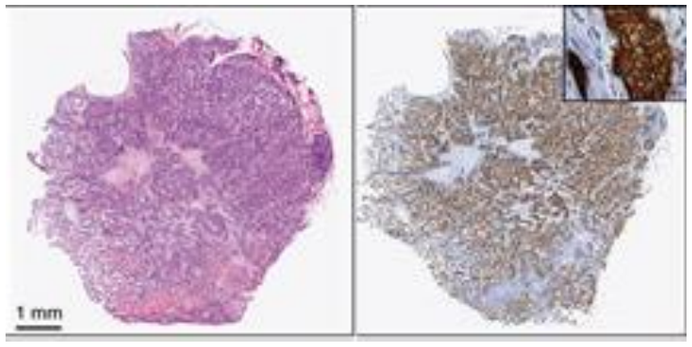
Bioimaging

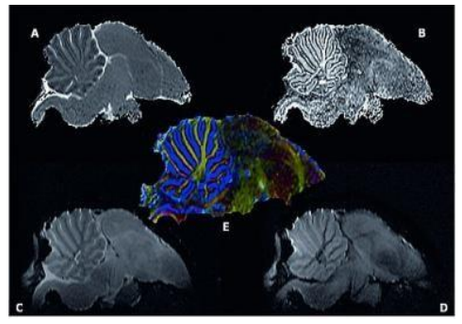
What is Imaging?

Imaging involves the visualisation of sample information in different ways

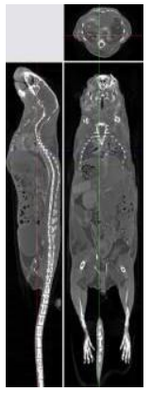
H&E **IHC**



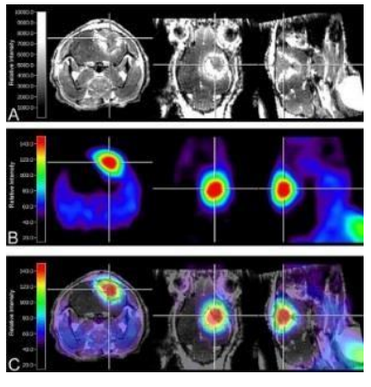
Nuclear Magnetic Resonance Imaging (MRI/MRS)



Nuclear computed tomography (CT)

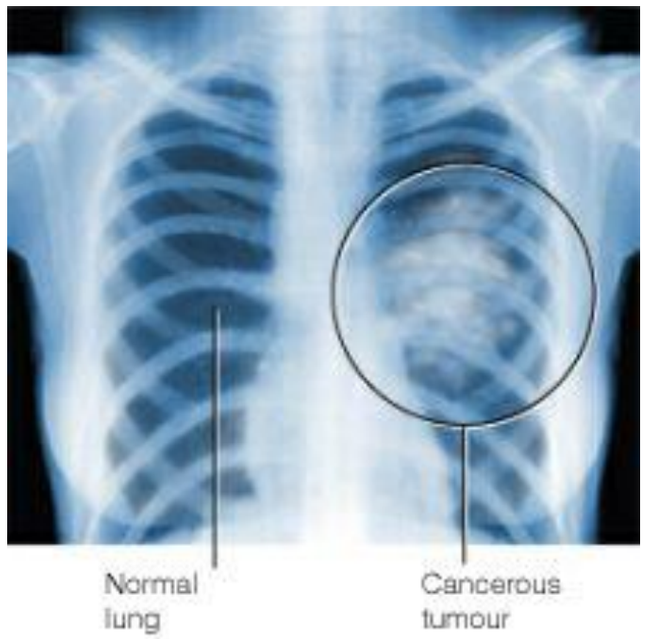


Positron Emission tomography (PET)`



Imaging Techniques

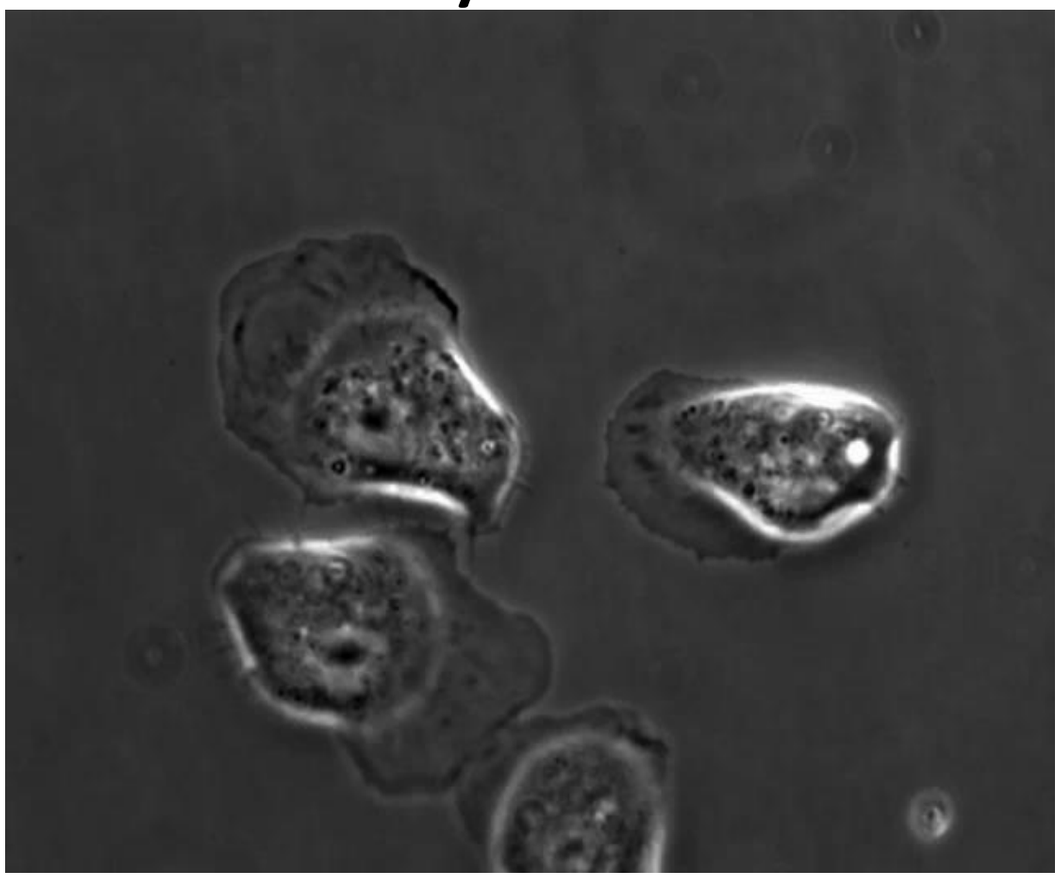
- Conventional Techniques:
  - X-ray, MRI, Fluoroscopy
  - CAT scan
- Limitations
  - Limited detail
  - Difficult to track movement



Taken from: http://www.besttreatments.co.uk/btuk/images/lung_cancer_xray.jpg

1. Imaging Cells in Culture

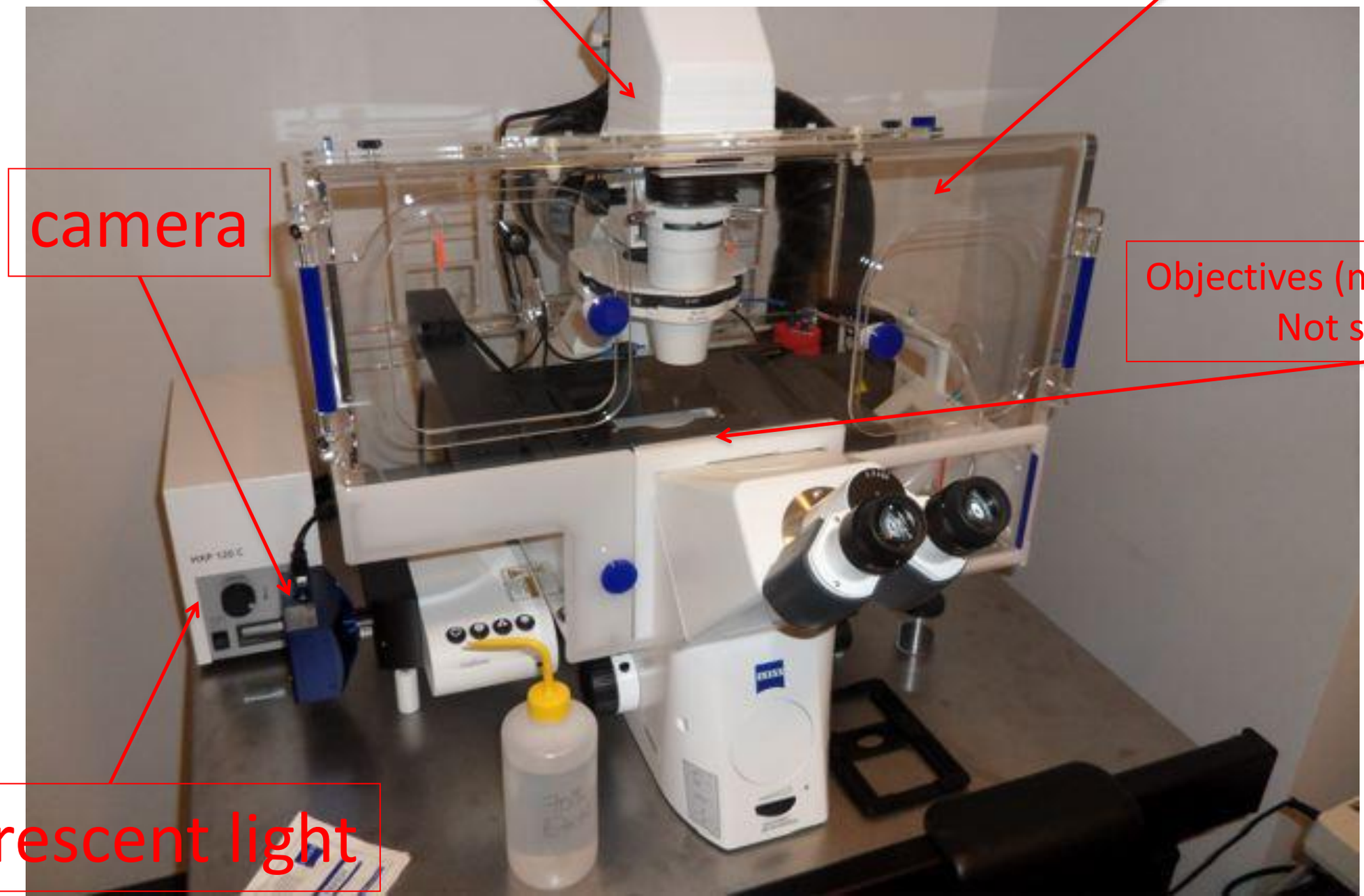
Rat mammary carcinoma cells



10 min, images every 20 seconds

Michele Balsamo, Gertler lab MIT

Quantifying Cell Migration: Live Microscopy



Magnification of signal

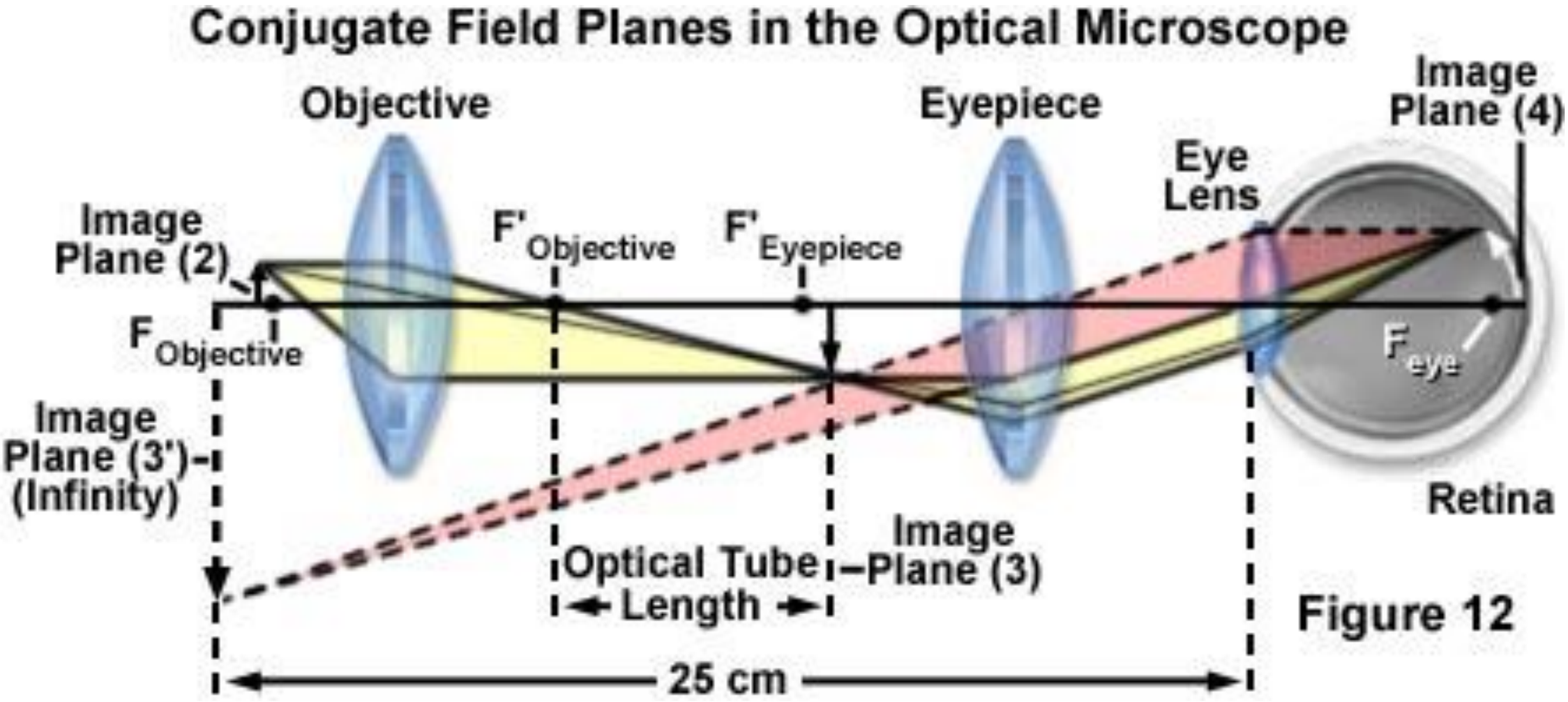
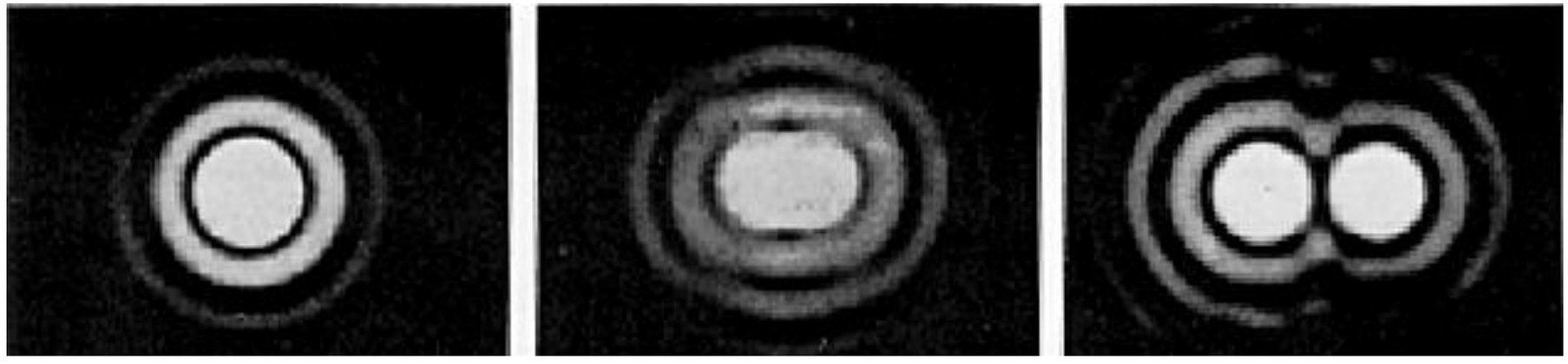
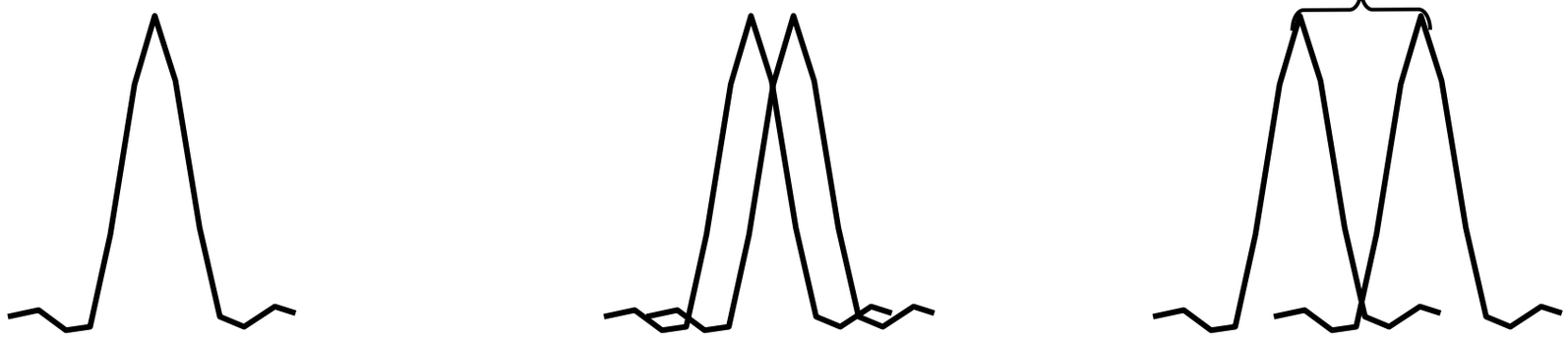


