Experience Simplified Imaging

- EVOS® FL Auto Imaging Systems

Tim, Tsui

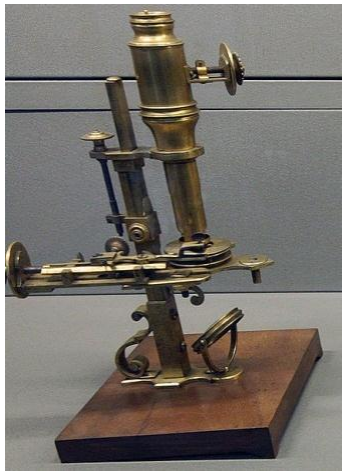
Cellular Imaging Analyzer

ThermoFisher SCIENTIFIC

金萬林企業股份有限公司
KIM FOREST ENTERPRISE CO.,LTD.

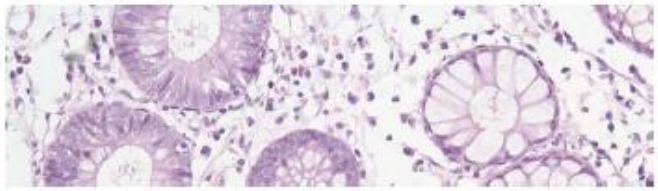
What is Microscope?

An instrument used to see the objects that are too small for the naked eye.



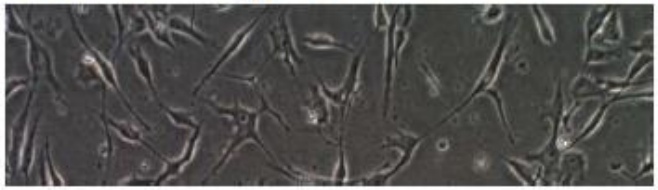
常見顯微影像系統的應用

明視野 (bright field)



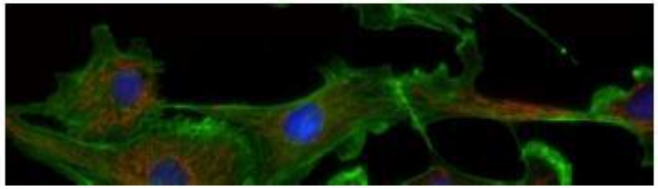
觀察染色的玻片樣本

相位差 (phase contrast)



觀察未經染色的透明樣本

螢光 (fluorescence)



觀察經螢光標定或
帶有螢光物質的樣本

相位差系統(Phase contrast)-觀察細胞的好幫手

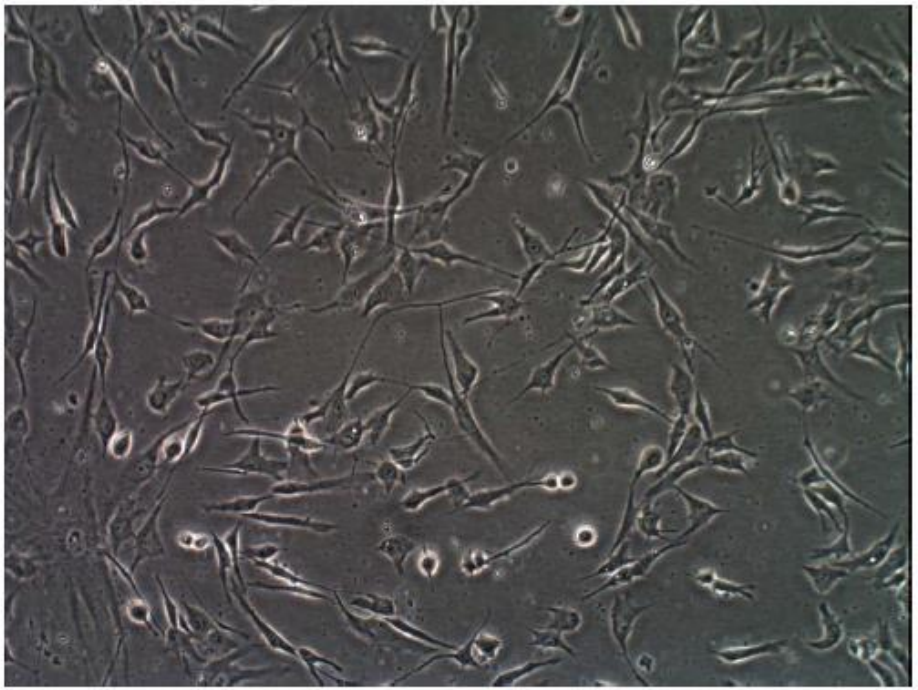
相位差板（輪）

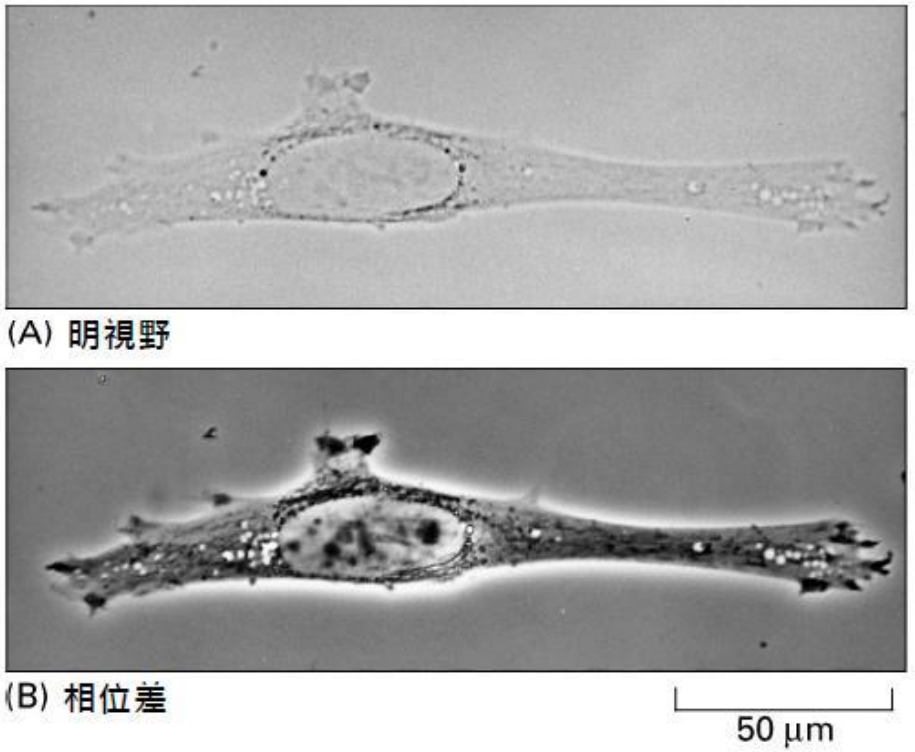
相位差物鏡

倒立顯微鏡

相位差系統(Phase contrast)-觀察細胞的好幫手

典型相位差影像

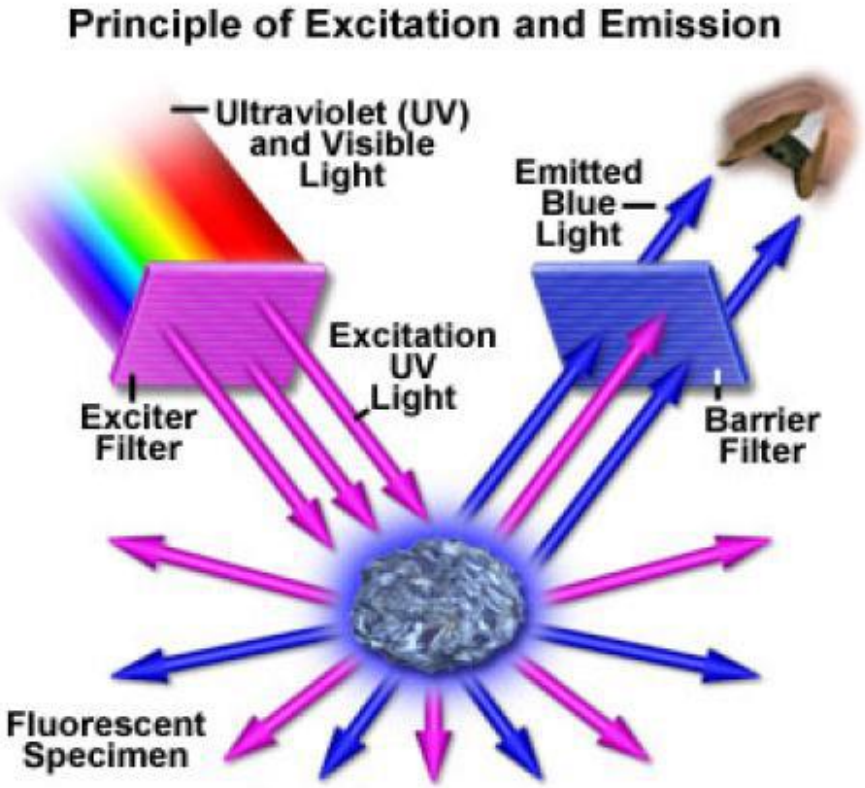




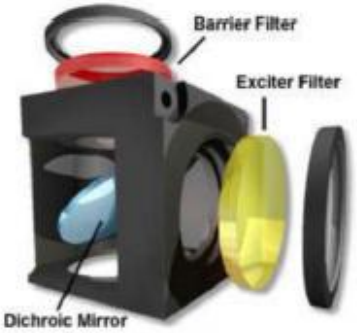
特點:

