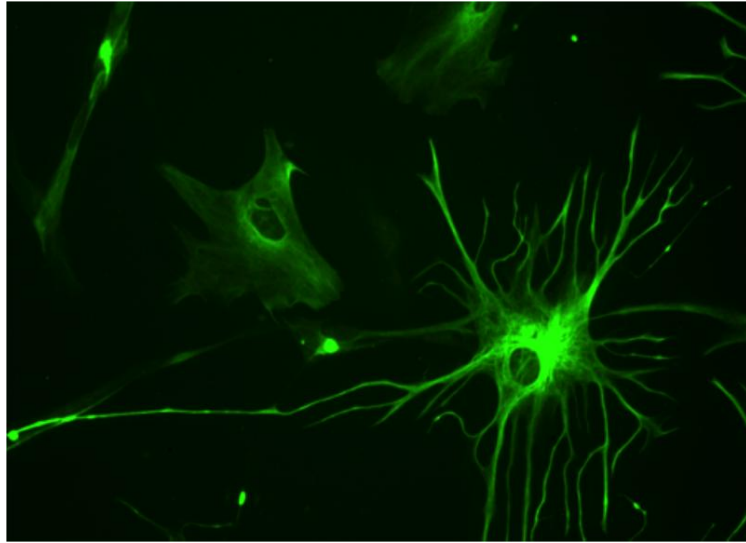


# **Fluorescent Cell Staining:**

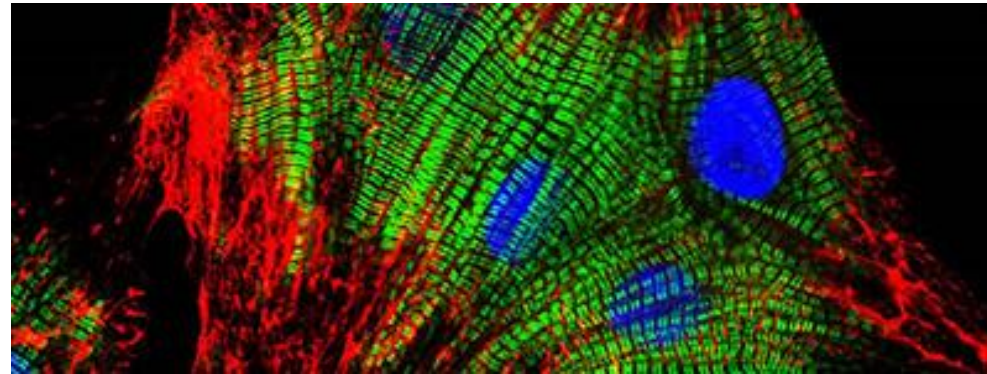
## **A Method to Explore Cell Structure and Function**

PROJECT BIOTECH  
Biotechnology and Cancer  
2018

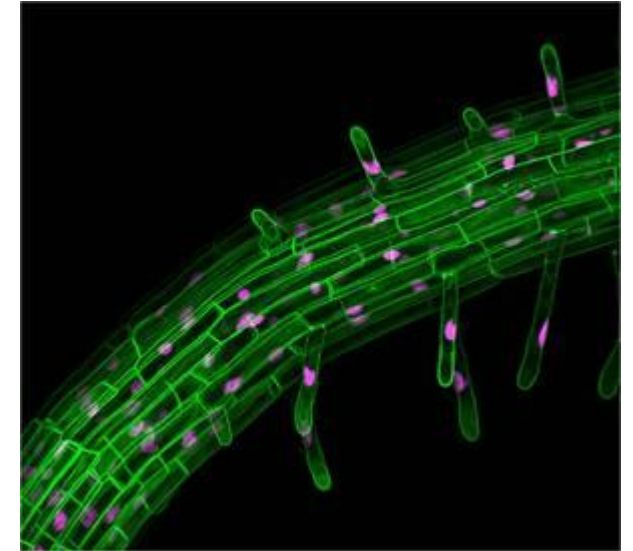
# Visualizing Cellular Structure with Fluorescent Staining