Figure 12

Resolution: a measure of how close two point images can come such that they are perceived as separate





Lord Rayleigh's criterion:

$$\delta^R = 0.61\frac{\lambda}{\mathrm{NA}}$$

The practical limit for \theta is about 70°. In an air objective or condenser, this gives a maximum NA of 0.95. In a high-resolution oil immersion lens, the maximum NA is typically 1.45, when using immersion oil with a refractive index of 1.52. Due to these limitations, the resolution limit of a light microscope using visible light is about 200 nm.

Link between resolution and pixel size: Magnification

$$p_x \leq \frac{\lambda \cdot M}{4NA}$$

Defined by camera

Defined by objective

$p_x < 8.9$ um

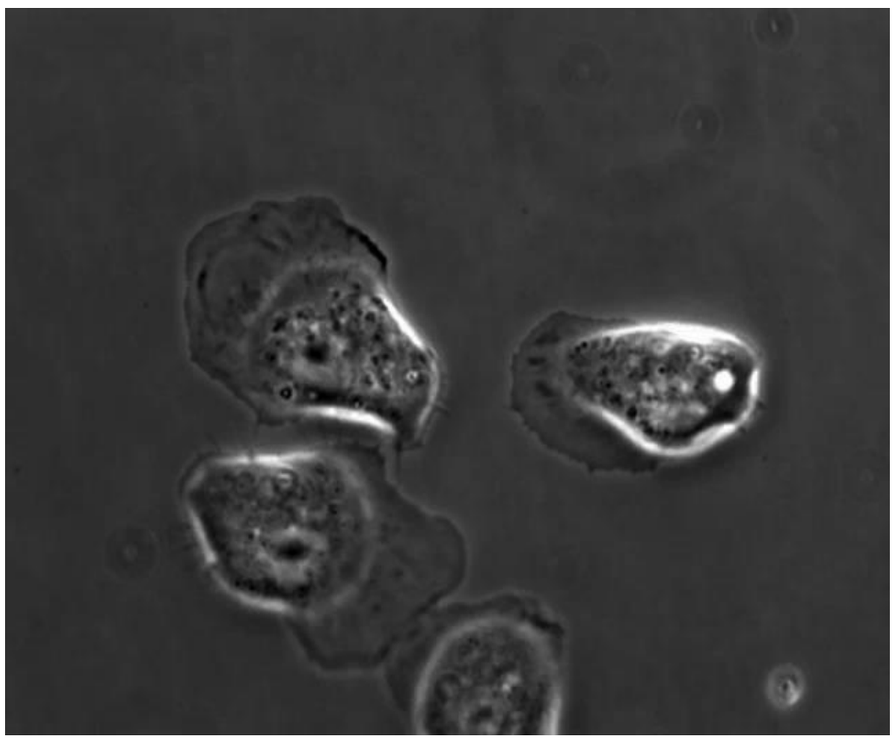
Interline transfer CCD	EM-CCD	sCMOS
6.4 um	12.4 um	6.5 um

Not an easy decision: decreasing pixel size means increasing $$

Pros and Cons of Standard LMs

Pros

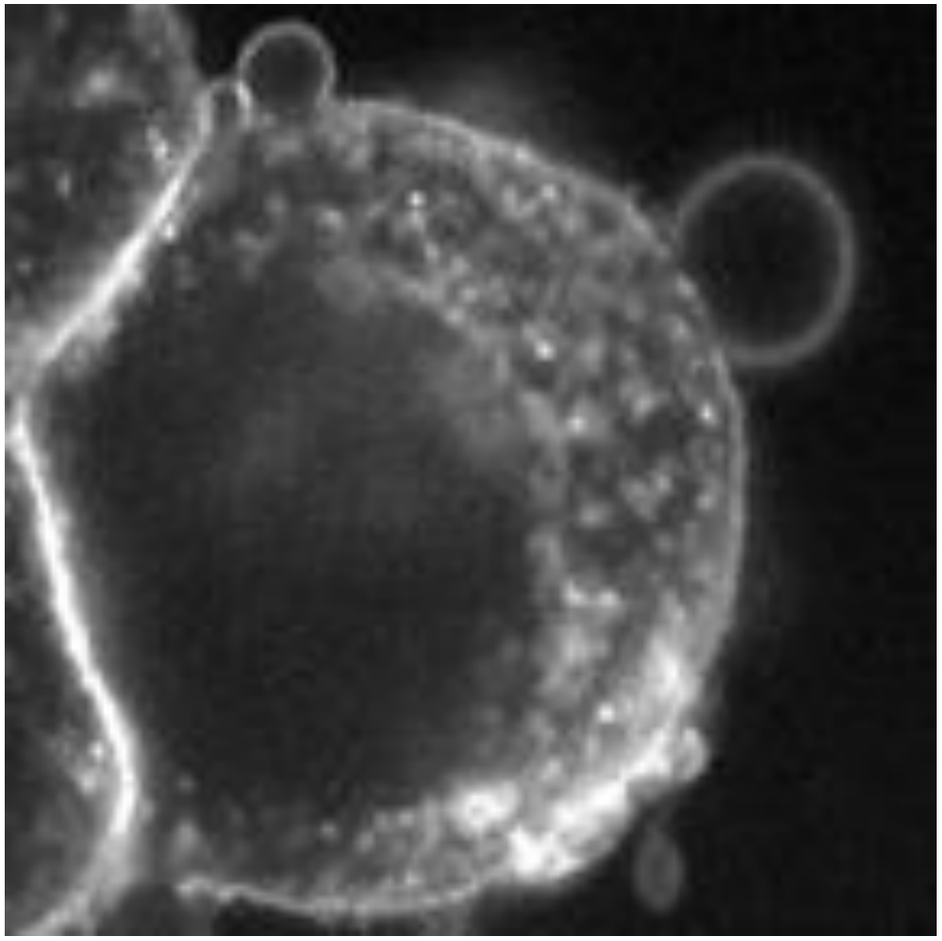
- Live imaging!
- Fairly quick: images every one second, if necessary (depends on camera speed)



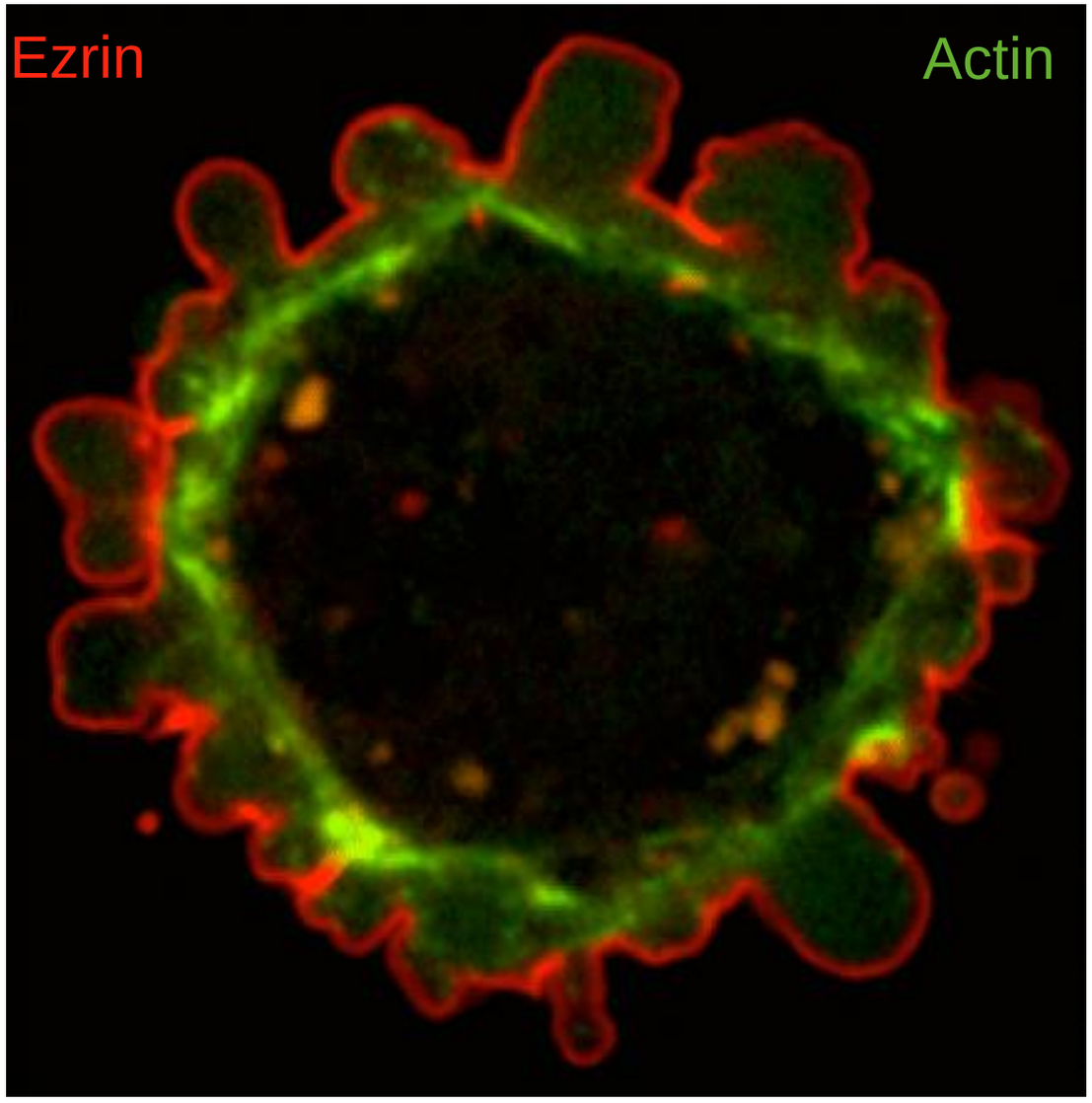
Cons

- Resolution limited at 200nm
- Increasing resolution, camera speed, light sources, depth of imaging == $$$.
  - Some examples: Peyton lab: $170K
  - Fancier, 3D microscopy: $1M +
- Can’t pick out individual proteins…..

