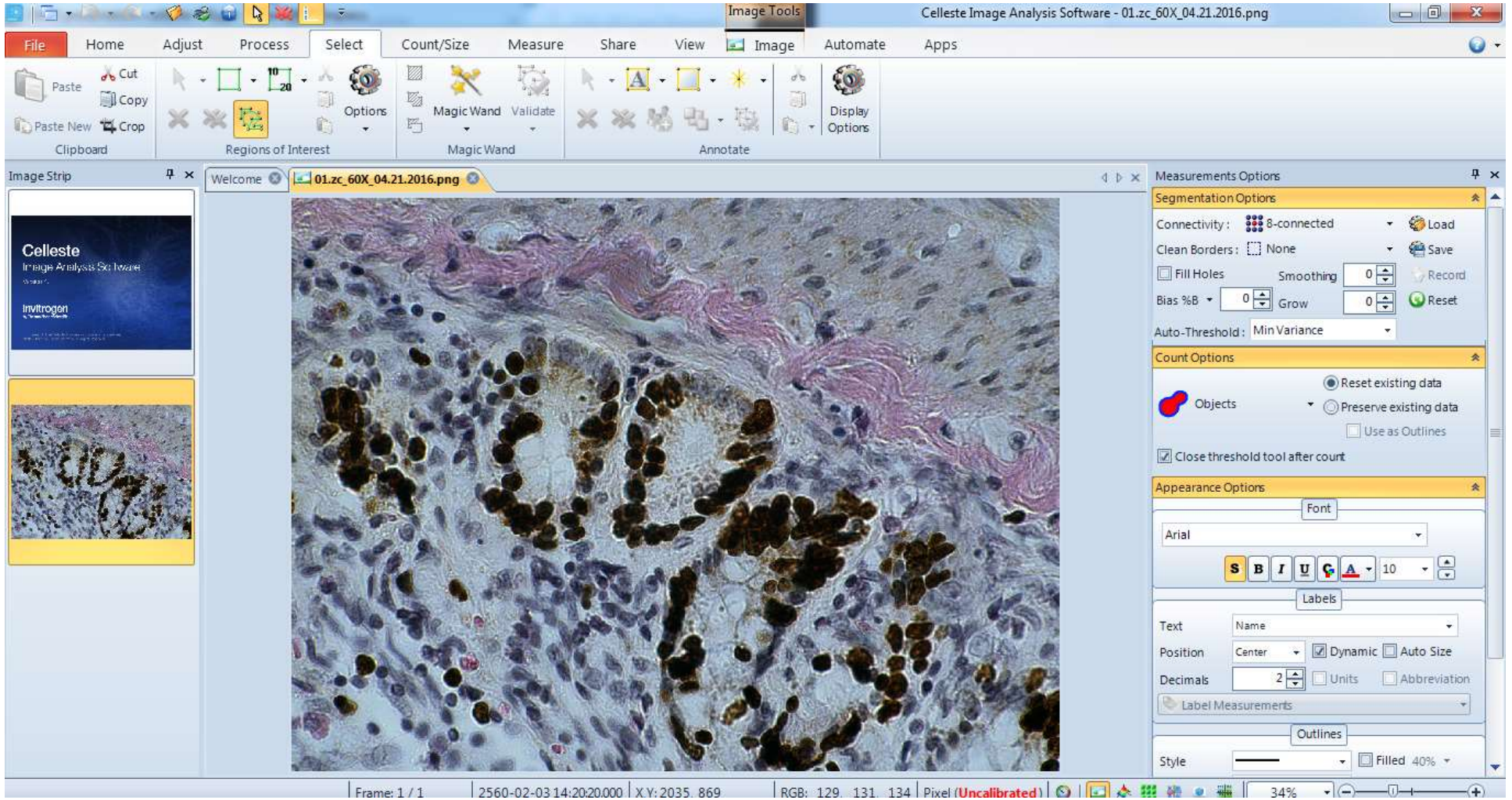


Select region in background to measure white balance correction factor

Data by: Trillium Blackmer
Celleste by: Oggie Golub

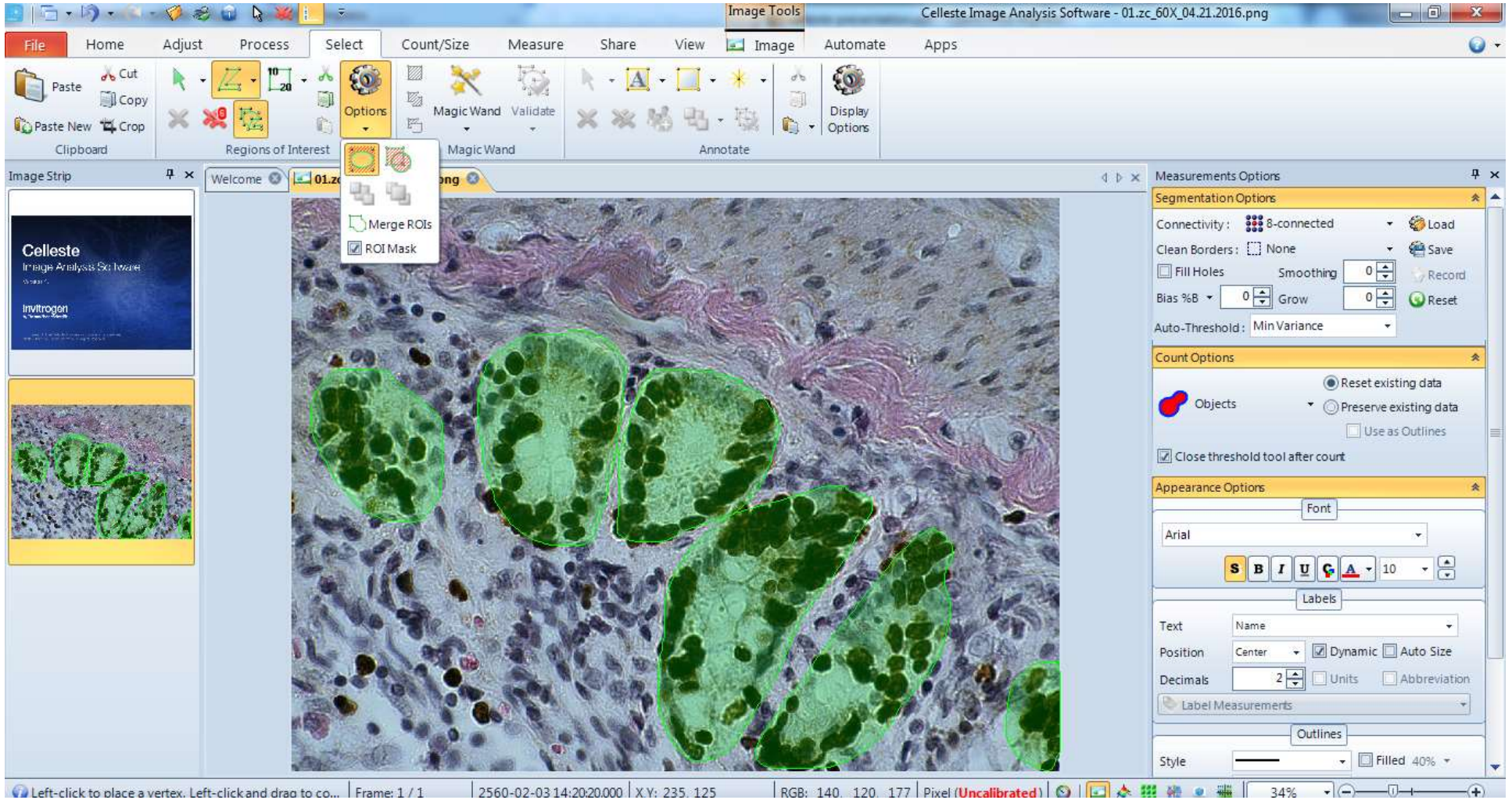
Celleste Image Analysis Software

Regions of Interest (ROI)



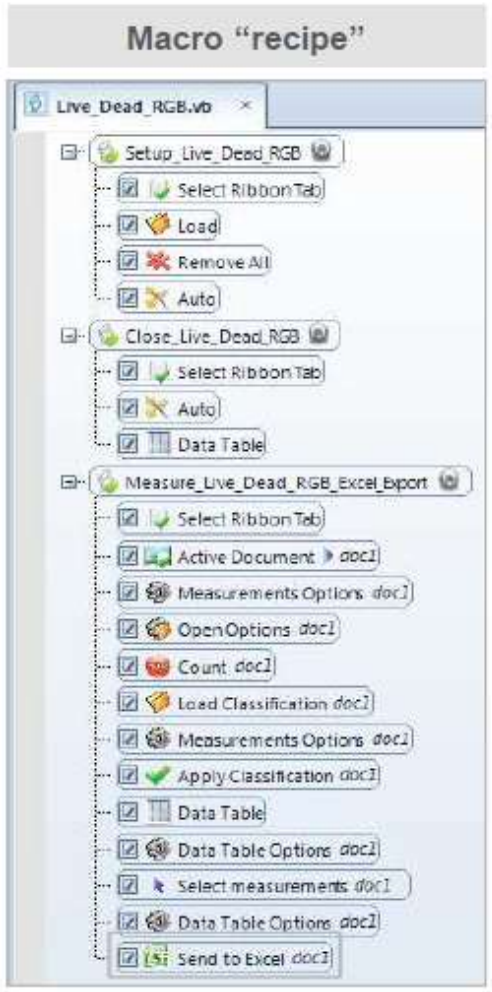
Celleste Image Analysis Software

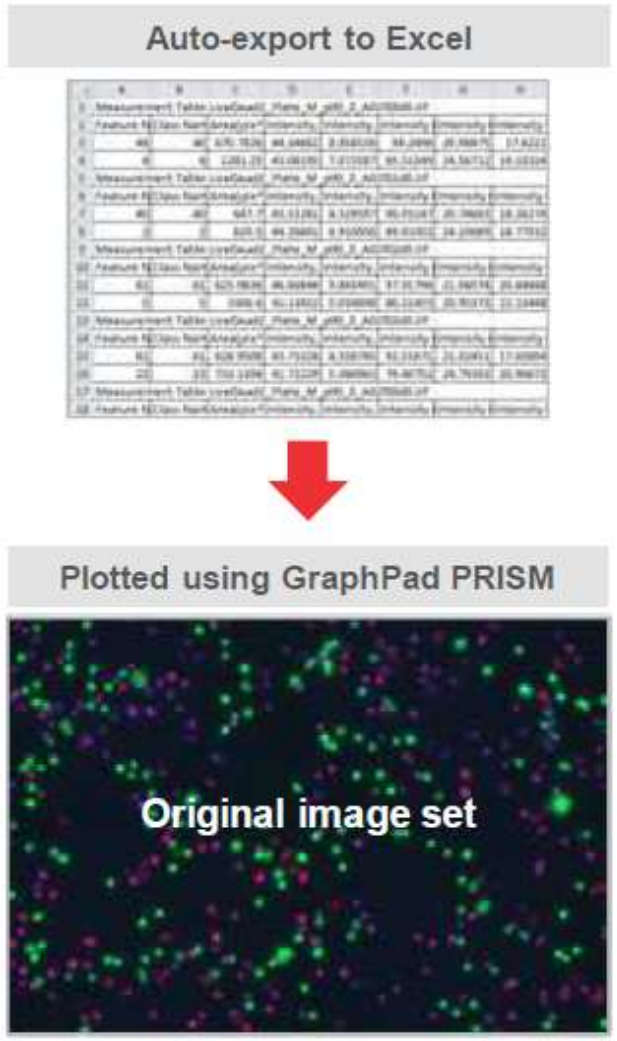
Regions of Interest (ROI)



