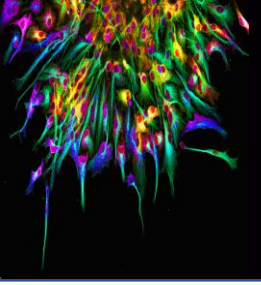
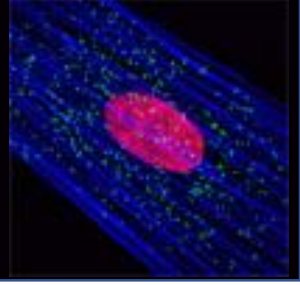


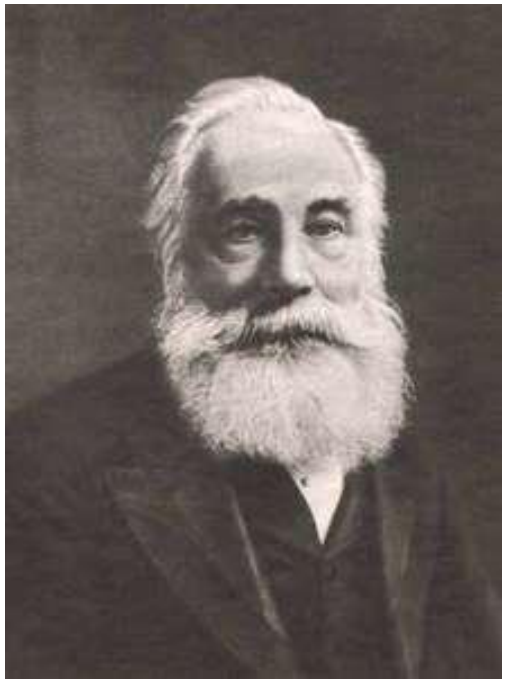


Imaging of Cells using fluorescents dyes

By: Josué A. Benjamín Rivera

September 27, 2018

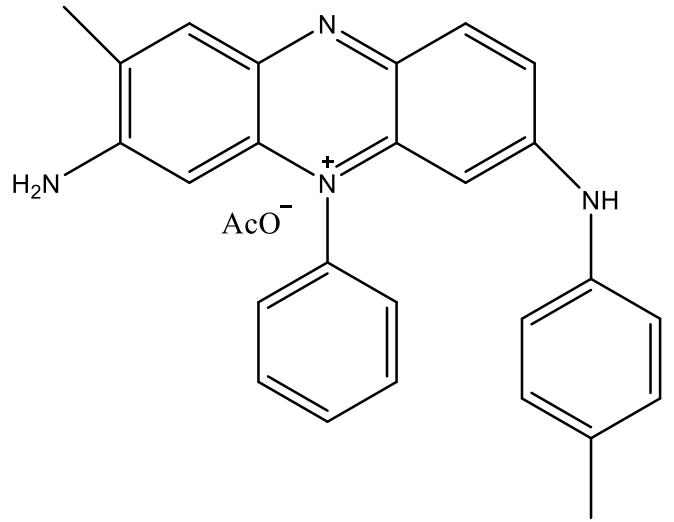
History



Sir William Henry Perkin
BRITISH CHEMIST

In 1856, at the age of 18, William Henry Perkin set out with idea of making quinine by oxidizing allytoluidine –instead he accidentally produced the synthetic dye, mauve, a derivative of coal tar with an aniline base

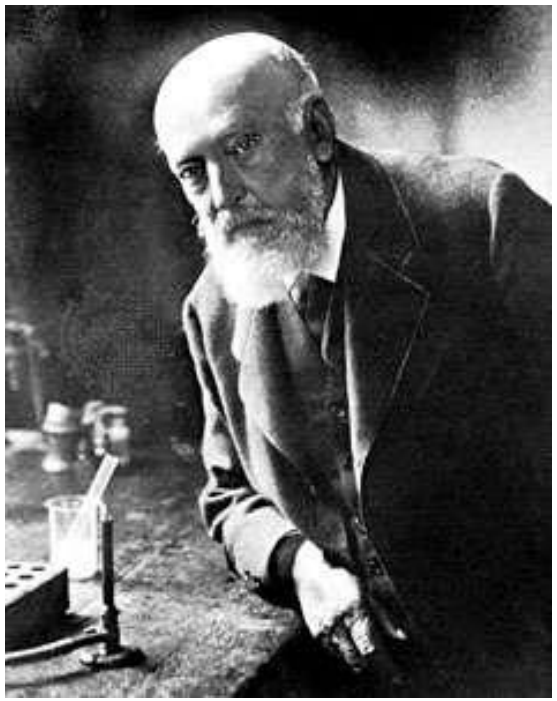
Tyrian purple



https://www.britannica.com/biography/William-Henry-Perkin/media/452128/207726 (access Sep 24,2018)

How synthesis the Fluorescein

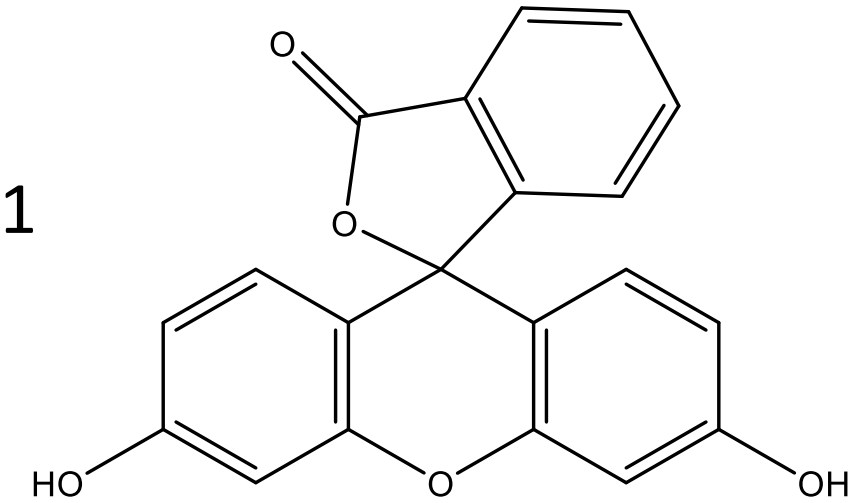
Adolf Von Baeyer
GERMAN CHEMIST



Nobel Prize at 1905

1871

FITC



Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy.

