# An introduction to Flow cytometry

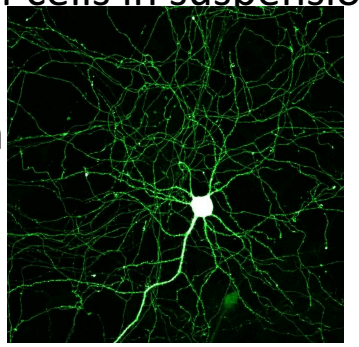
David K. Flaherty

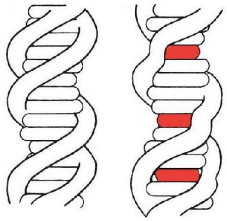
# The Goals of this course

- Gain an understanding of the functional components of flow cytometry
  - Basic Flow Cytometry
  - Fluorescence
  - Spectra viewers
  - Mirrors and Filters
  - Lasers
  - Fiber optic cables
  - Flow Cells
  - Photomultiplier Tubes (PMTs)
  - Example Experiments
  - Controls

# Fundamentals of flow cytometry

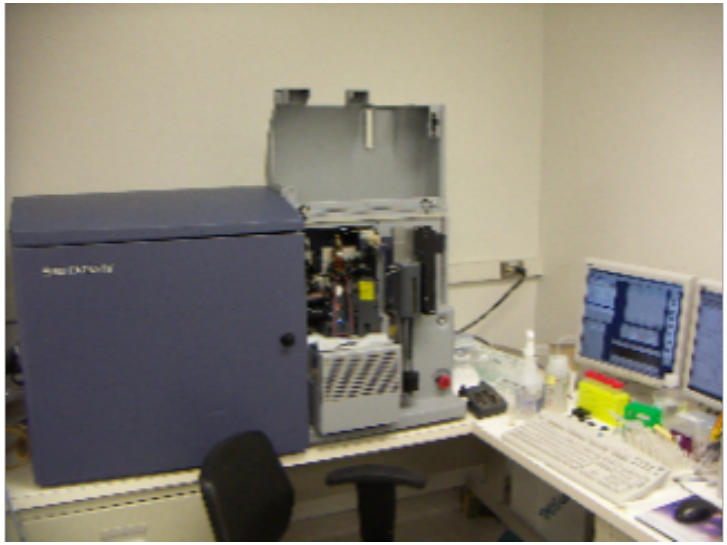
- What is Flow Cytometry?
  - Fluorescence based cellular analysis and / or sorting of cells in suspension
- The fluorescence can come from the following:
  - Antibodies with fluorescent conjugates or antibody pa
  - Endogenous fluorescent proteins (GFP)
  - Nuclear stains
  - qDots, nano particles, beads
  - Fluorescent dyes
- Why Use Flow Cytometry?
  - Measure Cell Cycle / proliferation
  - Measure Cell Death
  - Identify and differentiate various types of cells