**Human Astrocyte**  
(Part of the Nervous System)



**Rat Cardiomyocytes**  
(Heart Cells)



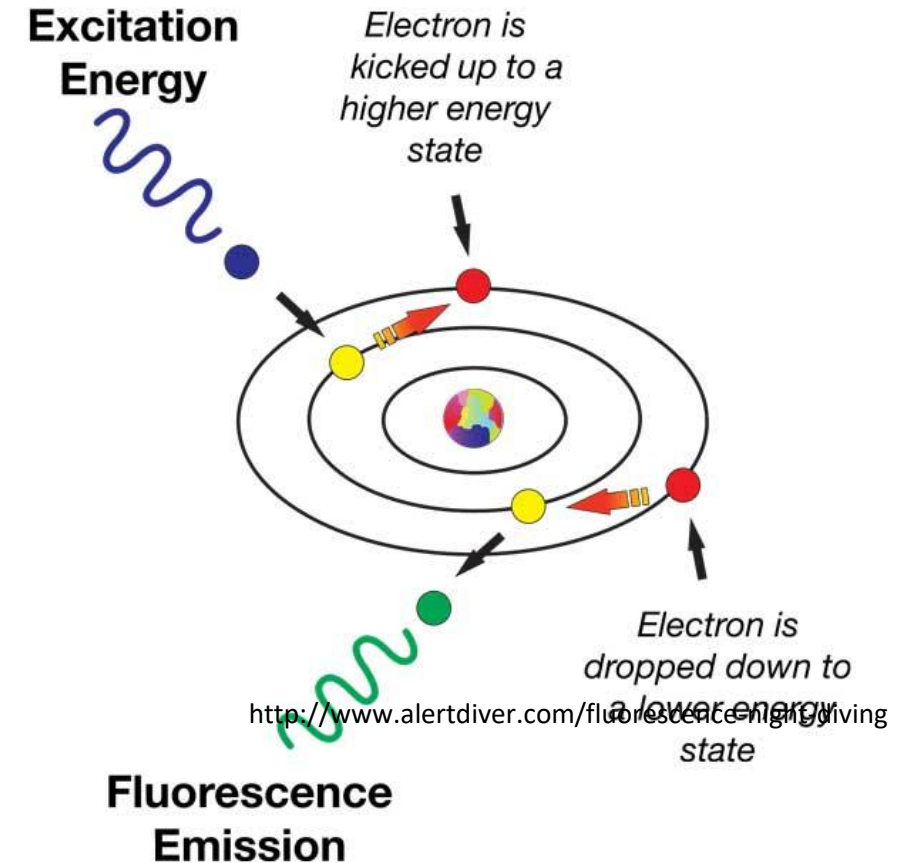
**Plant Cells**

# Fluorescent Cell Staining

- Multiple processing steps are required to prepare cells for fluorescence microscopy
- Can use either *live* or *fixed* cell microscopy
- Fluorescence microscopy of *live cells* uses:
  - Genetically-encoded fluorescent proteins (e.g. green fluorescent protein, GFP)
  - Cell membrane-permeable, non-toxic fluorescent stains
  - Antibodies conjugated to fluorescent proteins
- Appropriate sample preparation is needed for high quality images

# What is Fluorescence?

- When you shine light of one wavelength or color (***excitation wavelength***) on a fluorophore, light of a different wavelength or color (***fluorescence wavelength***) is emitted
- **How does this work?** When **blue light** strikes the protein GFP, the GFP absorbs the blue light's energy, electrons are excited and jump to a higher orbital, and as the energy decays, the electron drops back down to the original orbital and emits **green light**.



# Fluorescent Stains We Will Use

- **Antibody** – An antibody specific for membrane Estrogen Receptor (mER). This antibody is conjugated (chemically bound) to a fluorescent molecule.
- **Peptide** – Phalloidin, a toxin that binds to actin. The phalloidin is conjugated to a fluorescent molecule.
- **Dye** – Hoechst, a fluorescent dye that binds to nucleic acids.

# Cell Fixing

- Fixative agent kills cells, but maintains cellular structure, so antibodies and dyes can be used to see cell structure.
- Different fixation methods fall into two basic categories: *aldehyde* fixatives and *alcohol* fixatives
- Alcohols remove lipids and dehydrate the cells
- Aldehyde reagents cross-link proteins, forming intermolecular bridges
- Cross-linkers preserve cell structure better than alcohols, but may alter protein shape (and how antibodies bind to them), and require a permeabilization step to allow the antibody to get into the cell.

# Permeabilizing

- Fixation with cross linking agents leaves the cell membrane in tact
- Formaldehyde fixation requires the cells to be permeabilized to allow antibodies access into the interior of cells, because antibodies are quite large