One of the first uses of fluorescein was in 1877 in a major ground-water tracing experiment in southern Germany.

https://fluorescence-foundation.org/lectures/.../lecture1.pdf (access September 25,2018)

Fluorescents microscopy

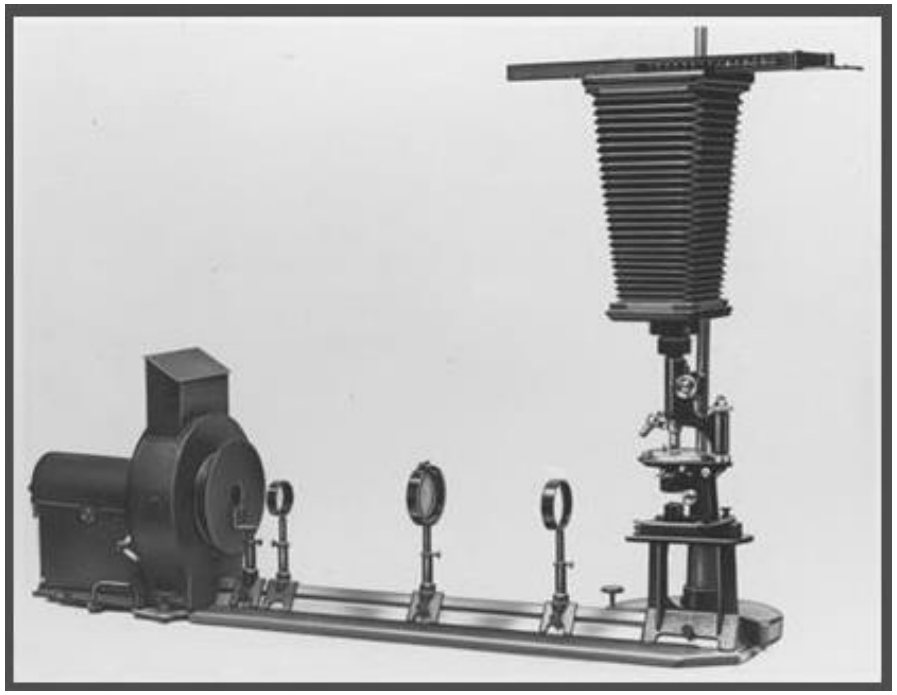


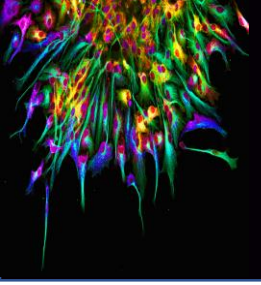
Image credit: corpotate.zeiss.com
"Technical Milestones of Microscopy"

Otto Heimstaedt and Heinrich Lehmann

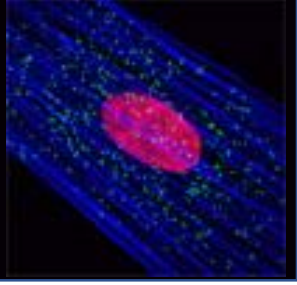
They developed the first fluorescence microscopes as an outgrowth of the UV microscope (1901-1904). The instrument was used to investigate the autofluorescence of bacteria, protozoa, plant and animal tissues, and bioorganic substances such as albumin, elastin, and keratin.

Stanislav Von Prowazek (1914) employed the fluorescence microscope to study dye binding to living cells.

https://fluorescence-foundation.org/lectures/.../lecture1.pdf (access September 25,2018)