1. 無須染色，可觀察樣品最原本的型態與樣貌
2. 可觀察樣品的細節，藉此能大致區別細胞種類與狀態
3. 搭配免疫染色法可分辨確切細胞內蛋白質表現與細胞種類

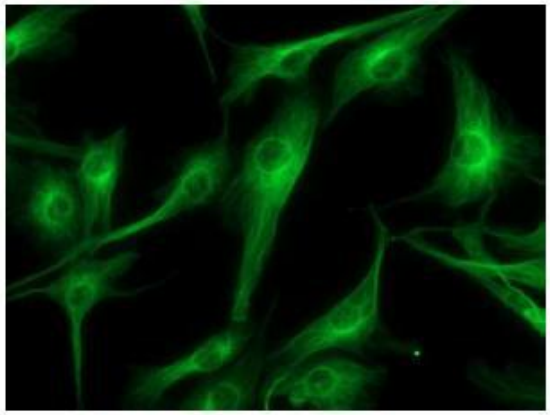
螢光顯微系統

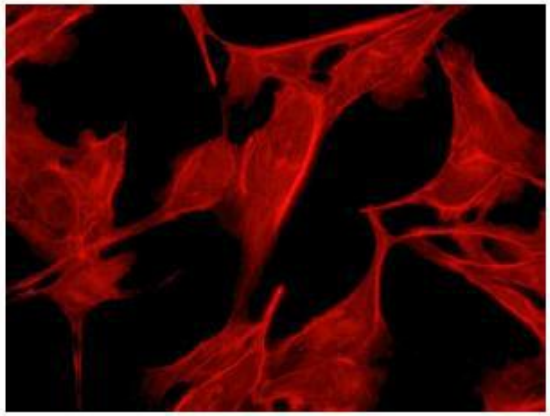


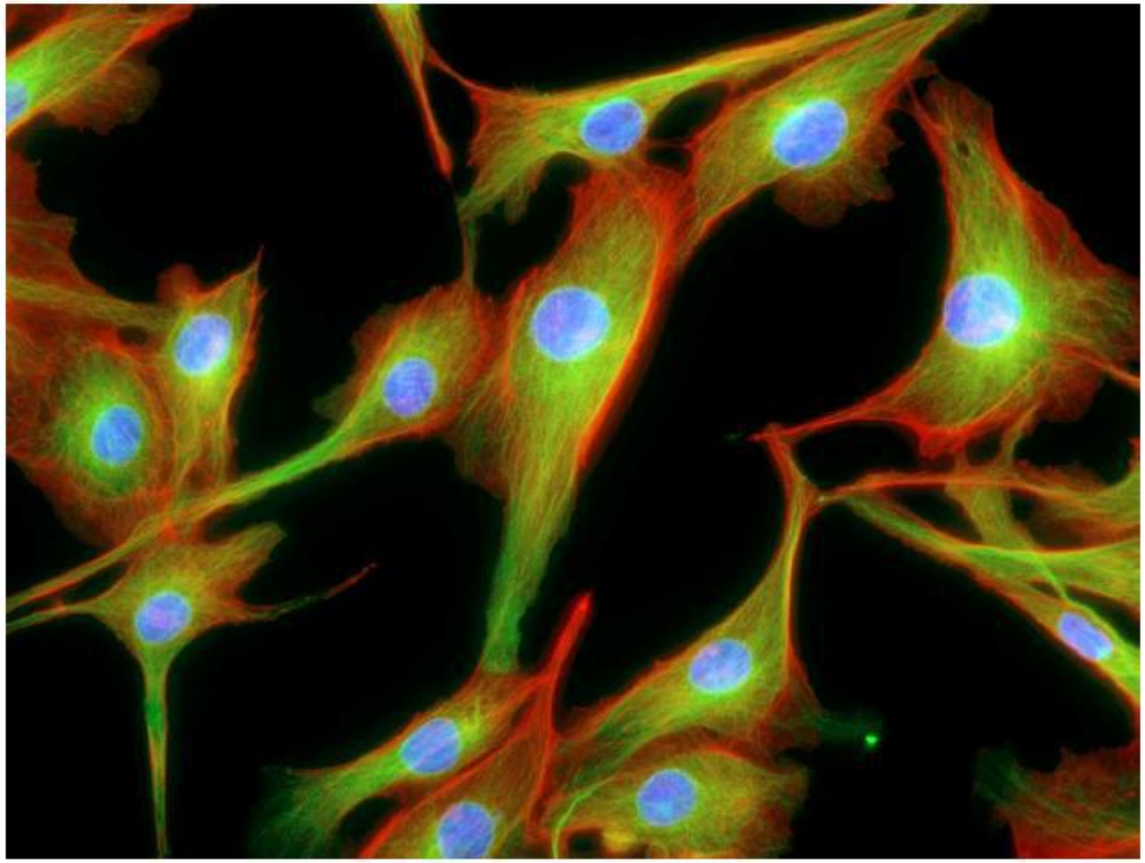
螢光顯微鏡必須有
螢光光源及濾鏡組



螢光顯微系統





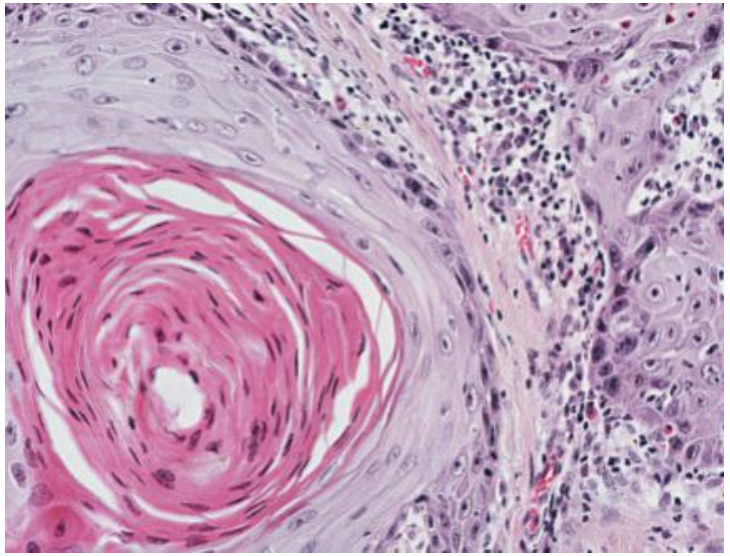


特點:

1. 影像對比明顯
2. 可使用免疫螢光染色法進行專一性標定
3. 需考量染劑與濾鏡搭配

Transmitted Light Applications

HeLa cells in culture

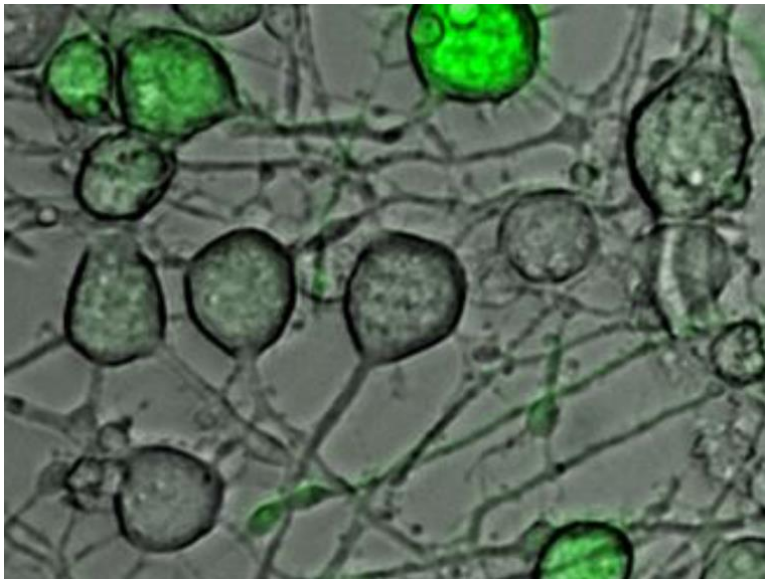


Immunohistochemical (IHC) staining of lung tissue with squamous cell carcinoma

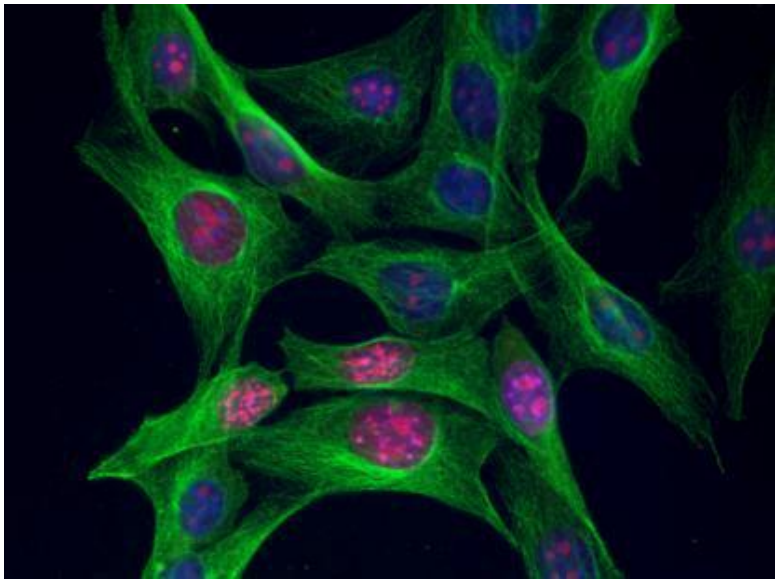
Cell culture: ***Are my cells confluent and healthy?***

IHC: ***Visual markers of disease***

Fluorescence Applications



Neuronal stem cells expressing GFP



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

Cell culture: *Are my cells expressing protein/ GFP?*

Cell health: *Do my cells look normal?*

EVOS Imaging Systems

次世代整合式顯微影像系統

1. 無須暗房
2. 無須校正
3. 無須暖機/冷卻
4. 快速觀察與紀錄

Why EVOS?