2. Imaging of Intracellular Proteins

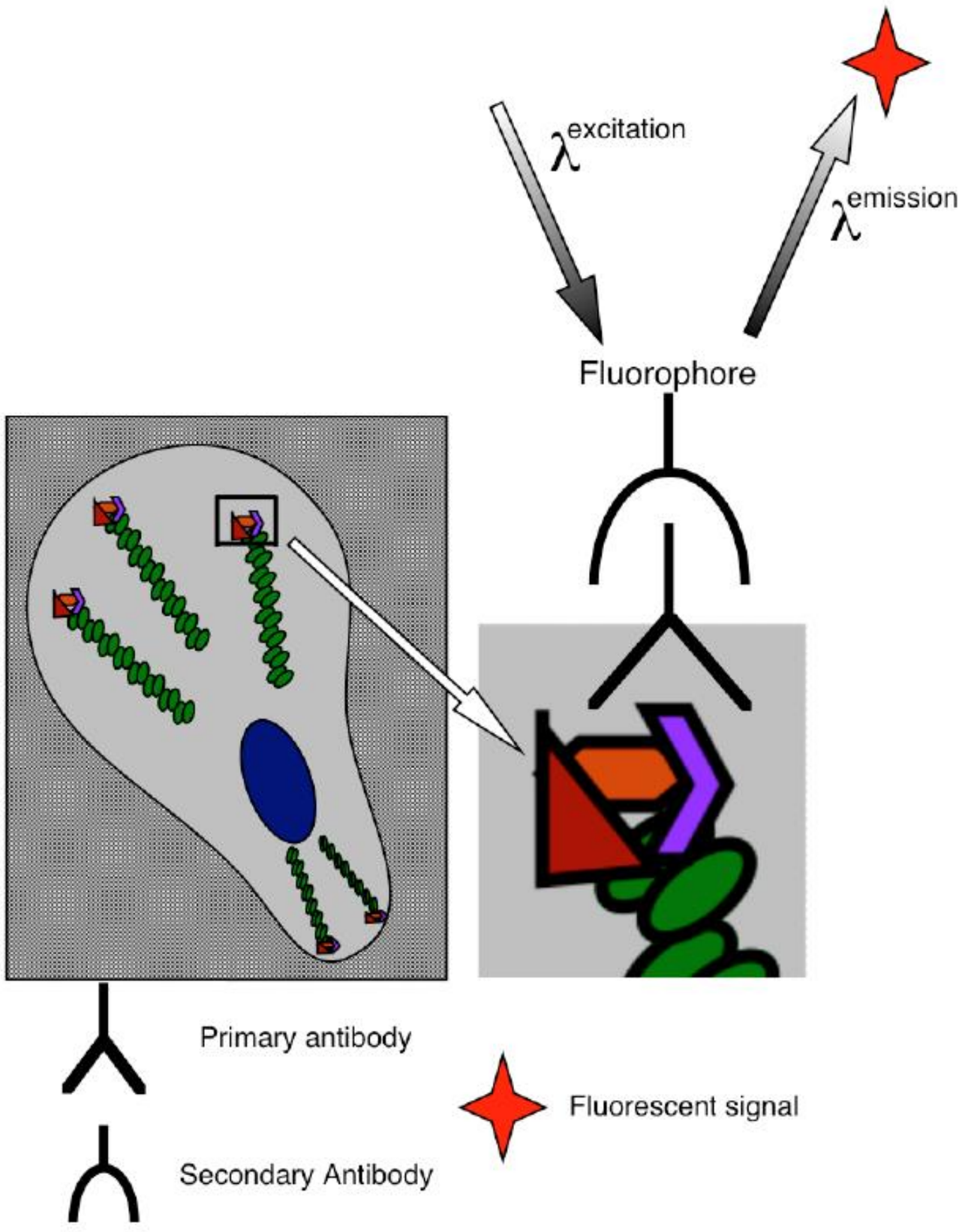


Susan Anderson, University of Washington



Charras, et al. JCB 2006

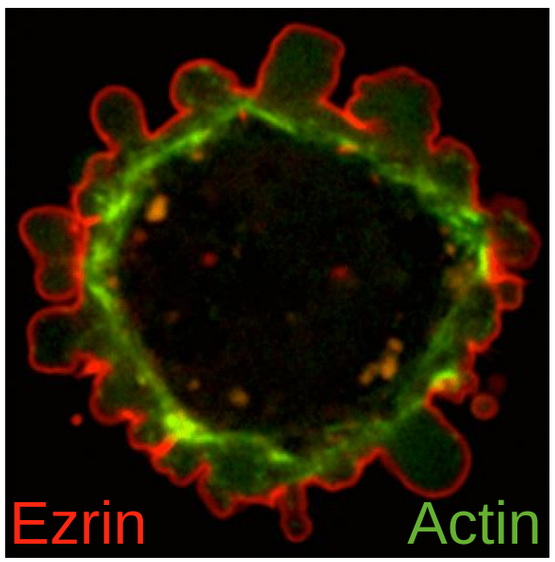
How immunofluorescence works



Pros and Cons of Fluorescent LM

Pros

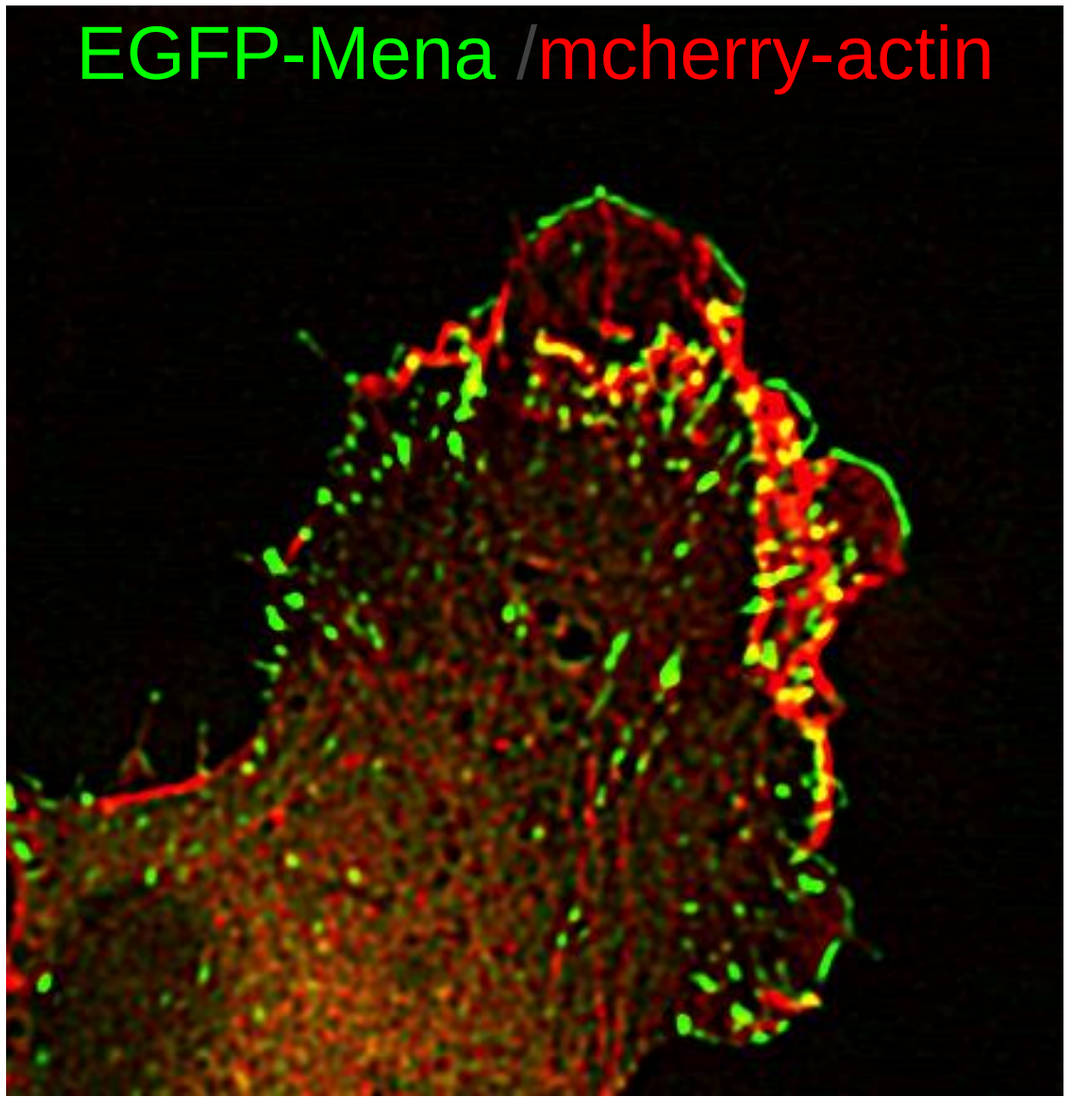
- Can visualize how what proteins a cell is expressing as a function of your material.
- Can visualize how the cells is organizing that protein, how much of the protein it's expressing at a given time, and where in the cell it is.



Cons

- Resolution limited at 200nm
- Increasing resolution, camera speed, light sources, depth of imaging == $$$.
  - Some examples: Peyton lab: $170K
  - Fancier, 3D microscopy: $1M +
- Sample prep can be time consuming.
- Cells are fixed, not live……

3. Live Imaging of Individual Proteins



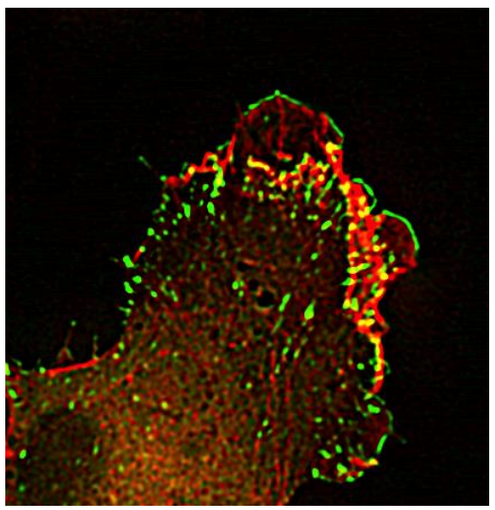
Gertler Lab, MIT

Tag protein with GFP
How: recombinant DNA technology

Pros and Cons of Live-fluorescent LM

Pros

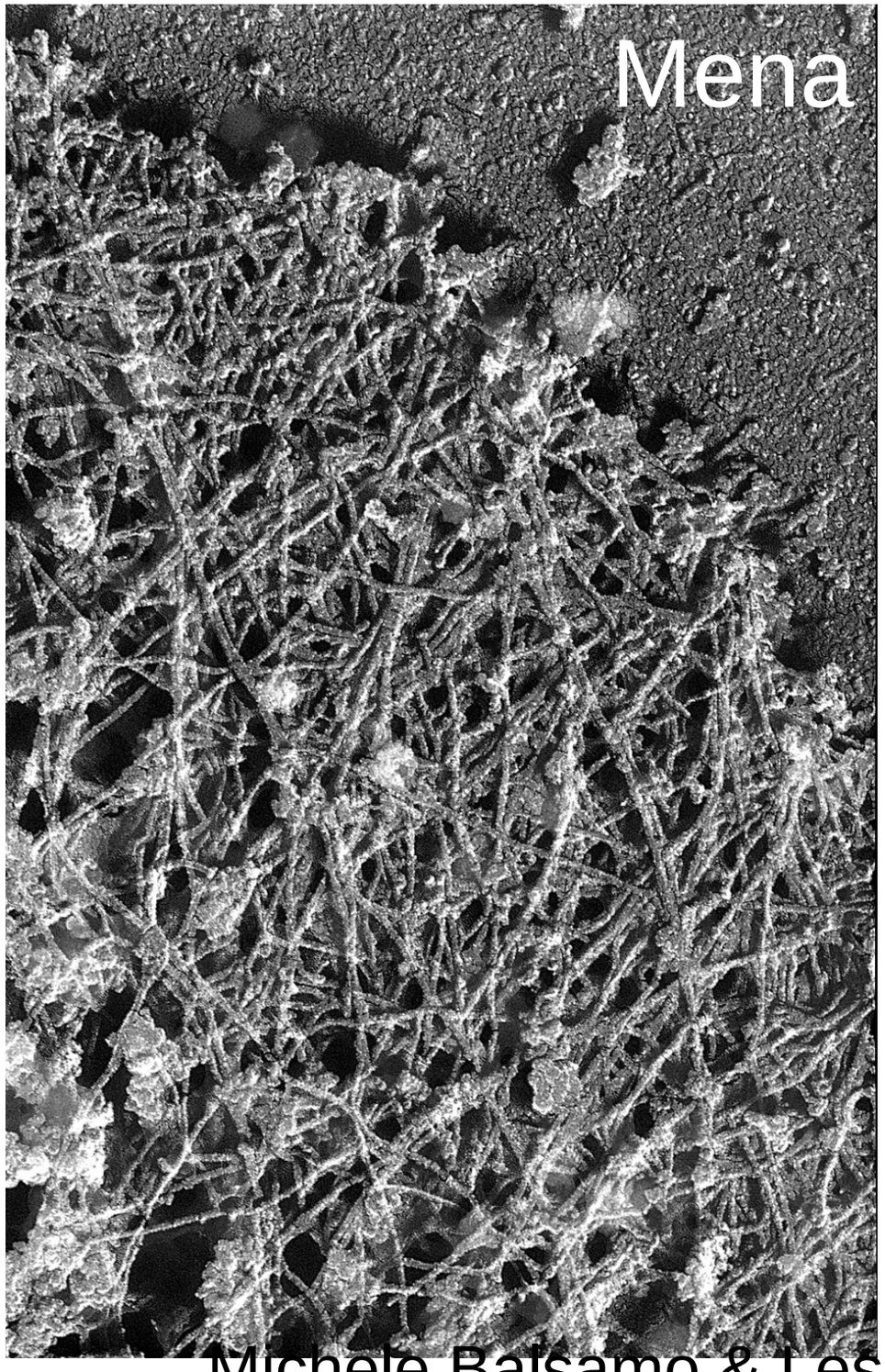
- Can visualize how what proteins a cell is expressing as a function of your material.
- Can visualize how the cells is organizing that protein, how much of the protein it's expressing at a given time, and where in the cell it is.
- Live microscopy!

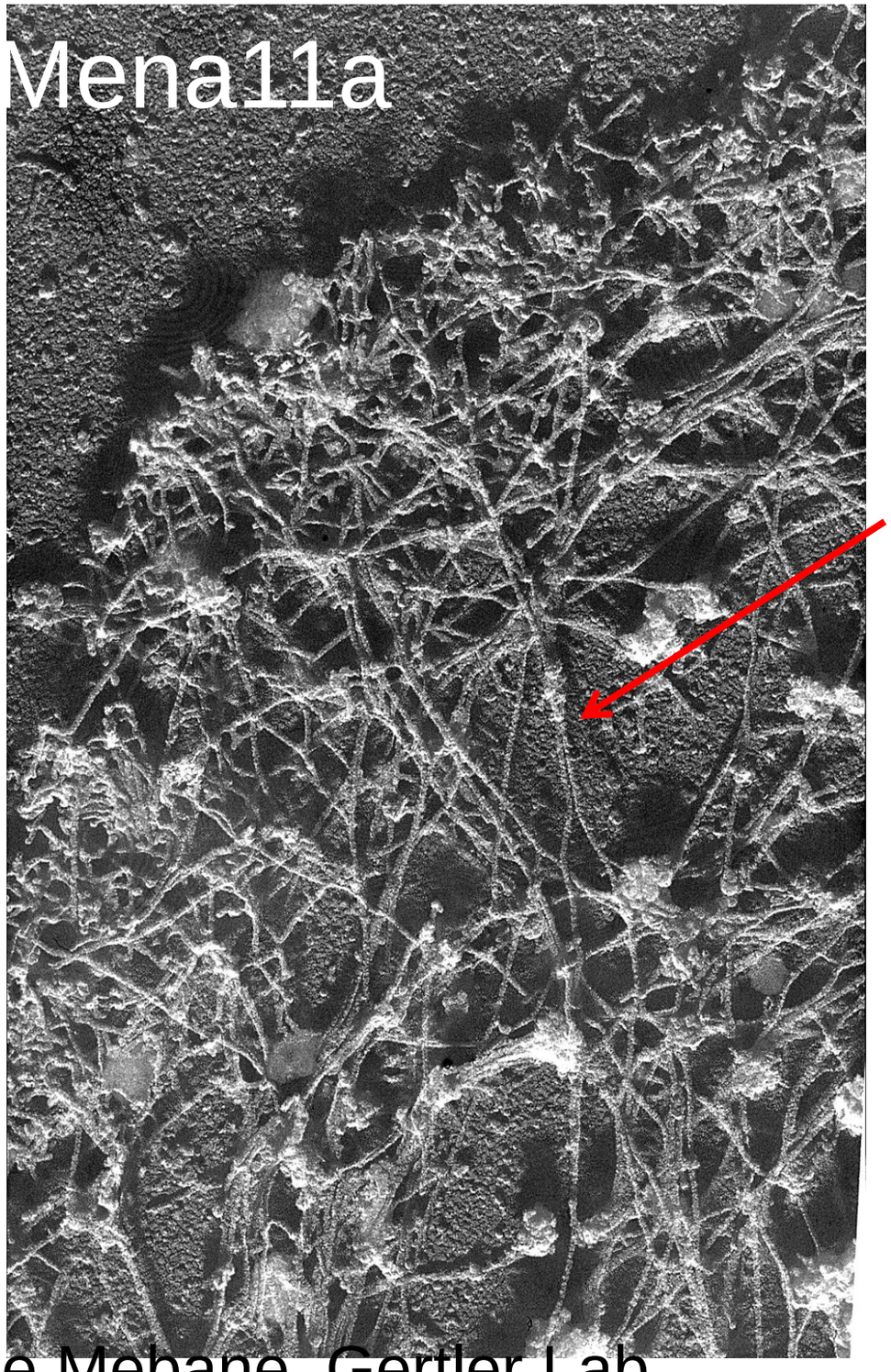


Cons

- Increasing resolution, camera speed, light sources, depth of imaging == $$$.
  - Some examples: Peyton lab: $170K
  - Fancier, high-resolution microscopy: $500K +
- Sample prep can be time consuming.
- Takes months to create a single recombinant protein.
- Still resolution limited at 200nm…