Celleste Image Analysis Software

Easily apply macros for automated analysis in batch process mode

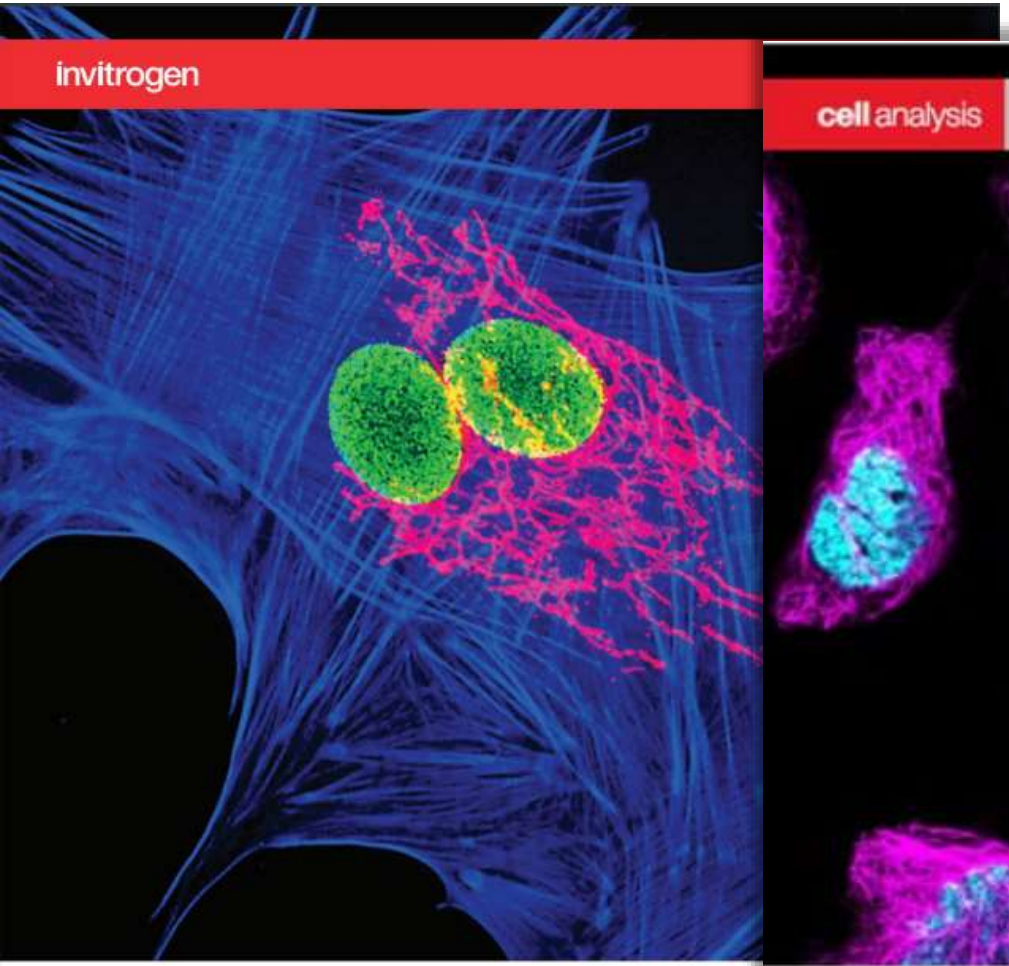




Celleste Image Analysis Software

Key capabilities:

- **Segment, count/size, classify, and analyze images**
- **Measure and analyze common assays**
- **Adjust, visualize, and share**
- **Easily generate quantitative data of commonly performed assays with flexible tools**
  - Cell counting
  - Live/dead analysis
  - Transfection/GFP expression
  - IHC
  - Intensity tracking
  - Wound healing



Fixed-cell imaging: five steps to publication-quality images

Follow this proven guide to capture the best possible fixed-cell images

5 steps to live-cell imaging

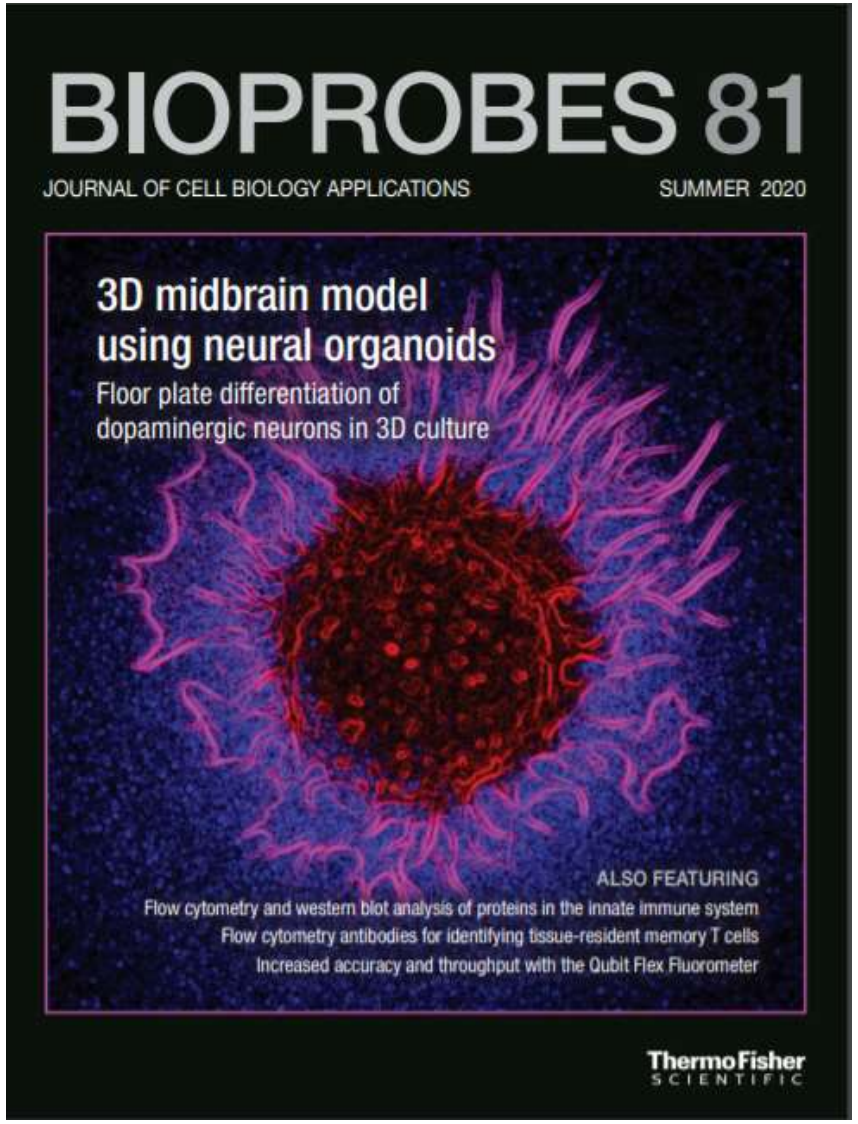
Follow this guide to capture the best possible images

ThermoFisher SCIENTIFIC

ohistochemistry: five steps lication-quality images

ThermoFisher SCIENTIFIC

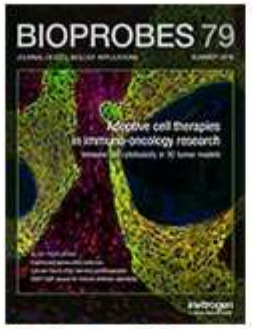
Molecular Probes Journal



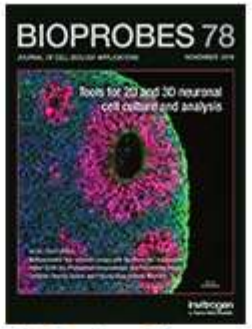
https://www.thermofisher.com/th/en/home/references/newsletters-and-journals/**bioprobes-journal-of-cell-biology-applications**.html

Back Issues

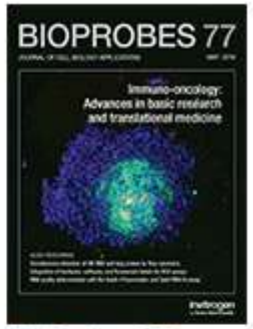
BioProbes 80



BioProbes 79



BioProbes 78



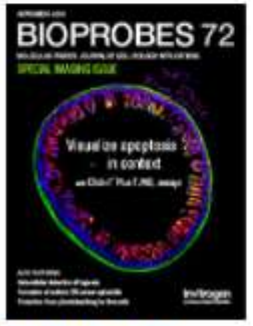
BioProbes 77

BioProbes 76

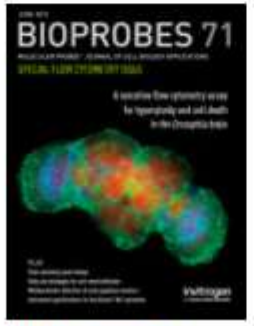
BioProbes 75

BioProbes 74

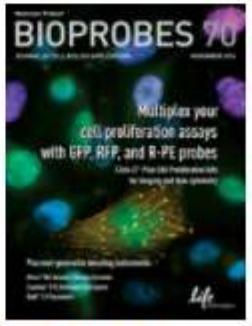
BioProbes 73



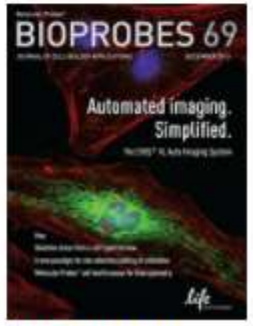
BioProbes 72



BioProbes 71



BioProbes 70



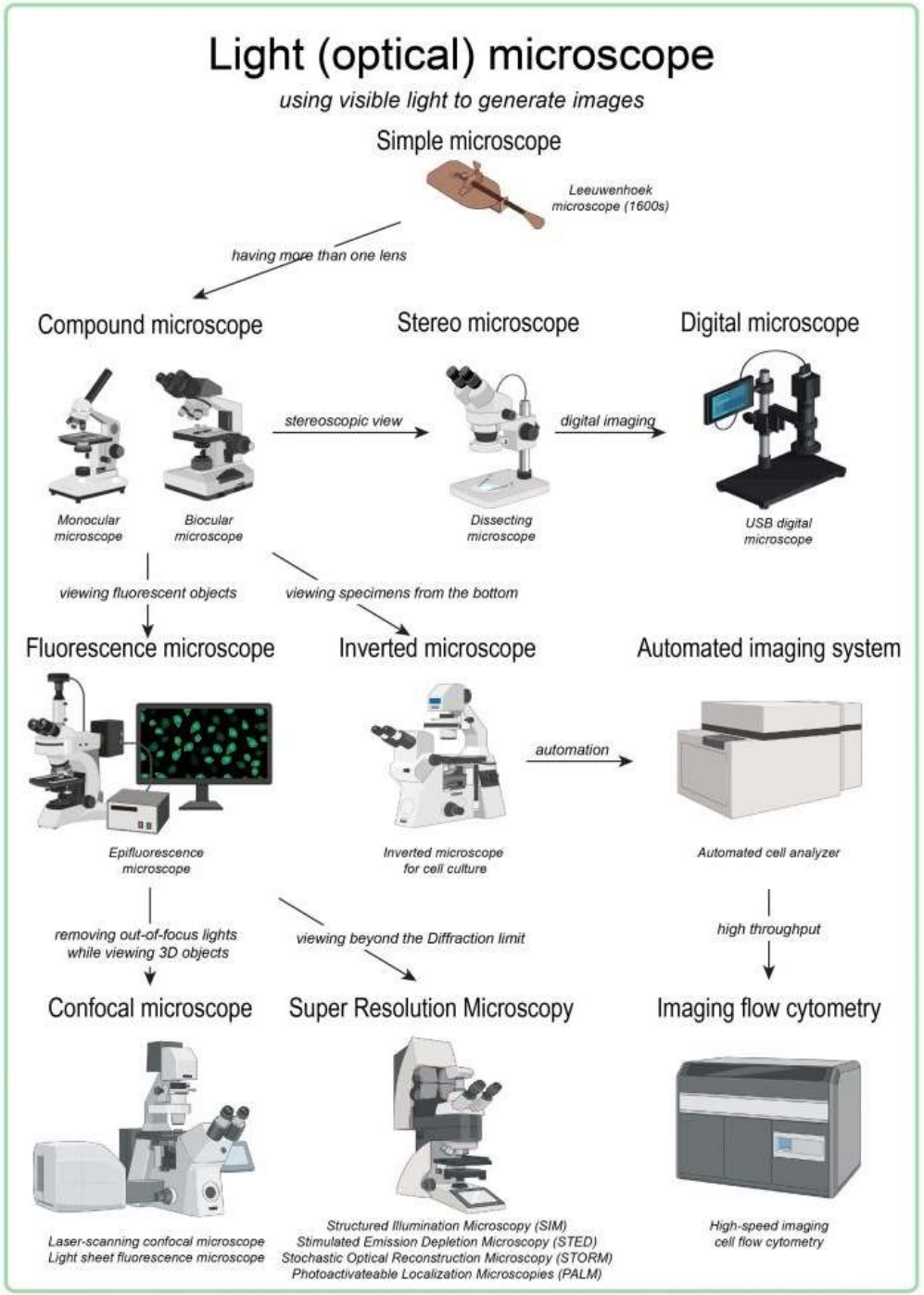
BioProbes 69

Basic Knowledge of Inverted Microscopy and Introduction of EVOS™ M7000 Fluorescent Imaging System

Chayaporn Subkamkaew
Technical Application Specialist
for Imaging and Cellular Analysis Product
Gibthai Co., Ltd.

