

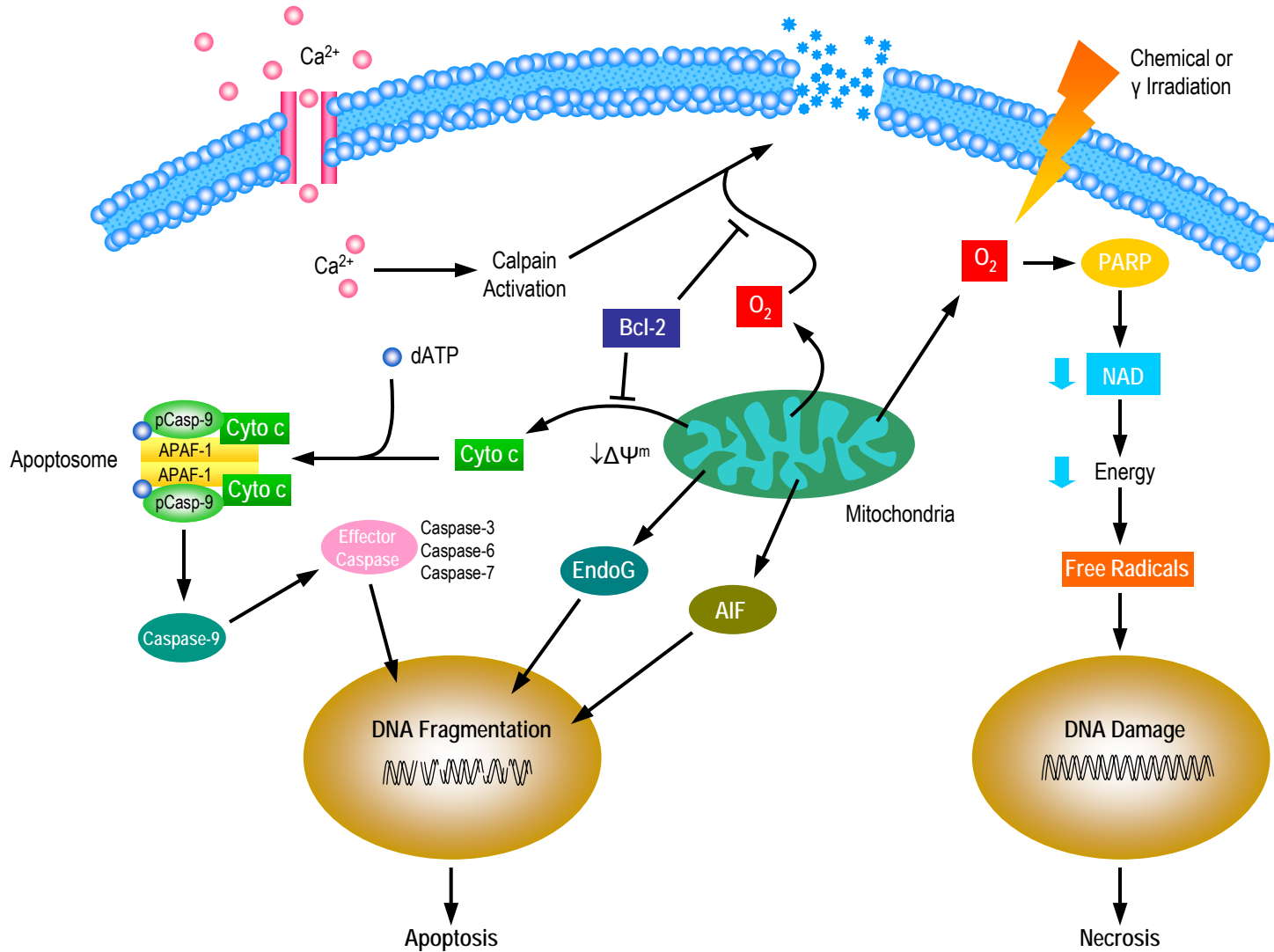
# *Fluorescence Tools for Analysis of Apoptosis*

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Technology Specialist**

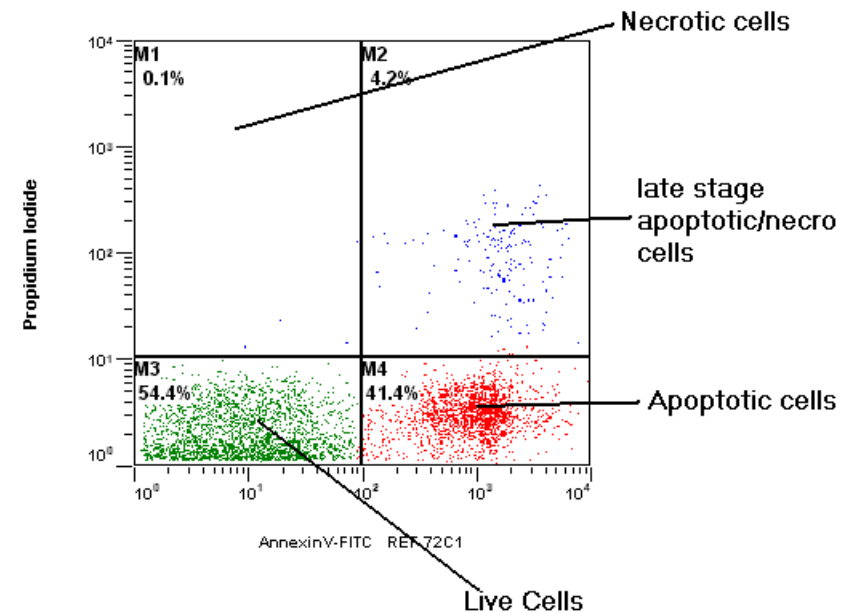


To discover how life works is the greatest scientific endeavor of our era, holding promise of fundamental improvement in the human condition. Our Quest is to accelerate this search through innovations in science and technologies that expand biological understanding. Success requires passion, intellectual curiosity and a sense of urgency. We will strive for excellence and act with unyielding integrity in everything we do so that we can serve as responsible stewards in the global life science community.