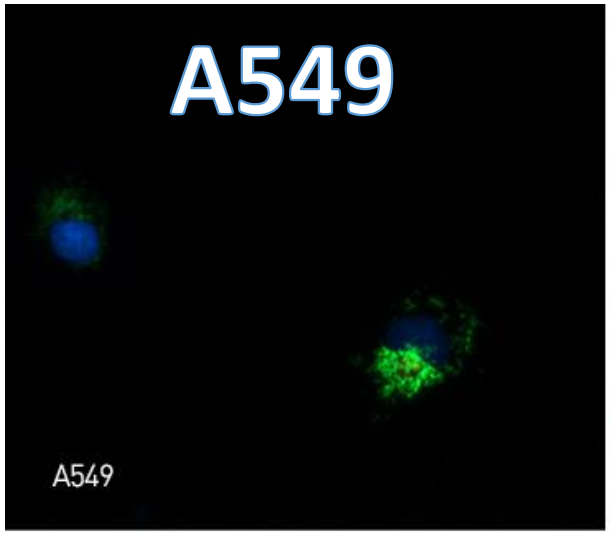
Experimental Considerations

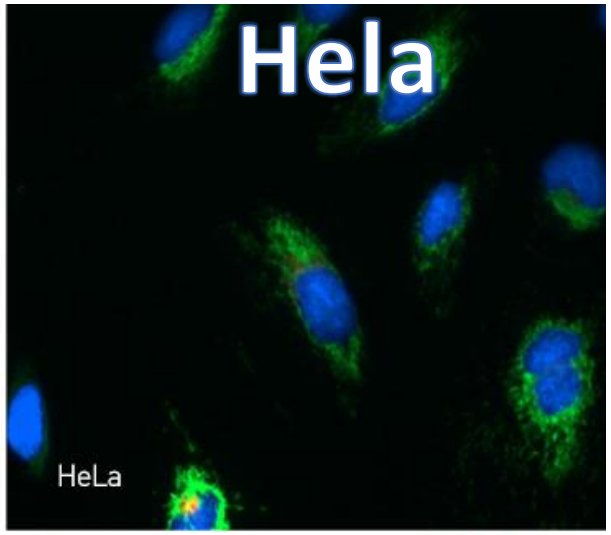


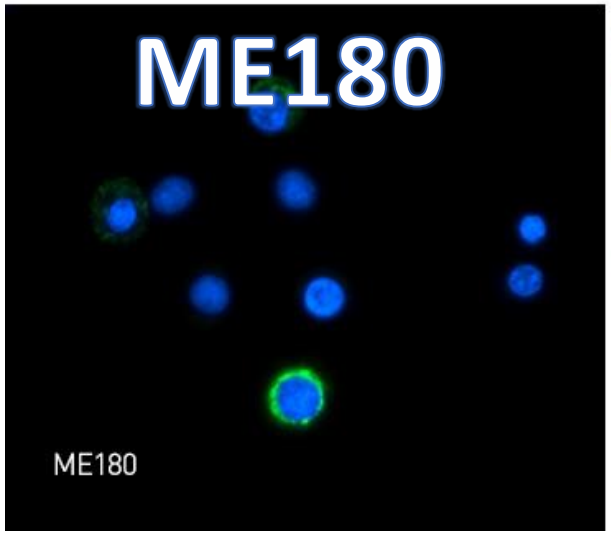
- Cell morphology
- Preparing fixed cells for labeling
- Preparing for live-cell imaging
- Choosing a vessel
- Dye selection

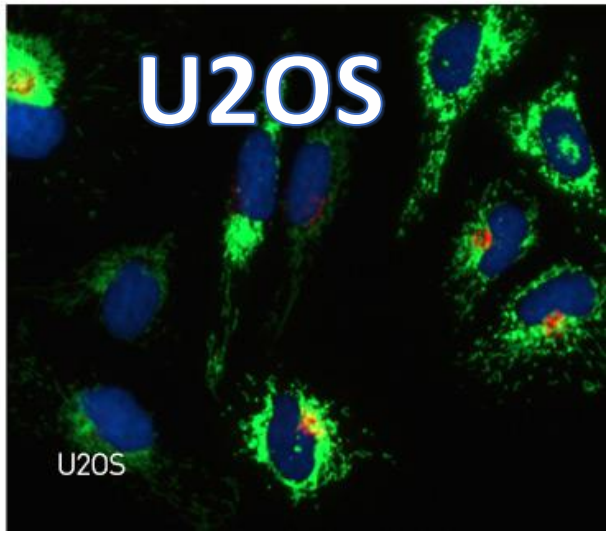
https://www.thermofisher.com/pr/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/imaging-basics/sample-considerations.html

Sample considerations









The cells all exhibit very different staining patterns for the same set of fluorescent reagents due to differences in their morphology and metabolic pathways.

NucBlue Live® (Nucleus/Blue)
CellLights®Golgi-RFP (Golgi /Red)
Mito-GFP reagents. (Mitochondria / Green)

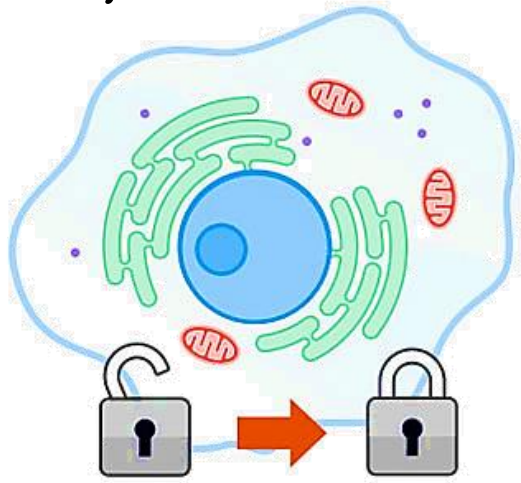
Preparing fixed cells for labeling

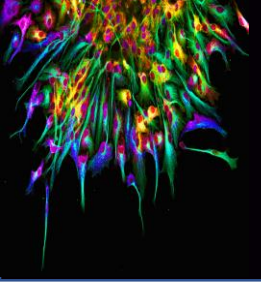
Pros

- Cells are preserved at a certain time point
- Cells become permeable to large molecules
- Gross cellular structures are protected and stabilized
- Targets are easier to image when they are not moving

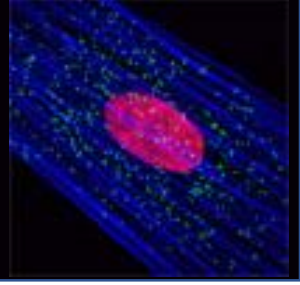
Cons

- Protein conformation may be changed by fixation
- Most enzymes are inactivated
- Soluble contents of the cell can be lost
- Dynamic processes give more functional information