THANK YOU

Gibthai Co. Ltd.
3N HOLDING HOUSE, 44/6 Suthisarnvinitchai Road, Samsennok, Huay Kwang, Bangkok 10310, Thailand

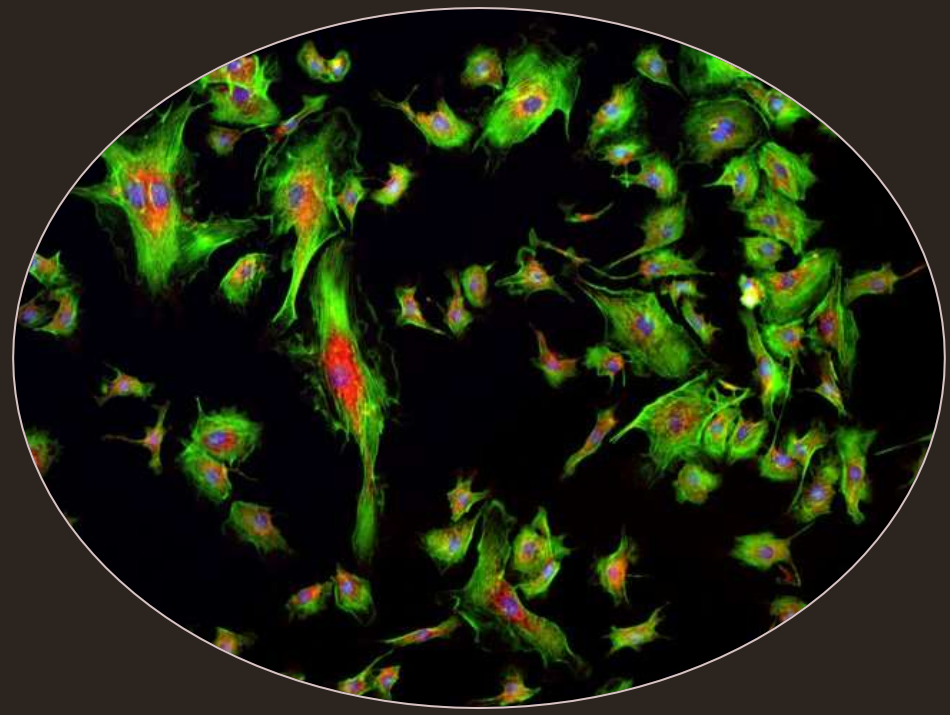


Inverted microscope for cell culture

Inverted microscope for cell culture

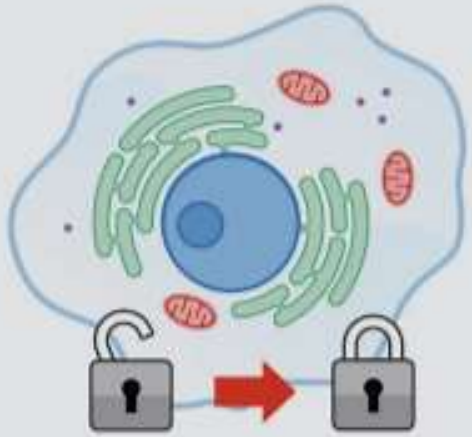
Fixed-cell imaging workflow : 5 Steps to quality image

Fixed-cell imaging workflow : 5 Steps to quality image

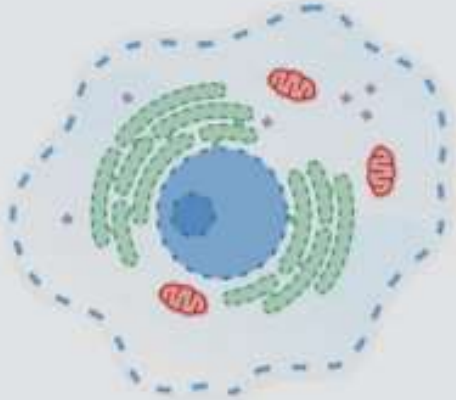


1. Fix, permeabilize, and block
2. Lebel
3. Detect
4. Protect and enhance
5. Image

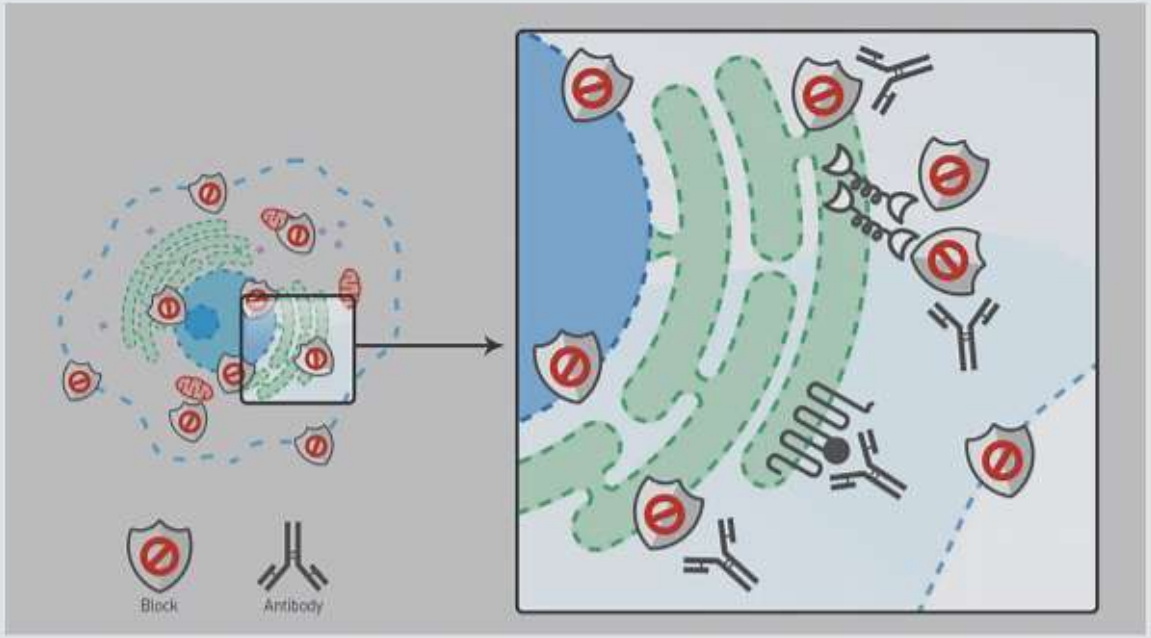
1 Fix, permeabilize, and block



Fixation locks cellular structures in place.



Permeabilization removes cellular membrane lipids—enabling labeling and detection reagents to reach the interior of the cells.

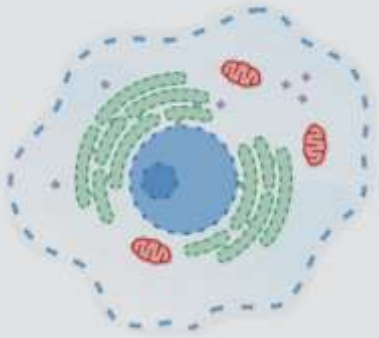


Protein-based blocking agents help reduce nonspecific staining. Antibodies are able to displace the blocking proteins to form high-affinity interactions with their epitopes, while blocking proteins prevent low-affinity antibody interactions elsewhere in the sample.

1

Fixation
Permeabilize

Fixation locks cellular structures in place.



Permeabilization removes cellular membrane lipids—enabling labeling and detection reagents to reach the interior of the cells.

Fixation solution

- Aldehydes
- Formalin
- Formaldehyde
- Paraformaldehyde
- Alcohol (Methanol)

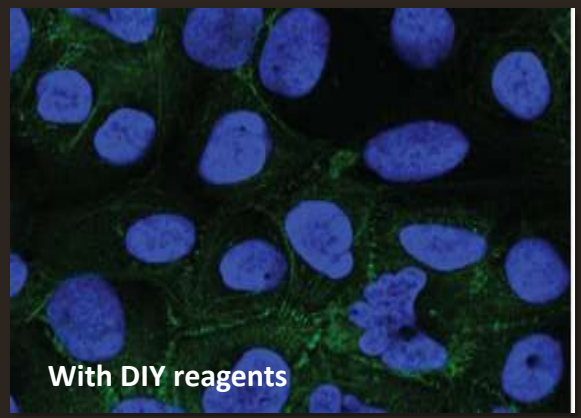
Image-iT™ Fixative Solution
(4% formaldehyde, methanol-free)

Permeabilization solution

- 0.1% Triton X-100 in PBS
- 0.1% NP-40 in PBS

Fixation and Permeabilize

Image-iT Fixation/Permeabilization Kit (R37602)

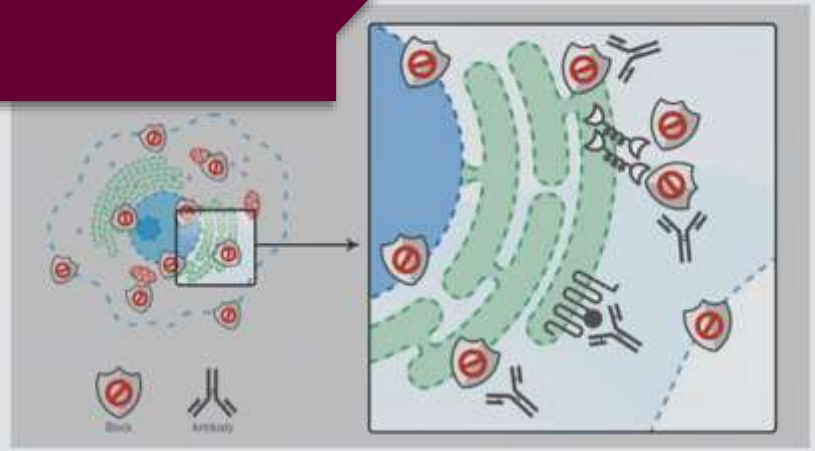


With DIY reagents

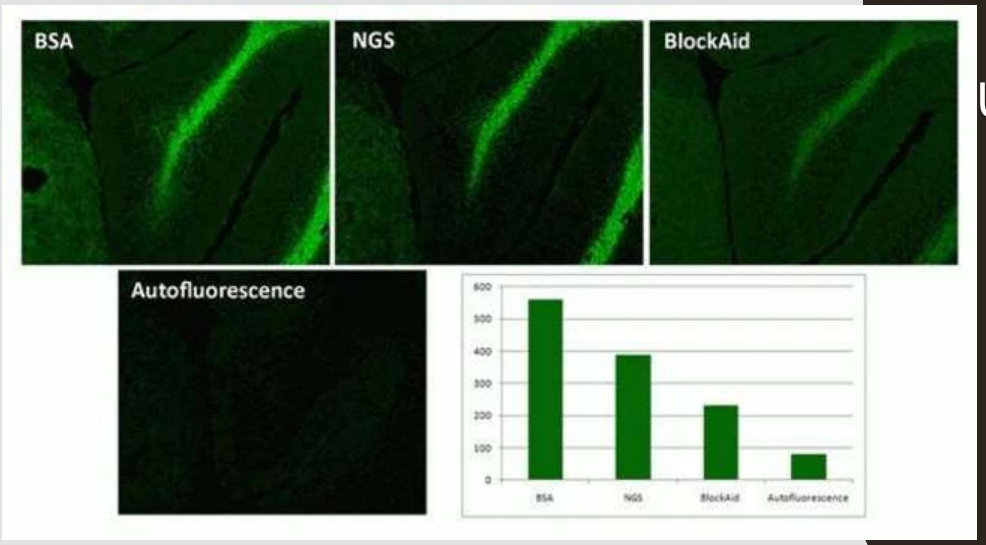
With Image-iT®
Fixation/Permeabilization Kit

1

Blocking



Protein-based blocking agents help reduce nonspecific staining. Antibodies are able to displace the blocking proteins to form high-affinity interactions with their epitopes, while blocking proteins prevent low-affinity antibody interactions elsewhere in the sample.



Non-specific Blocker Selection

Using 2nd antibodies from multiple host species

Use :

- 2-3% Fluorescence grade, BSA in PBS
- Dilute Blocker™ BSA (10%) in PBS Catalog number: 37525, to 3 times with PBS to make ~3% BSA solution.