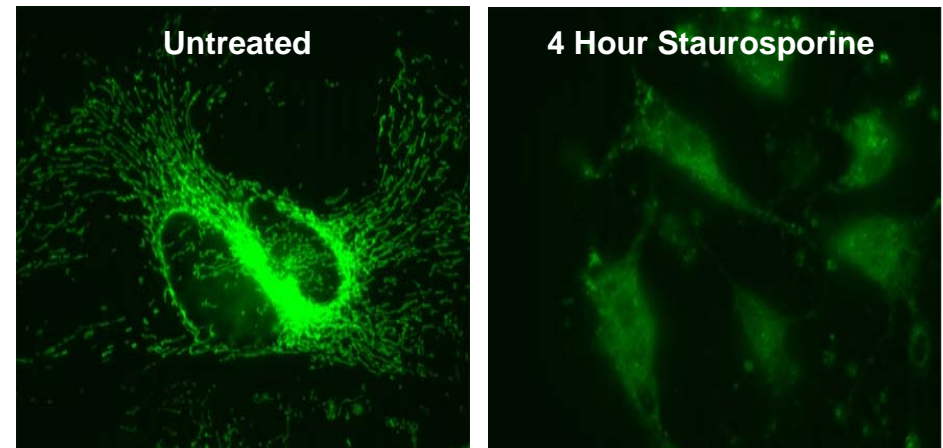
# Apoptosis – Programmed Cell Death



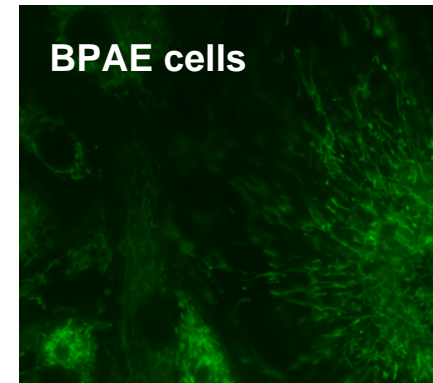
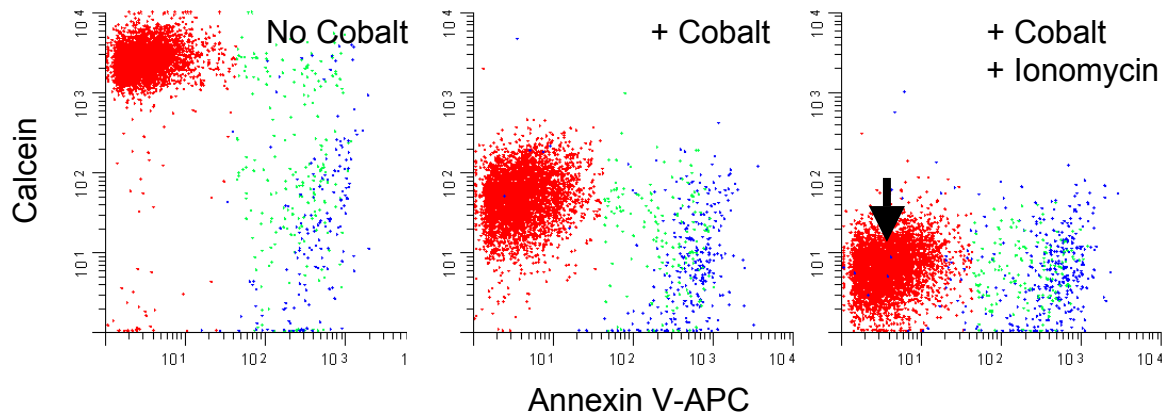
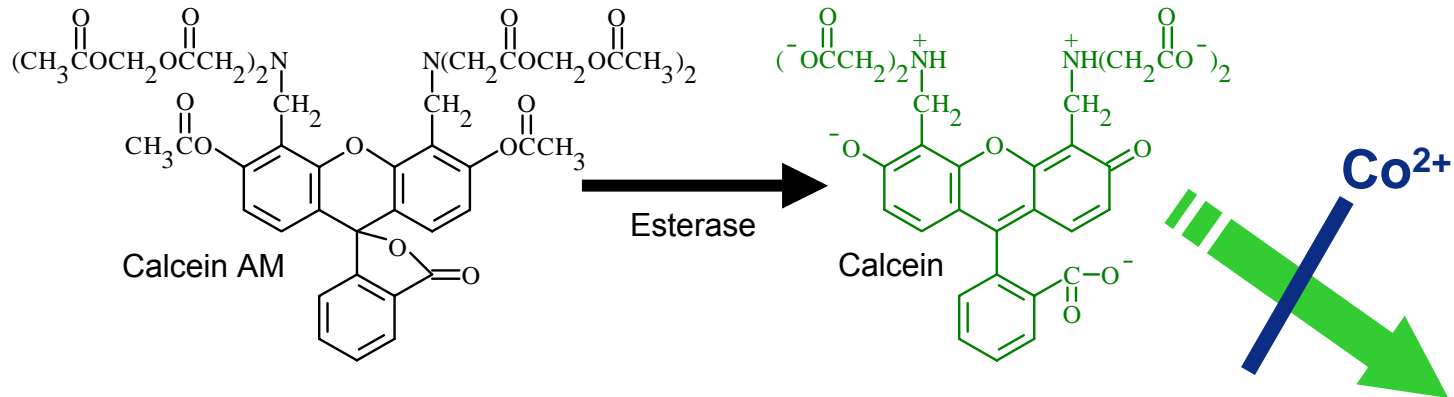
- **Annexin V Conjugates**  
(Alexa Fluor dyes, RPE, APC)
- **Vybrant® Apoptosis Kits**  
(annexin, viability, vitality)
- **MitoTracker® Dyes**
- **Caspase Substrates**
- **TUNEL**