Fully Integrated Design

Go from this

To this

EVOS Feature Overview

Dual camera system: monochrome and color CCD to fit experiment's need

Proprietary light Cubes

Simple and Powerful user interface: Minimize training – anyone can operate

Flexible of use: Wide range of objectives, sample/vessel types, image requirements

Touched screen display: Multiple users can view simultaneously

Get out of the darkroom: no need to stay in the dark anymore

EVOS® FL Auto

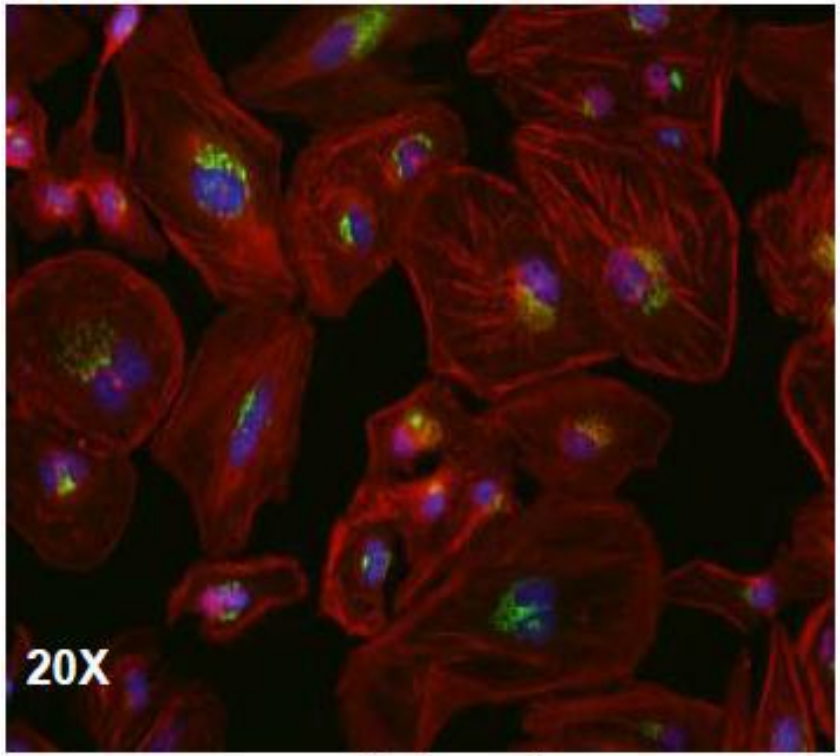
Fully automated, multi-channel fluorescence system

- 22” touch-screen LCD display
- 5 objectives (2x to 100x)
- 4 fluorescence colors
- Motorized scanning stage
- Color and B/W cameras
- Advanced software features

Dual Camera System

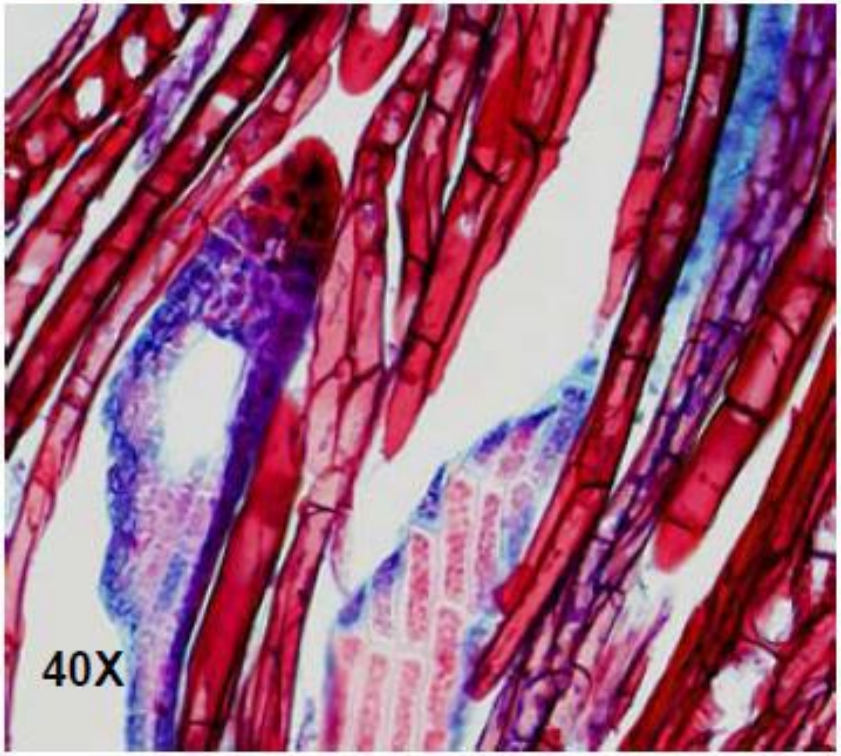
Applied to both fluorescent and colorimetric samples

Monochrome camera



HeLa cells
NucBlue® Fixed (nucleus, blue)
CellLight® Golgi-GFP (Golgi, green)
Ready Probes™ Actin Red 555 (actin, red)

Color camera



Moss antheridial head
polytrichum

Objectives

Objective 1: 4X

Objective 2: 20X

*May up to 5 objectives
(Air, oil objectives compatible)

*Internal Magnification:
Monochrome camera: 30X
Color camera: 18X

Plan Achromat

Magnification	NA	WD (mm)	Bright field	Phase	Long working distance	Coverslip corrected	Oil	Cat. No.
2x	0.06	5.10	•		•			AMEP4631
4x	0.13	16.90	•	•	•			AMEP4632
10x	0.25	6.90	•	•	•			AMEP4633
20x	0.40	6.80	•	•	•			AMEP4634
40x	0.65	3.10	•	•	•			AMEP4635
50x	0.95	0.19	•			•	•	AMEPOP050
100x	1.25	0.15	•			•	•	AMPFOP100

Plan achromat: Perfect for general applications; color and focus have standard correction.

Plan Fluorite

Magnification	NA	WD (mm)	Bright field	Phase	Long working distance	Coverslip corrected	Oil	Cat. No.
4x	0.13	19.70	•		•			AMEP4622
10x	0.30	8.30	•		•			AMEP4623
10x	0.25	9.20	•	•	•			AMEP4681
20x	0.45	7.10	•		•			AMEP4624
20x	0.40	3.10	•	•	•			AMEP4682
20x	0.50	2.50	•			•		AMEP4698
40x	0.65	2.80	•		•			AMEP4625
40x	0.65	1.60	•	•	•			AMEP4683
40x	0.75	0.72	•			•		AMEP4699
40x	1.30	0.20	•			•	•	AMEP4735
60x	0.75	2.20	•		•			AMEP4626
100x	1.28	0.21	•			•	•	AMEP4700

Plan fluorite: Excellent resolution resulting in brighter fluorescence signal and higher-contrast imaging.
Helps reduce optical aberrations; color and focus have a higher level of correction.

Plan Apochromat

Magnification	NA	WD (mm)	Bright field	Phase	Long working distance	Coverslip corrected	Oil	Cat. No.
1.25x	0.04	5.00	•		•			AMEP4736
20x	0.75	0.60	•			•		AMEP4734
60x	1.42	0.15	•			•	•	AMEP4694
100x	1.40	0.13	•			•	•	AMEP4733

Plan apochromat: Highest levels of resolution, fluorescence brightness, contrast, and chromatic correction.

Light Cube- FL light source

- Light cube1： DAPI
(Ex: 357/44 nm, Em: 447/60 nm)

- Light cube2： GFP
(Ex: 470/22 nm, Em: 510/42 nm)

- Light cube3： Texas Red
(Ex: 585/29 nm, Em: 624/40 nm)

*May up to 4 channel

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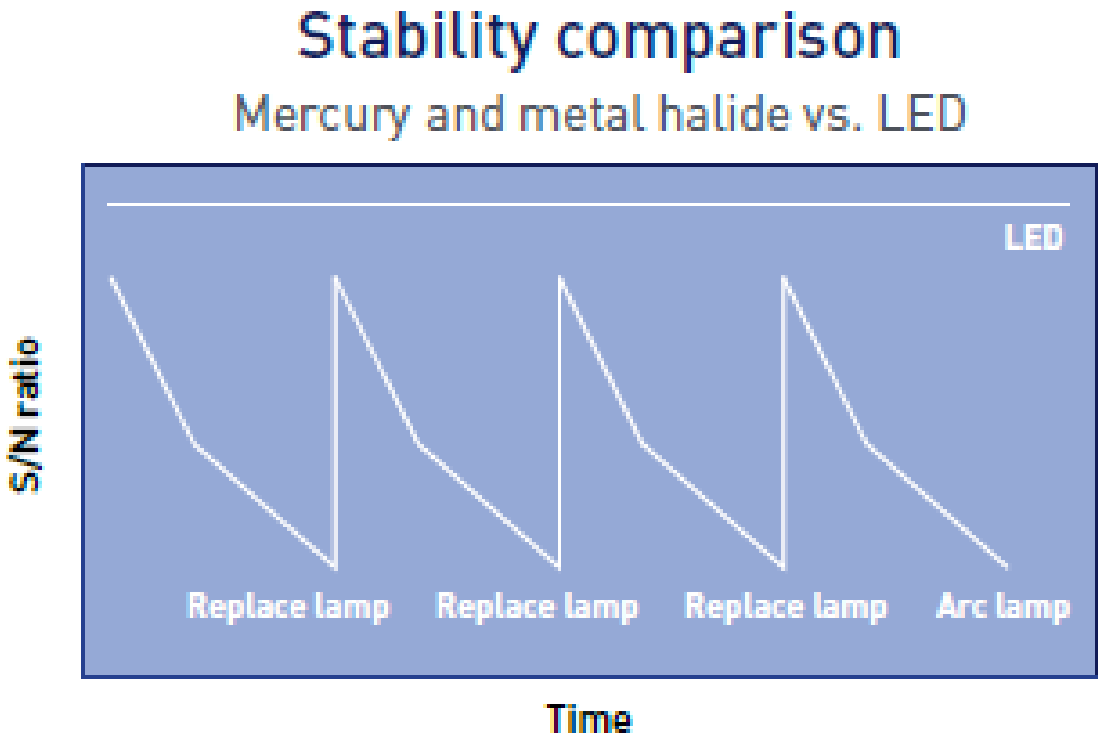
- Light cube2： GFP
(Ex: 470/22 nm, Em: 510/42 nm)

