



gibthai
A 3N HOLDING COMPANY

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.



Topics

1

Type of Microscopy

2

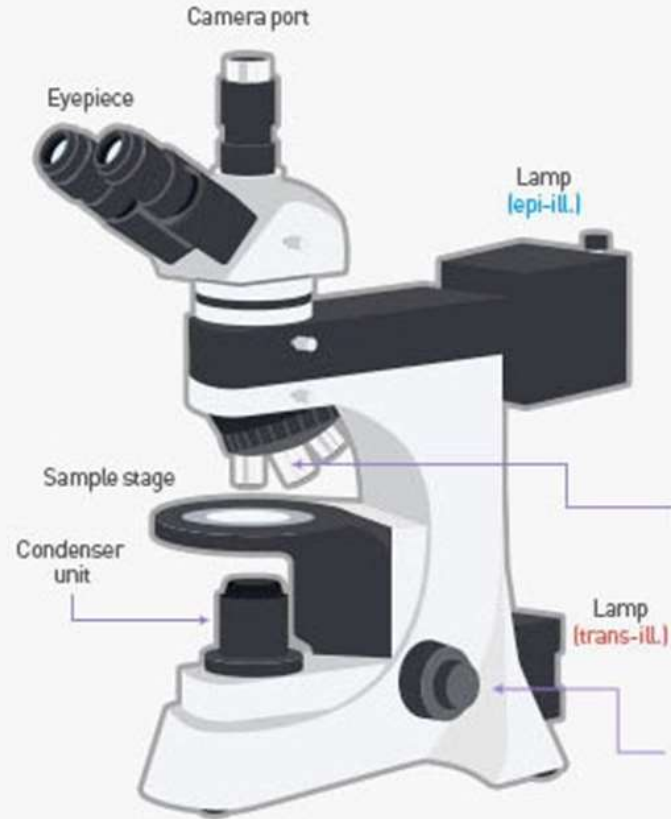
Principle of Fluorescent Microscopy

3

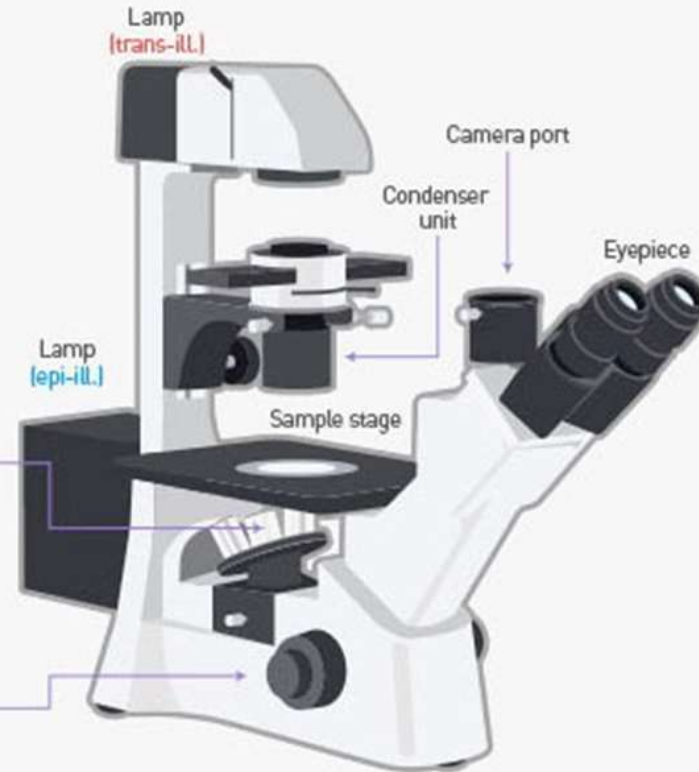
EVOS M7000 Imaging System

Why we use microscope ?

Upright microscope



Inverted microscope



Size and Scale

1 μm

10 μm

100 μm

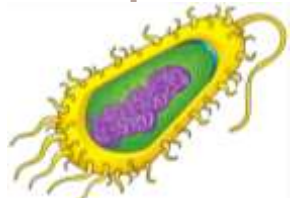
100 mm

1 m

Light Microscopy

Human eyes

Size and Scale



Bacteria cell



mitochonrion



White blood cells



Red blood cells



Animal cell



Chicken egg



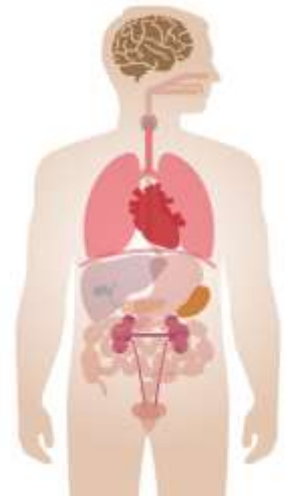
Stomach



Heart



Lung



1 μm

10 μm

100 μm

100 mm

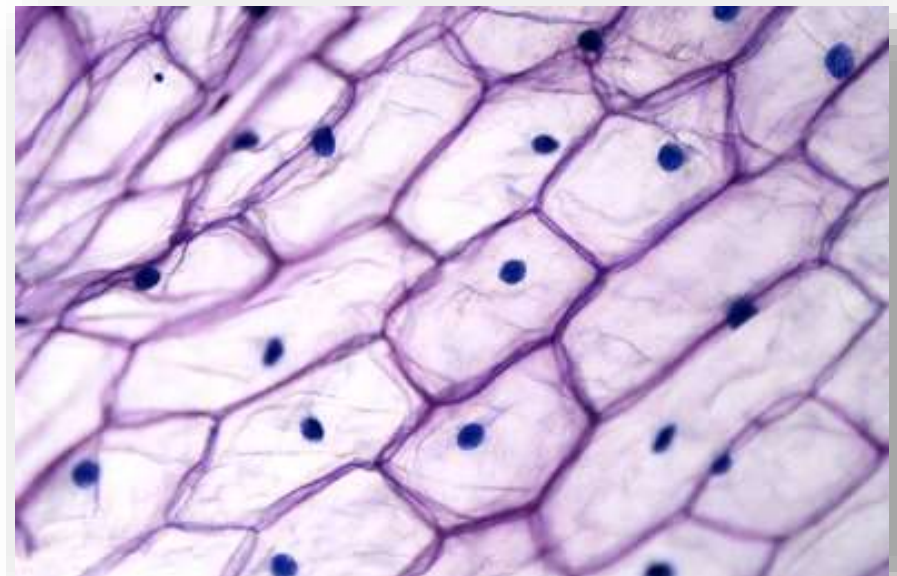
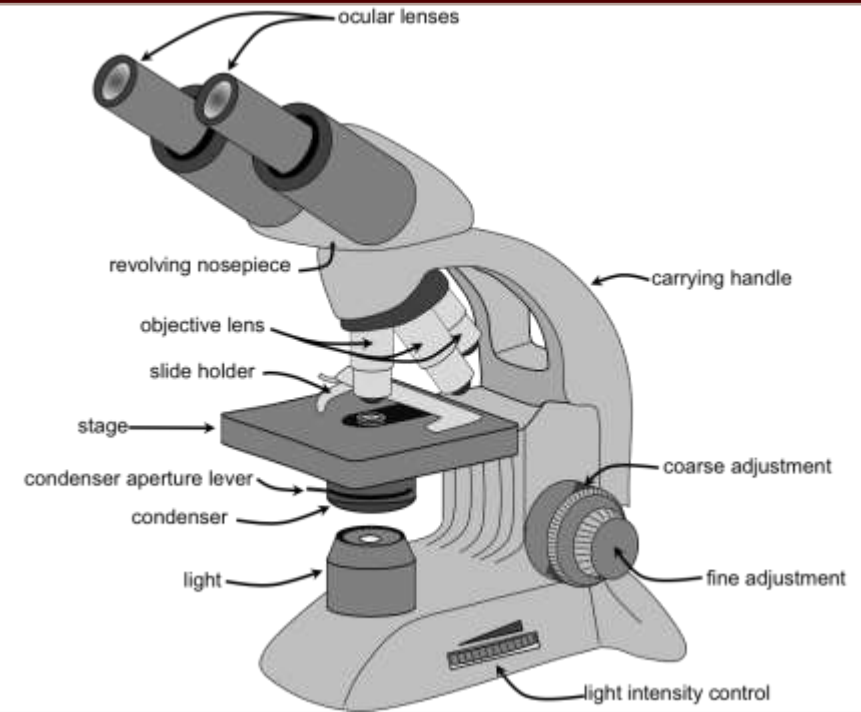
1 m

Light Microscopy

Human eyes

Microscope

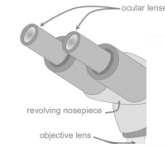
- Compound Microscope



Microscope

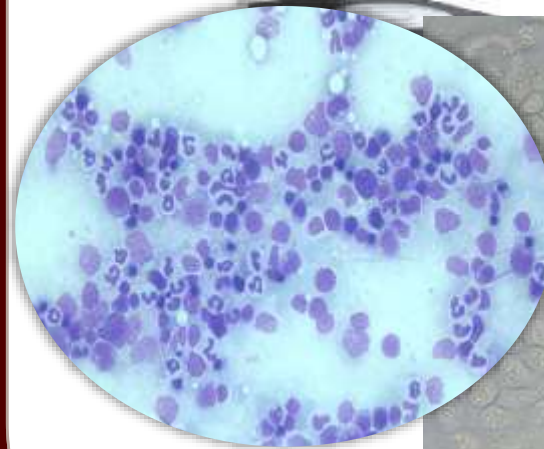
- Compound Microscope

- **Inverted Microscope**

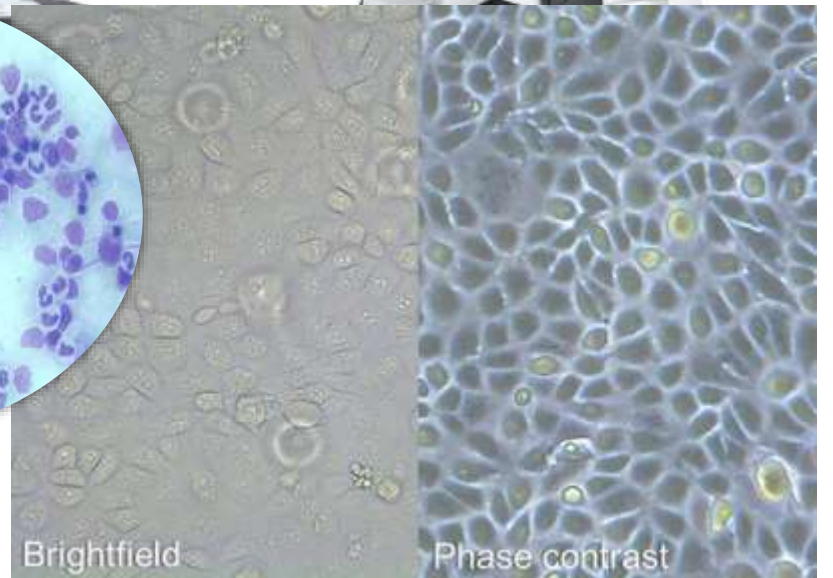


Inverted microscope for cell culture

cont.



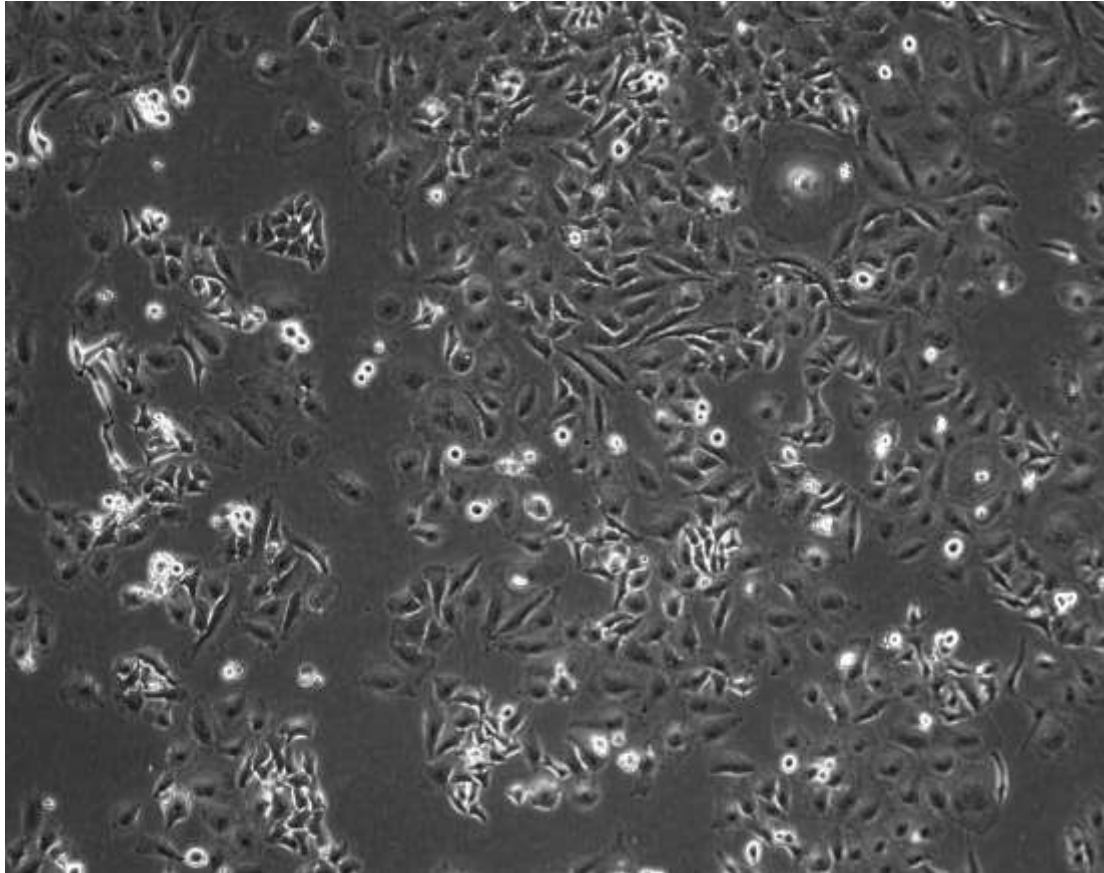
Brightfield



Phase contrast

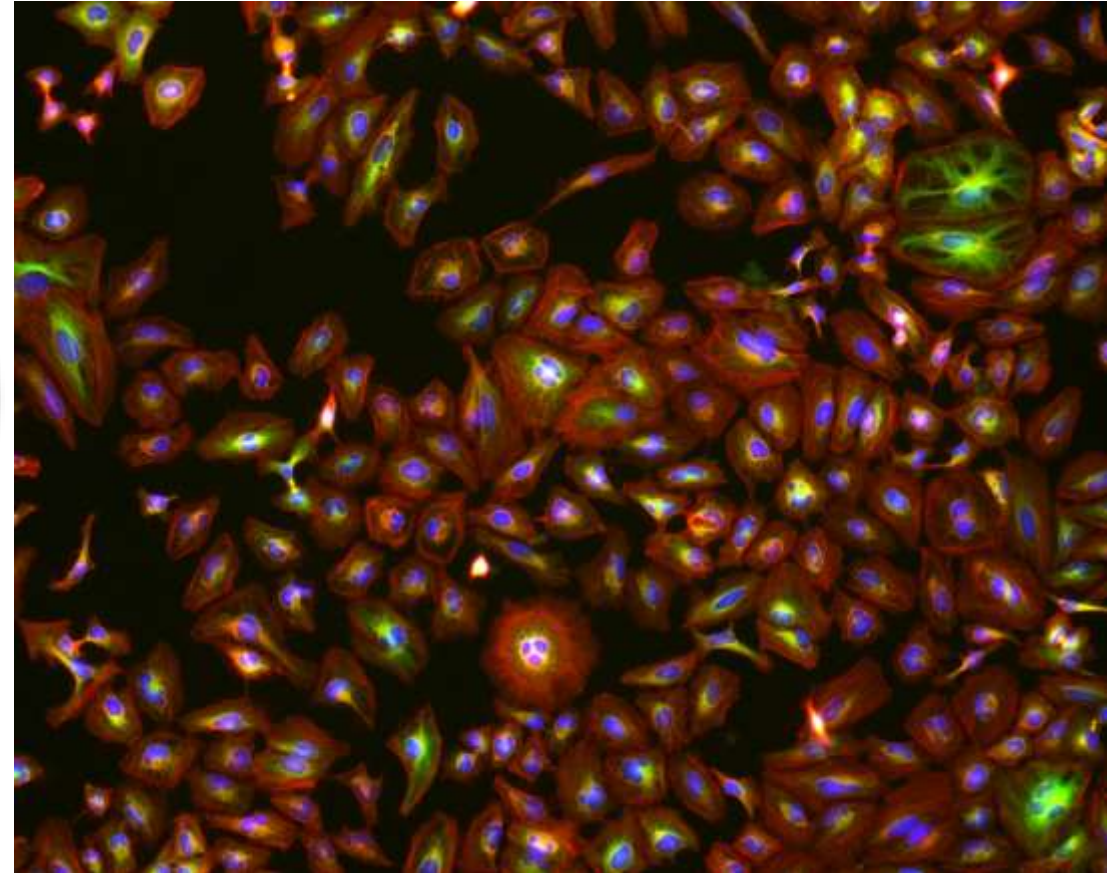
Why Fluorescence in Microscopy?

Brightfield



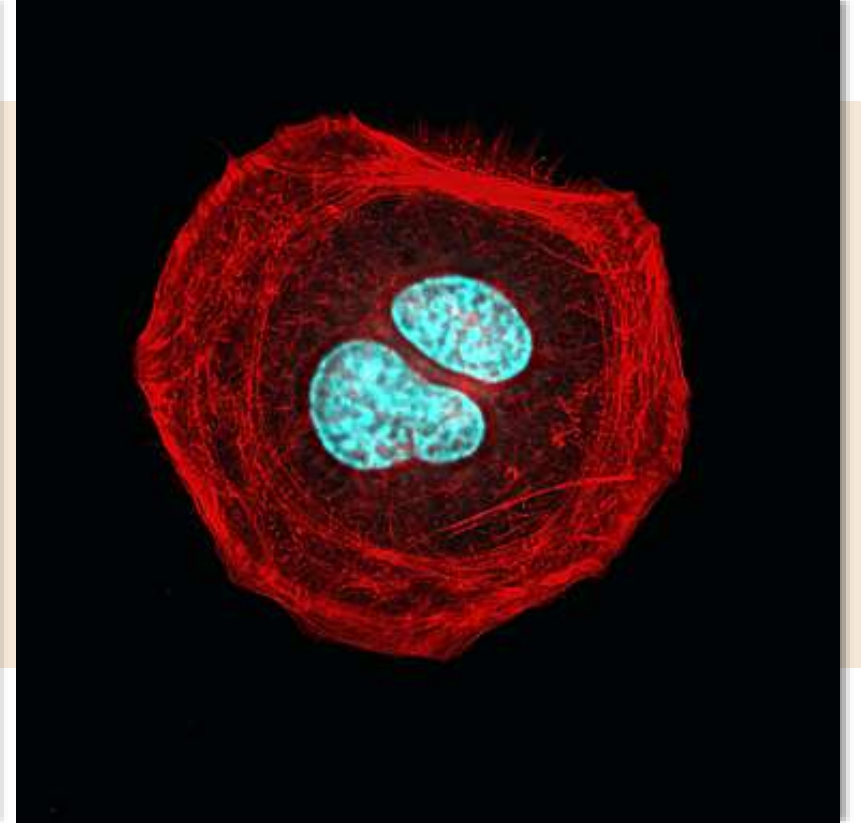
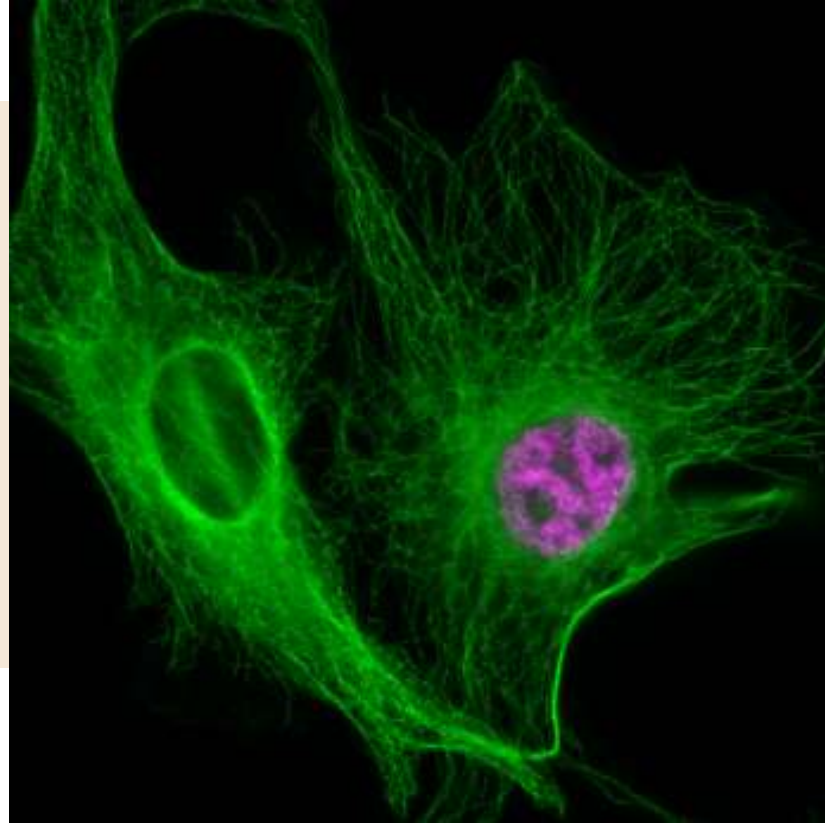
VS.

Fluorescence



Why Fluorescence in Microscopy?

**Beautiful images
tell a story**



Microscope

- Compound Microscope

- Inverted Microscope

- **Fluorescent Microscope**



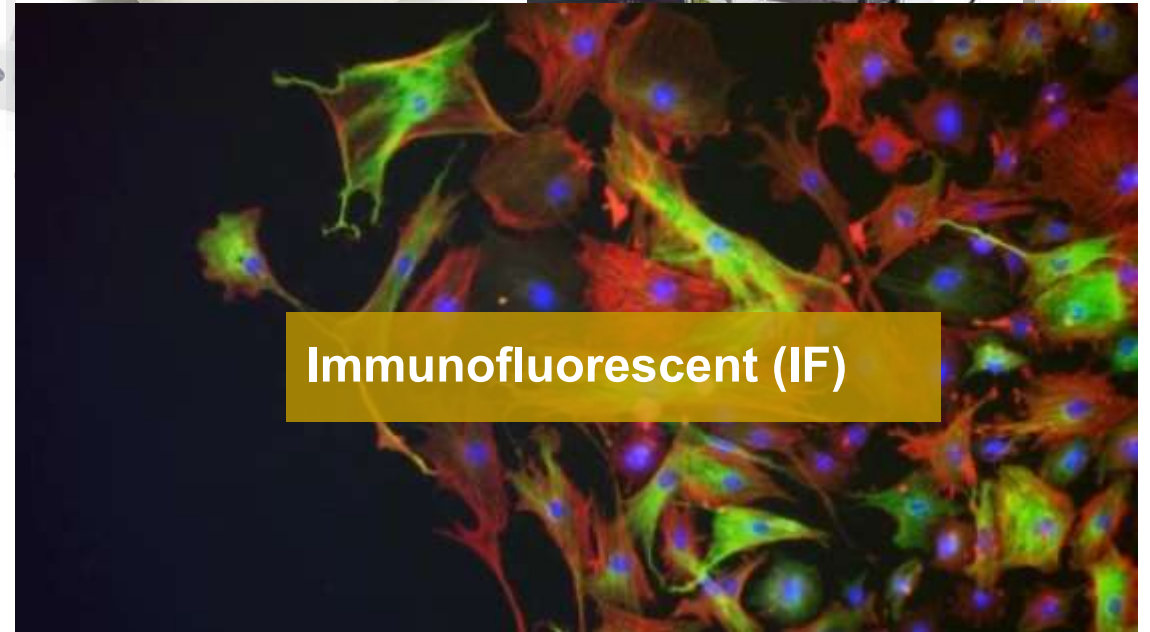
Epifluorescence microscope

Microscope

- Compound Microscope

- Inverted Microscope

- **Fluorescent Microscope**



Epifluorescence microscope

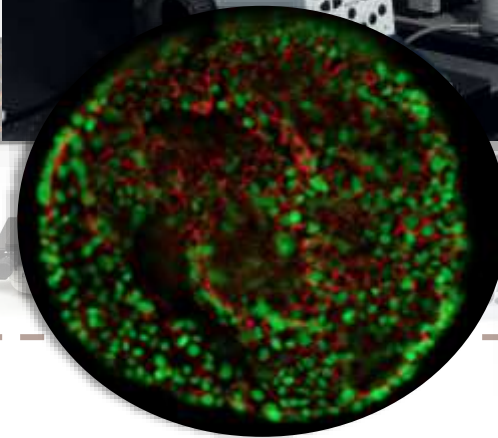
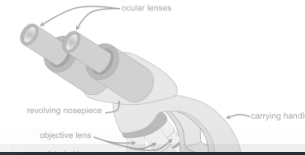
Microscope

- Compound Microscope

- Inverted Microscope

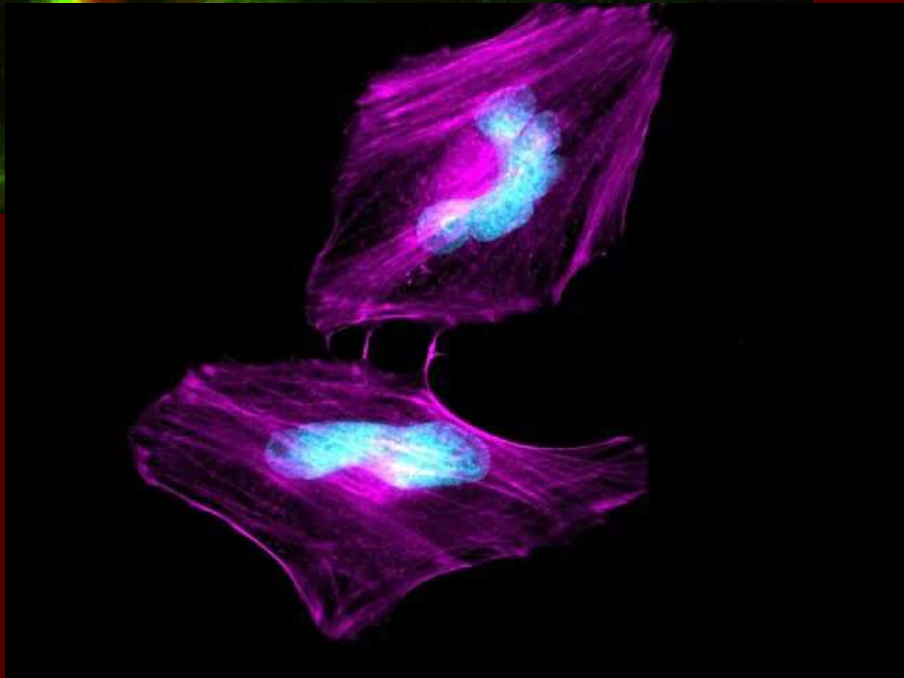
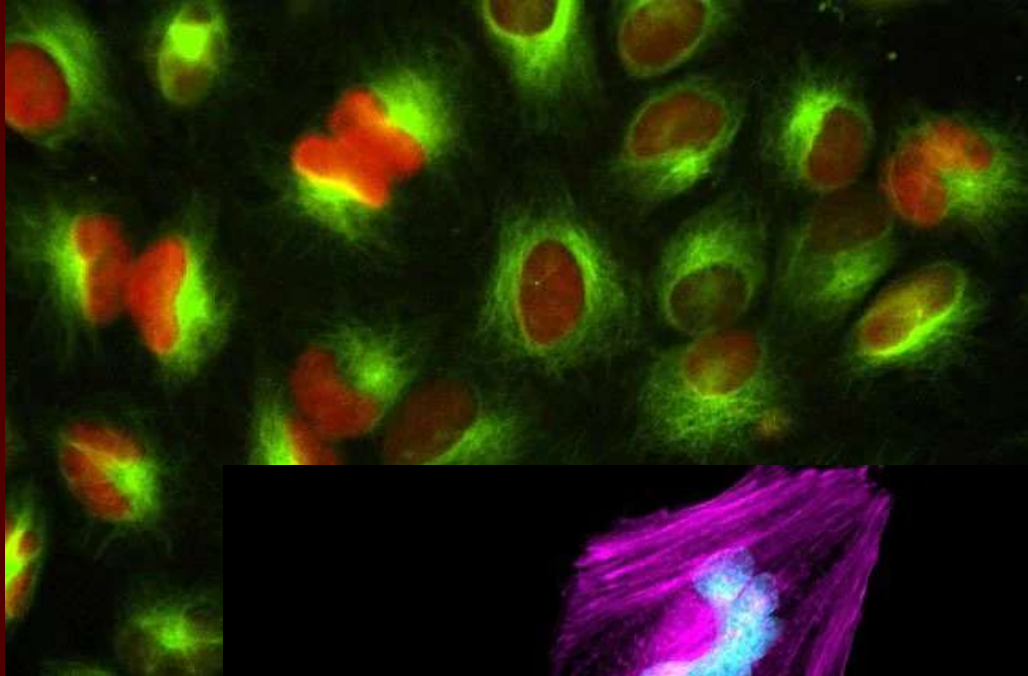
- Fluorescent Microscope

- Confocal Microscope



3D objects
Laser-scanning confocal microscope
Light-sheet fluorescence microscope

Fluorescent Microscope



Epifluorescence microscope



Topics

1

Type of Microscopy

2

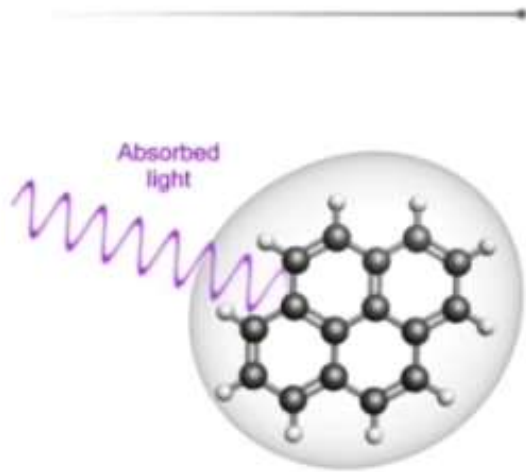
Principle of Fluorescent Microscopy

3

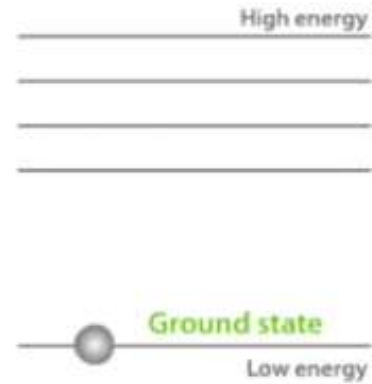
EVOS M7000 Imaging System

The Principle of Fluorescence

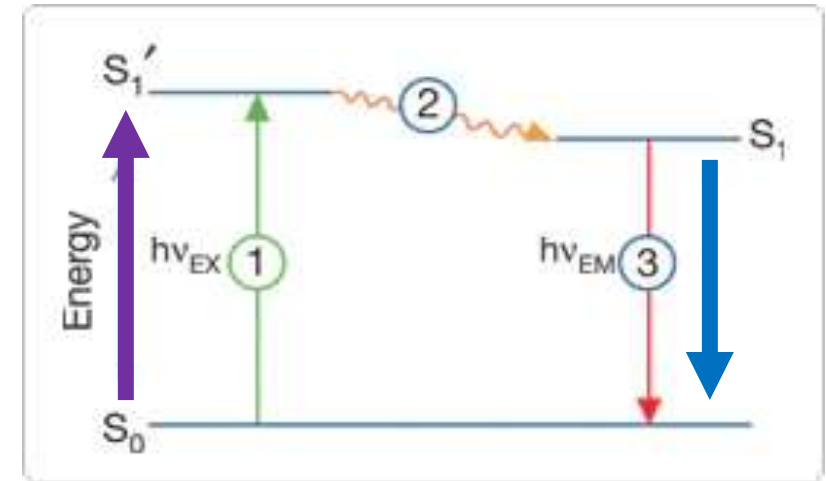
Absorption of Light Energy



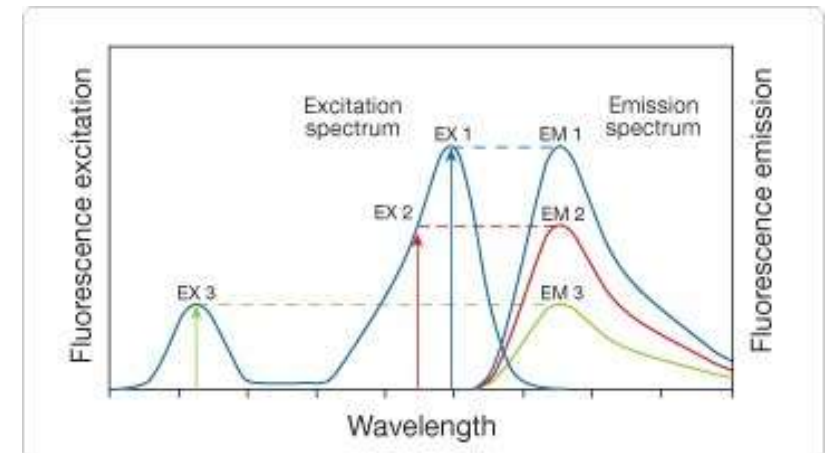
Fluorophore



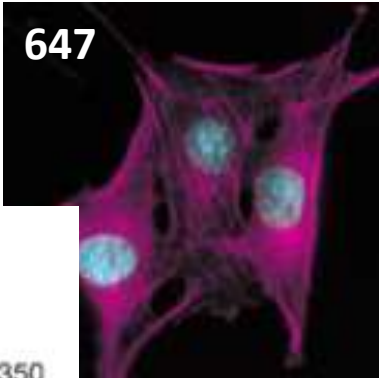
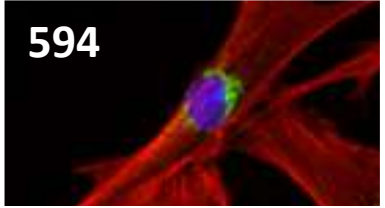
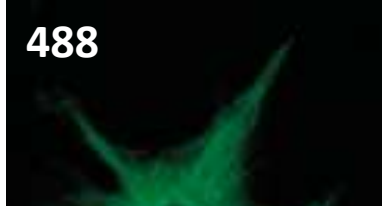
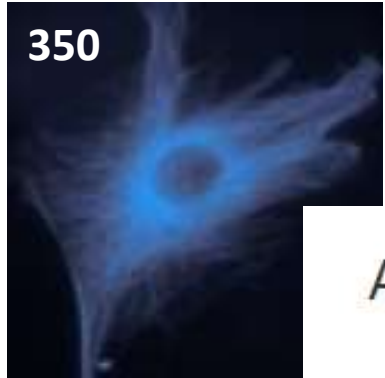
Energy levels