Preparing for live-cell imaging



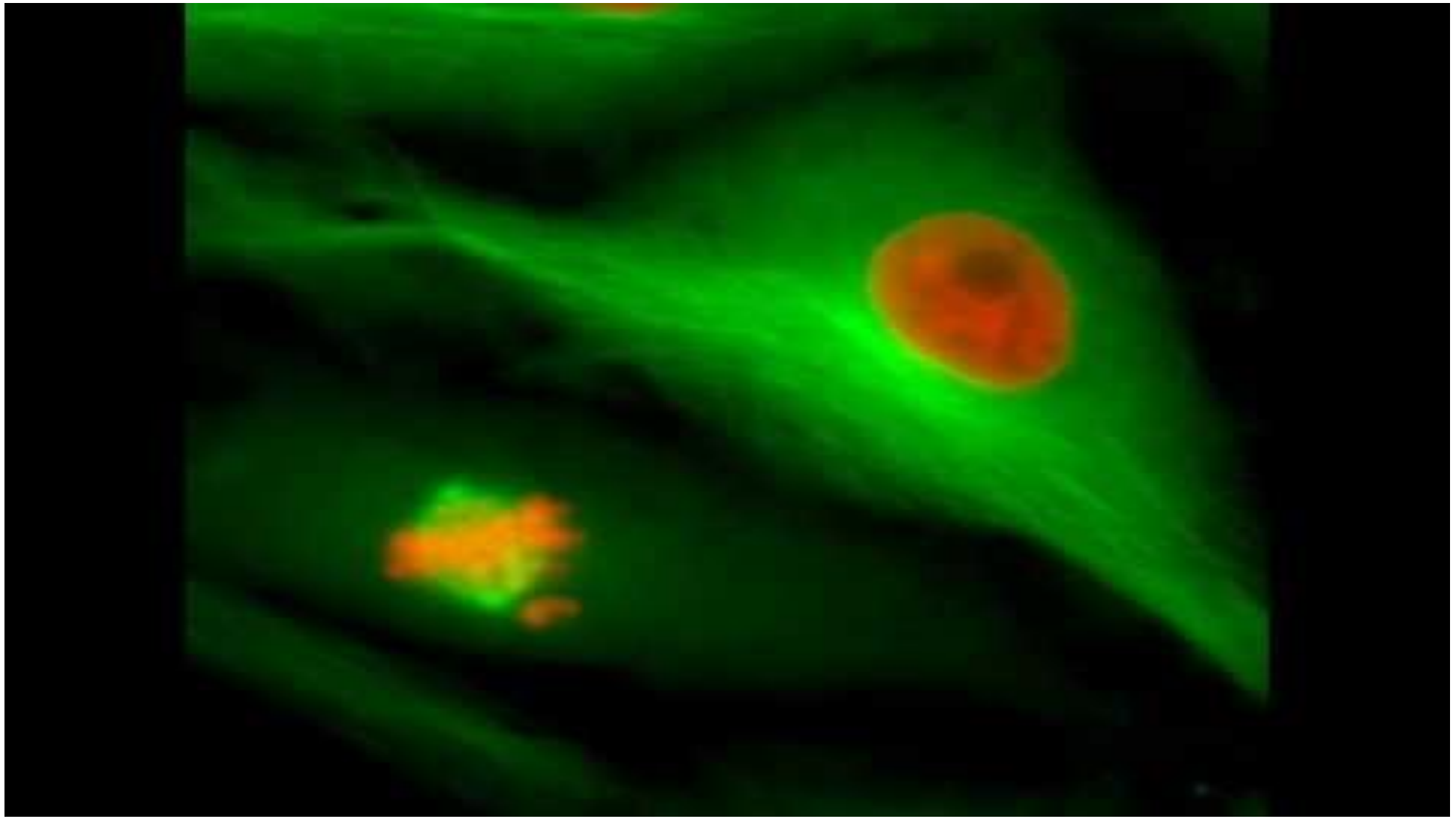
Pros

- Cellular enzymes and other cytosolic biomolecules remain in the cell
- Can observe dynamic cellular processes as they happen
- Cellular structures can be studied in their native environment, so you get less experimental artifact
- Cellular biomolecules and structures can be tracked over time
- Interactions between cells can be observed

