CytoPainter imaging products

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Distributor Webinar

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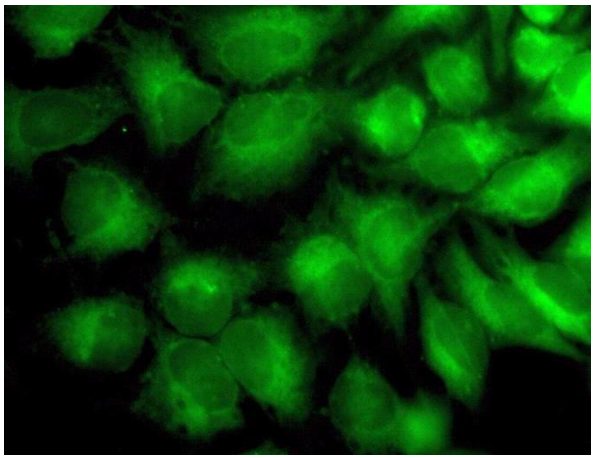

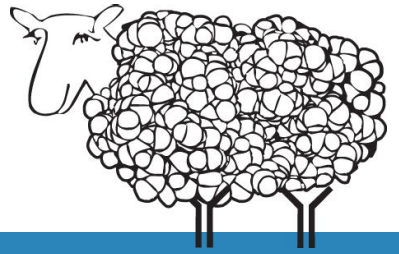

Overview

1. **Fluorescence microscopy**
   - Introduction
   - Why use fluorescence microscopy
   - What can we study with fluorescence microscopy
   - Things to consider
2. **CytoPainter**
   - Product range
   - Why/ when use CytoPainter
   - Details and supplier comparison
3. **General Protocols**
4. **Troubleshooting**
5. **Selling tips**
6. **Summary**
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1. Fluorescence microscopy

Fluorescence microscopy

- **A fluorescence microscope is an optical microscope that uses fluorescence instead of (or in addition to) reflection and absorption to study specimens.**

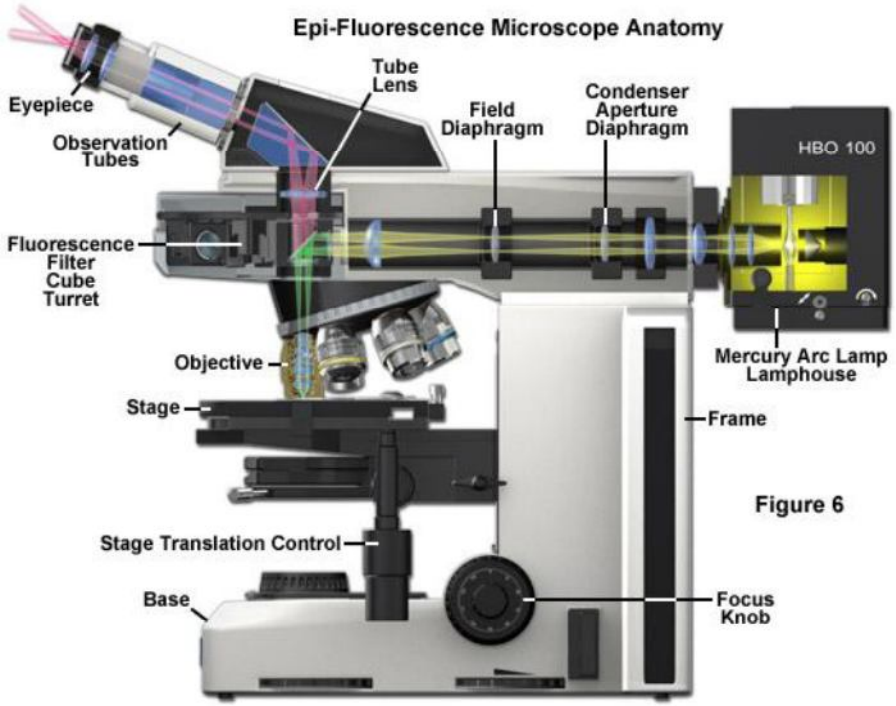
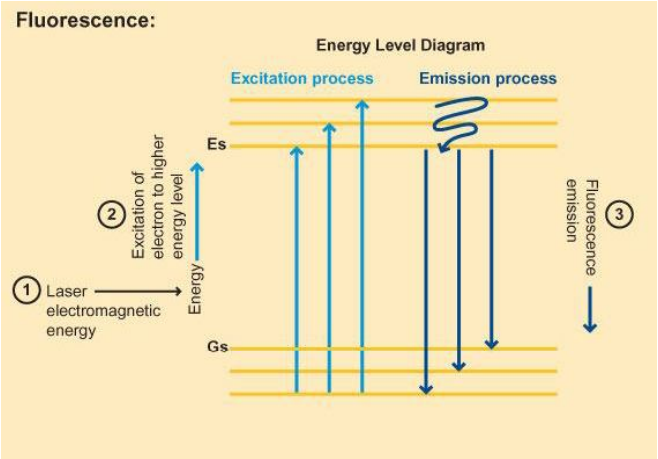


Figure 6

Fluorescence microscopy

- **How does fluorescence works:**
  - Electromagnetic energy from a laser set at the correct wavelength will provide the right amount of energy to an electron in the fluorescent dye molecule. This is the signature **excitation wavelength** for the molecule. The energy is absorbed by this electron.
  - On absorption of this energy, the electron moves to an excitation state at the next energy level (Es).