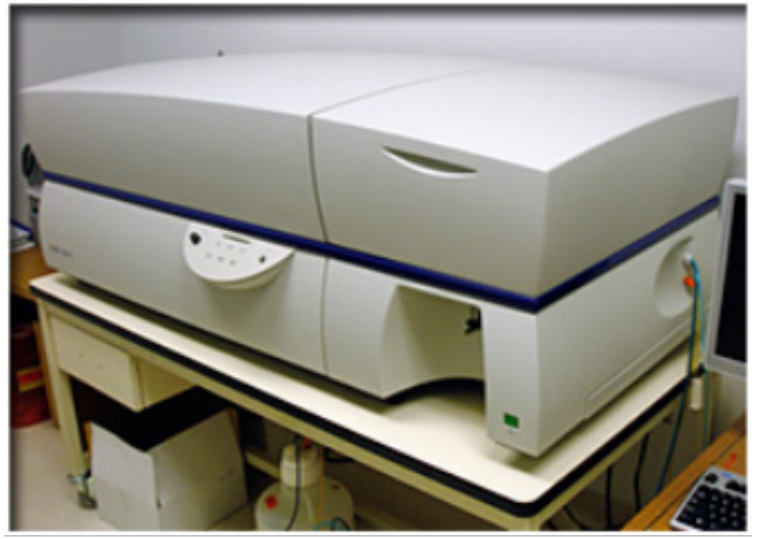


# Fundamentals of flow cytometry

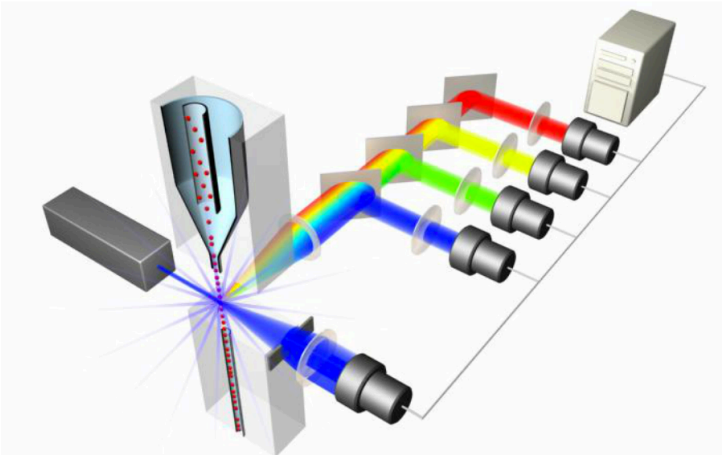
- Cytometers that we will demonstrate today:
  - Analyzers (LSRII)
  - Cell Sorters (FACSAria III)

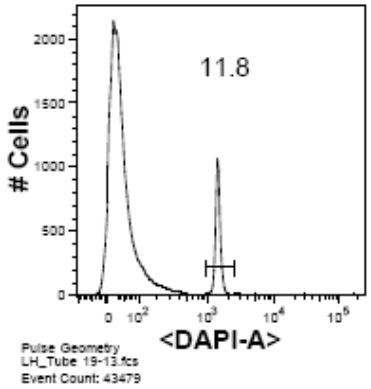


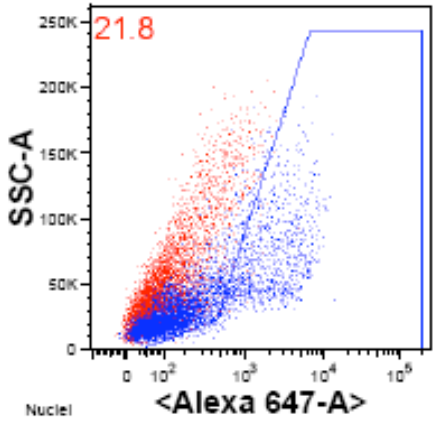


# Fundamentals of flow cytometry

- Basic concepts of Flow Cytometry
  - The instrument passes your sample through the path of a laser in a single cell manner
  - The laser excites the fluorescent agent on, or within, the cells
  - Photons are emitted from the fluorescent agents and captured by detectors

