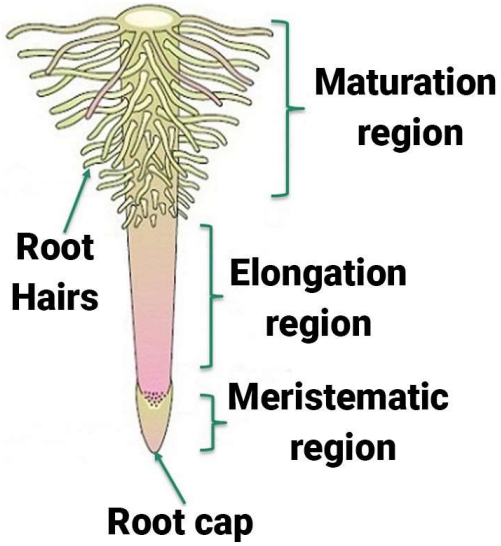
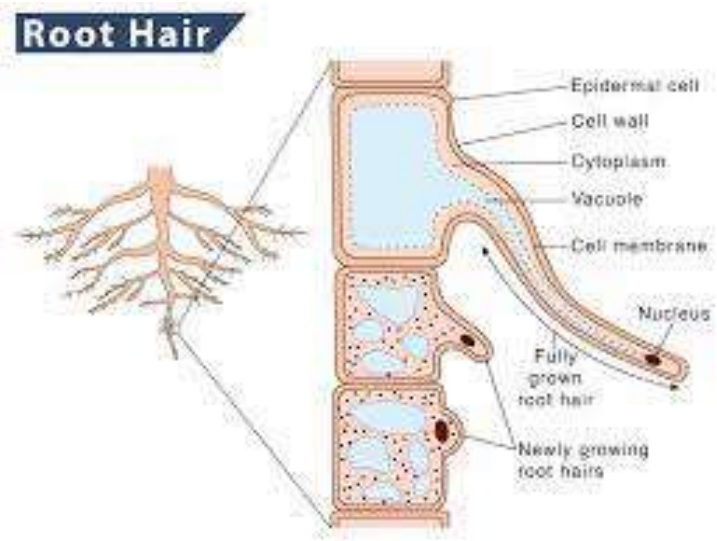
# DiOC6 /AO