  - Finally, this energy is released in the form of a photon (fluorescence) and the electron moves back to the lower energy level. The amount of energy released will be determined by how far the electron drops down the energy levels which will always be the same in the same fluorescent molecule. This will determine the wavelength of the photon, and the "color" observed giving the fluorescent dye its signature **emission wavelength**.
- **Excitation (Ex) and emission (Em) wavelengths of a dye are essential information prior any fluorescent experiment.**

Why use fluorescence microscopes:

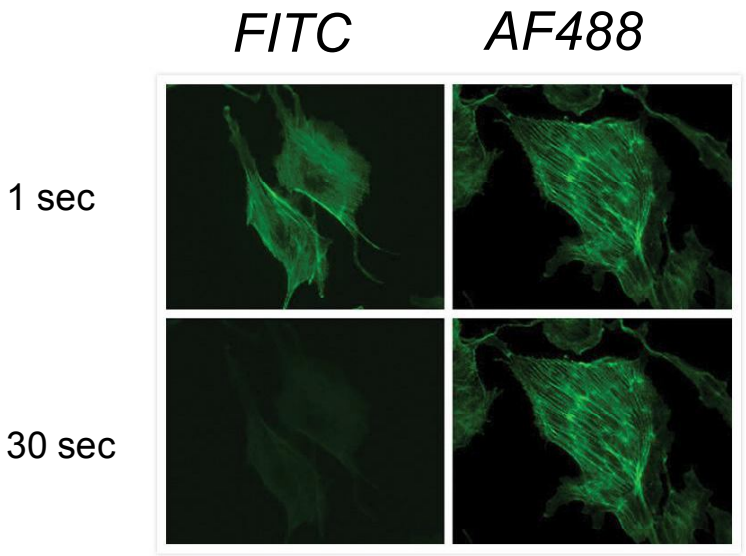
- Fluorescence microscopes reach higher resolution than “light” microscopes.
- Because of the variety of fluorochromes available nowadays, it is easy to look at different processes/ proteins at the same time (multicolor staining).
- Fluorescence microscopy allows to look at:
  - Fixed sections: cells or tissues are fixed/embedded to stabilize the structure at a specific time point.
  - Live sections: cells or tissues or embryos (*C.elegans* or *Drosophila*) are kept in growing media. This allows visualization of events and structures in real time.

What can we study with fluorescence:

What to study	Detection method	Sample type
Proteins	Antibody	Fixed cells & tissues
	Linked to Fluorescent Protein (GFP, BFP, mCherry, ...)	Live cells & tissues Fixed cells & tissues
Cellular structures or events (vesicle movement)	Antibody (to protein in the structure)	Fixed cells & tissues
	Specific dye	Live cells & tissues Fixed cells & tissues

Things to consider:

- **Photobleaching:** after a number of excitation/emission cycles, fluorochromes start to “bleach” and loose signal intensity. Some fluorochromes bleach easier than others, and in general, new generation dyes are more resistant to photobleaching.

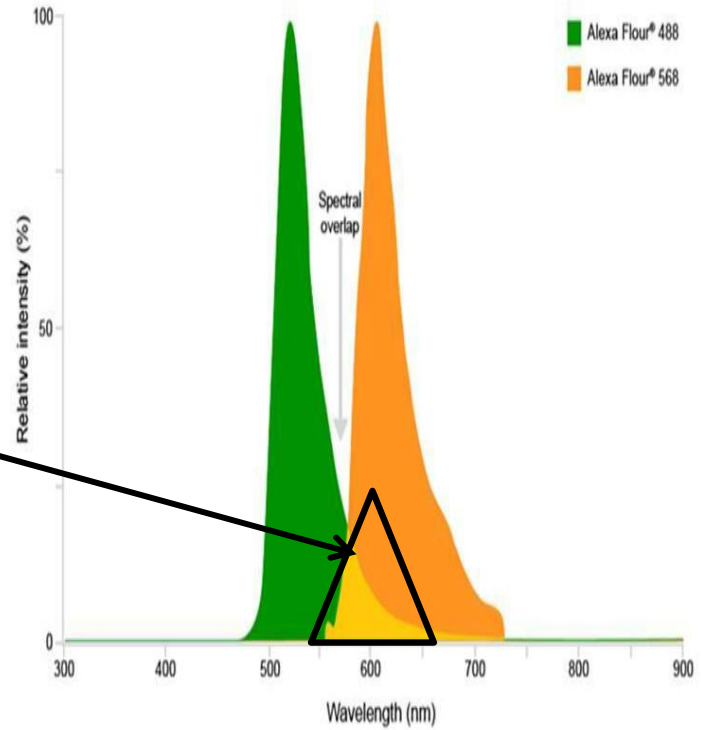


- **Intensity:** some fluorochromes are more intense than others and will show brighter on the microscope. New generation dyes are brighter.