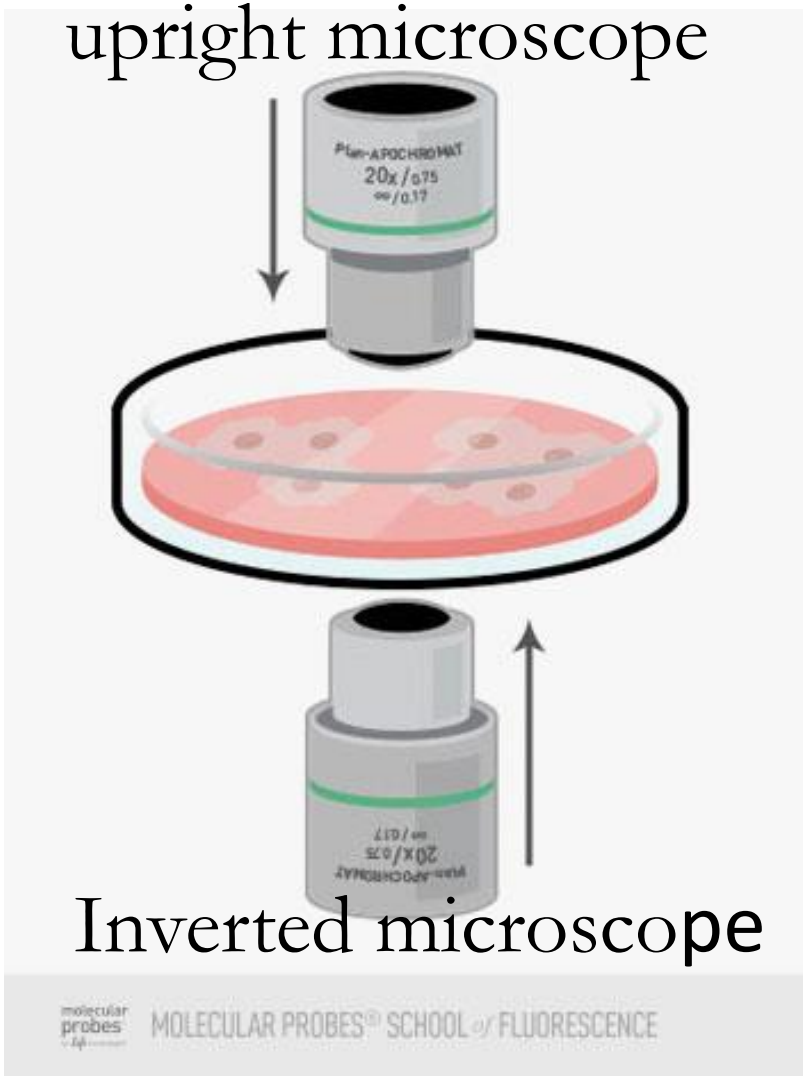
Cons

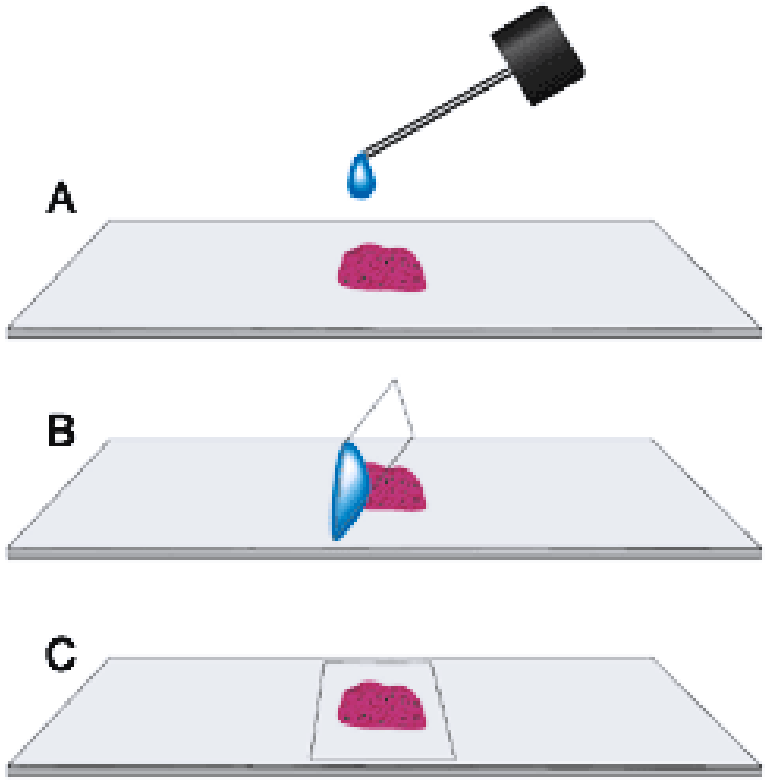
- Cells must be kept in their natural physiological ranges for pH, temperature, and osmolarity
- Must have a specific way to label your target whether it is a molecule, a cellular function, or a cellular state—and illuminate it with minimum toxicity
- Living cells are not generally permeable to large molecules (i.e., antibodies)
- Moving objects can be more difficult to keep in focus

Live-cell imaging examples



Choosing a vessel





To mount a slide, (A) Apply a single drop of mounting medium upon tissue section. (B) Hold coverslip at 45° allowing the drop to spread along the edge of the slip. (C) Let go of slip and allow medium to spread slowly.

https://www.nationaldiagnostics.com/histology/article/mounting-tissue-sections (acesses September 25,2018)

Dye selection

https://www.thermofisher.com/pr/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html

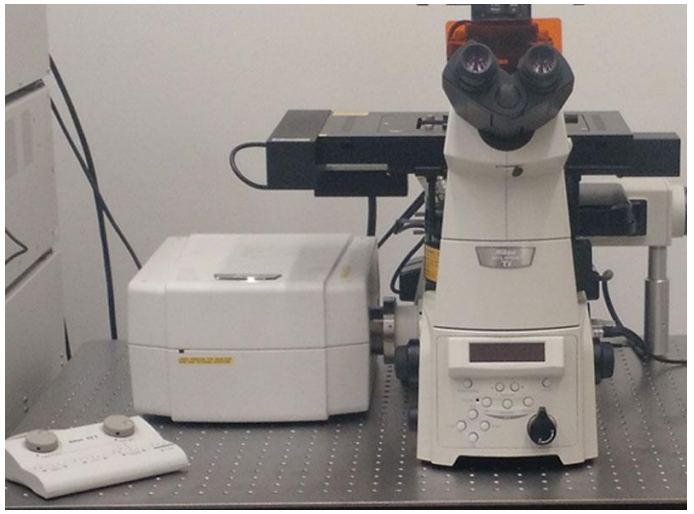
Cell Imaging
Invitrogen Corporation

Instruments available

Nikon Eclipse Ti-E Inverted Microscope

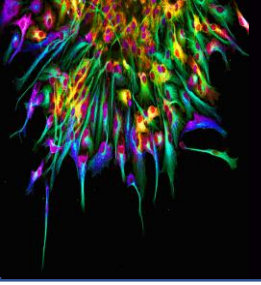
A1R laser scanning confocal system
LU-N4/N4S 4-laser unit (405 nm, 488 nm, 561 nm, 640 nm)
Ultrafast resonant scanner (Up to 512 x 32 pixels at 420 fps)
High-resolution galvano scanner (Up to 4096 x 4096 pixels)
A1-DUS spectral detector unit (Up to 32-channel spectral image at 24 fps)

ww.nief-upr.com/instrumentation/

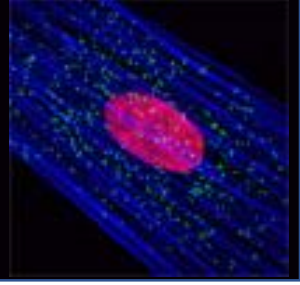


Inverted Microscope Eclipse TS100

High luminescent white LED illuminator (Eco-illumination)
6V30W halogen lamp
T1-FM Epi-fluorescence Attachment, with field diaphragm,
Fluorescence filter cube holder (2 filter cubes AT-EGFP, FITC/TEXAS RED/ DAPI),UV-cut filter (detachable)
Mercury lamp to fluorescents experiments