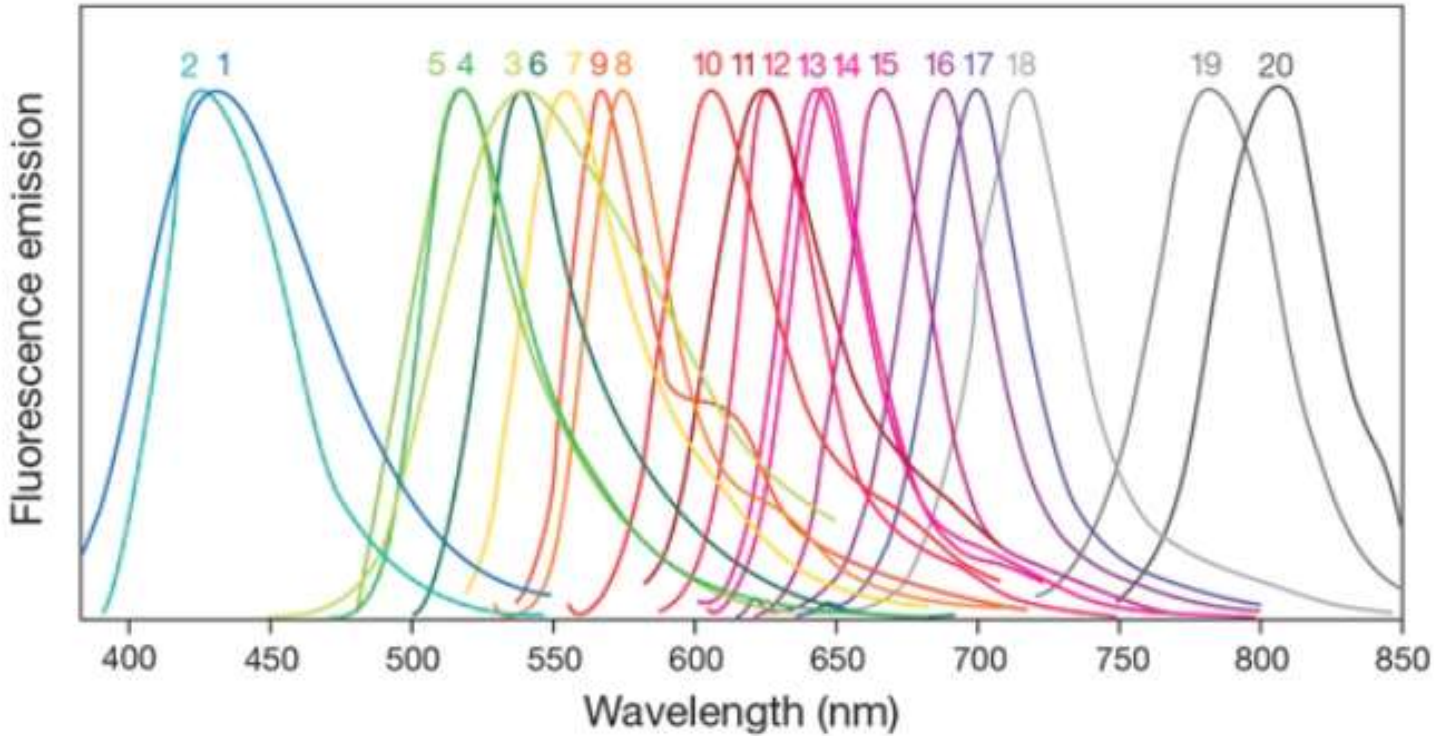
molecular probes™



Fluorescence: The Main Colors



Alexa Fluor dyes—Emission spectra



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

Blue

350

Alexa Fluor 405

DAPI

Hoechst

CFP

Far red

650

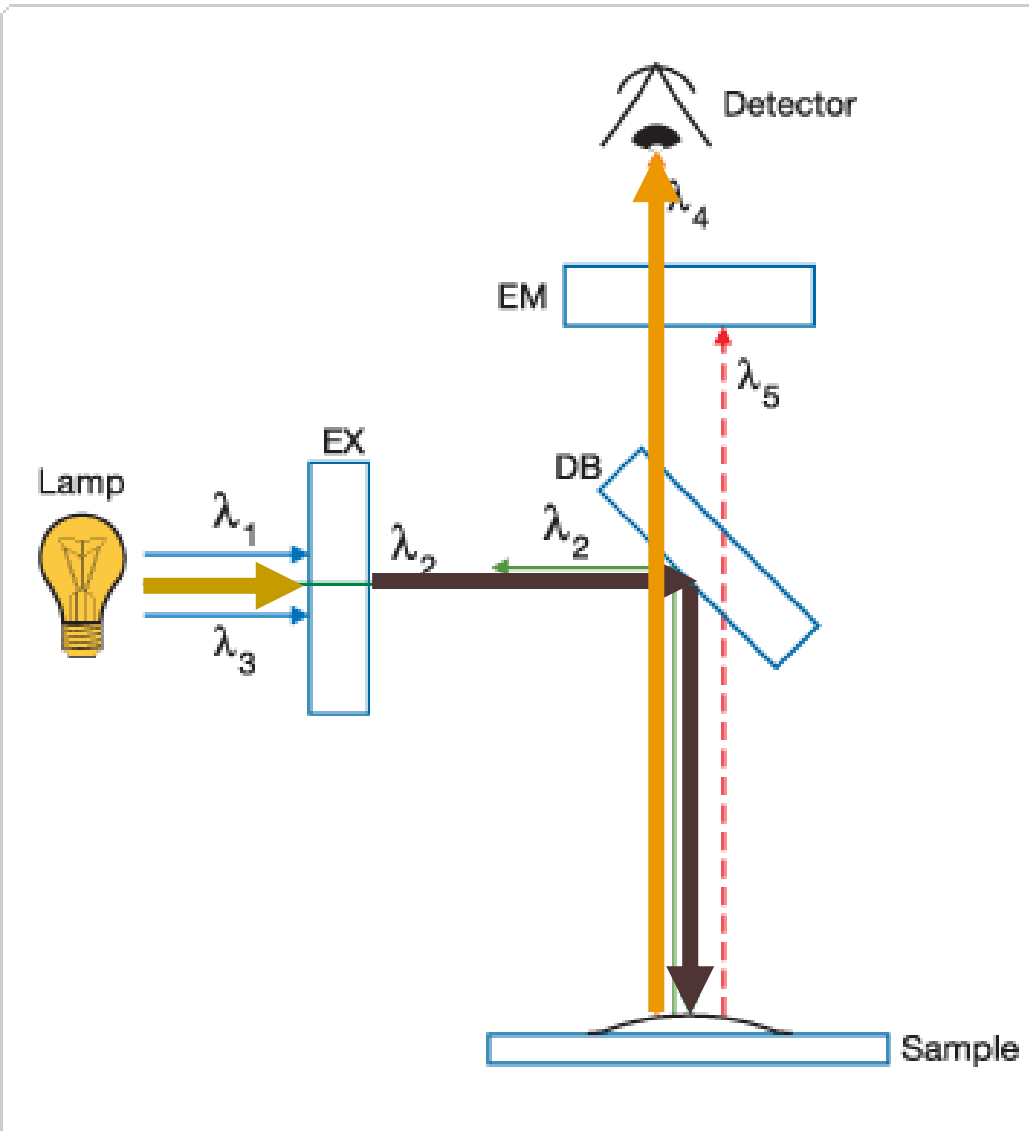
Alexa Fluor 647

Cy5

mPlum

Figure 1. Emission spectra for Alexa Fluor dyes.

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

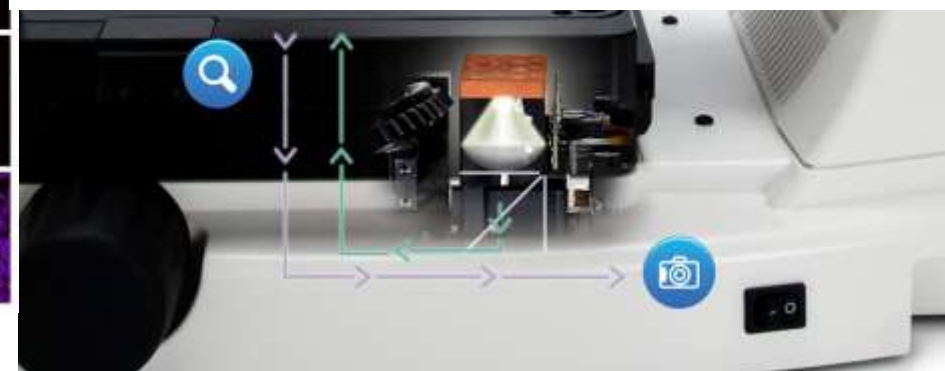
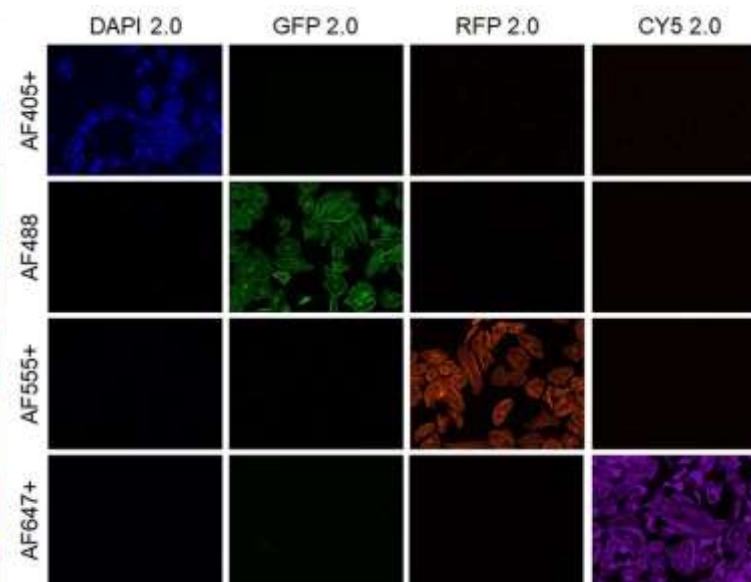
EVOS Light Cube

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

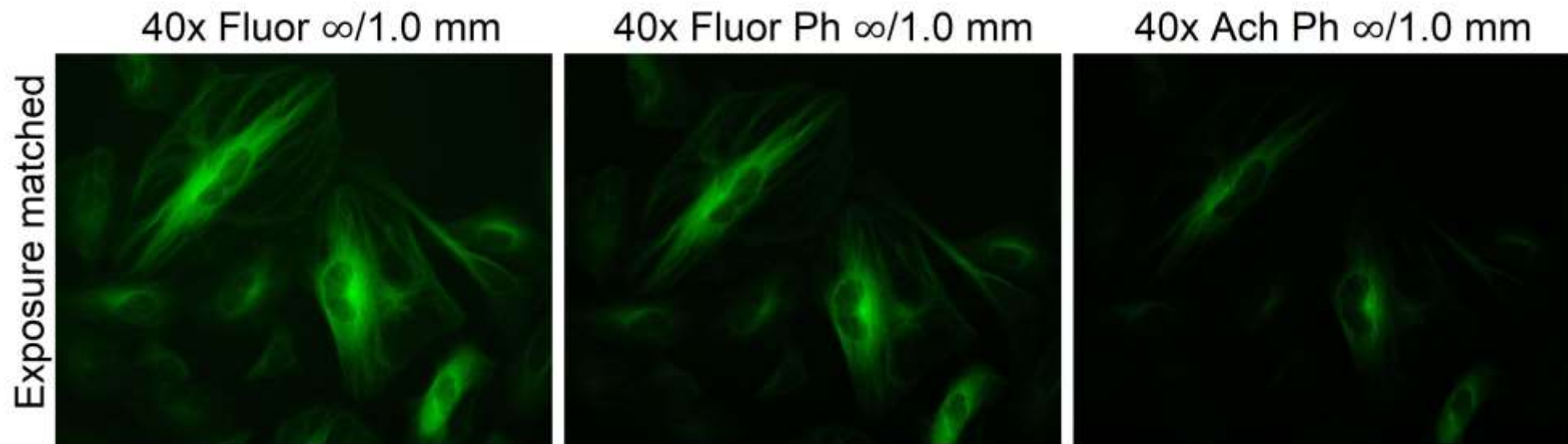
| |
|-----------|
| DAPI |
| TagBFP |
| CFP |
| GFP |
| YFP |
| RFP |
| Texas Red |
| Cy5 |
| Cy5.5 |
| Cy7 |



| 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

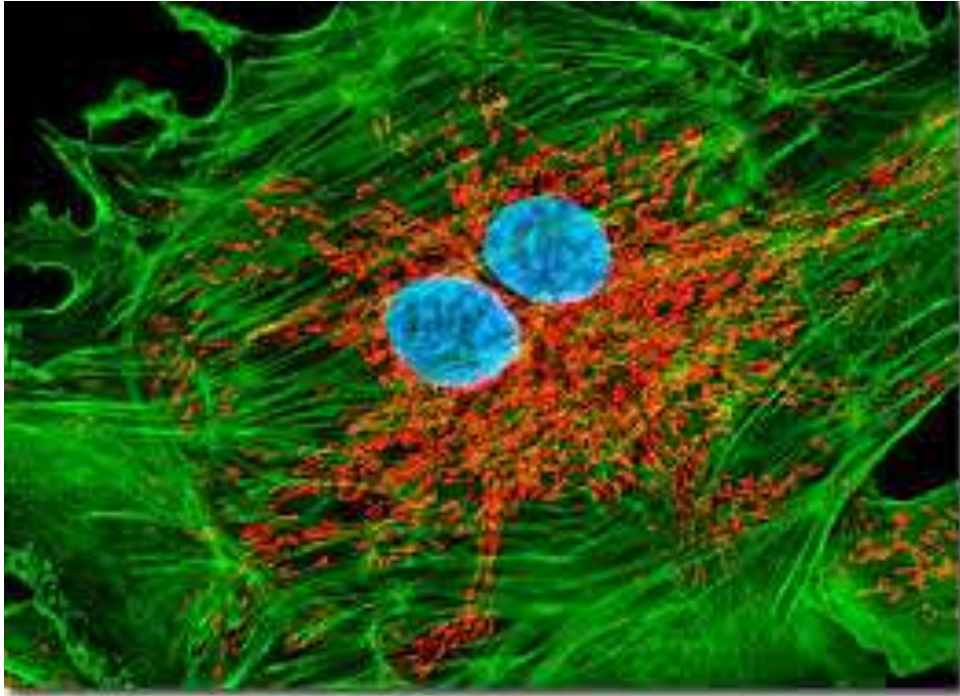
- Most important part of the microscope because it forms the image
- Nomenclature indicates things like field flatness and color correction
- Resolution is defined by numerical aperture (NA)
- Working distance generally is shorter for high resolution lenses
- It is important to match refractive indexes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



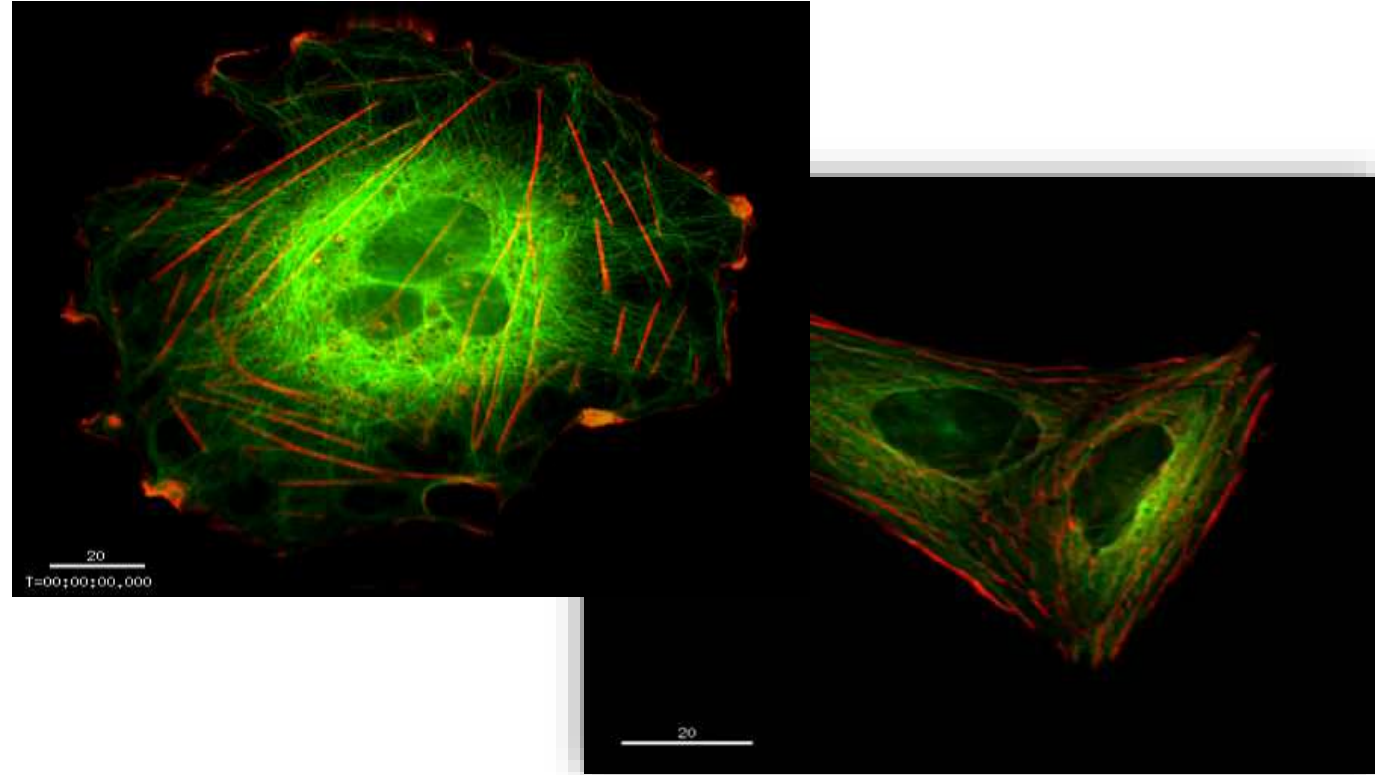
Impact of image quality comparing three types of LWD objectives with the same sample and exposure settings.

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.....

...to this



'Typical' epifluorescence system



Compact design

Sample holders for slides or almost any vessel type

Automated operation

Interchangeable LED light cubes

High-resolution camera (monochrome and/or color)

EVOS M7000

EVOS™ M7000 Imaging System

FAST scan speed

Powerful analysis software option

***Four changeable light cubes PLUS
transmitted light***

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

Large bright monitor



Outstanding image quality

Powerful image analysis options

Robust and fast autofocus

Instrument installation AND training included

Automated acquisition routines

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



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



Designed for use on the bench
get out of the darkroom



EVOS vessel holders and stage plates

All models

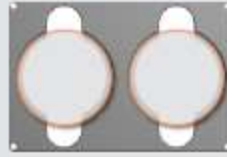
AMEPVH009

Universal stage insert



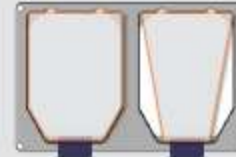
AMEPVH003

Holds two 60 mm Petri dishes



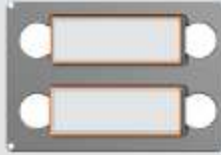
AMEPVH005

Holds two 25 cm² flasks (rectangular or triangular)



AMEPVH001

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



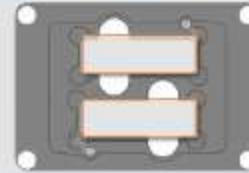
AMEPVH006

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



AMEPVH021

Holds two microscope slides or chamber slides with retention clip



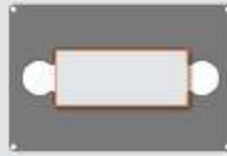
AMEPVH004

Holds one 100 mm Petri dish



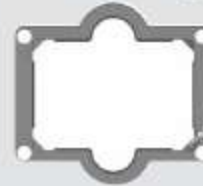
AMEPVH007

Holds one hemocytometer



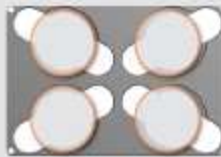
AMEPVH022

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

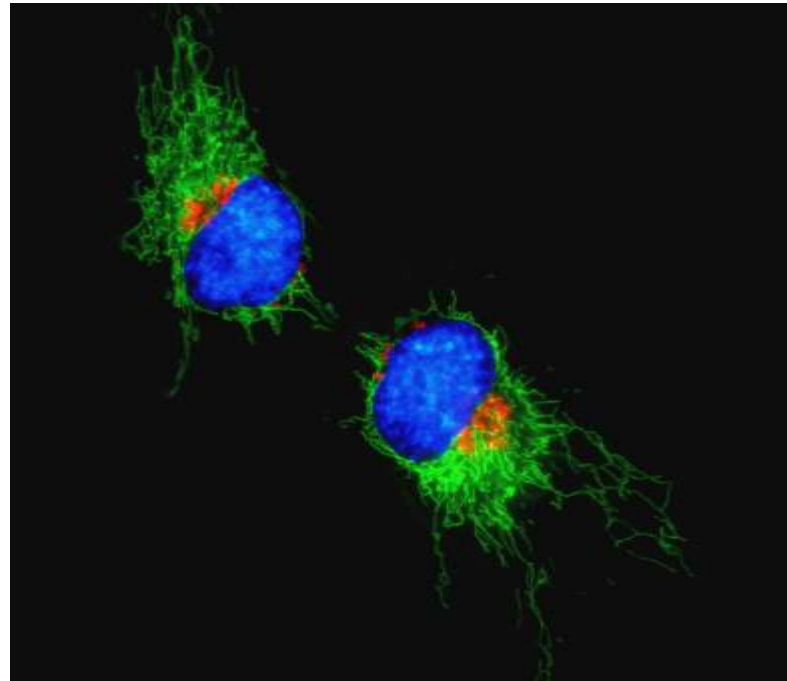
Holds two 35 mm Petri dishes



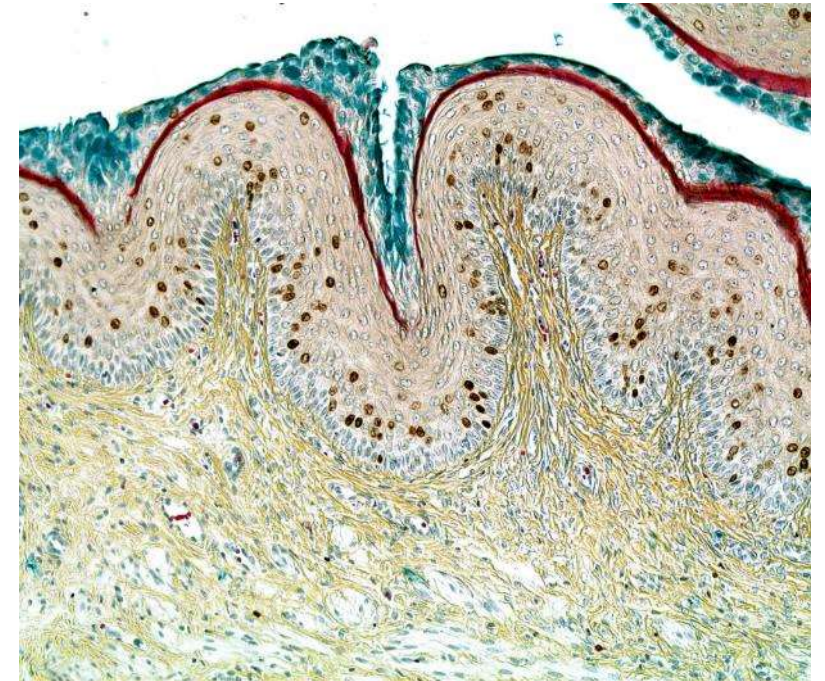
EVOS M7000 System—One Microscope, Two Cameras

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



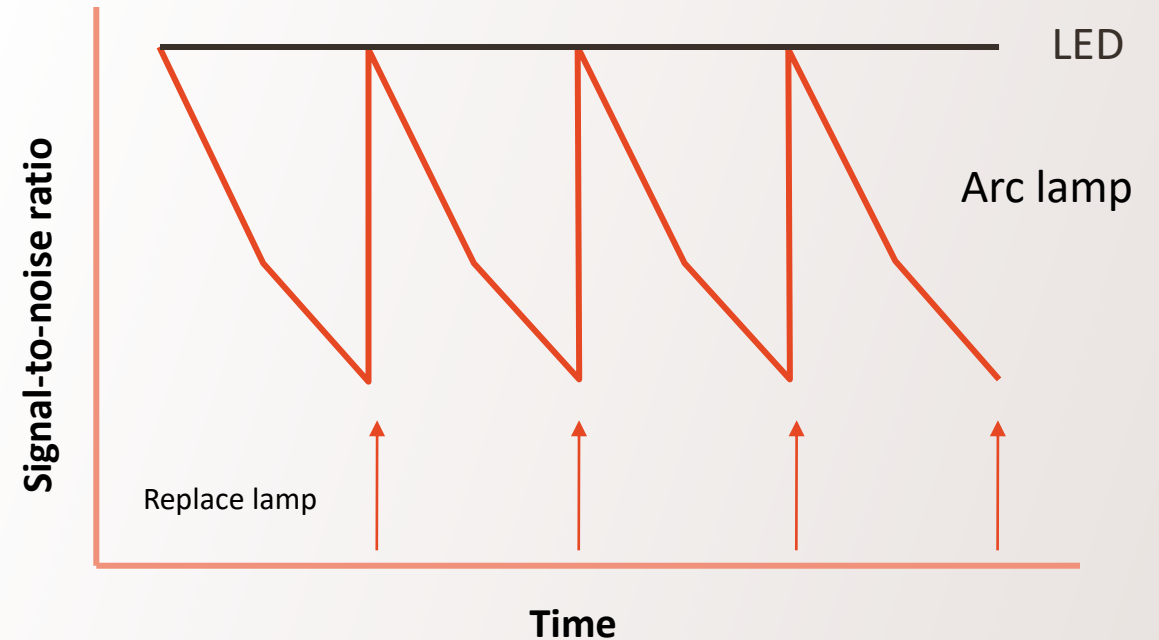
Better detection of weak fluorescence signals

Illumination Stability

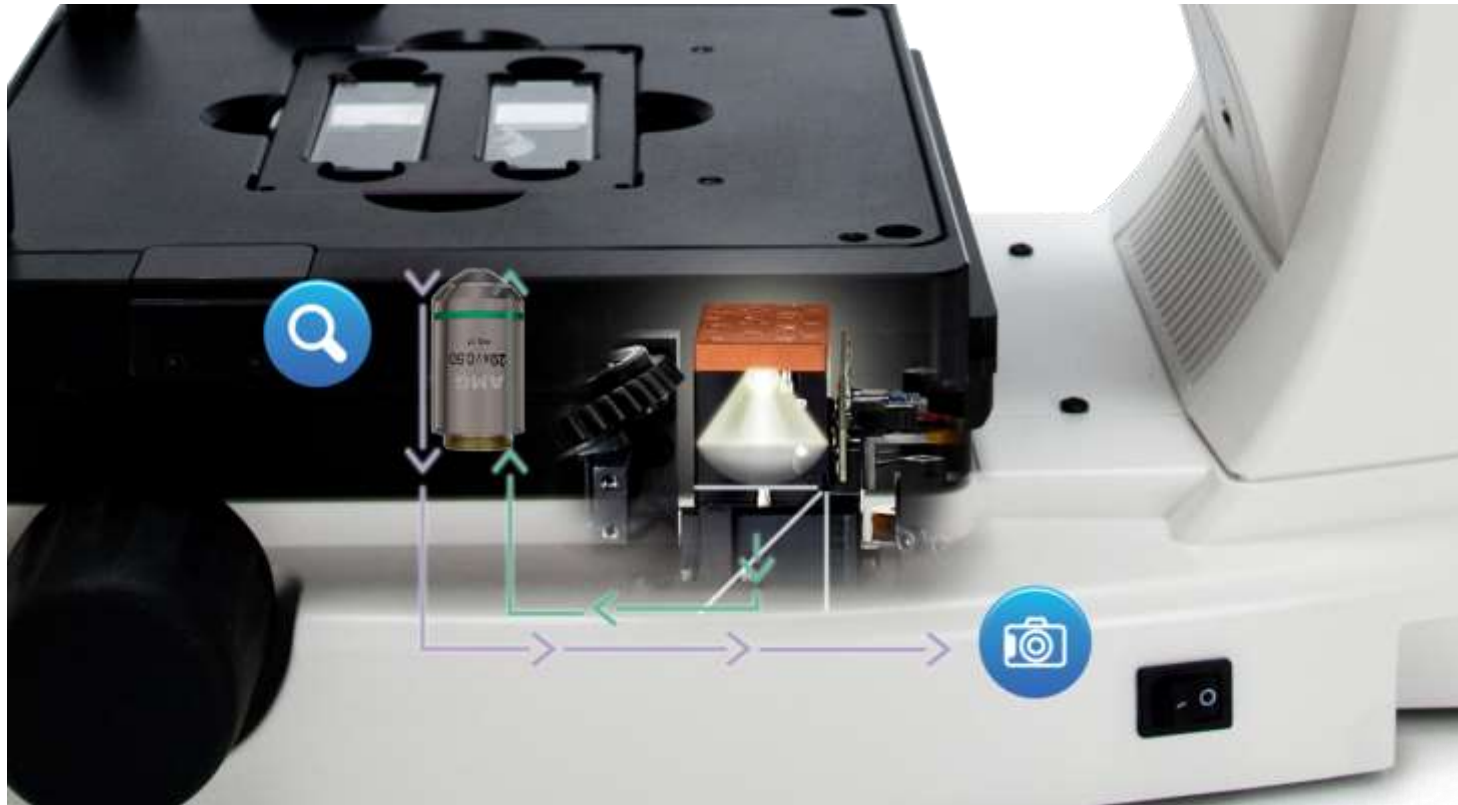
Mercury arc lamps lose 0.5% intensity per hour of use, resulting in 50% reduction in only 100 hours of use

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

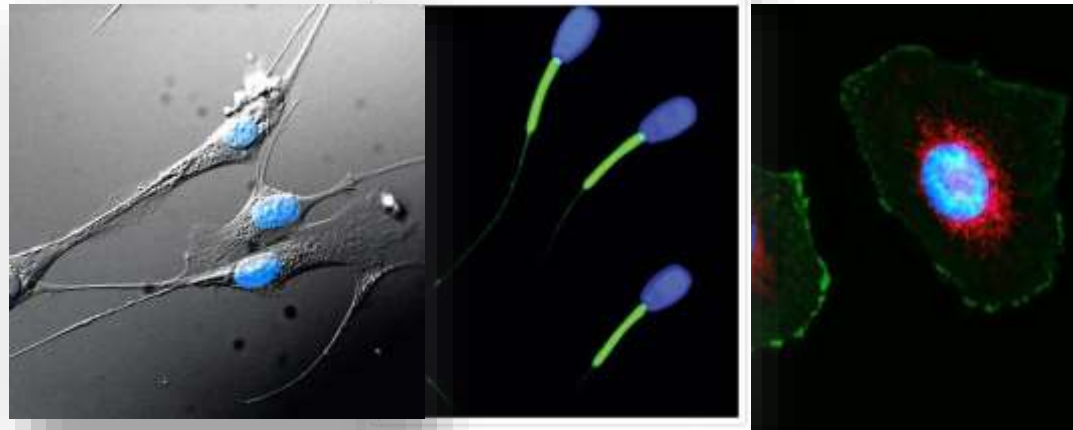
**No
photobleaching**

**No
photo toxicity**

**No warm up/cool
down**



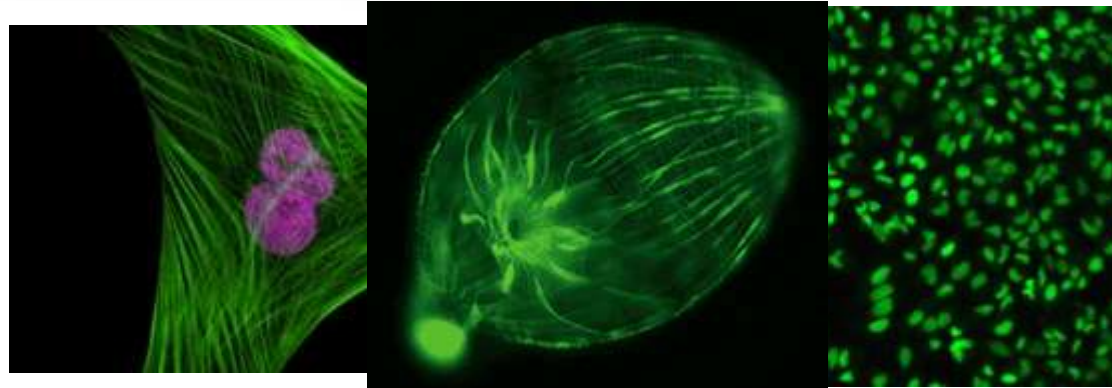
EVOS™ Light Cube, DAPI 2.0
(357/44 nm Excitation;
447/60 nm Emission)



Alexa Fluor 350
DAPI
Hoechst 33342
LysoTracker Blue
NucBlue
Etc.



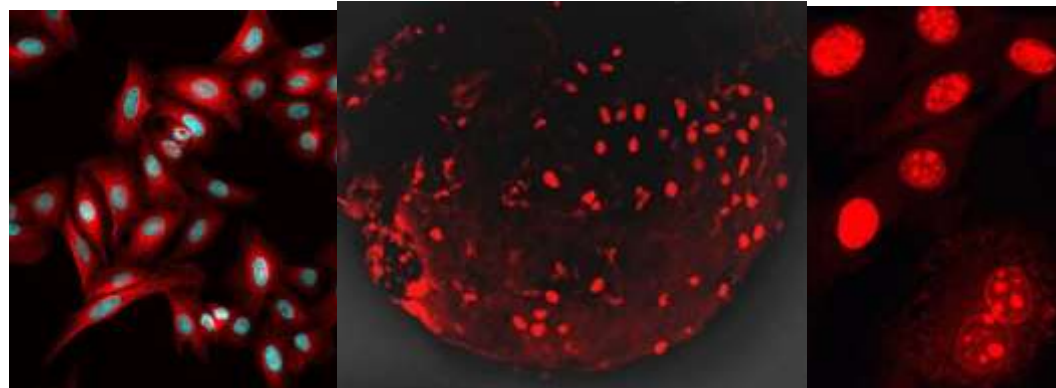
EVOS™ Light Cube, GFP 2.0
(482/25 nm Excitation;
524/24 nm Emission)



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

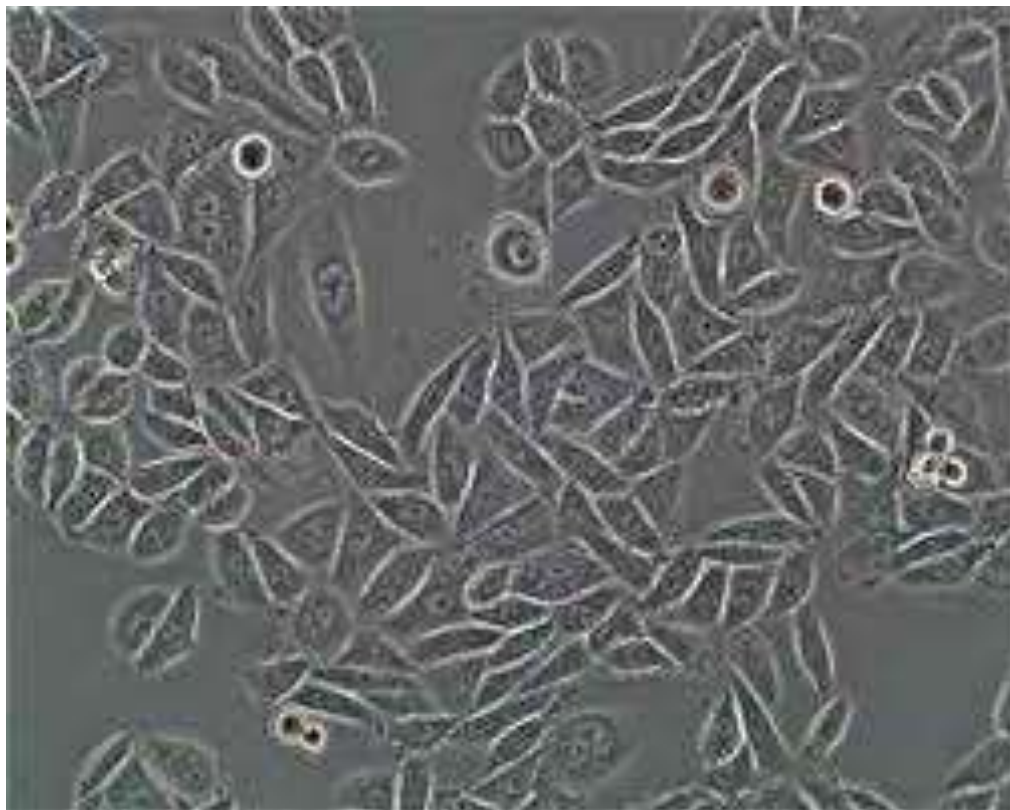


EVOS™ Light Cube, Texas Red 2.0
(585/29 nm Excitation;
628/32 nm Emission)



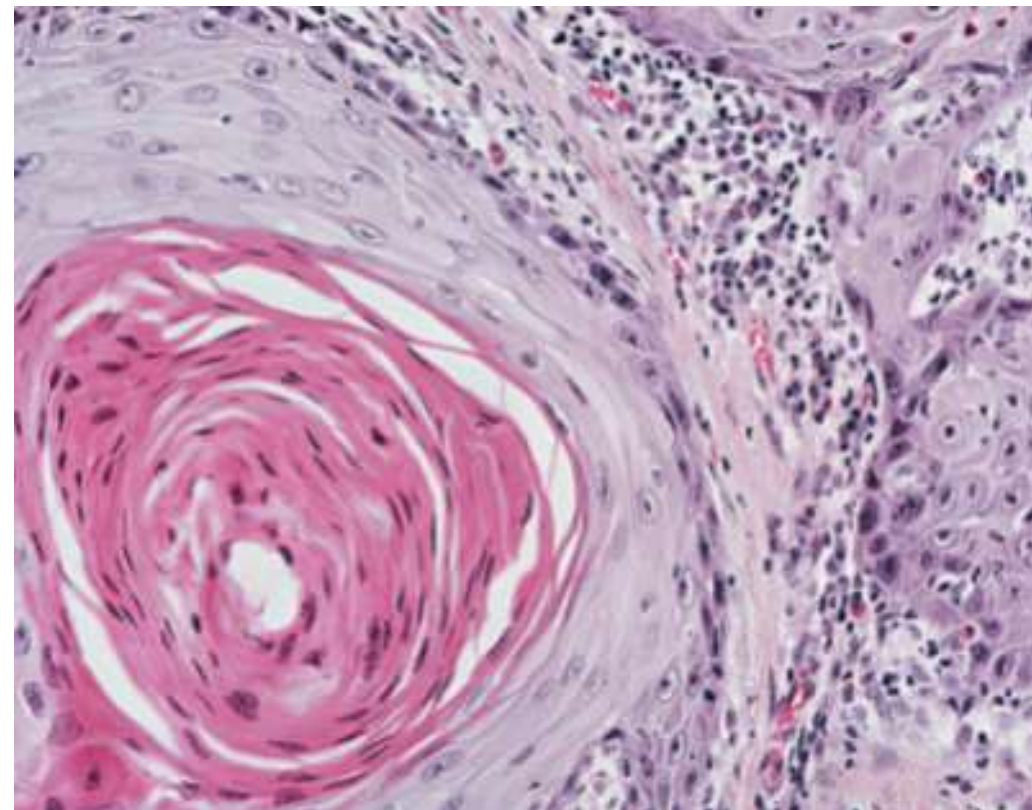
Texas Red
Alexa Fluor 594
mCherry
pHrodo Red
Cy3.5
Etc.