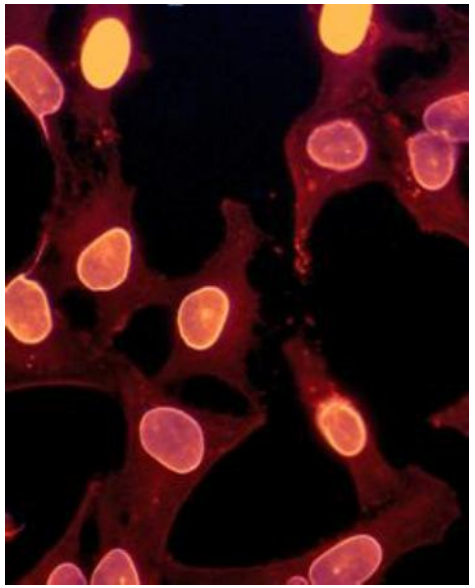
Things to consider:

- **Fluorescence bleeding**: fluorochromes that have a close spectra to each other may “bleed through” into other channels and mask the initial signal in that channel.

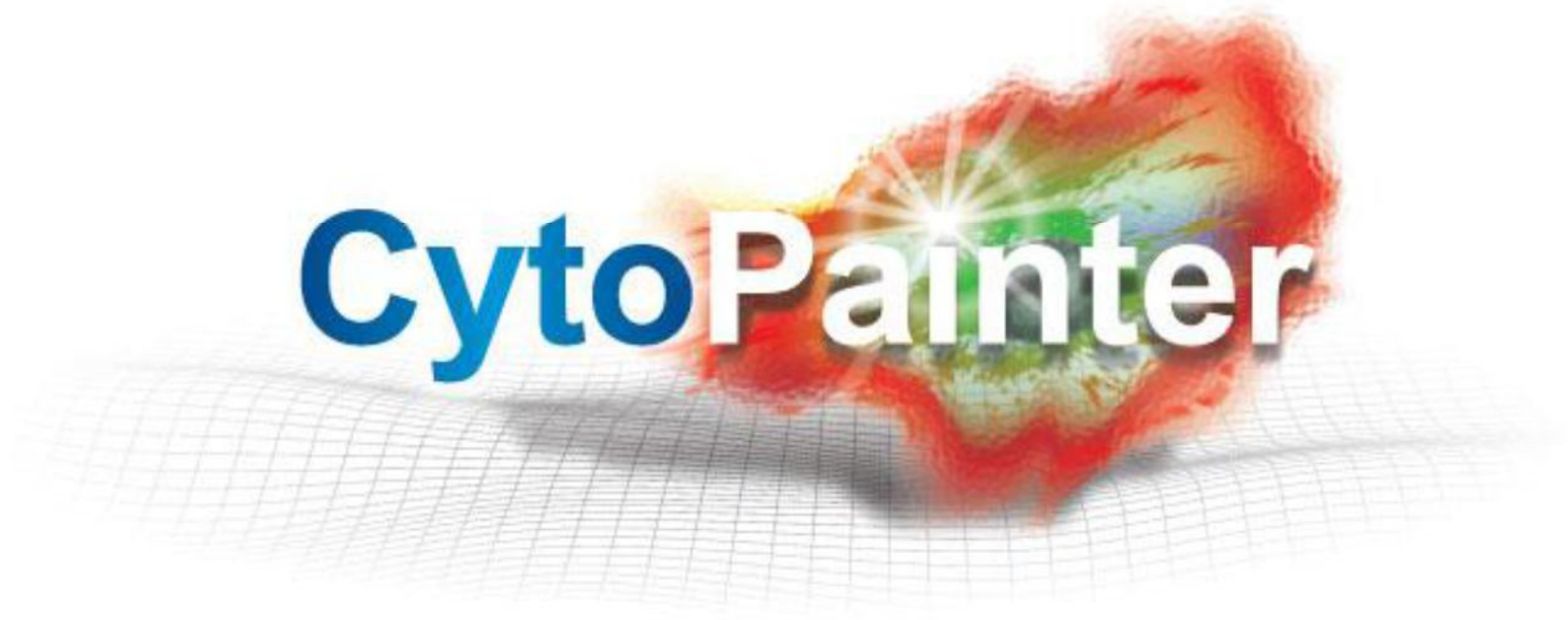
AF488 bleeds through into AF568 channel



Cy3 ® bleed through into Texas Red®



2. CytoPainter



What is CytoPainter

- **CytoPainter is a range of staining products that allow researchers to effectively visualize cellular components and perform cellular tracking**
- **Main Characteristics:**
  - High photostability – minimal photobleaching
  - Compatible with most common fluorescence microscope filters
  - Available in a variety of colors – ideal for co-localization studies
  - Suitable for proliferating and non-proliferating cells growing in suspension or adhesion
- **CytoPainter range includes products for:**
  - Cell Tracking – visualization of cells through several generations
  - Cellular staining – visualization of main subcellular components and structures

Why use CytoPainter?