- Light cube3： Texas Red
(Ex: 585/29 nm, Em: 624/40 nm)

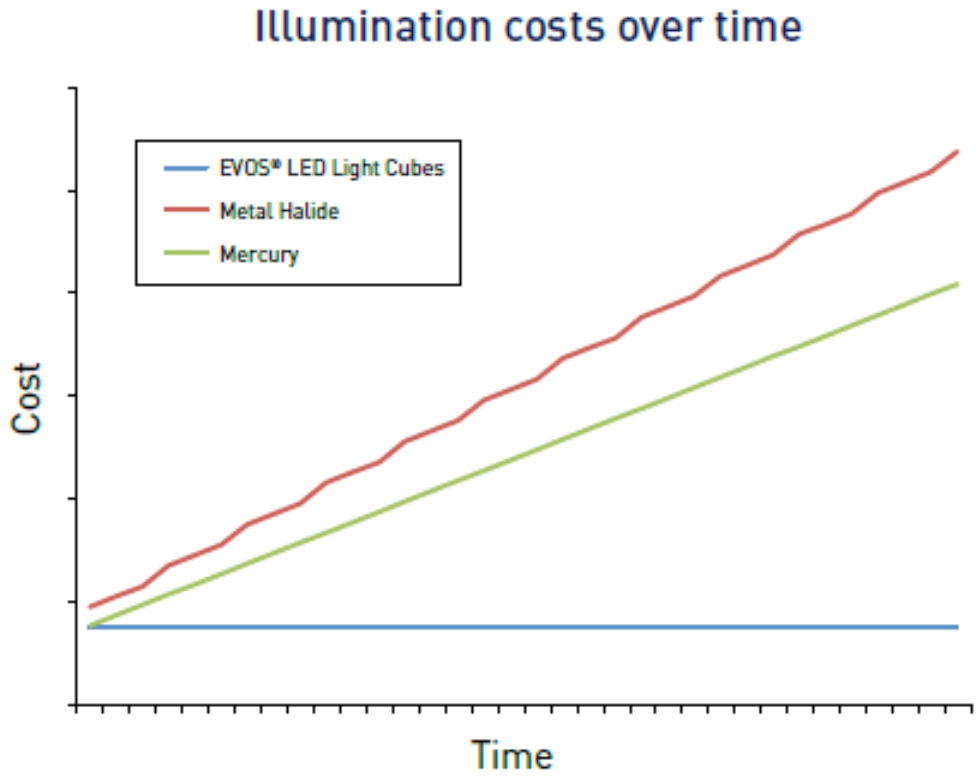
*May up to 4 channel

Light cube keep continuous light intensity



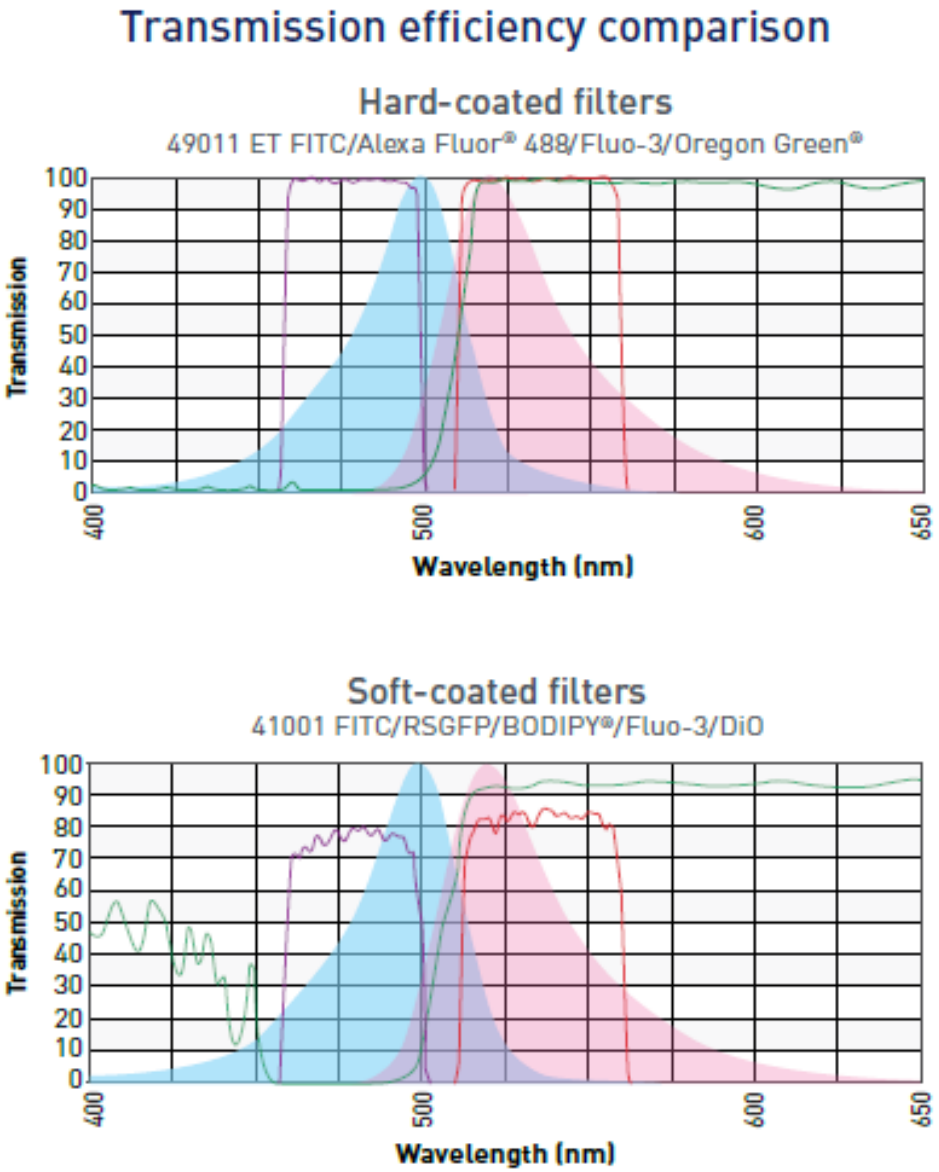
- Mercury arc lamps can decrease in intensity by 50% in the first 100 hours of operation.
- EVOSR systems have continuous light cube intensity, users can rely on consistent illumination.

Less expensive to own and maintain



- The LED bulbs on the EVOS are rated for >50,000 hours.
- Typical mercury bulb: 300 hours
- Metal halide bulb: 1500 hours.

Hard-coated filter sets for higher transmission efficiencies



- **Increase >25% light transmission than traditional soft-coated filters.**

- **Better to detect faint fluorescence signals, better S/N ratios.**

- Excitation filter (purple), emission filter (red), dichroic mirror (green); Alexa FluorR 488 excitation (blue), Alexa FluorR 488 emission (pink).

LED vs Mercury Arc Lamp illumination

Traditional Hg Lamp House

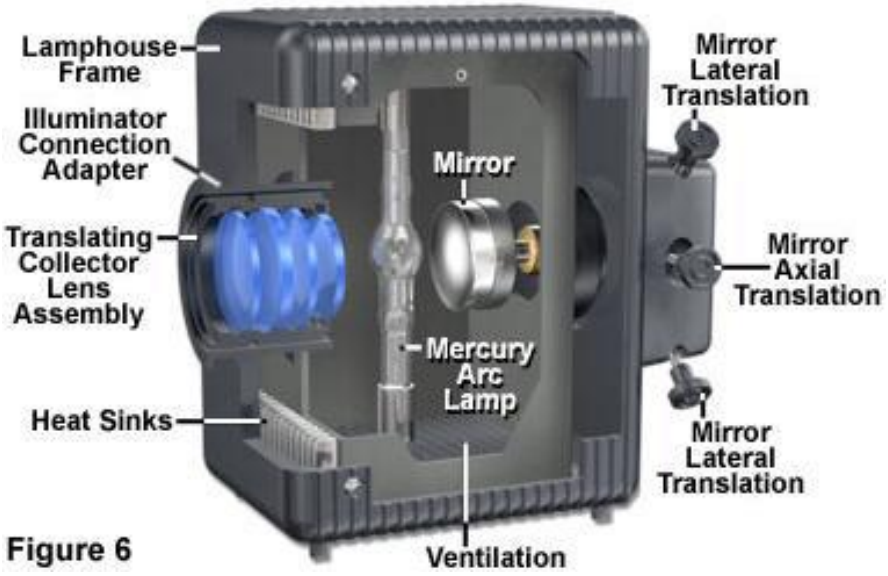


Figure 6

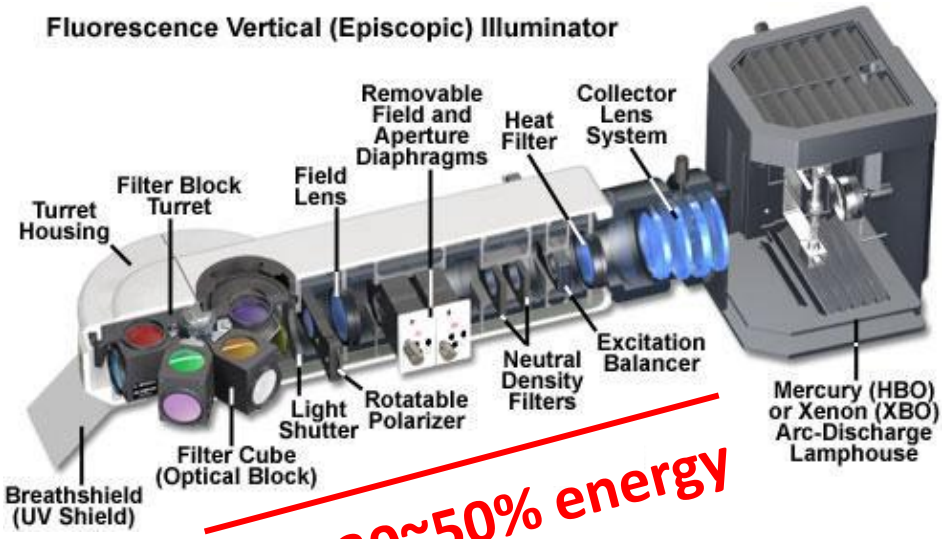
- ✓ 5-10 Adjustments need to be done
- ✓ 15-30 min for warm up/ cool down
- ✓ 300 hr life time

