Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

Consortium leader

PETER PAZMANY CATHOLIC UNIVERSITY

Consortium members

SEMMELWEIS UNIVERSITY, DIALOG CAMPUS PUBLISHER

The Project has been realised with the support of the European Union and has been co-financed by the European Social Fund ***

**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

***A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

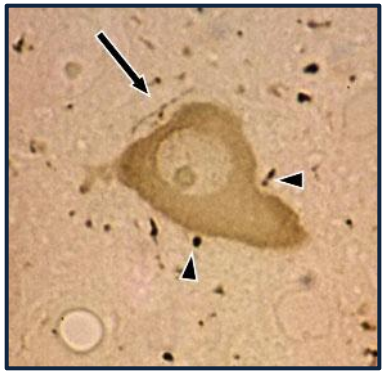
Neurobiológia alapjai - Módszerek

BASICS OF NEUROBIOLOGY - Methods

By Imre Kalló

Methods in Neurobiology I.
Histology techniques: light microscopic studies

Imre Kalló
Pázmány Péter Catholic University, Faculty of Information Technology



I. Histology techniques: light microscopic studies
II. Applications using fluorescent dyes
III. Histology techniques: electron microscopic studies
IV. Techniques to map neuronal connections
V. Molecular biological techniques
VI. Living experimental models
VII. Electrophysiological approaches
VIII. Behavioral studies
IX. Dissection, virtual dissection, imaging techniques

Techniques based on invivo or post mortem sampling - HISTOLOGY

I. PRESERVATION OF THE SAMPLE

1. Aim of this procedure
    a. Advantages
    b. Disadvantages
2. Preservation techniques
    a. Heat, microwaves
    b. Chemical preservation (e.g. 4% PFA, 0.1-10% GA, Acrolein etc.)
        - Perfusion of the fixation solution
        - Immersion of sample in the fixation solution

II. CUTTING SECTIONS

1. Aim of this procedure
2. Prerequisites of precise cutting
   - Embedding
   - Sharp knives
3. Cutting tools

Scalpel, razor blade – free hand cutting		200-500 μm
Microtome		5- 20 μm
Freezing microtome		10- 50 μm
Vibratome, vibroslicer		20-300 μm
Cryostat		10-300 μm
Ultratome	0.5-2 μm	40- 80 nm

III. STAINING TECHNIQUES (in order to localise and identify tissue components, cells, cellular organelles or molecules)

Histochemistry:

Dyes – binding to molecules according to their physico-chemical properties

- Proteins
- Lipids
- Carbohydrites
- Nucleic acides

Enzim histochemistry - demonstration of endogenous enzymes

- Acidic and alkaline phosphatases
- Dehidrogenases etc.

Autoradiography

Binding or incorporation of radioactively labelled ligands, molecules

Immunohistochemistry – detection of molecules with antigenic properties

direct labelling

indirect labelling

enzyme - labels

fluorescent - labels

intensification techniques

In situ hybridization histochemistry - detection of DNA, heteronuclear and messenger RNA