Fixed HeLa cells  
anti-cytochrome c  
Alexa Fluor 488–goat anti-mouse IgG

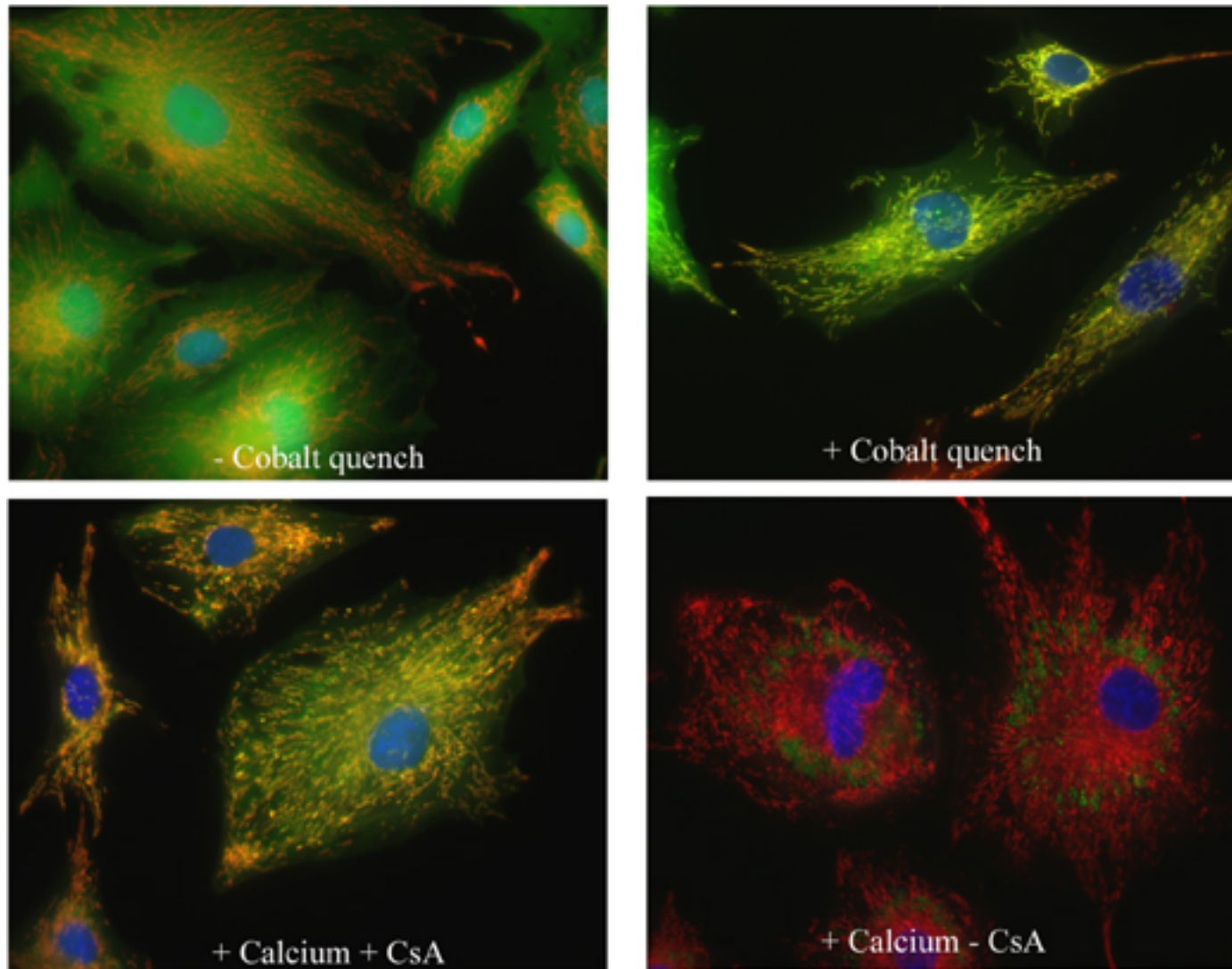


- **Cytochrome c translocation:** mitochondrial transition pore assay
- **Mitochondrial function:** JC-1, MitoTracker® Red dye, DiOC<sub>2</sub>(3), DiIC<sub>1</sub>(5)
- **Membrane structure:** annexin V, YO-PRO®-1 dye
- **Proteolysis:** caspase activity
- **Cellular metabolism:** C<sub>12</sub>-resazurin
- **Membrane integrity:** propidium iodide, SYTOX® Green dye
- **DNA fragmentation:** Hoechst dyes, TUNEL

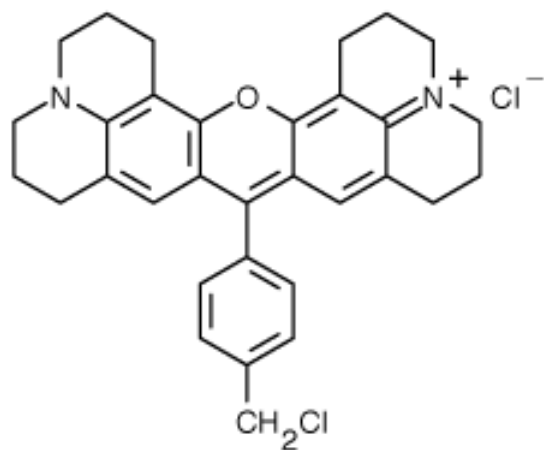


**488 nm & 633 nm Excitation**

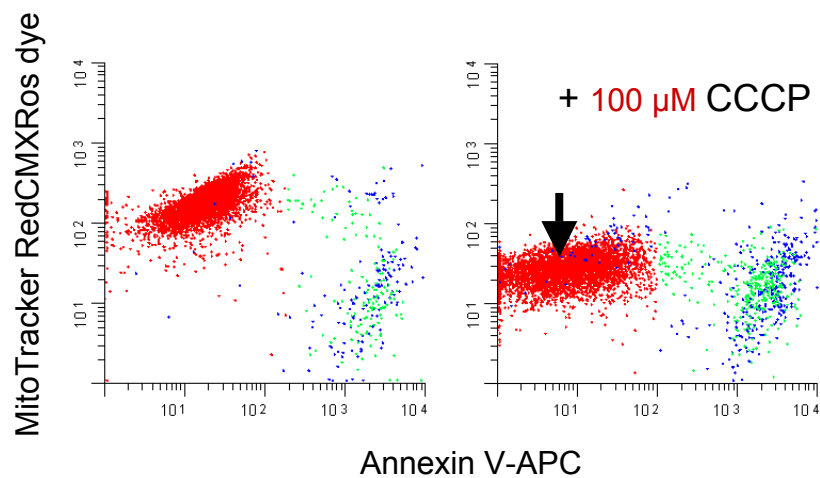
10 nM calcein AM  
400  $\mu\text{M}$   $\text{CoCl}_2$