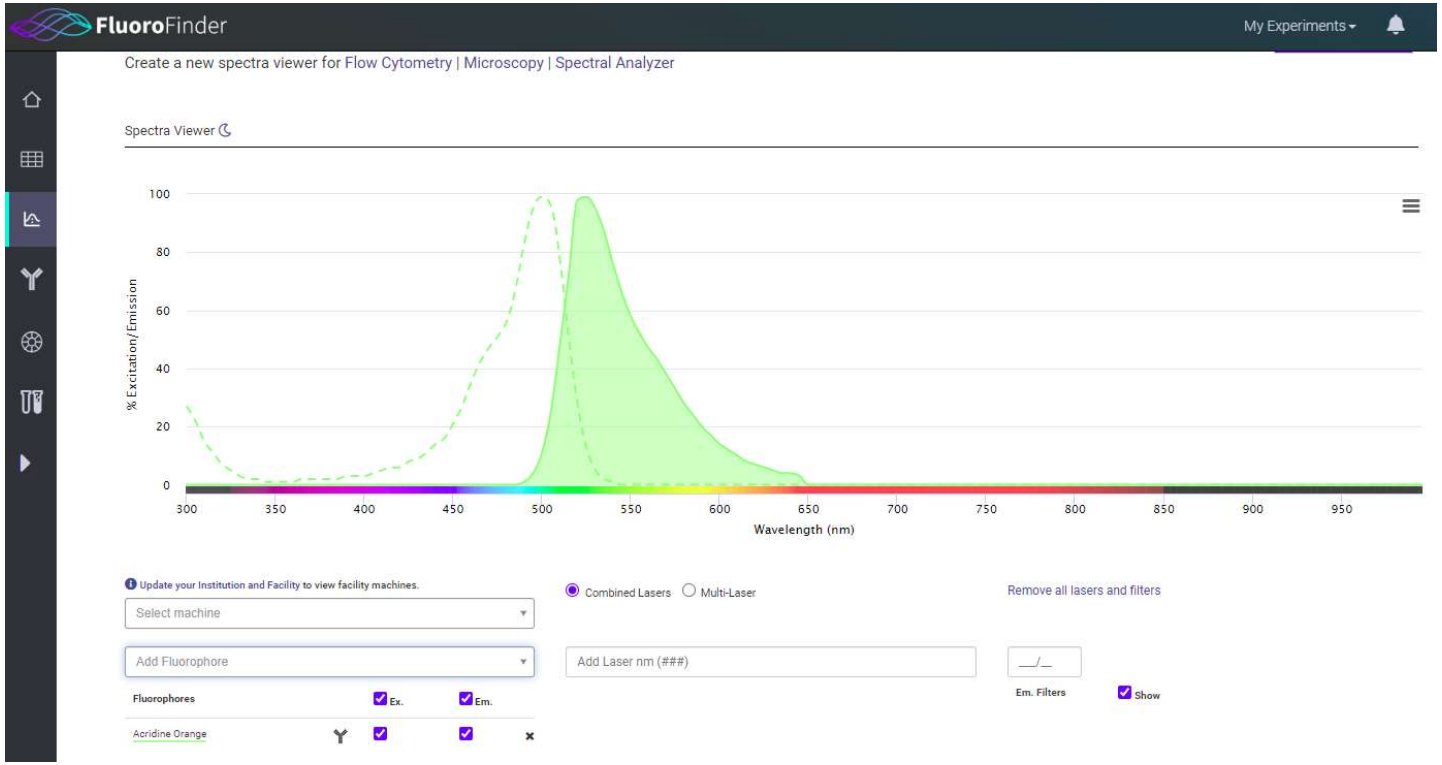

**Root hairs:**

- Unicellular.
- Hairlike.
- Present on the outer surface of plant roots.
- Root hairs are continually being sloughed off by the soil and regrown.
- Absorb water and nutrients from the soil.