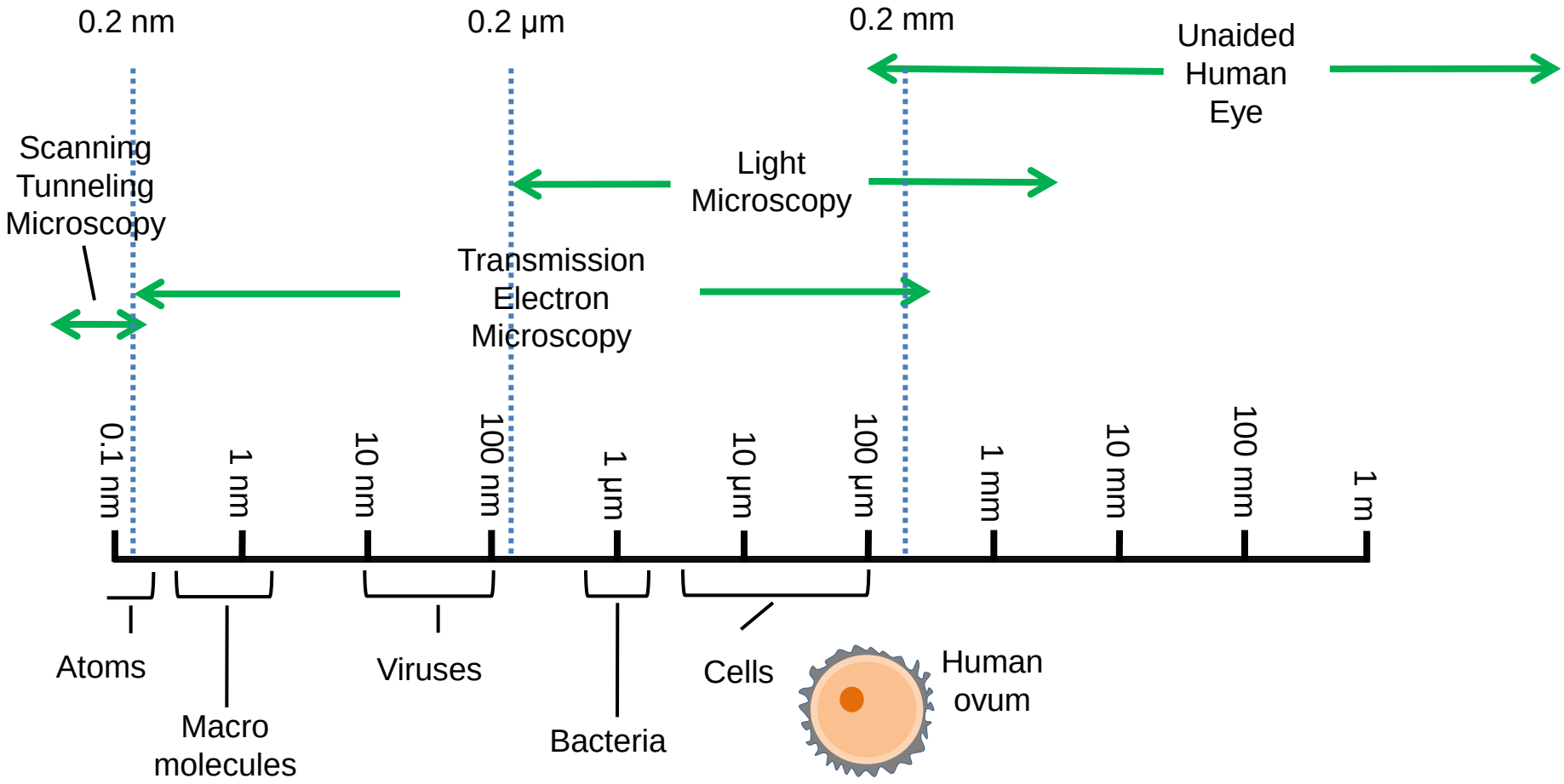
riboprobes

oligoprobes

radioactively labelled probes

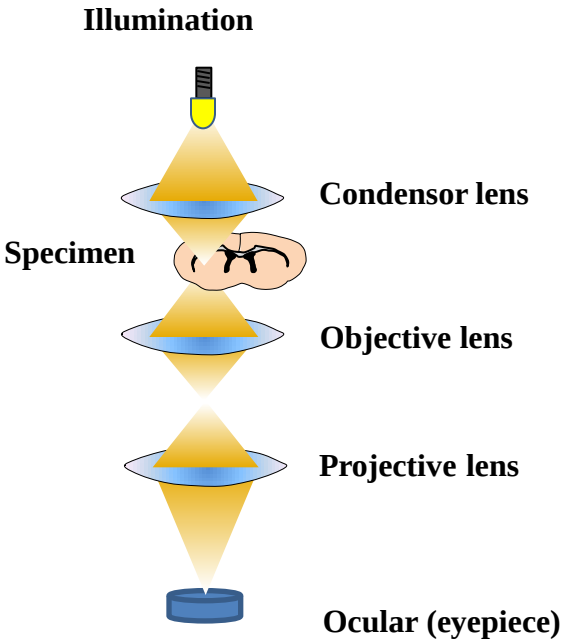
non-radioactively labelled probes

IV. OBSERVATION POSSIBILITIES



LIGHT MICROSCOPE

Principle



XVII. century

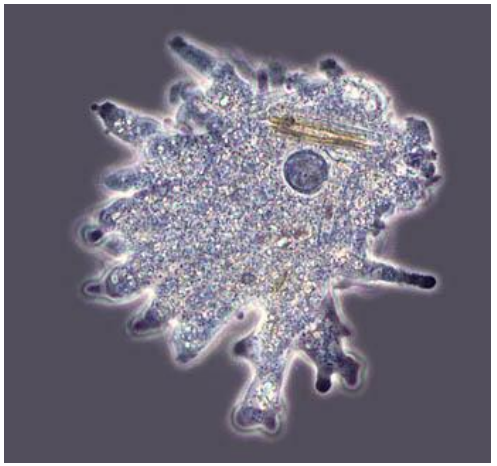
XXI. century

Current microscopes use **multiple, variously filtered and targeted illuminations, corrected lens systems, automated, motorized units, digital recording techniques, computerized image processing and analyzing support.**

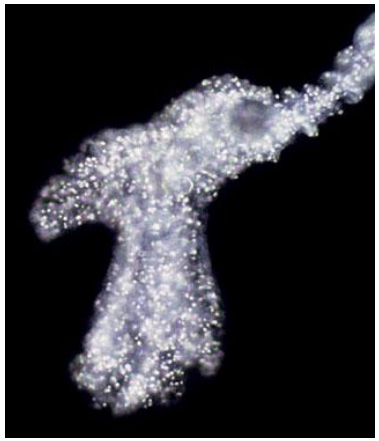
NATIVE PREPARATIONS ARE SUFFICIENTLY VISIBLE AT SPECIAL ILLUMINATON AND CONTRAST MODES

