Microscopy I
Fluorescence
Confocal
Multiphoton
Flow Cytometry

By
Luis Filgueira

Fluorescence
Three-stage process

1. Excitation
Photon of energy
\( h_{\text{EX}} \)

2. Excitation-state
Lifetime
(1-10 \times 10^{-9} \text{ seconds})

3. Emission
Photon of energy
\( h_{\text{EM}} \)

Difference in energy
\( = h_{\text{EX}} - h_{\text{EM}} \)
Difference in wave length

Fluorophore = fluorescent dye

polyaromatic hydrocarbons
heterocycles
Fluorophore = fluorescent dye

Binding specifically to biochemical structures
- Protein
- DNA
- RNA
- Lipids
- Ca
- K
- H+

Fluorophore = fluorescent dye

Fluorescence labelled macromolecules
- Antibodies
- Lipids
- Nucleotides
- Oligo-DNA
- Oligo-RNA
- Peptides
- Lectines
- Phalloidin

Fluorophore = fluorescent dye

Detection of Organelles
- Nucleus
- Golgi
- Lysosomes
- RER
- Mitochondria
- Cytoskeleton
- Membranes

Fluorophore = fluorescent dye

Enzymes and Enzyme Substrates and Cell Function
- Glycosidases
- Phosphatases
- Proteases/Peptidases
- Peroxidases
- Lipases
- Membrane potential

Applications for fluorescent dyes
- Microscopy
- Flow Cytometry
- Spectrofluorometric Assays
- Molecular Biology

Molecular Biology
- Gel-Electrophoresis
- Real time/quantitative RT-PCR
- DNA
- RNA
- Protein
Spectrofluorometric Assays
- Cell Viability
- Cytotoxicity
- Enzymatic Assays
- Ca+ Measurement
- Protein quantification

Flow Cytometry
- Quantification of Surface marker expression
- Intracellular Protein expression
- Beads assays
- Cell Cycle Analysis
- Cell Viability
- Cytotoxicity
- Protein quantification

Microscopes
- Axial
- Invert

Fluorescence Microscopy
- Mercury Vapor lamp = Arc Lamp

Confocal Microscopy
- Ar UV 351, 364nm
- HeCd 442nm
- Ar 457nm, 488nm, 514nm
- ArKr 488nm
- Kr 568nm
- HeNe 633nm
Axial optical sectioning of the object is possible in two ways. Either by:
- direct acquisition of x/z- or y/z-optical cross sections, or
- calculation of vertical optical sections using the normal x/y-image stack (as shown here).

Rotation and volume rendering:
Rotation of the image stack and projection into one plane allows viewing the object from different angles.

Stereoscopic imaging:
The combination of a left and a right image either as:
- stereo pairs or
- red/green anaglyphs
allows easy visualisation of 3D-structures!
Use red/green glasses for stereoscopic viewing of this red/green anaglyph.