Use : BlockAid™ Blocking Solution, Catalog number: B10710

Using 2nd antibodies from same host species

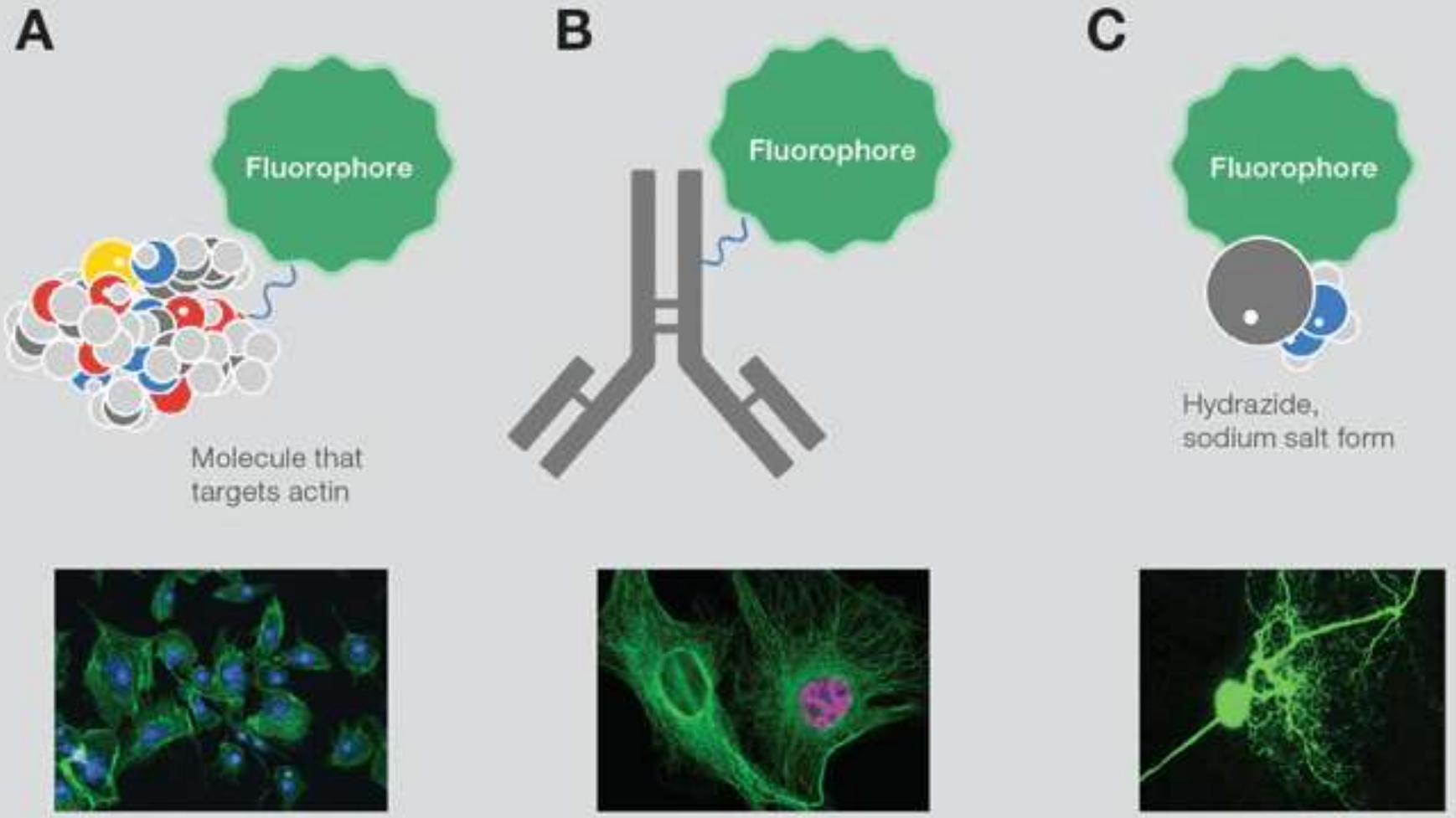
It is highly recommended that for best blocking, use the blocker from same or similar specie as the host of secondary antibodies

2nd AB from Goat:	ReadyProbes™ 2.5% Normal Goat Serum (1X) Catalog number: R37624
2nd AB from Horse or Donkey:	Ready Probes™ 2.5% Normal Horse Serum (1X) Catalog No: R37625
2nd AB from Chicken:	Ready Probes™ 2.5% Normal Chicken Serum (1X) Catalog No: R37626
2nd AB from Mouse:	Normal Mouse Serum (Dilute 1:20 to make 5%)Catalog # 31880
2nd AB from Rat:	Normal Rat Serum (Dilute 1:20 to make 5%)Catalog # 31888
2nd AB from Rabbit:	Normal rabbit Serum (Dilute 1:20 to make 5%) Catalog # 01-6101

2

Lebel

Label—target cell structures and proteins with selective dyes and primary antibodies



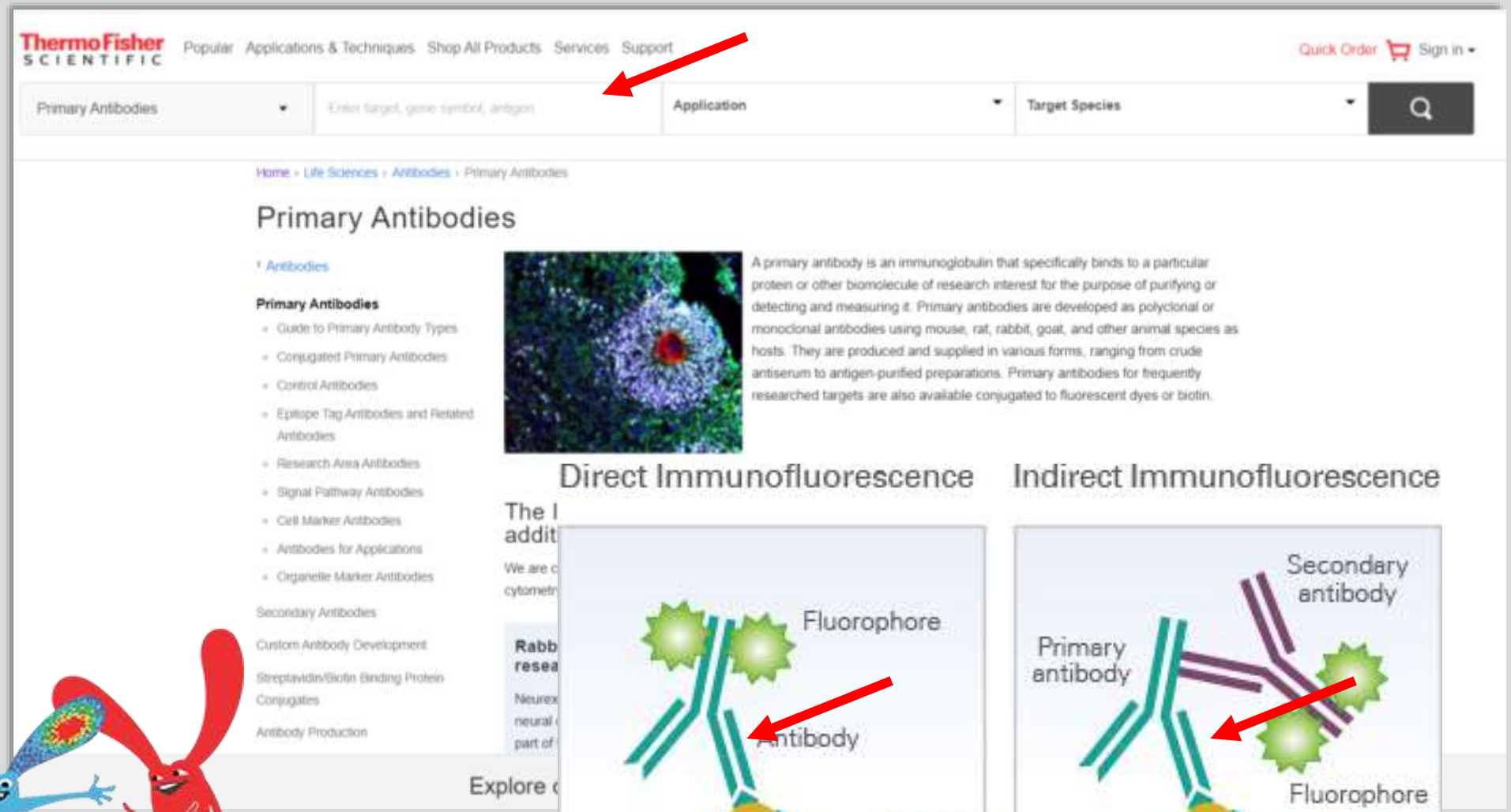
A single fluorophore can be modified to carry out any number of labeling jobs, including functionalized forms for labeling cell structure components such as (A) actin, (B) tubulin, and (C) salt forms for whole-cell staining.

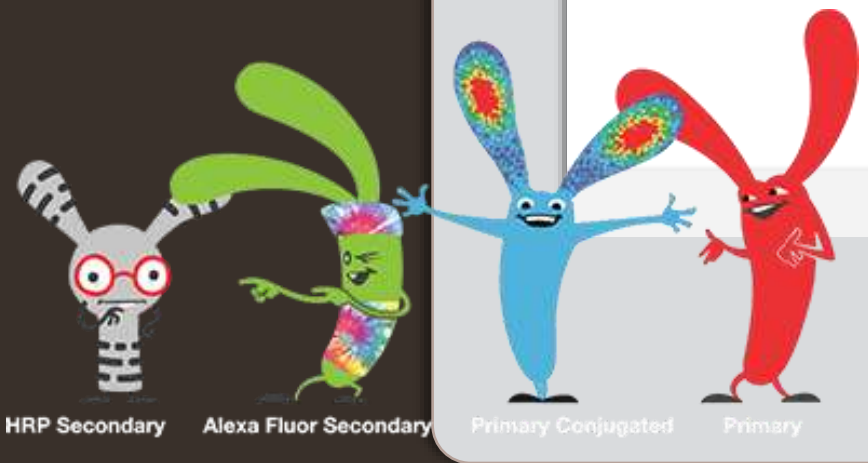
2

Lebel

Primary Antibodies

Label—target cell structures and proteins with selective dyes and primary antibodies



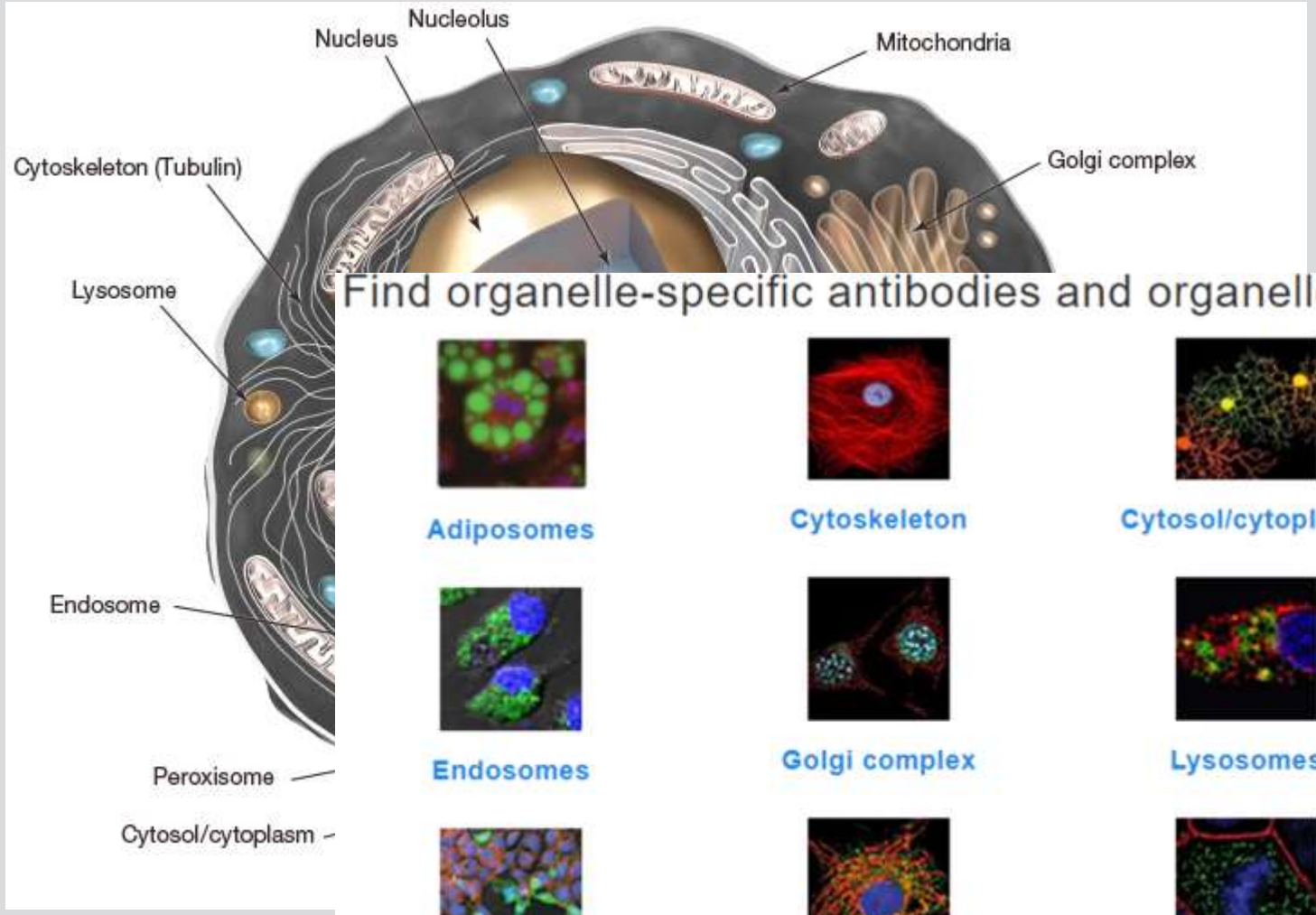


2

Lebel

Labeling or staining dyes

Label—target cell structures and proteins with selective dyes and primary antibodies



2

Lebel

Labeling or staining dyes

Label—target cell structures proteins with selective dyes

- **Nucleus :**
  - DAPI (4',6-Diamidino-2-Phenylin
  - **NucBlue Fixed Cell ReadyPro**

ReadyProbes reagents | CellLight reagents | Nuclear dyes, live cells | Nuclear dyes, fixed cells | Nucleoli stains | HCS