LED Light Cube

- ✓ No adjustment or alignment
- ✓ No warm up/ cool down time
- ✓ 50,000 hr life time
- ✓ Adjustable light intensity

Revolutionary Light Path

Traditional light path

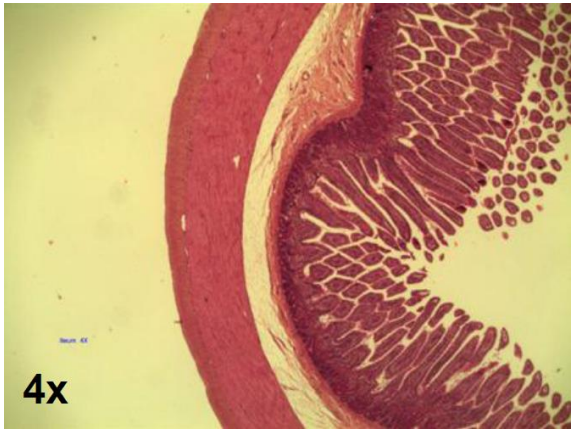


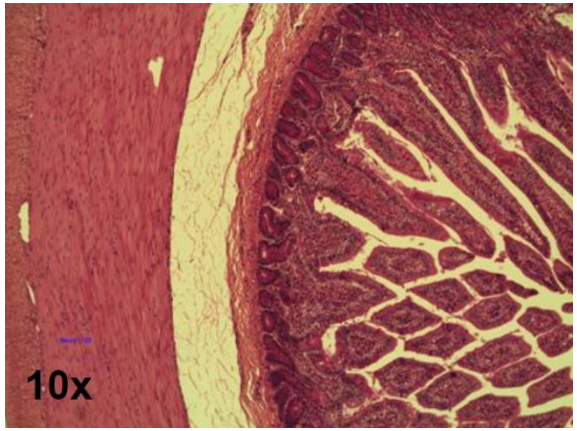
EVOS light path

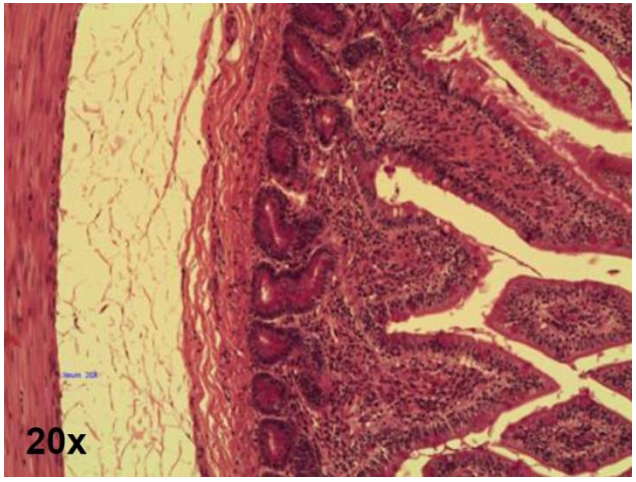
- ✓ Less optical elements!
- ✓ 1/10~1/5 energy need