Bright field

Phase contrast

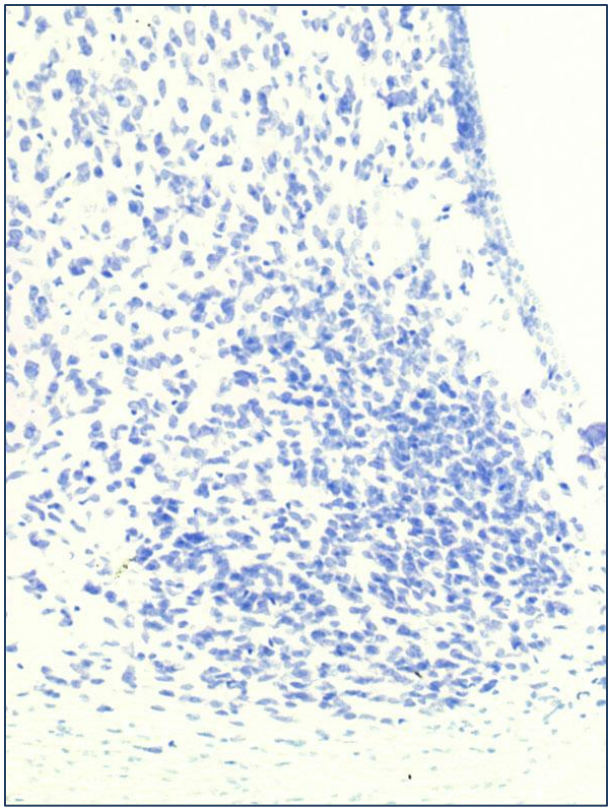


Dark field

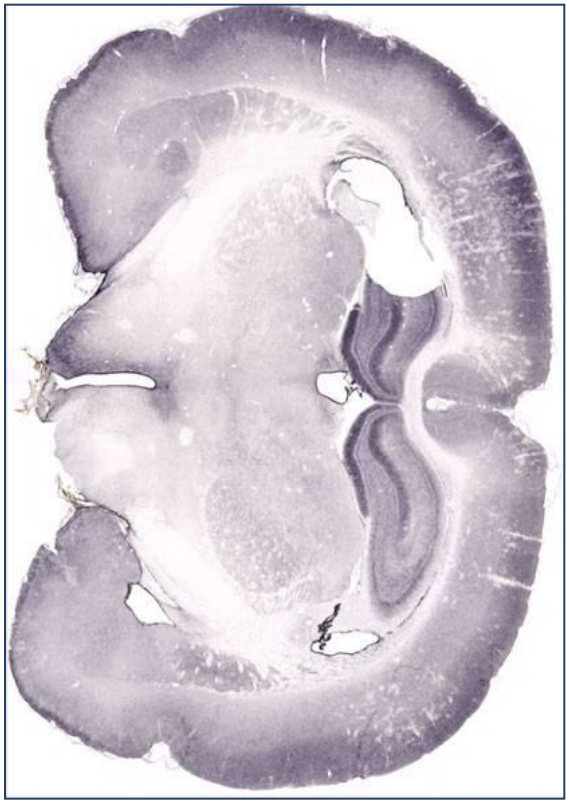


Differential interference contrast

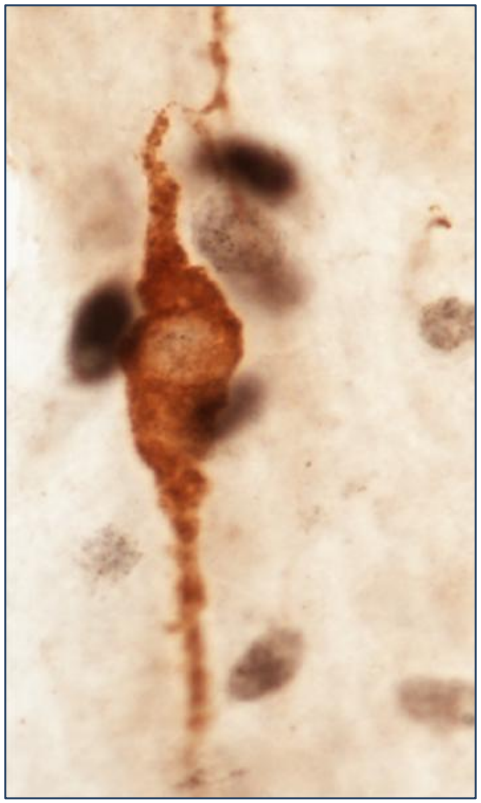