	NucBlue Live ReadyProbes Reagent	NucRed Live 647 ReadyProbes Reagent	NucBlue Fixed Cell ReadyProbes Reagent
Readout	Fluorescent staining of nucleic acids		
Target	Membrane-permeable dyes targeting RNA and DNA		Membrane impermeable dye targeting RNA and DNA
Common filter set	DAPI	Cy5	DAPI
Labels	Hoechst 33342	NucRed Live	DAPI
Ex/Em (nm)	360/460	638/686	360/460
Signal-to-noise ratio	■■■□□	■■■■□	■■■■□
Photostability	■■■■□	■■■■□	■■■■□
Multiplexing	Yes	Yes	Yes
Live cells	Yes	Yes	No
Fixed cells	No	No	Yes
Fixable	Yes	No	No
Platform	Imaging	Imaging	Imaging
Protocol	Microscopy protocol	Microscopy protocol	Microscopy protocol
Format	6 x dropper bottles	6 x dropper bottles	6 x dropper bottles
Cat. No.	R37605	R37106	R37606

2

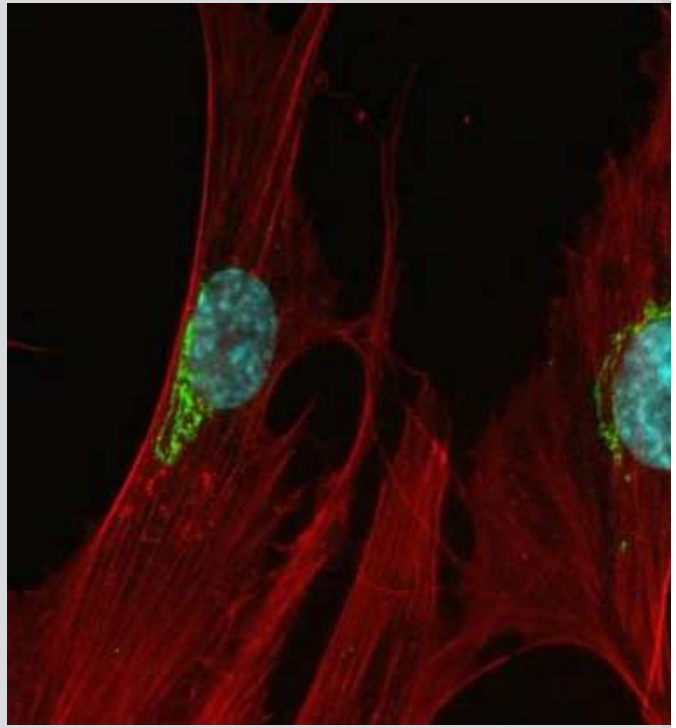
Lebel

Labeling or staining dyes

Label—target cell structure… proteins with selective dye…

- **Cytoskeleton :**

- Alexa Fluor Plus 555 Phalloidi…
- **Alexa Fluor 594 Phalloidin (A…**



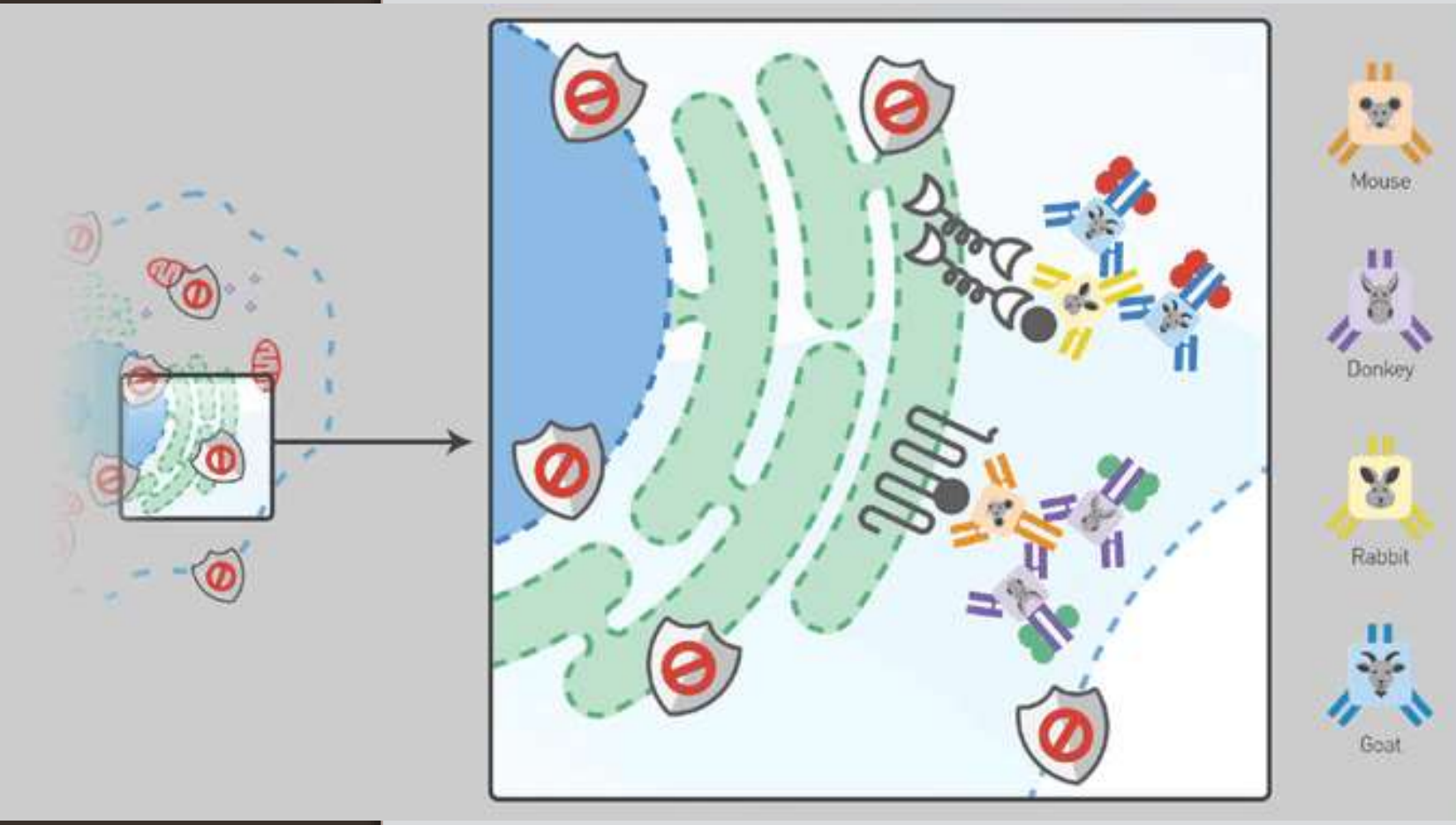
Cells were also stained with Alexa Fluor® 594 phalloidi…
Fixed (R37606) to label nuclei. Finally, cells were mour…

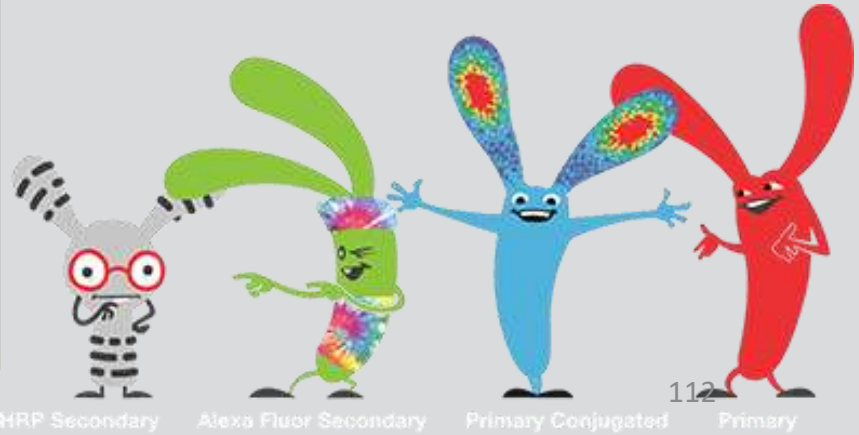
	Alexa Flour 568 Phalloidin	Alexa Fluor 594 Phalloidin	Alexa Fluor Plus 647 Phalloidin	Alexa Fluor 680 Phalloidin	Alexa Fluor Plus 750 Phalloidin
Readout	High-performance fluorescent label with stable signal, resistant to photobleaching				
Range	F-actin				
Common filter set	Texas Red	Texas Red	Cy5	Cy5.5	Cy7
Labels	Alexa Fluor 568	Alexa Fluor 594	Alexa Fluor Plus 647	Alexa Fluor 680	Alexa Fluor 750
Ex/Em (nm)	578/600	590/617	650/665	679/702	758/784
Signal-to-noise ratio	■■■■■	■■■■■■	■■■■■■	■■■■■■	■■■■■
Photostability	■■■■■	■■■■■■	■■■■■■	■■■■■■	■■■■
Bibliography	Citations				
Multiplexing	Yes	Yes	Yes	Yes	Yes
Live cells	No	No	No	No	No
Fixed cells	Yes	Yes	Yes	Yes	Yes
Fixable	Yes	Yes	Yes	Yes	Yes
Platform	Imaging	Imaging	Imaging	Imaging	Imaging
Format	300 units	300 units	300 units	300 units	300 units
Cat. No.	A12380	A12381	A30107	A22286	A30105

3

Detect

Detect—Fine-tune the fluorescence signal by using fluorophores and methods optimal for target abundance



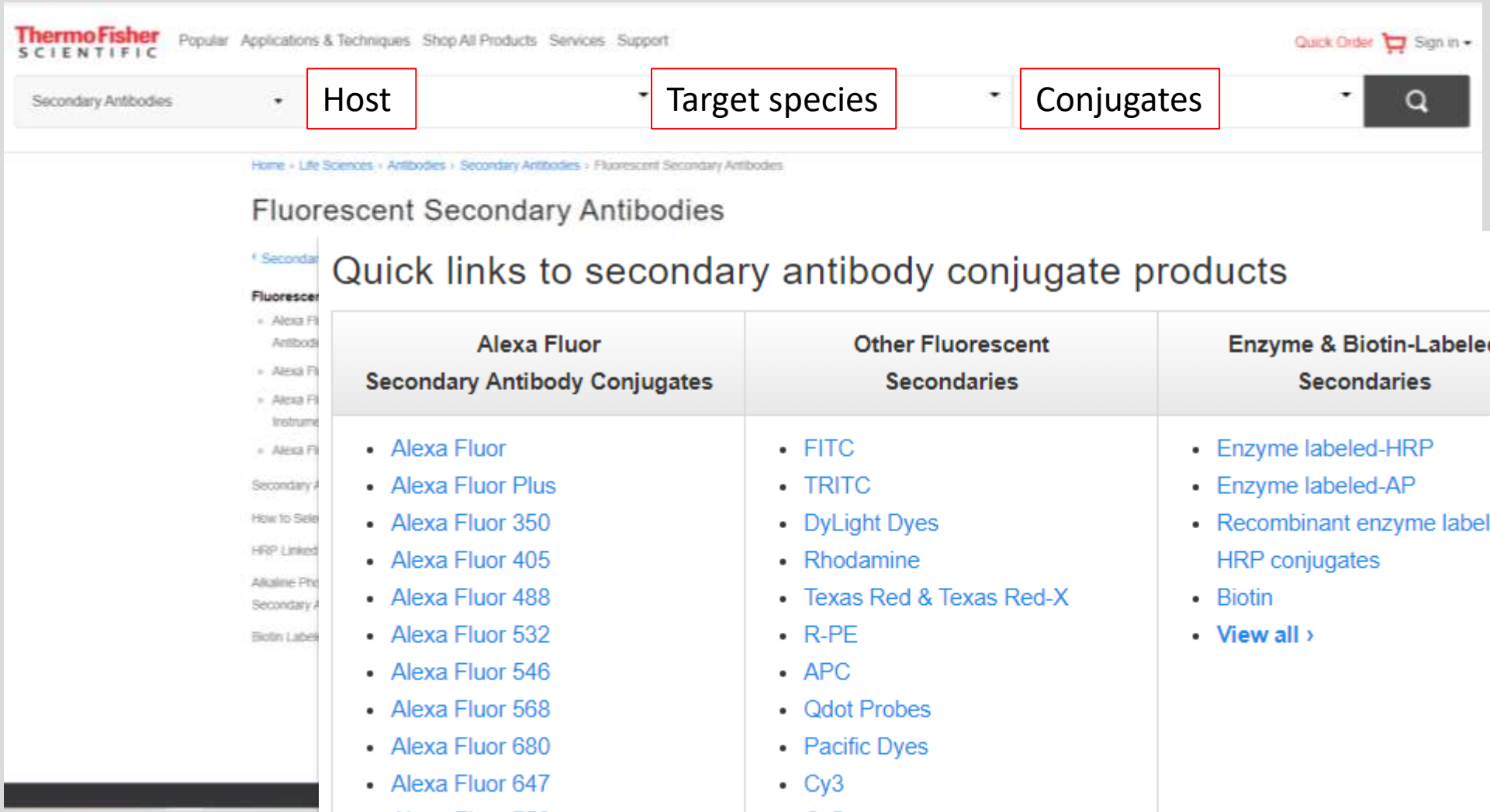


3

Detect

Secondary Antibodies

Detect—Fine-tune the fluorescence signal
by using fluorophores and methods optimal for target abundance



