Problem:

Conventional stains allow observation of only 1 structure:

- Where are the centromeres?
- Where are the telomeres?
- How are satellites/transposons distributed in the genome?
- Where are proteins/organelles located?

Solution: label different structures for simultaneous viewing!

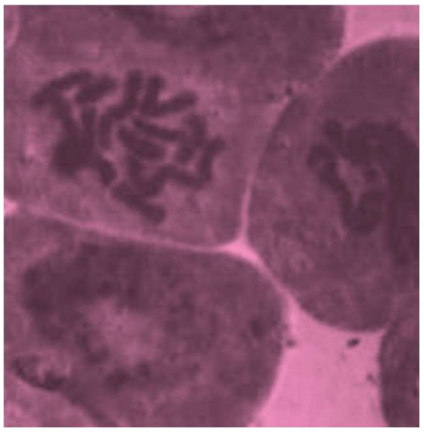
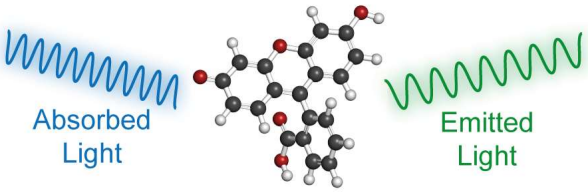
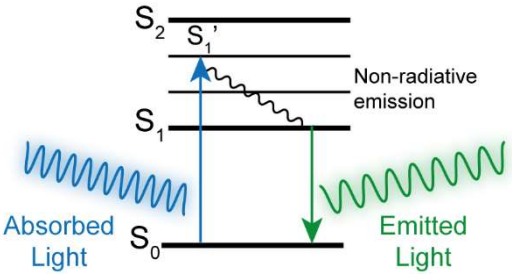


Figure 1. Former 8890 student: Mary Sledge, alfalfa

Stoke's law





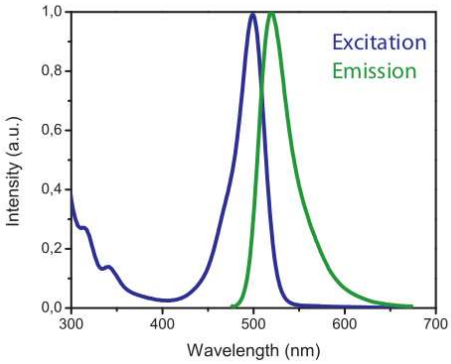


Figure 2. https://biotium.com/; Scientific Volume Imager

Fluorochromes & emission spectra

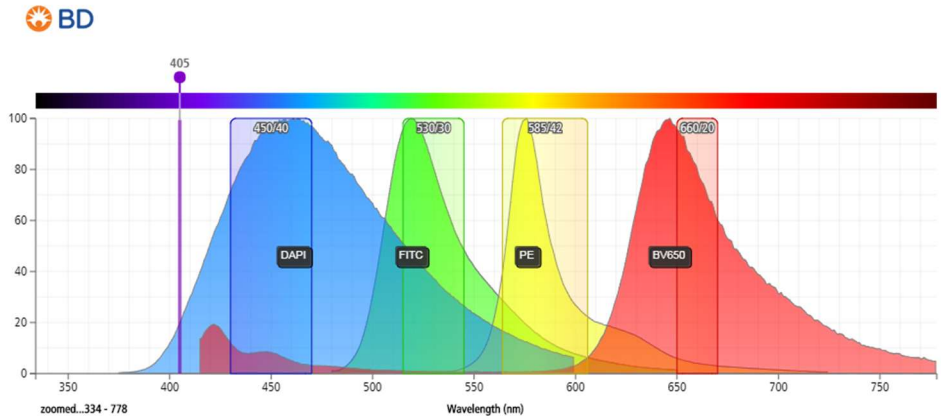
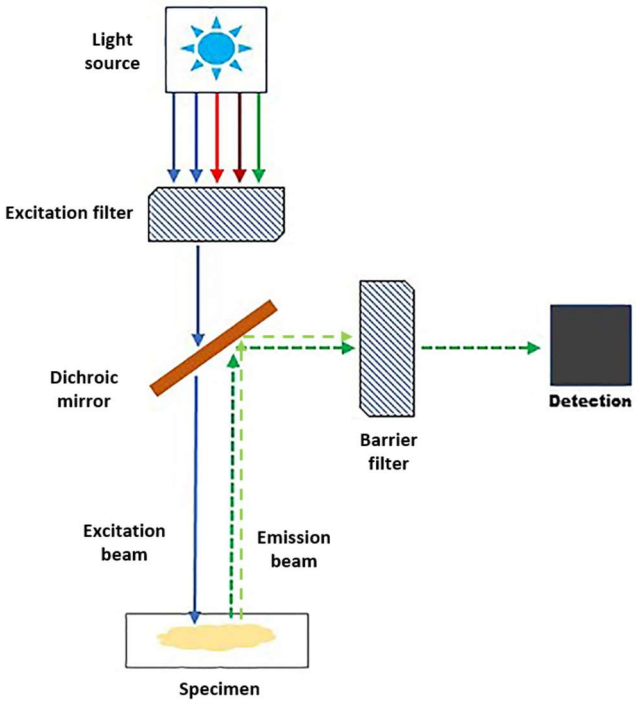