COLOURED PREPARATIONS AT BRIGHT FIELD CONDITIONS



Toluidin blue

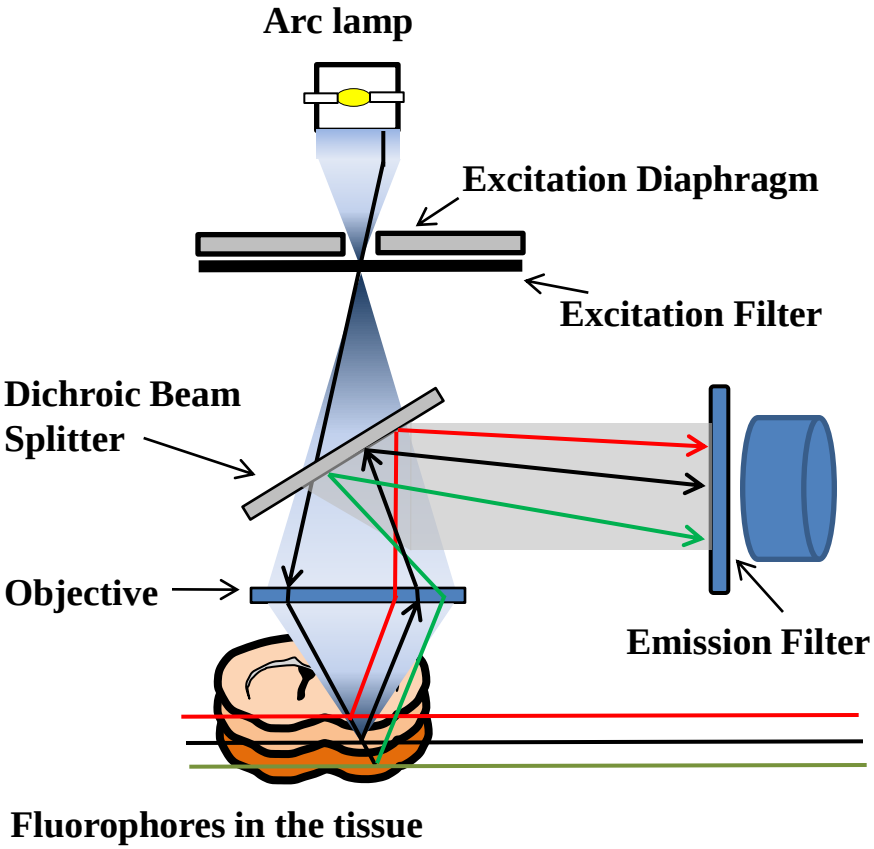


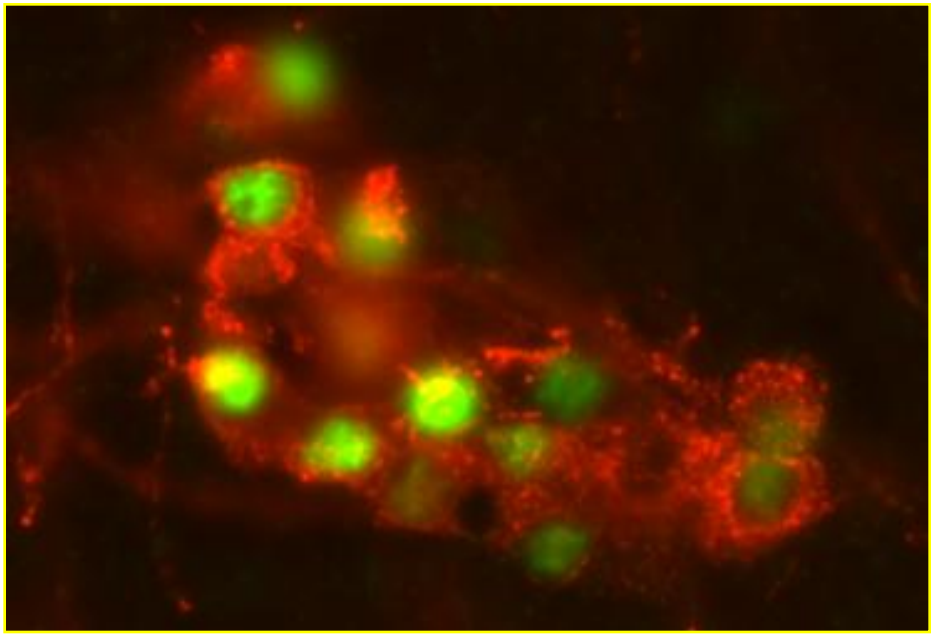
Nickel (Ni) enhanced DAB