EVOS® M7000: Transmitted Light Applications



HeLa cells in culture

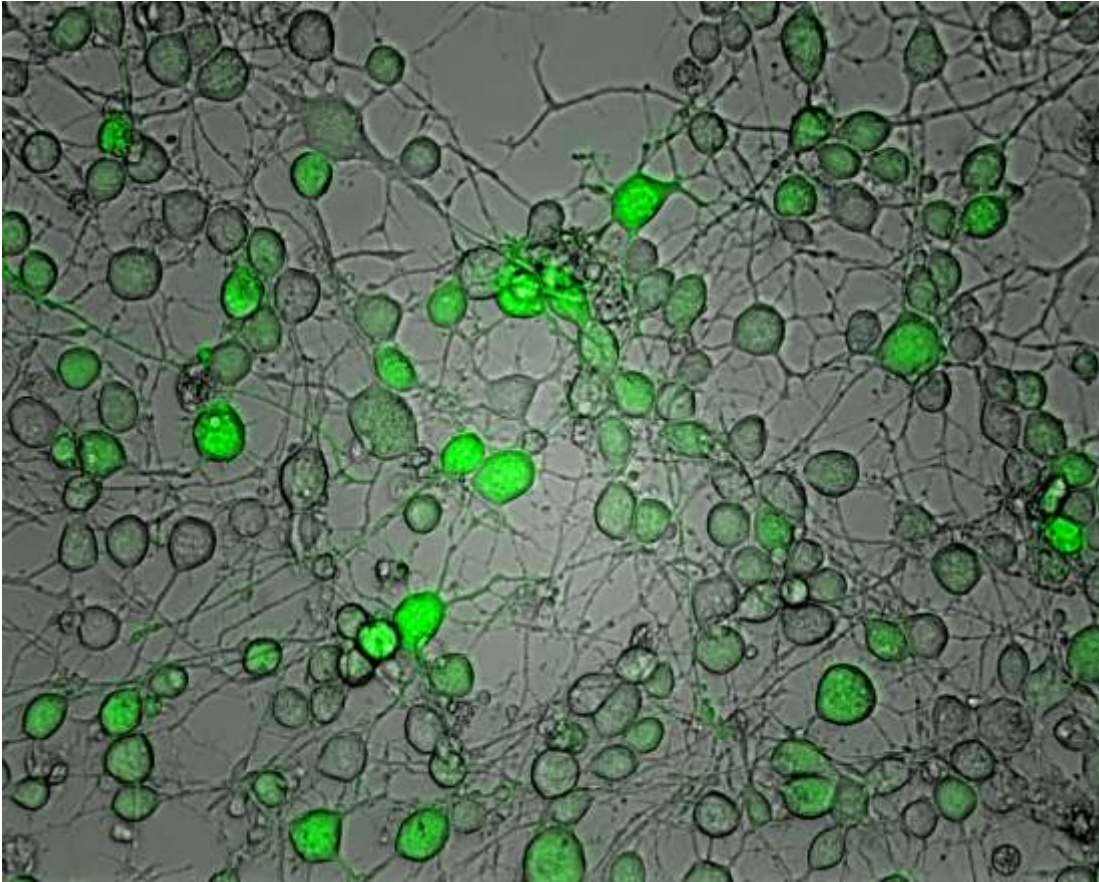
Cell culture: *Are my cells confluent and healthy?*



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

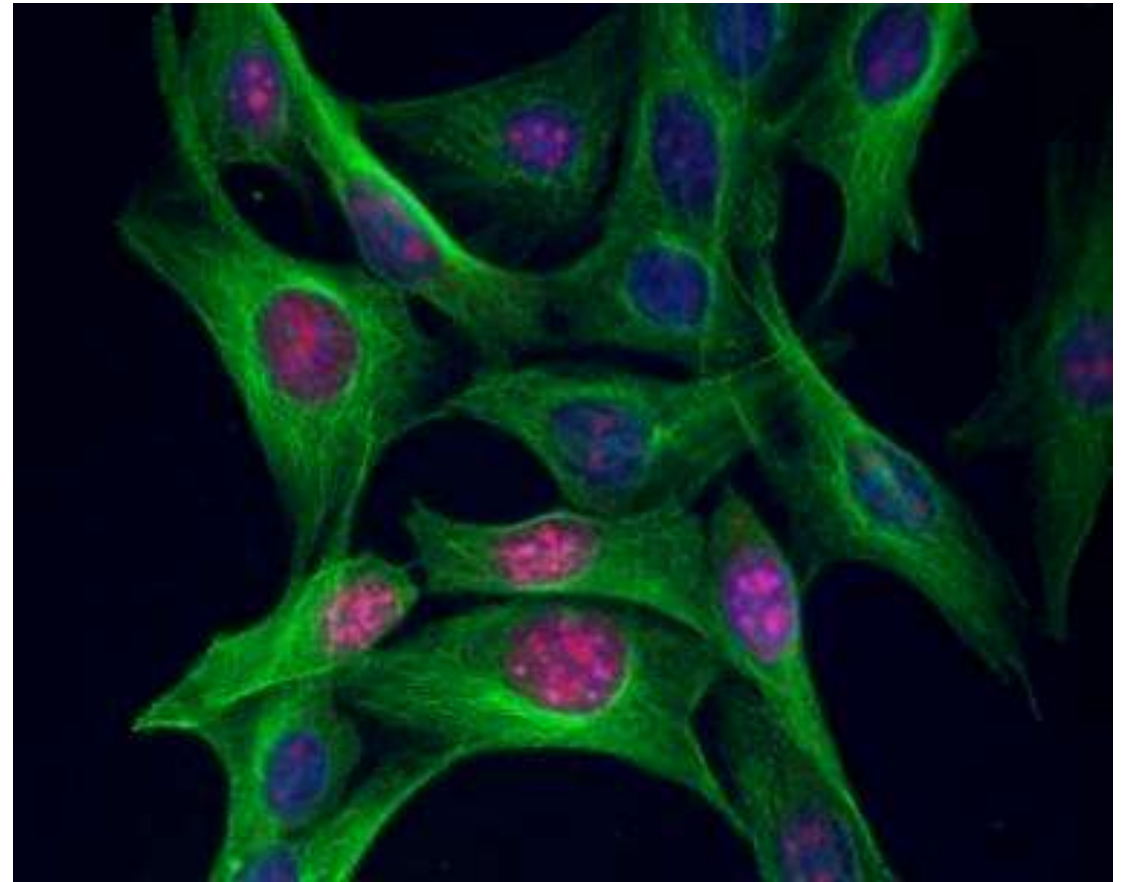
IHC: *Visual markers of disease*

EVOS® M7000: Fluorescent Applications



Neuronal stem cells expressing GFP

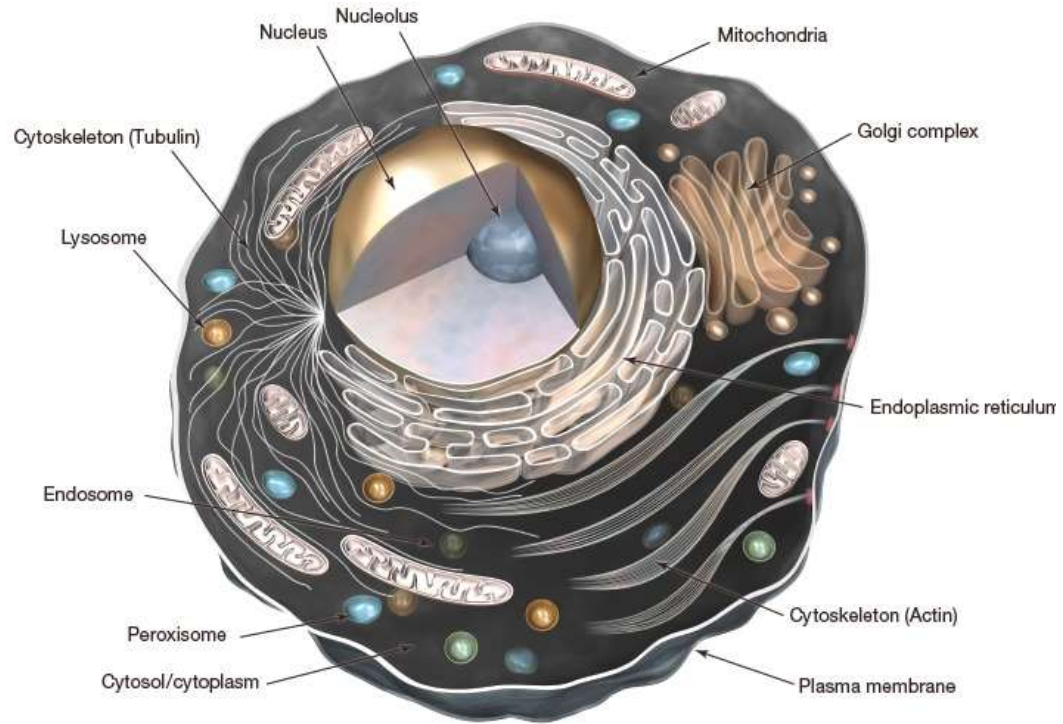
Cell culture: *Are my cells expressing GFP?*



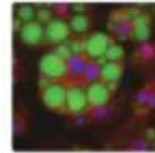
NIH 3T3 cells: mRNA (red), tubulin (green), and nucleus (blue) are clearly visualized

Cell health: *Do my cells look normal?*

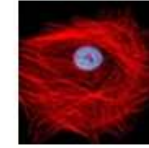
EVOS® M7000: Fluorescent Applications



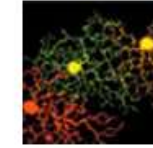
Find organelle-specific antibodies and organelle stains



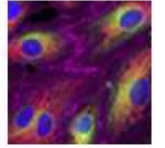
Adiposomes



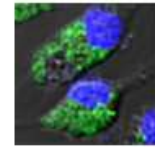
Cytoskeleton



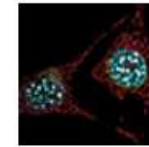
Cytosol/cytoplasm



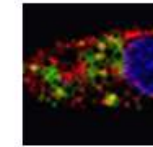
Endoplasmic reticulum



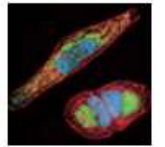
Endosomes



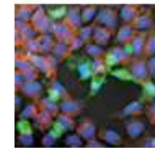
Golgi complex



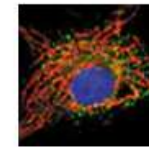
Lysosomes



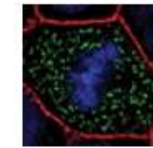
Mitochondria



Nucleus/nucleolus



Peroxisomes



Plasma membrane

Live Cell Imaging Solution

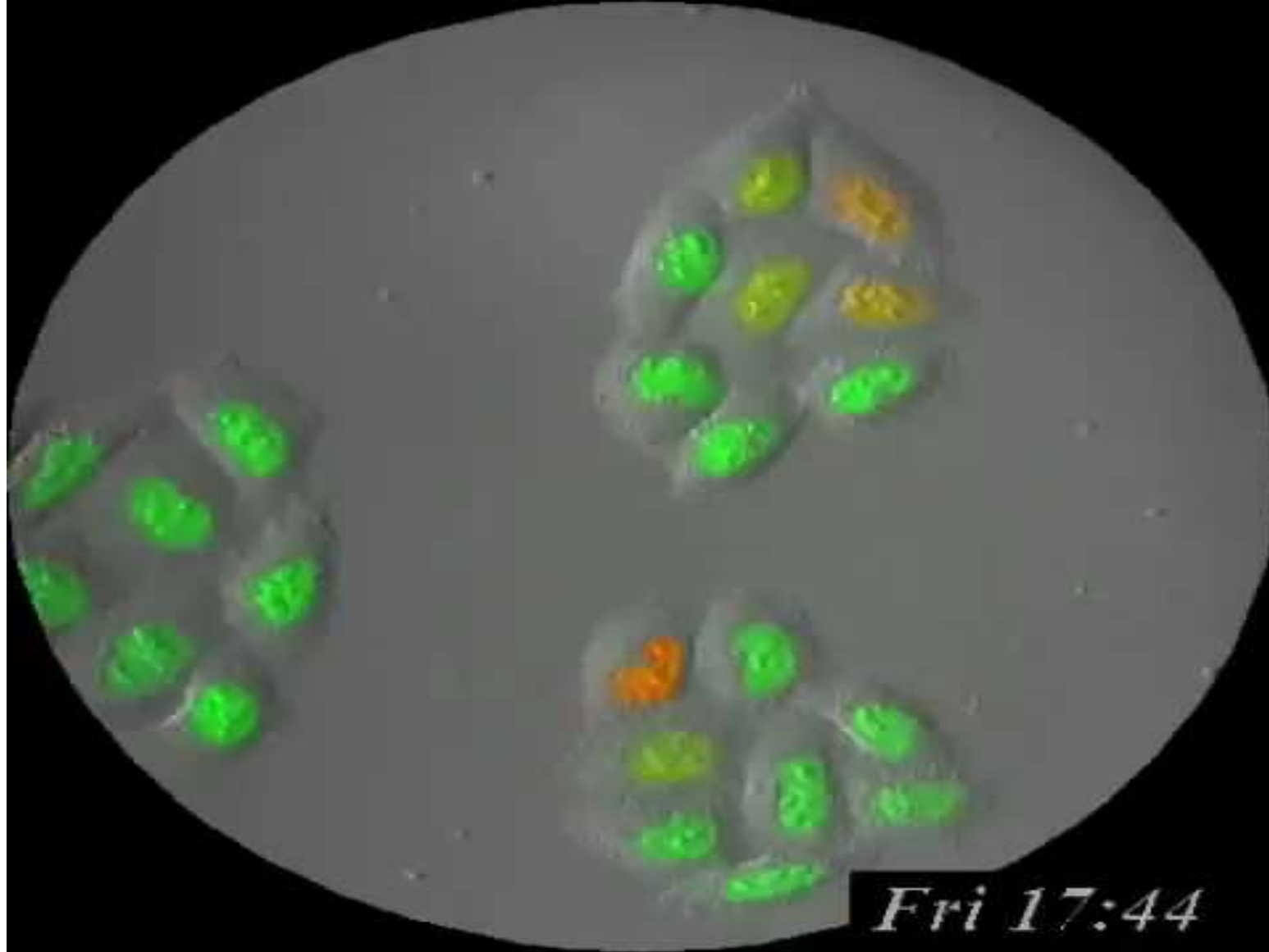
Complete solution to environmental control during live cell and time-lapse imaging studies



- 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 3M 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 (accommodates 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



Invitrogen EVOS™ M7000 Imaging System

Locations

Capture Automate Review Settings

Vessel

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

Zoom: Generic 96 Well Glass Bottom

Objective: 2x 4x 10x 20x 40x

Light source: DAPI GFP RFP TX Red Trans

Light: Bright: 0.00013

AutoFocus: Coarse: 13.7 Fine: 15.7

Capture

Record Video Capture Z Stack Capture Channels

Save... 42

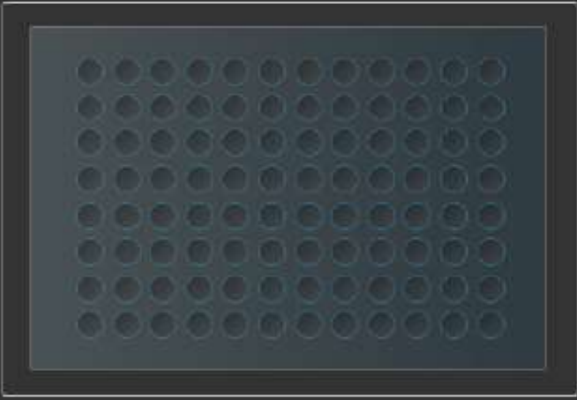


Vessel Selection

Well Plates Flasks Dishes Slides

Holder: Well Plate | Generic Plate

Plate: 96 Well | NUNC | 167311

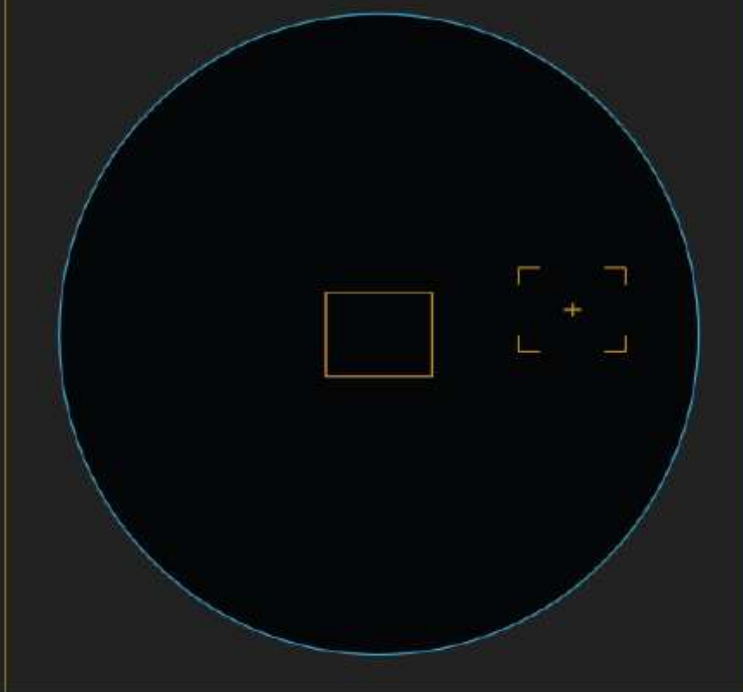


Cancel Done



Leibniz EVO5™ M7000 Imaging System

Locations



Capture Automate Review Settings

Vessel

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | | | | | | | | | | | |
| B | | | | | | | | | | | |
| C | | | | | | | | | | | |
| D | | | | | | | | | | | |
| E | | | | | | | | | | | |
| F | | | | | | | | | | | |
| G | | | | | | | | | | | |
| H | | | | | | | | | | | |

Zoom: 4x 10x 20x 40x 7x

Objective: 4x 10x 20x 40x 7x

Light source: DAPI GFP RFP CF3 Trans

Light: Bright: 0.25

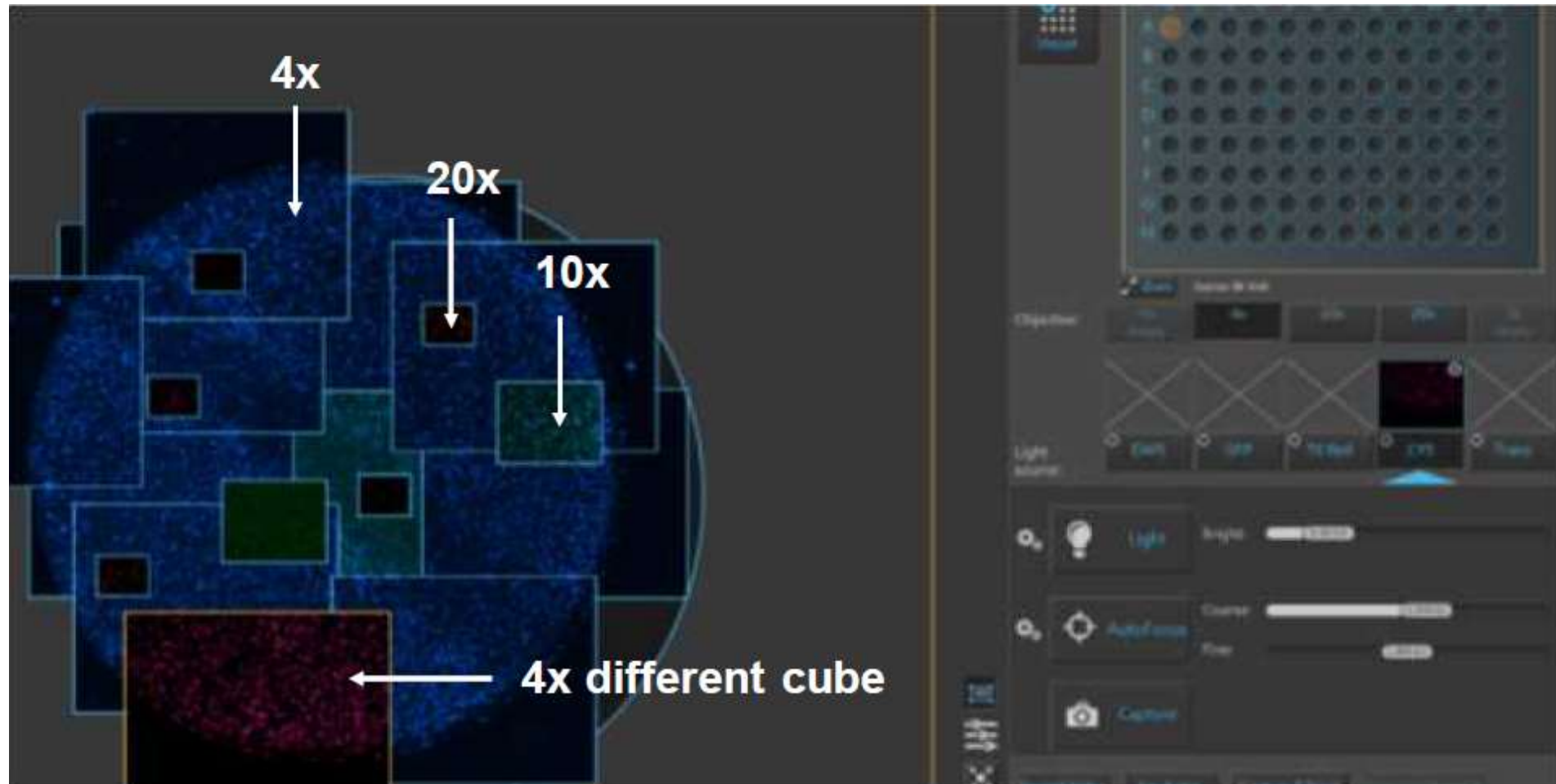
AutoFocus: Coarse: 0.100 Fine: 0.100

Capture

Record Video Capture Z-Stack Capture Channels

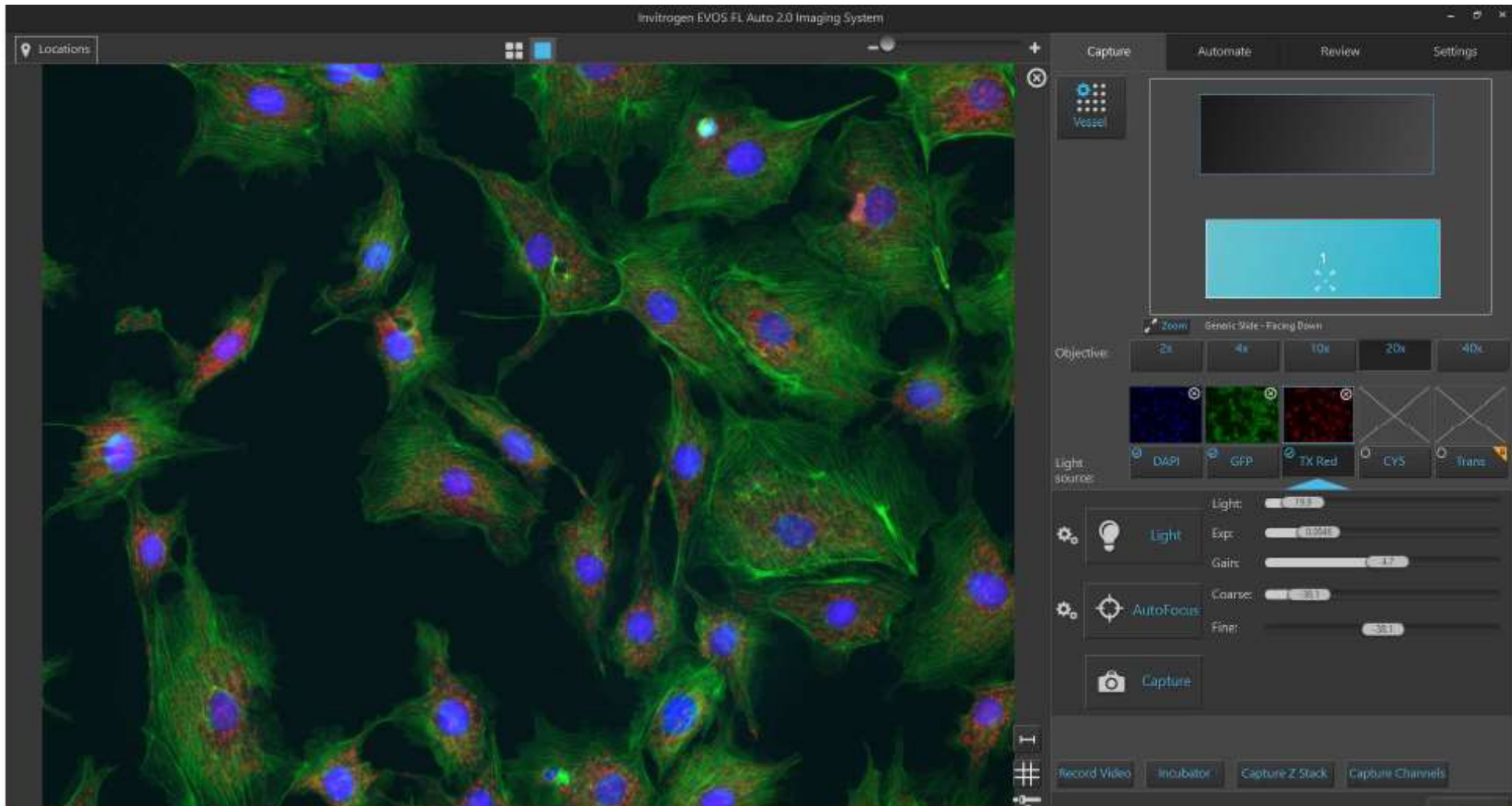
Done

Area XY Location (micrometers): (-3296.87, 265.74)



Area view mode

Capture tab



Field view mode

Invitrogen EVOS FL Auto 2.0 Imaging System

Locations

Autofocus: three modes

- Small structure (filaments)
- Large structure (whole cell stain)
- Small bright objects (nuclear stain)

Autofocus Method: Large Structure Lock Z Offset Clear Z Offsets

Capture Automate Review Settings

Vessel

Zoom: 2x 4x 10x 20x 40x

Objective: 2x 4x 10x 20x 40x

Light source: DAPI GFP TX Red CYS Trans

Light: Bright: 0.000

AutoFocus: Coarse: 0.000 Fine: 0.000

Capture

Record Video Capture Z Stack Capture Channels

Save...

46

Capture tab

InVivoGen EVOS FL Auto 2.0 Imaging System

Locations

Capture Automate Review Settings

Vessel

Zoom Genet. Slide - Facing Down

Objective 2x 4x 10x 20x 40x

Light source DAPI GFP TX Red CY5 Trans

Light Bright: 510

AutoFocus Coarse: 311 Fine: 311

Capture

Record Video Incubator Capture Z Stack Capture Channels

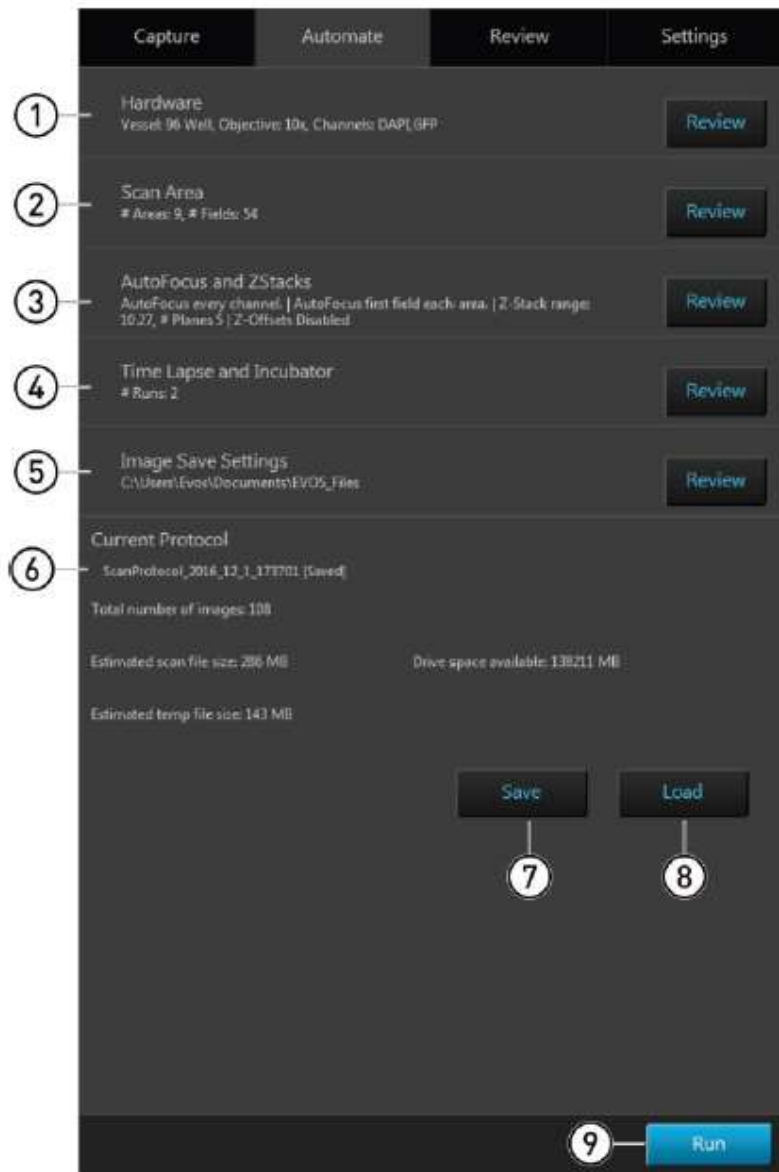
Brightness **Contrast** **Gamma**

GFP Brightness: 0.50 Contrast: 0.50 Gamma: 1.00 [Reset]

DAPI Brightness: 0.50 Contrast: 0.50 Gamma: 1.00 [Reset]

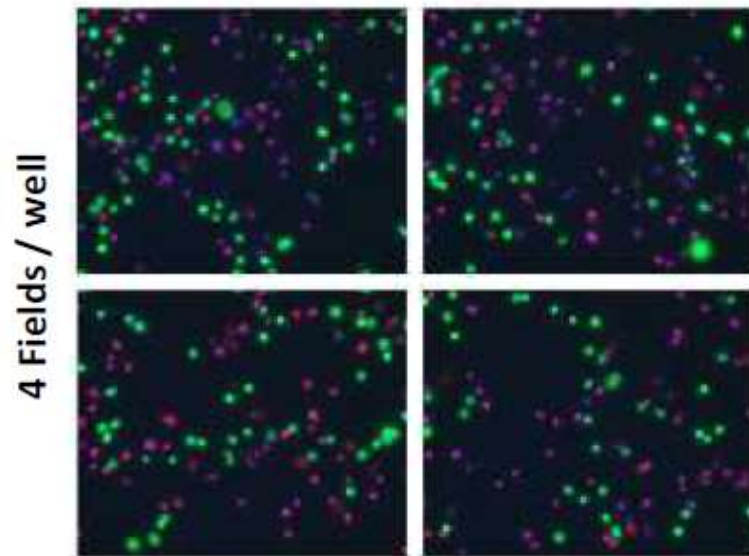
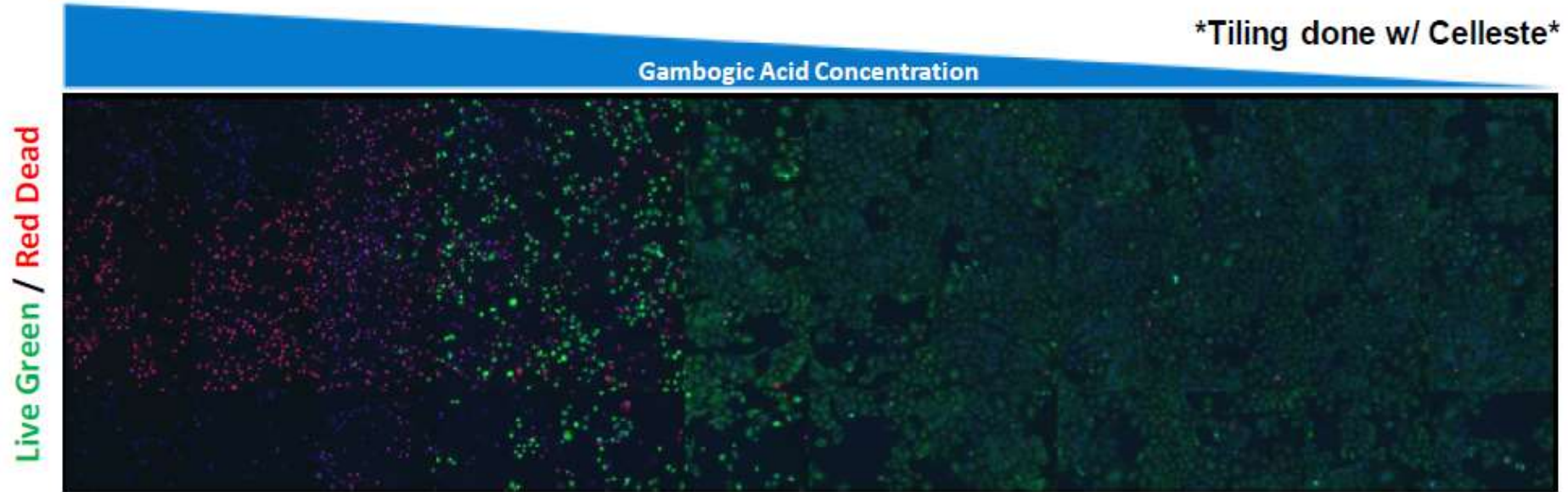
TX Red Brightness: 0.50 Contrast: 0.50 Gamma: 1.00 [Reset]