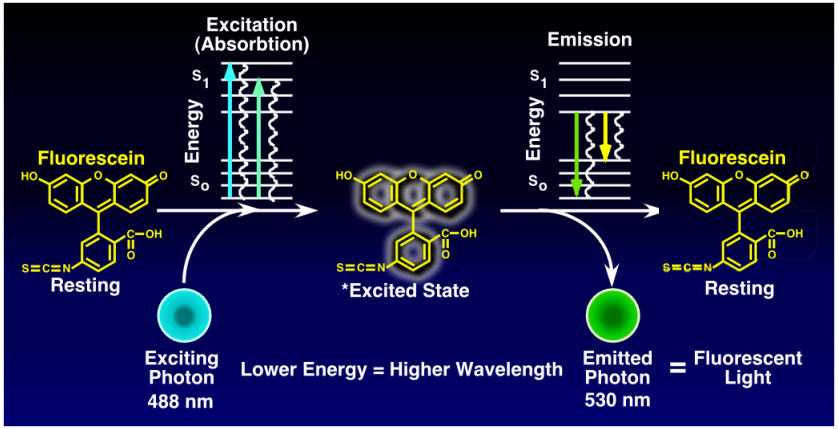




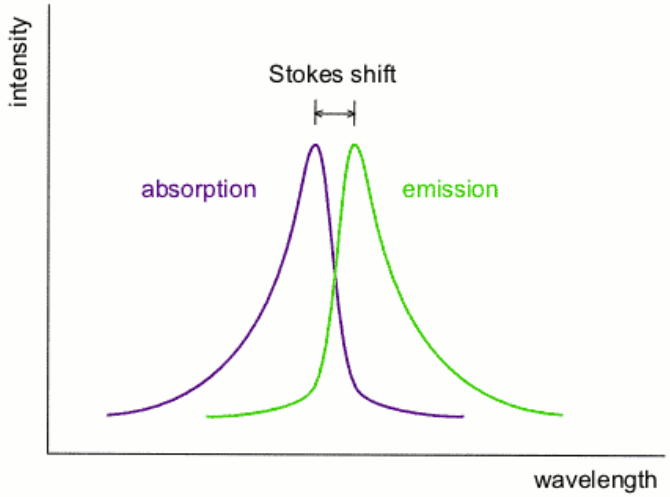
# Basic concepts of Fluorescence

- Fluorescence is a form of luminescence
- Fluorescence is dependent on the ability of a light source to excite a molecule to a higher energy state
- One example of a fluorophore (molecule with fluorescent potential) is Fluoresce



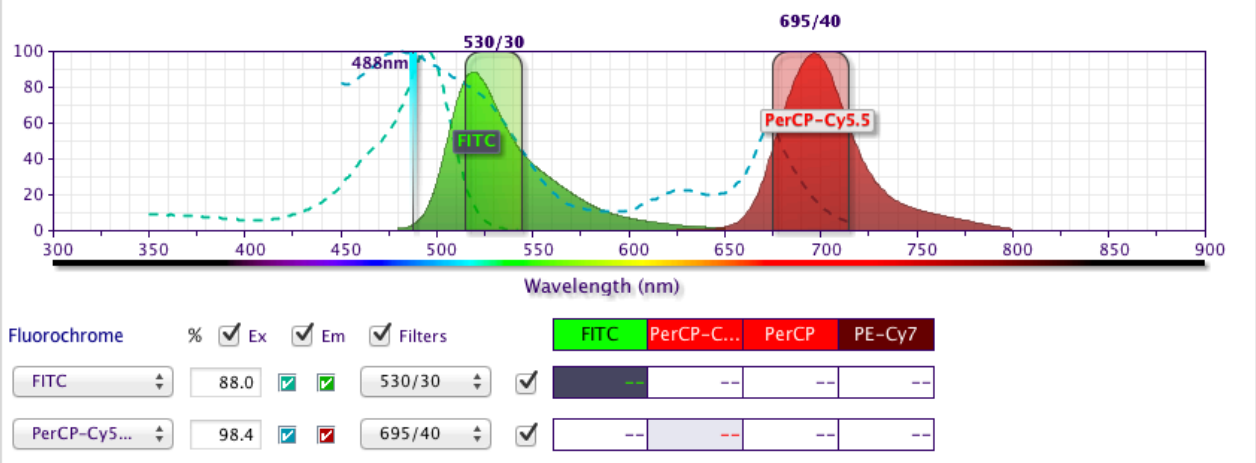
# Stokes shift

- Named after the Irish Physicist George Stokes
- Describes the difference in excitation wavelengths of the energy source (laser) and emission wavelengths of the photon (fluorescence)
- The longer wavelength of the emitted photon is due to the inefficient transfer of energy



# Fluorescent spectrum viewers

- Spectrum viewers are a valuable tool when planning an experiment
- Excitation and emission information can be obtained
- This is an example from the BD biosciences website:
- http://www.bdbiosciences.com/research/multicolor/spectrum_viewer/index.jsp