# Fluorochromes

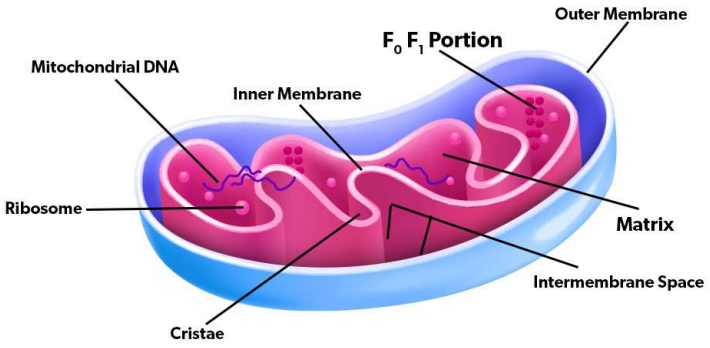
## Acridine Orange

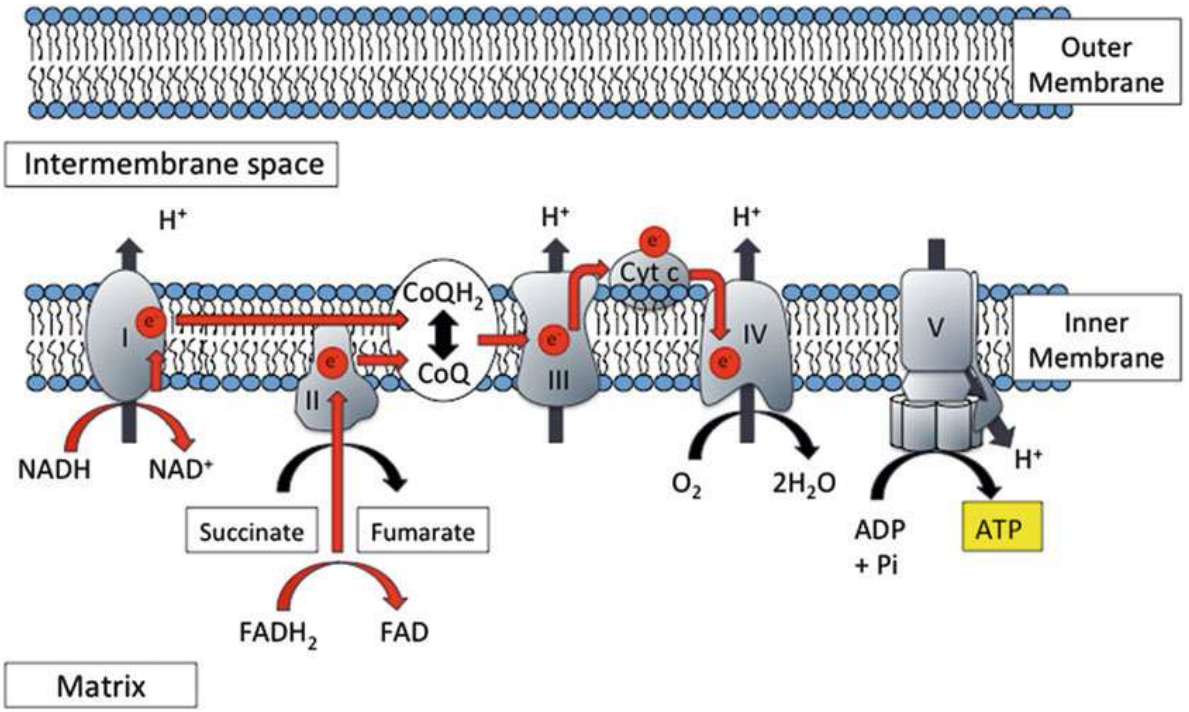
- Cell-permeant nucleic acid binding dye that emits green fluorescence when bound to dsDNA and red fluorescence when bound to ssDNA or RNA.
- has also been used as a lysosomal dye.



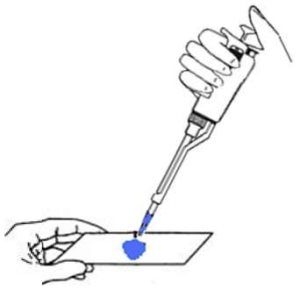
# DiOC6(3) (3,3'-Dihexyloxacarbocyanine Iodide)

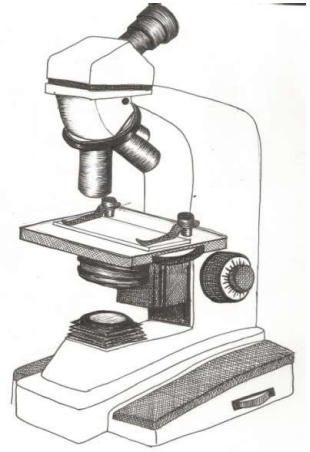
- cationic probe.
- cell-permeant.
- accumulates in the negatively charged mitochondria.
- green-fluorescent.
- lipophilic dye that is selective for the mitochondria of live cells, when used at low concentrations.
- At higher concentrations, can be used to stain other internal membranes, such as the endoplasmic reticulum.
- Excitation 482nm, Emission 504nm