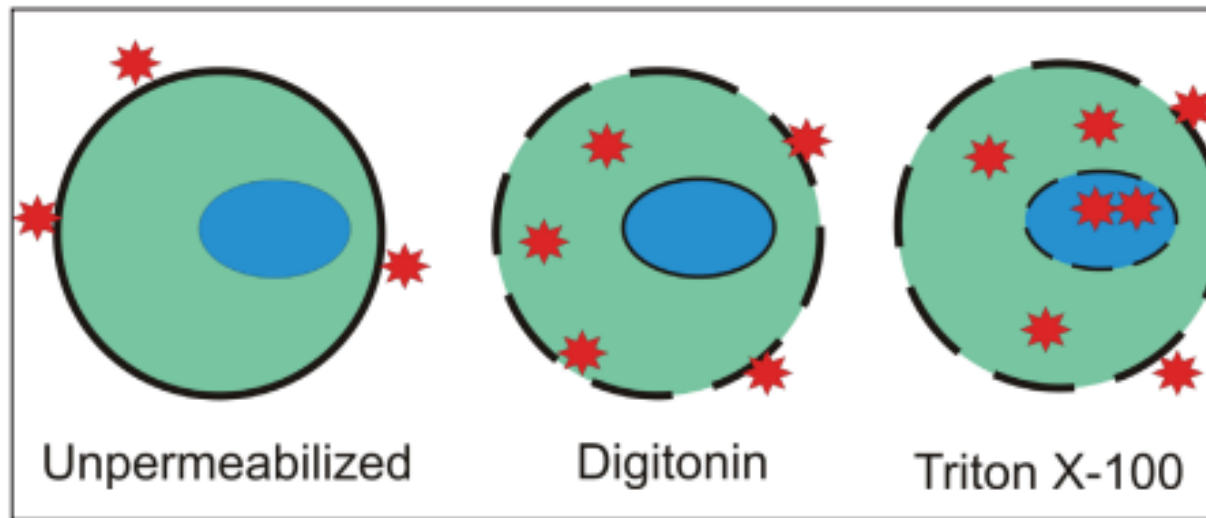
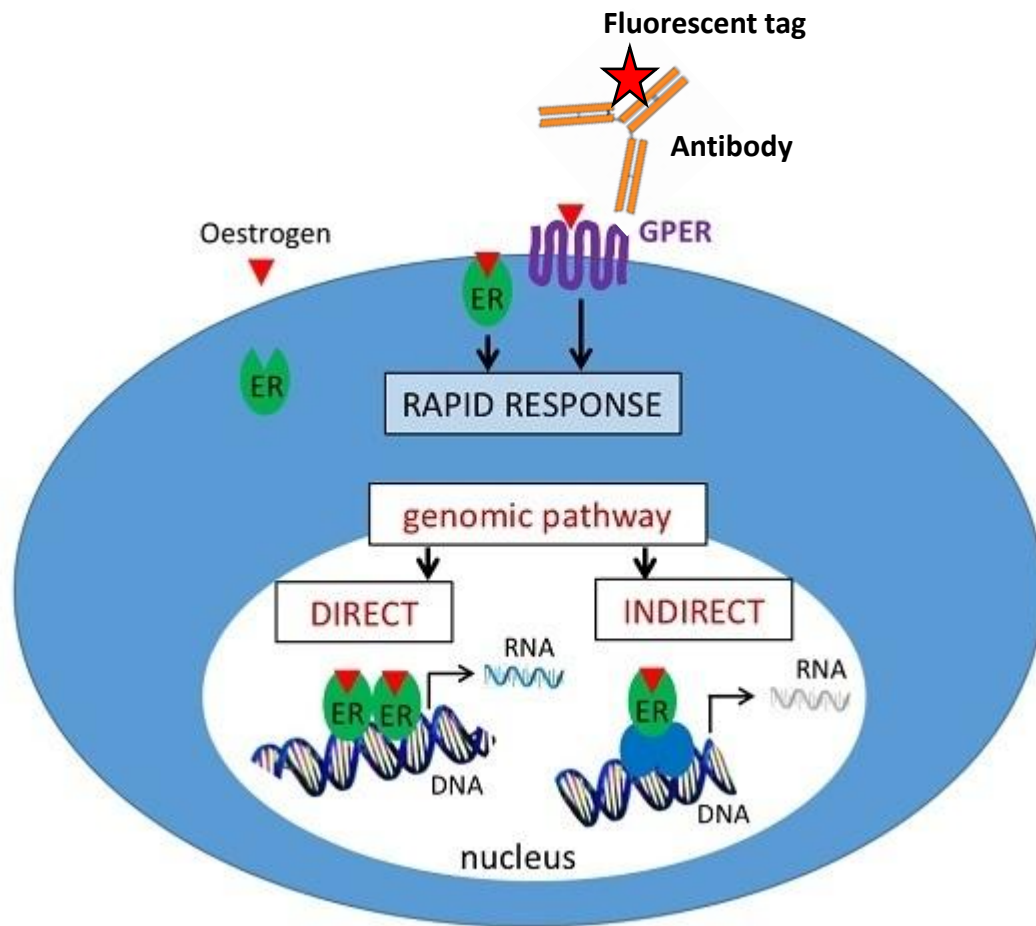


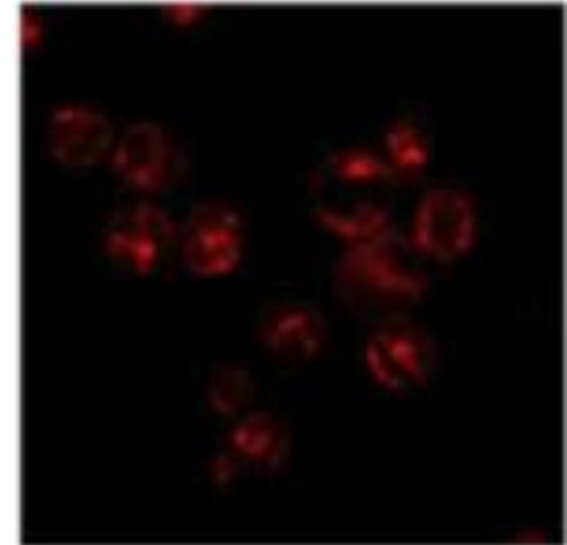
Figure 1. Antibody Accessibility with Unpermeabilized and Digitonin or Triton X-100 Permeabilized Cells. Antibodies can only access the exterior of aldehyde fixed unpermeabilized cells, while mild agents such as digitonin will permeabilize the plasma membrane allowing access to cytoplasmic interior, but not interior membrane bound organelles such as the nucleus or mitochondria. Stronger nonionic detergents, such as Triton X-100, permeabilize both the plasma membrane and interior membranes, allowing full access while still preserving cell structure.