Live BPAE cells labeled with Calcein AM, MitoTracker® Red dye, and Hoechst 33342



**MitoTracker Red CMXRos**

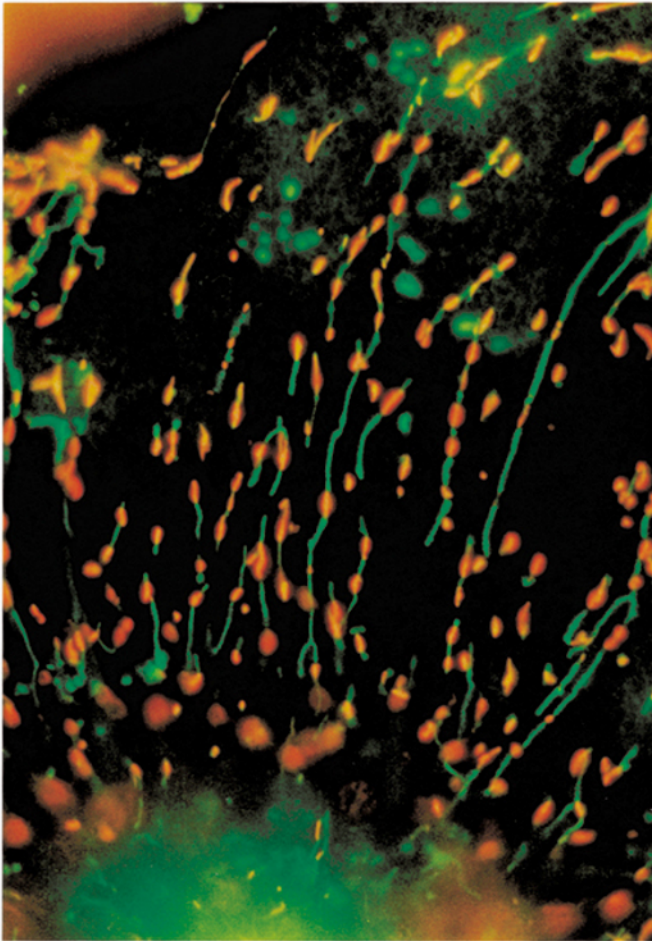


**488 nm & 633 nm Excitation**

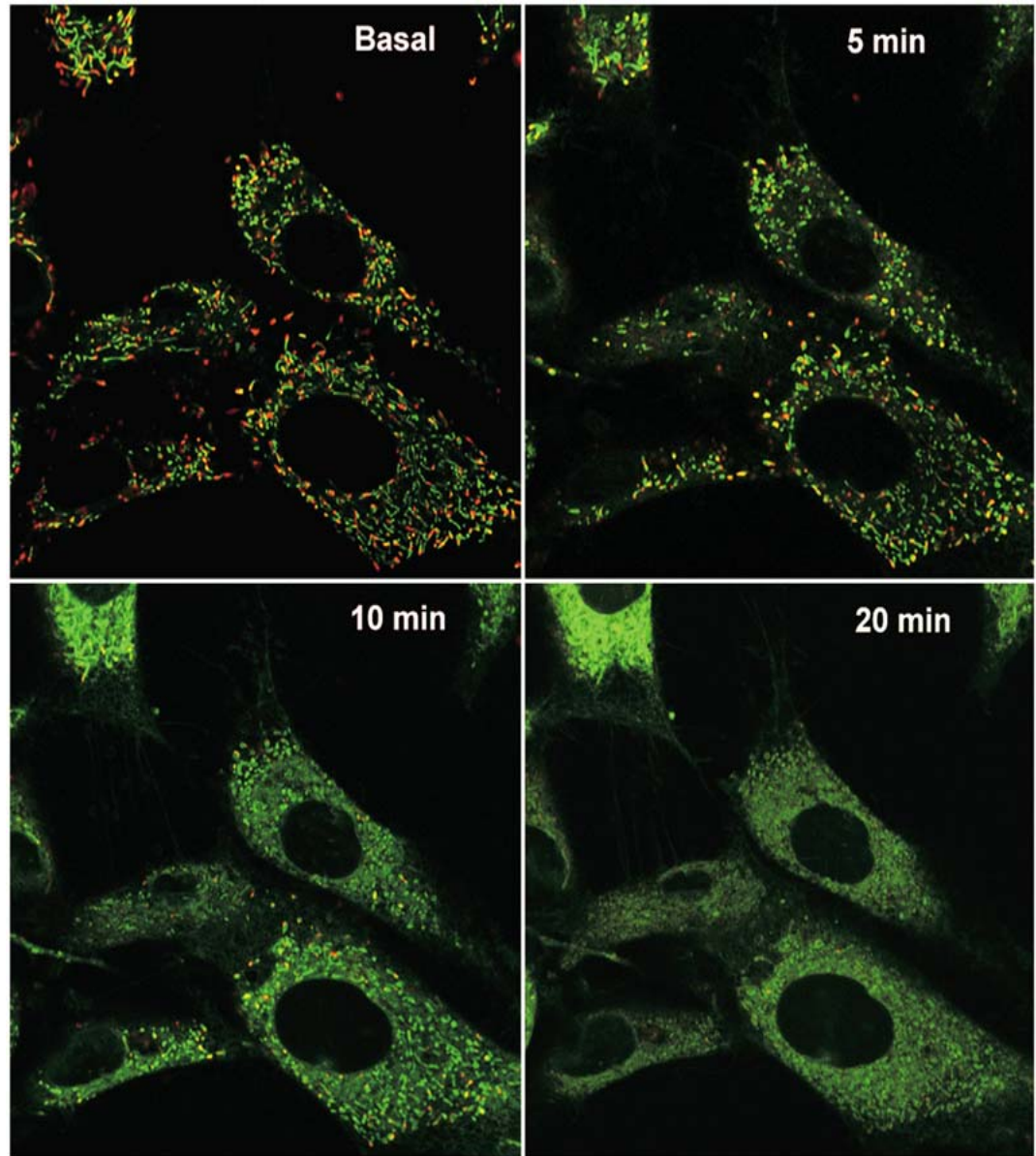
40 nM MitoTracker Red CMXRos

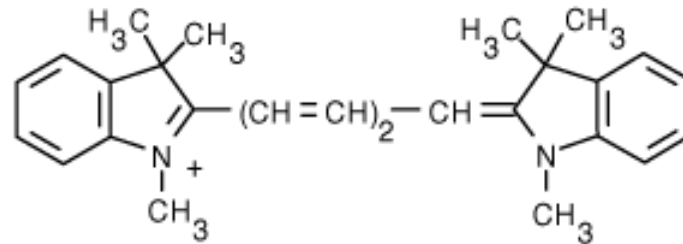


## JC-1: Change after hydrogen peroxide

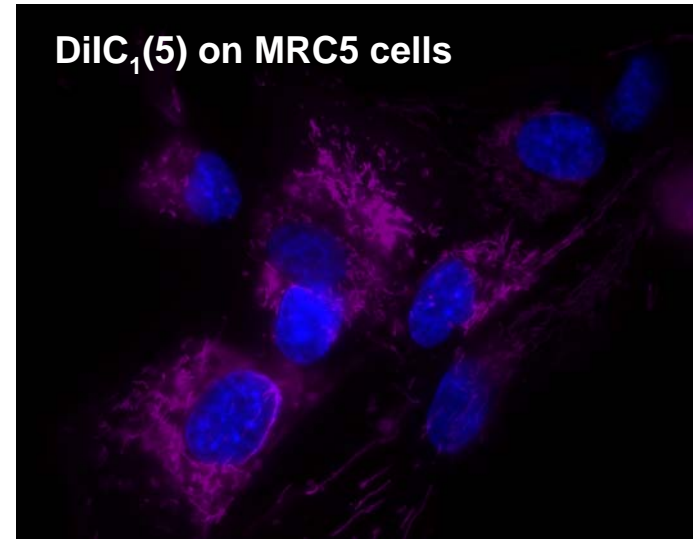
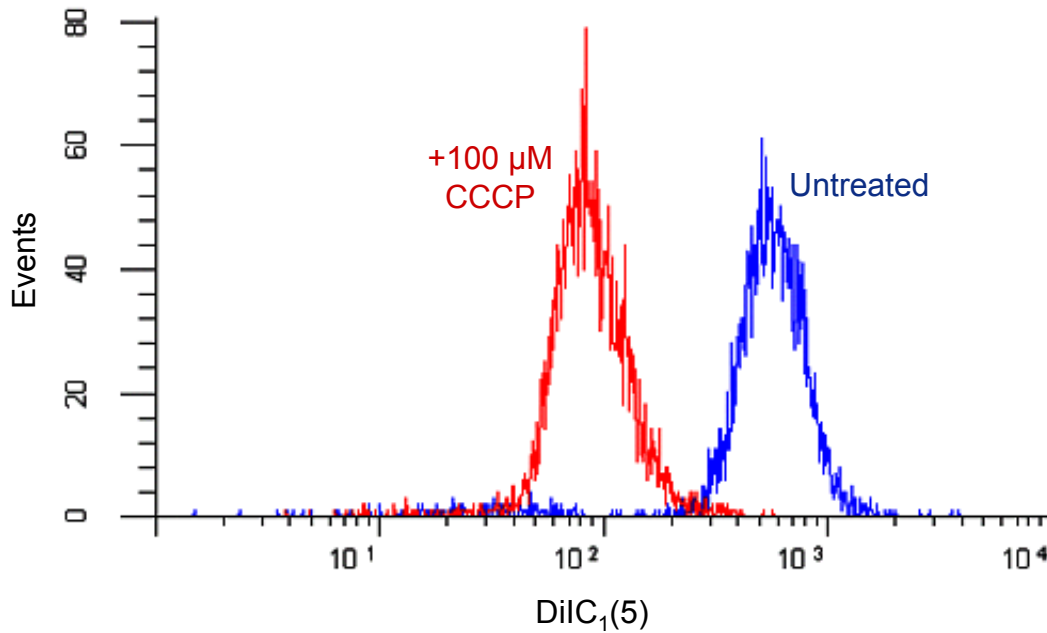