# Experimental Design





**40 µl $H_2O$ + 10 µl fluorochrome (10x diluted)**

**+**

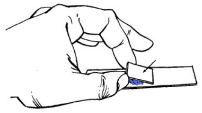
**Roots (*Tradescantia fluminensis*)**

Observe with the appropriate emission cubes

**AC**
**[stock 50 µM]**

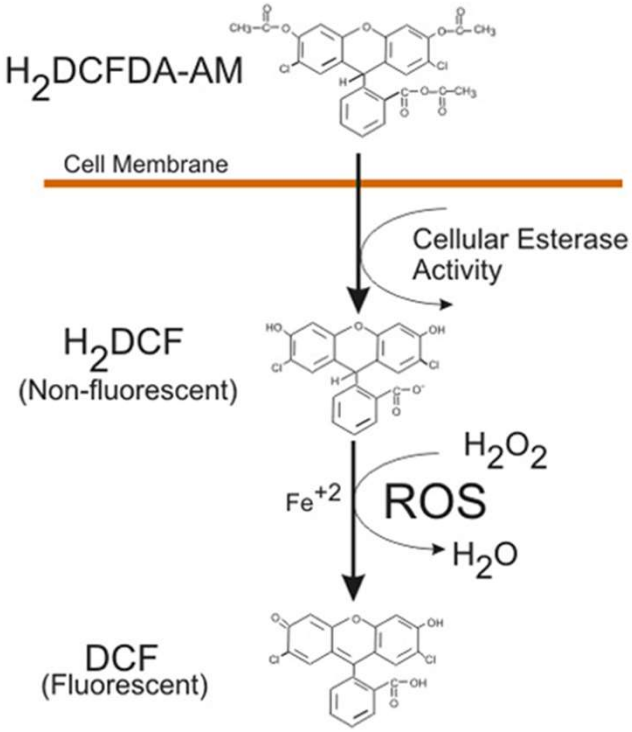
**DiOC6**
**[STOCK 10 µM]**

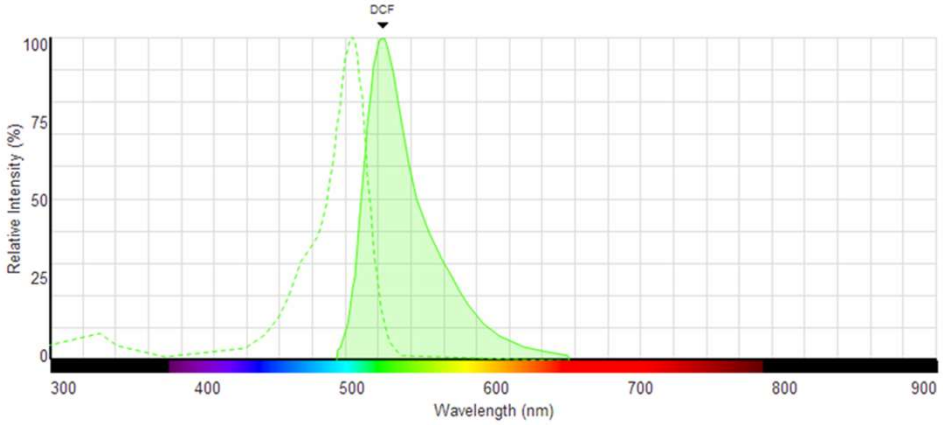
**CTC**
**(cálcio associado a membranas)**
**[stock $10^{-2}$ M]**



cover slip

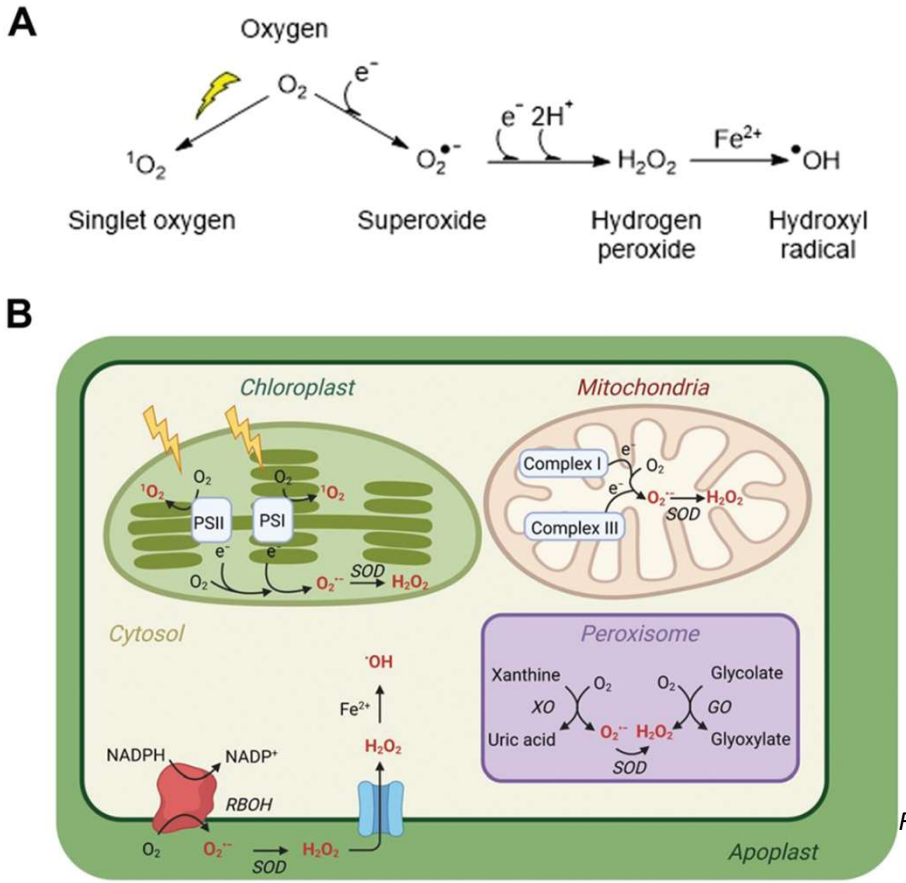
# 6-carboxy-2´,7´-dichlorodihydrofluorescein diacetate ($H_2$DCFDA)