High brightness: Increases sensitivity and decreases dynamic range

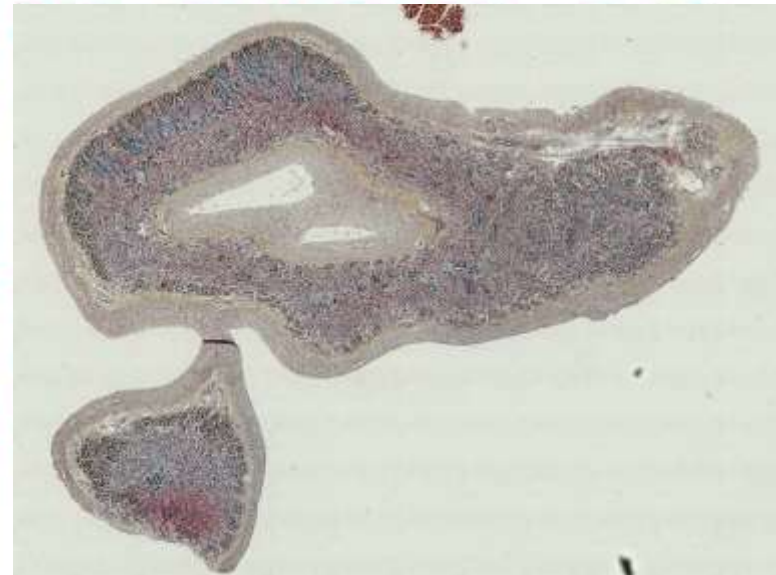


- 1 Hardware:** Allows you to configure hardware options (such as sample vessel, objective, light source etc.) for the scan protocol
- 2 Scan Area:** Allows you to specify the scan areas and fields to capture for the scan protocol
- 3 AutoFocus and Z Stacks:** Allows you to configure AutoFocus options 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)

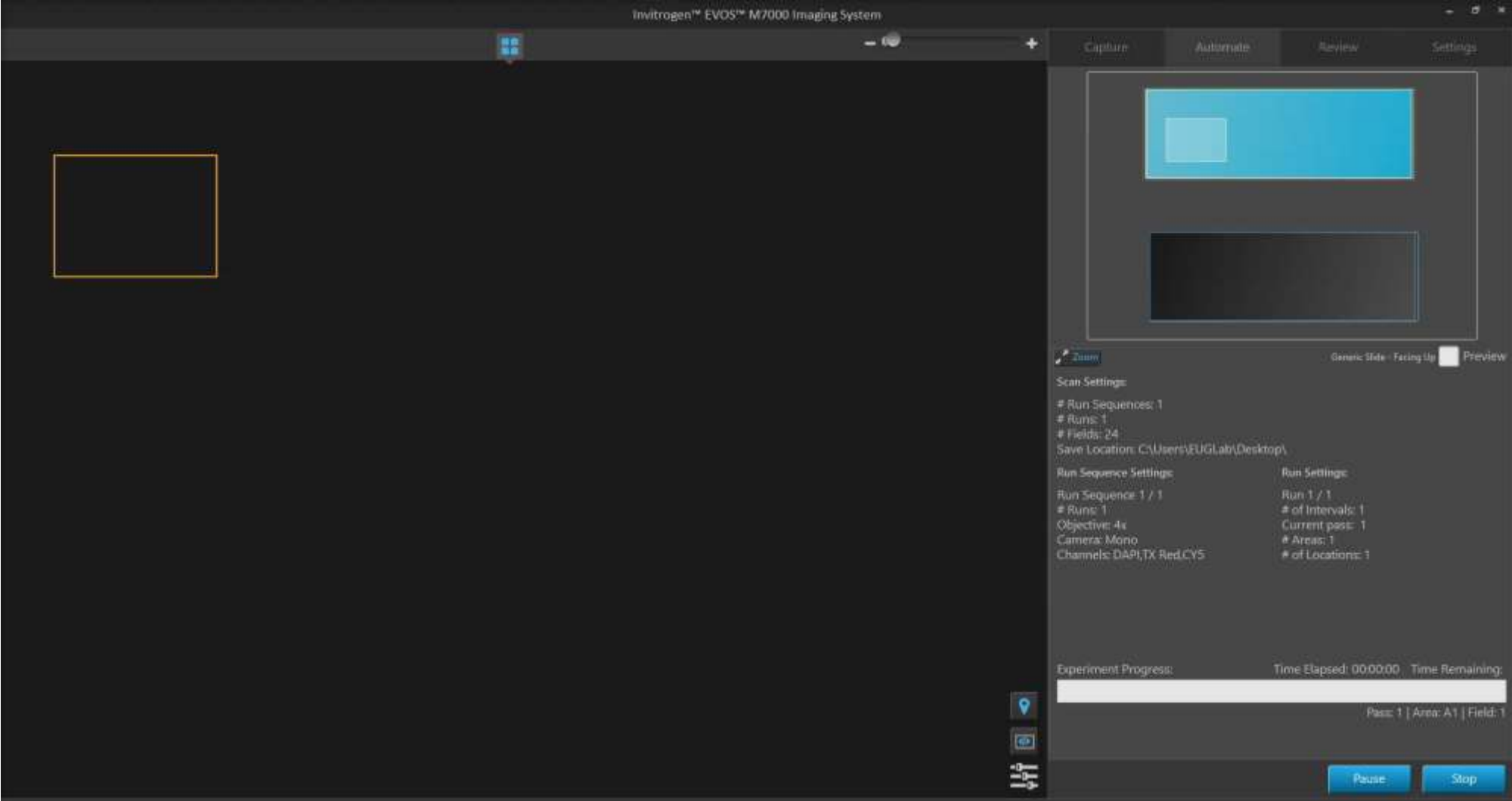


Stitch



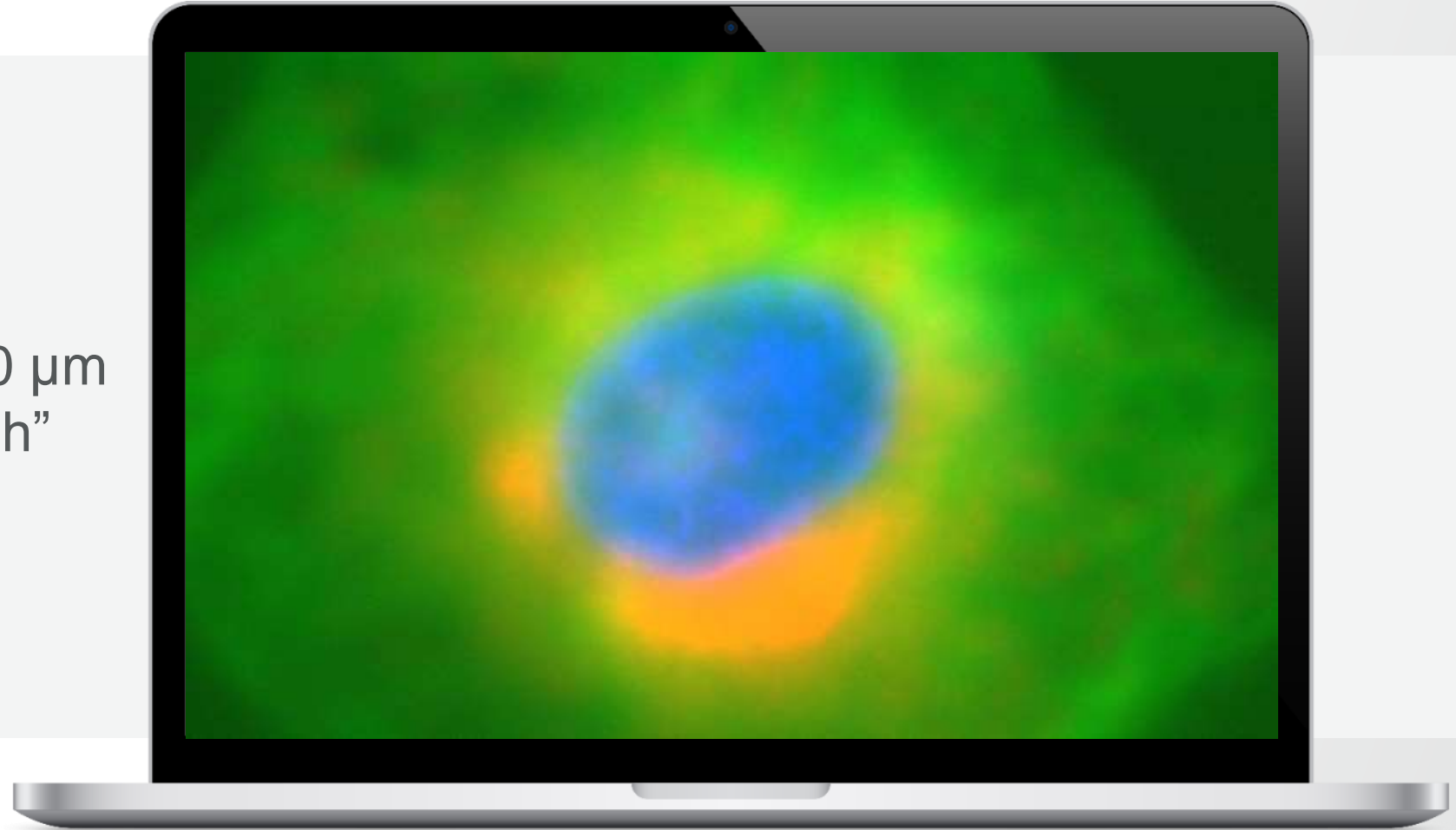
Imaging Over Space: X and Y Scanning

Blazingly fast acquisition and tile-stitching speed



Imaging Through Space: Z-Stack

Z-stacks: Collect layers in step sizes down to $0.150\ \mu\text{m}$ thickness and “walk through” an object



Automate tab : Time Lapse and Incubator

Capture Automate Review Settings

Time Lapse and Incubator

Use Time Lapse Use Incubator

Run 1

Duration: 8 Hours : 30 Minutes : 0 Seconds

Delay Start: 0 Hours : 30 Minutes : 0 Seconds

Image capture frequency:

Frequency 8 Hours : 30 Minutes : 0 Seconds

1 Intervals

As fast as possible

Incubator:

Temperature: 37.00 °C Use humidity

Co2: 5.00 %

Oxygen: 0.00 %

Shutdown:

Turn off manually

Turn off at end of experiment

Turn off after: 0 hr 30 min

Add run +

Autofocus Settings

First time point only

Every time point

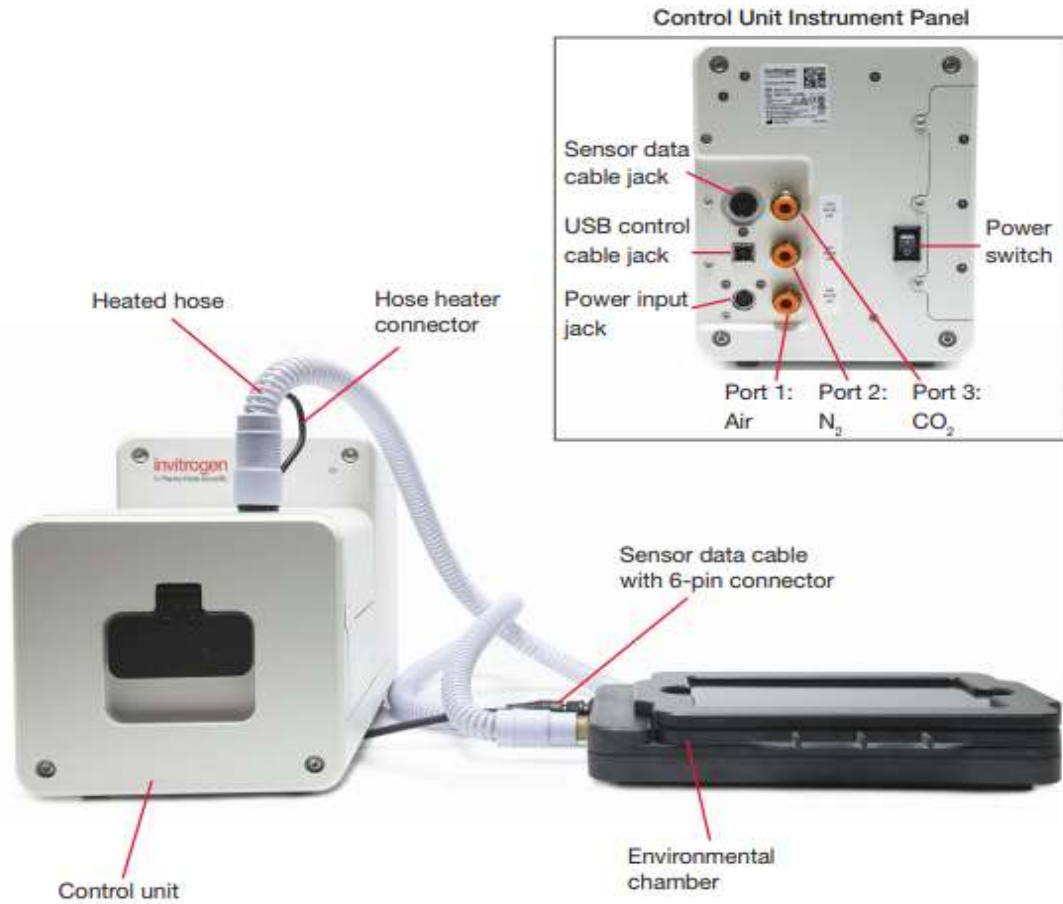
In event of autofocus fail, skip autofocus for location at subsequent time points? Yes No

Cancel Done

- 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

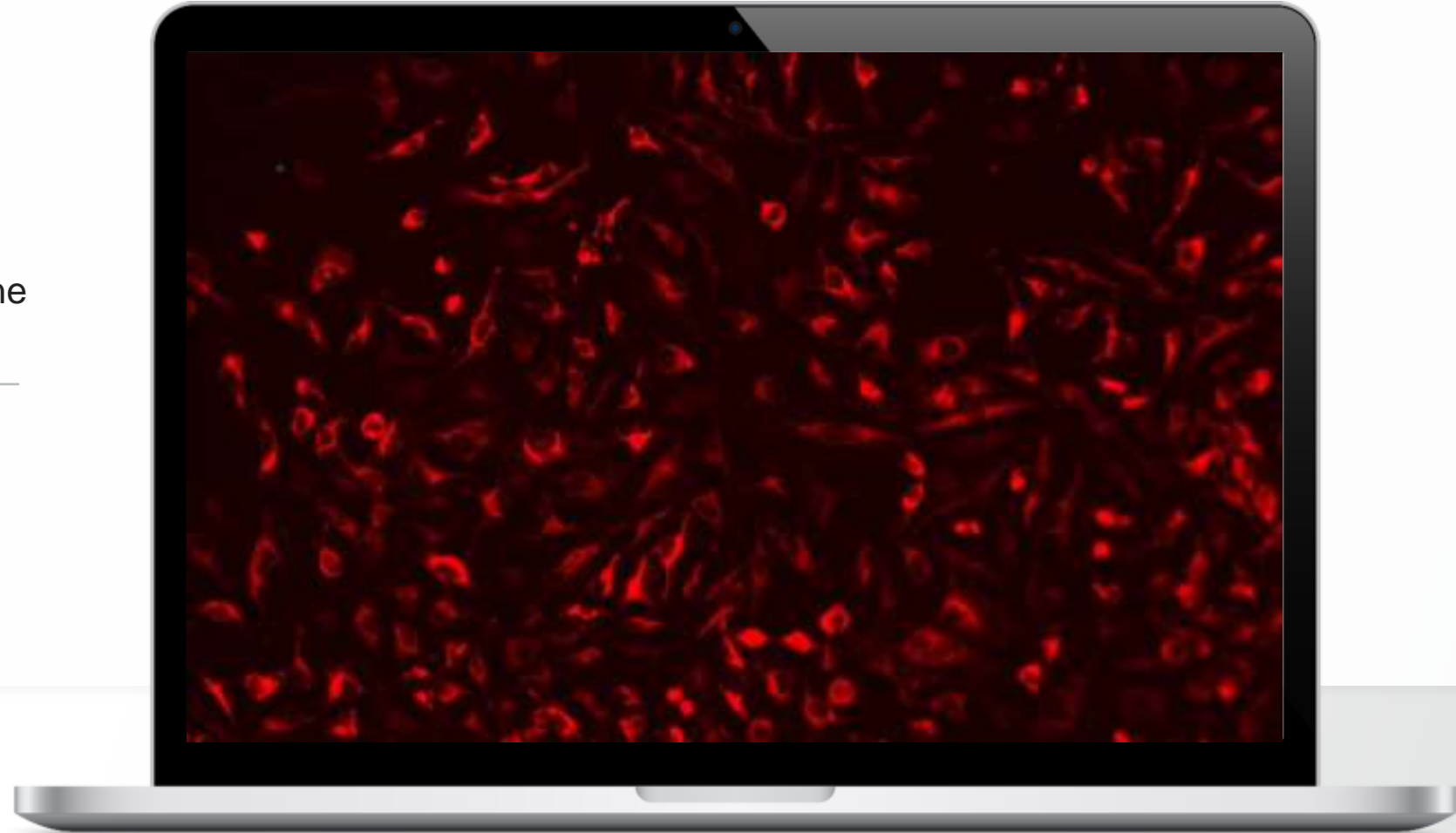
Apoptosis and Toxicity

Red: TMRM (mitochondrial membrane potential indicator)

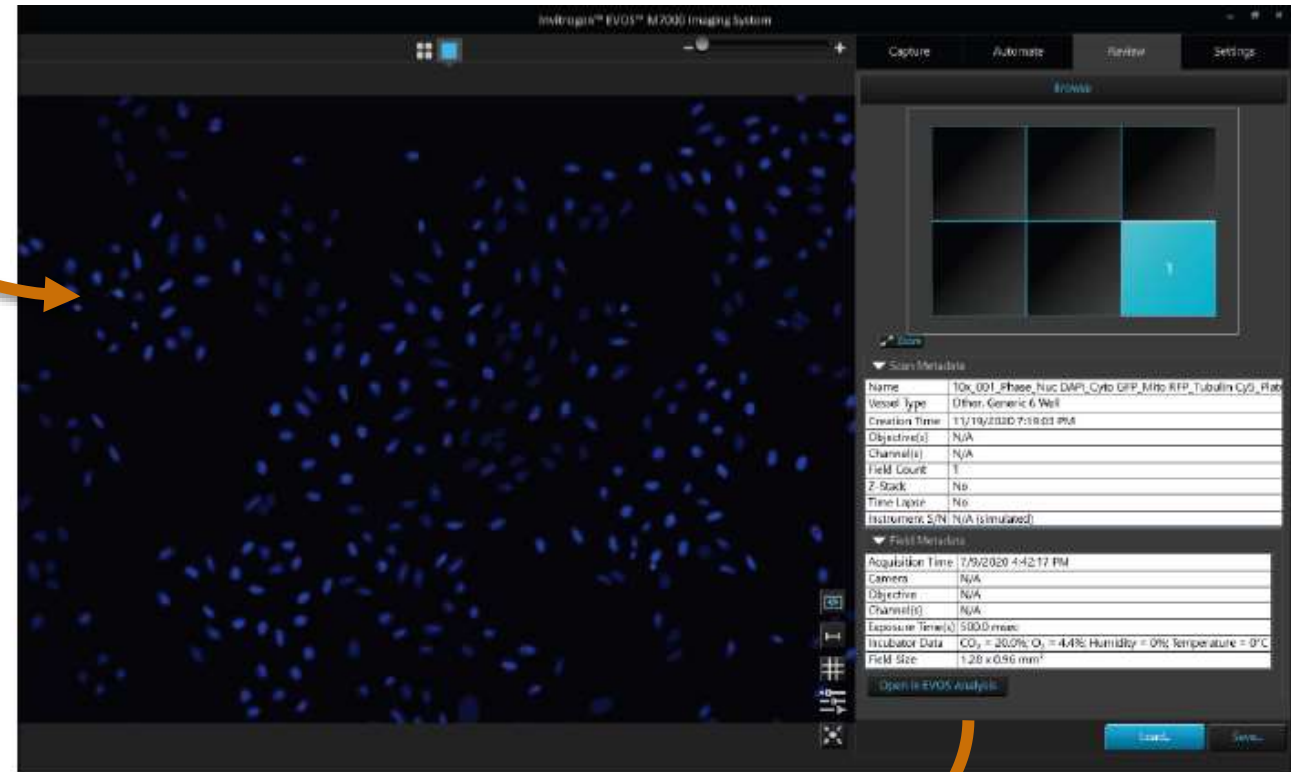
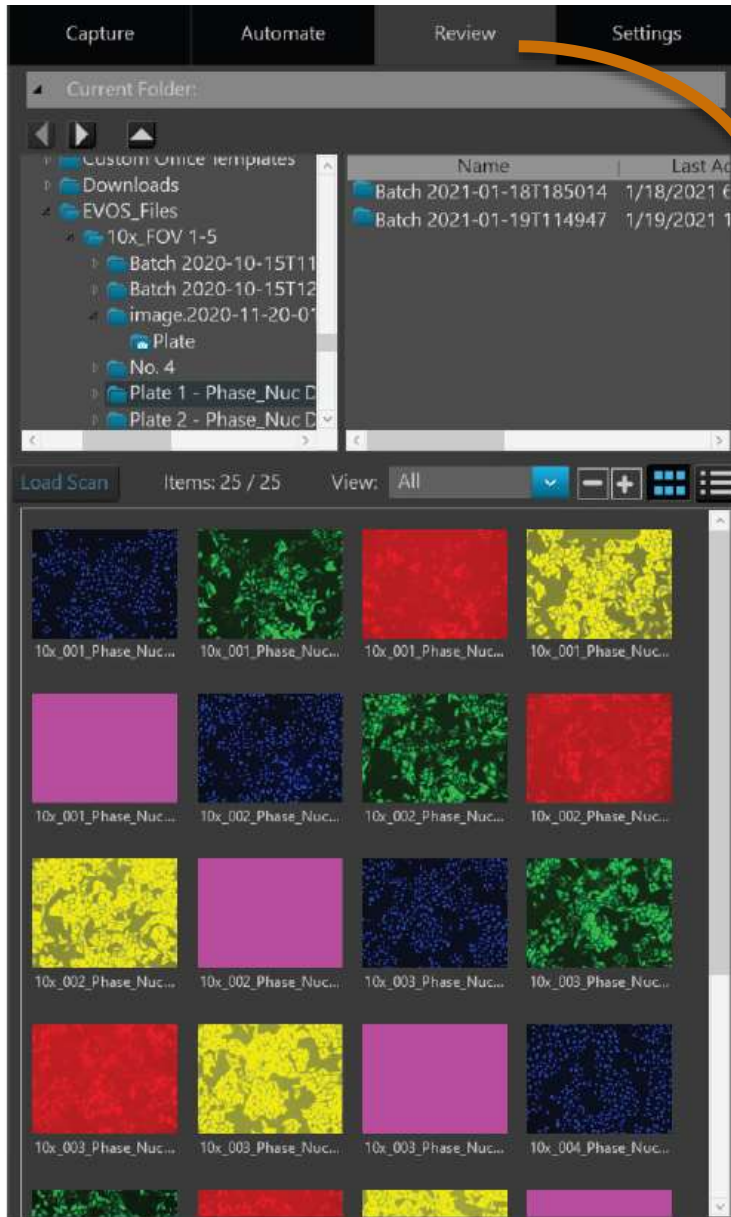
Fading red signal indicates loss of membrane potential and pre-lethal toxicity

Green: Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent

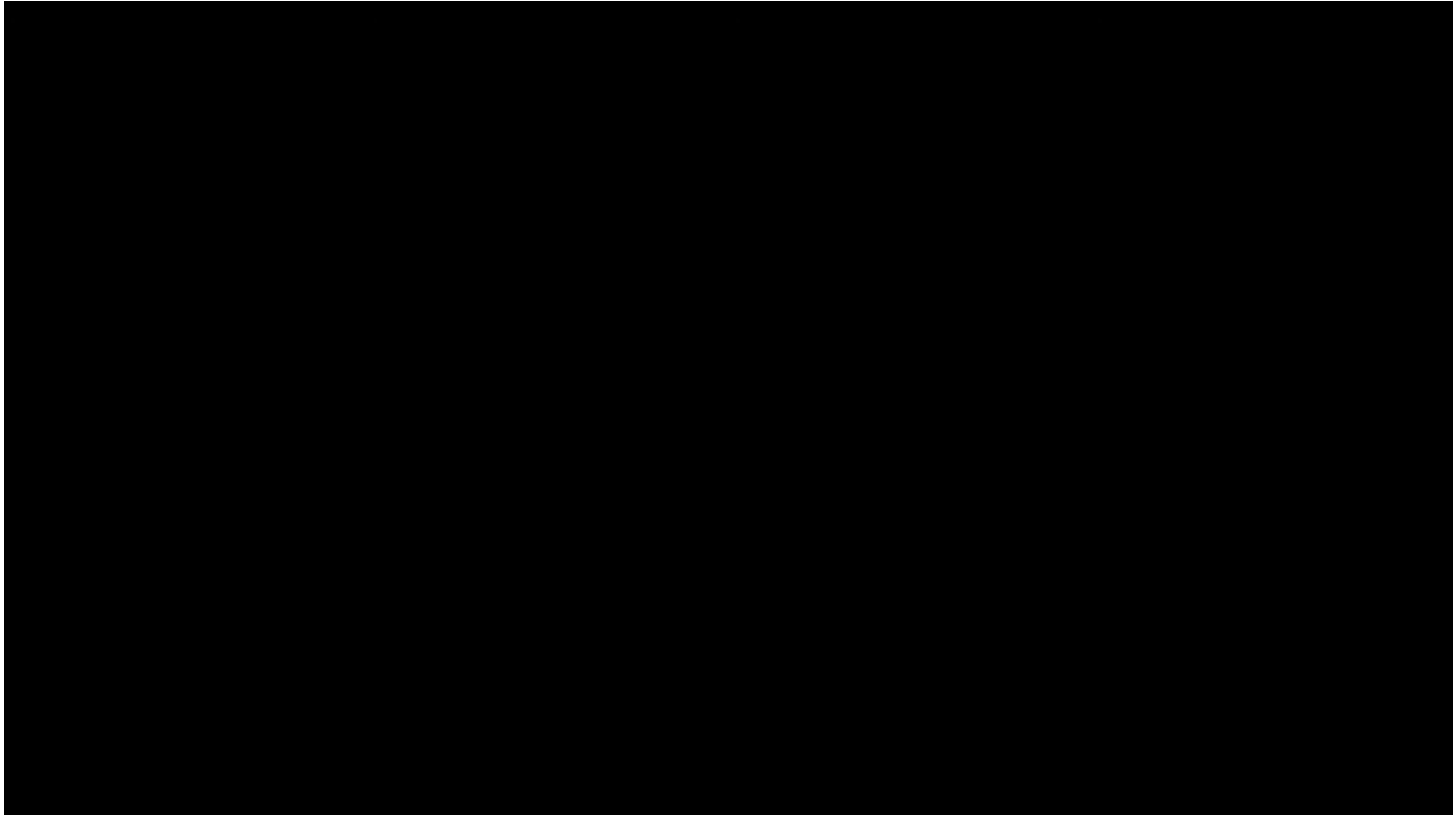
Green signal increases with onset of apoptosis



EVOS Analysis



Open in EVOS Analysis



Celleste Image Analysis Software

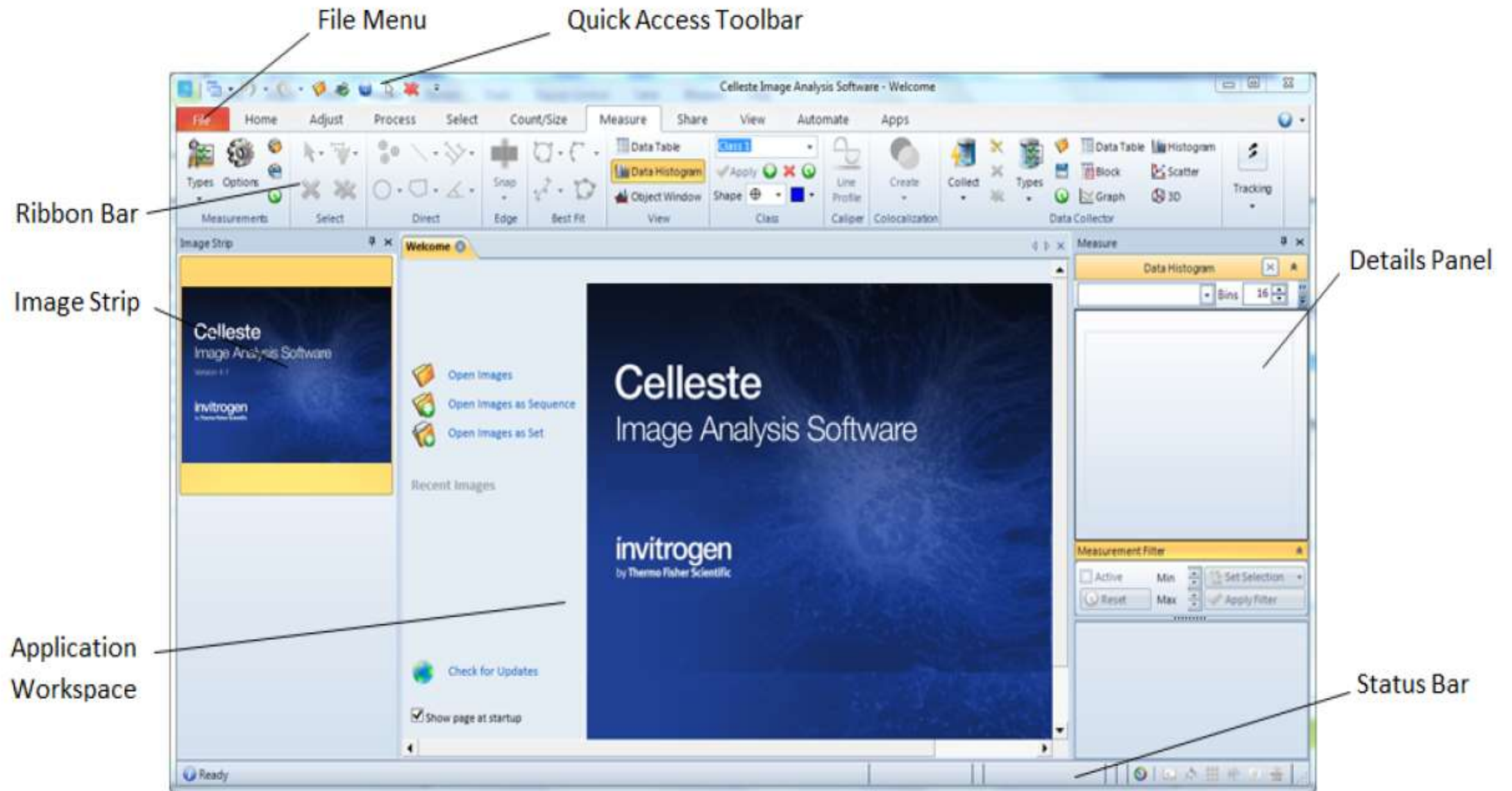
What is Celleste software?

