The CNR Biological Imaging Facility

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Fluorescence Microscopy
The visible spectrum spans from 400-700nm
Epifluorescence Microscopy

**Diagram: Epifluorescence Microscopy**

- **Arc light source**
- **Excitation filter**
- **Dichroic mirror**
- **Objective**
- **Barrier filter**
- **Specimen**

**Light Path:**
1. Arc light source
2. Excitation filter
3. Dichroic mirror
4. Objective
5. Fluorescent light from sample
Confocal Laser Scanning Microscope

- Laser
- Dichroic Beam Splitter
- Scanner
- Objective
- Lens
- Pinhole
- Detector 1
- Detector 2
- Z-Focus Motor
Deconvolution Microscopy

Spreading of light from 0.1µm fluorescent beads
Bacillus subtilus as Imaged Using Deconvolution Microscopy

DAPI and FtSZ localization
Fluorophores

Figure 1: Common Fluorophores in Widefield and Confocal Microscopy

(a) fluorescein
(b) DAPI
(c) Texas Red

Figure 2: Spectral Profiles of Popular Traditional Fluorophores

Absorption and Emission Spectra for DAPI, FITC, Texas Red.
Fluorescence Emission Properties

DAPI

Syto 11
Fluorescence filter sets

“Rhodamine Long Pass set”

“Fluorescein Band Pass set”
Fluorescence Filter Arrangement

Fluorescence Microscope Illumination System

The Application of Fluorescence Microscopy in Biology

Fluorescence probing

Gene localization

DNA sequencing
Fluorescence of Applied Dyes

Hemp stem transverse section

Brightfield

Fluorescence
Confocal microscopy of intact maize ear tissue reveals the organization of the embryo sac.

Fluorescence Resulting from Chemical Reactions

Fluorescent Schiff’s Reagent stains Carbohydrates and Chromatin

Confocal microscopy of intact maize ear tissue reveals the organization of the embryo sac.
Probing with Fluorescent Molecules: DAPI targets DNA

Maize pachytene chromosomes

Fluorescence labeling of interphase cells

Fluorescence emission
Absorbance

Wavelength (nm)

300 350 400 450 500 550 600 650
Mitotracker Red targets mitochondria
Fluorescent Metabolic Indicators

Fluorescein diacetate tests for viability

Onion epidermal cells
Fluorescent Dyes to Determine Cell Morphology

Lucifer Yellow Microinjected into a Neuron
Fluorescent Dyes to Determine Tissue Morphology

Identifying new osteoblasts in scintillated bone

DAPI

Eosin
Targeting with Multiple Fluorescent Probes

DAPI, Hoechst 33258

Rhodamine phalloidin

Fluorescinated Ab
Drosophila Ovary as Imaged by Confocal Microscopy

Nuclei localize ovule and nurse cells
GFP: Green Fluorescent Protein
GFP transformed *Erwinia* bacteria
**Targeting DNA Sequences: Fluorescence in situ Hybridization**

**Molecular Biology**
- Subclone gene sequence into Bluescript or an equivalent plasmid
- Linearize DNA template
- Do in vitro transcription using labeled UTP
- Hydrolyze RNA probe to the appropriate size
  - i. Check size using formaldehyde gel
  - ii. Check concentration using dot-blot

**Tissue Preparation**
- Fix tissue in paraformaldehyde
- Dehydrate tissues in EtOH
- Infiltrate tissues with paraffin
- Embed tissue samples
- Section material and mount on treated glass slide

Proceed with *in situ* hybridization
- Prehybridize tissues
- Heat denature probe
- Hybridize RNA probe to DNA/RNA sequence in tissues
- Detect RNA probe
  - Use anti-Dig to visualize sequences
  - Detect $^{35}$S with NTB photoemulsion
12 kb gene probe hybridizes to 4 pachytene strands
3D Image Processing and Analysis

Yeast Golgi
The Golgi Apparatus

Localization of Golgi and trafficking molecules
The Mechanics of Epifluorescence Microscopy

- HBO Lamp
- Fluorescence light stop
- Fluorescence “slider”
Zeiss 3FL slider

Endow GFP set

3x Bandpass set

DsRed set
Zeiss Axiophot slider positions

Blue Ex ("fluorescein set")

Green Ex ("Rhodamine set")