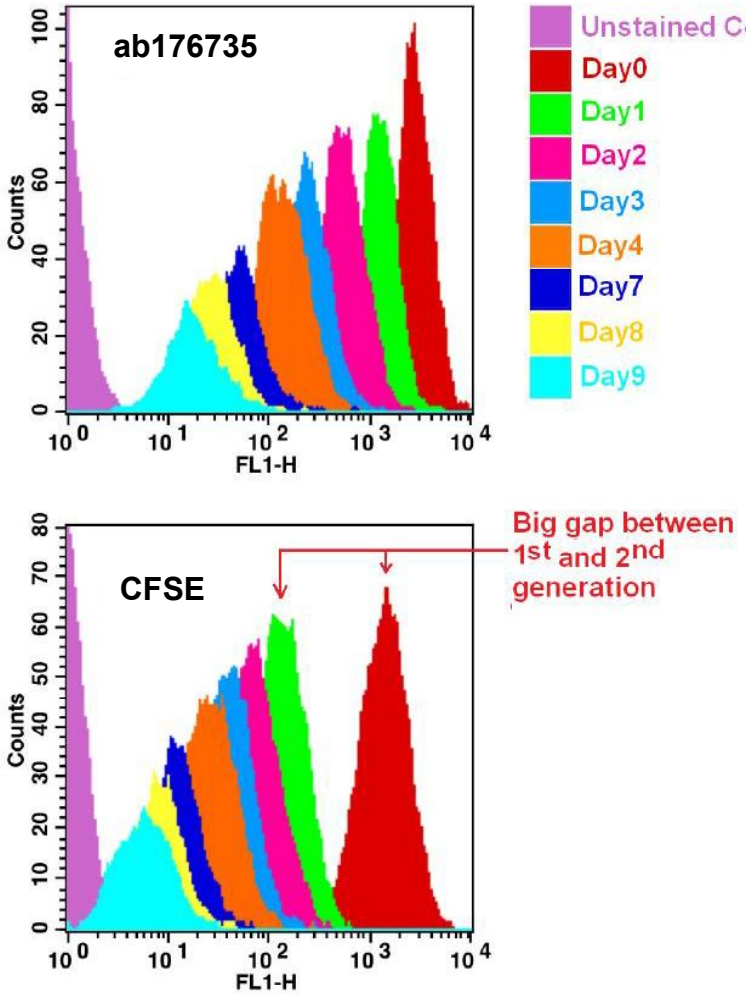
- **It is not an antibody**
  - Direct staining of structure
  - Can be used in conjunction with antibody detection without worrying about cross-detection
- **Can be added at any stage of the staining procedure (if doing multi-color staining)**
- **Minimal hands-on time**
  - Simply add dye to cells, incubate and analyze
- **Minimal photobleaching**
  - Staining can sustain long exposures on the microscope (ideal for live cell staining)
- **Compatible with most common fluorescence microscope or FACS filters**
  - No need to buy new and expensive filters

Cell Tracking

- No efflux from cell – more stable inside cell than CFSE
- Can be used in fluorescence microscopy, flow cytometry and microplate reader assays – mainly flow cytometry
- Available in 500 or 1000 tests size

Structure	Dye information	Live/Fixed cells	Ex/Em (nm)	AbID
Cell Tracking and labeling	Non-fluorescent dye with a cell—retaining moiety. Upon entering cells, dye becomes fluorescent and trapped in cells. Adduct formed in labelling cells in retained by cells through development, and is inherited by daughter cells after cell division	Live cells	**405/545**	**176726**
			511/528	**176735**
			542/556	**176737**
			628/643	**176736**