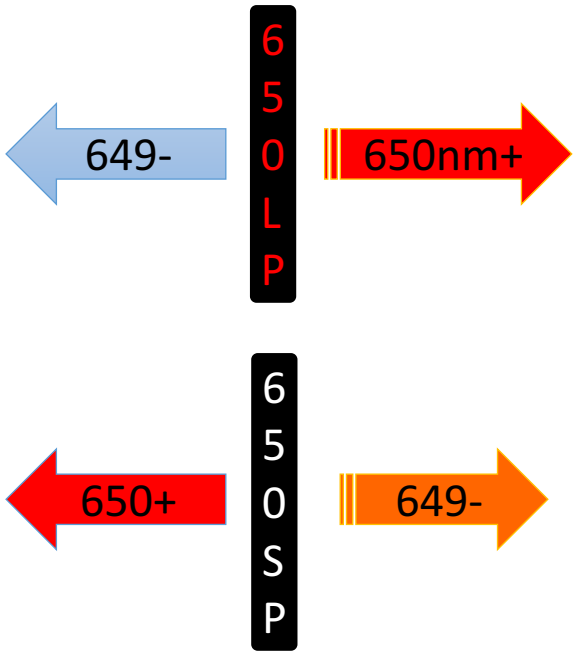
# Long pass and short pass mirrors

- These mirrors are used in various places throughout the cytometer
- They are used to direct the lasers to the interrogation point
- They are used to separate various fluorescent signals from a single cell

# Long pass and short pass Dichroic filters (mirrors)

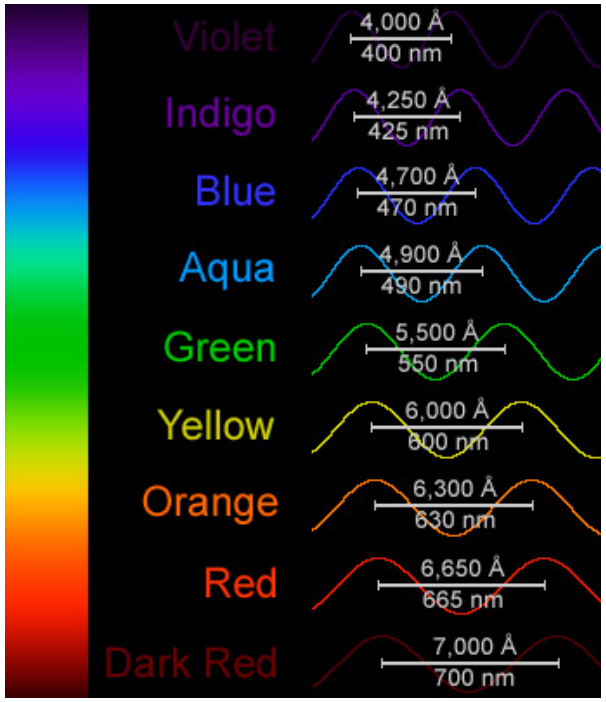
- Long pass mirrors are laminated with a proprietary coating that only allows light longer than a certain wavelength through them, reflecting all others

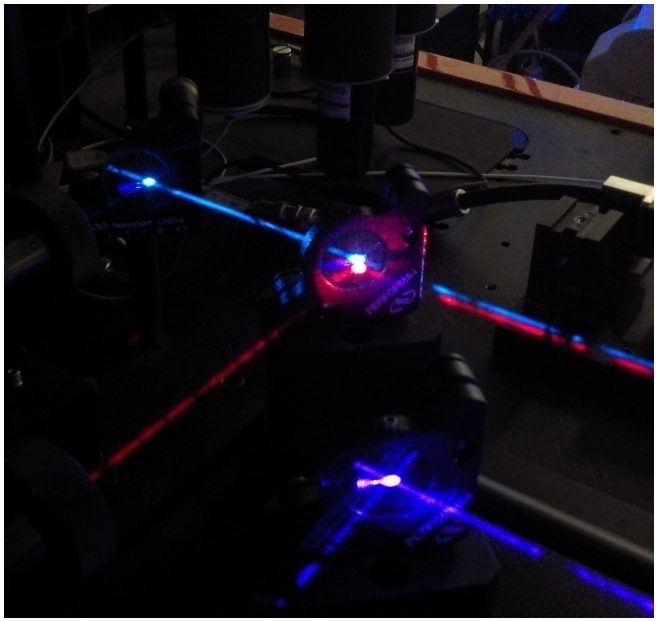
- Short pass mirrors allow light shorter than a certain wavelength to pass through them reflecting all wavelengths that are longer