# Cell Membrane Stain for Estrogen Receptor (GPER)

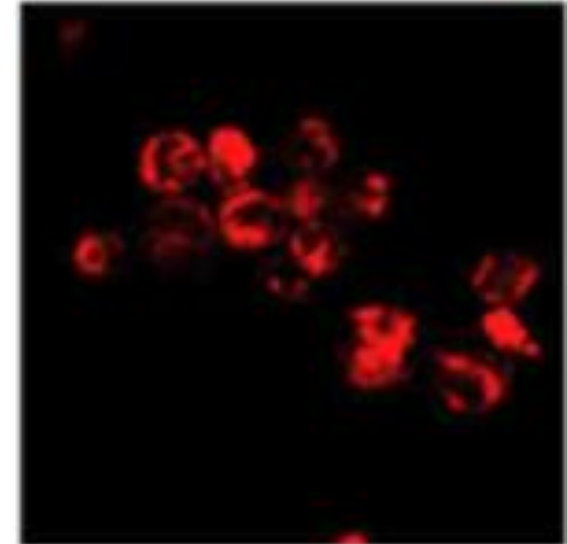


<http://www.mdpi.com/1422-0067/18/5/904/htm>

Low expression



High expression

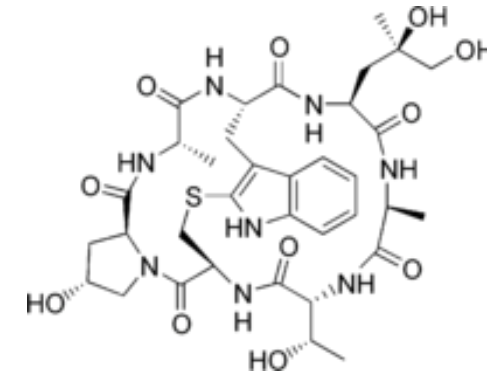


<https://www.researchgate.net/publication/273956708>

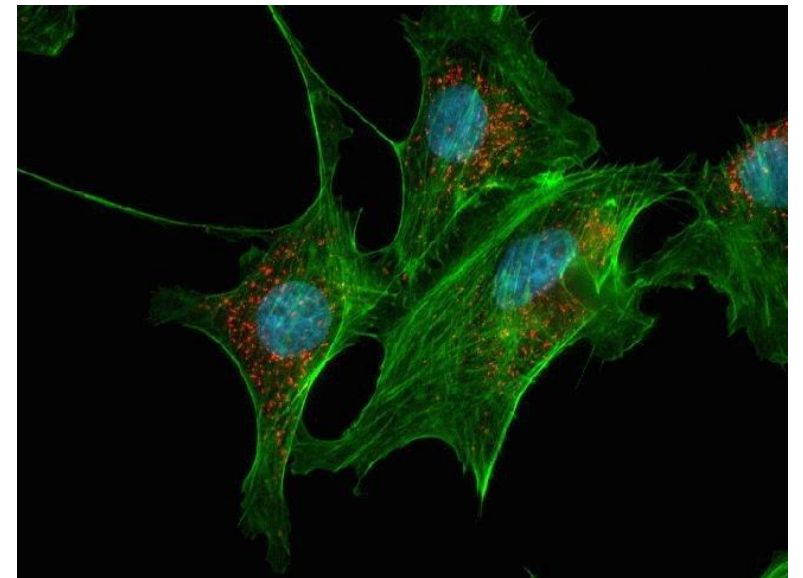


# Actin Green Stain Cytoskeleton: Phalloidin & Green Fluorescent Protein (GFP)

- Phalloidin belongs to a class of toxins called phallotoxins, found in the death cap mushroom (*Amanita phalloides*)
- Ingestion is can be fatal
- The major symptom of phalloidin poisoning is acute hunger due to the destruction of liver cells.
- It functions by binding and stabilizing actin microfilaments, preventing depolymerization of actin fibers.
- Used in microscopy to see the cytoskeleton (actin microfilaments)