Cell Tracking



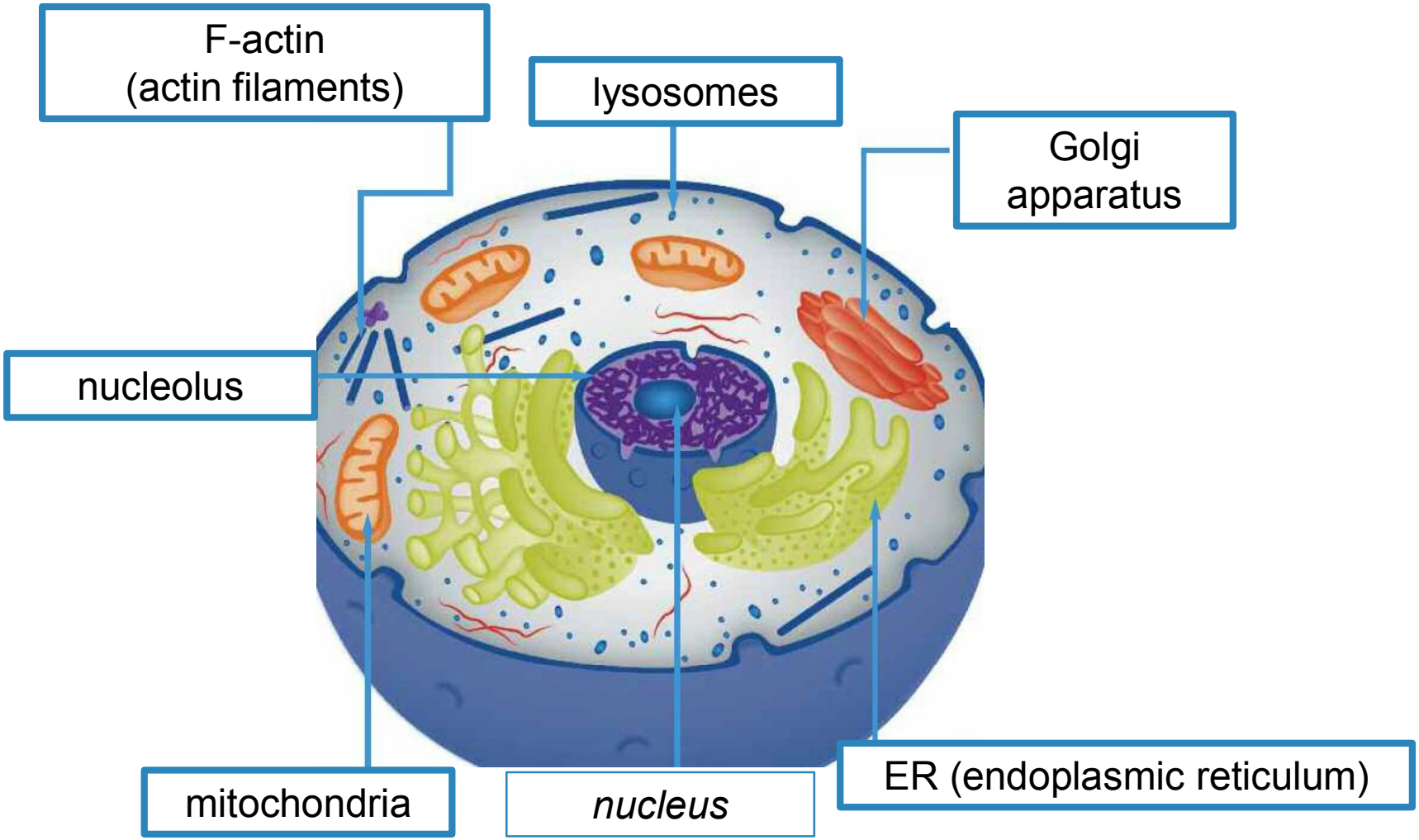
- **Key features:**
  - Spectrally similar to CFSE and FITC
  - Faster response to cell proliferation than CFSE
  - More sensitive than CFSE
  - More stable than CFSE

Supplier comparison

Product	Supplier	Cat Nb	Product Name	Characteristics	Nb tests	USD
Cell Labelling	Abcam	Ab176735	CytoP Cell Labeling Green Reagent	Up to 9 generations	500 1000	99 149
	Life Tech	C34554	CellTrace CFSE Cell Prolifer kit for Flow	Up to 9 generations	1 kit (~200-500)	159
	Life Tech	C2925	CellTracker Green CMFDA	Up to 5 generations	1mg (~1000)	251
	eBio	65-0842-85	Cell Proliferation Dye eFluor 450	Up to 7 generations	500ug (~200 - 500)	101

- **Available colors:**
  - eBio: 2 colors = Blue (450nm) – Red (647nm)
  - Life Tech: 2 colors = Green – Violet (405nm exc)
  - Abcam: 4 colors = Blue – Green – Red – Orange

Cellular structures



Cellular structures

Structure	Dye information	Live/Fixed cells	Ex/Em (nm)	AbID
ER	Cell-permeable dyes that specifically localize to endoplasmic reticula (SER & RER).	Live/ Fixed cells (aldehyde)	**441/551**	139481
			580/677	139482
Golgi	Cell-permeable dye that specifically localizes to Golgi aparatus	Live/ Fixed cells (aldehyde)	**473/534**	139483
ER/Golgi	Combination of cell-permeable probes that specifically localize to Golgi & ER.	Live cells only	**473/534 (G)** **580/677 (E)**	139485
Nucleolus	Cell-permeable dye that specifically accumulates in the nucleolus.	Live cells	**450/481**	139475
F-actin (actin filaments)	Phalloidin-conjugated dye that specifically binds to actin filaments (not to actin monomers or dimers).	Fixed cells or tissues (aldehyde)	**350/450**	112124
			500/520	112125
			550/575	112126
			594/610	112127

Cellular structures

Structure	Dye information	Live/Fixed cells	Ex/Em (nm)	AbID
Lysosome	Cell-permeable dyes that specifically accumulates in the lysosome via the lysosome pH gradient (pH 4.5 – 4.8)	Live/ Fixed cells (aldehyde)	350/445	112135
			500/520	112136
			542/560	138895
			575/597	112137
			596/619	138896
			630/650	176746
Mitochondria	Cell-permeable dye that specifically accumulates in the mitochondria via the mitochondrial membrane potential gradient.	Live/ Fixed cells (aldehyde)	480/520	112143
			545/575	138897
			580/600	112145
			640/660	176747

Dyes for cellular structures

- The dyes for F-actin, mitochondria and lysosomal staining are now available as separate reagents.
- Ideal products for core facility users and CROs – not interested in using buffers.

- **F-actin (phalloidin):** conjugated to iFluor (equivalent to Alexa Fluor® dyes), covering 13 different colors. Includes blue and IR dyes.

- **Lysosomes**: 6 different Lyso Indicator reagents available in 6 different colors. Fixable after staining.