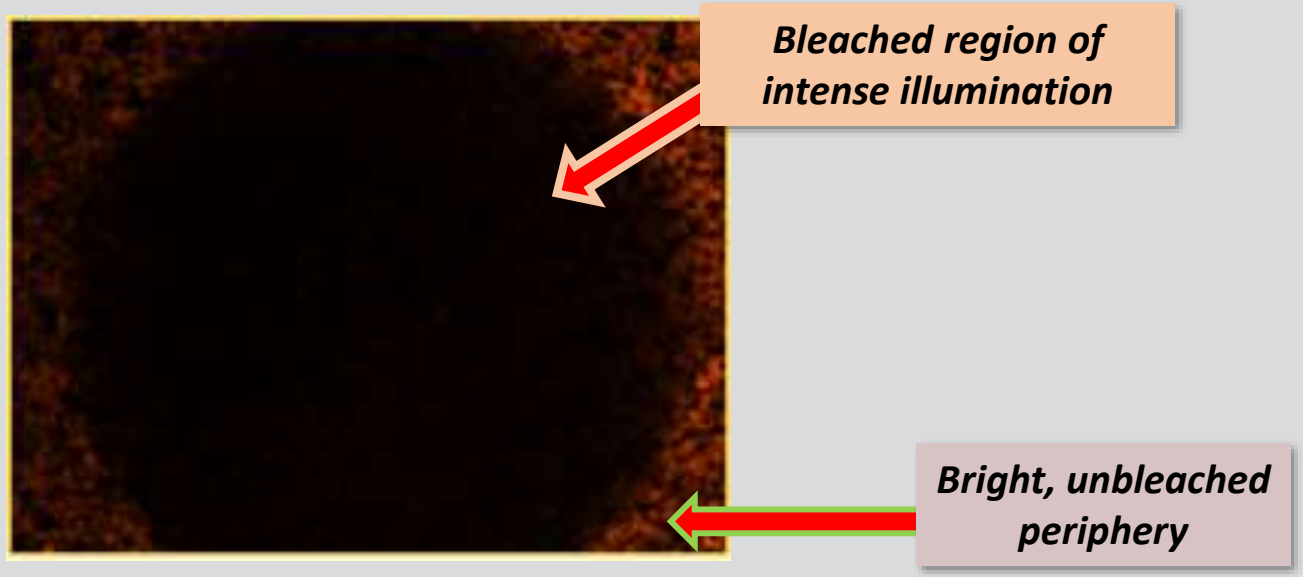
4

Protect and enhance

Protect—Protect the signal from photobleaching and enhance the image quality.

Under the high intensity illumination of a microscope, fluorescent dyes are prone to

photobleaching – irreversible fading of signal

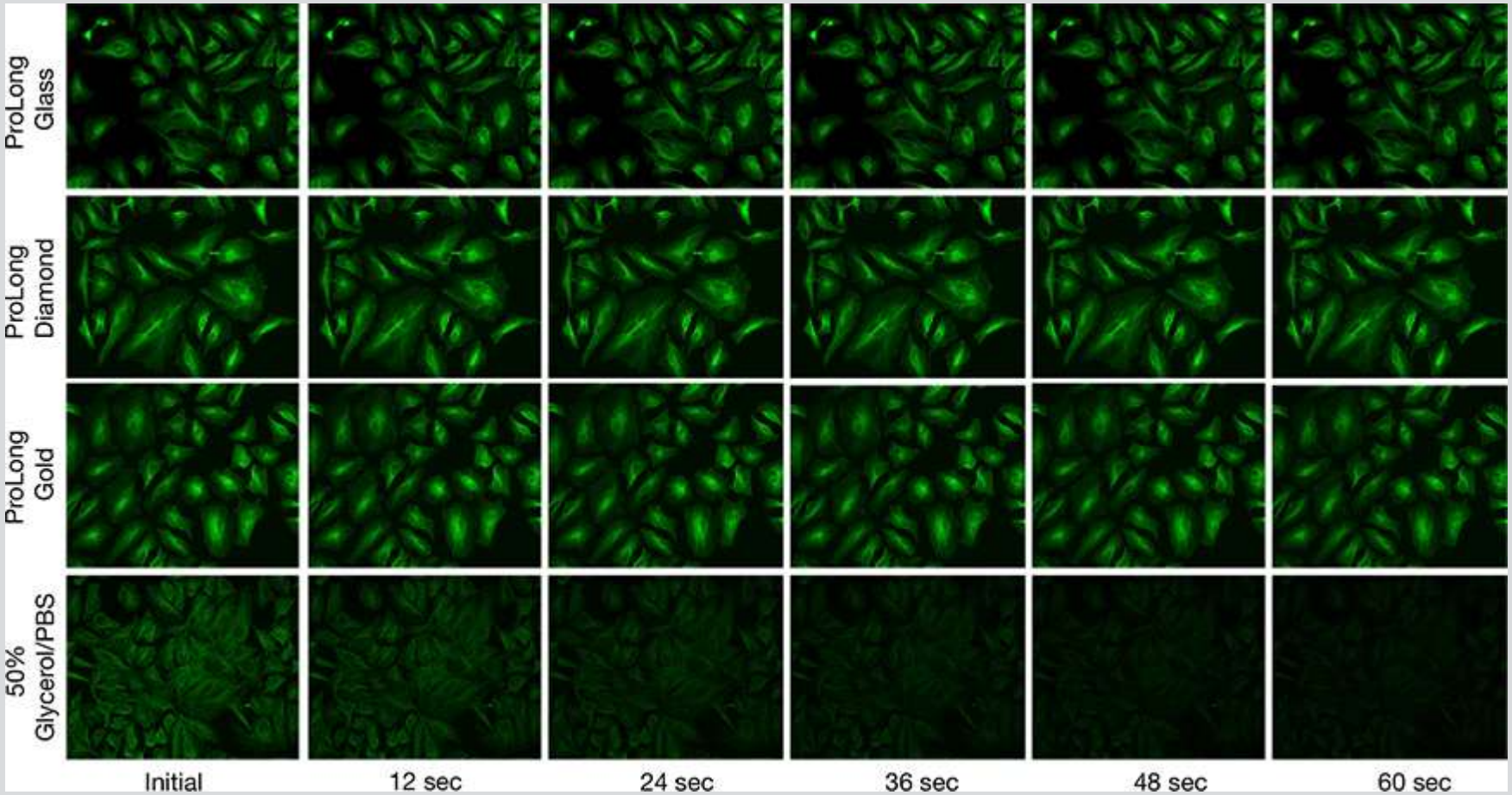


4

Protect and enhance

Antifade Mounting media

Protect—Protect the signal from photobleaching and enhance the image quality.

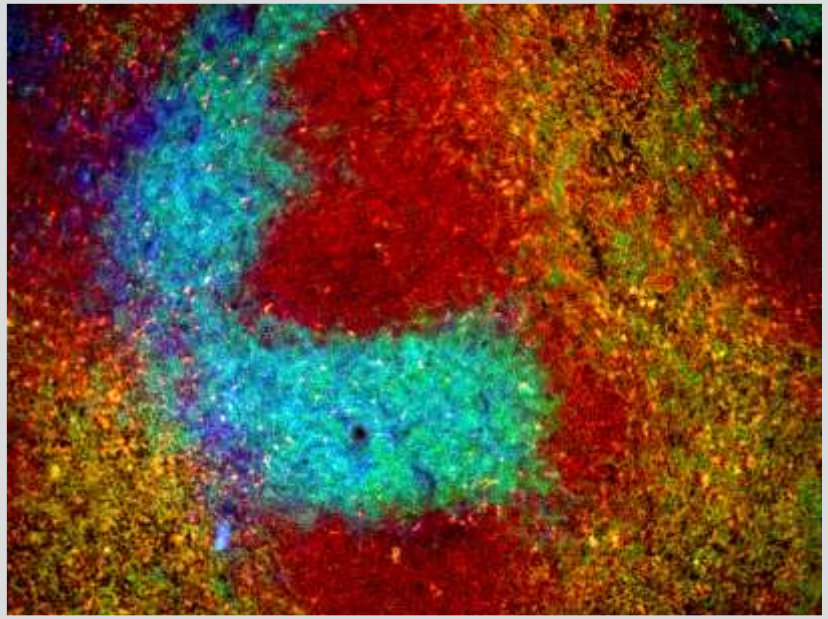


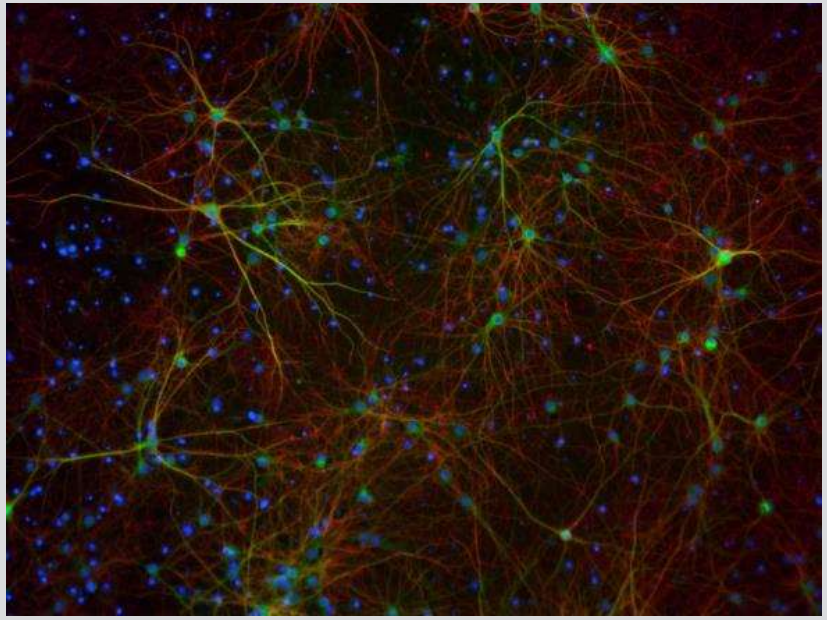
Protect the signal from photobleaching and enhance the image quality with the best resolution. Fluorophores are ideal for high-quality cell imaging but are inevitably prone to photobleaching, which is a photochemical degradation or fading of fluorescence signals. Antifade mountants are designed to protect the photostability of fluorescently labeled proteins and maintain image integrity from several weeks to months.