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



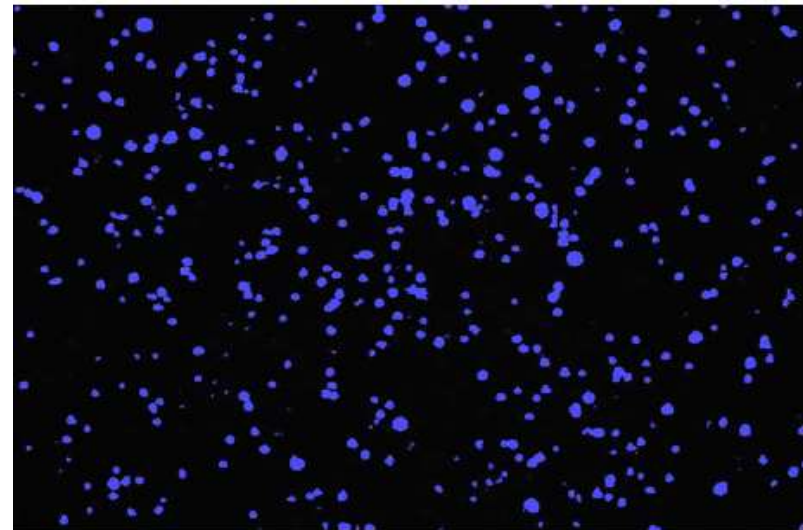
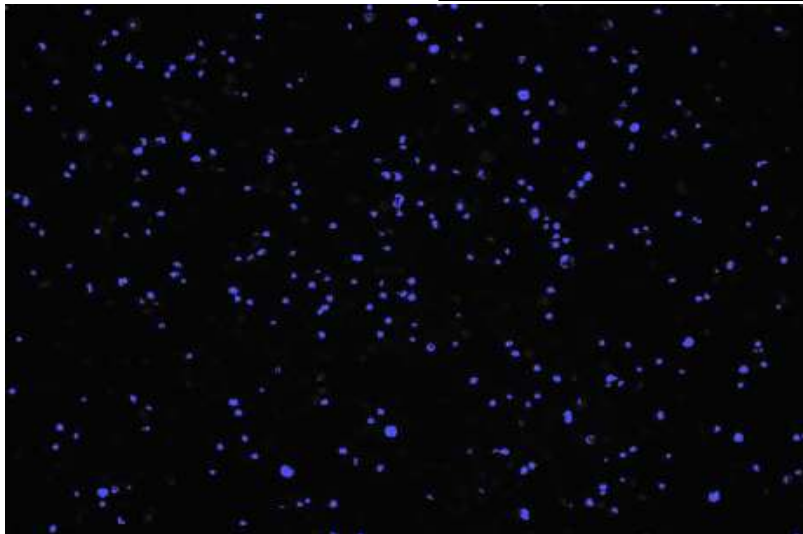
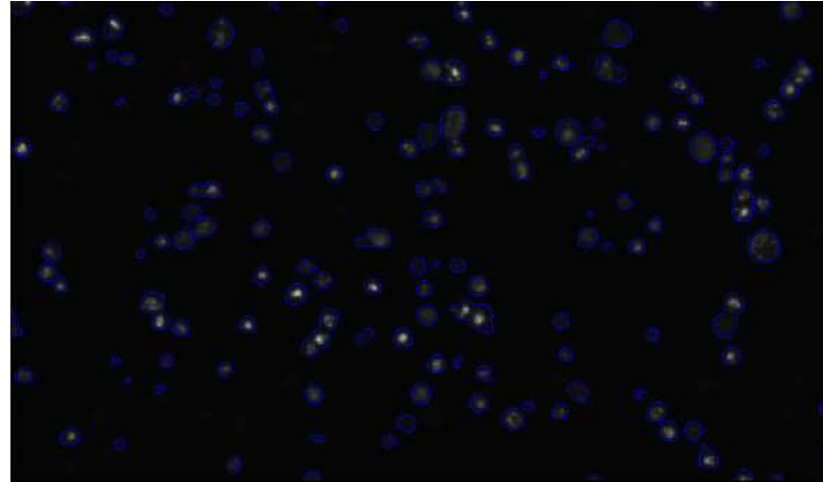
Celleste Image Analysis Software



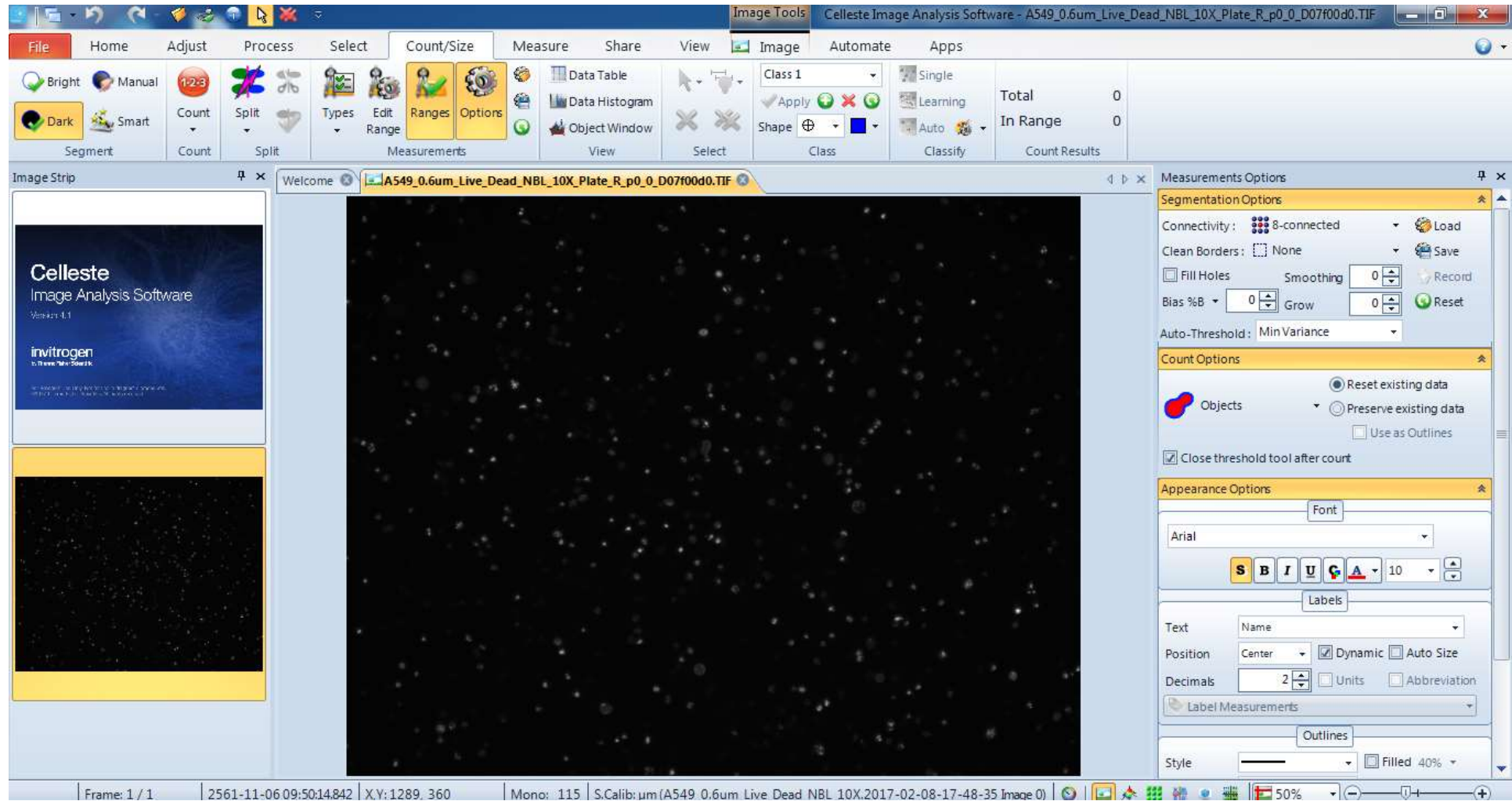
Celleste Image Analysis Software

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

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a microscopy image of a cell culture plate with numerous bright spots. The software is in the 'Image Tools' tab, and the 'Threshold Tool' is active. The 'Threshold Tool' panel on the right shows a histogram of the image's pixel intensity, with a threshold range set from 457 to 4095. The 'Count' button is highlighted, indicating that the software is counting the objects within the selected threshold range. The status bar at the bottom shows the current frame (1/1), date and time (2561-11-06 09:50:14.842), coordinates (X,Y: 1293, 388), and other parameters (Mono: 71, S.Calib: µm (A549_0.6um Live_Dead_NBL_10X.2017-02-08-17-48-35 Image 0)).

| Class | Total | In Range |
|---------|-------|----------|
| Class 1 | 0 | 0 |

| Start | End |
|-------|------|
| 457 | 4095 |

Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a microscopy image of cells with a threshold applied, resulting in blue highlights on a black background. The software's ribbon menu includes File, Home, Adjust, Process, Select, Count/Size, Measure, Share, View, Image, Automate, and Apps. The 'Count/Size' tab is active, showing a 'Count' button and a 'Measure' button. The 'Measure' panel on the right is open, displaying a histogram and a 'Threshold Tool' window. The histogram shows a distribution of pixel intensities, with a vertical line indicating the current threshold. The 'Threshold Tool' window has a 'Count' button and a 'Measure' button. A red box labeled 'Rename' points to the 'Class 1' dropdown menu in the 'Threshold Tool' window. Another red box labeled 'Click to count' points to the 'Count' button in the 'Measure' panel. The status bar at the bottom shows 'Frame: 1 / 1', '2561-11-06 09:50:14.842', 'X,Y: 1116, 26', 'Mono: 75', 'S,Calib: µm (A549_0.6um Live Dead NBL 10X.2017-02-08-17-48-35 Image 0)', and '50%'.

Rename

Click to count

Frame: 1 / 1 | 2561-11-06 09:50:14.842 | X,Y: 1116, 26 | Mono: 75 | S,Calib: µm (A549_0.6um Live Dead NBL 10X.2017-02-08-17-48-35 Image 0) | 50%

Celleste Image Analysis Software

Result

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a segmented image of cells, with each cell labeled with a unique ID (e.g., P1R1, P1R2, P1R7, P1R5, P1R6, P1R3, P1R13, P1R14, P1R16, P1R8, P1R10, P1R18, P1R19, P1R23, P1R12, P1R9, P1R22, P1R25, P1R24, P1R27, P1R21, P1R20, P1R26, P1R29, P1R65, P1R44, P1R46, P1R35, P1R28, P1R42, P1R41, P1R62, P1R48, P1R59, P1R38, P1R64, P1R51, P1R75, P1R66, P1R52, P1R83, P1R72, P1R63, P1R76, P1R89, P1R71, P1R85, P1R69, P1R68, P1R56, P1R94, P1R102, P1R88, P1R90, P1R128, P1R91, P1R117, P1R98, P1R97, P1R95, P1R113, P1R104, P1R129, P1R122, P1R111, P1R99, P1R106, P1R110, P1R105, P1R131, P1R120, P1R129, P1R127, P1R123, P1R125, P1R133, P1R160, P1R138, P1R148, P1R164, P1R139, P1R154, P1R146, P1R147, P1R173, P1R176, P1R165, P1R170, P1R177, P1R172, P1R179, P1R187, P1R196, P1R224, P1R180, P1R189, P1R201, P1R205, P1R200, P1R215, P1R215, P1R229, P1R211, P1R223, P1R227, P1R217, P1R214, P1R233, P1R221, P1R231, P1R238, P1R248, P1R242, P1R239, P1R243, P1R235, P1R246, P1R275, P1R234, P1R252, P1R258, P1R273, P1R266, P1R256, P1R263, P1R268, P1R278, P1R267, P1R270, P1R272, P1R283, P1R281, P1R265, P1R271, P1R282, P1R270, P1R272, P1R297, P1R310, P1R291, P1R295, P1R289, P1R282, P1R270, P1R272, P1R317, P1R310, P1R322, P1R311, P1R306, P1R301, P1R300, P1R299, P1R302, P1R334, P1R330, P1R333, P1R326, P1R345, P1R337, P1R324, P1R351, P1R358, P1R363, P1R339, P1R343, P1R341, P1R353, P1R354, P1R350, P1R361, P1R357, P1R355, P1R348, P1R366, P1R356, P1R367, P1R375, P1R383, P1R370, P1R395, P1R389, P1R387, P1R381, P1R373, P1R367, P1R375, P1R383, P1R389, P1R395, P1R387, P1R381, P1R373, P1R367, P1R375, P1R383, P1R427, P1R407, P1R398, P1R422, P1R416, P1R406, P1R384, P1R397, P1R409, P1R418, P1R413, P1R421, P1R403, P1R411, P1R432, P1R418, P1R426, P1R411).

The interface includes a menu bar (File, Home, Adjust, Process, Select, Count/Size, Measure, Share, View, Image, Automate, Apps), a toolbar with various analysis tools, and a central image strip. On the right, there are panels for 'Measurements Options' (Segmentation and Count Options) and 'Appearance Options' (Font, Labels, Outlines). A 'Count Results' table is visible in the top right corner:

| Count Results | Total | In Range |
|---------------|-------|----------|
| | 576 | 448 |

The status bar at the bottom shows: Left: Frame: 1 / 1, 2561-11-06 09:50:14.842, X,Y: 1231, 493, Mono: 114, S.Calib: µm (A549_0.6um_Live_Death_NBL_10X.2017-02-08-17-48-35_Image_0), 50% zoom.

Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a segmented image of cells, each labeled with a unique ID (e.g., P1R1, P1R2, etc.). The software includes a comprehensive toolbar with options for image adjustment, segmentation, and measurement. A 'Data Table' window is open at the bottom, displaying a list of measured cells with their respective area and percent area.

Measurement Table

| Feature Name | Area(μm ²) | Class Name | Percent Area Pa... |
|------------------------------|------------------------|------------|--------------------|
| Class Name: Cells Count: 448 | | | |
| P1R1 | 122.09 | Cells | 0.01 |
| P1R2 | 67.49 | Cells | 0.01 |
| P1R3 | 138.77 | Cells | 0.01 |
| P1R4 | 134.22 | Cells | 0.01 |
| P1R5 | 117.54 | Cells | 0.01 |

An arrow points to the 'Data table' window, which is highlighted in red.

Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a microscopy image of cells. A 'Split' menu is open, showing options: 'Split', 'Split Selected', 'Split Objects with Count' (checked), 'Separation Method' (with 'Watershed' selected), 'Boundary Shape' (checked), and 'Ridge size' (set to 20). The software has a ribbon-style menu with tabs: File, Home, Adjust, Process, Select, Count/Size, Measure, Share, View, Image, Automate, and Apps. The 'Count/Size' tab is active, showing a 'Count Results' table with 'Total' 576 and 'In Range' 462. The 'Image' tab is also visible, showing 'Apply', 'Shape', and 'Class' options. On the right, the 'Measurements Options' panel is open, showing 'Count Options' (with 'Reset existing data' selected), 'Appearance Options' (with 'Font' set to Arial and size 10), 'Labels' (with 'Text' set to None and 'Position' set to Center), 'Outlines' (with 'Style' set to a solid line and 'Width' set to 1), and 'Cursor Options' (with 'Cross Bars' and 'Crosshair' unchecked, and 'Tooltip' checked). The status bar at the bottom shows 'Frame: 1 / 1', '2561-11-06 09:50:14.842', 'X,Y: 450, 383', 'Mono: 123', 'S.Calib: μm (A549_0.6um_Live_Death_NBL_10X.2017-02-08-17-48-35_Image_0)', and '144%' zoom.

Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a microscopy image of blue fluorescent spots. The software is running the 'Measure' tab, with the 'Image Tools' ribbon active. The 'Available Measurements' panel on the left lists various measurement categories: All Types, Object, Point, and Region. The 'Selected Measurements / Filters' table shows the following data:

| Measurement | Minimum | Maximum |
|------------------------|---------|---------|
| Object:Class Name | | |
| Region:Area | 0 | 1E+308 |
| Region:Intensity, M... | -1E+308 | 1E+308 |
| Region:Roundness | 0 | 1000000 |

The 'Measurements Options' panel on the right is configured with the following settings:

- Auto-Threshold: Min Variance
- Count Options: Reset existing data, Preserve existing data, Use as Outlines
- Appearance Options: Font: Arial, Size: 10
- Text: None, Position: Center, Dynamic, Auto Size, Decimals: 2, Units, Abbreviation
- Outlines: Style: Solid, Filled 40%, End Points: None, Pixel Aligned, Width: 1, Outline Pixels
- Cursor Options: Cross Bars, Crosshair, Tooltip

The status bar at the bottom indicates: Left-... Frame: 1 / 1 | 2561-11-06 09:50:14.842 | X,Y: 218, 28 | Mono: 69 | S.Calib: µm (A549_0.6um_Live_Dead_NBL_10X.2017-02-08-17-48-35_Image_0) | 47%

Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a histogram of intensity values for a selected region. The histogram has a red bar chart with a vertical dashed line at the maximum value. The x-axis ranges from 0 to 500, and the y-axis ranges from 0 to 100. The histogram is titled "Edit Measurement Range" and shows the following data:

| Region | Area | Intensity, Mean | Roundness |
|------------------------|------|-----------------|-----------|
| Region:Area | 16 | | |
| Region:Area | | | Max |
| Region:Intensity, Mean | | | |
| Region:Roundness | | | |

The histogram also shows the following statistics:

- Objects in range: 410/587 (69.85%)
- Min: 32.91
- Max: 1.00E+308

The software interface includes a menu bar (File, Home, Adjust, Process, Select, Count/Size, Measure, Share, View, Image, Automate, Apps) and a toolbar with various tools. The main image area shows a dark field with red spots. The status bar at the bottom displays the following information: Ready, Frame: 1 / 1, 2561-11-06 09:50:14.842, X,Y: 1324, 30, Mono: 63, S.Calib: μm (A549_0.6um_Live_Dead_NBL_10X.2017-02-08-17-48-35_Image_0), 47% zoom.

Celleste Image Analysis Software

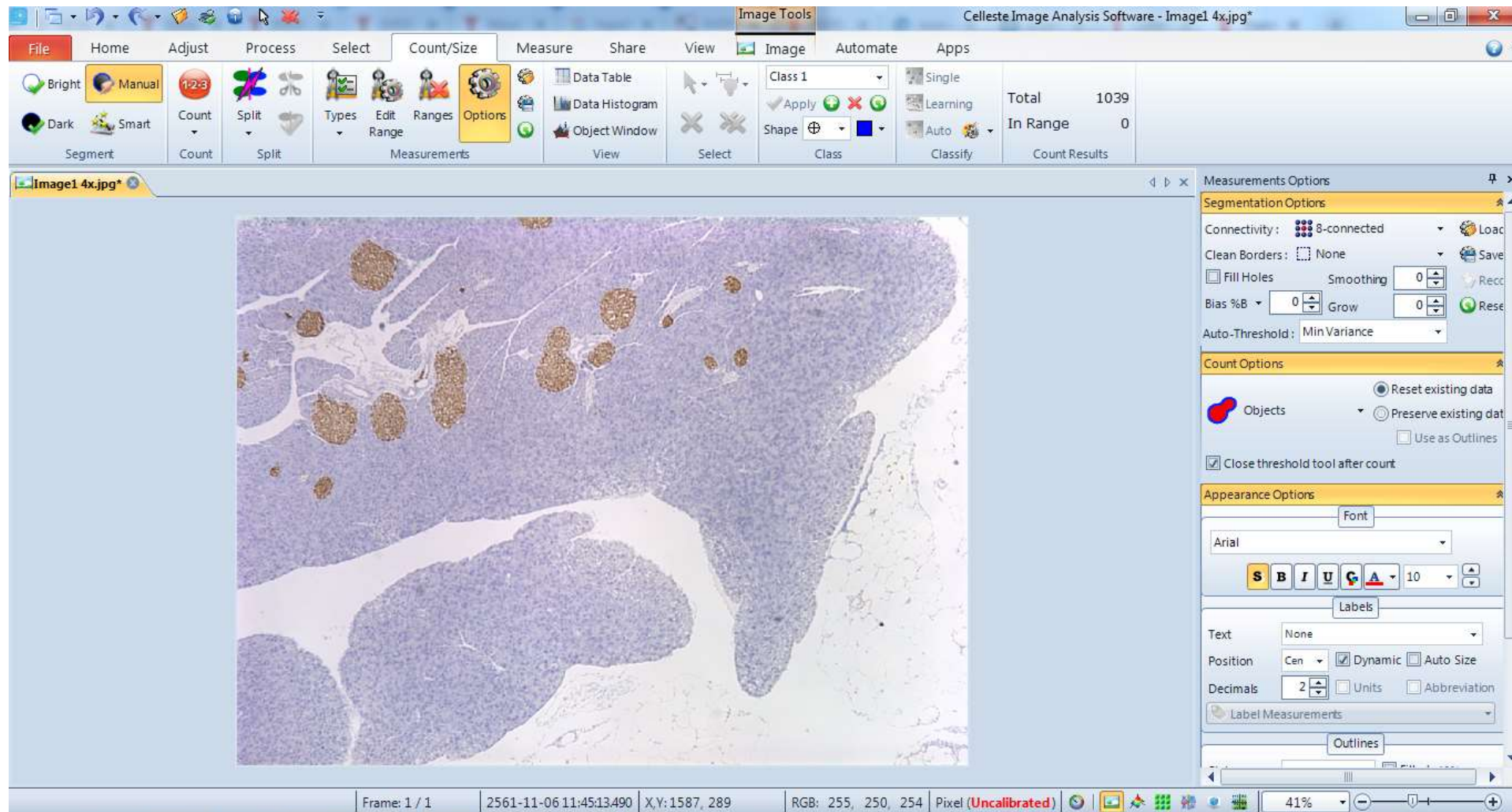
auto-threshold

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a microscopy image of cells with blue nuclei. The 'Measurements Options' panel on the right is open, showing 'Segmentation Options' with 'Auto-Threshold' set to 'Min Variance'. The 'Count Options' panel shows 'Objects' set to 'Reset existing data'. The 'Measurement Table' at the bottom displays the following data:

| Feature Name | Class Name | Area(μm²) | Intensity, Mean... | Roundness |
|------------------------------|------------|-----------|--------------------|-----------|
| Class Name: Cells Count: 344 | | | | |
| Class Name: Cells | | | | |
| Mean value | 0.00 | 177.26 | 412.93 | 1.16 |
| Standard Devia... | 0.00 | 93.45 | 111.75 | 0.26 |
| Minimum | 0.00 | 53.08 | 218.35 | 1.00 |
| Maximum | 0.00 | 551.30 | 754.91 | 2.63 |
| Range | 0.00 | 498.22 | 536.57 | 1.63 |
| Sum | 0.00 | 60977.38 | 142046.59 | 399.36 |
| Number of Ele... | 344.00 | 344.00 | 344.00 | 344.00 |

Celleste Image Analysis Software

Classification and Counting Multiple Classes



Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a histology image with red and green segmentation. The 'Image Tools' menu is open, and the 'Classify' button is highlighted with a red box and an arrow pointing to it. The 'Measure' panel on the right shows a histogram with a red peak and a green peak, and the 'Threshold Tool' is set to 'Hematoxylin'. The status bar at the bottom shows 'Pixel (Uncalibrated)' and '41%' zoom.

Classify

Color image usually use Hue

Ready | Frame: 1 / 1 | 2561-11-06 11:45:13.490 | X,Y: 1160, 398 | RGB: 185, 182, 199 | Pixel (Uncalibrated) | 41%

Celleste Image Analysis Software

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a histology image with segmented regions in red and purple. The software includes a menu bar (File, Home, Adjust, Process, Select, Count/Size, Measure, Share, View, Image, Automate, Apps) and a toolbar with various analysis tools. A 'Count/Size' panel on the right shows 'Total' and 'In Range' counts of 955. A 'Measurement Table' window is open, displaying a table with columns for 'Class Name', 'Feature Name', and 'Area(pix^2)'. The table contains two rows: 'Class Name: DAB Count: 81' and 'Class Name: Hematoxylin Count: 874'. A red box with the text 'Count >> result show' is overlaid on the table, with an arrow pointing to the 'Count' column. The 'Measurements Options' panel on the right shows 'Segmentation Options' (Connectivity: 8-connected, Clean Borders: None, Fill Holes: checked, Smoothing: 0, Bias %B: 0, Grow: 0, Auto-Threshold: Min Variance) and 'Count Options' (Reset existing data: selected, Objects: selected, Preserve existing data: unselected, Use as Outlines: unselected, Close threshold tool after count: checked). The 'Appearance Options' panel shows font settings (Arial, size 10) and label settings (Text: None, Position: Cen, Decimals: 2). The status bar at the bottom shows 'Frame: 1 / 1', '2561-11-06 11:45:13.490', 'X,Y:1434, 355', 'RGB: 243, 242, 240', 'Pixel (Uncalibrated)', and '42%' zoom.

| Class Name | Feature Name | Area(pix^2) |
|-------------------------|--------------|-------------|
| Class Name: DAB | Count: 81 | |
| Class Name: Hematoxylin | Count: 874 | |

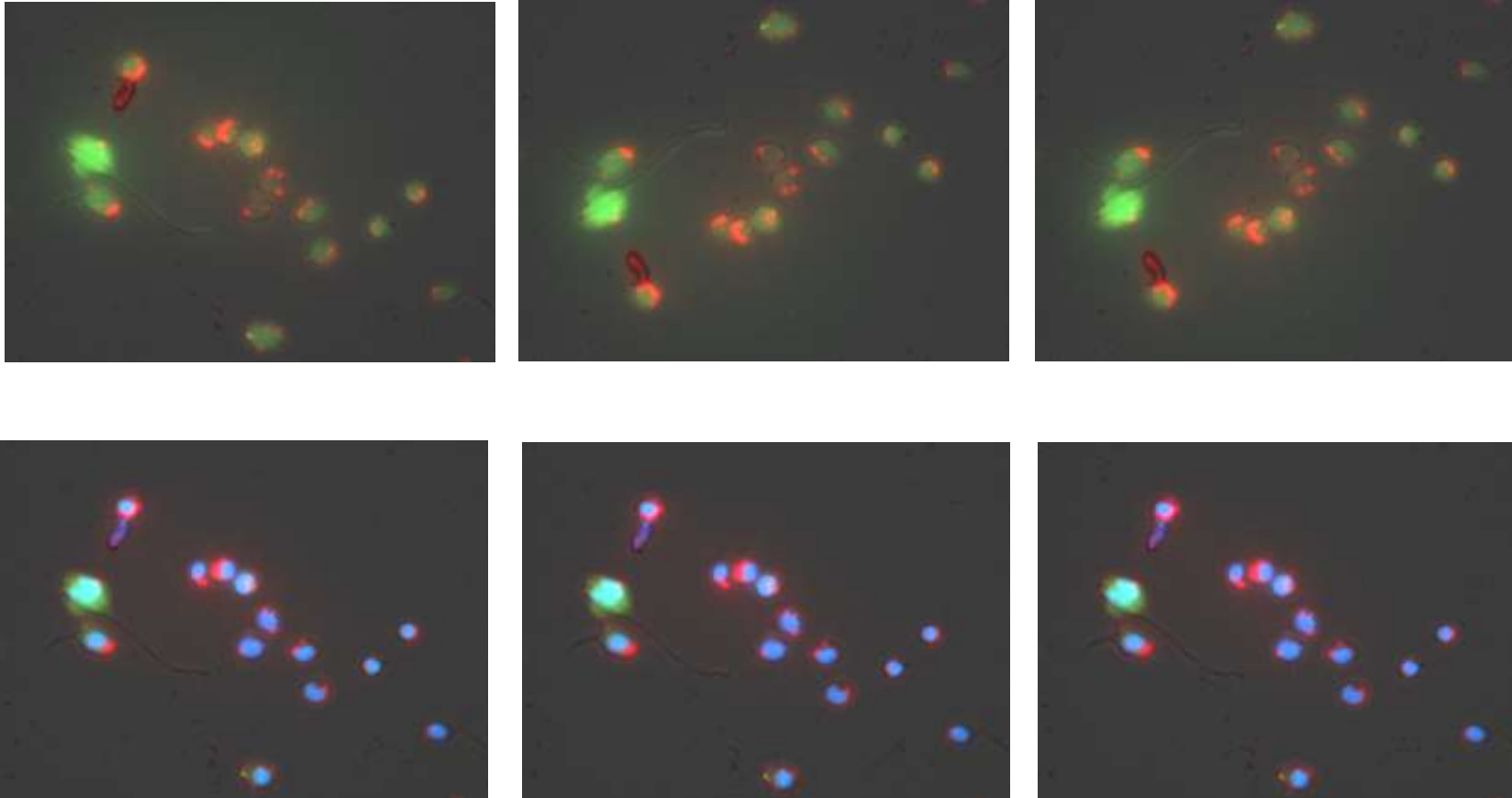
Count >> result show

Celleste Image Analysis Software

Intensity Tracking

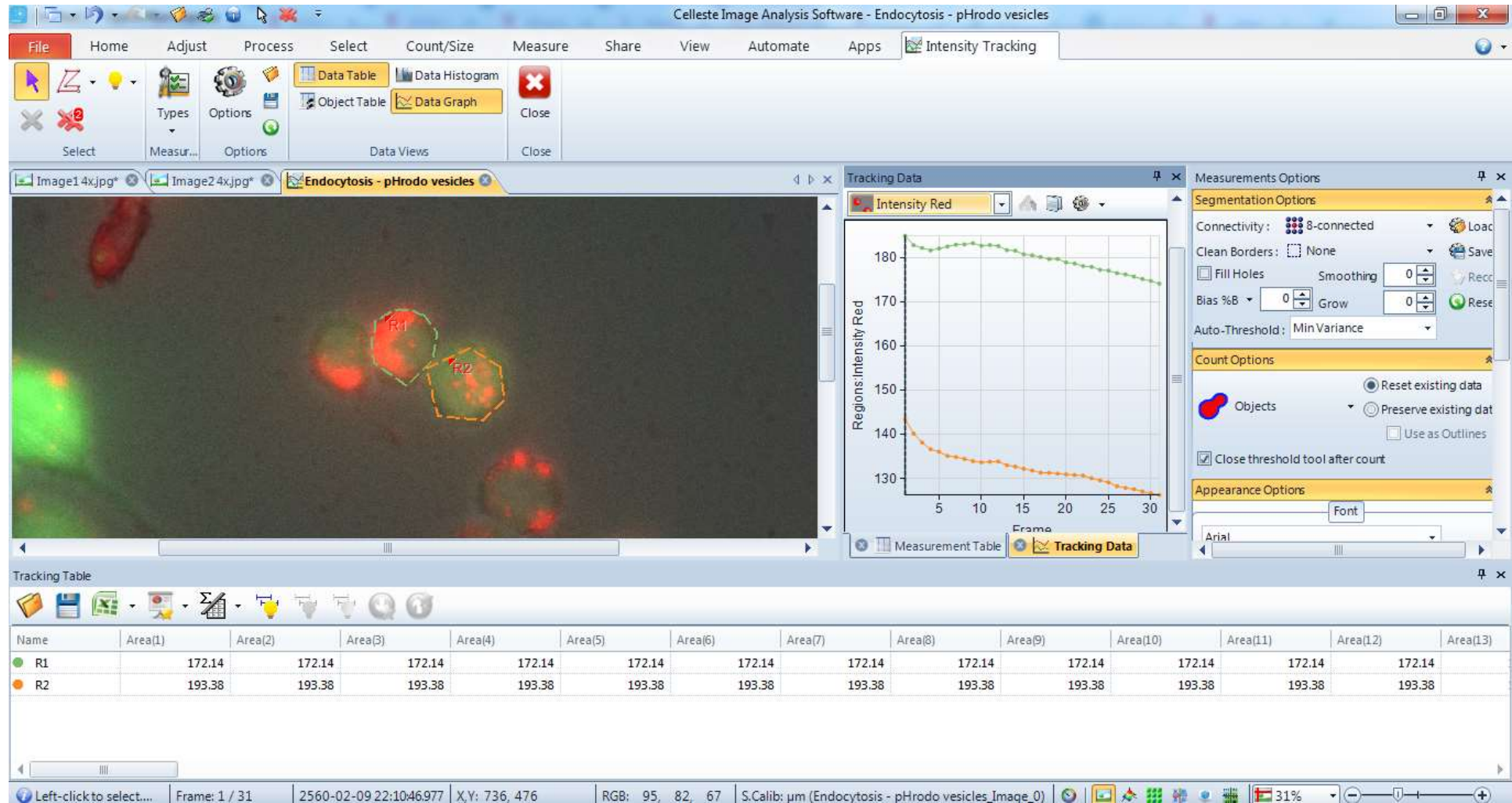
pHrodo Indicators for pH Determination

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

Tracking Data

Regions: Intensity Blue

| Frame | R1 Intensity | R2 Intensity |
|-------|--------------|--------------|
| 1 | 170 | 170 |
| 2 | 170 | 170 |
| 3 | 170 | 170 |
| 4 | 170 | 170 |
| 5 | 170 | 170 |
| 6 | 170 | 170 |
| 7 | 170 | 170 |
| 8 | 170 | 170 |
| 9 | 170 | 170 |
| 10 | 170 | 170 |
| 11 | 170 | 170 |
| 12 | 170 | 170 |
| 13 | 170 | 170 |
| 14 | 170 | 170 |
| 15 | 170 | 170 |
| 16 | 170 | 170 |
| 17 | 170 | 170 |
| 18 | 170 | 170 |
| 19 | 170 | 170 |
| 20 | 170 | 170 |
| 21 | 170 | 170 |
| 22 | 170 | 170 |
| 23 | 170 | 170 |
| 24 | 170 | 170 |
| 25 | 170 | 170 |
| 26 | 170 | 170 |
| 27 | 170 | 170 |
| 28 | 170 | 170 |
| 29 | 170 | 170 |
| 30 | 170 | 170 |

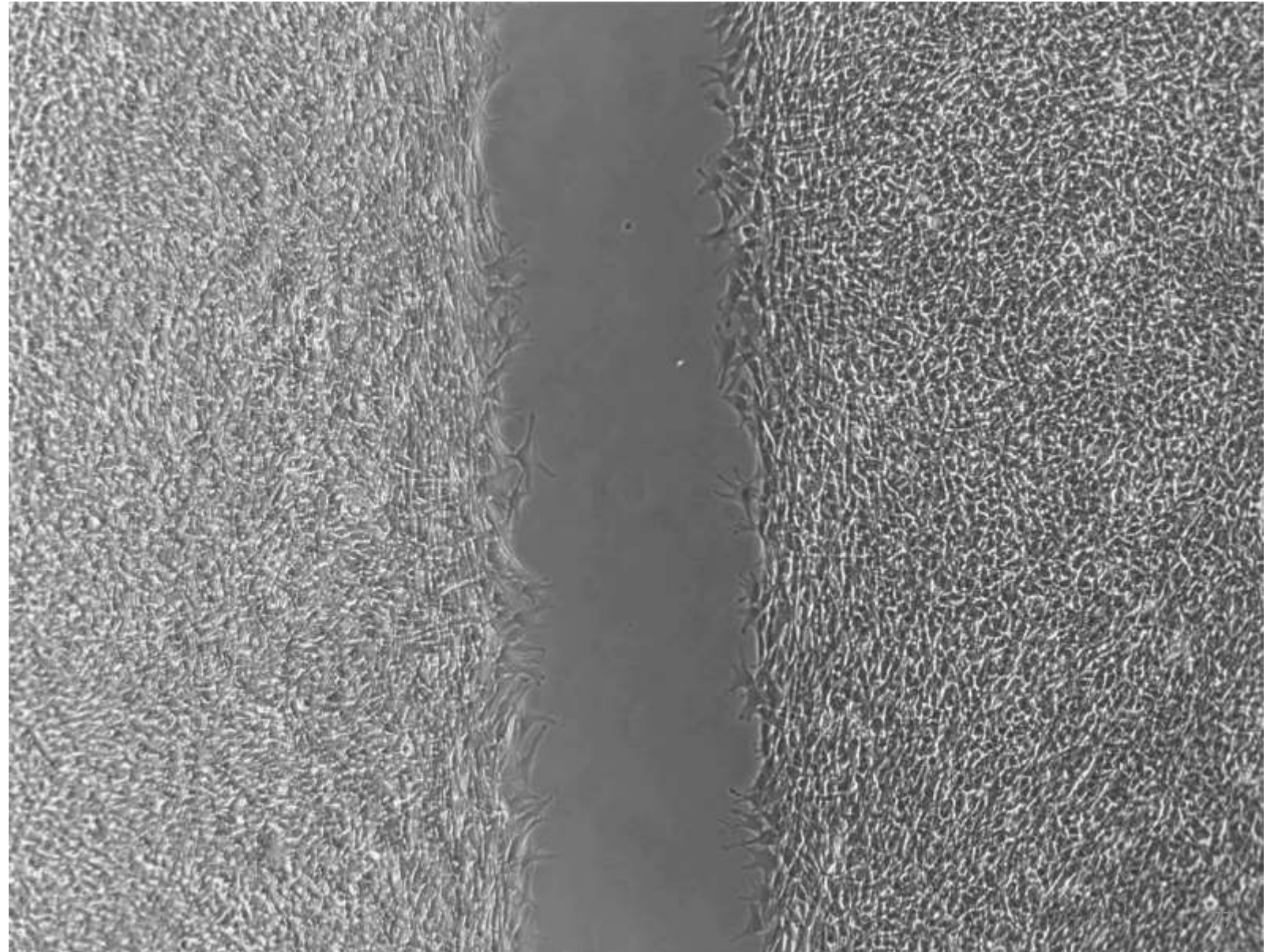
Tracking Table

| Name | Area(1) | Area(2) | Area(3) | Area(4) | Area(5) | Area(6) | Area(7) | Area(8) | Area(9) | Area(10) | Area(11) | Area(12) | Area(13) |
|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|----------|----------|
| R1 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 | 172.14 |
| R2 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 | 193.38 |

Left-click to select... | Frame: 5 / 31 | 2560-02-09 22:10:47.128 | X,Y: 1008, 492 | RGB: 58, 58, 58 | S:Calib: µm (Endocytosis - pHrodo vesicles_Image_0) | 31% | Speakers / Headphones: Muted

Celleste Image Analysis Software

Wound Healing



Celleste Image Analysis Software

Wound Healing

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a grayscale image of a wound with a blue outline. The software is running the 'Wound Healing' module. The 'Auto Threshold (%)' is set to 100, highlighted with a red circle and a pink label 'Auto threshold'. The 'Measure Wound Healing' button is also highlighted with a red circle. The 'Results' section shows the following data:

| Parameter | Value |
|----------------------|--------|
| Wound Area(%) | 42.756 |
| Area Rate(%/frame) | 4.403 |
| Edge Rate(pix/frame) | 6.359 |
| Time to 50%(frames) | 11.355 |

The 'Measure Wound Healing' button is highlighted with a red circle. The 'Batch Processing' section is visible at the bottom left. The 'Measurements Options' panel on the right shows various settings for segmentation, count, and appearance. The status bar at the bottom indicates 'Frame: 1 / 20', '2555-09-22 12:55:44.000', 'X,Y: 1171, 581', 'RGB: 166, 166, 166', 'Pixel (Uncalibrated)', and '52%' zoom.

Celleste Image Analysis Software

Wound Healing

The screenshot displays the Celleste Image Analysis Software interface. The main window shows a grayscale image of a wound with a blue outline. The software is running a 'Wound Healing' analysis on the image 'beacon 65 run two* (11/20)'. The 'Wound Healing' panel on the left shows the 'Auto Threshold (%)' set to 70. The 'Options' section includes checkboxes for 'Prompt to set Calibration', 'Auto Save File Name', 'Save Data', and 'Save Snapshot Sequence'. The 'Results' section displays the following data:

| Results | Value |
|----------------------|--------|
| Wound Area(%) | 42.756 |
| Area Rate(%/frame) | 4.403 |
| Edge Rate(pix/frame) | 6.359 |
| Time to 50%(frames) | 11.355 |

The 'Batch Processing' section includes a 'Folder' field and an 'Extension' field set to '*.tif;*.tiff'. The 'Measure Wound Healing' button is visible. The 'Measurements Options' panel on the right shows 'Segmentation Options' with 'Connectivity' set to 8-connected, 'Clean Borders' set to None, and 'Auto-Threshold' set to Min Variance. The 'Count Options' section includes 'Reset existing data' and 'Preserve existing data' options. The 'Appearance Options' section shows 'Font' set to Arial, 'Text' set to None, 'Position' set to Center, and 'Decimals' set to 2. The 'Outlines' section shows 'Style' set to Filled 40%.