# WHAT TYPE OF MIRROR IS THIS?

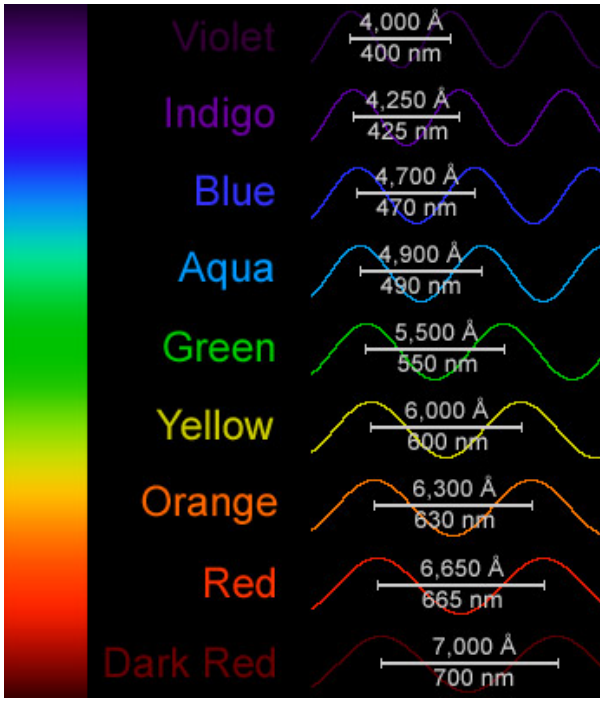
- Is this an example of a long pass or short pass mirror ?



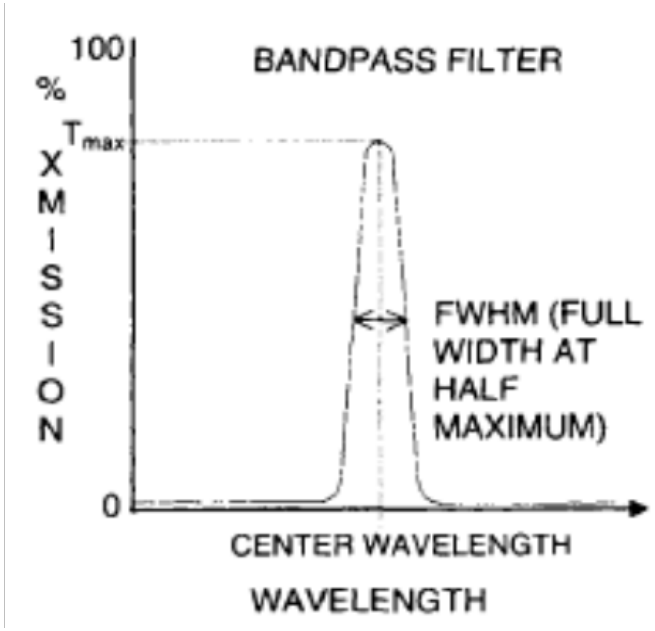
# Long pass mirrors (photon separation)