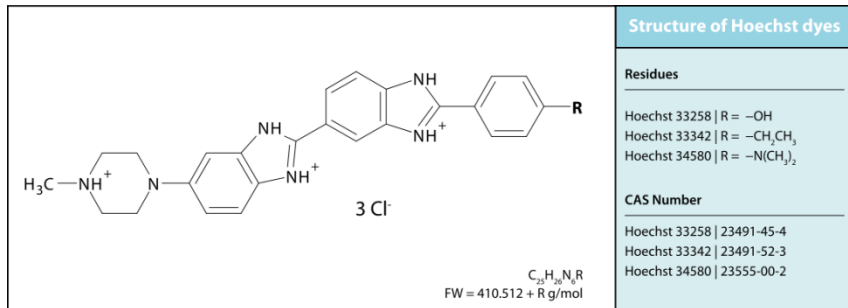


**Phalloidin  
chemical  
structure**

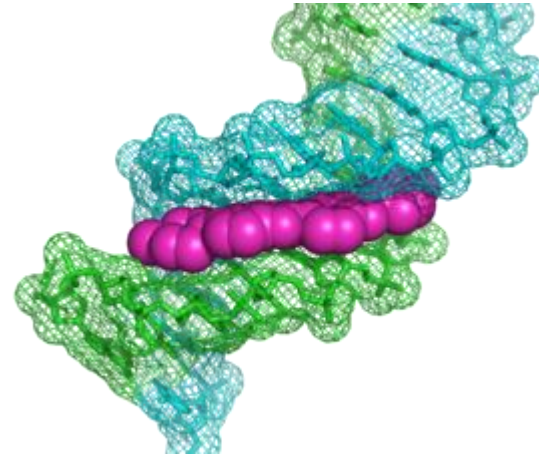


# Hoechst Stains the Nucleus Blue

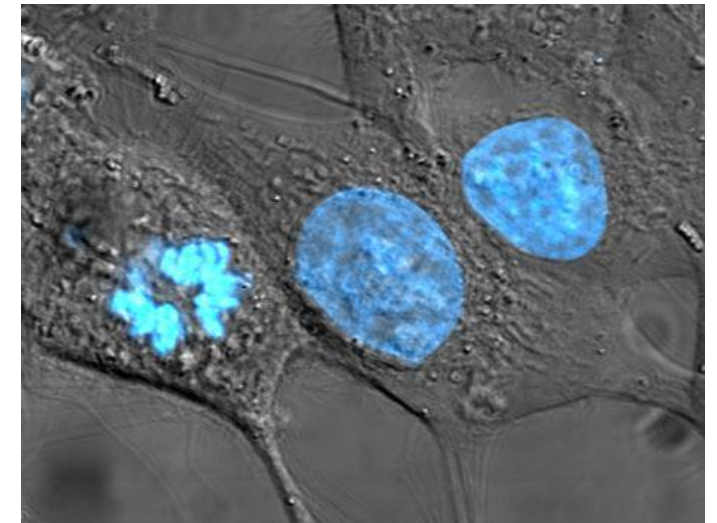
Hoechst stains are part of a family of blue fluorescent dyes that are used to stain DNA.



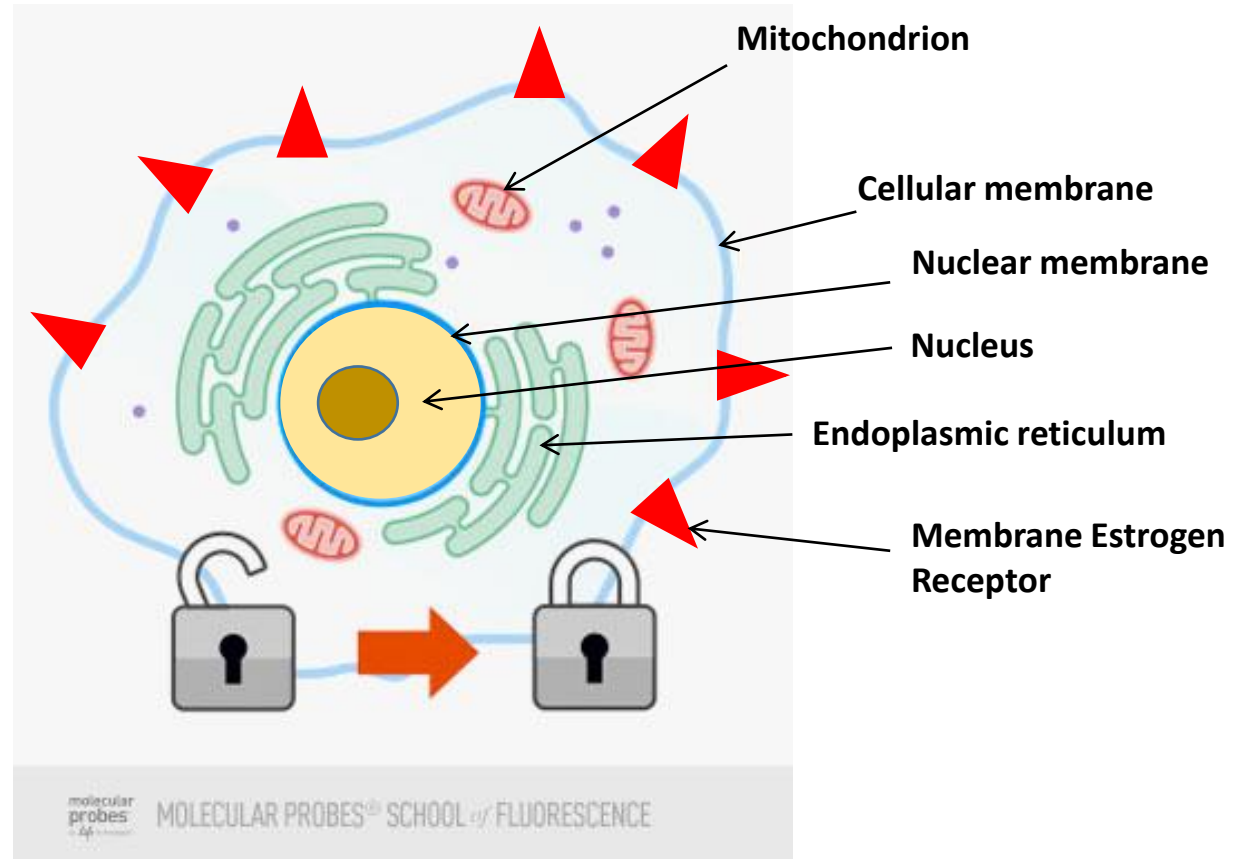
Hoechst chemical structure



Hoechst (magenta) bound to  
the minor groove of DNA  
(green and blue)  
From [PDB: 264D](https://www.rcsb.org/structure/264D)