3. Beat the Resolution Limits with Scanning Electron Microscop

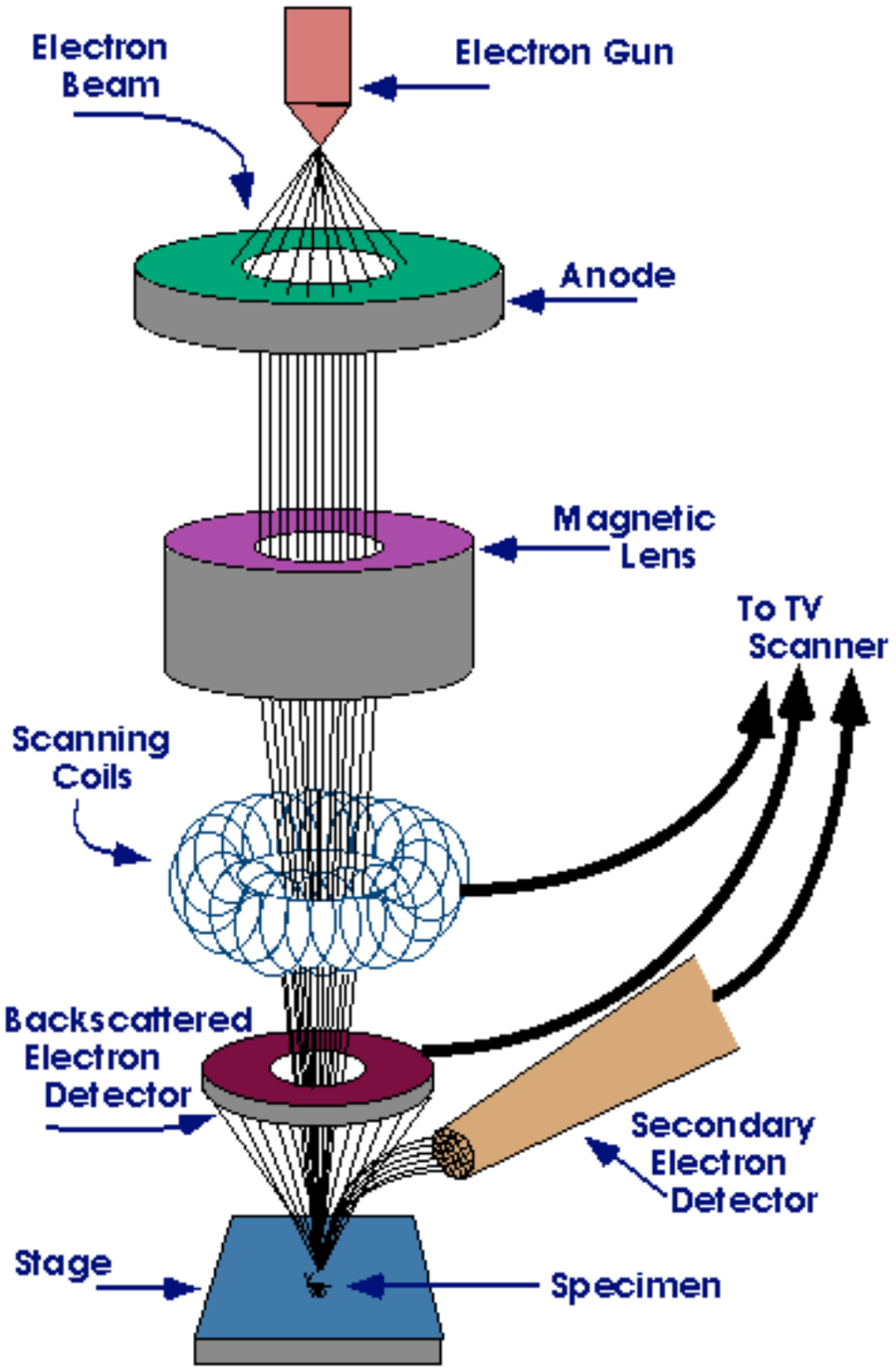




These filamentous structures are less than 100nm wide!

Michele Balsamo & Leslie Mebane, Gertler Lab, MIT

How SEM works



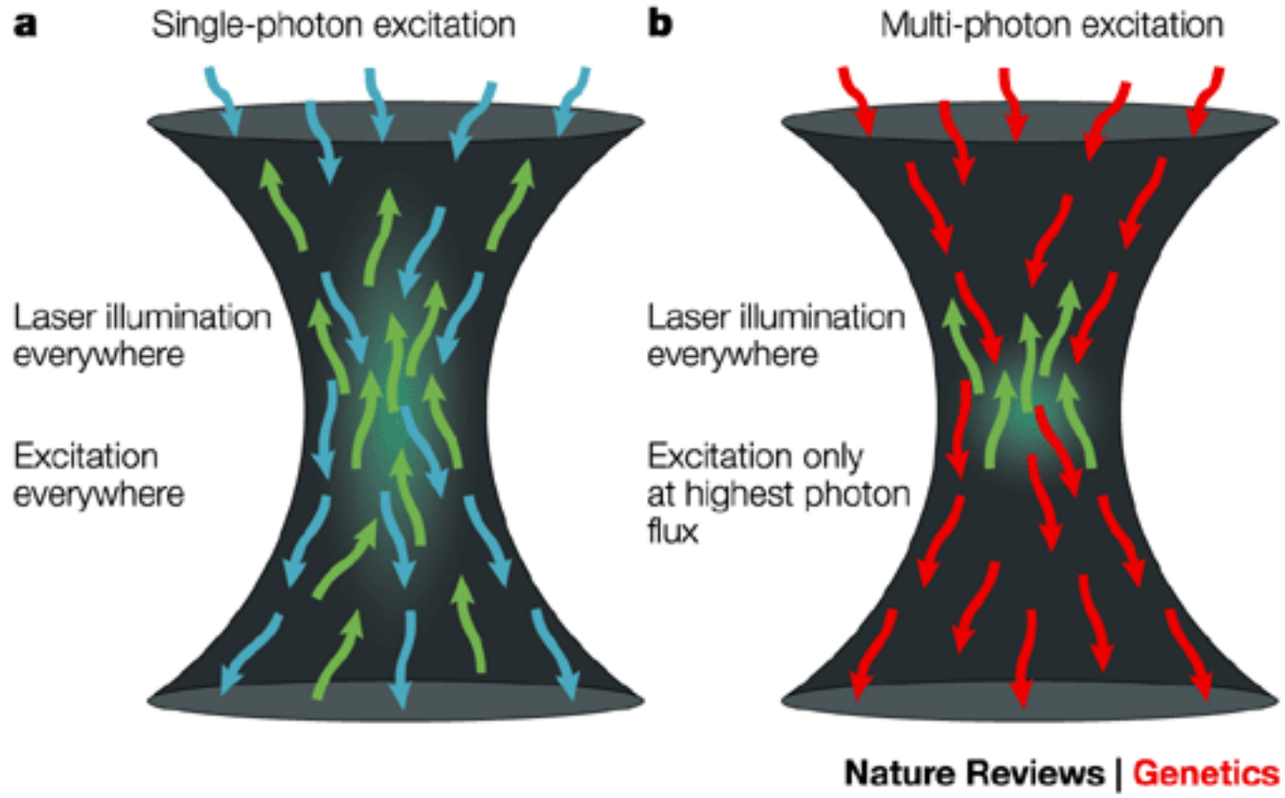
Pro: sub-visible light wavelength imaging

Con: fixed samples only, everything is under super vacuum.

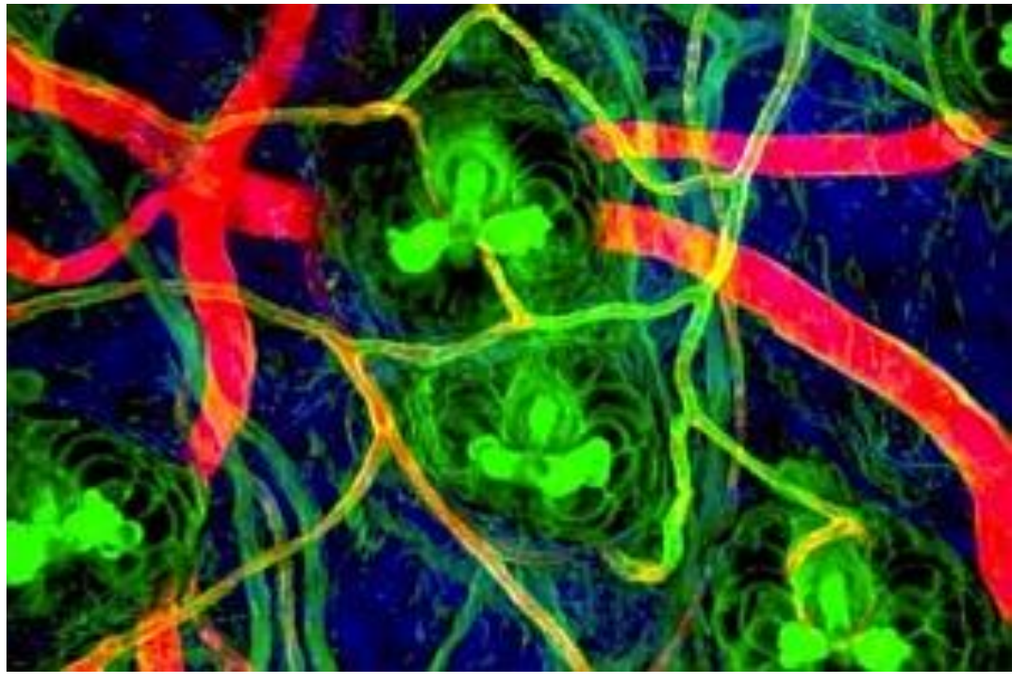
Con: sample preparation can be destructive, no water!!!

Con: sample must be conductive!

4. Get Deep into tissue with Multiphoton Imaging

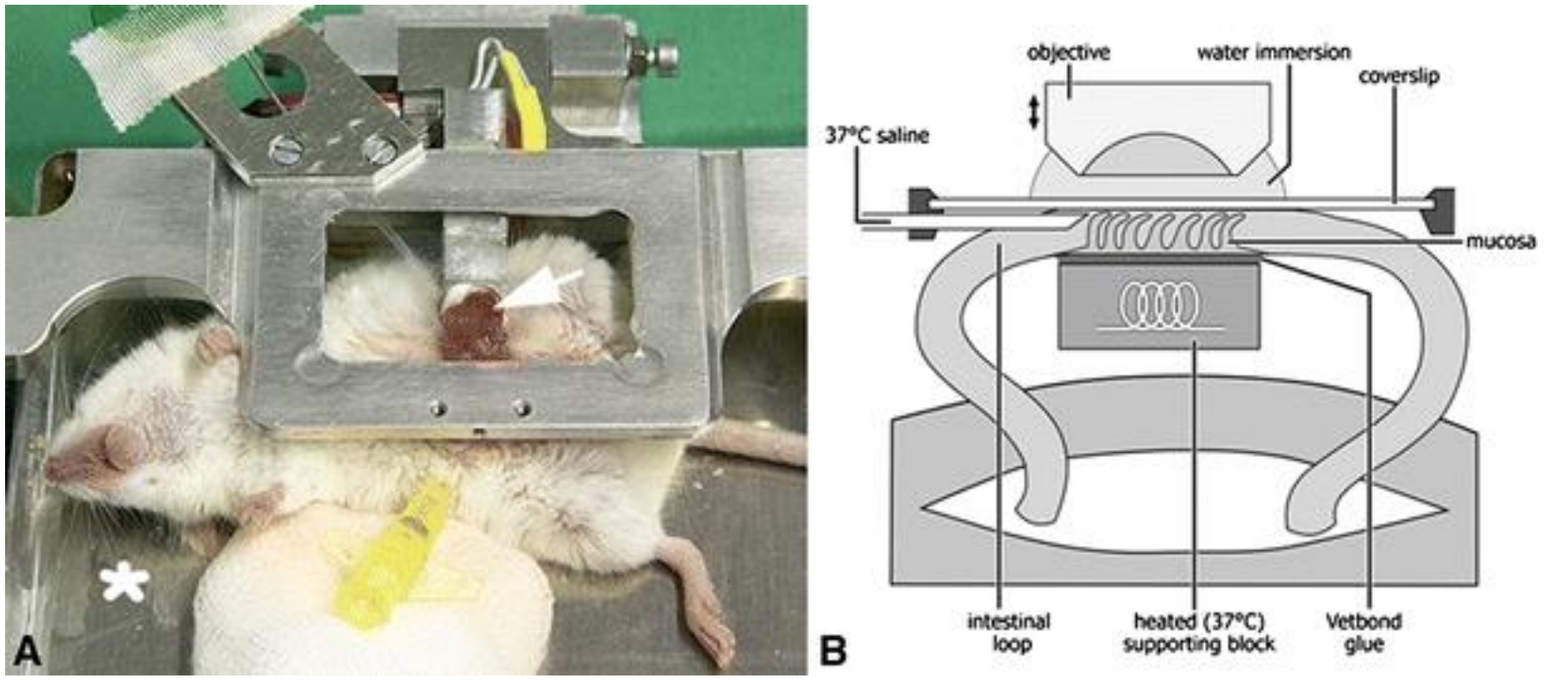


Pros: Deep into tissues, no photobleaching

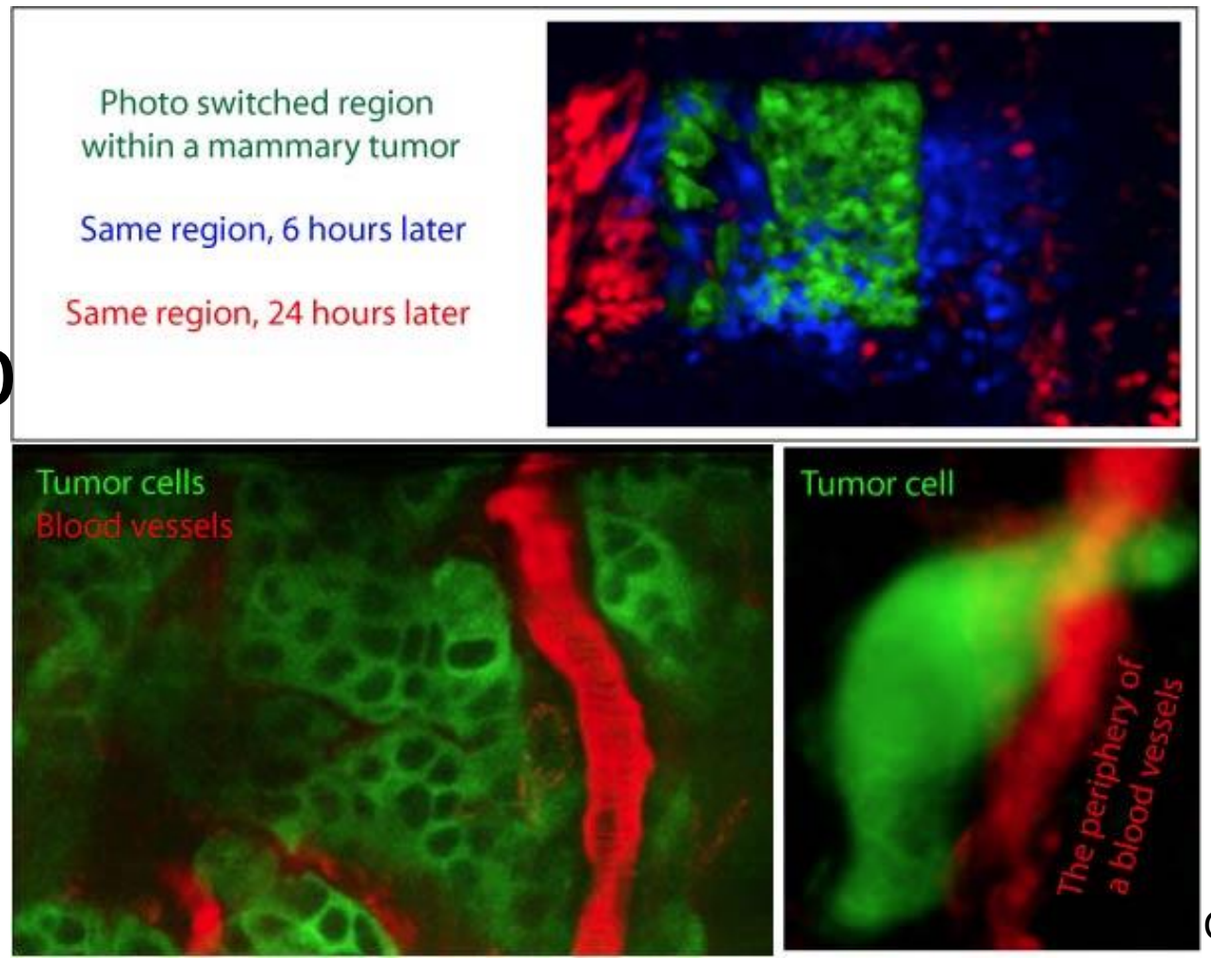


Cons: $1M+.