NIH 3T3 mouse fibroblasts + JC-1  
Progressive loss of red J-aggregate  
fluorescence and cytoplasmic diffusion of  
green monomer fluorescence following  
exposure to hydrogen peroxide





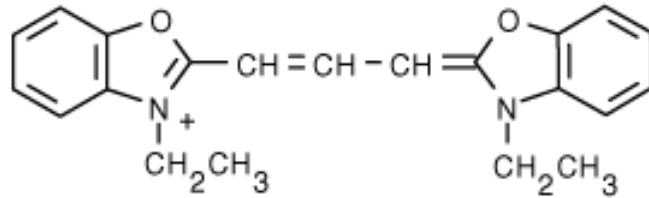
**DiIC<sub>1</sub>(5)**



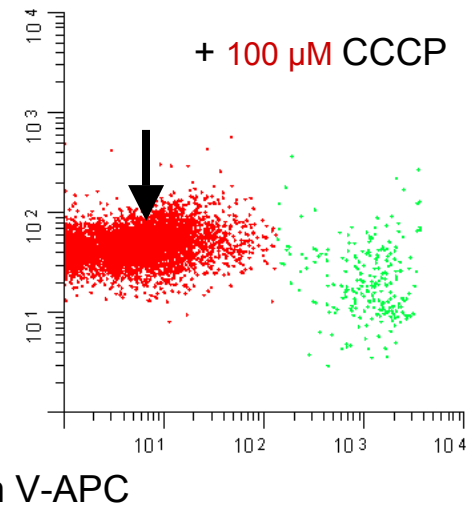
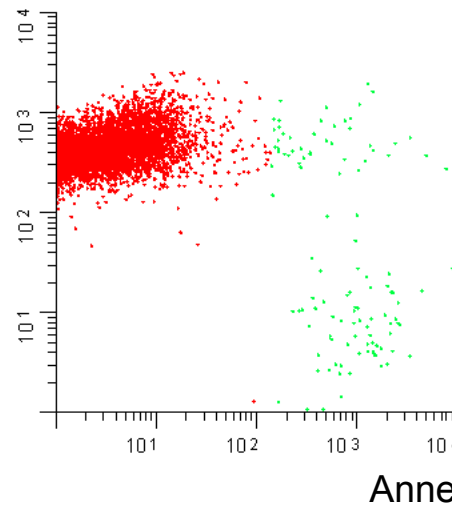
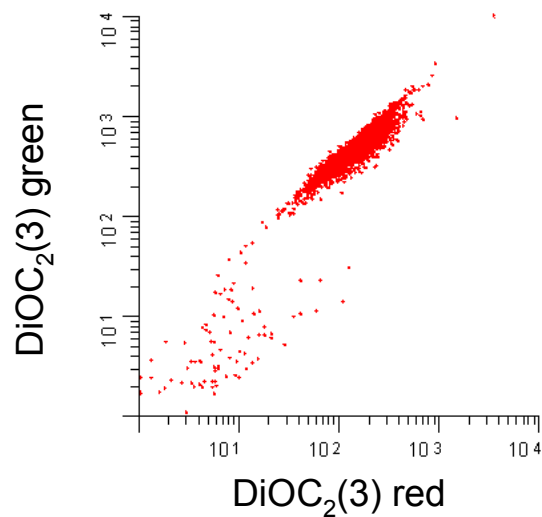
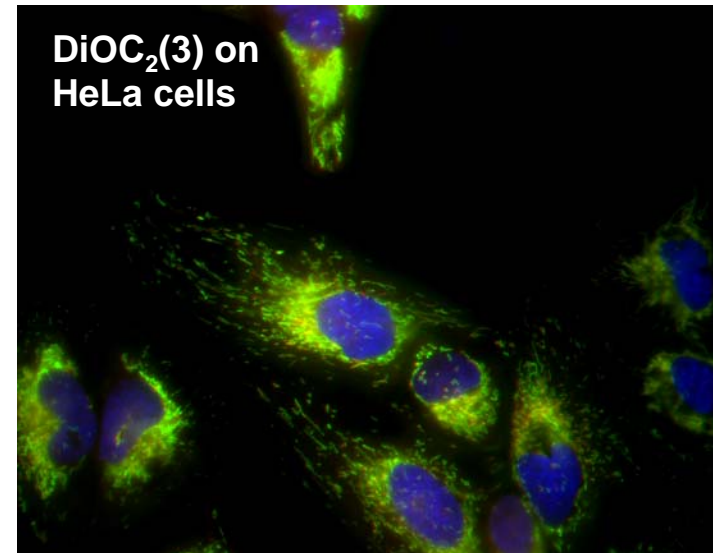
**633 nm Excitation**

50 nM DiIC<sub>1</sub>(5)

## Other Cyanine-based Membrane Potential Dyes

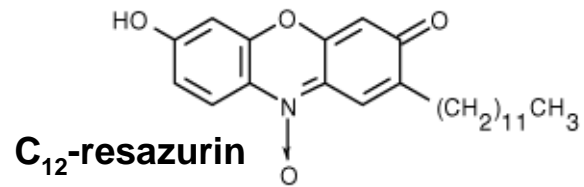


**DiOC<sub>2</sub>(3)**



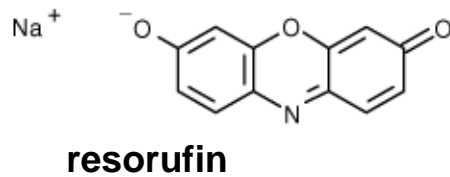
**488 nm & 633 nm Excitation**

50 nM DiOC<sub>2</sub>(3)