The status bar at the bottom indicates: Left-click to select. Ctrl-left-click to add to or remov... Frame: 11 / 20 | 2555-09-22 12:55:54.000 | X,Y:1297, 825 | RGB: 108, 108, 108 | Pixel (Uncalibrated) | 52%

Celleste Image Analysis Software

Tile Image

FL Auto 2 Stitch



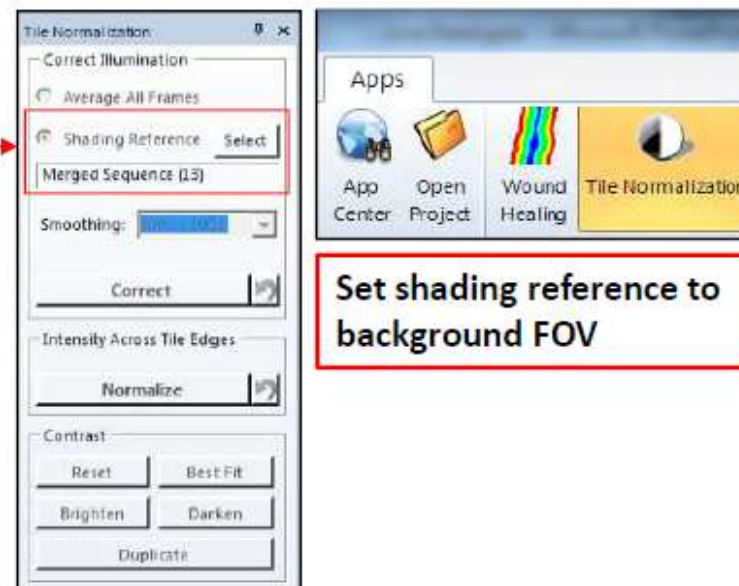
Celleste Corrected



1. Assemble overlapping images



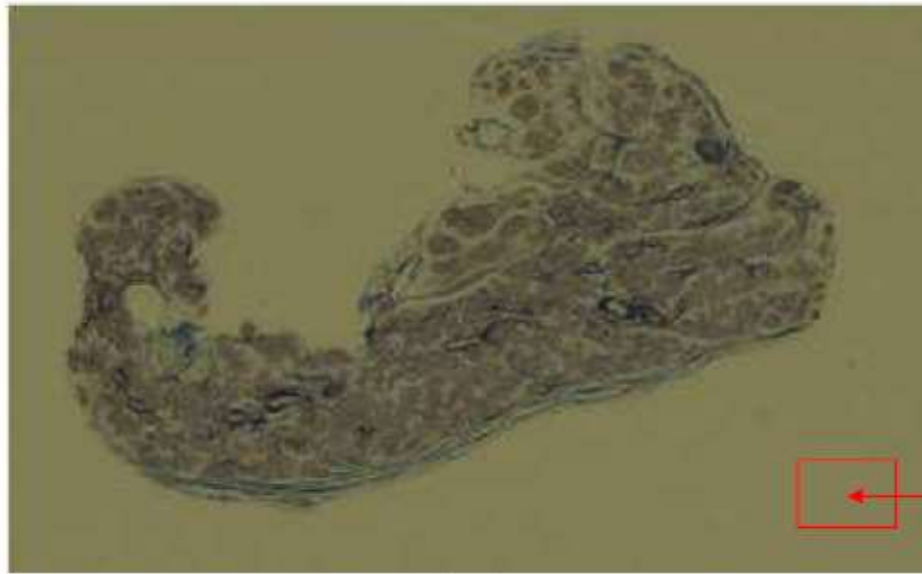
2. Correct edges using Tile App



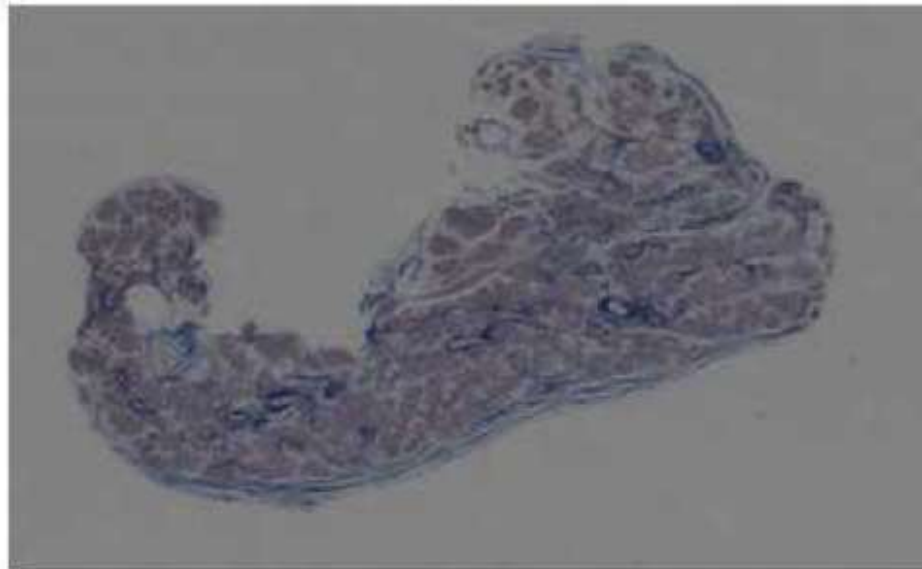
Data by: Trillium Blackmer
Celleste by: Oggie Golub

Celleste Image Analysis Software

Celleste Stitched



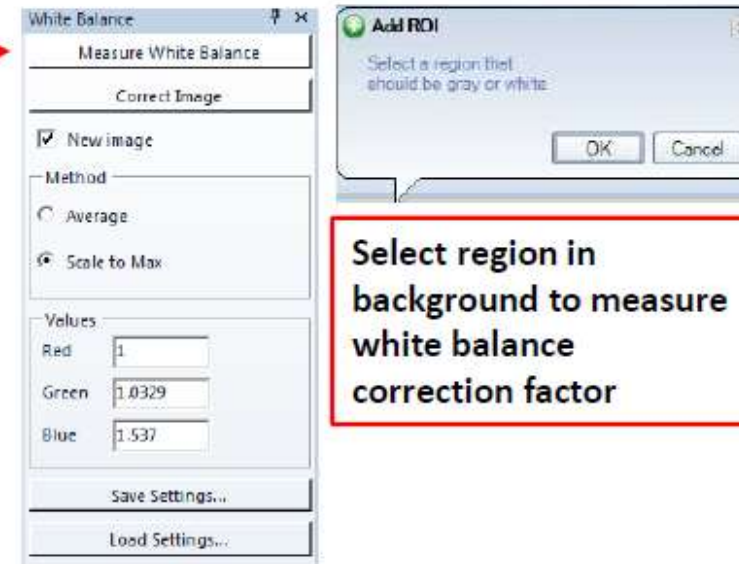
White Balance Corrected



1. Activate White Balance App



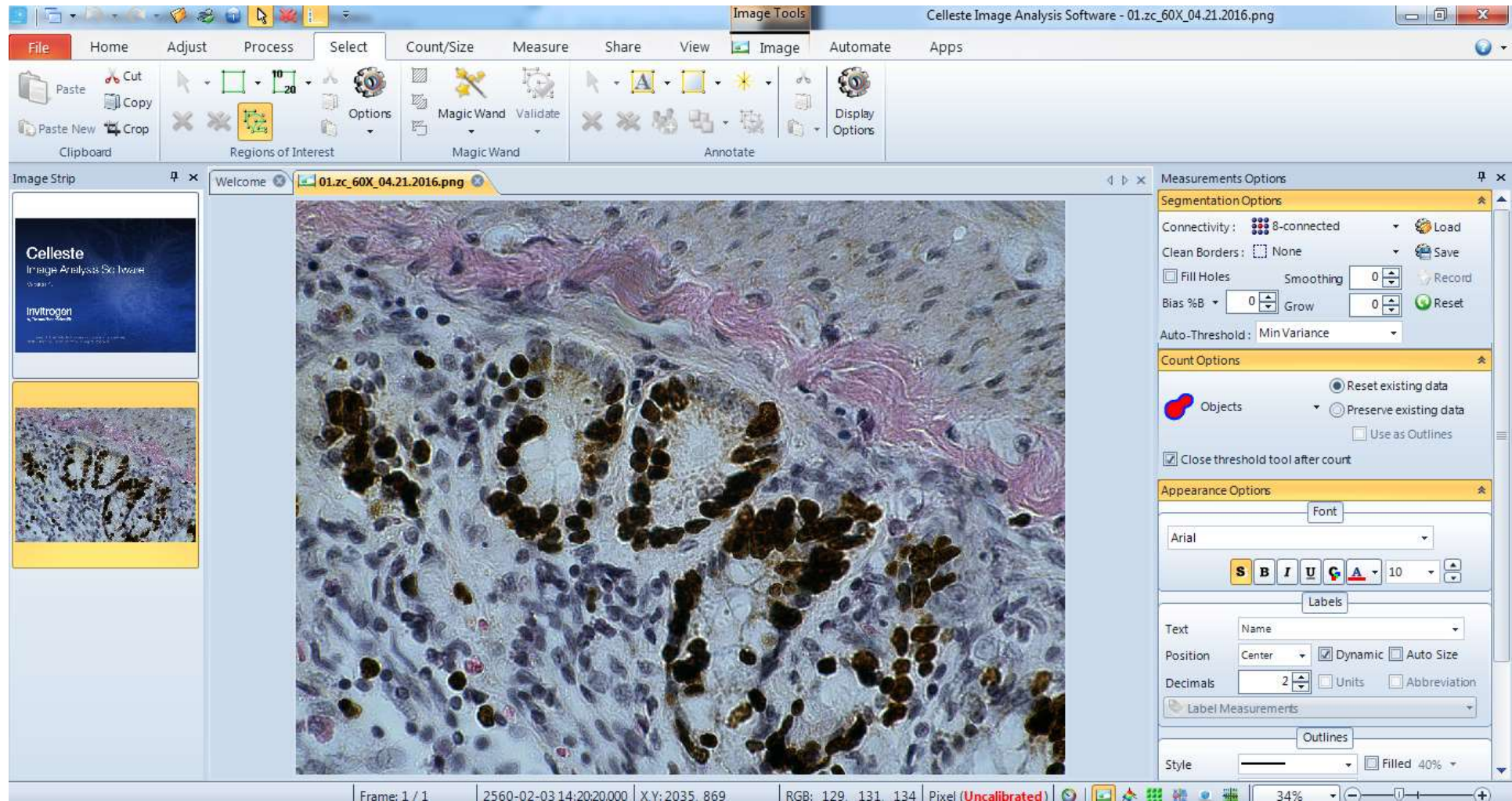
2. Measure White Balance & Correct



Data by: Trillium Blackmer
Celleste by: Oggie Golub

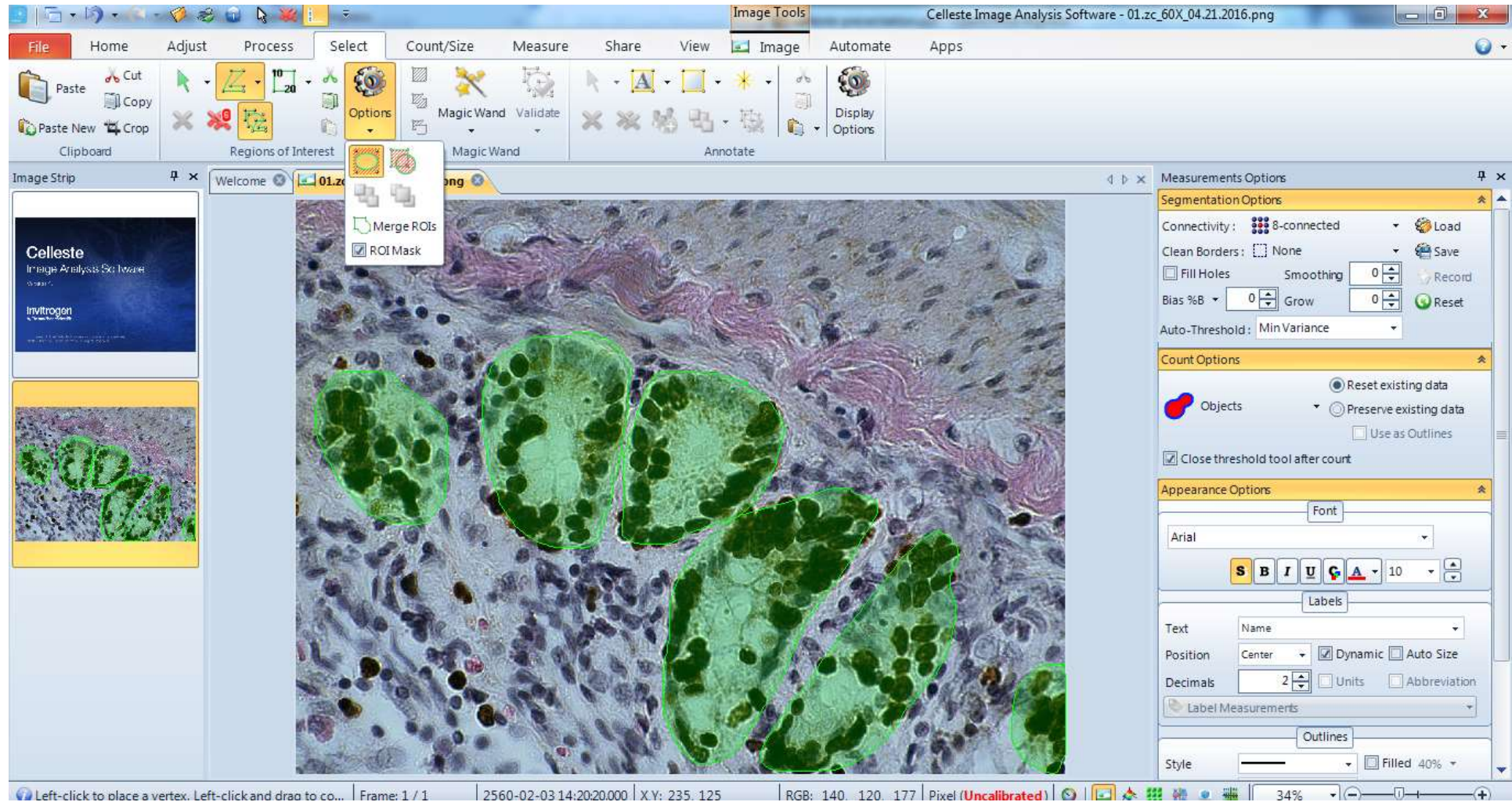
Celleste Image Analysis Software

Regions of Interest (ROI)



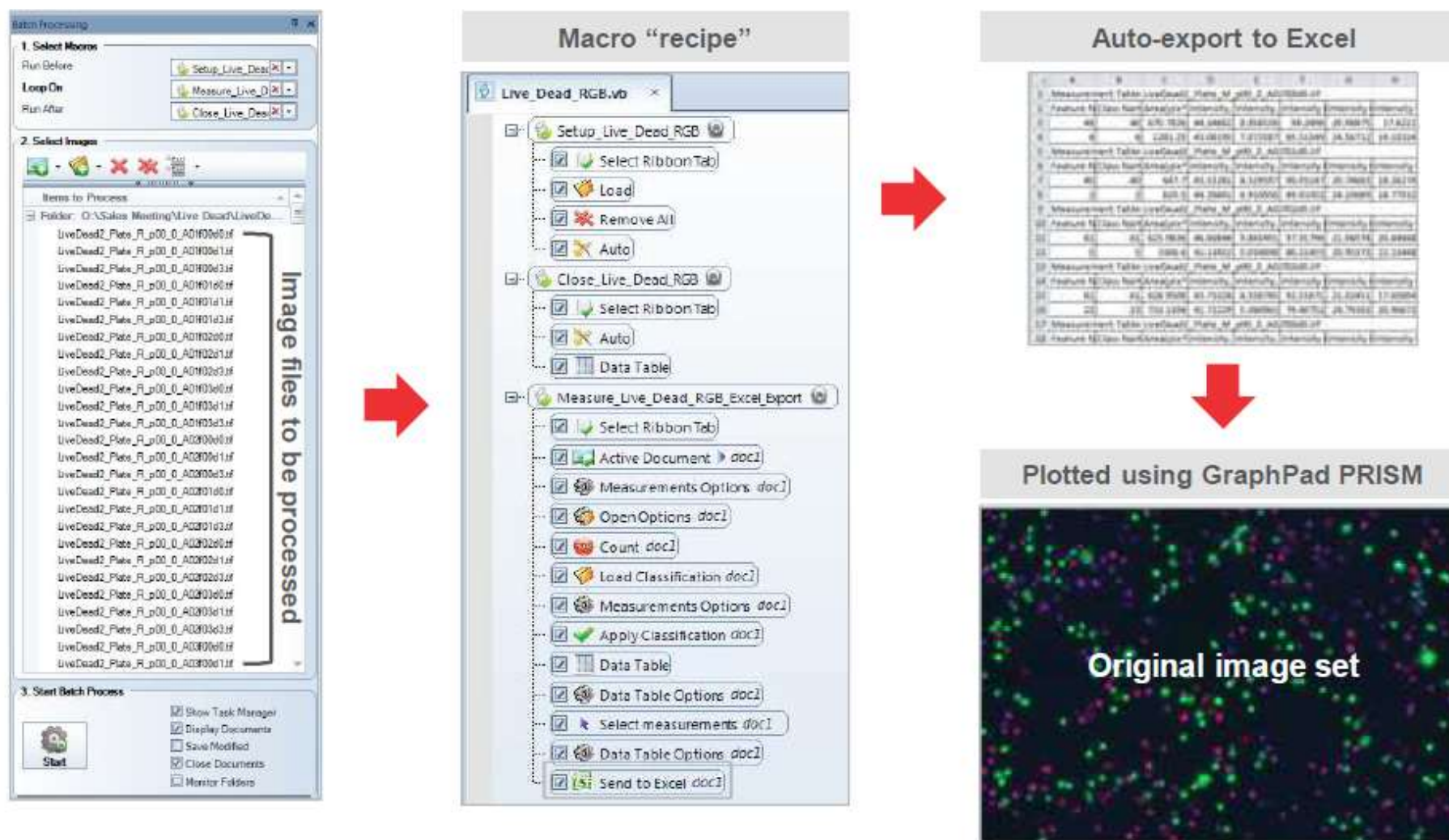
Celleste Image Analysis Software

Regions of Interest (ROI)



Celleste Image Analysis Software

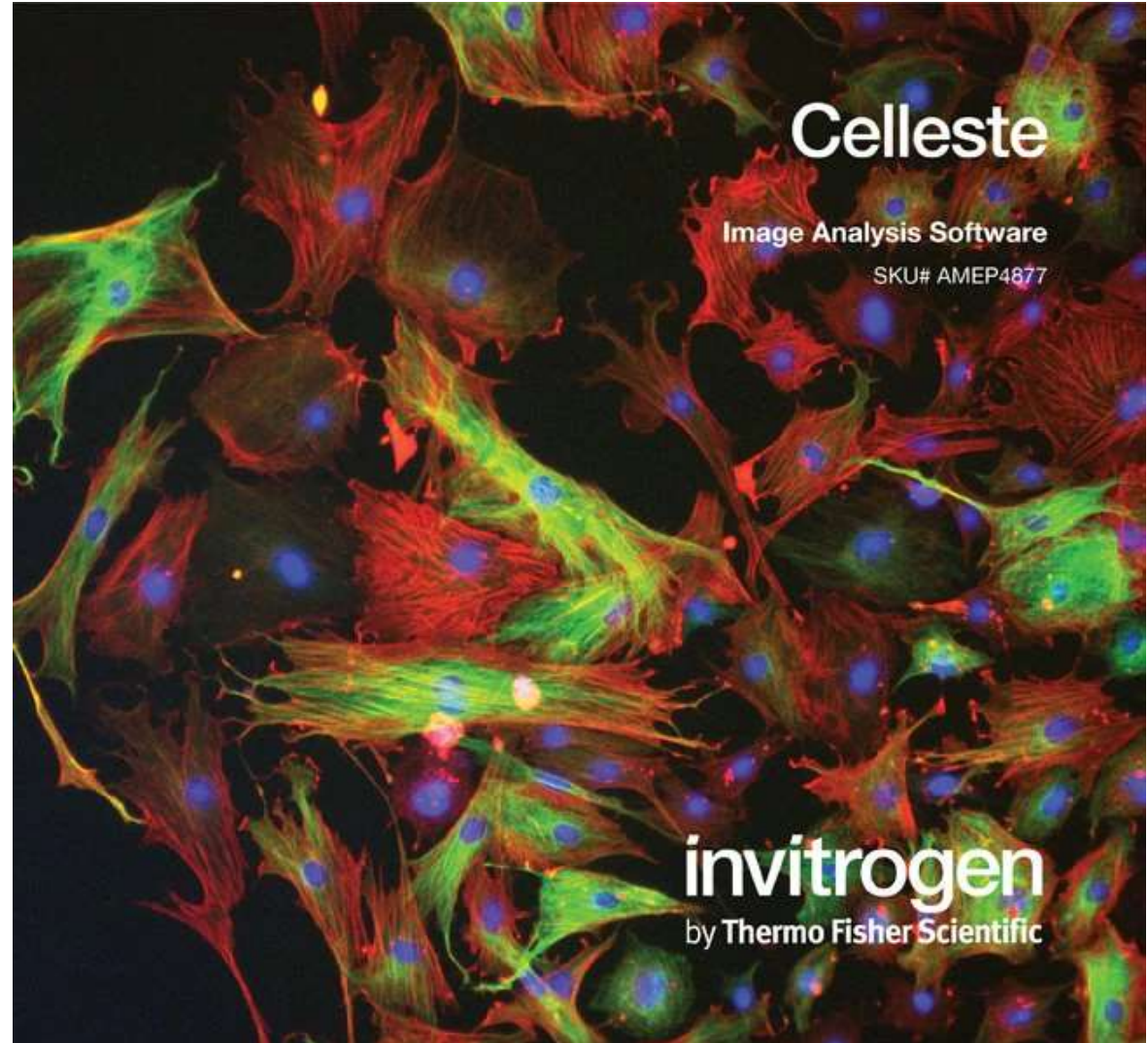
Easily apply macros for automated analysis in batch process mode



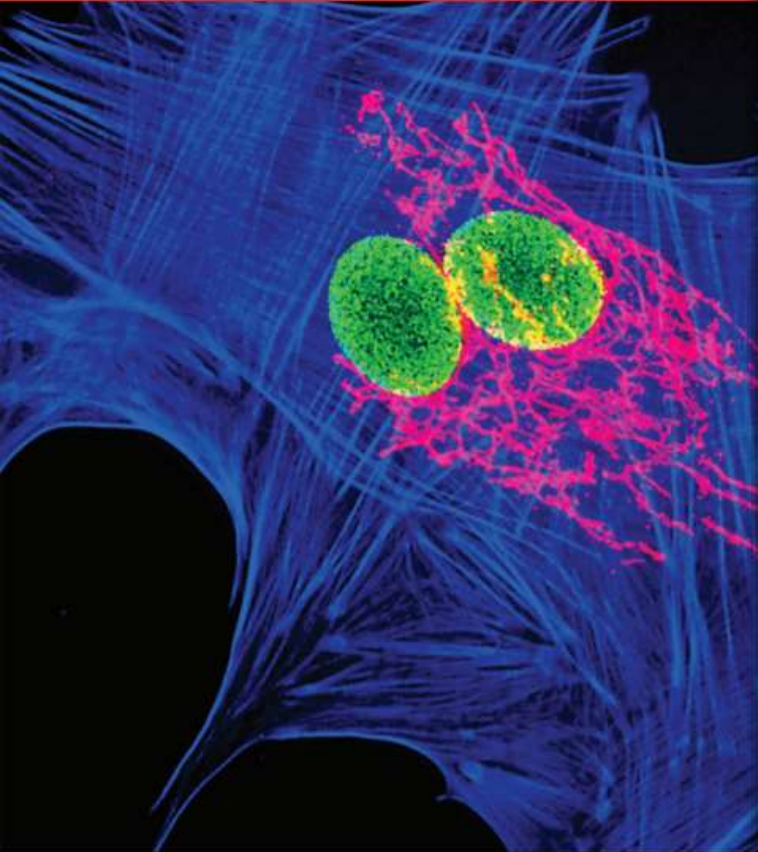
Celleste Image Analysis Software

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



invitrogen

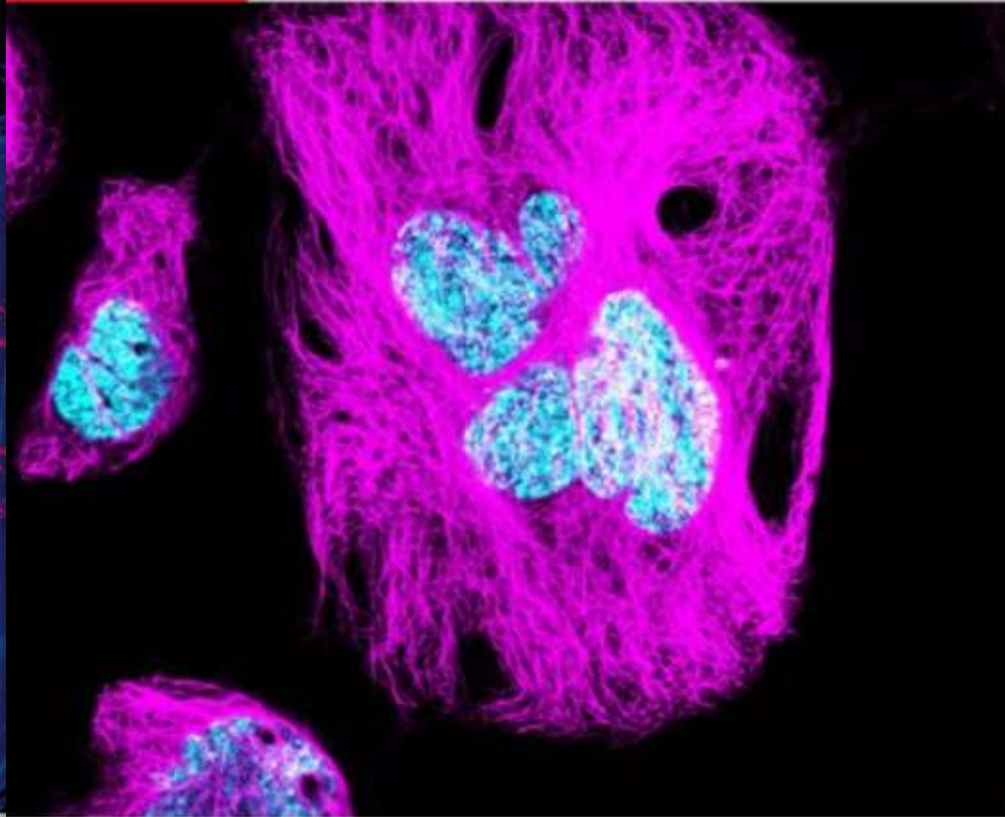


Fixed-cell imaging: five steps to publication-quality images

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

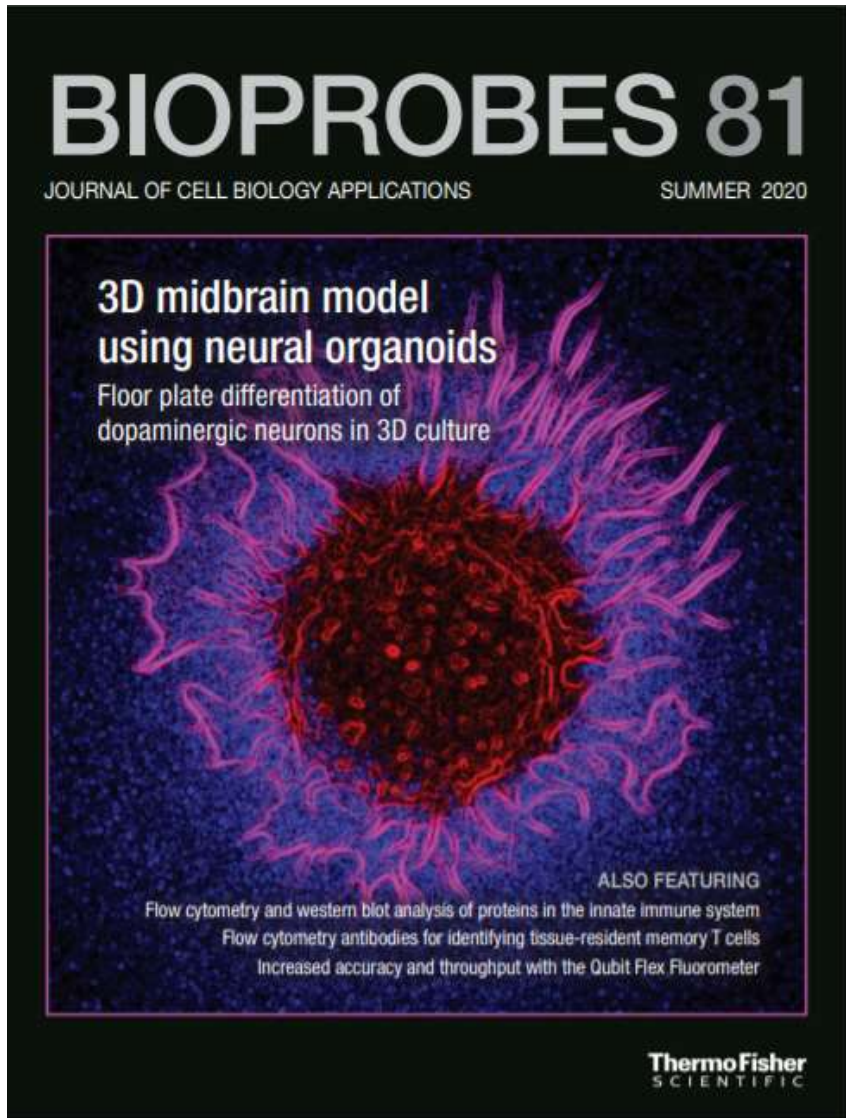
ThermoFisher
SCIENTIFIC

ohistochemistry: five steps to publication-quality images

ThermoFisher
SCIENTIFIC

Molecular Probes Journal

Back Issues



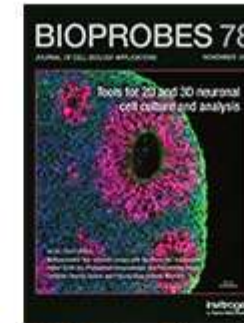
<https://www.thermofisher.com/th/en/home/references/newsletters-and-journals/bioprobres-journal-of-cell-biology-applications.html>