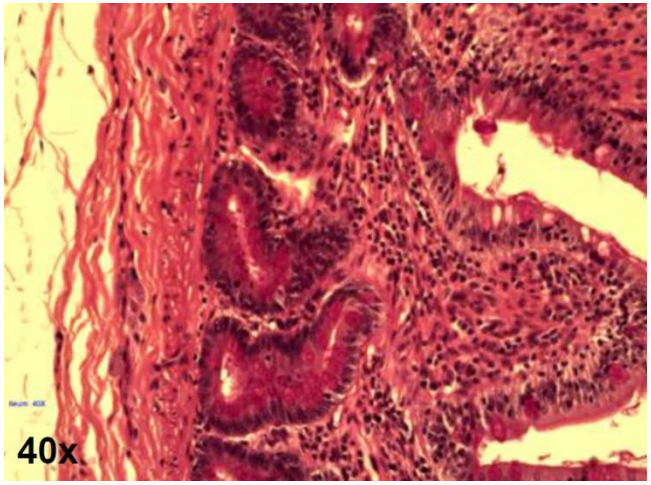
Automated Optical System

Automated switching objective, light cubes and cameras



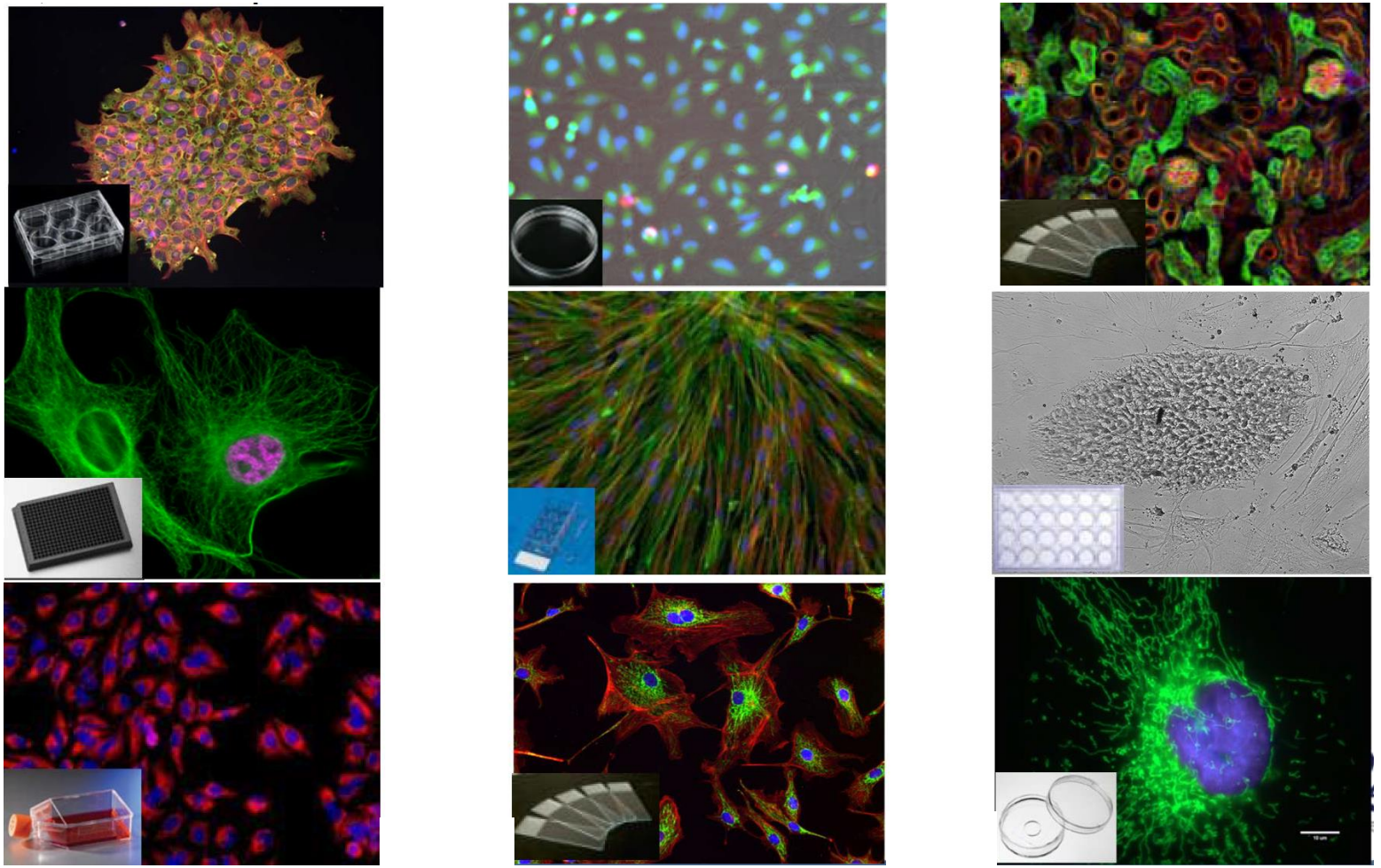






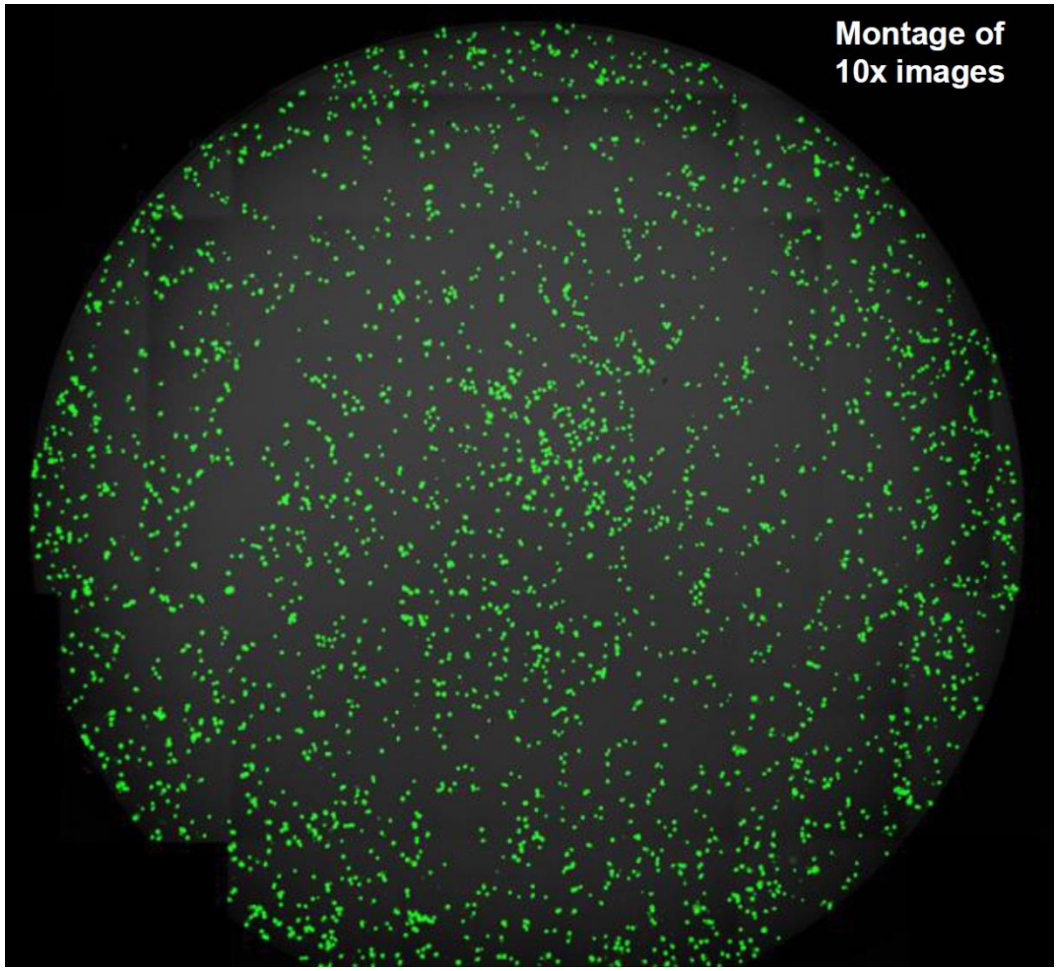
Various vessel holders

Multi-well plate, 10/6/**3.5 cm plate**, T25 flask, regular slide (25mmx75mm)



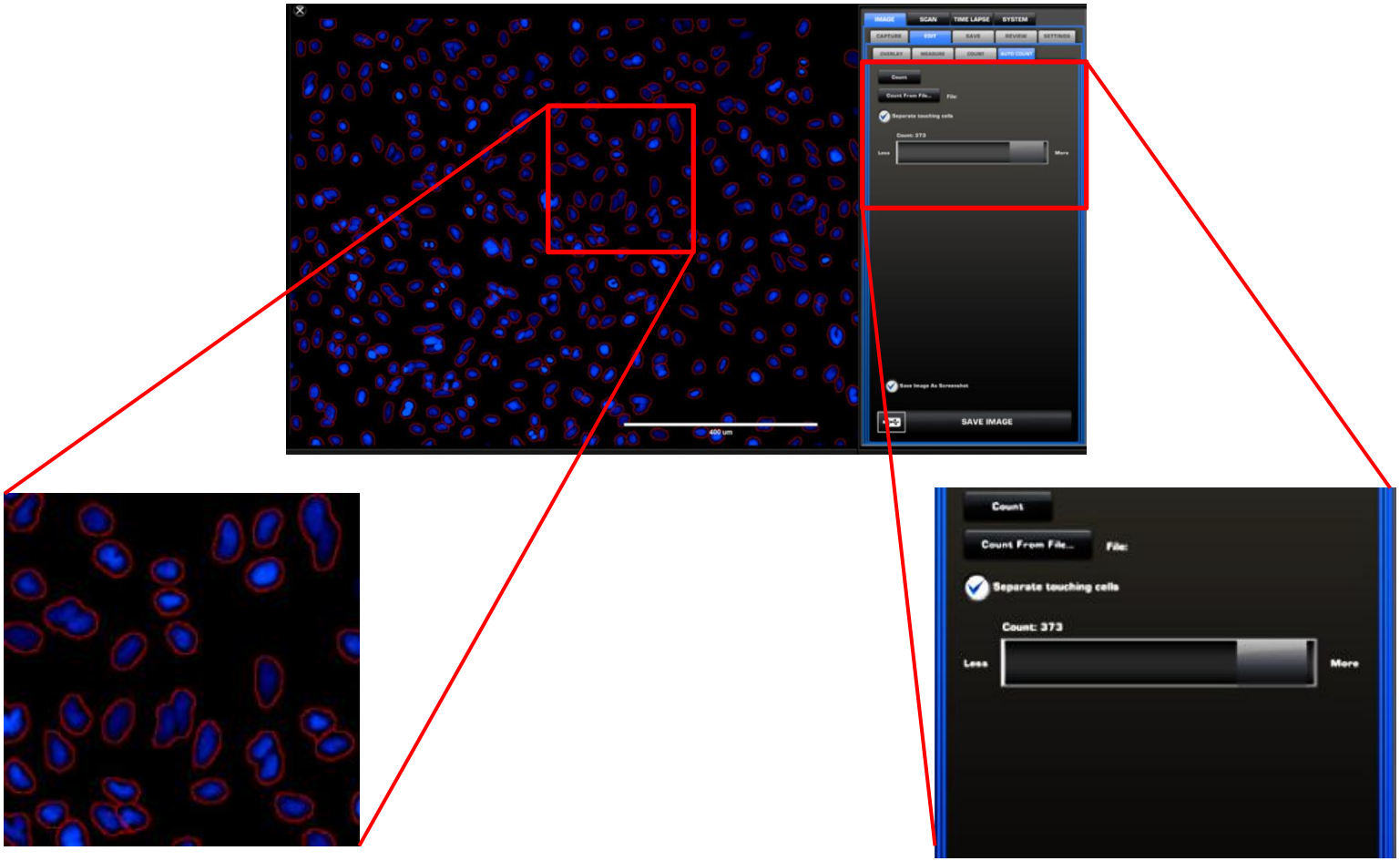
Software Features

Image Stitching: Capture multiple images with overlapping fields and use mosaic tiling to stitch a high-resolution image of a large area.



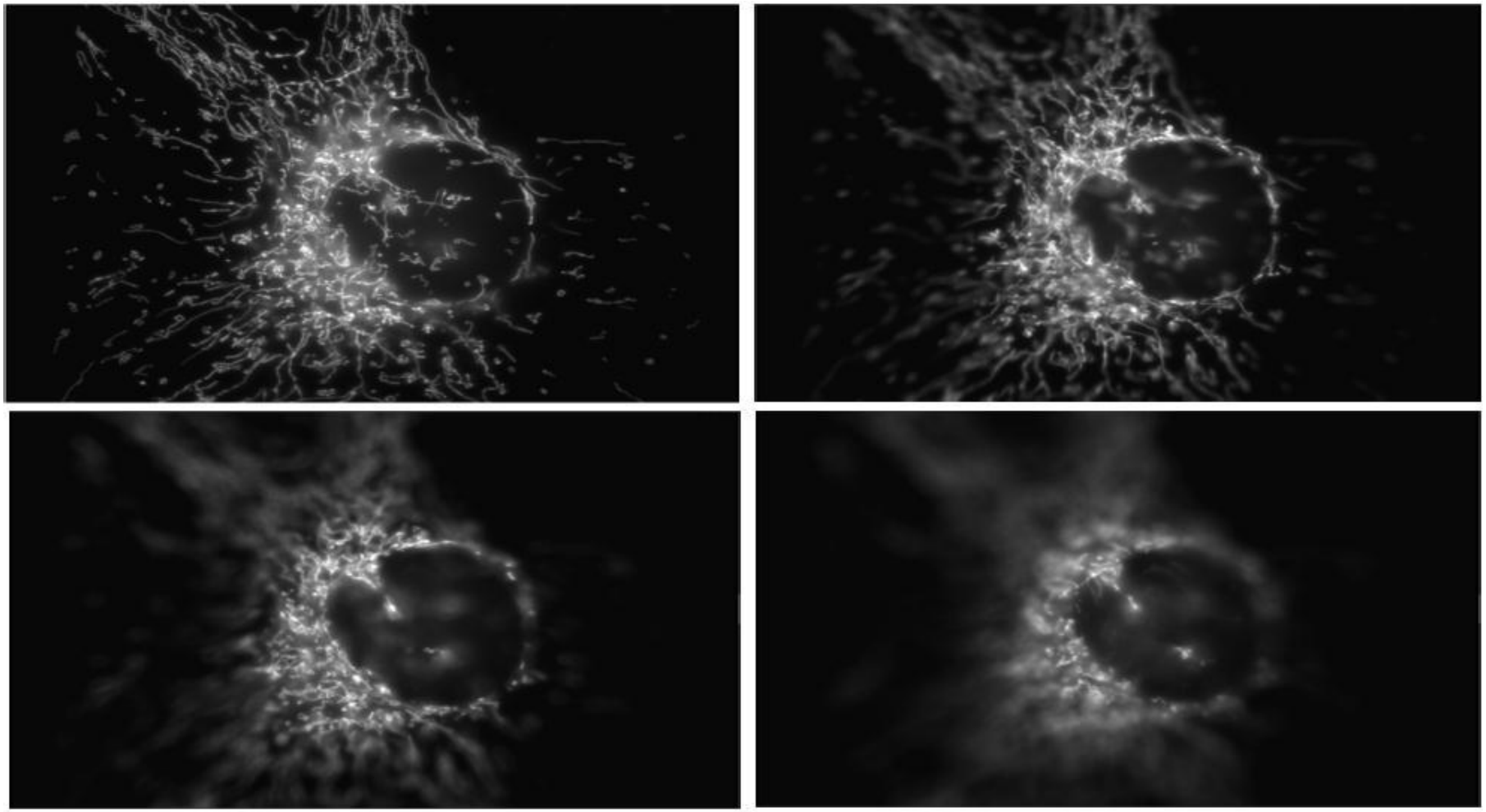
Software Features

Auto Cell Counting: Nucleus (DAPI/ Hoechst) staining and counted by area, intensity, and roundness automatically.