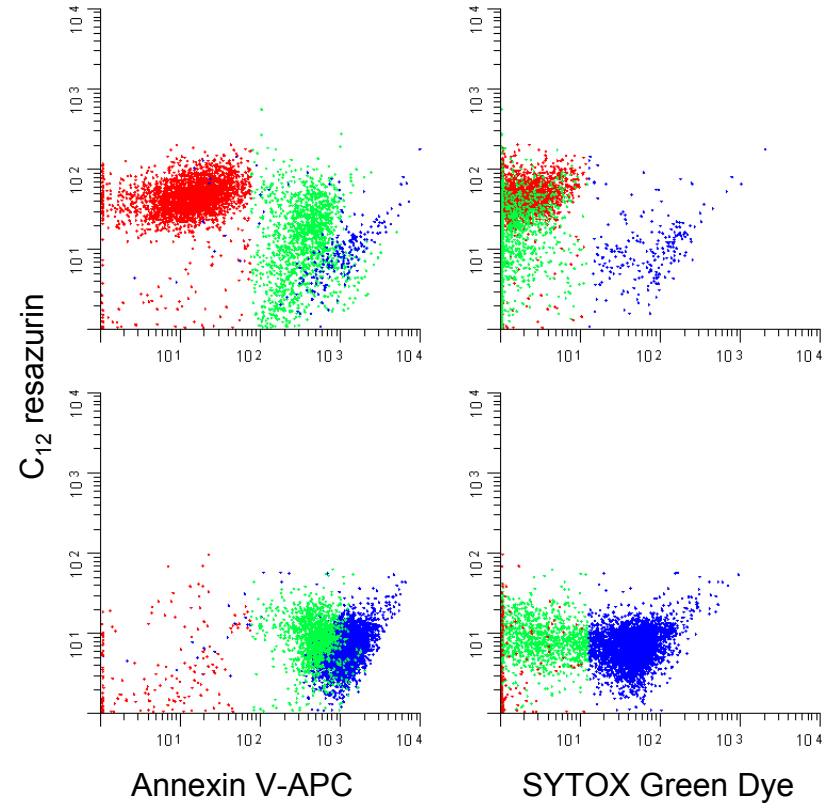


4 hours

**Reductase**

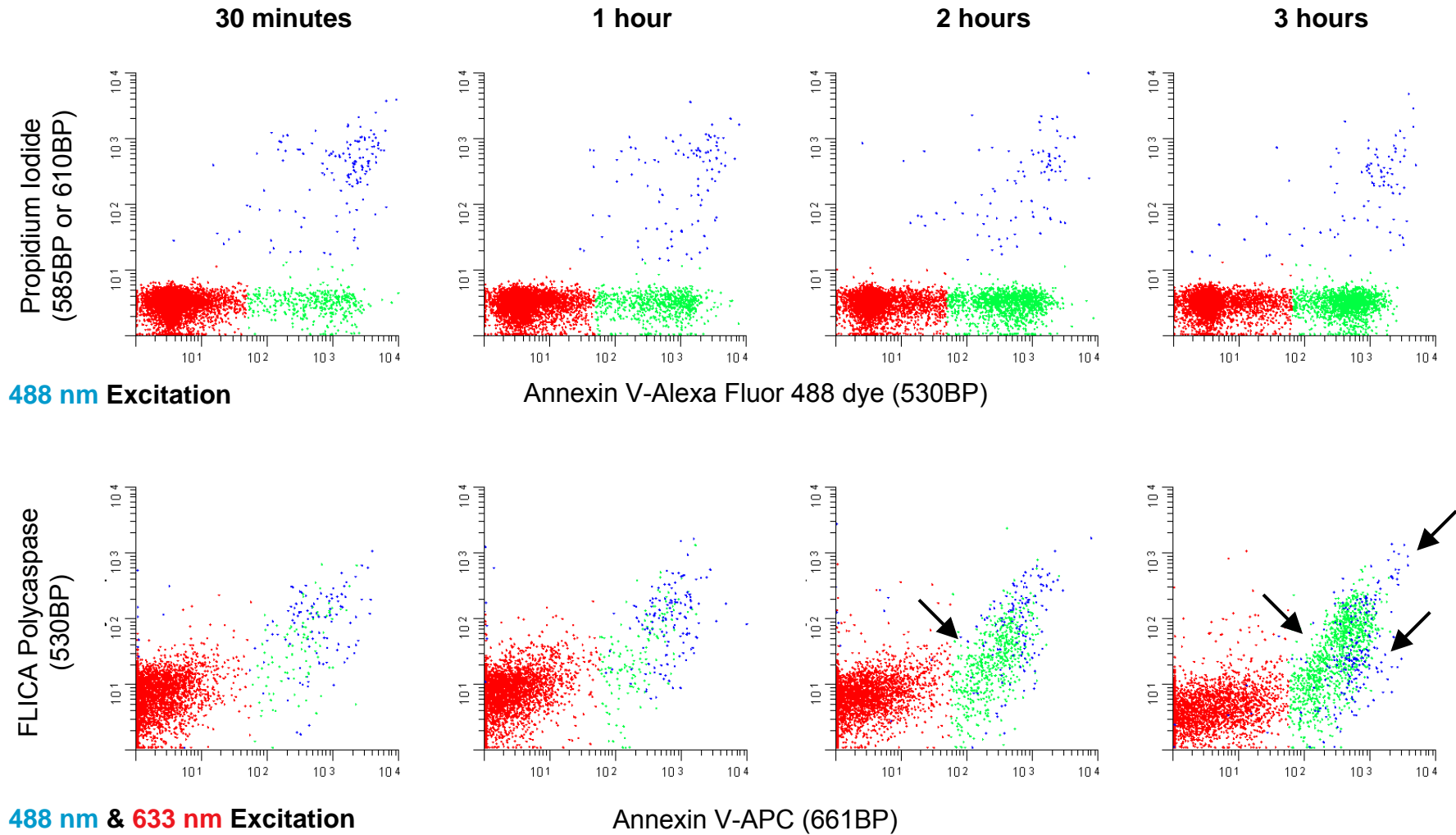


Necrotic

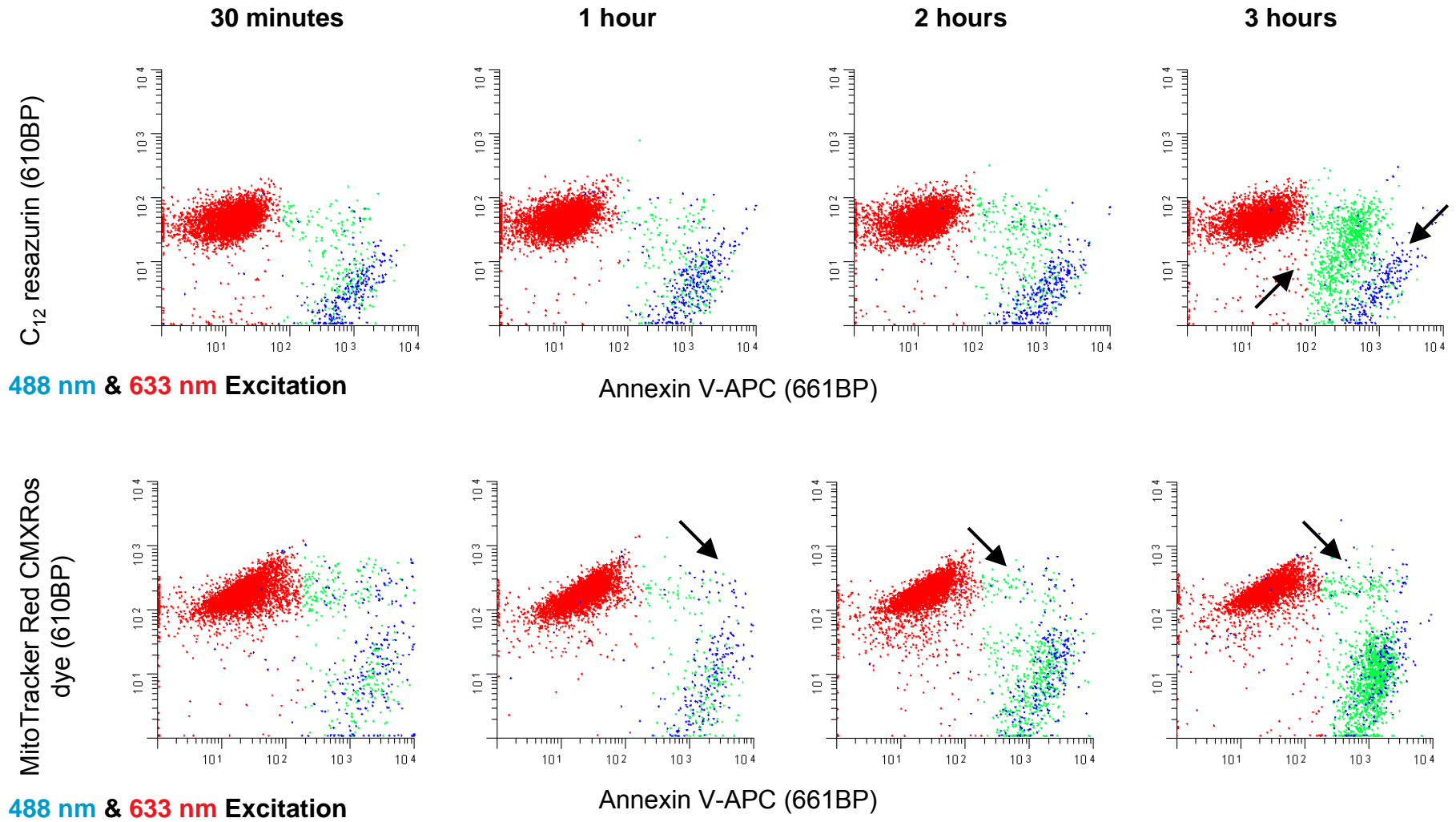