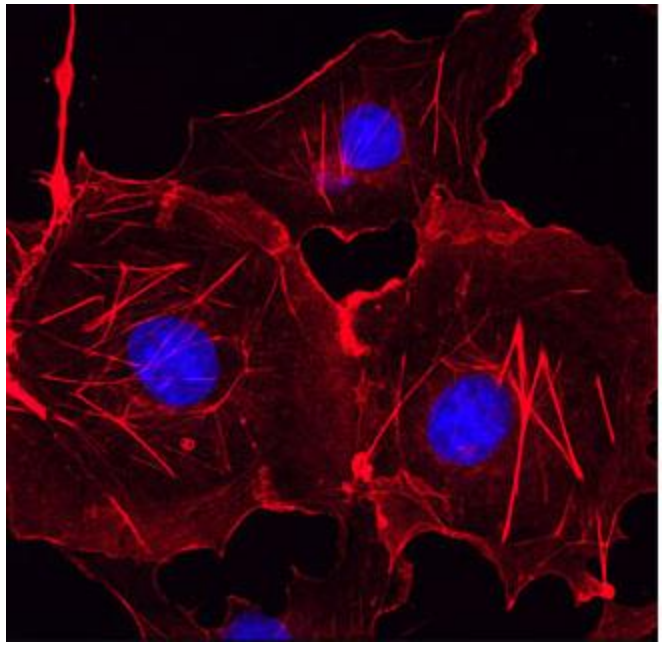
Dyes available in our laboratory

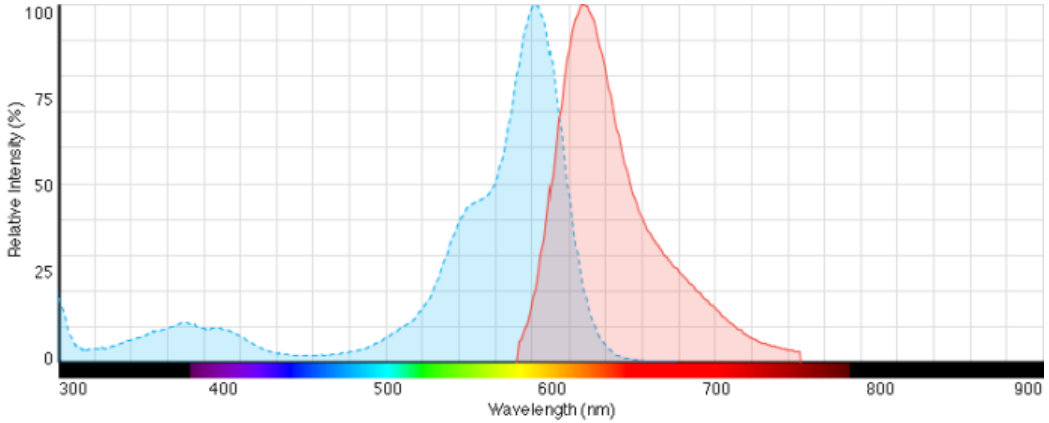


- Alexa Fluor 594 dye
- NucBlue Fixed Cell (Dapi)
- Hoechst 33342
- Propidium Iodide (PI)
- FITC (5-animno fluorescein)
- Apoptosis Kit :
  - Annexin V
  - Alexa Fluor 488
  - Propidium Iodide

Alexa Fluor 594

- Target : plasma membrane labeling
- Color : Red
- Excitation wavelength : 590 nm
- Emission wavelength 617 nm