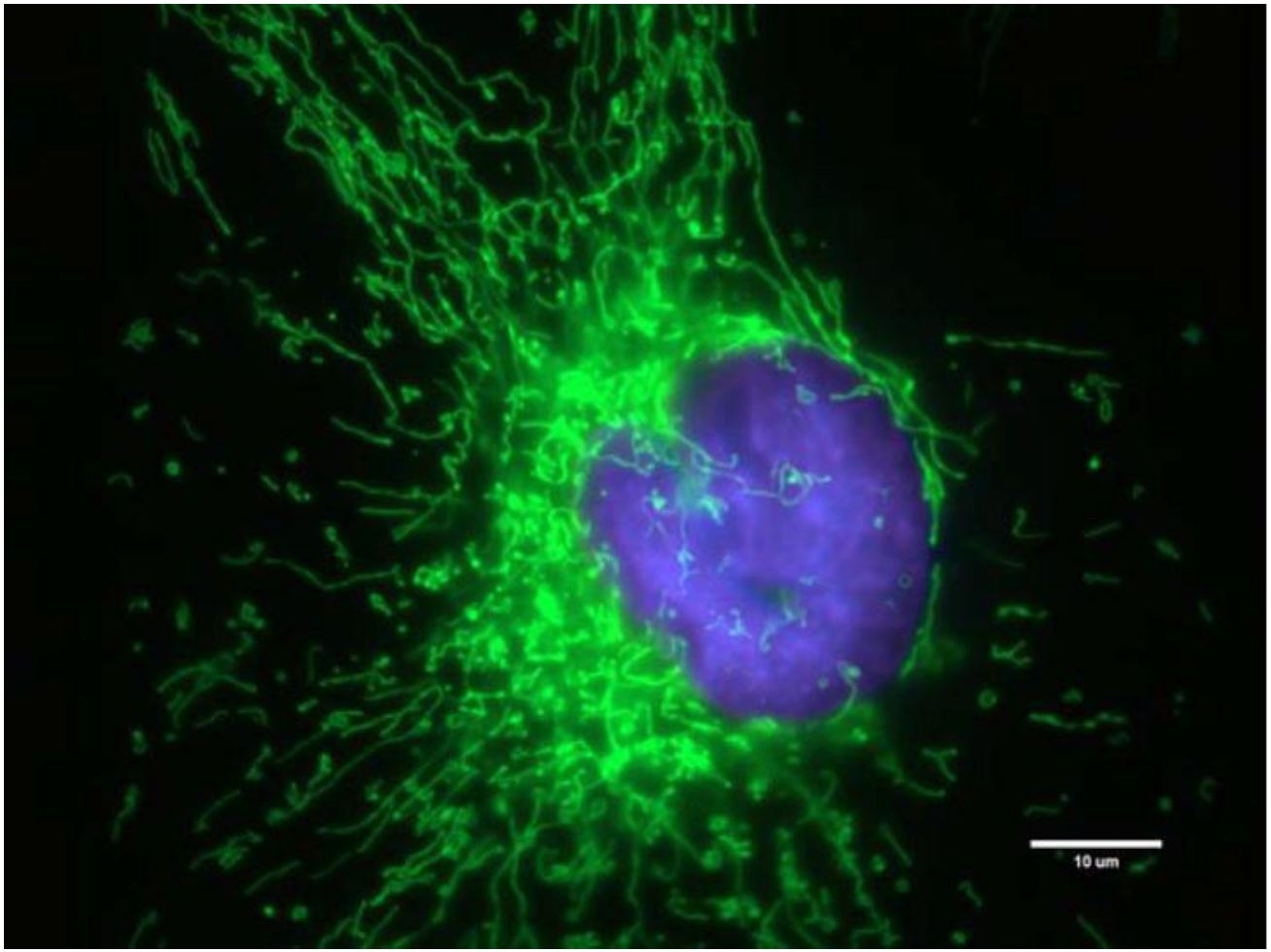
Software Features

Z-Stacks: Collect layers in the z-dimension down to 0.12 um thickness and “walk through” an object.



Software Features

Z-Stacks Flat Focus: Collect series of images, extract the most "in focus" pixels to create a focused image even from thick samples



Environmental Controler

Precise environmental control

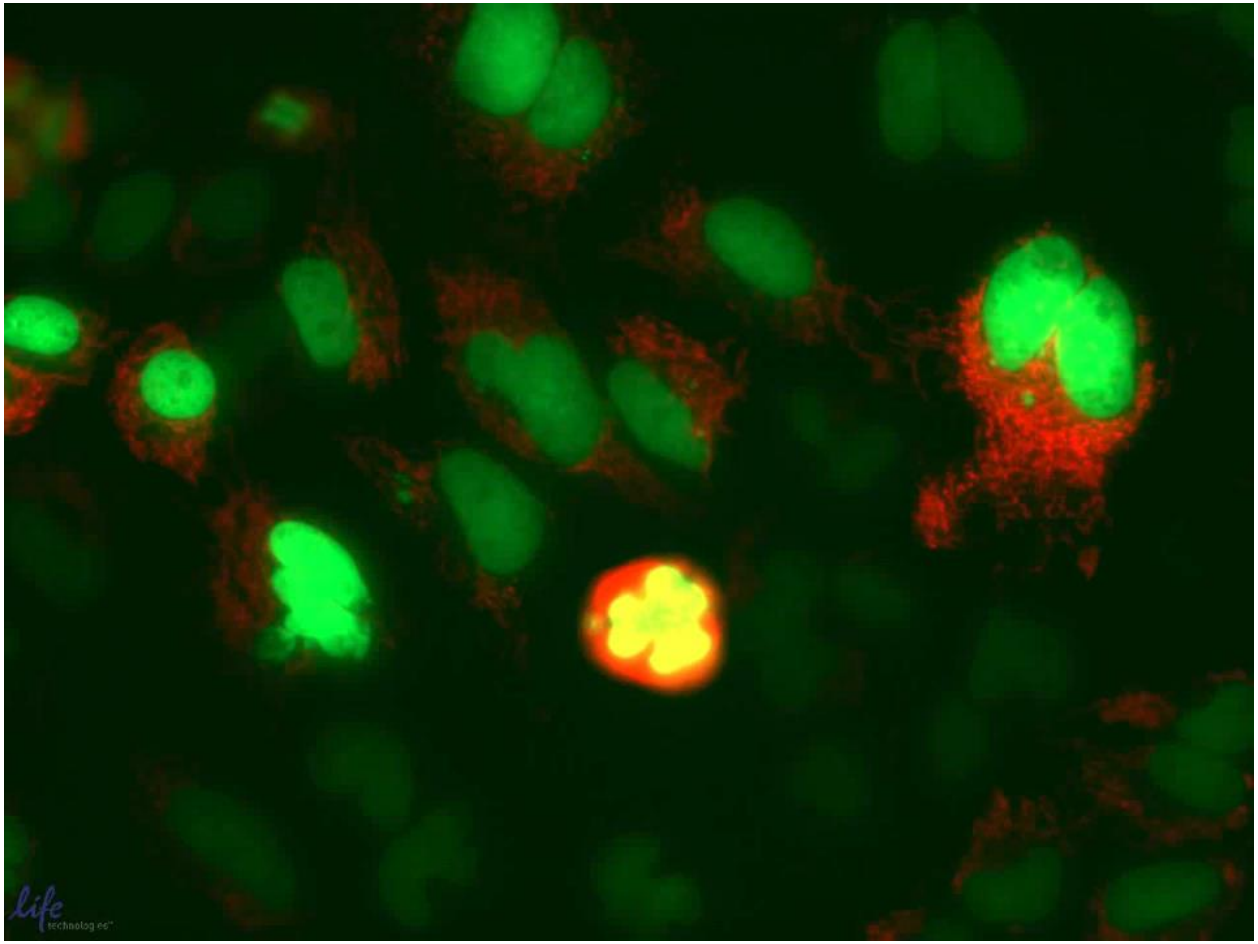
- Compatible vessels: Multi-well plates, 35 mm Petri dishes, 60 mm Petri dishes, T-25 flasks
- Temperature range: Ambient to 40 °C (+/- 0.1 °C)
- CO_2 range: 0% to 20%
- O_2 range: 0% to ambient
- Humidity: >80% relative humidity (RH) at 37 °C

Key applications:

Dynamic live cell events, including cell division, cell cycle, apoptosis and cytotoxicity, stem cell differentiation, cell migration and wound healing, membrane and organelle dynamics

Software Features

Time Lapse: Use up to 96 beacons to record events in multiple microplate wells over time and then seamlessly create movies