# Basic Cell Structure



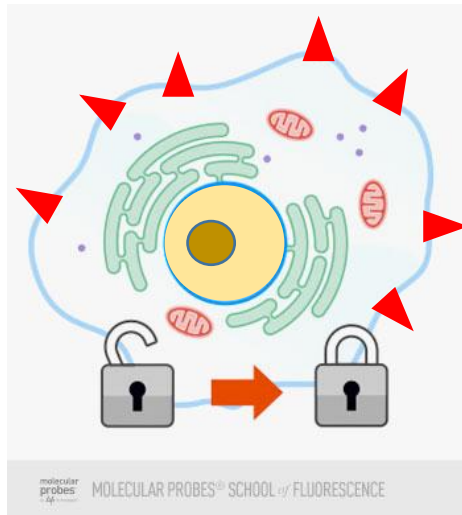
Adapted from:

<https://www.thermofisher.com/us/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/imaging-basics/sample-considerations/preparing-fixed-cells-imaging.html>

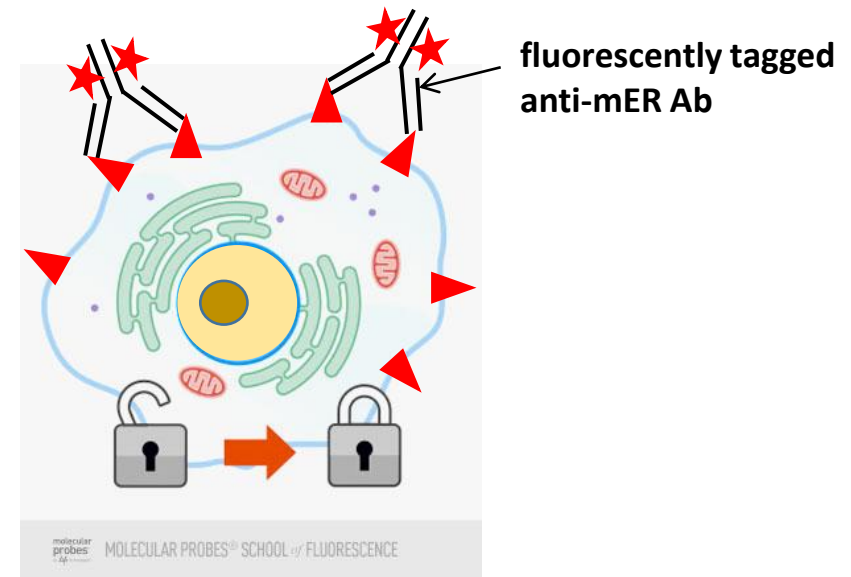
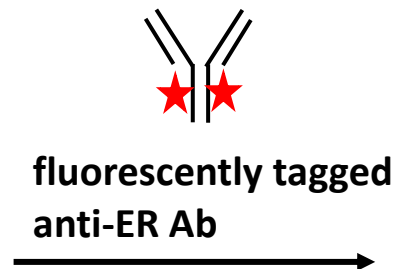
# Our Staining Protocol

## 1. Fixation

## 2. Membrane Stain



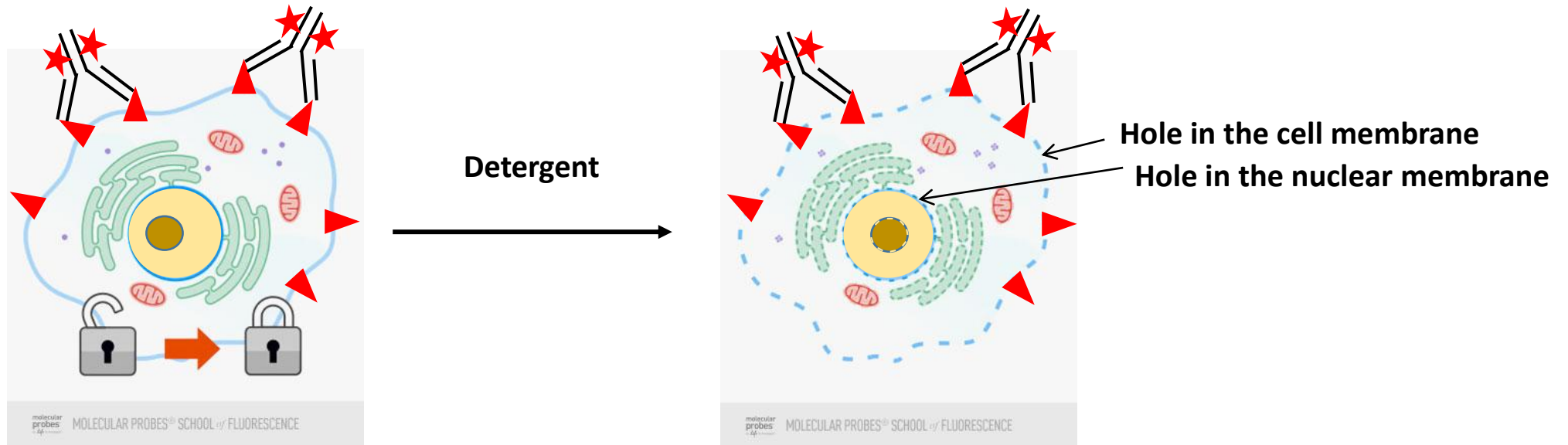
**Fig 3. Fixation locks cellular structures in place.**