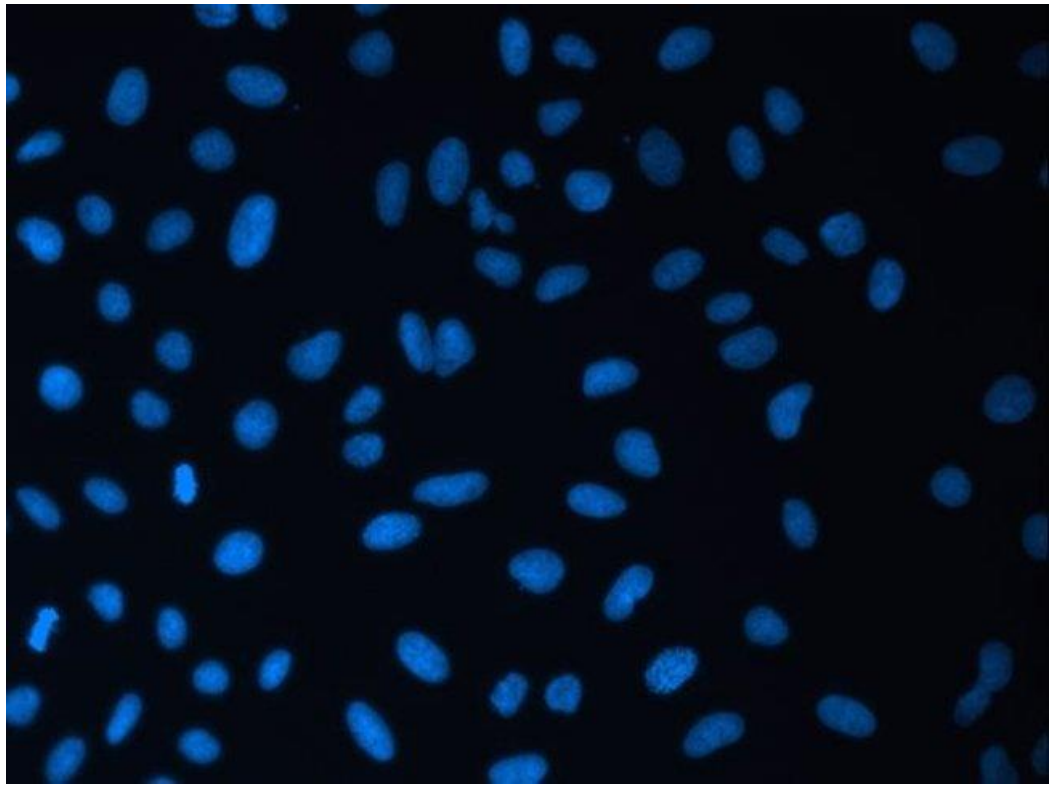


Confocal immunofluorescent analysis of COS-7 cells using β-Actin (13E5) Rabbit mAb (Alexa Fluor® 594 Conjugate) (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

https://www.cellsignal.com/products/antibody-conjugates/b-actin-13e5-rabbit-mab-alexa-fluor-594-conjugate/9470

NucBlue Fixed Cell (Dapi)

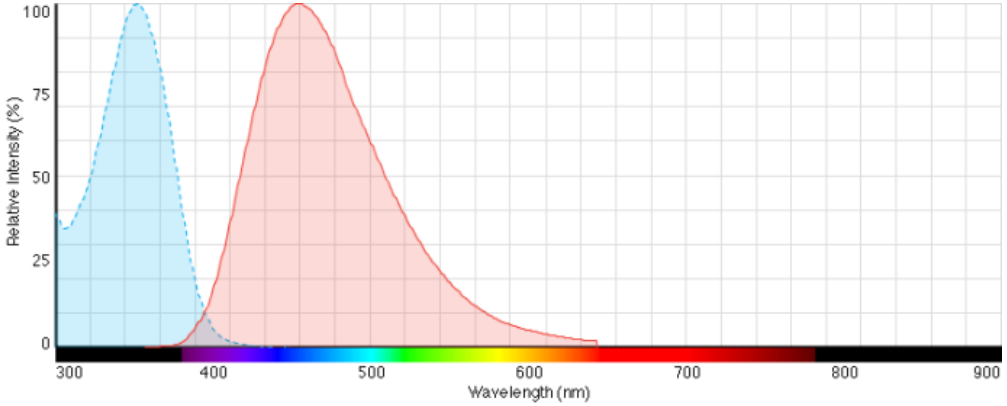
- Target : Nucleus
- Color Blue
- Excitation wavelength : 360 nm
- Emission wavelength 460 nm

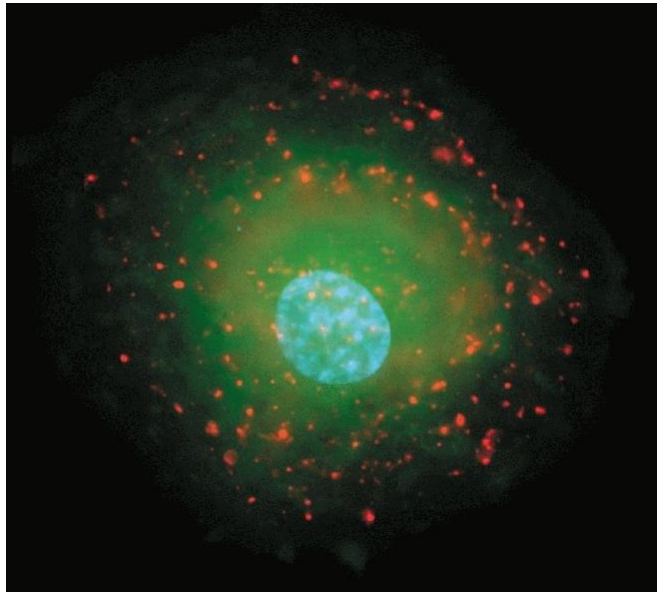


U-2 OS Cells Stained with NucBlue™ Fixed Cell Stain

Hoechst 33342

- Target : Nucleic Acids, Nucleus
- Color Blue
- Excitation wavelength : 350 nm
- Emission wavelength 461 nm