**Ex: 492–495 nm**
**Em: 517–527 nm**

# Reactive Oxygen Species - ROS




*RSC Chem. Biol.*, 2021, 2, 1384

ROS in plant cells. (A) Common ROS production from atmospheric oxygen; (B) primary ROS production sites in a plant cell; PSI/II, photosystem I/II; SOD, superoxide dismutase; XO, xanthine oxidase; GO, glycolate oxidase; RBOH, respiratory burst oxidase homolog

**ROS are by-products of electron transfer processes.**

**Under normal conditions, ROS are scavenged by the cell maintaining a redox equilibrium.**

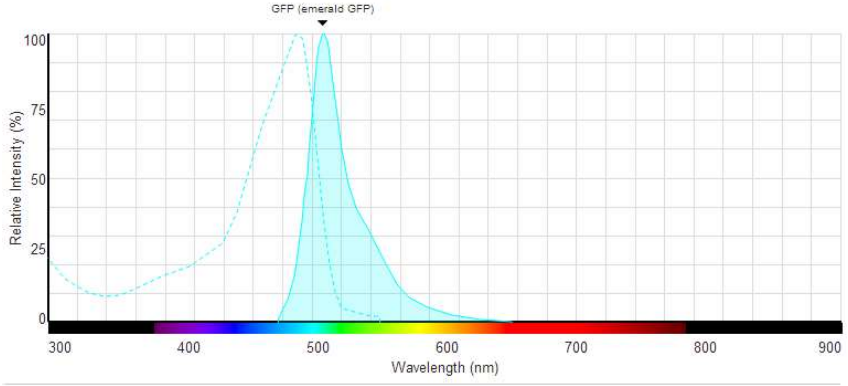
- **ROS scavengers: small molecules buffers – glutathione, ascorbate**
  **enzymes – superoxidase dismutase, catalase**

**Under stress, ROS levels can increase which works as useful stress signals but in excess results in cell damage and death.**

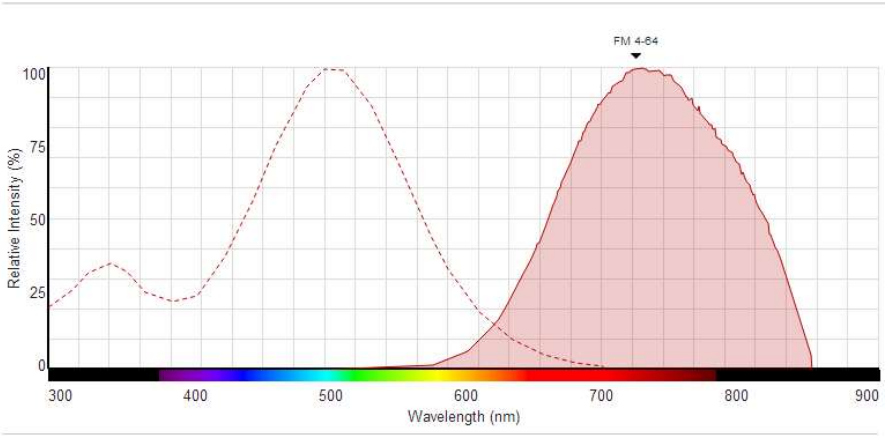
- **biomolecules affected by high levels of ROS: proteins**
  **lipids**
  **nucleic acids**