Intravital Imaging



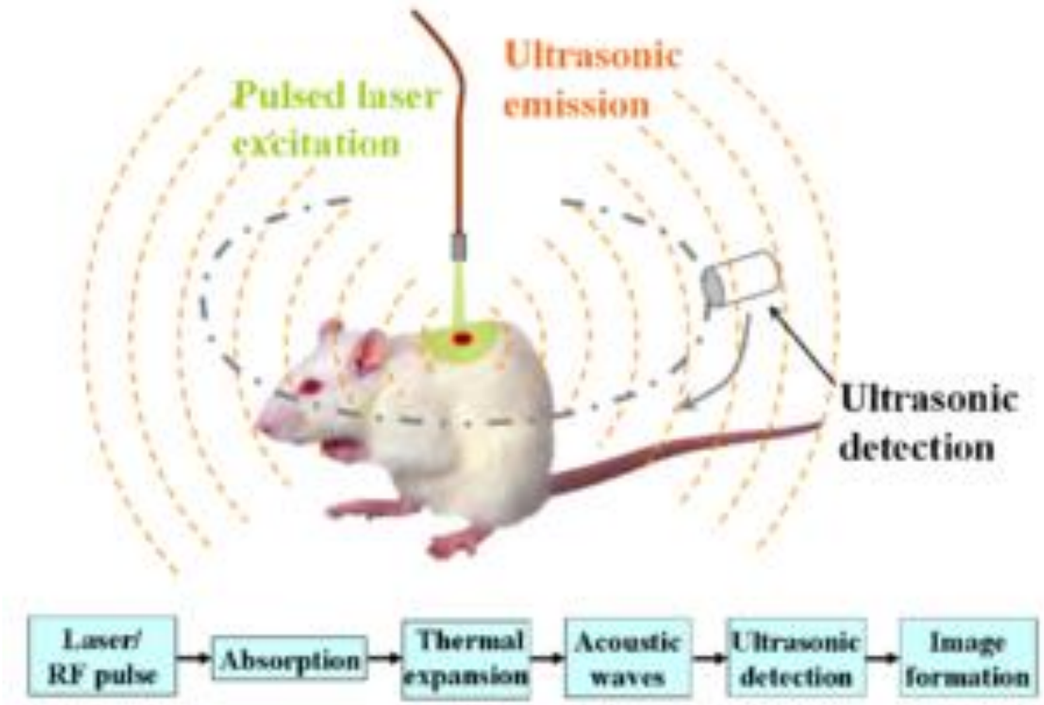
Pros: Deep into tissues, also, live imaging

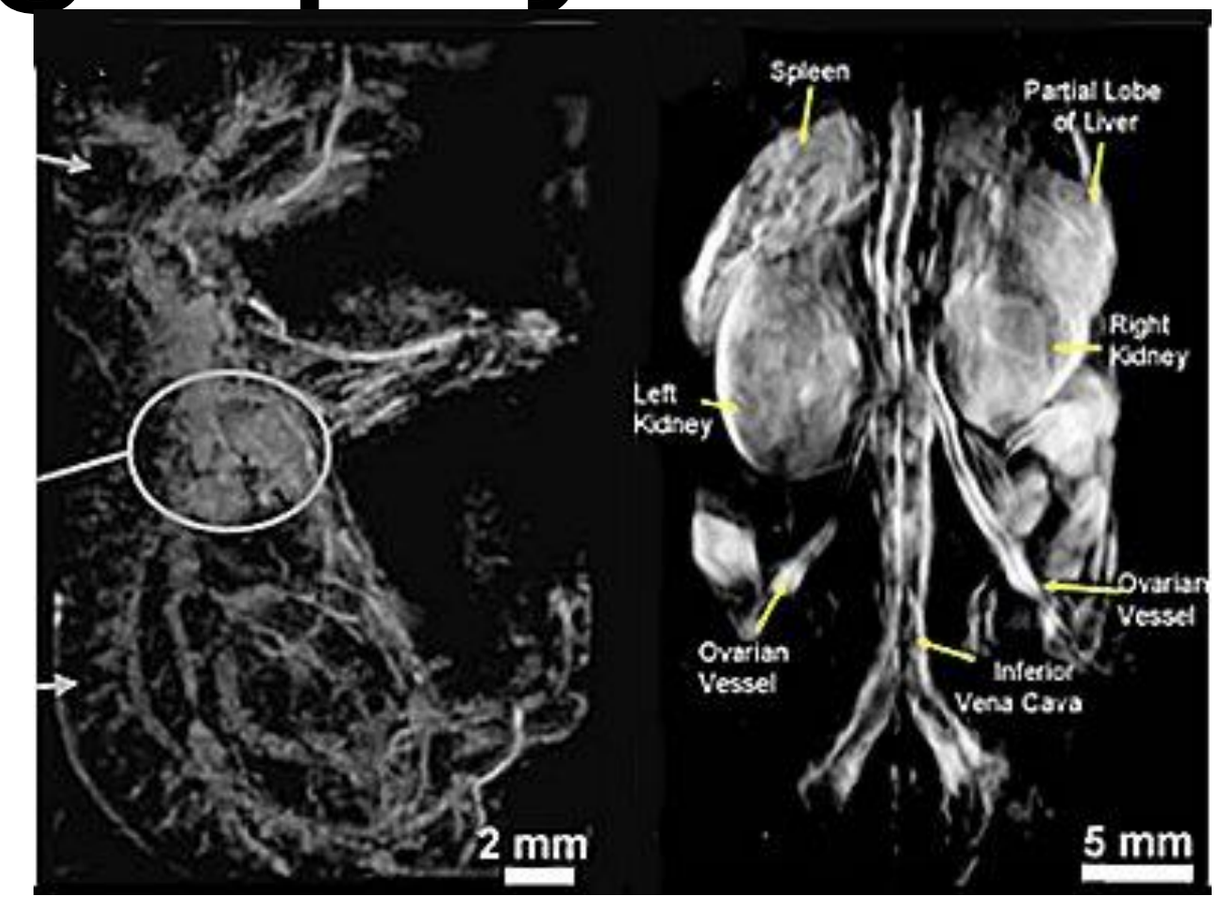


Cons: $1M+.

Cancer biophysics, Hubrect Institute

Photoacoustic Tomography

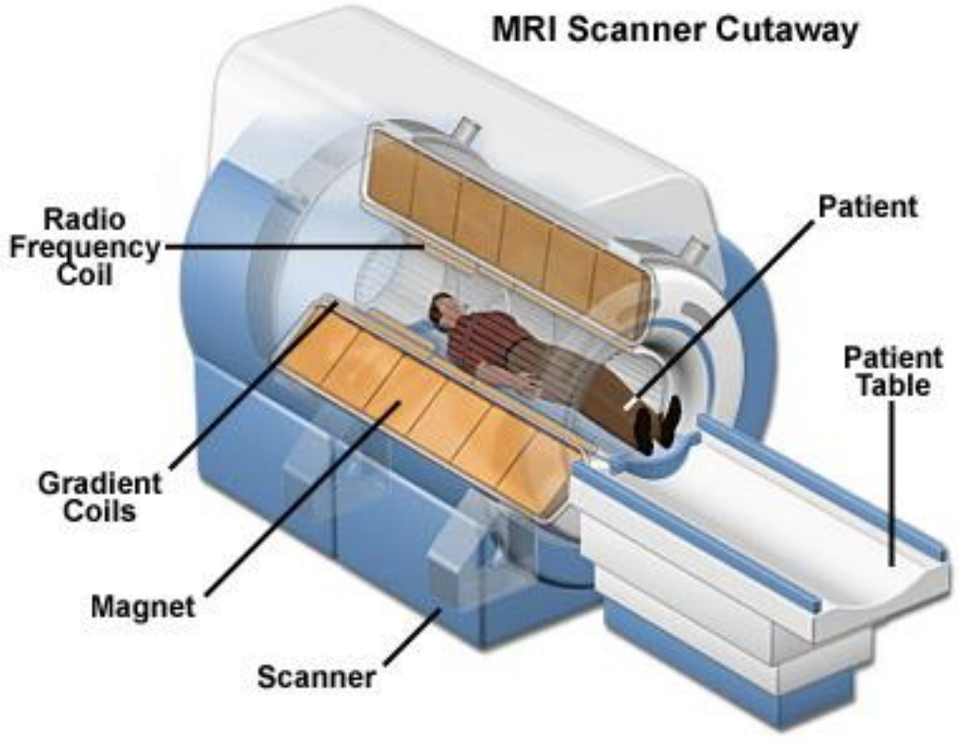


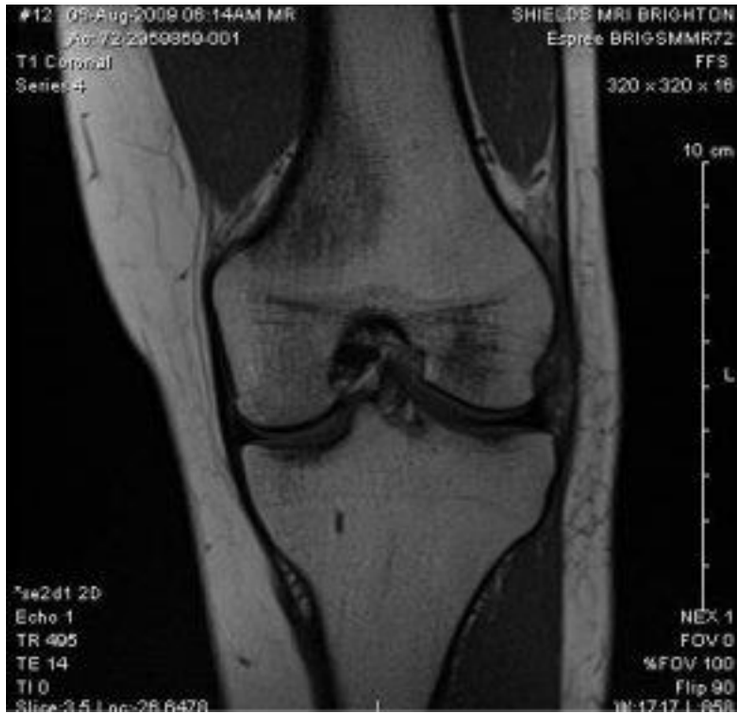


Pros: Deep into tissues, also, live imaging, non destructive. No staining needed. quick and noninvasive.

Cons: Low Resolution
Still reliant on wave reflection (limits depth)

MRI (Magnetic Resonance Imaging)

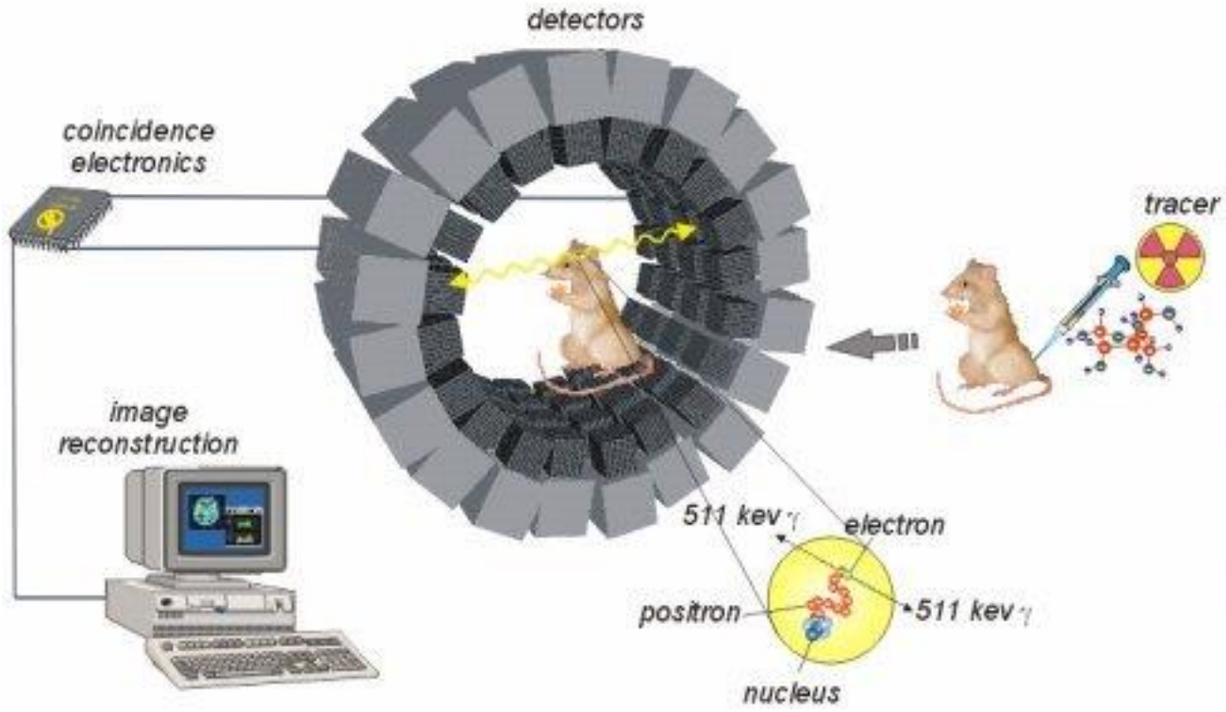




Strong magnetic fields cause nuclei in body to align, then rotate, which is detected.
Used to detect differences between soft tissues.
Pros: Deep into tissues, also, live imaging

Cons: Low Resolution
Long imaging times
expensive

PET (Positron Emission Tomography)