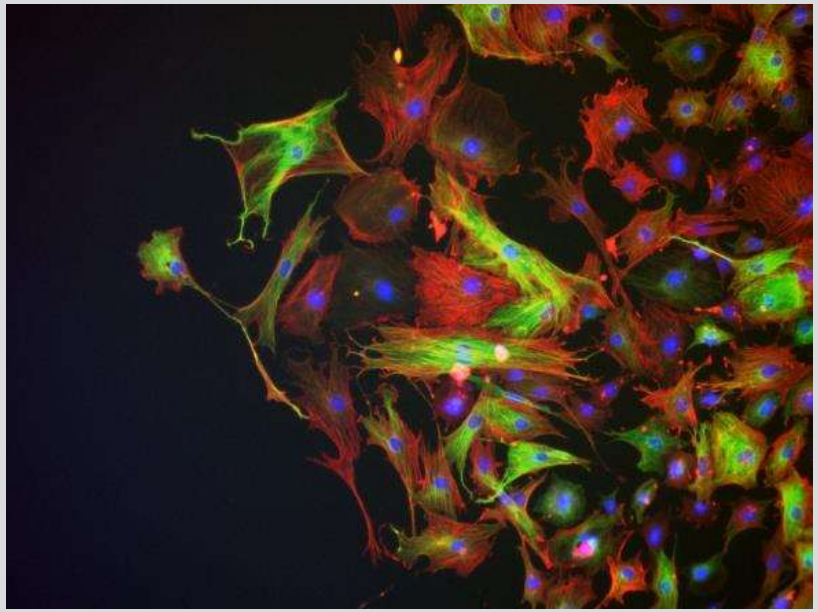
5

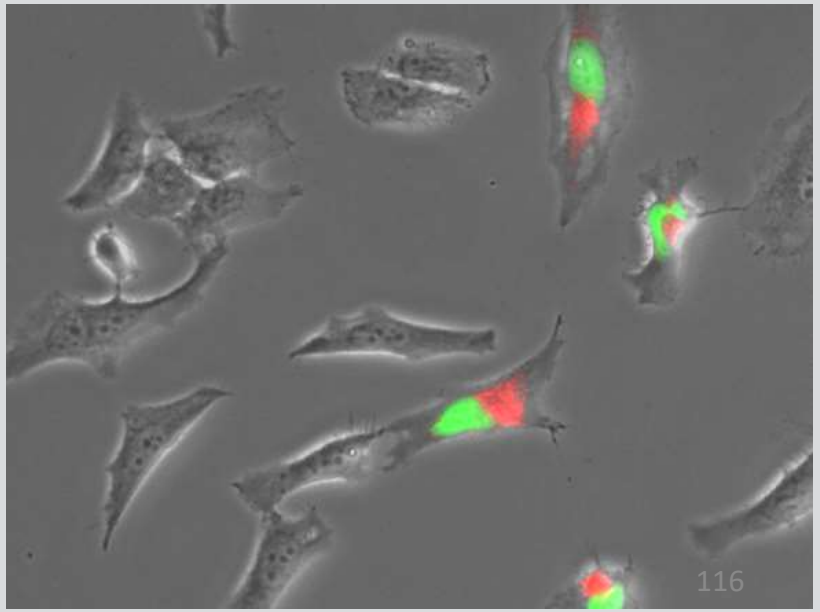
Image

Image— capture imaging discoveries with maximum clarity and definition









5

Image

Image— capture imaging discoveries with maximum clarity and definition

To this……

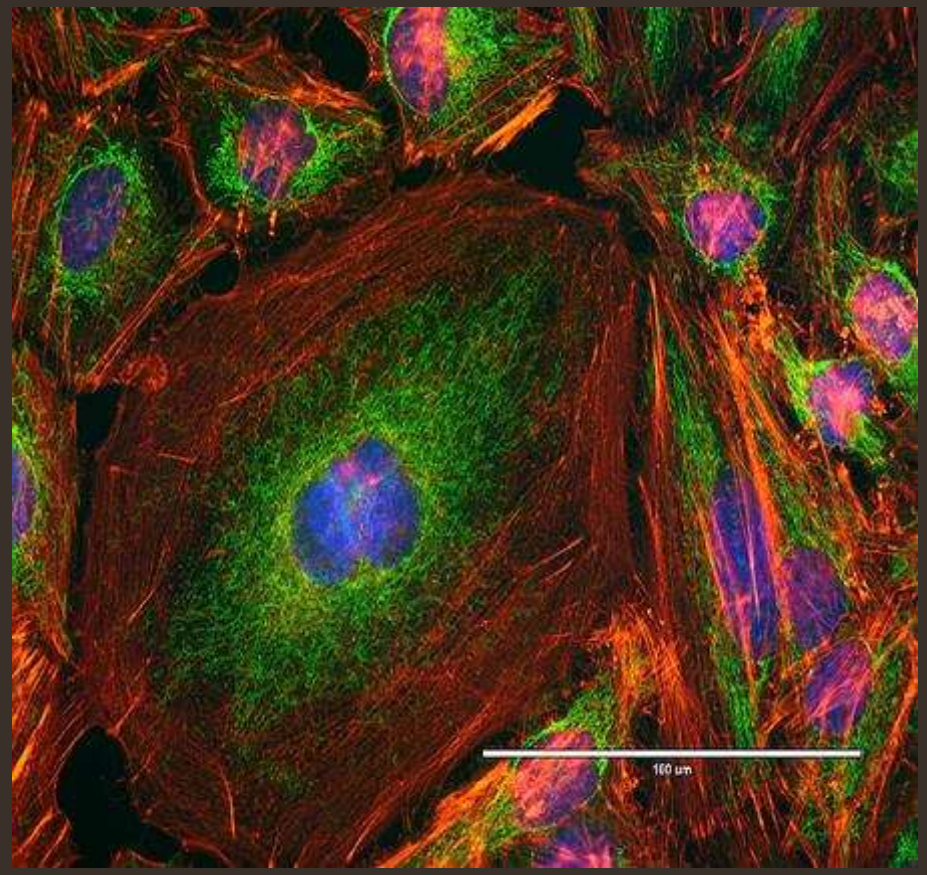
EVOS M5000

Go from all this……

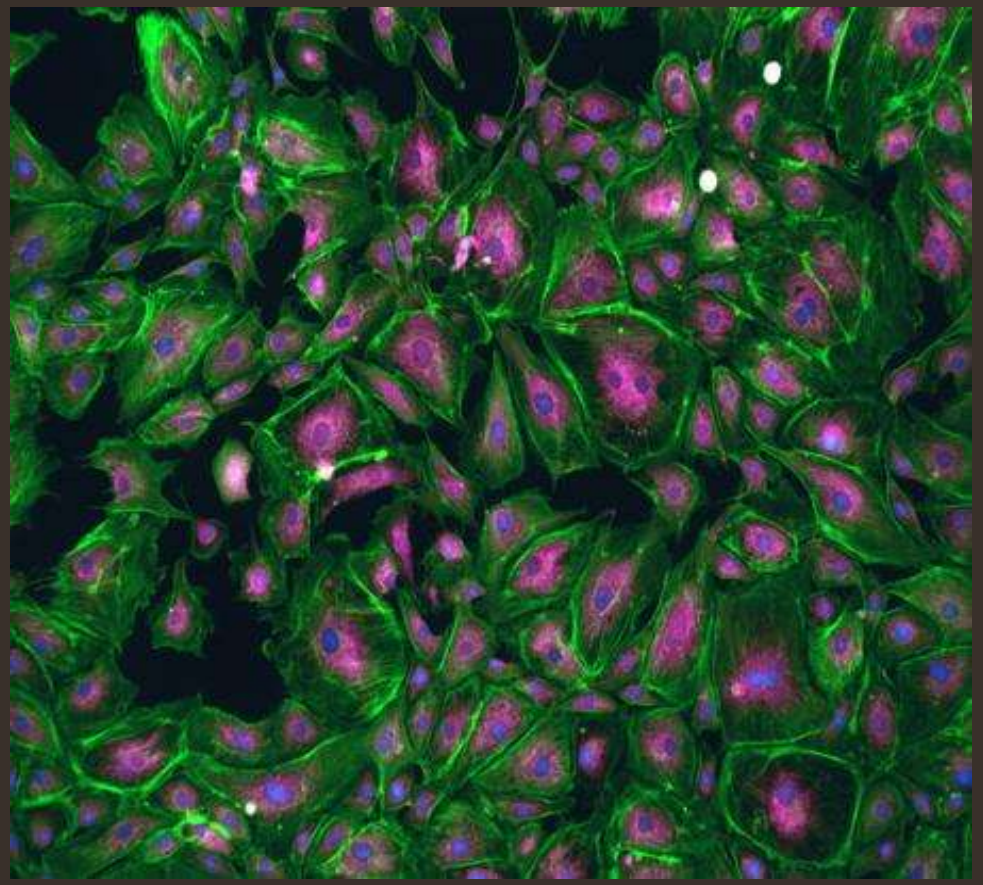
‘Typical’ epifluorescence system

EVOS M7000

Examples of Image-based Contextual information



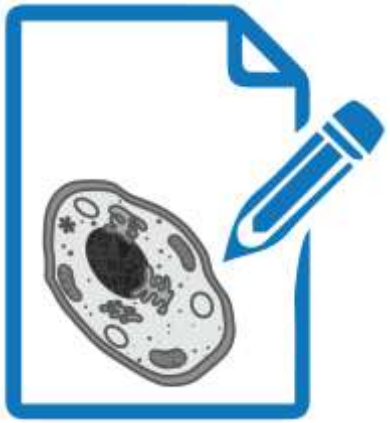
1. Image-iT® Fixation/Permeabilization Kit/ BlockAid
2. Primary antibody to mitochondria,
   NucBlue Cell Stain (**Blue**)
   ReadyProbes ActinRed 555 (**Red**)
3. FITC Secondary Antibody (**Green**)
4. Mounted with Prolong Antifade Reagent
5. Detected – EVOS Imaging System



1. Image-iT® Fixation/Permeabilization Kit/ BlockAid
2. Primary antibody to mitochondria
   NucBlue Cell Stain (**Blue**)
   ReadyProbes ActinGreen 488 (**Green**)
3., Alexa Fluor 750 Secondary Antibody (**Purple**)
4. Mounted with ProLong Antifade Reagent
5. Detected –EVOS Imaging System

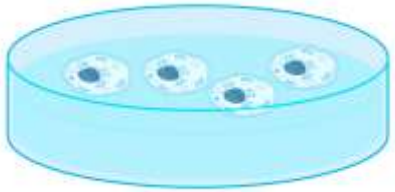
Five Steps to Live-Cell Imaging

Step 1: Plan



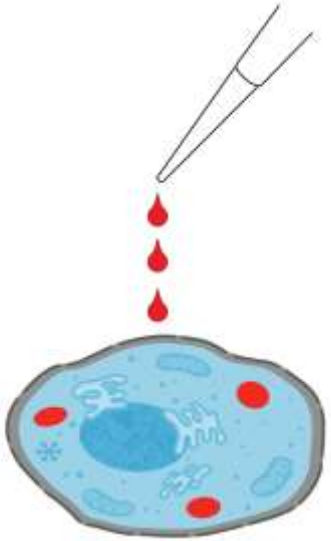
Plan—design your experiment carefully considering the tools and resources needed for each step

Step 2: Culture



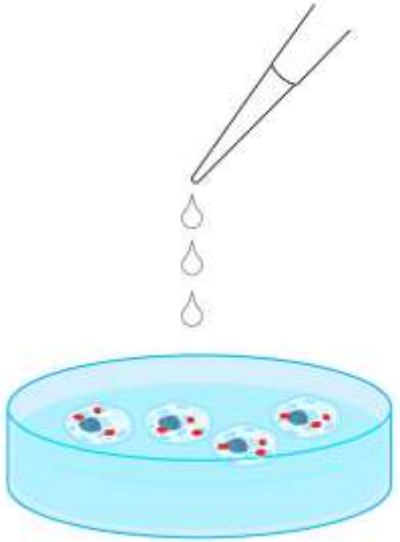
Culture—maintain or grow your cells under optimum conditions

Step 3: Label



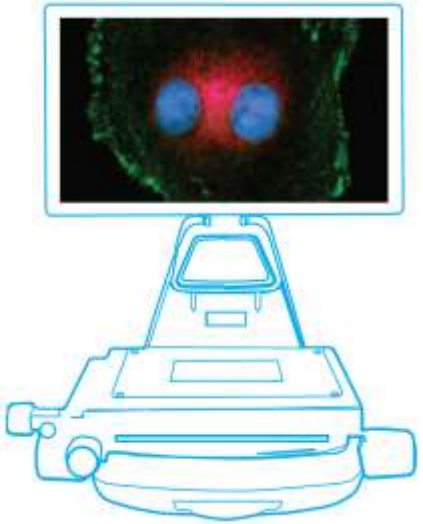
Label—target cell structures and functions with selective dyes and stains

Step 4: Optimize



Optimize—minimize background and maintain photo-stability of fluorescence signals