- **Mitochondria**: 5 different Mito Indicator reagents available in 4 different colors. Fixable after staining.

Supplier comparison

Product	Supplier	Cat Nb	Product Name	Characteristics	Nb tests	USD
Phalloidin (F-actin staining)	Abcam	Ab176753	CytoP phalloidin iFluor488	iFluor dye is brighter and more stable than AF	300	149
	Life Tech	A12379	Alexa Fluor 488 Phalloidin	Most established dye in the market	300	367
	Sigma	49409	Phalloidin-Atto488		10nmol (~150-200)	344.50
	CST	8878S	Alexa Fluor 488 Phalloidin	Distributing Life Tech products	300	387
	Thermo Sci	21833	Phalloidin DyLight488	Thermo claims that DyL are more stable than AF	300	360

- **Available colors:**
  - Life Tech: 12 colors = AF350– 488–532–546–555–568–594–633–635–647– 660 – 680
  - Thermo Scientific: 8 colors = DyL350–488– 500–554–594–633–650–680
  - Abcam: 13 colors = iFluor350–405–488–514–532–555–594–633– 647–680–700 –750–790

Supplier comparison

Product	Supplier	Cat Nb	Product Name	Characteristics	Nb tests	USD
Lysosome staining	Abcam	Ab176826	CytoP LysoGreen Indicator Reagent	Fixable after staining	500	149
	Life Tech	L7525	LysoTracker Green DND-26	Most established stain in the market. Not fixable	20 x 50µl (~1000/vial)	282
	CST	8873S	LysoTracker Green DND-26	Distributing Life Tech products. Not fixable	10 x 50µl (~1000/vial)	195

- **Abcam Lyso Indicators can be fixed after staining**
- **Available colors:**
  - Life Tech: 5 colors = Blue – Green – Yellow – Red – Deep Red
  - Abcam: 6 colors = Blue – Green – Orange – Red – Deep Red - NIR

Supplier comparison

Product	Supplier	Cat Nb	Product Name	Characteristics	Nb tests	USD
Mitochondrial staining	Abcam	Ab176830	CytoP MitoGreen Indicator Reagent	Fixable after staining	500	99
	Life Tech	M7514	Mitotracker Green FM	Most established stain in the market. Not fixable (other MitoTracker dyes are)	20 x 50µl (~500/vial)	282
	CST	9074S	Mitotracker Green FM	Distributing Life Tech products. Not fixable	10 x 50µl (~500/vial)	199

- **Abcam Mito Indicators can be fixed after staining**
- **Available colors:**
  - Life Tech: 4 colors = Green – Orange – Red – Deep Red (some are available as fixable dyes)
  - Abcam: 4 colors = Green – Orange – Orange 405 – Red - NIR

3. General staining protocols

Staining procedure: single vs multiple

- **Single staining:**
  - CytoPainter dye
  - Nuclear counterstaining – not necessary *per se* but gives information about state of the cells and it's a good control for staining procedure

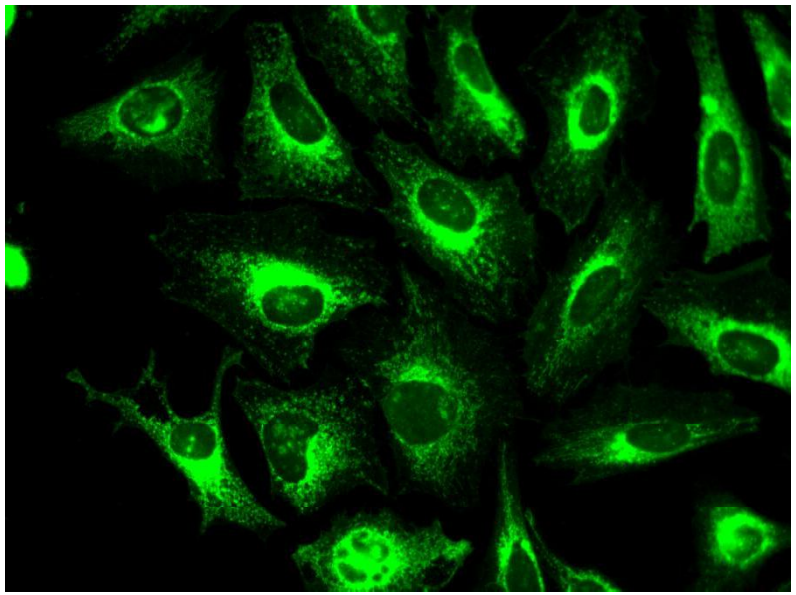
- **It might be possible to use multiple CytoPainter dyes on the same sample, but incubation times may vary.**

- **Multiple staining:**
  - CytoPainter
  - Antibody staining (+ 2ry Ab conjugated to fluorochrome) – could use more than 1 antibody
  - Nuclear counterstaining