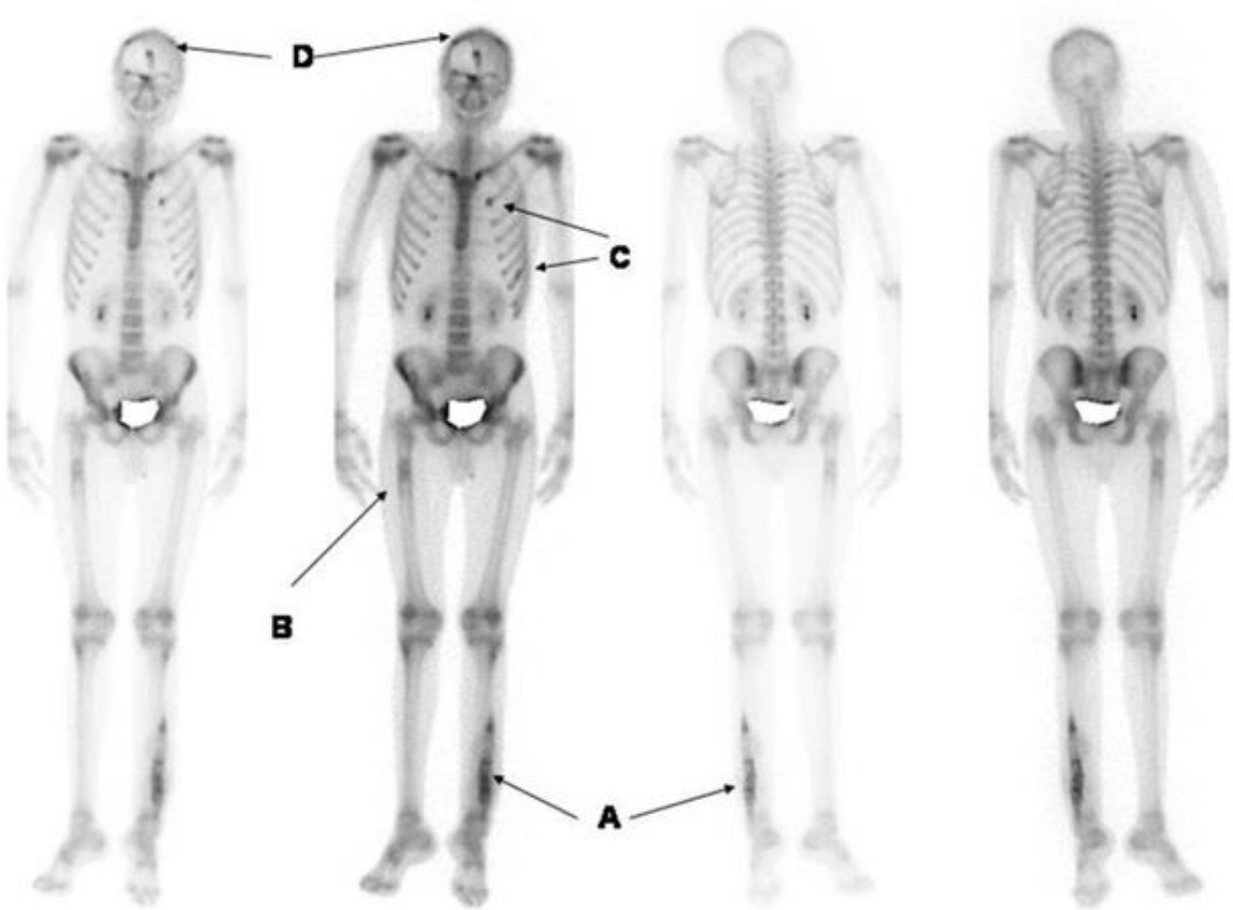
Patient takes tracer dye, picked up by highly metabolic tissues

Pros: Deep into tissues,

Cons: low resolution
Long imaging times
expensive

Bone density scan

A bone density scan is a low-dose x-ray which checks an area of the body such as the hip, hand or foot for signs of mineral loss and bone thinning



Uses X-ray to find areas of bone thinning. Typically used for osteoporosis or cancer patients.
Pros: Deep into tissues, also, live imaging

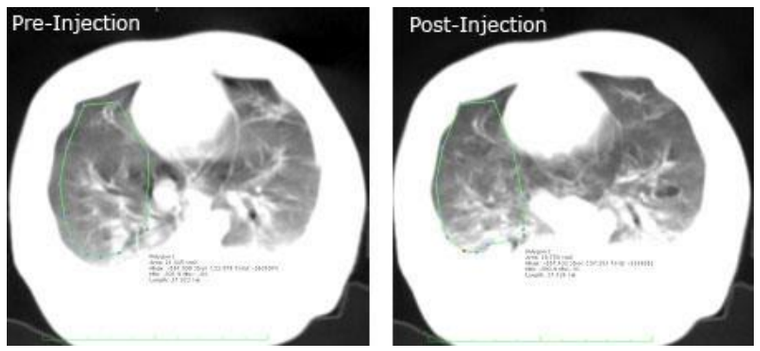
Cons: Low resolution.
Long imaging times
Expensive.

Imaging Applications

- Molecular Tracking:
  - Use Quantum Dots as labels
    - Dots attached to molecules before injection
- Fluoroscopy used to track movement
  - Colors from dots seen and imaged

Imaging Applications

- Tracking blood flow:
  - Tag proteins of cells with gold nanoparticles
    - View process of angiogenesis
      - Important for cancer detection and imaging
- Cancer Imaging:
  - Injection of gold nanoparticles
  - Localization around tumors



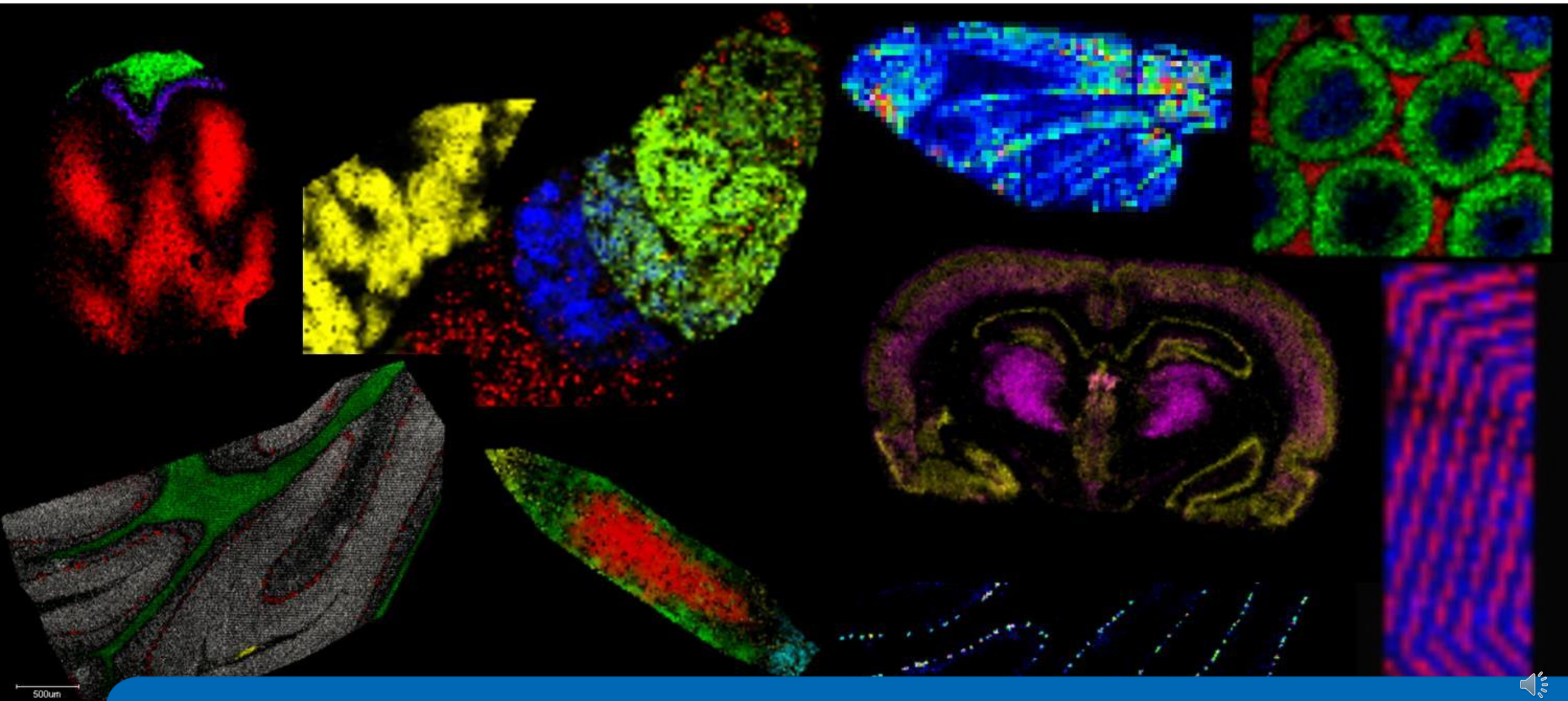
Taken from http://www.rsna.org/Publications/rsnanews/oct05/nanoparticles.cfm

Possible Concerns

- Negative biological side-effects:
  - Toxicity of quantum nanodots
  - Effects on living organisms not well known
- Gold nanoparticles safer:
  - Biologically inert
  - Won’t interact with other chemicals

MALDI Imaging

– Looking beyond Classical Histology



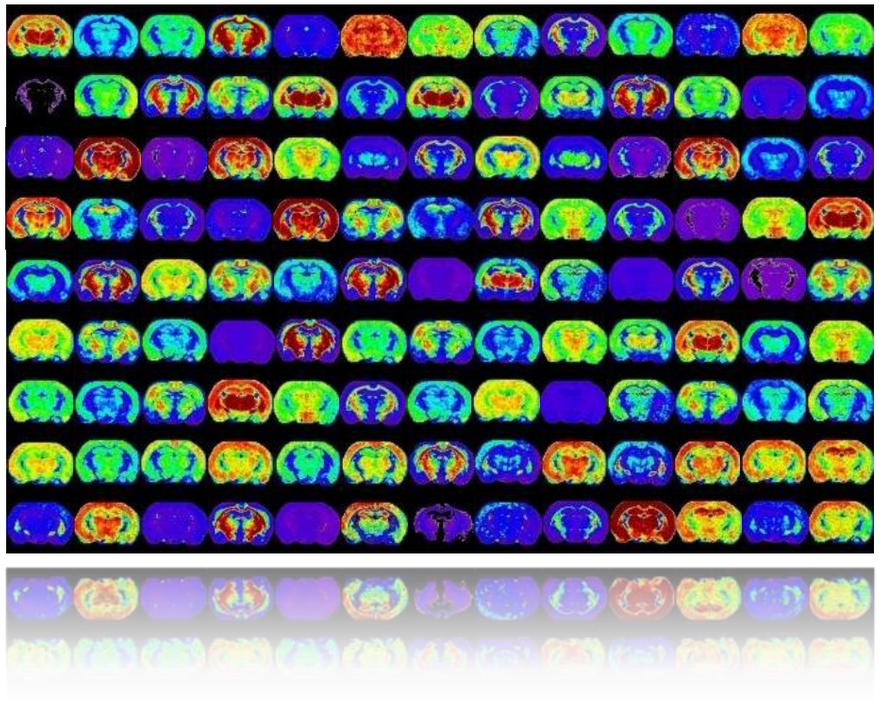
Imaging Mass Spectrometry

Exploiting Mass Spectrometry for the analysis of multiple analytes, from small molecules and metabolites to lipids, peptides and proteins

MALDI (Matrix-Assisted Laser Desorption Ionization)

SIMS (Secondary Ion Mass Spectrometry)

DESI (desorption electro tion)



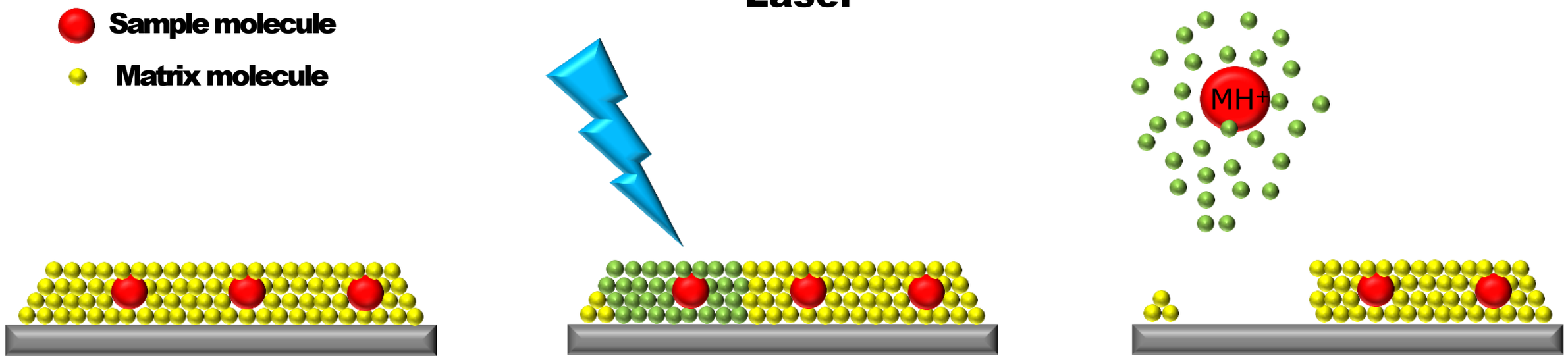
MALDI stands for…
Matrix-Assisted Laser Desorption/Ionization

- **invented by Tanaka (Nobel prize award in 2002), Hillenkamp and Karas in the mid 80s**

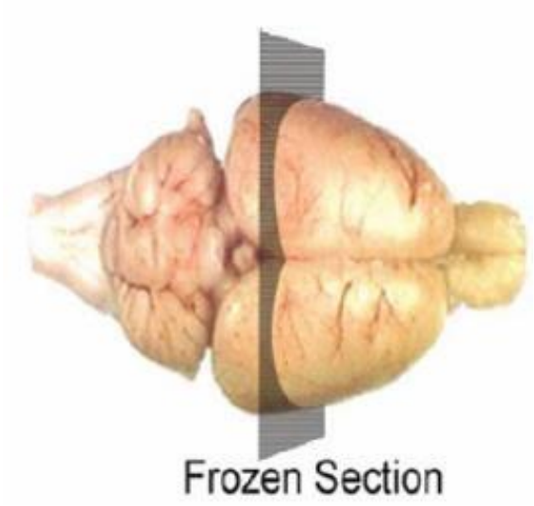
Sample embedded in light-absorbing matrix

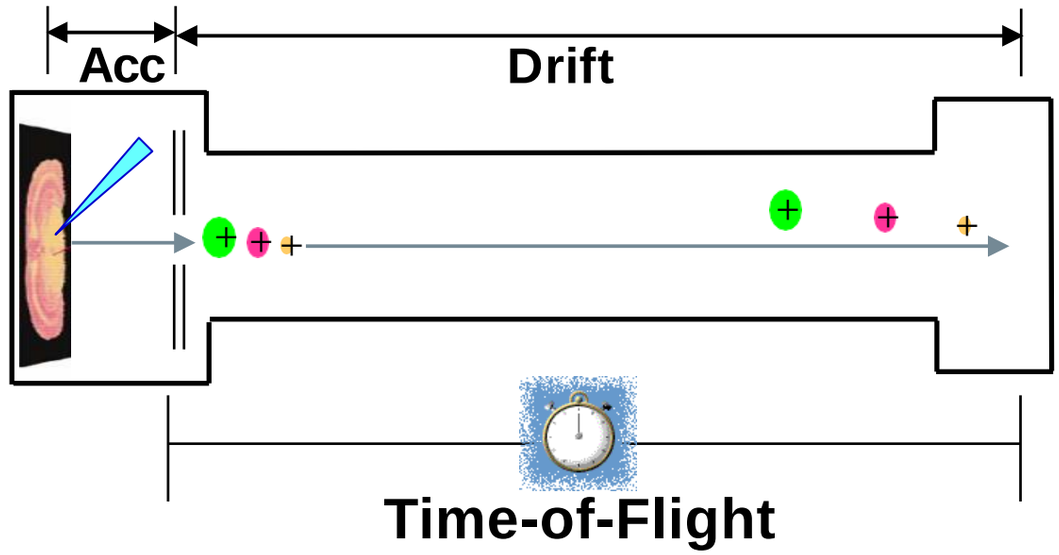
Excitation of matrix molecules by laser light