Figure 3. http://flowcytometry.utoronto.ca/applications/cell-viability/viabilty_unfixed/

Light path of a fluorescence microscope

- Band pass filter -
- Long/short pass filter –
- Excitation filter –
- Barrier filter –
- Dichroic mirror –



The filter cube

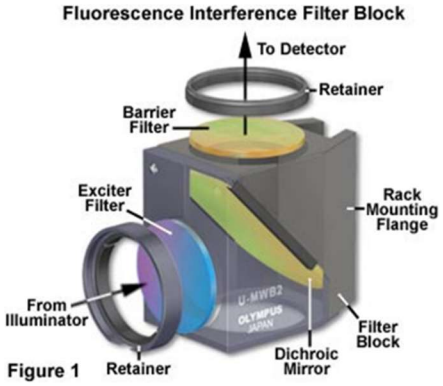


Figure 1

A typical fluorophore set allows simultaneous detection of four colors

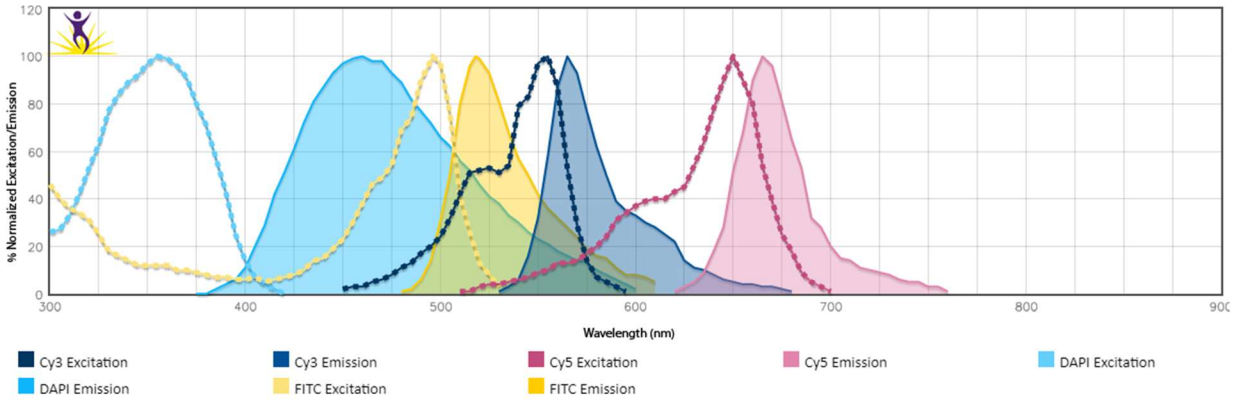


Figure 4. BioLegend

Fluorescence microscopy allows simultaneous viewing of different cellular features