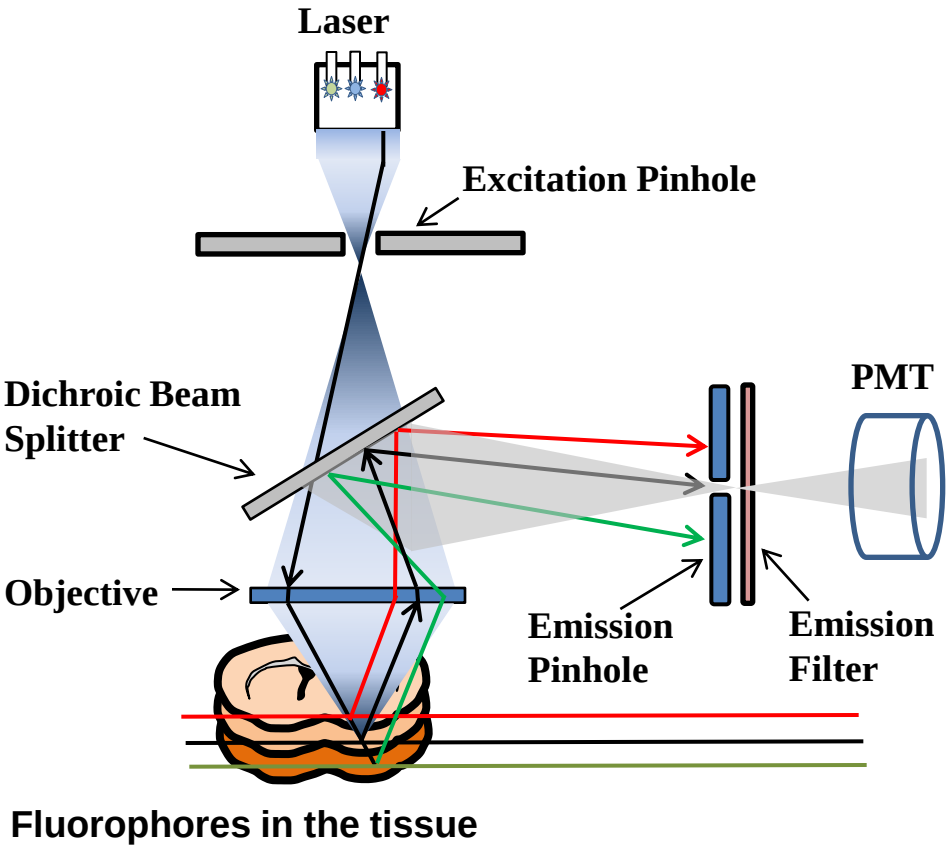
Ni-DAB/DAB

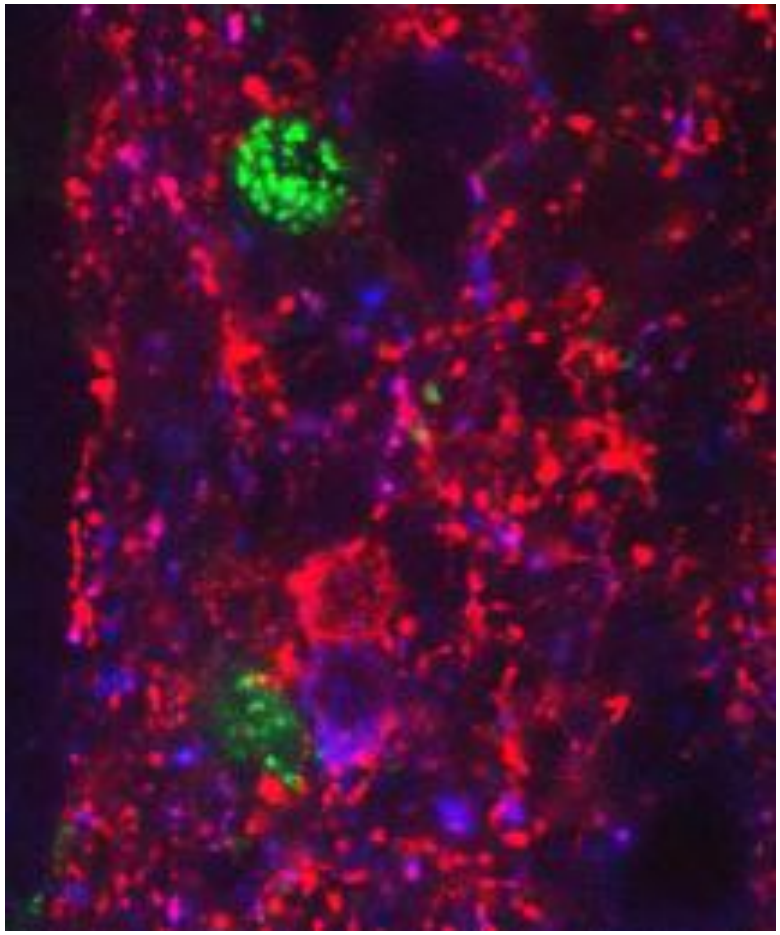
FLUORESCENCE MICROSCOPY



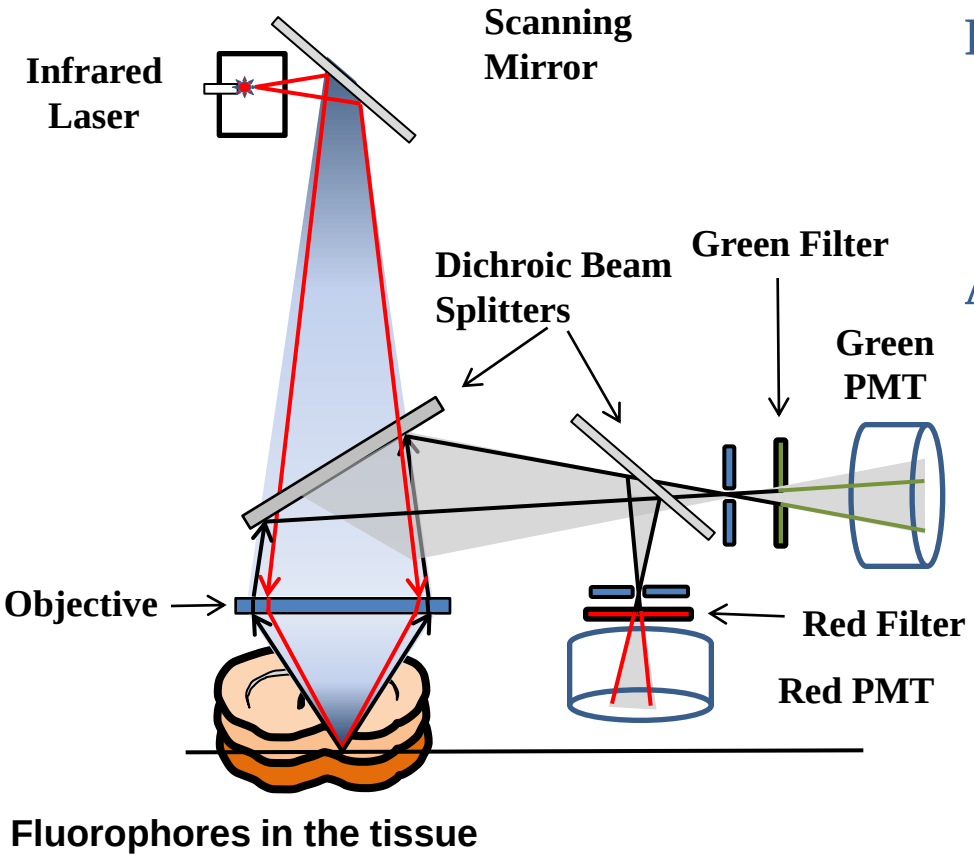


CONFOCAL LASER MICROSCOPY





TWO-PHOTON MICROSCOPY



Principle :

Dyes in the tissue absorb two, small energy (infrared) photons almost concurrently.

Advantages:

- no fading
- no phototoxicity
- increased focal depth