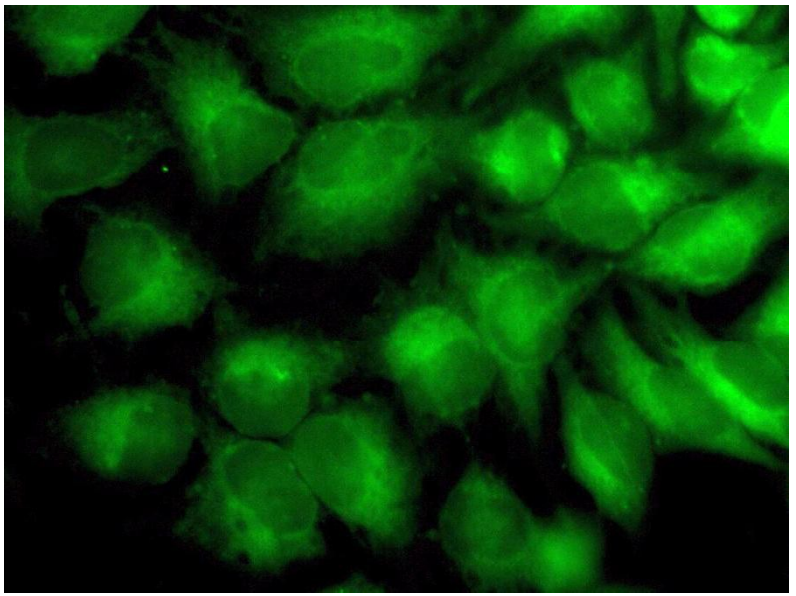
Single Staining

Live cells	Fixed cells
Grow cells (±treatment)	Grow cells (±treatment)
Wash (HBSS or PBS)	Wash (HBSS or PBS)
Add dye + incubate	Fix cells – PFA (DO NOT USE METHANOL)
Wash	Wash
Visualize cells in microscope	Add dye (+ nuclear counterstain) + incubate
Opt: fix cells + add mounting media	Wash
	Add mounting media
	Visualize cells in microscope

Single staining



Ab139483 – Golgi staining



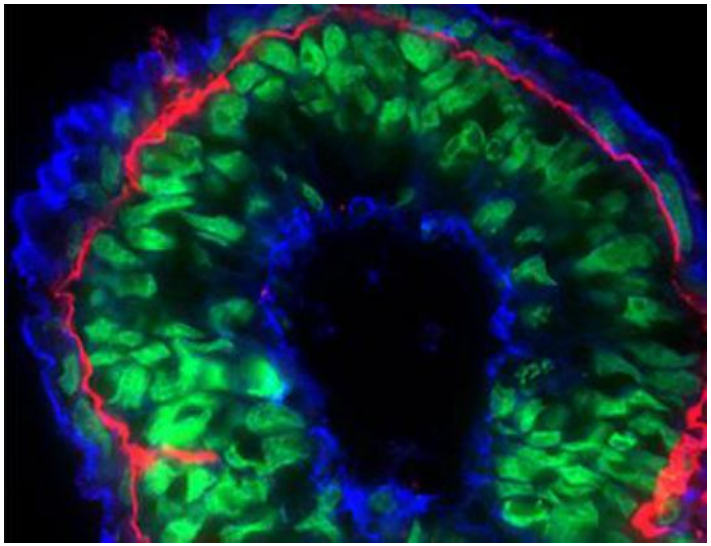
Ab112143 – mitochondria staining

Multiple Staining

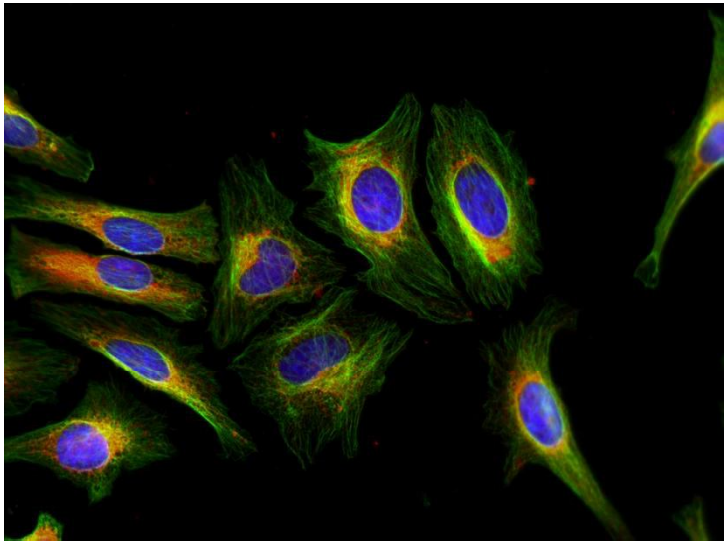
Fixed cells	"Live" cells
Grow cells (±treatment)	Grow cells (±treatment)
Wash (HBSS or PBS)	Wash (HBSS or PBS)
Fix cells – PFA (do not use MetOH)	Add CytoPainter dye
Wash	Fix cells – PFA (do not use MetOH)
Add 1ry Antibody + incubate	Wash
Wash	Add 1ry Antibody + incubate
Add blocking agent (serum, BSA, etc ...)	Wash
*Add 2ry Ab + CytoPainter dye + nuclear stain + incubate	Add blocking agent (serum, BSA, etc ...)
Wash	*Add 2ry Ab + nuclear stain + incubate
Add mounting media	Wash
Visualize cells in microscope	Add mounting media
	Visualize cells in microscope