**488 nm & 633 nm Excitation**

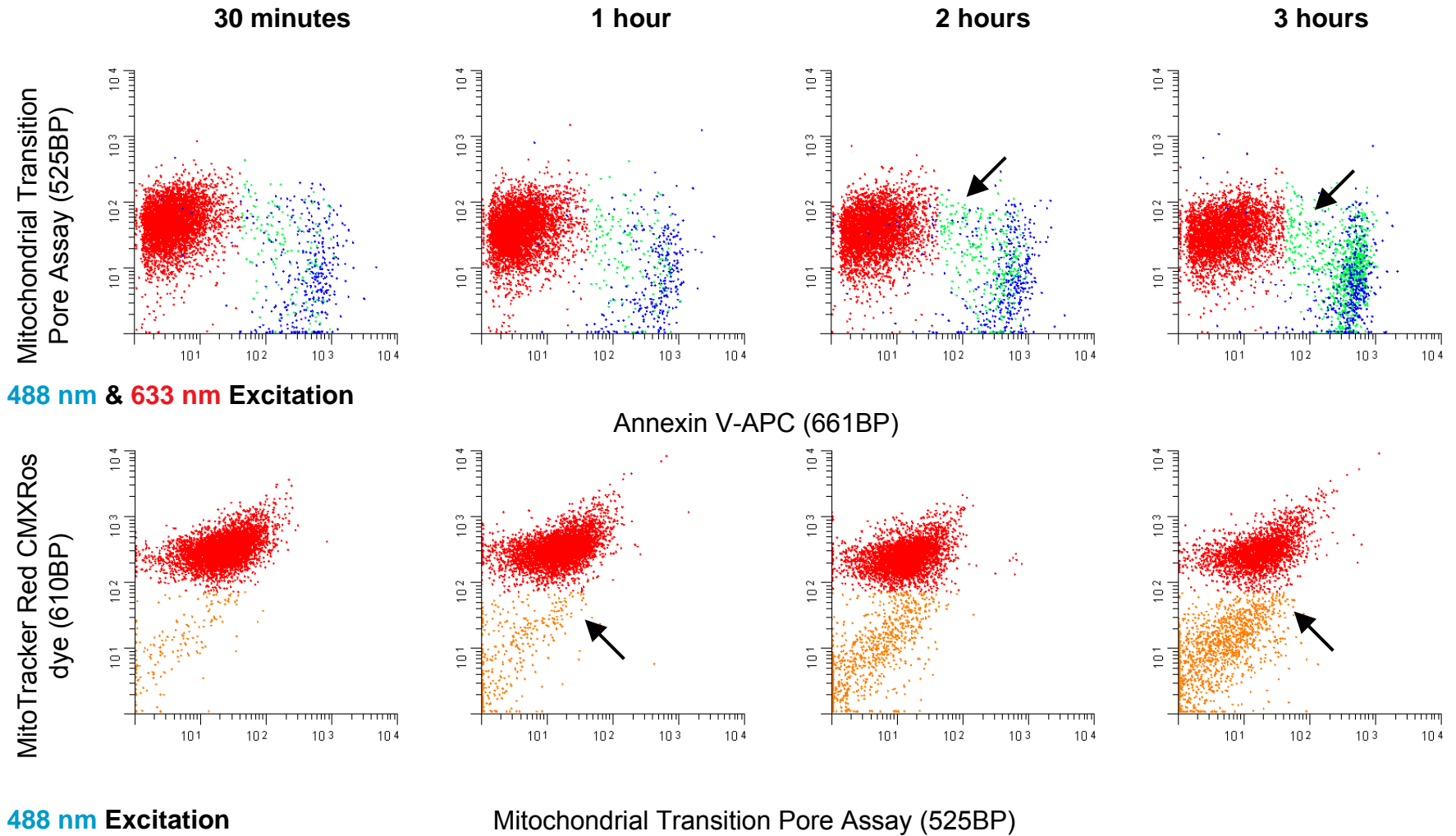
# Jurkat Cells: Time After Camptothecin Induction



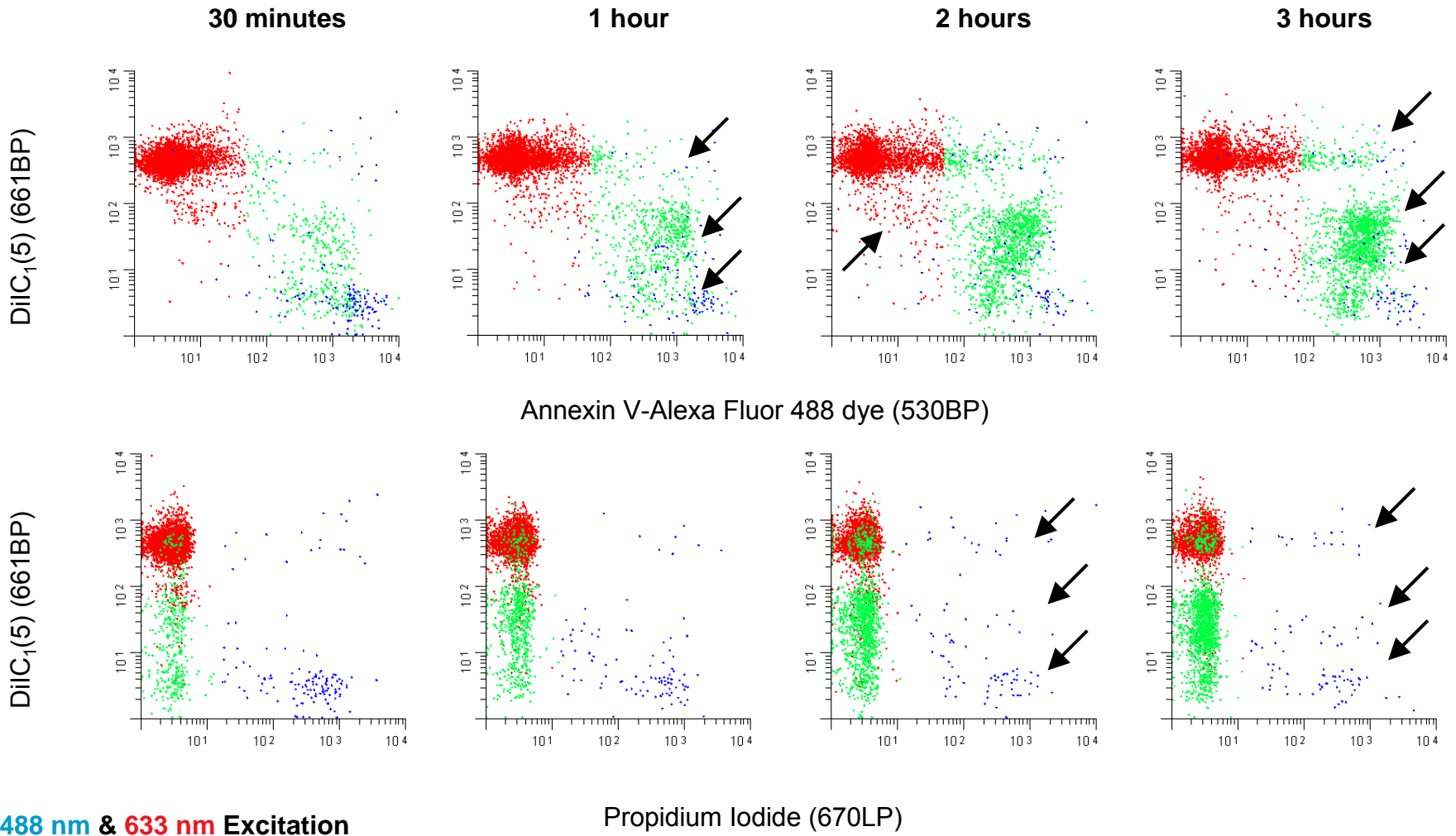
# Jurkat Cells: Time After Camptothecin Induction