**Fig 4. Fluorescently labeled antibodies can be used to detect expression of proteins on the cell membrane.**

# Our Staining Protocol

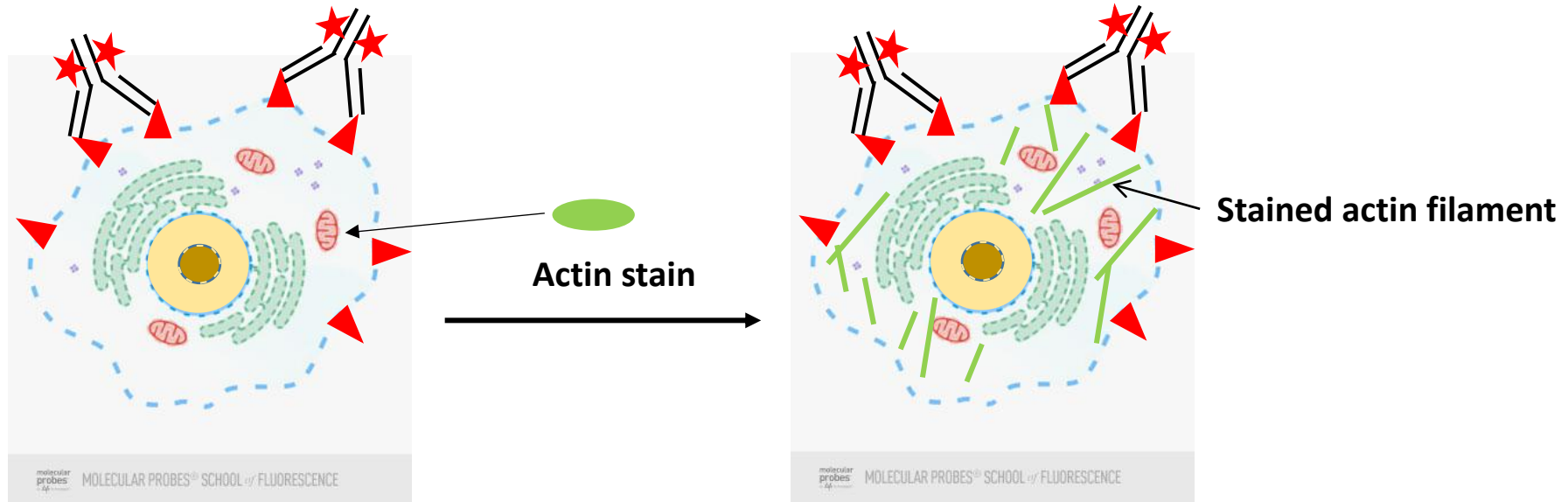
## 3. Permeabilization



**Fig 5. Permeabilization creates holes in the membranes, allowing other molecules to enter the cell.**

# Our Staining Protocol

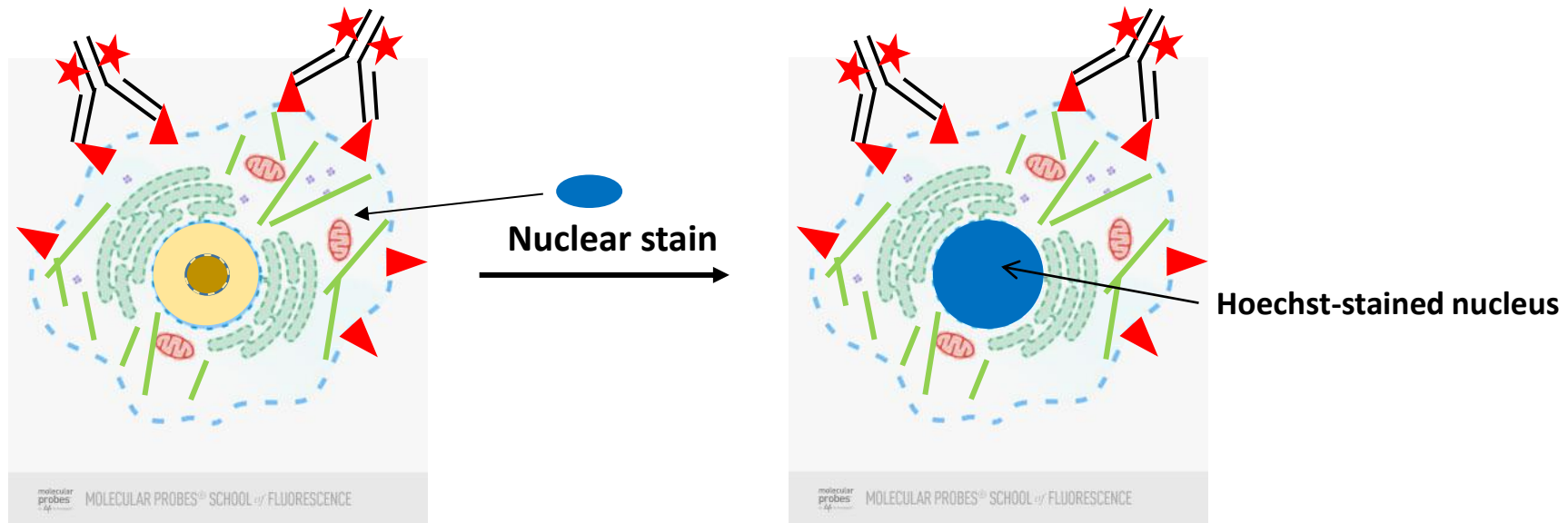