**ROS as biotic and/or abiotic stress signal: hormonal**
**physiological**
**development**

# FM 4-64 Dye (*N*-(3-Triethylammoniumpropyl)-4-(6-(4-(Diethylamino) Phenyl) Hexatrienyl) Pyridinium Dibromide)

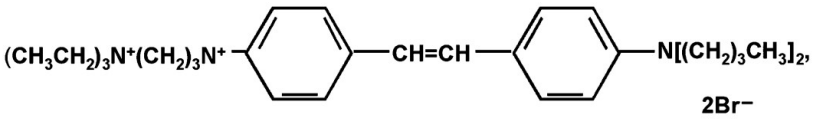



GFP

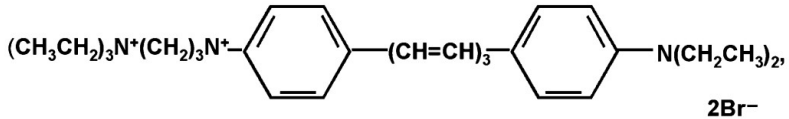


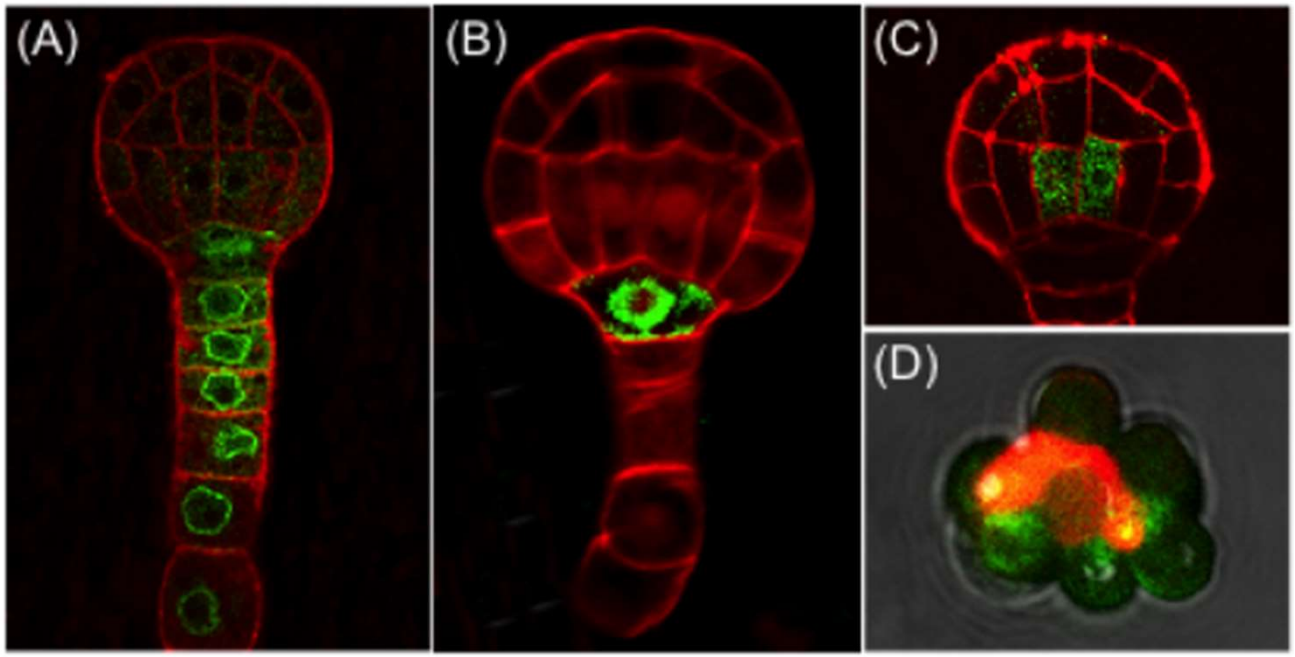

FM1-43 / FM4-64

**FM1-43** (MW 612)
N-(3-triethylammoniumpropyl)-4-(4-(dibutylamino)styryl)pyridinium dibromide



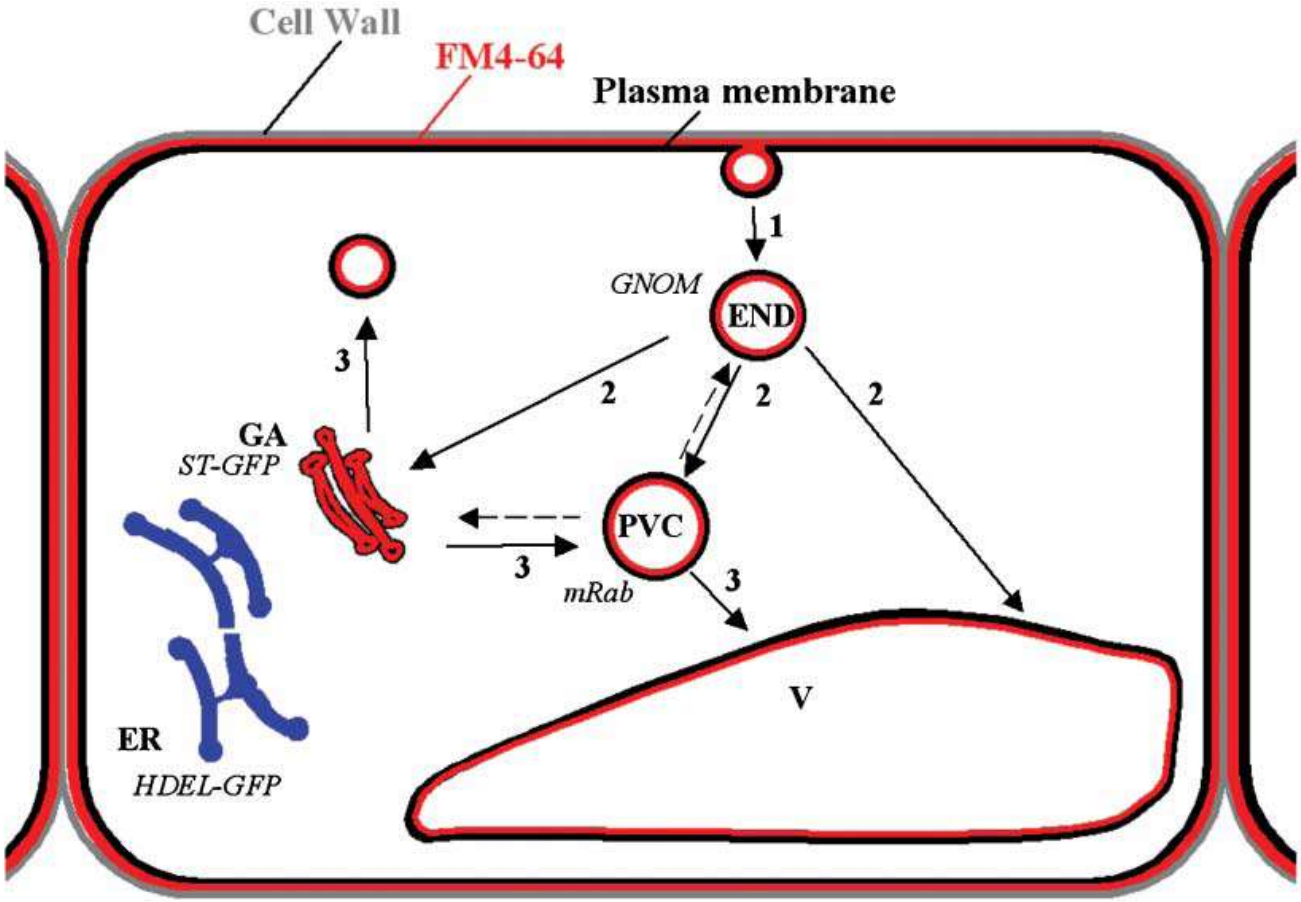

**FM4-64** (MW 608)
N-(3-triethylammoniumpropyl)-4-(6-(4-(dibutylamino)phenyl)hexatrienyl)pyridinium dibromide