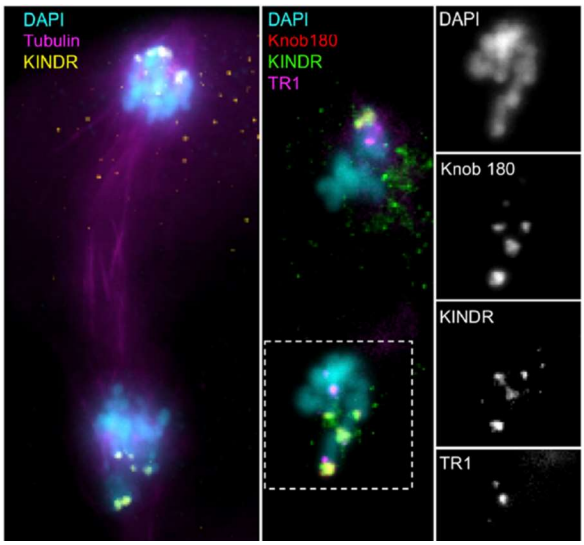


Figure 5. Dawe et al. (2018) Cell

What can be labeled with fluorochromes?

- Whole chromosomes (DAPI, PI, Hoechst, SYTO)
- DNA sequences (FISH probes, Oligo Paints, RGEN-ISL)
- Proteins (Immunofluorescence, GFP)
- Natural autofluorescent compounds (cellulose, lignin)
- Organelle/molecule-specific markers (many)

Example: FISH can be used for karyotyping

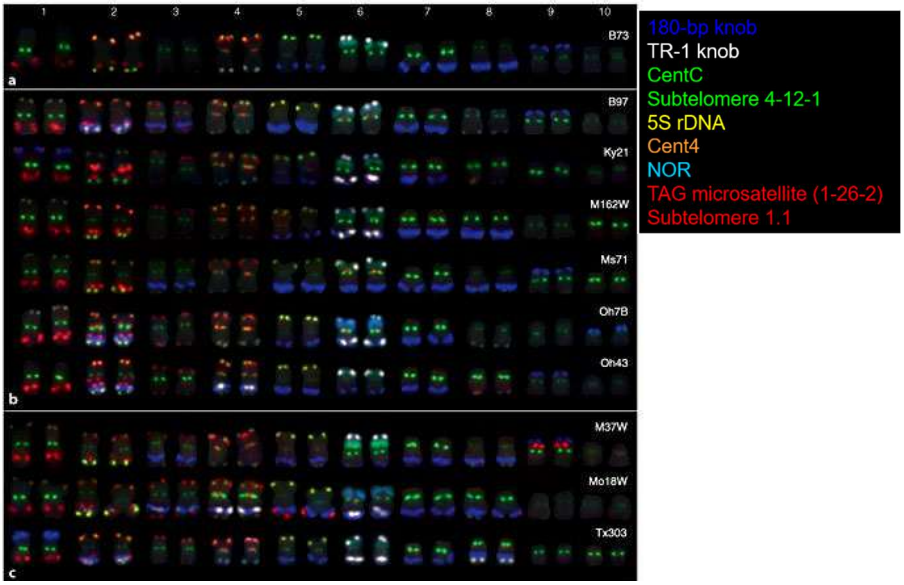


Figure 6. Albert et al. (2010) Cytogenetic and Genome Research

Example: Chromosome paints can label whole chromosomes

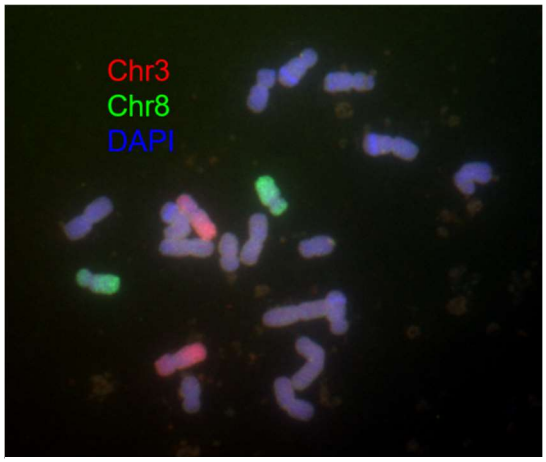


Figure 7. Kyle Swentowsky @ Birchler lab