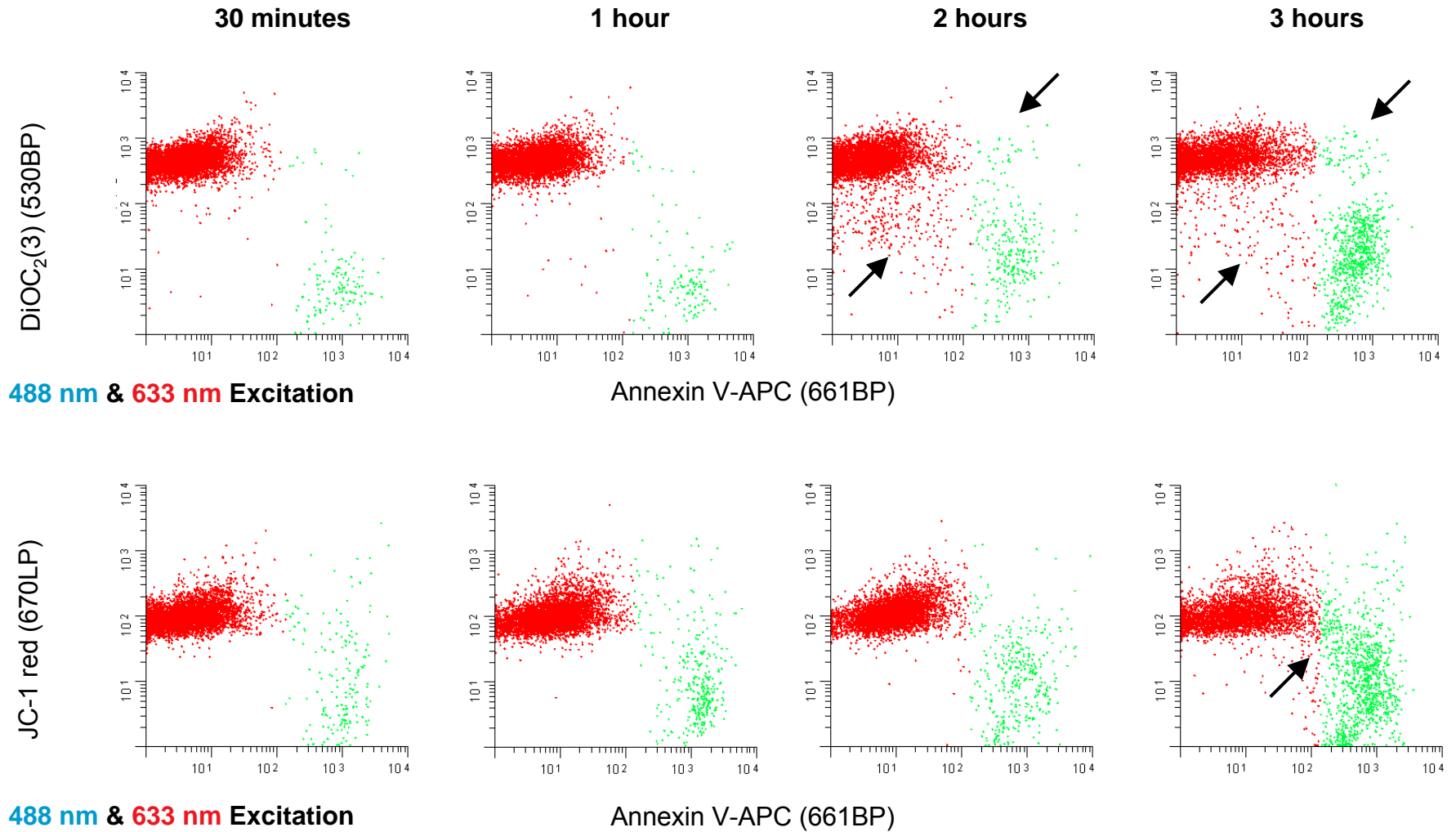
# Jurkat Cells: Time After Camptothecin Induction



# Jurkat Cells: Time After Camptothecin Induction



# Jurkat Cells: Time After Camptothecin Induction



- **Before annexin V binding**
    - DiIC<sub>1</sub>(5), DiOC<sub>2</sub>(3), MitoTracker Red CMXRos dye detect dim annexin V-negative populations
  - **Concurrent with annexin V binding**
    - DiIC<sub>1</sub>(5), DiOC<sub>2</sub>(3), MitoTracker Red CMXRos dye show bright and dim annexin V-positive populations
    - JC-1 & mitochondrial transition pore assays decreases as annexin V staining increases
    - Caspase activity increases after annexin V-positivity;
    - C<sub>12</sub>-resazurin activity decreases after annexin V-positivity.
  - **After loss of membrane integrity**
    - DiIC<sub>1</sub>(5) shows a dramatic crash after membrane integrity fails
    - Resazurin activity shows a further drop at least concurrently with the failure of membrane integrity
-

- **Mitochondrial activity appears to change in parallel with cytoplasmic and plasma membrane events, although not in “lock step”**
- **Reagents that signal changes in mitochondrial activity can detect early stress**
- **Reagent combinations can provide a window on intracellular processes not available with the much-used pairing of annexin V and propidium iodide**

## **Flow Cytometry Group**

- **Jolene Bradford**
- **Gayle Buller**
- **Bill Godfrey**

## **Cell Biology Group**

- **Mike Janes**
  - **Jerrod Salisbury**
-