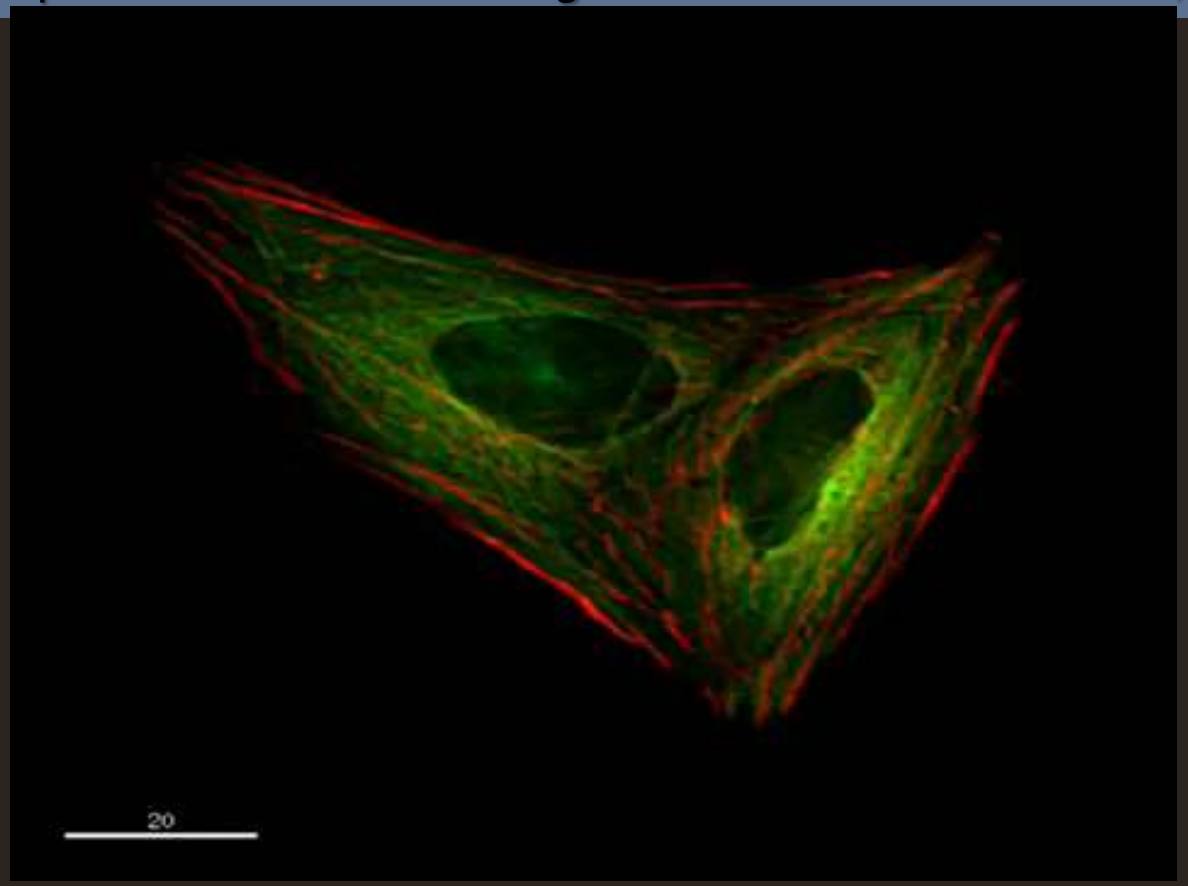
Step 5: Image

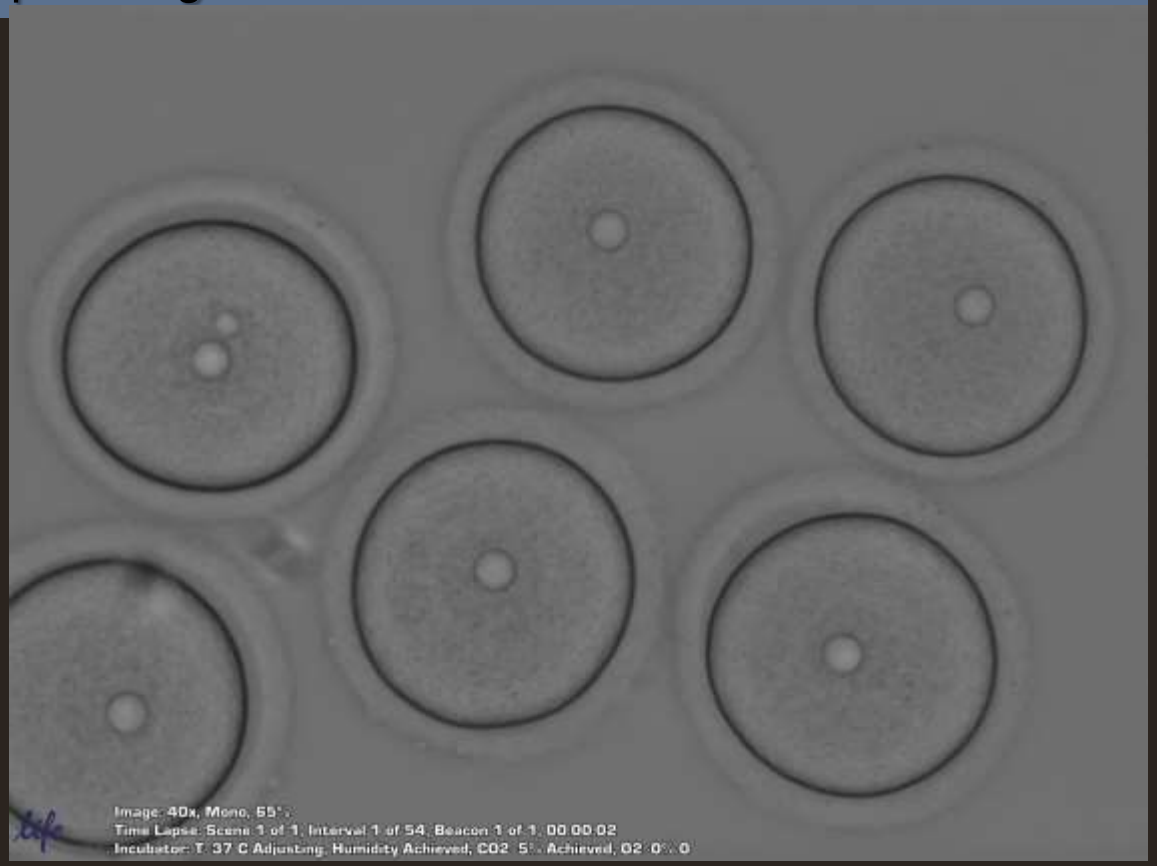


Image—capture discoveries as they happen with the highest reproducibility and definition

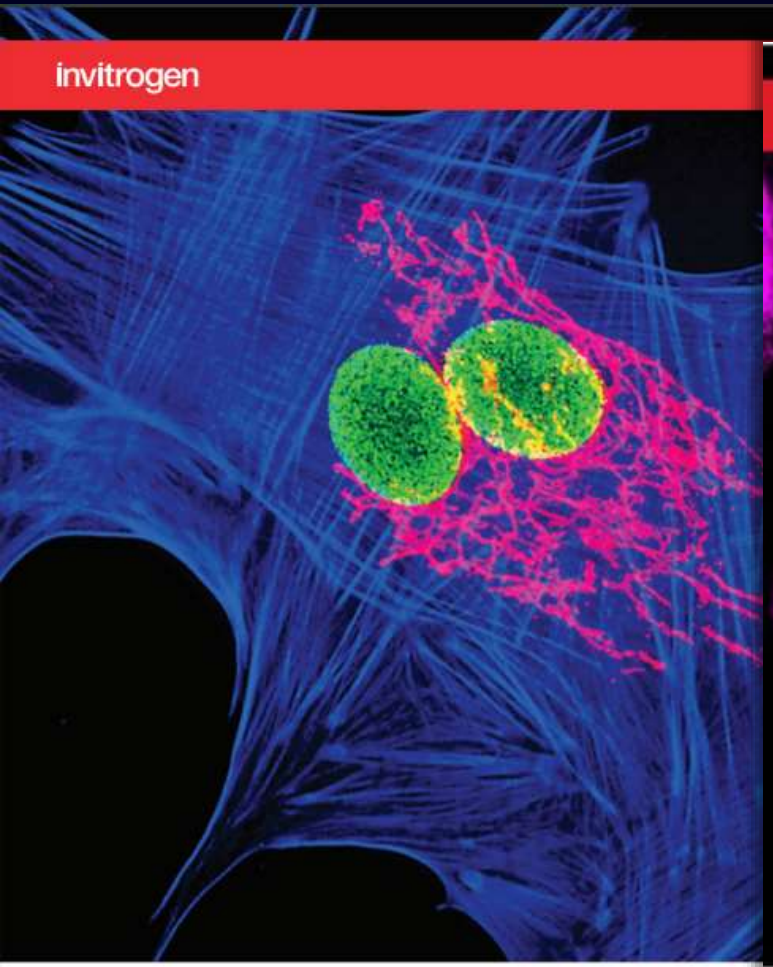
Five Steps to Live-Cell Imaging

Optimized live-cell staining will result in healthier cells, sharper images and better data





invitrogen



Fixed-cell imaging: five steps to publication-quality images

Follow this proven guide to capture the best possible fixed-cell images

cell analysis

thermo scientific invitrogen gibco

5 steps to live-cell imaging

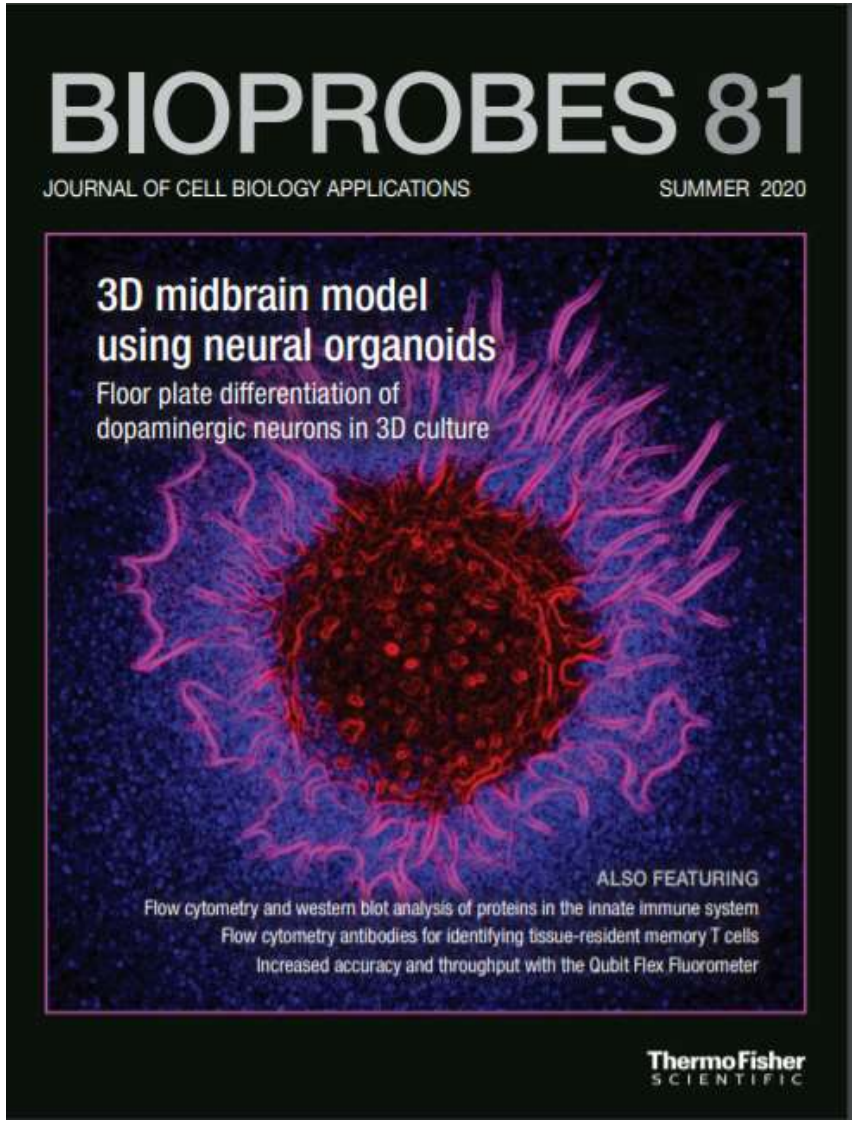
Follow this guide to capture the best possible images

ThermoFisher SCIENTIFIC

ohistochemistry: five steps lication-quality images

ThermoFisher SCIENTIFIC

Molecular Probes Journal



https://www.thermofisher.com/th/en/home/references/newsletters-and-journals/**bioprobes-journal-of-cell-biology-applications**.html

Back Issues

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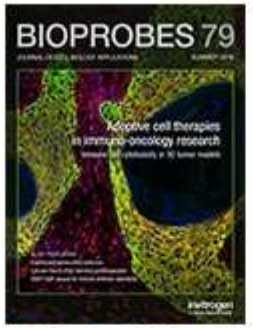

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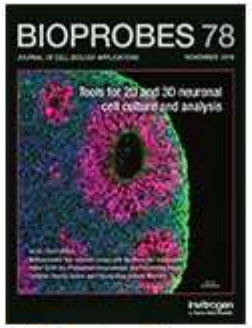

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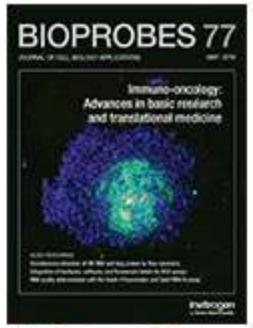

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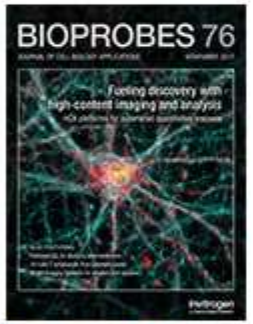

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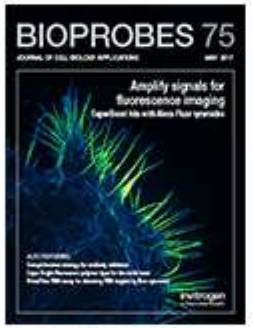

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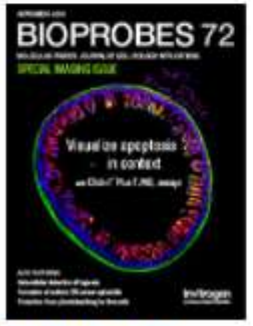

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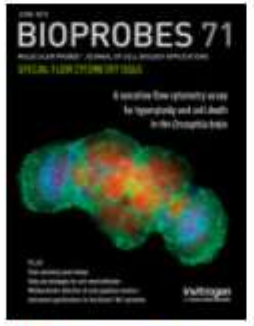

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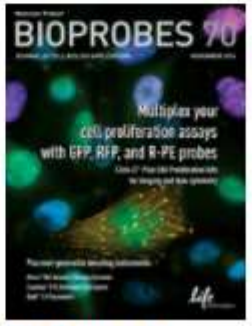

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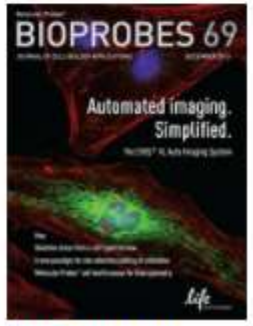

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