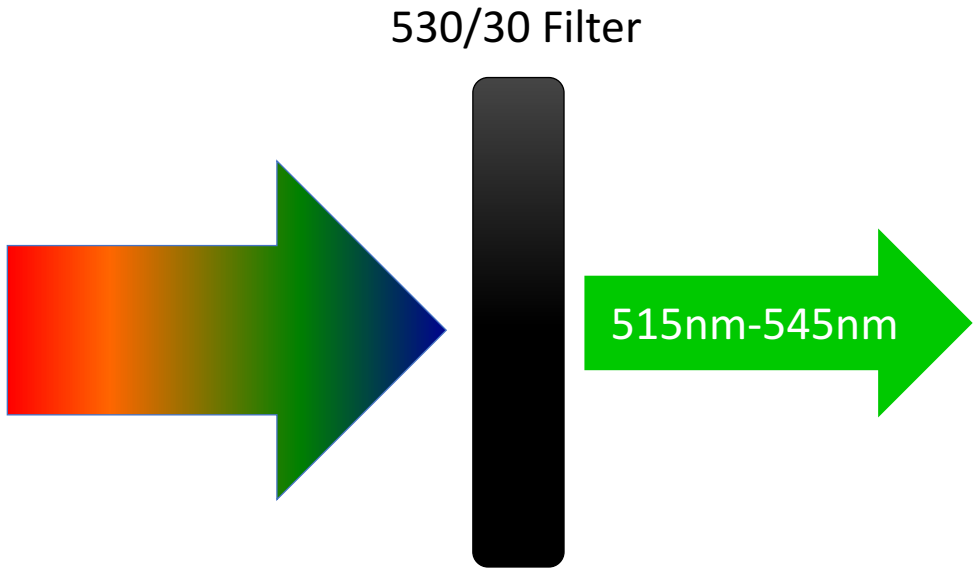
- Example of a long pass mirror used to separate fluorescent photons into the detectors




# Bandpass filters

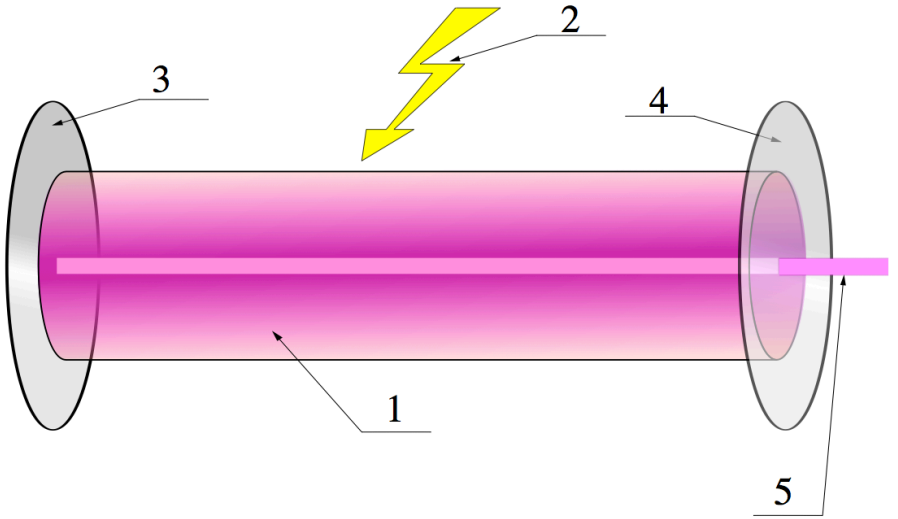
- These filters are absorption filters that only allow a certain bandwidth of light to pass through them



# Lasers

- Light Amplification by Stimulated Emission of Radiation
- 1) Gain Medium
  - Argon ion Gas
  - HeNe
- 2) Energy source
  - Light or electricity
- 3) Reflectors
- 4)Output Coupler
  - Semi-transparent / reflective
- 5) Laser Beam



# Path of the lasers

- Lasers can be aligned in in instrument to be collinear (BD FACSCaliber)
  - Collinear laser alignment can cause a bunch of problems with compensation and multi-color assays
  - No distinction can be made between the excitation wavelengths of various fluorphores
- They can also be aligned in parallel (BD LSR…)
  - Allowing the instrument to differentiate between different excitation wavelengths as well as emission wavelengths