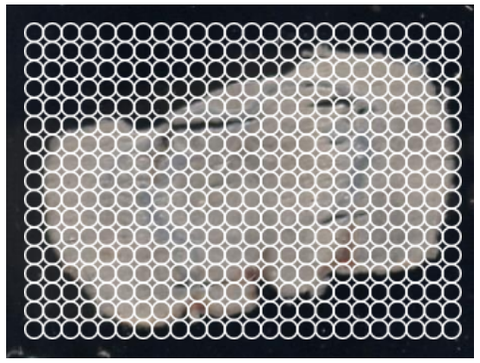
Desorption/protonation of sample molecules

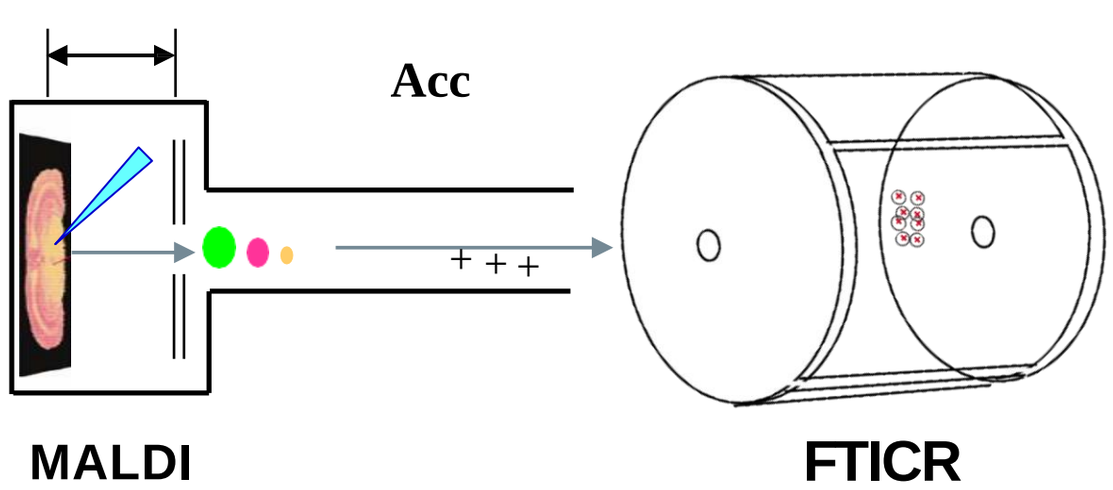


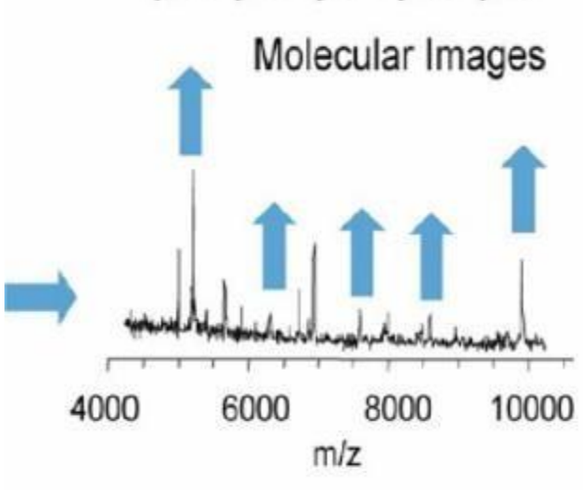
MALDI-Imaging Mass Spectrometry (IMS)





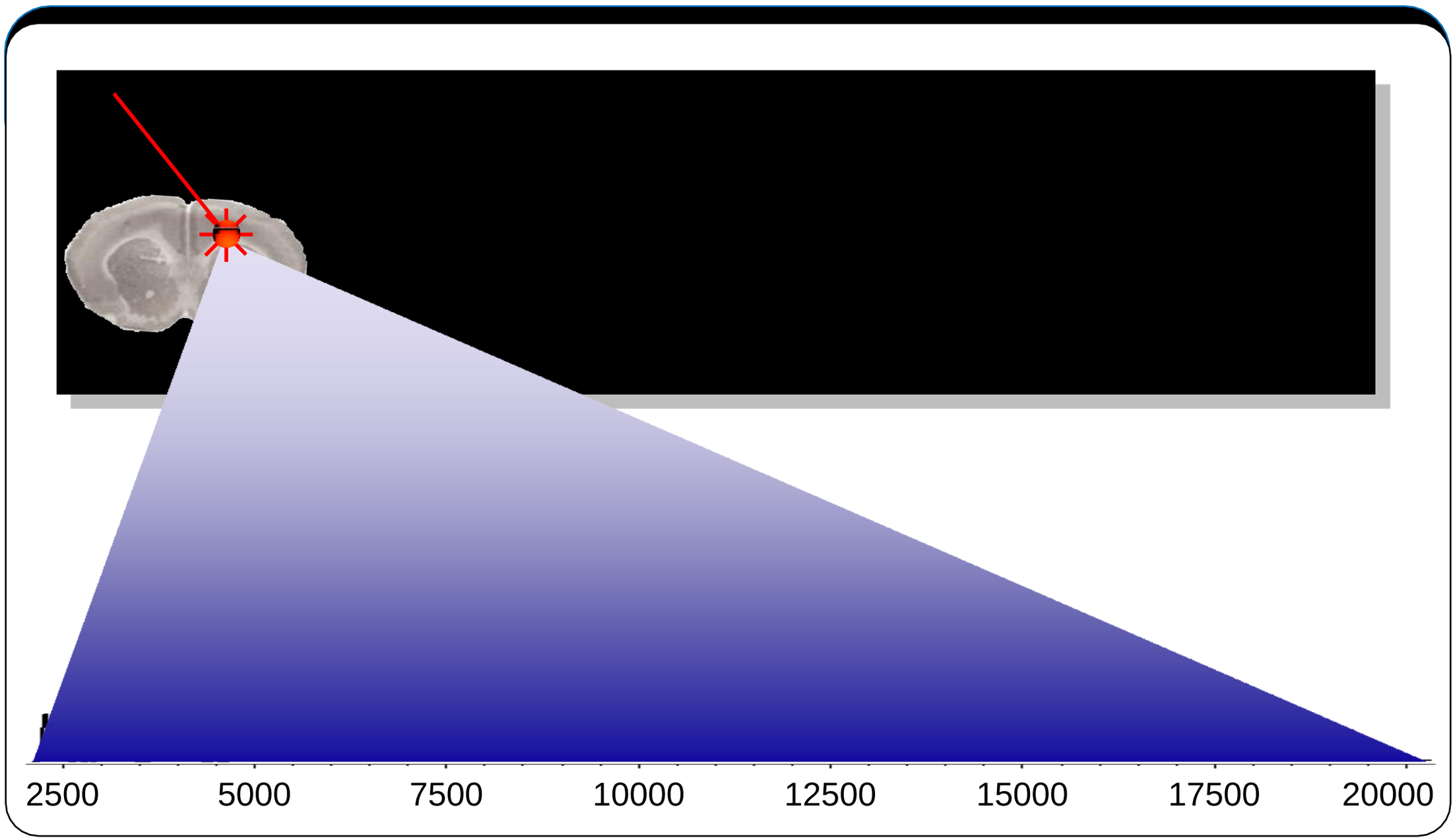




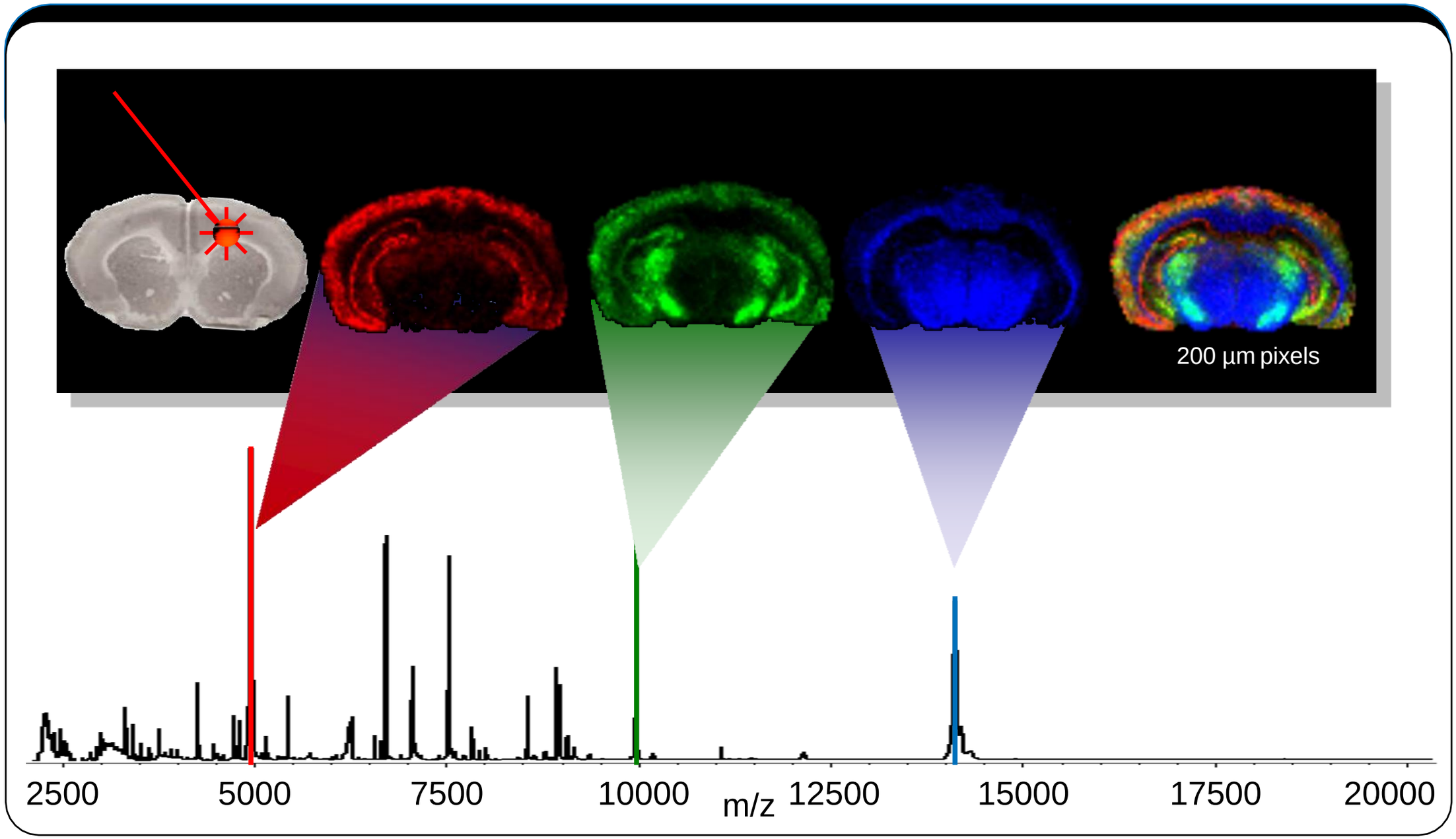


Modified from: Lottspeich, Zorbas, eds“Bioanalytik”, Spektrum Akademischer Verlag, 1998
and M.Stoeckli et al. Analytical BioChemistry 311 (2002)33-39