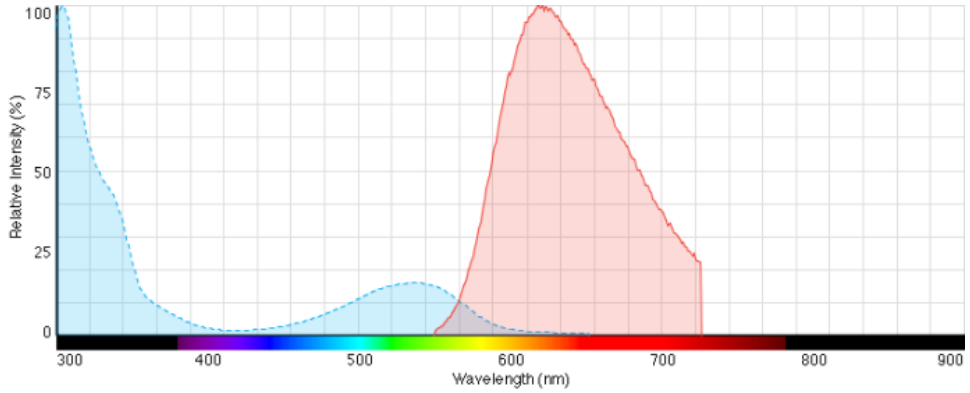


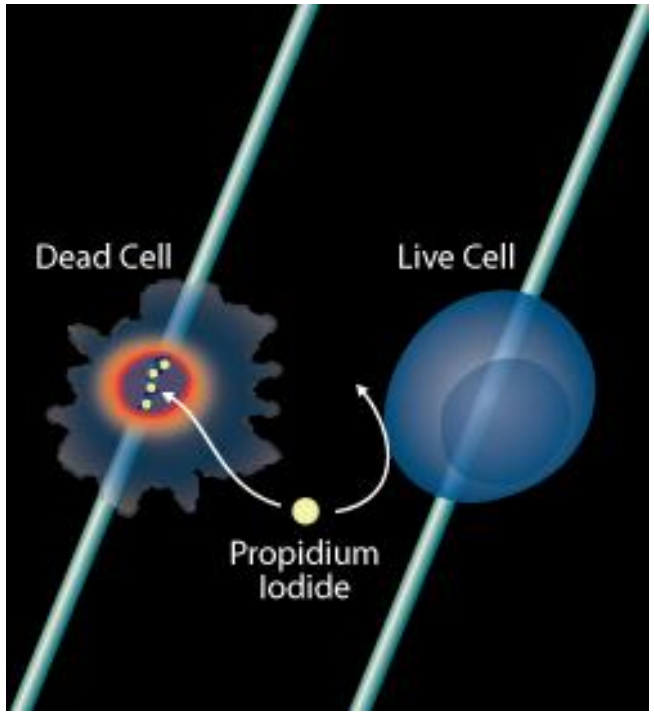


Bovine pulmonary artery endothelial cells (BPAEC) stained with 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, FM 5-95 and Hoechst 33342.

Propidium Iodide

- Target : DNA
- Color Red
- Excitation wavelength : 535 nm
- Emission wavelength 617 nm

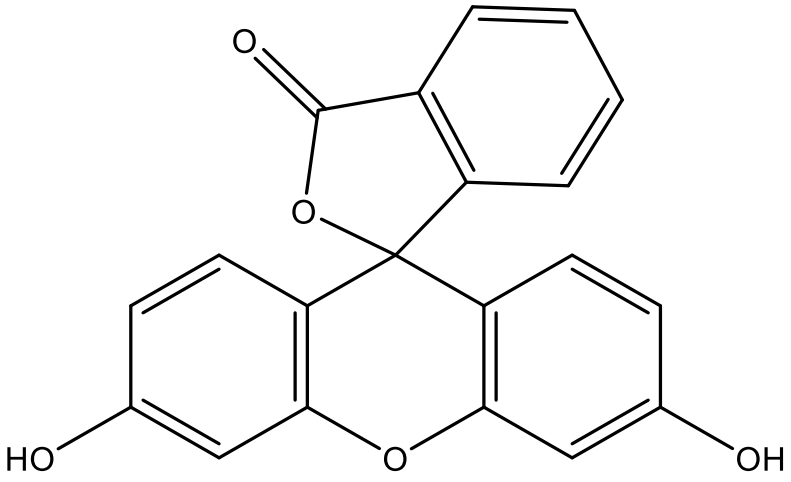


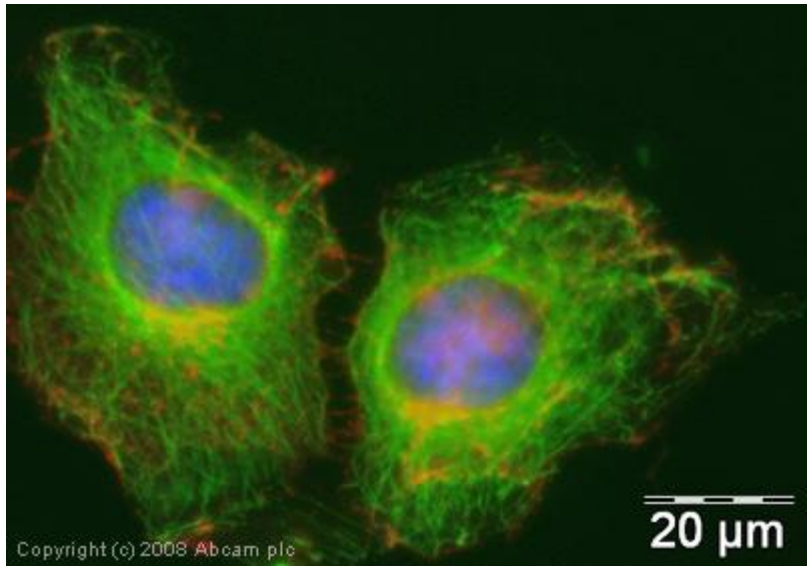


Propidium Iodide (PI) is a standard reagent used for assessing cell viability and exclusion of non-viable cells in flow cytometry. PI binds to double stranded DNA, but is **excluded** from cells with intact plasma membranes.

FITC (5-animno fluorescein)

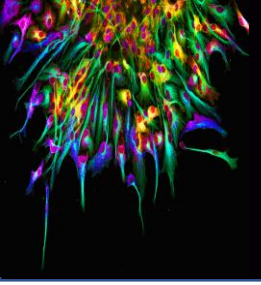
- Target : Depends of the application
- Color Green
- Excitation wavelength : 490 nm
- Emission wavelength 525 nm

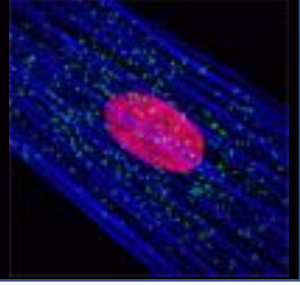




Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (FITC)

https://www.abcam.com/alpha-tubulin-antibody-dm1a-microtubule-marker-fitc-ab64503.html





Preguntas ?