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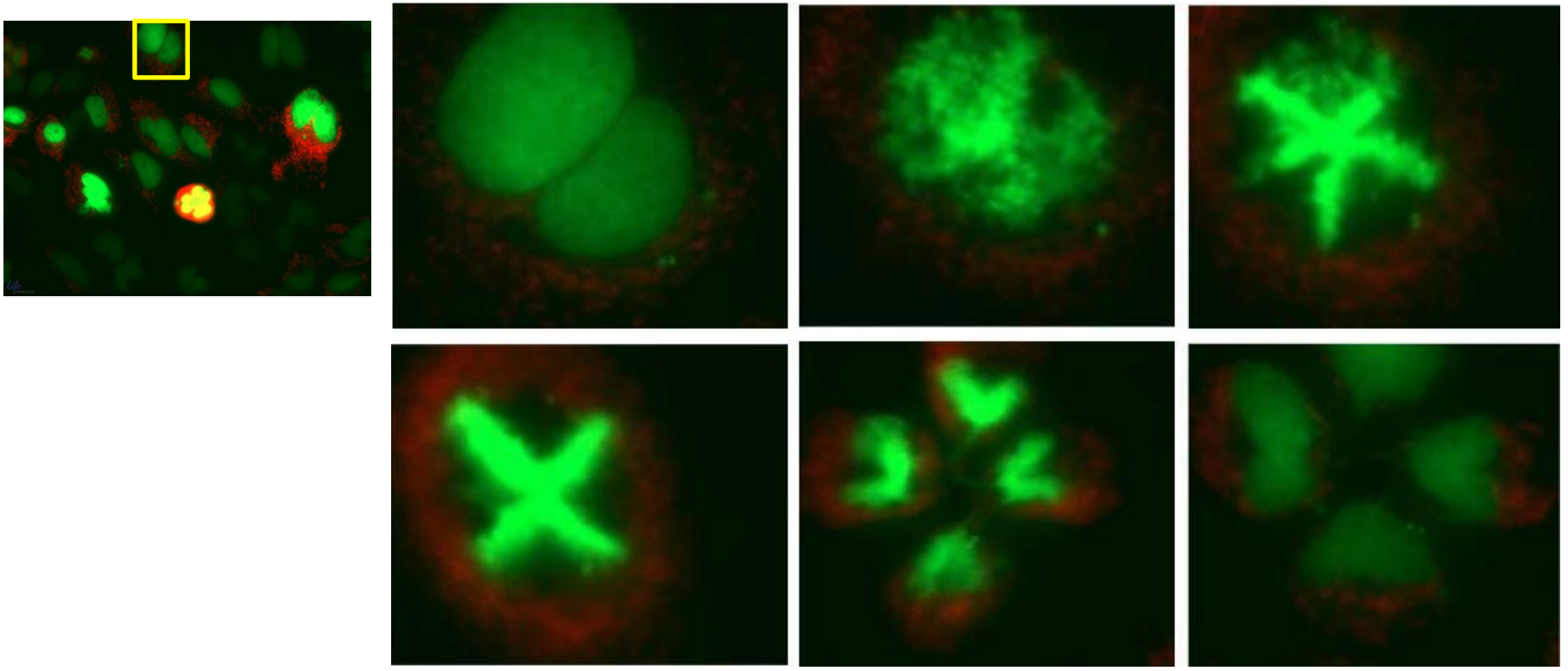
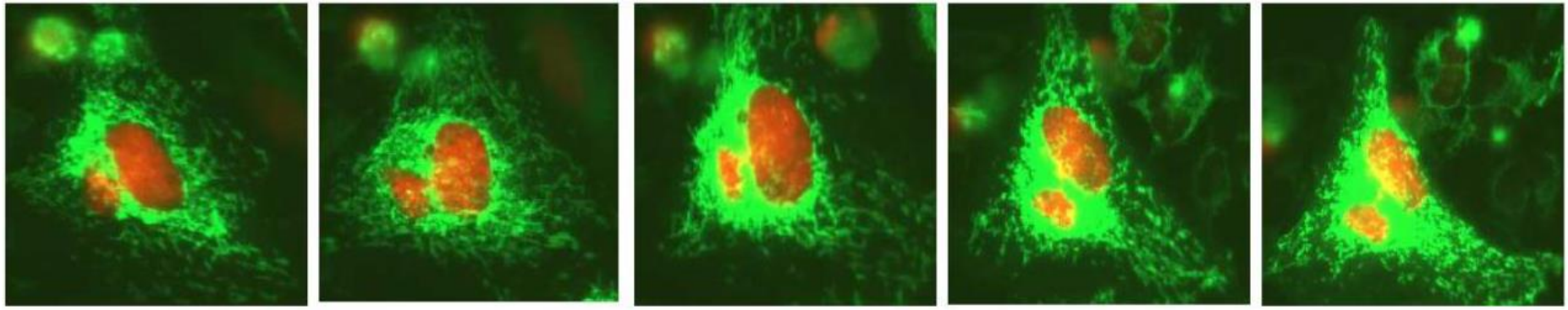
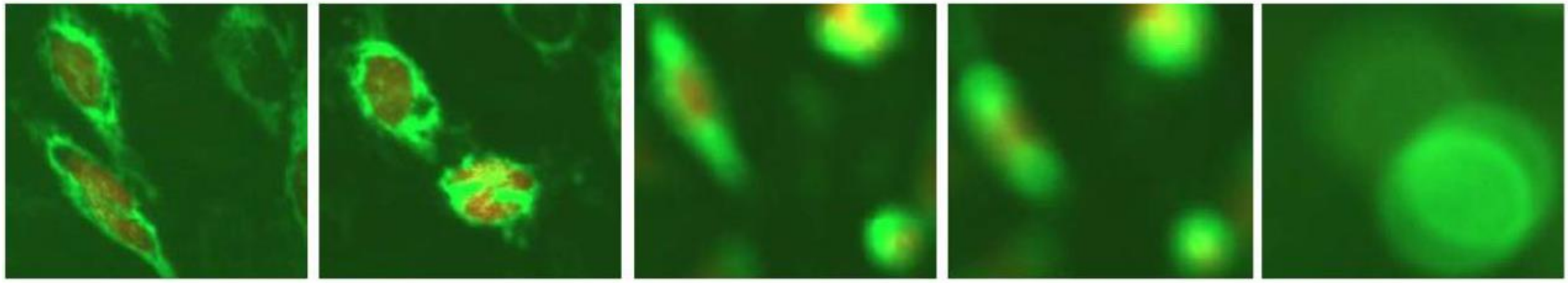


Software Features

Auto focus: Set autofocus to keep all the image in focus during image acquisition



Autofocus set: Focus maintained



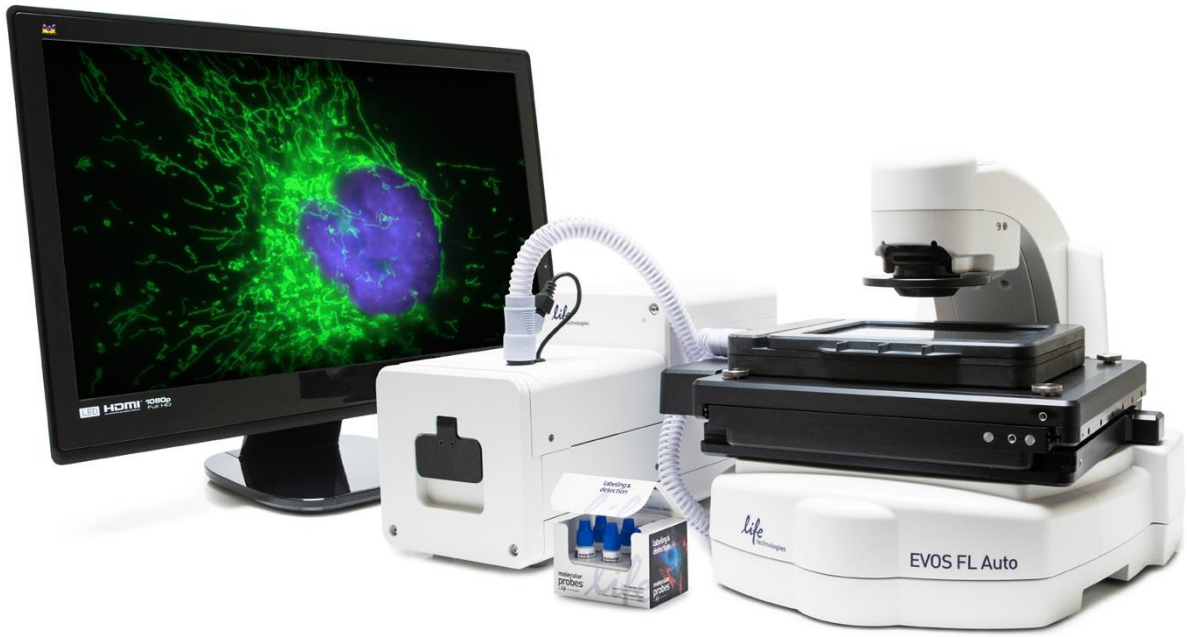
No autofocus: Out-of-focus drift

Features Review

Research areas	Key applications
Cell Culture	
– Cell maintenance	– **Cell confluence, counting & viability**
Molecular Biology	
– Gene expression	– **Transf. efficiency / RNAi (GFP)**
Cell Biology	
– Stem cell research	– **Differentiation markers, morphology**
– Embryonic development	– **Protein expression, localization**
Neurobiology	
– Neuronal development	– **Neurite outgrowth, differentiation**
Oncology & Immunology	
– Cancer and metastasis	– **Vascularization & invasion**
– Infection & immunity	– **Cell migration & proliferation**
– Apoptosis & autophagy	– **Cell death and cytotoxicity**

Experience Simplified Imaging

- Meet EVOS® Cell Imaging Systems



Thanks for your attention!

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