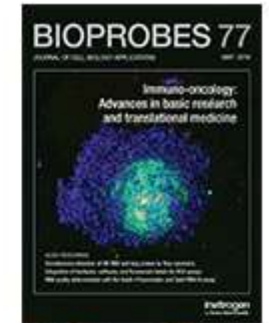
BioProbes 80



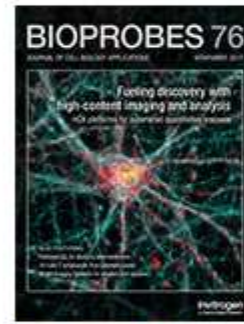
BioProbes 79



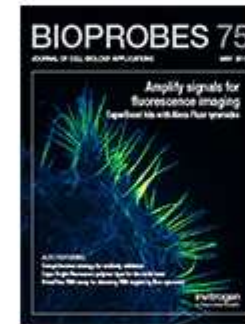
BioProbes 78



BioProbes 77



BioProbes 76



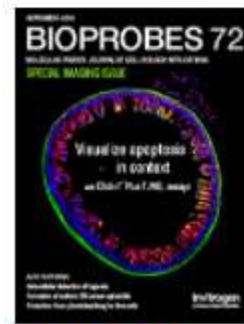
BioProbes 75



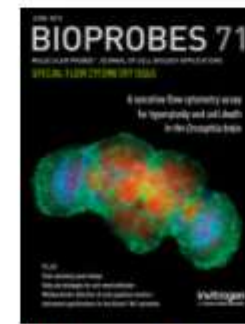
BioProbes 74



BioProbes 73



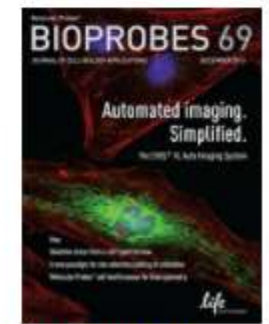
BioProbes 72



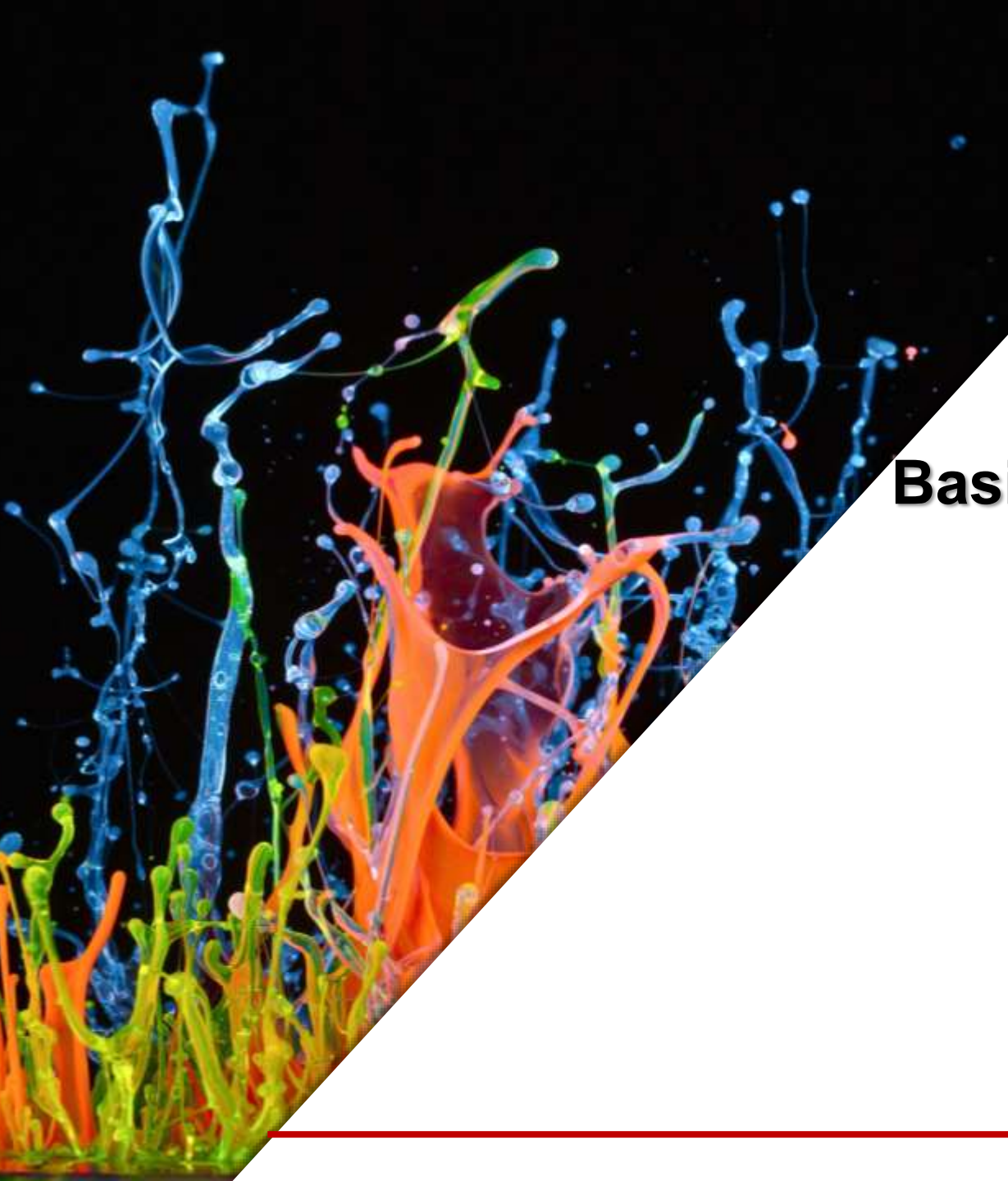
BioProbes 71



BioProbes 70



BioProbes 69



gibthai
A 3N HOLDING COMPANY

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

Light (optical) microscope

using visible light to generate images

Simple microscope



having more than one lens

Compound microscope

Stereo microscope

Digital microscope



stereoscopic view



digital imaging



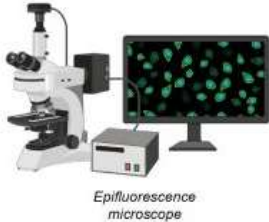
viewing fluorescent objects

viewing specimens from the bottom

Fluorescence microscope

Inverted microscope

Automated imaging system



automation



removing out-of-focus lights while viewing 3D objects

viewing beyond the Diffraction limit

high throughput

Confocal microscope

Super Resolution Microscopy

Imaging flow cytometry



Light sheet fluorescence microscope

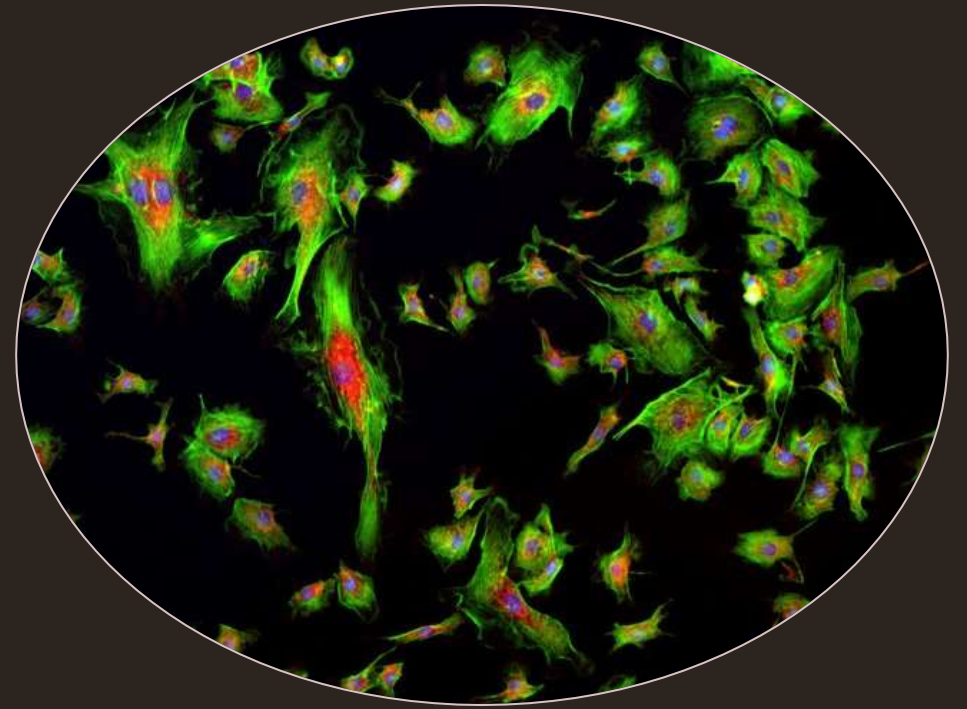
High-speed imaging cell flow cytometry

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

Label

3

Detect

4

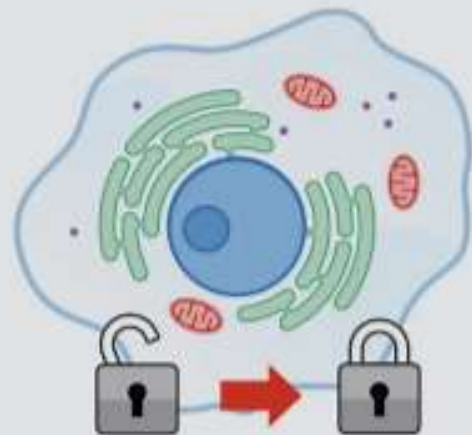
Protect and
enhance

5

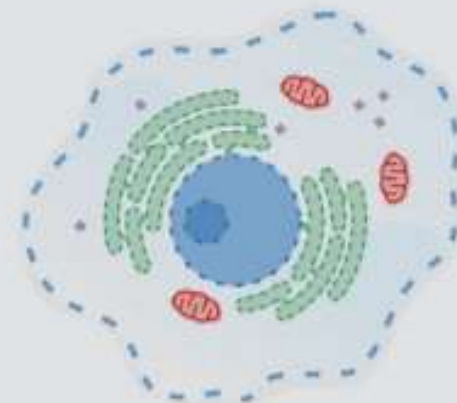
Image

1

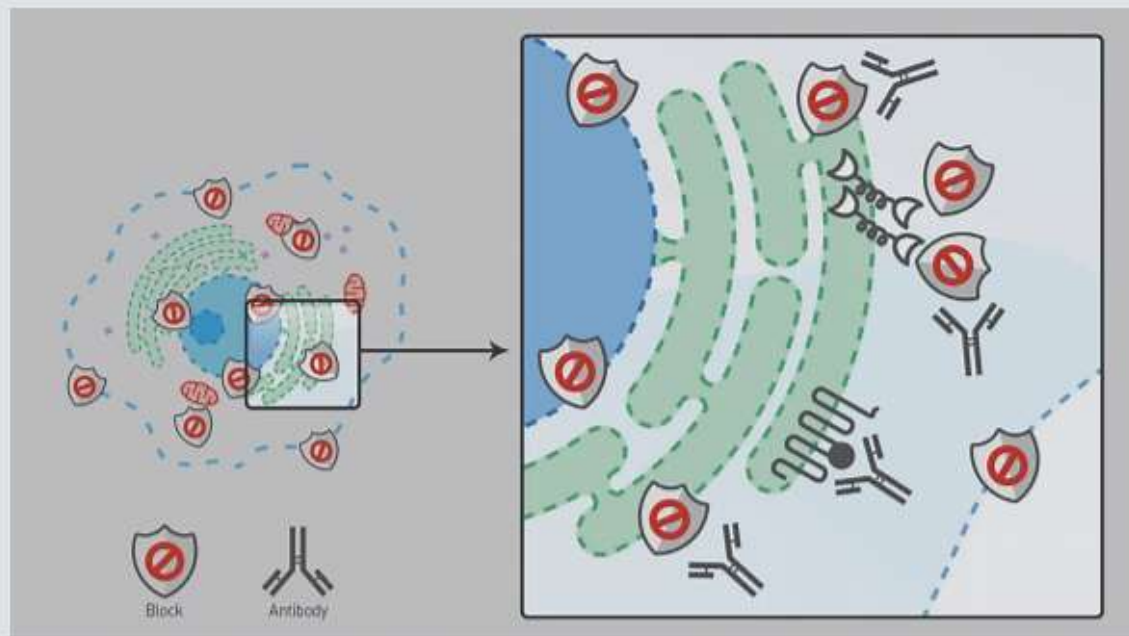
Fix,
permeabilize,
and block



Fixation locks cellular structures in place.



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



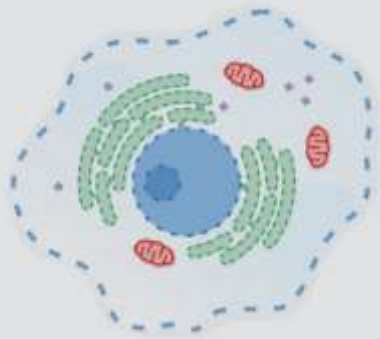
Protein-based blocking agents help reduce nonspecific staining. Antibodies are able to displace the blocking proteins to form high-affinity interactions with their epitopes, while blocking proteins prevent low-affinity antibody interactions elsewhere in the sample.

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)



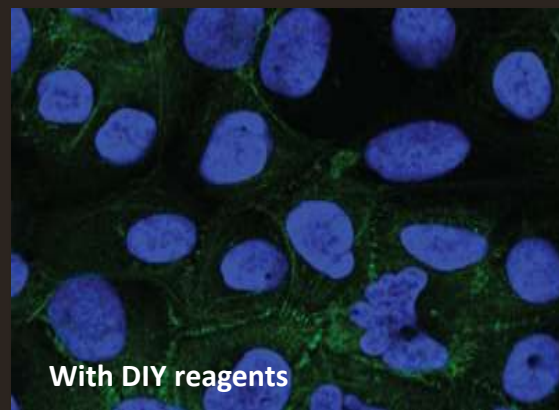
Image-iT™ Fixative Solution
(4% formaldehyde, methanol-free)

Permeabilization solution

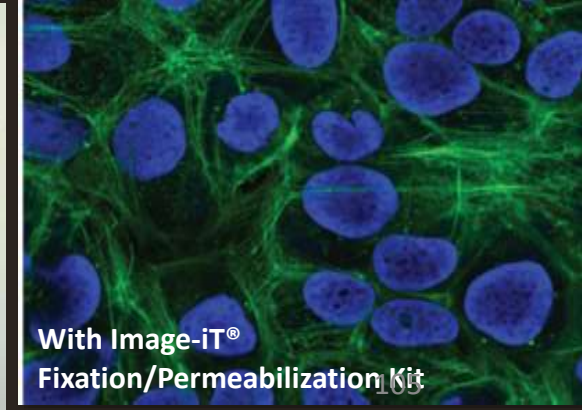
- 0.1% Triton X-100 in PBS
- 0.1% NP-40 in PBS

Fixation and Permeabilize

Image-iT Fixation/Permeabilization Kit (R37602)



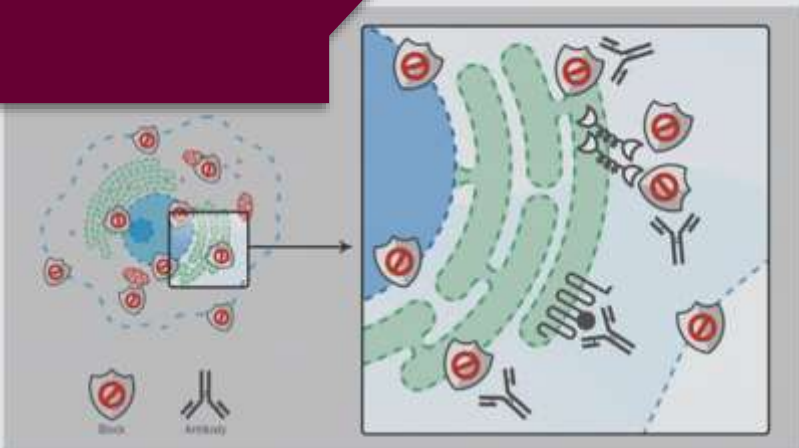
With DIY reagents



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.

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

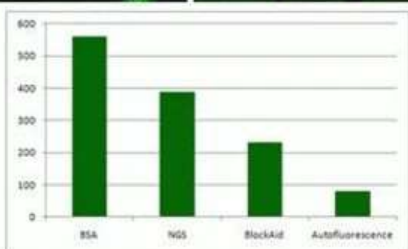
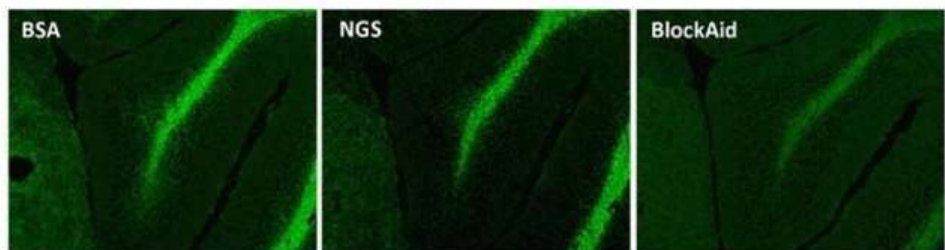
2nd AB from Chicken: Ready Probes™ 2.5% Normal Chicken Serum (1X) Catalog No: R37626

2nd AB from Mouse: Normal Mouse Serum (Dilute 1:20 to make 5%)Catalog # 31880

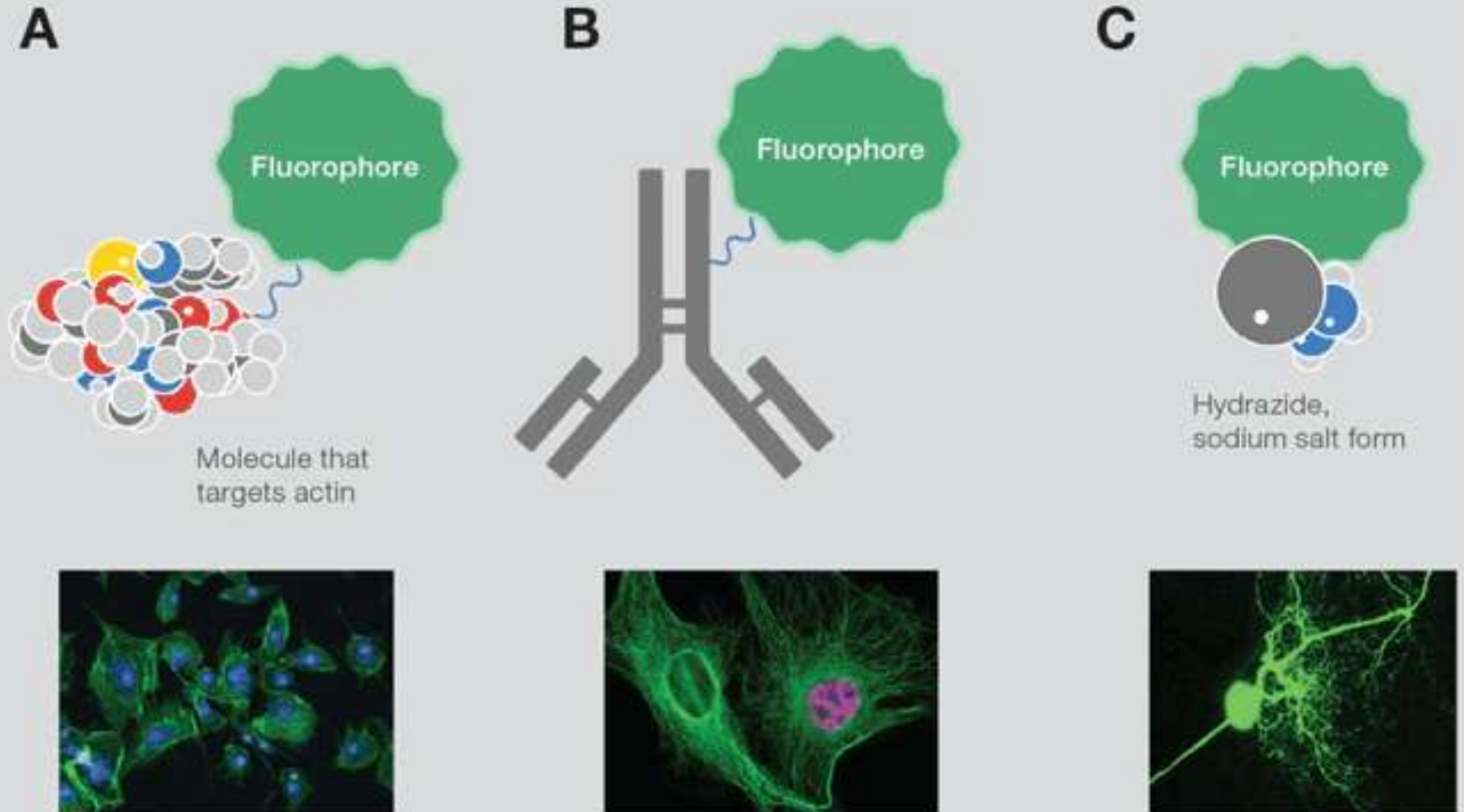
2nd AB from Rat: Normal Rat Serum (Dilute 1:20 to make 5%)Catalog # 31888

2nd AB from Rabbit: Normal rabbit Serum (Dilute 1:20 to make 5%) Catalog # 01-6101

Use : BlockAid™ Blocking Solution, Catalog number: B10710



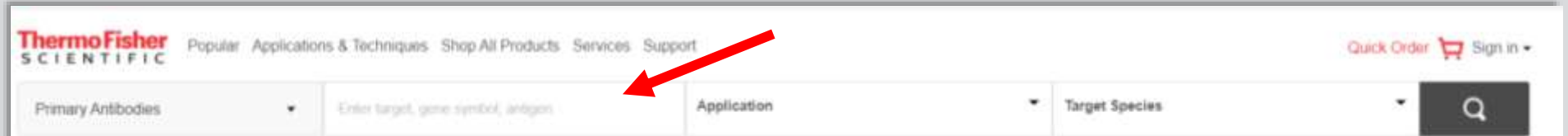
Label—target cell structures and proteins with **selective dyes** and **primary antibodies**



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.¹⁰⁷

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

Primary Antibodies



Home > Life Sciences > Antibodies > Primary Antibodies

Primary Antibodies

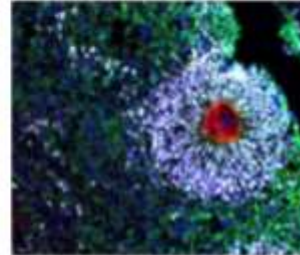
Antibodies

Primary Antibodies

- Guide to Primary Antibody Types
- Conjugated Primary Antibodies
- Control Antibodies
- Epitope Tag Antibodies and Related Antibodies
- Research Area Antibodies
- Signal Pathway Antibodies
- Cell Marker Antibodies
- Antibodies for Applications
- Organelle Marker Antibodies

Secondary Antibodies

- Custom Antibody Development
- Streptavidin/Biotin Binding Protein Conjugates
- Antibody Production



A primary antibody is an immunoglobulin that specifically binds to a particular protein or other biomolecule of research interest for the purpose of purifying or detecting and measuring it. Primary antibodies are developed as polyclonal or monoclonal antibodies using mouse, rat, rabbit, goat, and other animal species as hosts. They are produced and supplied in various forms, ranging from crude antiserum to antigen-purified preparations. Primary antibodies for frequently researched targets are also available conjugated to fluorescent dyes or biotin.

Direct Immunofluorescence

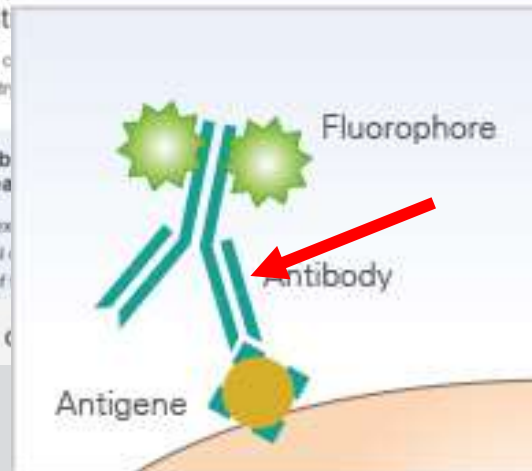
The I
addit

We are c
cytometry

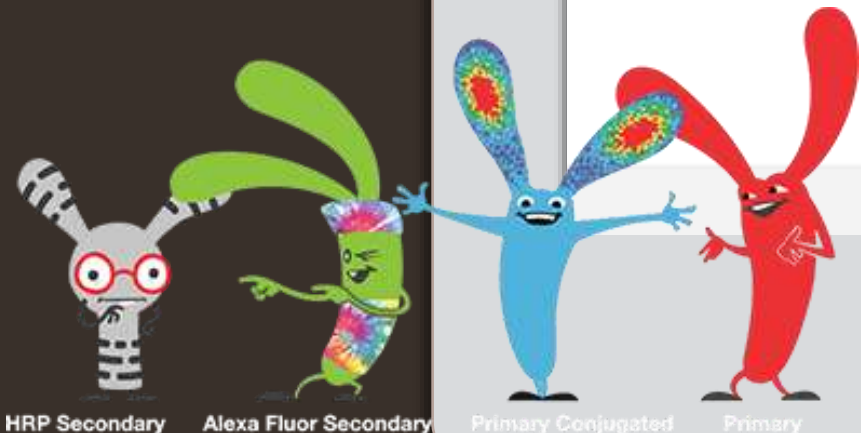
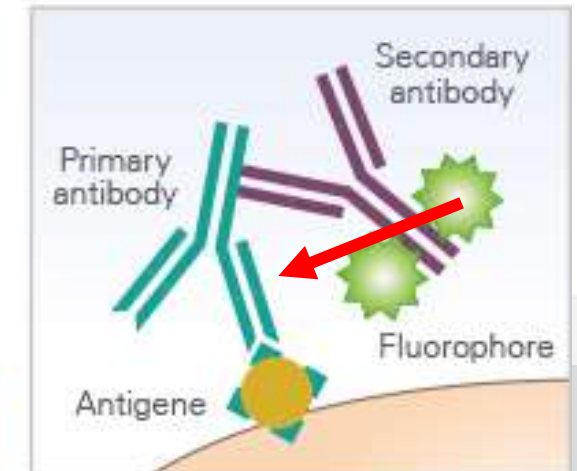
Rabb
resea

Neure
neural c
part of

Explore c



Indirect Immunofluorescence

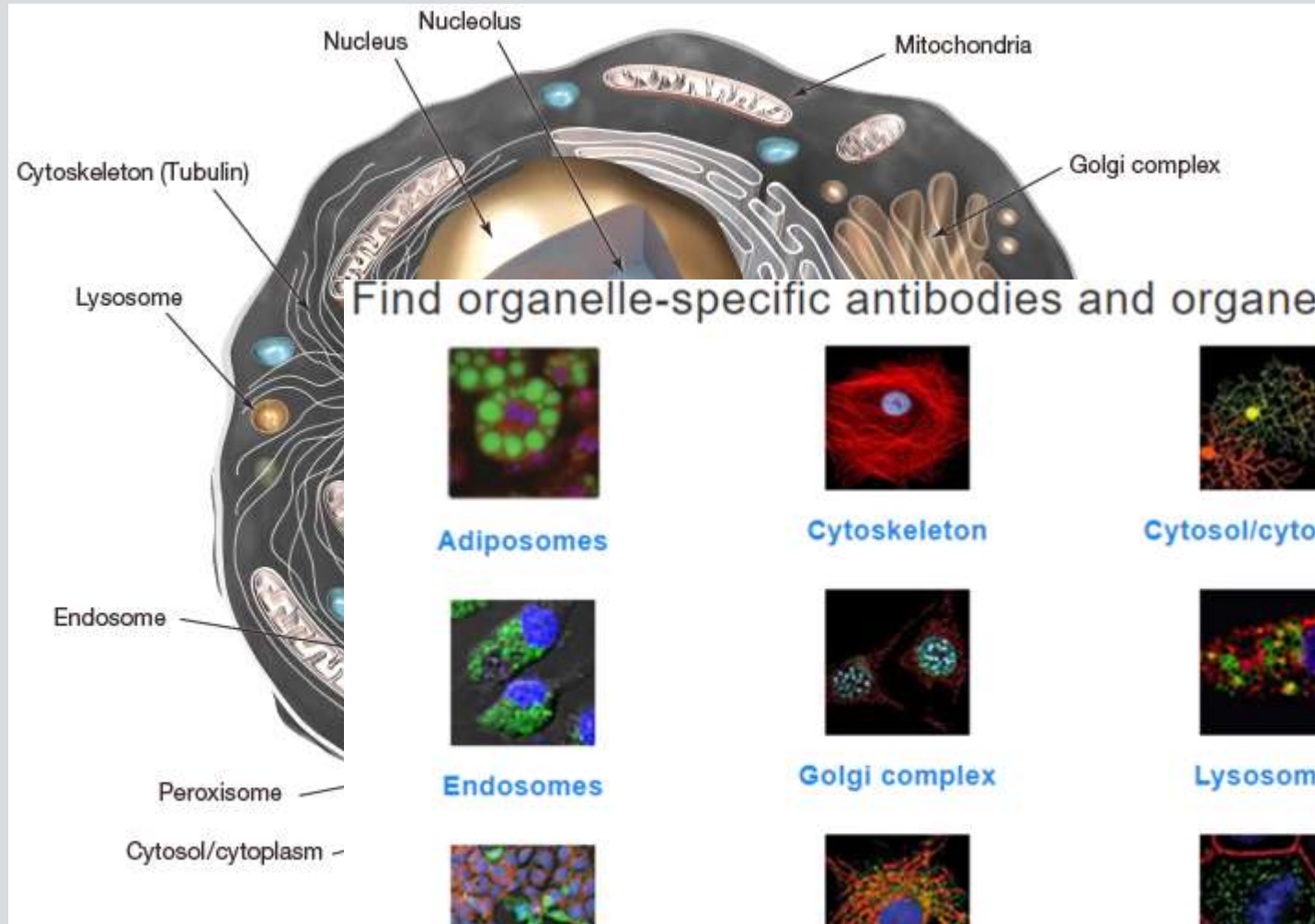


2

Label

Labeling
or staining
dyes

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



Find organelle-specific antibodies and organelle stains

Labeling or staining dyes

Label—target cell structures proteins with **selective dyes**

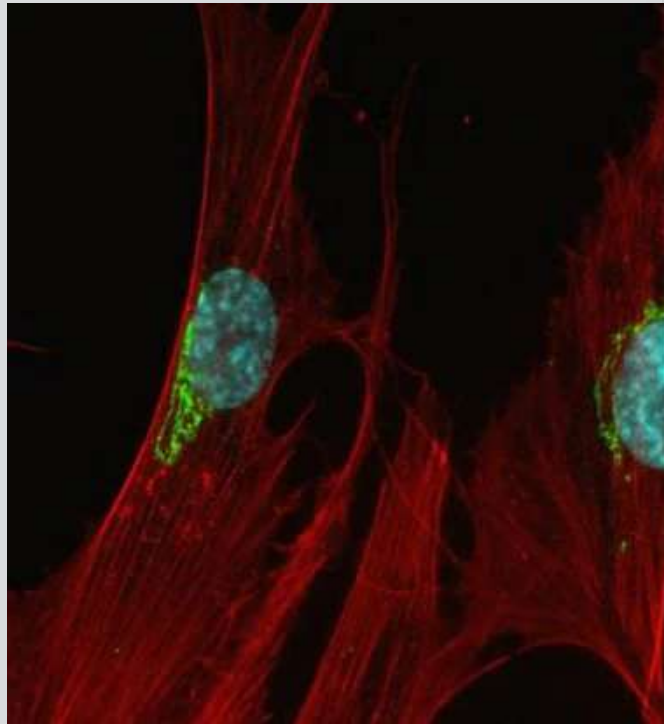
- **Nucleus :**
 - DAPI (4',6-Diamidino-2-Phenylindole)
 - **NucBlue Fixed Cell ReadyProbes**



| 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 | | |

Label—target cell structure proteins with **selective dyes**

- **Cytoskeleton :**
 - Alexa Fluor Plus 555 Phalloidin
 - **Alexa Fluor 594 Phalloidin (A12381)**



Cells were also stained with **Alexa Fluor® 594 phalloidin** (A12381) to label F-actin. Finally, cells were mounted in DAPI (R37606) to label nuclei.

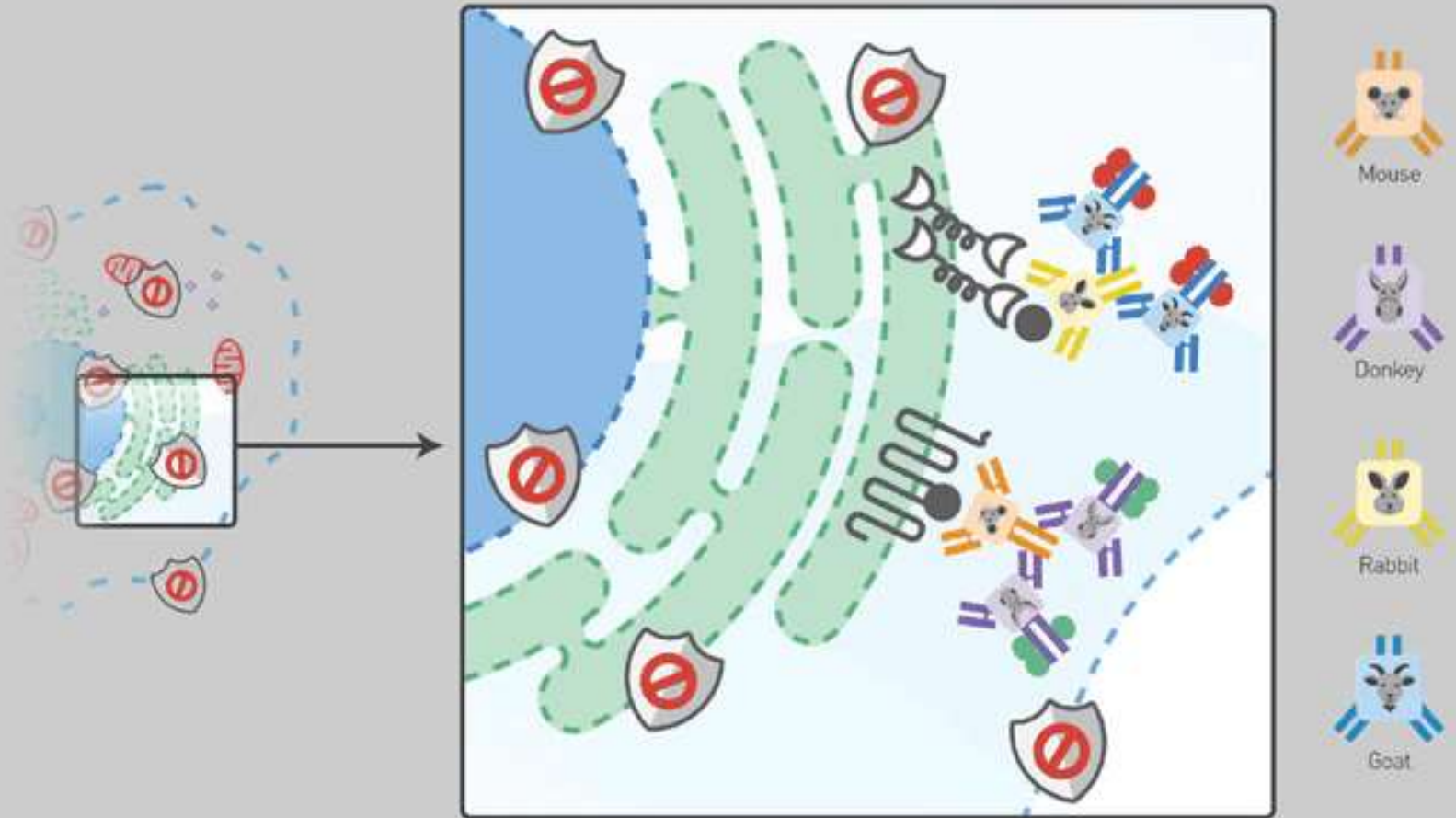
| | Alexa Fluor 568 Phalloidin | Alexa Fluor 594 Phalloidin | Alexa Fluor Plus 647 Phalloidin | Alexa Fluor 680 Phalloidin | Alexa Fluor Plus 750 Phalloidin |
|-----------------------|--|----------------------------|---------------------------------|----------------------------|---------------------------------|
| 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 |

Labeling
or staining
dyes

3

Detect

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



3

Detect

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

Secondary Antibodies

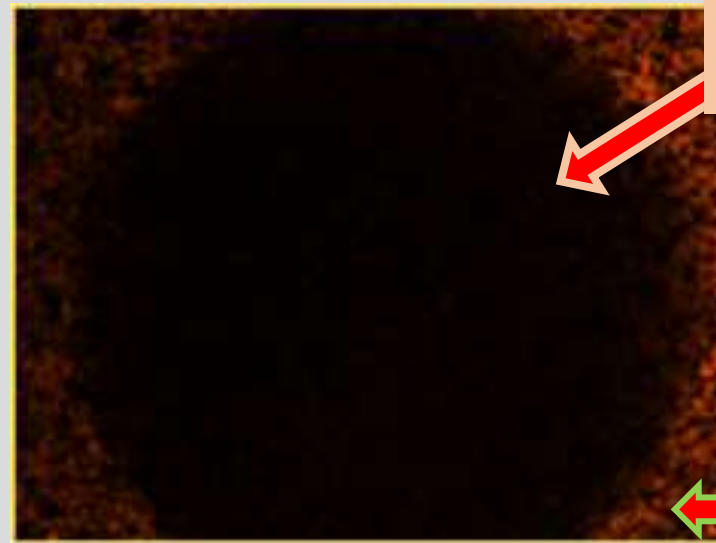
The screenshot shows the ThermoFisher Scientific website. At the top, there is a navigation bar with the ThermoFisher logo and links for 'Popular Applications & Techniques', 'Shop All Products', 'Services', and 'Support'. On the right, there are links for 'Quick Order' and 'Sign in'. Below the navigation bar is a search bar with the text 'Secondary Antibodies' and three dropdown menus: 'Host', 'Target species', and 'Conjugates', each highlighted with a red box. A search icon is on the right of the search bar. Below the search bar is a breadcrumb trail: 'Home > Life Sciences > Antibodies > Secondary Antibodies > Fluorescent Secondary Antibodies'. The main heading is 'Fluorescent Secondary Antibodies'. Below this is a section titled 'Quick links to secondary antibody conjugate products' which contains a table with three columns: 'Alexa Fluor Secondary Antibody Conjugates', 'Other Fluorescent Secondaries', and 'Enzyme & Biotin-Labeled Secondaries'. Each column contains a list of product links and a 'View all' link.

| Alexa Fluor Secondary Antibody Conjugates | Other Fluorescent Secondaries | Enzyme & Biotin-Labeled Secondaries |
|--|---|---|
| <ul style="list-style-type: none">Alexa FluorAlexa Fluor PlusAlexa Fluor 350Alexa Fluor 405Alexa Fluor 488Alexa Fluor 532Alexa Fluor 546Alexa Fluor 568Alexa Fluor 680Alexa Fluor 647Alexa Fluor 750View all > | <ul style="list-style-type: none">FITCTRITCDyLight DyesRhodamineTexas Red & Texas Red-XR-PEAPCQdot ProbesPacific DyesCy3Cy5View all > | <ul style="list-style-type: none">Enzyme labeled-HRPEnzyme labeled-APRecombinant enzyme labeled HRP conjugatesBiotinView all > |

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

Protect and
enhance

Under the high intensity illumination of a microscope, fluorescent dyes are prone to **photobleaching – irreversible fading of signal**