*Use fluorochromes that do not overlap to avoid bleed through

Multiple staining



F-actin (blue) – ab112124
Laminin a + Cy5 (red)- ab97077
Nuclei (green) – SYTO16



ER (red) – ab139482
alpha-tubulin + AF488 (green)- ab150113
Nuclei (blue) – DAPI

4. Troubleshooting

4. General Troubleshooting

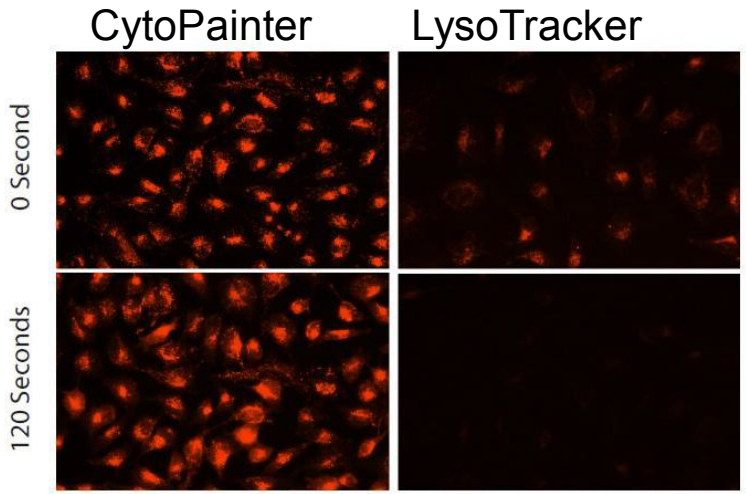
- **No/ Weak staining of CytoPainter dye:**
  - Increase incubation time/concentration – every dye needs optimization for each cell type.
  - Only use formaldehyde/paraformaldehyde as fixative. Do not use methanol as it will destroy the native conformation of the structure.

- **Antibody/ Nuclear counterstain too bright compared to CytoPainter:**
  - Reduce concentration or shorten exposure time (different filters or camera settings may make some signal appear very bright).

- **Cells are not looking healthy:**
  - Check cells before starting experiment.
  - Some cells may need serum in washing and incubation solutions

5. Selling tips

- **Why use CytoPainter dyes instead of antibodies?**
  - There is no problem of reactivity or epitope exposure.
  - You can then use antibodies to detect other specific structures/proteins, widening your selection range.
  - Can be used for live cell imaging – ideal for co-localization in transfected cell lines.
- **Why CytoPainter dyes are better than Mitotracker/ Lysotracker?**
  - More photostable.
  - Increased signal intensity: brighter dyes.
  - Excellent cellular retention.
  - All dyes can be fixed after staining.



6. Useful resources

- **Imaging (general):**

http://www.abcam.com/imaging

- **CytoPainter range:**

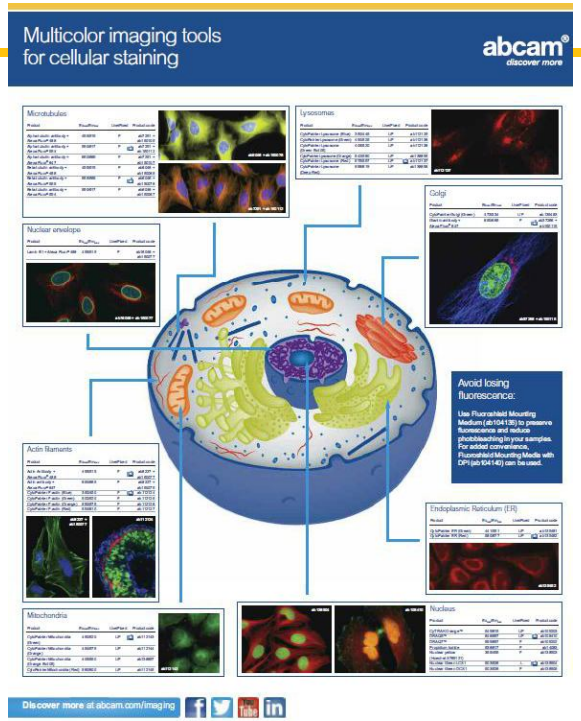
http://www.abcam.com/cytopainter

- **Alexa Fluor range:**

http://www.abcam.com/alexa

- **Fluorescent dyes range:**

http://www.abcam.com/dyes



- **Multicolor imaging poster:**

http://docs.abcam.com/pdf/general/Multicolor-imaging-tools-for-cellular-staining.pdf

- **Guide to Fluorochromes:**

http://www.abcam.com/index.html?pageconfig=resource&rid=14321

- **Webinars: Introduction to ICC/IHC & Optimization of ICC/IHC staining**

http://www.abcam.com/index.html?pageconfig=resource&rid=14535

Summary

- **CytoPainter is a range of multicolor staining products that allows researchers to easily visualize cellular components and to track cells during development.**
- **Can be used alone or in immunostaining procedures.**
- **Can be used in live cell imaging experiments – can be fixable.**
- **Comparable to other stainings available in the market.**
- **Take home message:**
  - Easy and simple to use.
  - Easy to incorporate in any established fluorescent staining protocol.
  - Can be used in live and fixed cells.