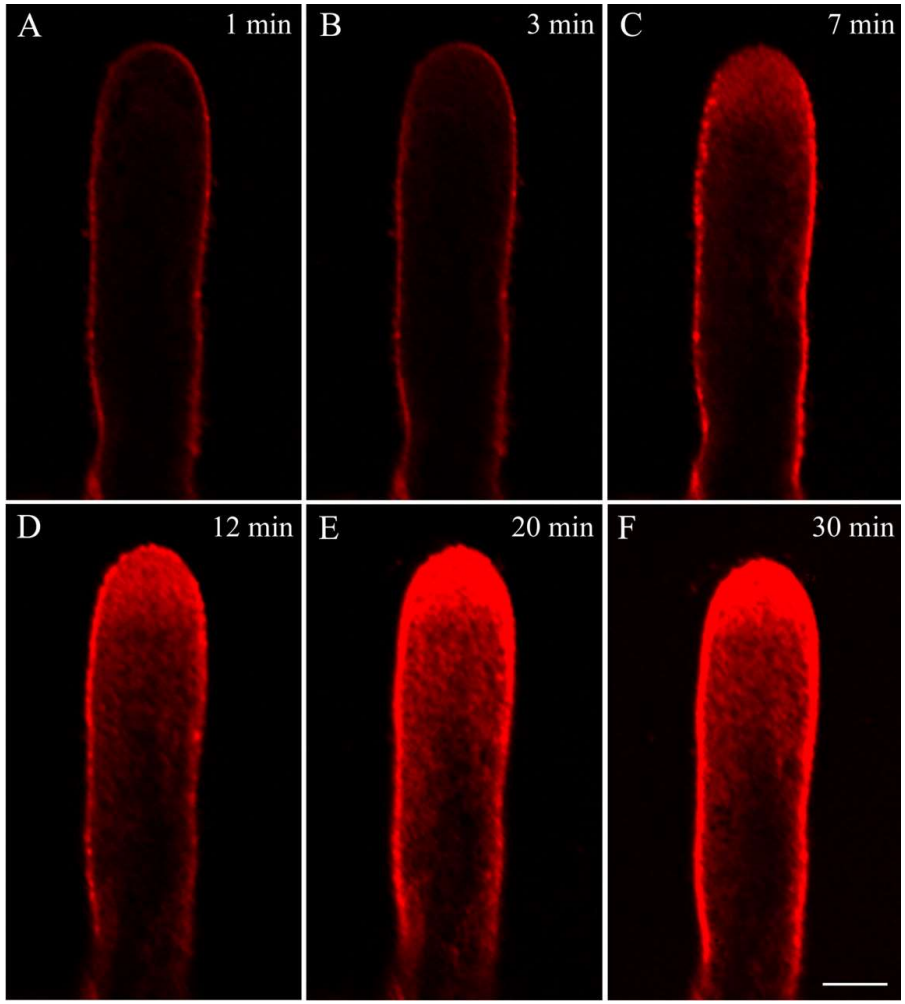



Transcriptomics approaches in the early Arabidopsis embryo
DOI: http://dx.doi.org/10.1016/j.tplants.2013.04.011

*Confocal images of suspensor-specific (A), hypophysis-specific (B), and vascular-specific (C) NTF expression in the Arabidopsis embryo. (D) Biotin-tagged nucleus (red) bound to streptavidin-coated beads. GFP fluorescence in (A-C) is in green while membranes are counterstained with FM4-64 (red).*



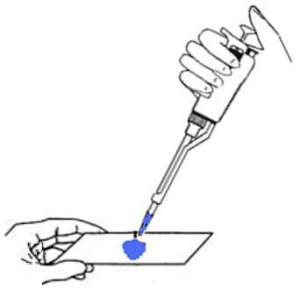

Model of possible pathways of membrane staining by FM4-64 in plant cells. FM4-64 immediately stains the plasma membrane by becoming inserted and anchored in the outer leaflet of the plasma membrane lipid bilayer. After internalization by endocytosis, the dye becomes localized to the inner leaflet of endocytic vesicles and all other organelles, which FM4-64 subsequent stains.




FM4-64-uptake time course in a growing *P. meyeri* pollen tube.

P Physiology, 2005, 139: 1692–1703
http://dx.doi.org/10.1104/pp.105.069765

# Experimental Design



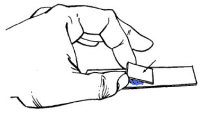
**40 µl $H_2O$ + 10 µl fluorochrome**

**+**

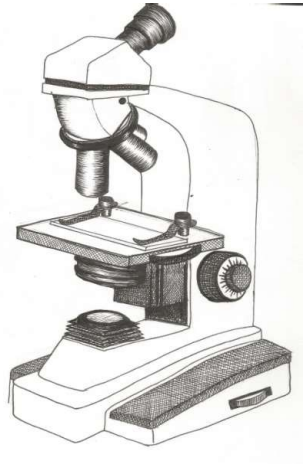
**roots**

**FM4-64***
**[50 µM]**

**H2DCFDA**
**[STOCK 10 µM]**



cover slip



observe