*Bleached region of
intense illumination*

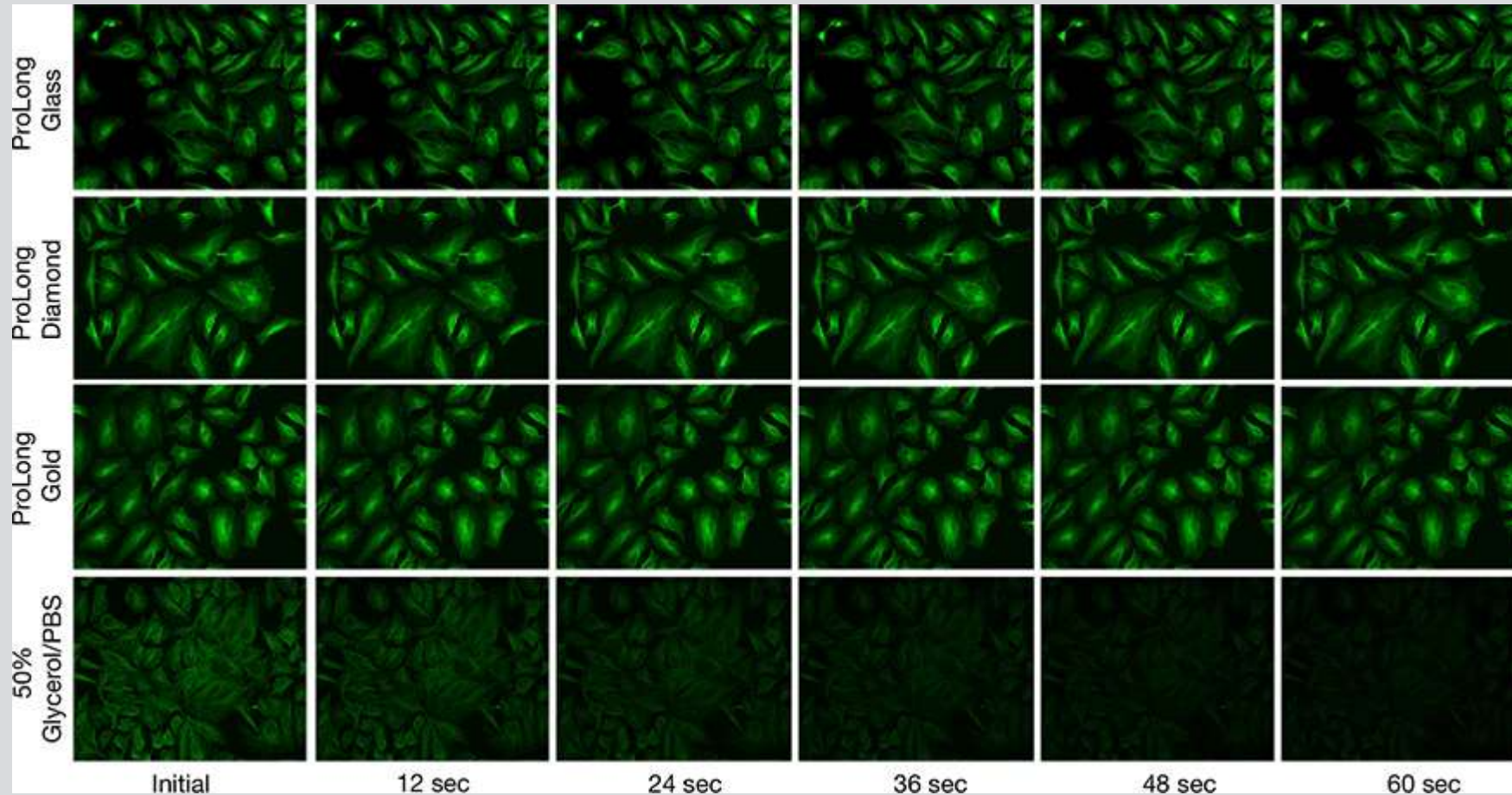
*Bright, unbleached
periphery*



4

Protect and
enhanceAntifade
Mounting
media

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



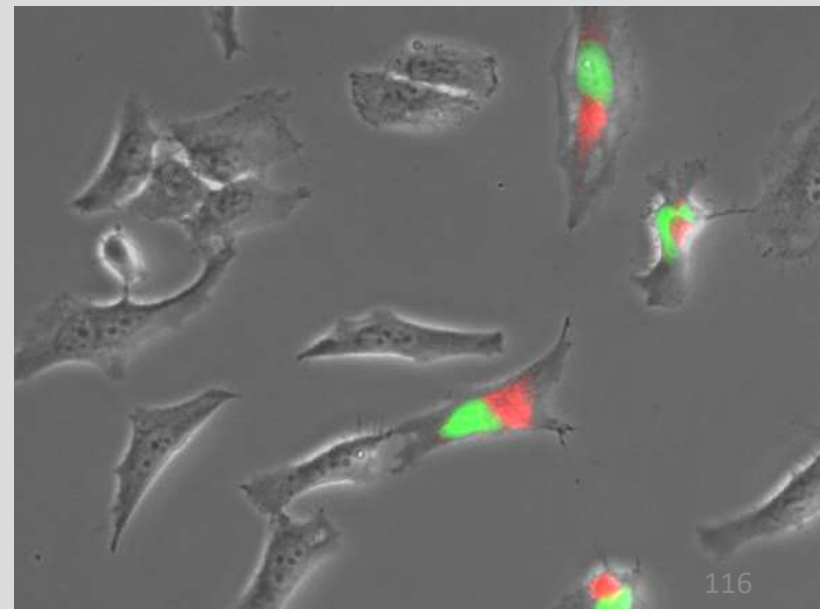
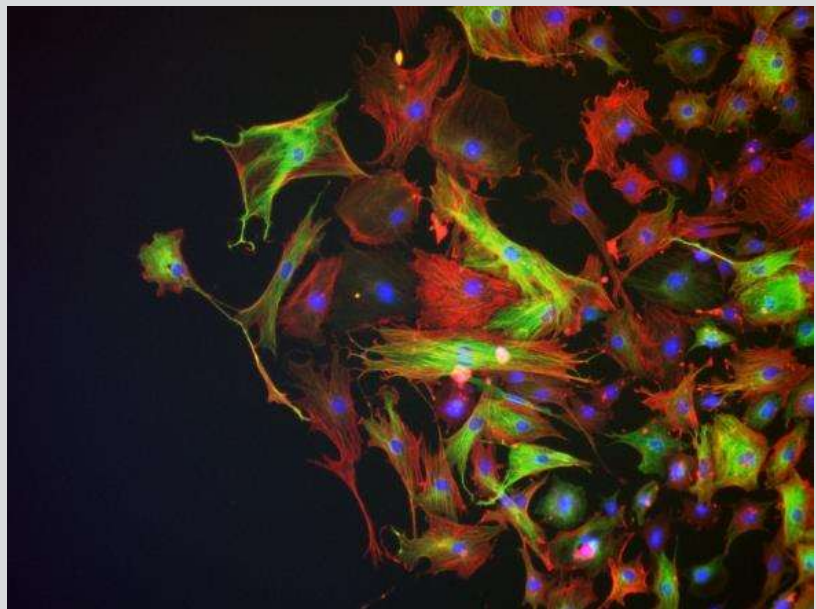
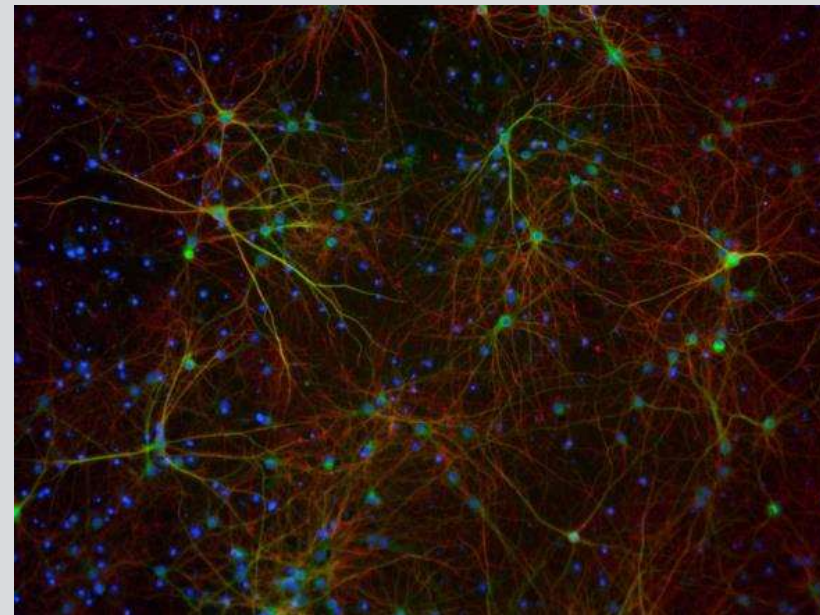
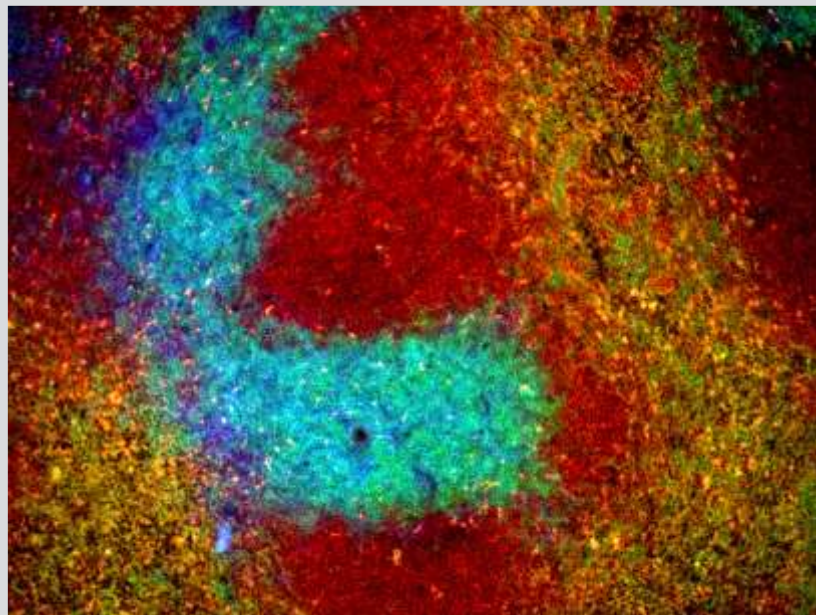
Protect the signal from photobleaching and enhance the image quality with the best resolution. Fluorophores are ideal for high-quality cell imaging but are inevitably prone to photobleaching, which is a photochemical degradation or fading of fluorescence signals. Antifade mountants are designed to protect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



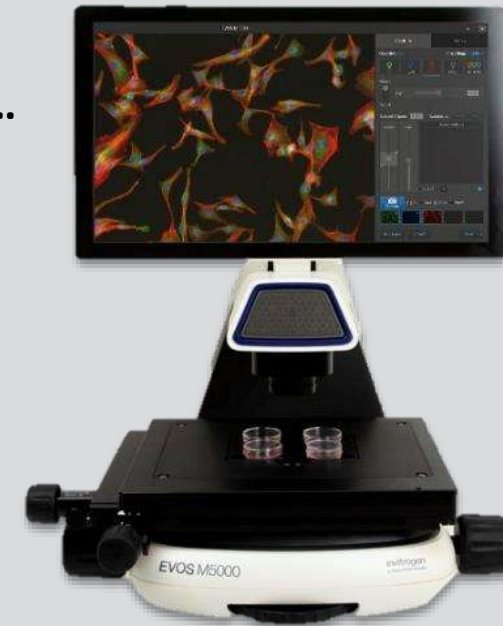
Image— capture imaging discoveries with maximum clarity and definition

Go from all this.....



'Typical' epifluorescence system

To this.....

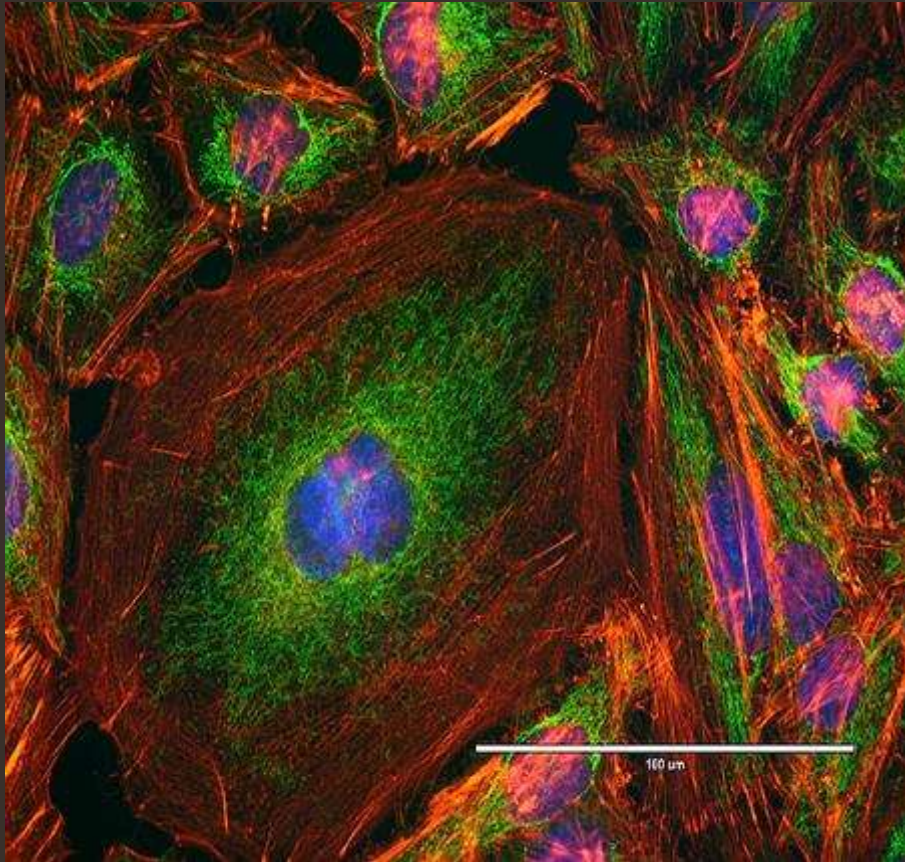


EVOS M5000

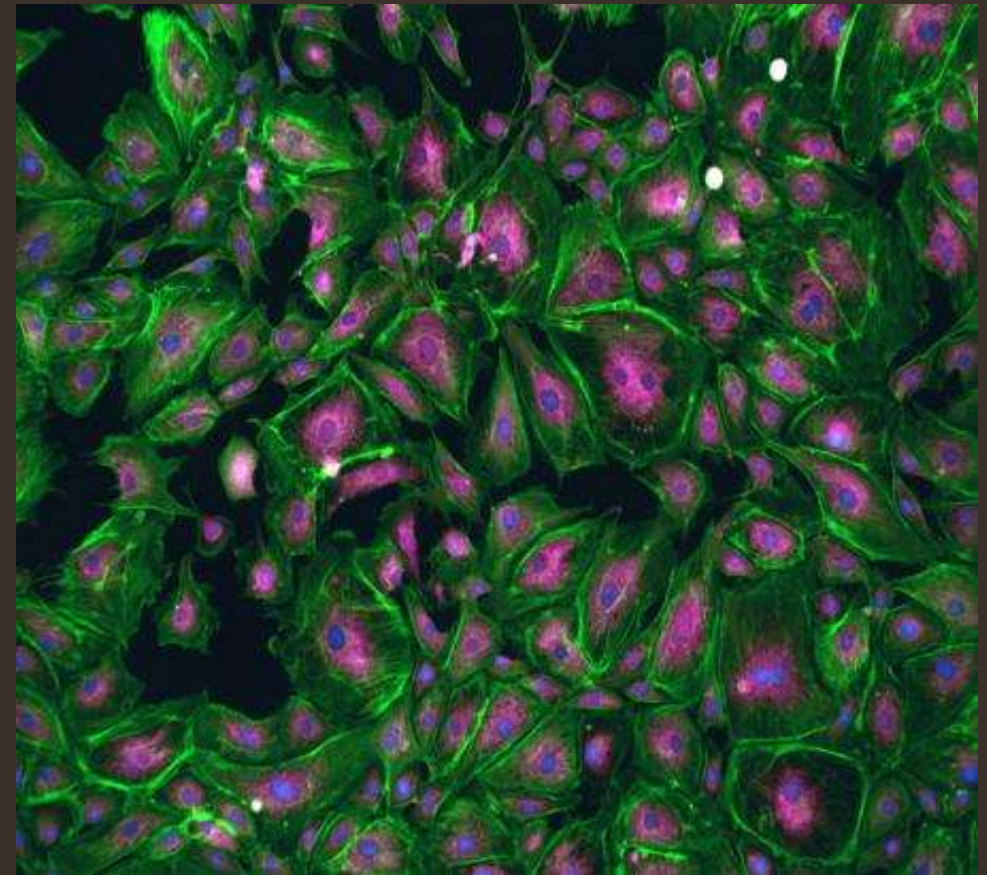


EVOS M7000

Examples of Image-based Contextual information



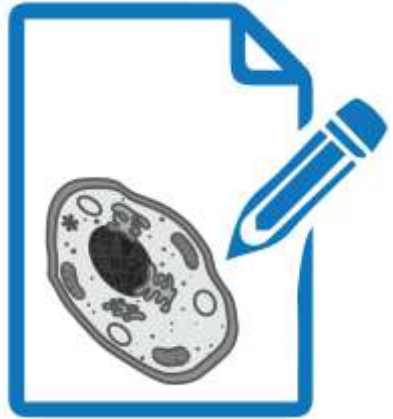
1. Image-iT® Fixation/Permeabilization Kit/ BlockAid
2. Primary antibody to mitochondria,
NucBlue Cell Stain **(Blue)**
ReadyProbes ActinRed 555 **(Red)**
3. FITC Secondary Antibody **(Green)**
4. Mounted with Prolong Antifade Reagent
5. Detected – EVOS Imaging System



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

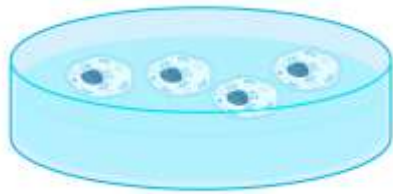
Five Steps to Live-Cell Imaging

Step 1:
Plan



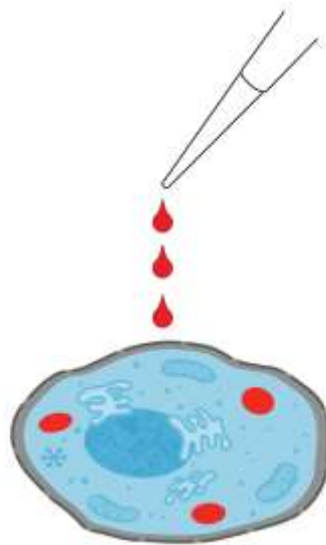
Plan—design your experiment carefully considering the tools and resources needed for each step

Step 2:
Culture



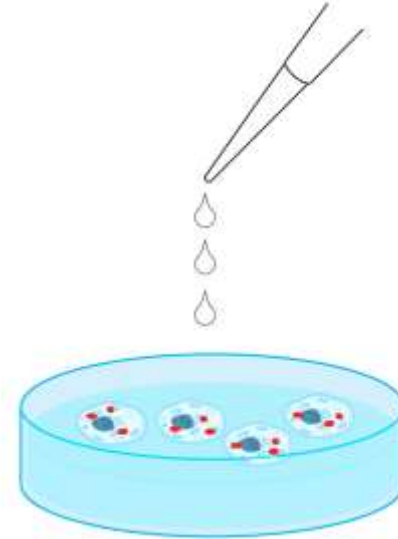
Culture—maintain or grow your cells under optimum conditions

Step 3:
Label



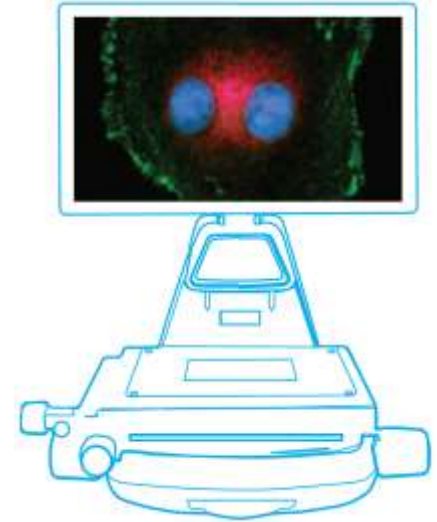
Label—target cell structures and functions with selective dyes and stains

Step 4:
Optimize



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

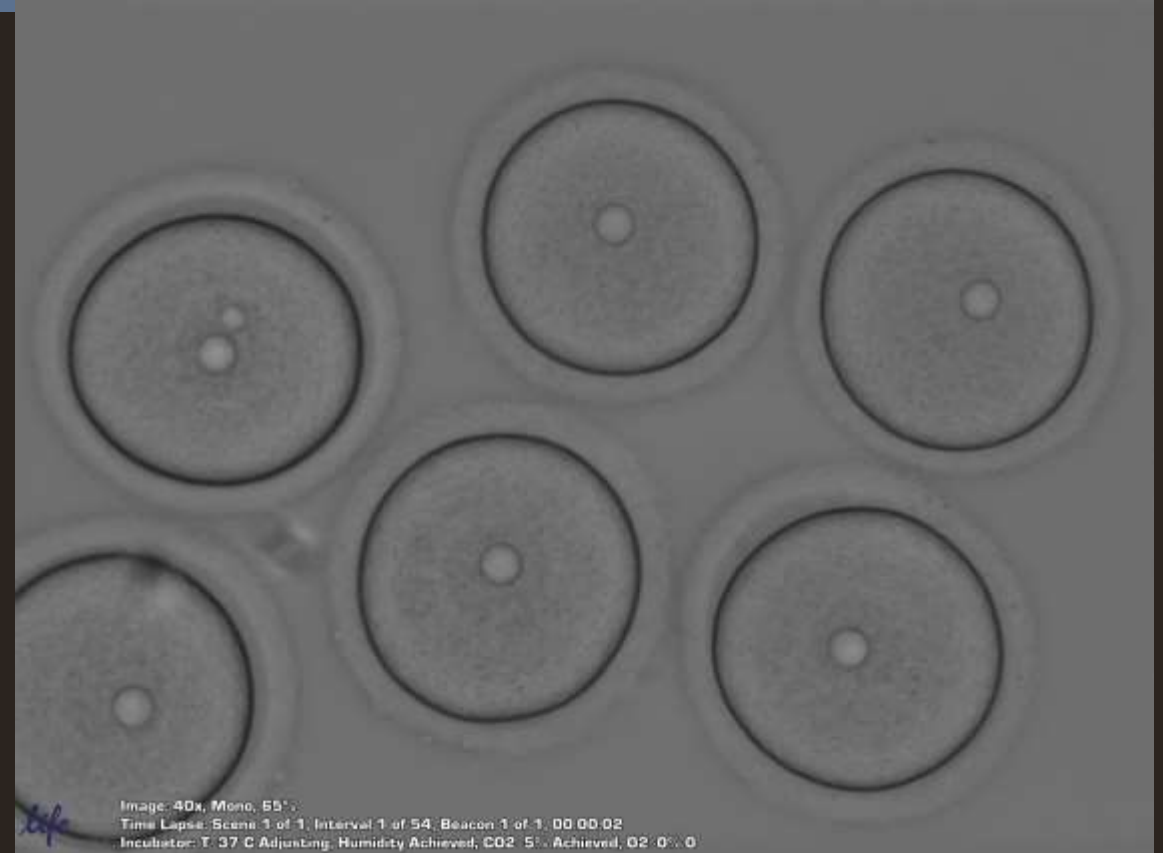
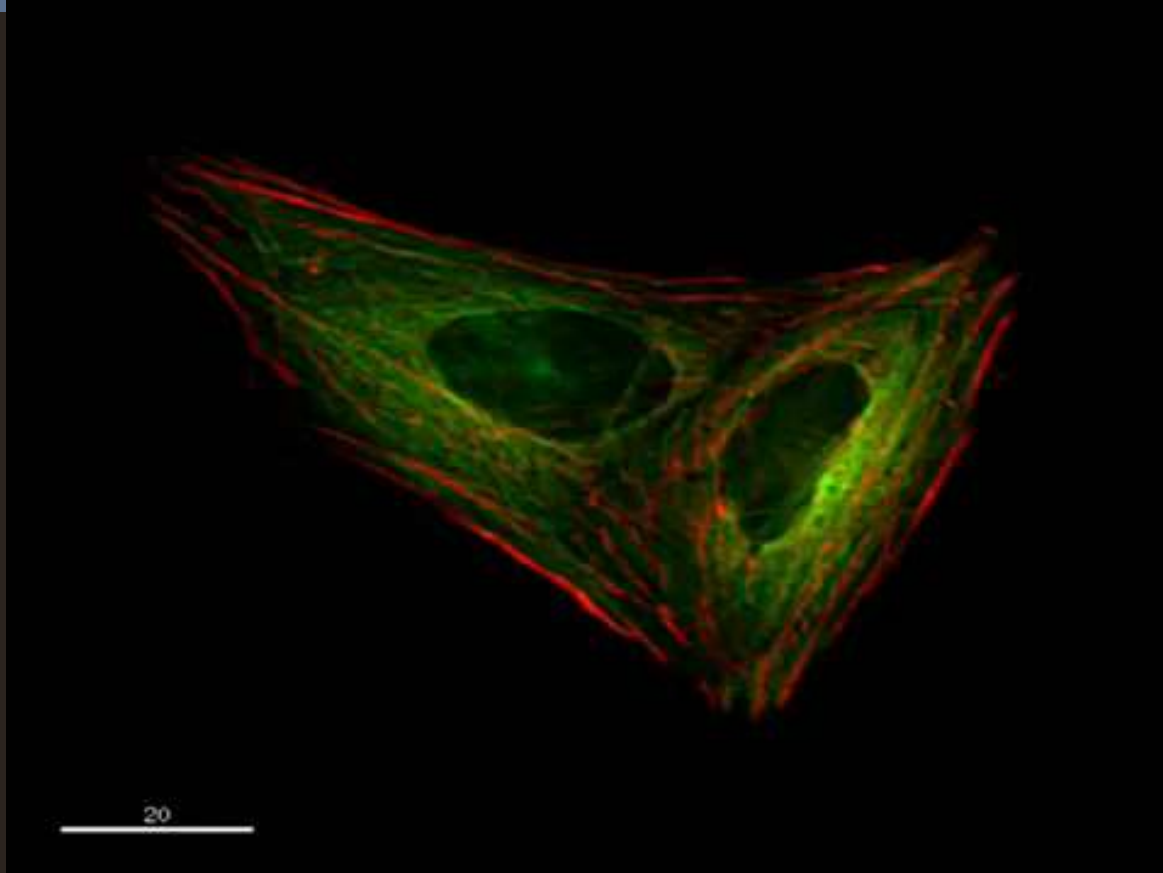
Step 5:
Image



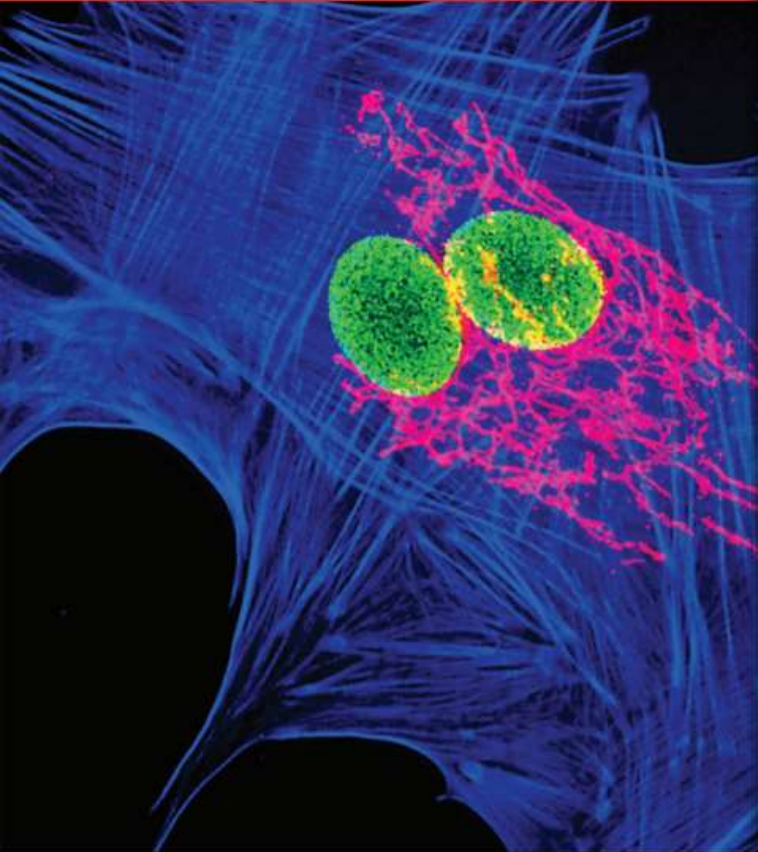
Image—capture discoveries as they happen with the highest reproducibility and definition

Five Steps to Live-Cell Imaging

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



invitrogen

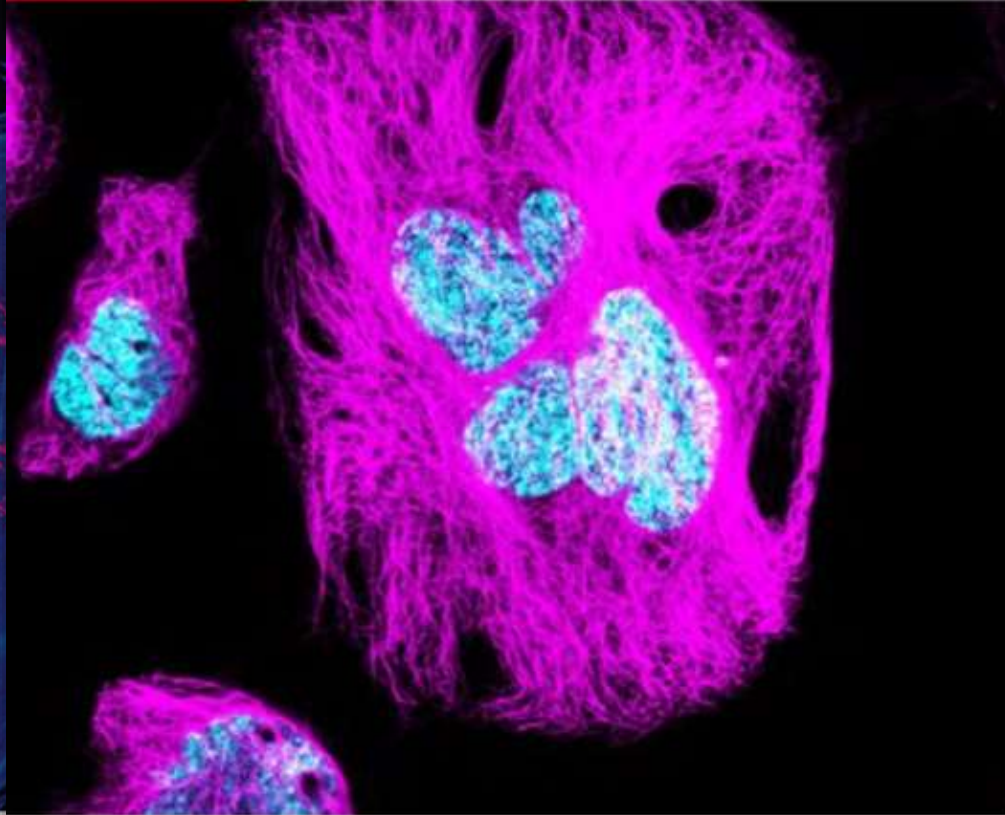


Fixed-cell imaging: five steps to publication-quality images

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

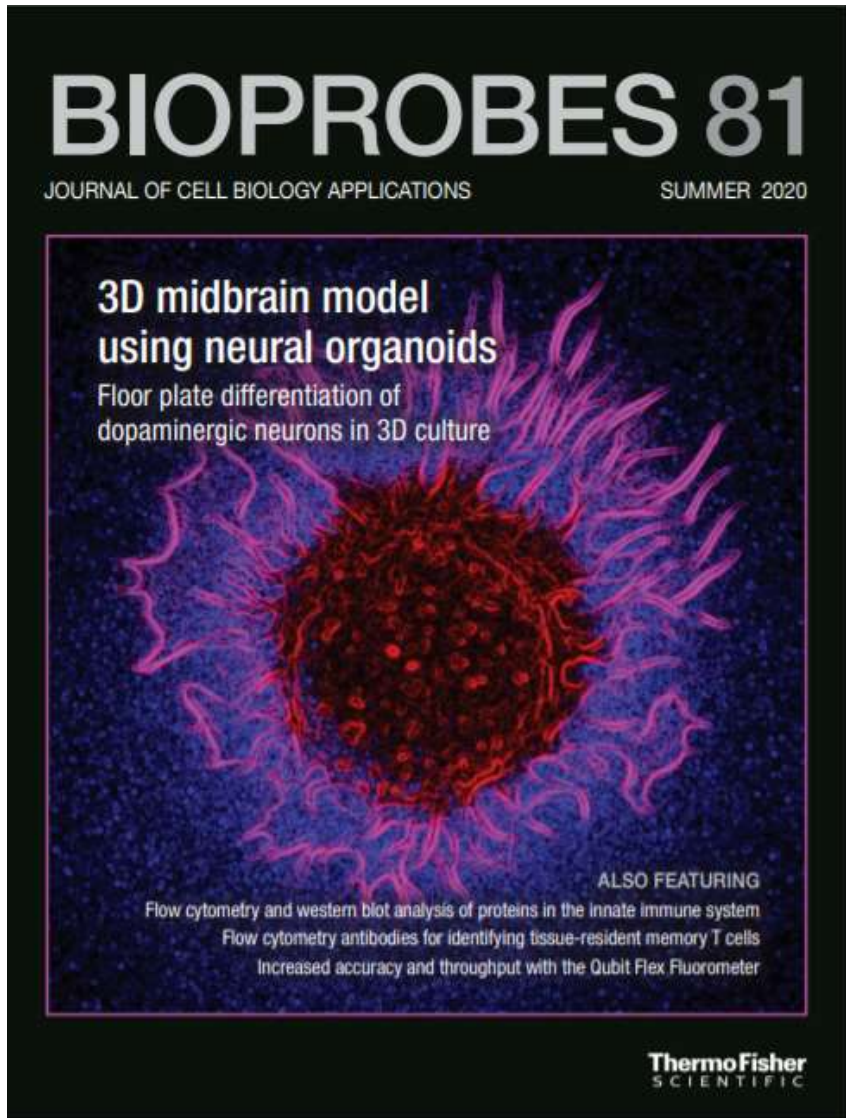
ThermoFisher
SCIENTIFIC

ohistochemistry: five steps to publication-quality images

ThermoFisher
SCIENTIFIC

Molecular Probes Journal

Back Issues



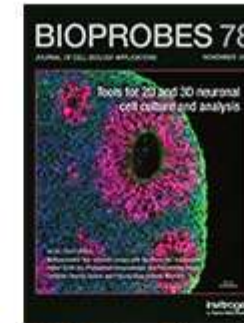
<https://www.thermofisher.com/th/en/home/references/newsletters-and-journals/bioprobres-journal-of-cell-biology-applications.html>



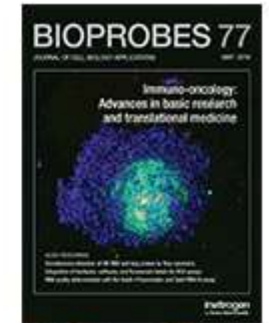
BioProbes 80



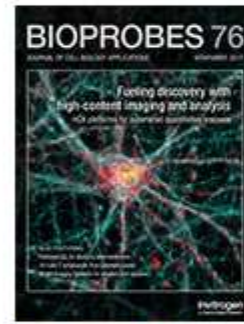
BioProbes 79



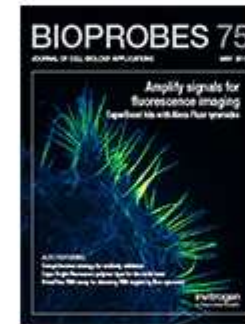
BioProbes 78



BioProbes 77



BioProbes 76



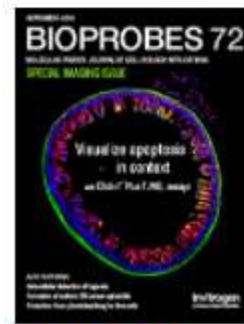
BioProbes 75



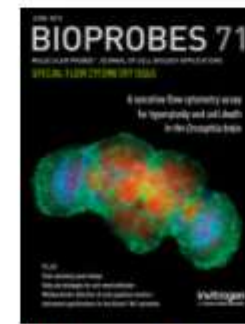
BioProbes 74



BioProbes 73



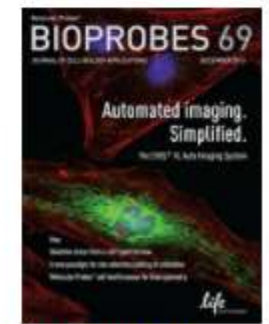
BioProbes 72



BioProbes 71



BioProbes 70



BioProbes 69

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