## 4. Actin Stain



**Fig 6. ActinGreen can enter through the holes in the cell membrane to detect the actin filaments of the cytoskeleton.**

# Our Staining Protocol

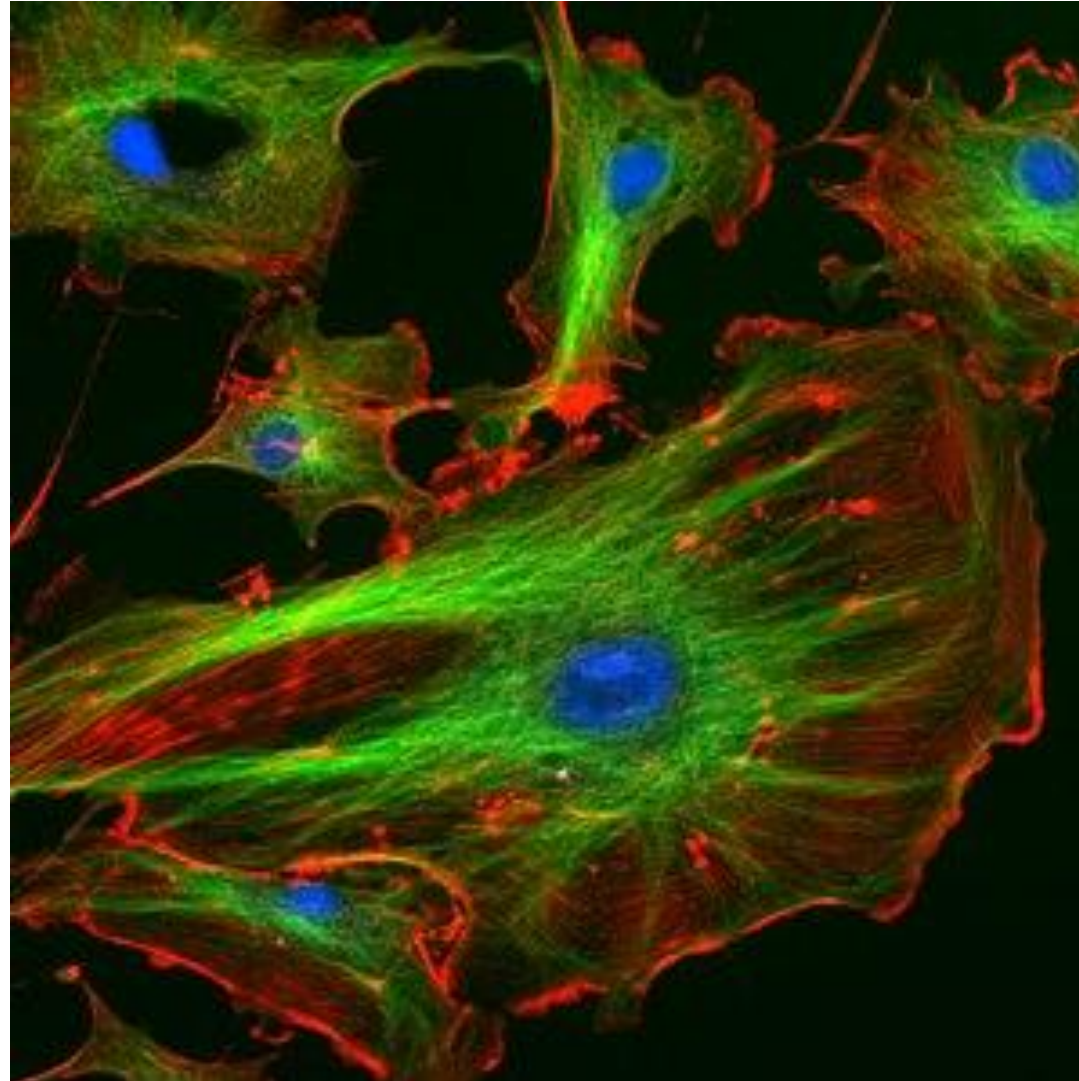
## 5. Nuclear Stain



**Fig 7. Hoechst stain can enter through holes in the cell and nuclear membranes and bind to the nucleic acids (DNA) in the nucleus.**



# Cell Staining: Membrane, Nucleus, Cytoskeleton



<https://en.wikipedia.org/wiki/Phalloidin>