The Principles



The Principles



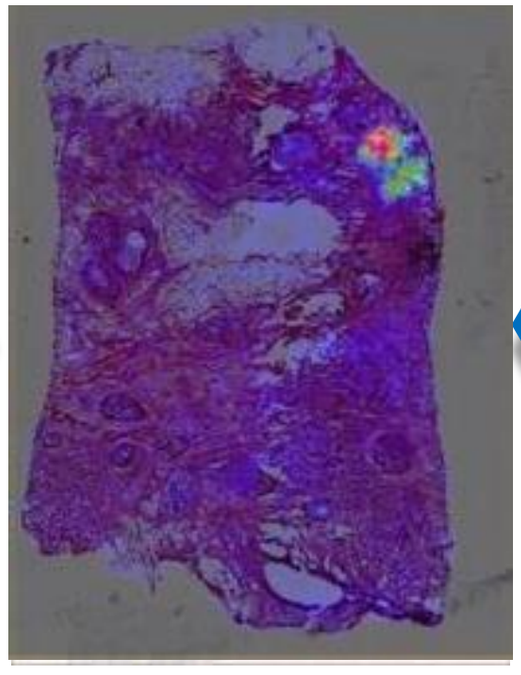
MALDI Imaging Workflow

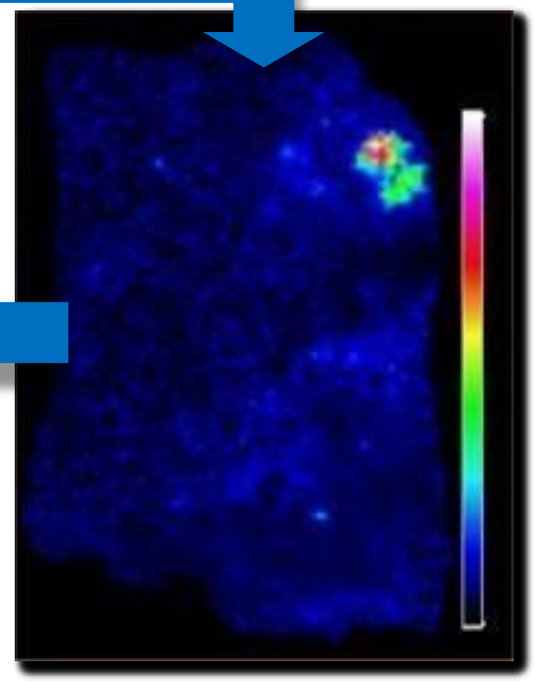
Tissue

Cryosection

Matrix coating

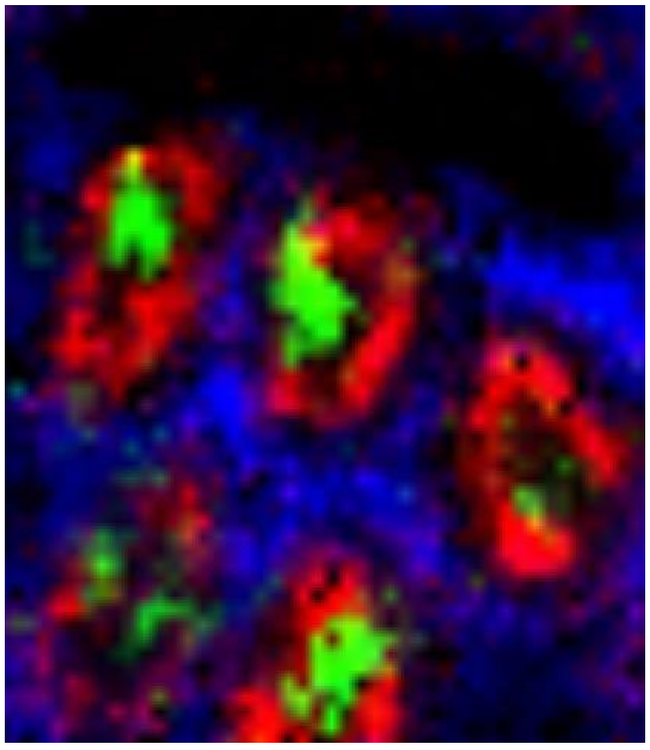
MALDI Mass Spectrometry



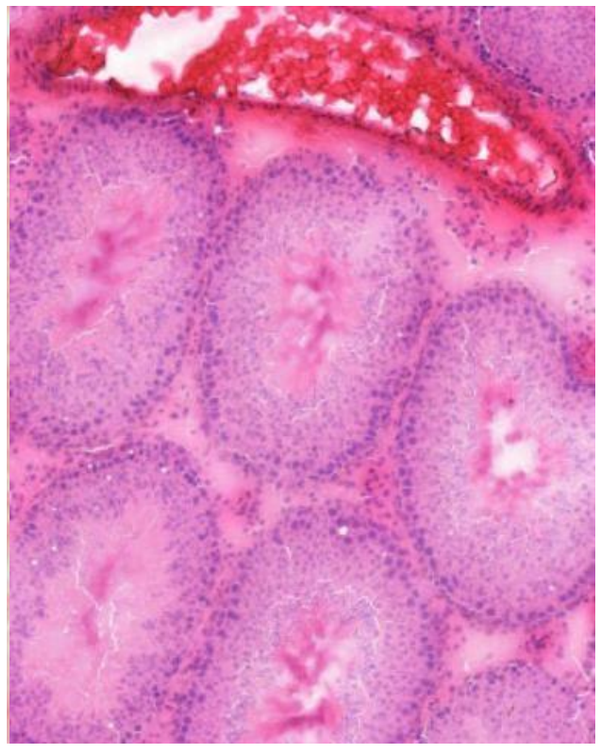


Co-registered Image & Virtual Microscopy

Sample image



Molecular image from MALDI imaging



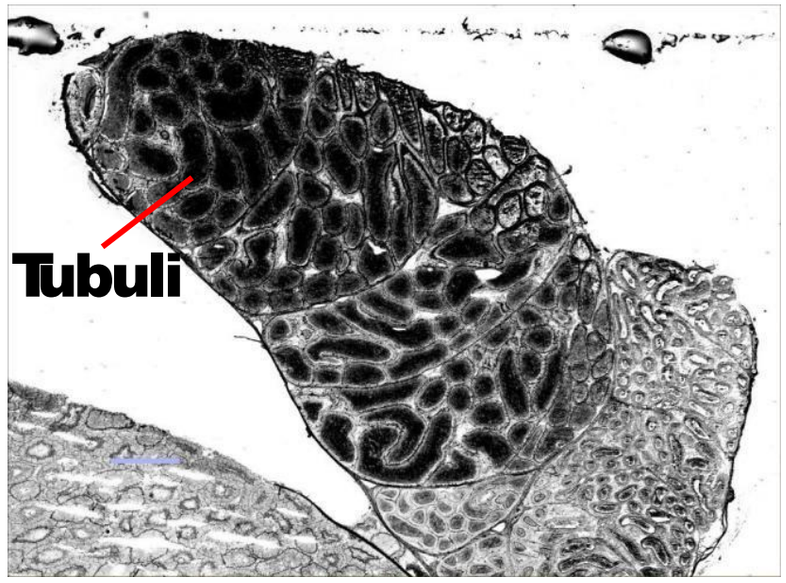
H&E image from virtual microscope

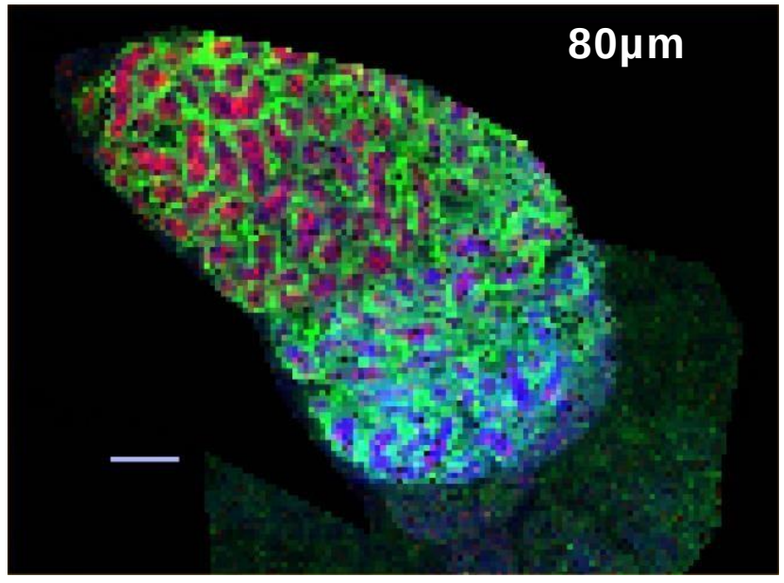
- H&E staining after MALDI measurement on the same sample: Unambiguous correlation of optical and molecular imaging modalities
- High-resolution virtual slide provide full access to molecular and histological information

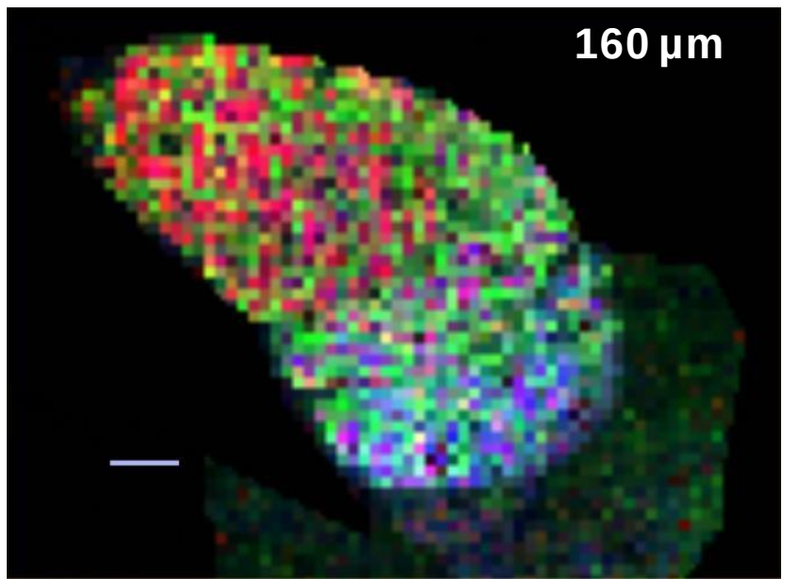
MALDI Imaging Applications

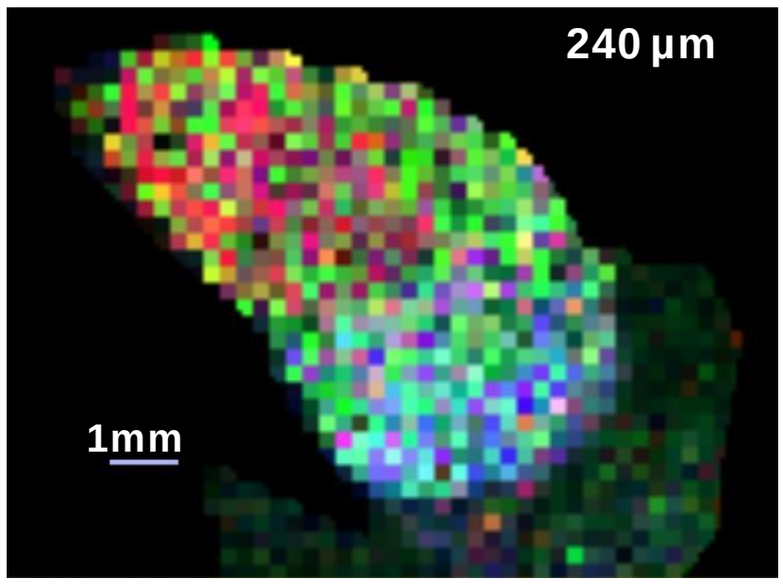
	Small molecules (<2-4 kDa)	Large molecules (>2-4 kDa)
Discovery "Profiling"	**Lipidomics, Metabolomics and peptide profiling** **FTICR**	**Proteomic Biomarker Discovery** **TOF**
Targeted	**Drug & Metabolite Detection** **FTICR**	**Protein Detection**

Lateral Resolution is Important to Understand Tissue Morphology









Rat testis sample: Charles Pineau, Univ. Rennes, France

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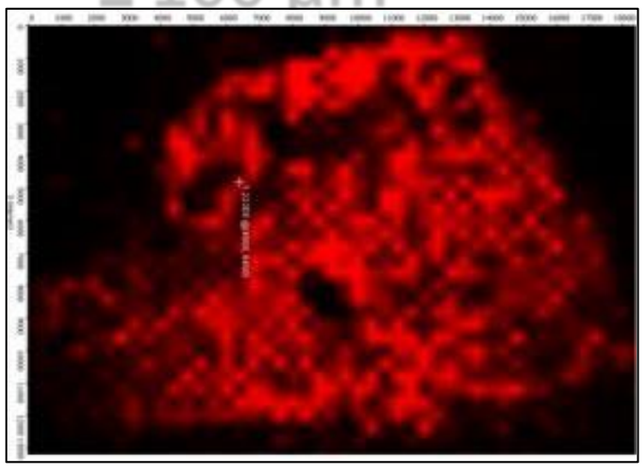
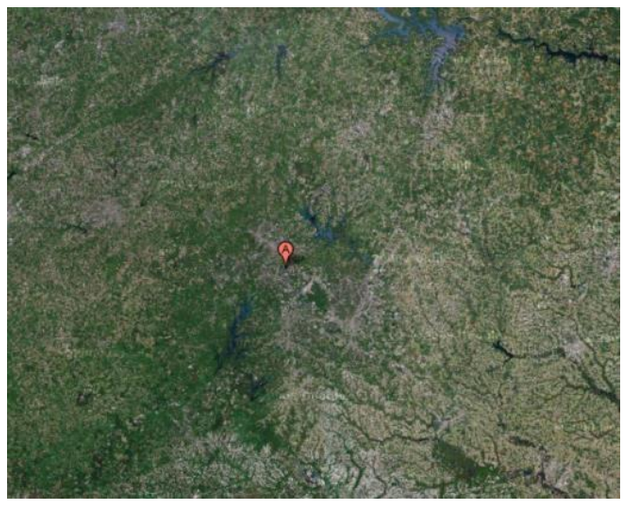
GlaxoSmithKline

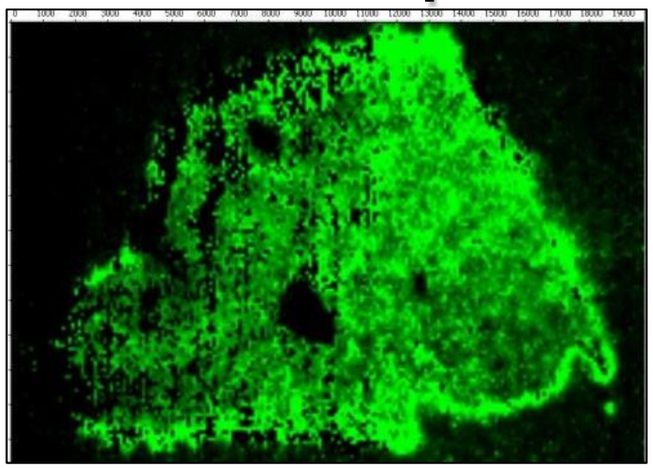
≥ 100 µm



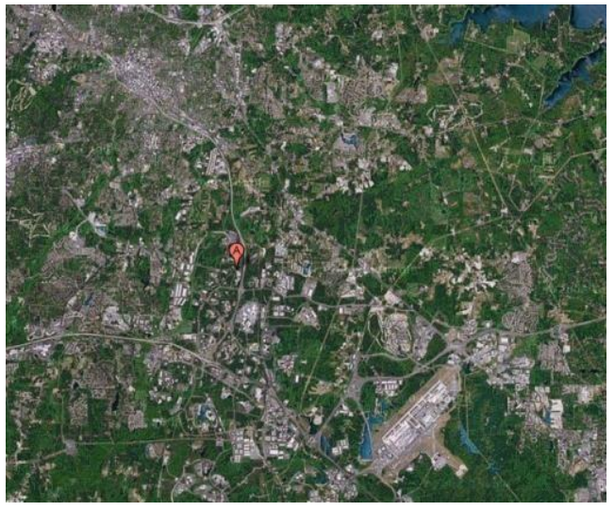
- Cover whole tissue
- Hot spots
- Good Sensitivity
- Similar to QWBA



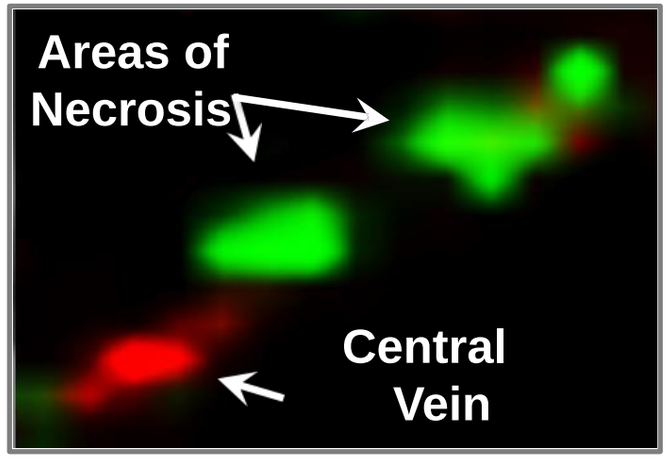
100 <-> 50 µm



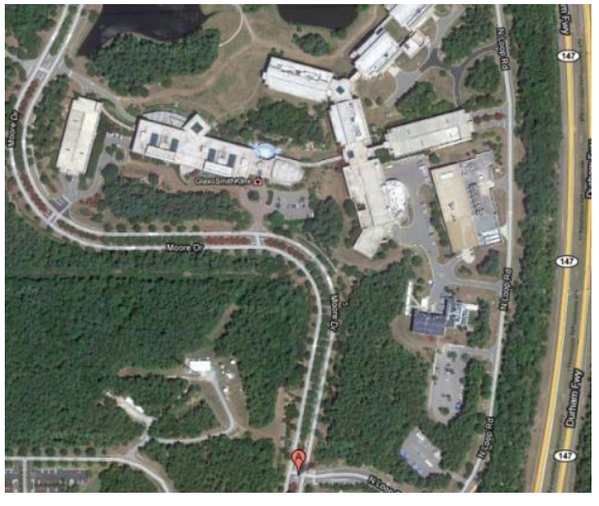
- Large portion tissue
- Some gross tissue features
- Compare Distributions
- Medium Sensitivity



< 50 µm



- Small portion of tissue
- Histology overlays
- Explore mechanisms
- Compare Distributions
- Low Sensitivity



S Castellino, et al, *Bioanalysis*. Nov. 2011

Questions for you:

- Do you need to do imaging in your grant to show that something is working?
- What imaging modality will you choose and why?
- Address both advantages and limitations.
- Can you design or use a new, cheap imaging method? (see assigned reading)