Optical Tomography (overview)

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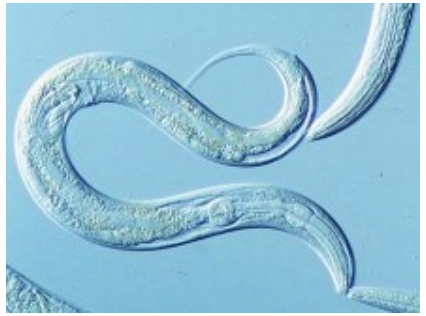
Optical microscopy vs. Optical and Optoacoustic Imaging

-In vivo tissue imaging capacities of optical microscopy are limited

Images: www.socmucimm.org



- Dynamic interactions of cellular processes at different system levels are reachable with optical and optoacoustic methods

- Promising photonic methods: micro-, meso- and macroscopic

(according to the tissue depth at which they operate)

Imaging Limit of Conventional Microscopy 1/2

- MFP *(Mean Free Path)* of a photon

- Of the order 100 µm in tissue (depends on the tissue type)

- Shorter in lungs or muscle, longer in semi-transparent organisms

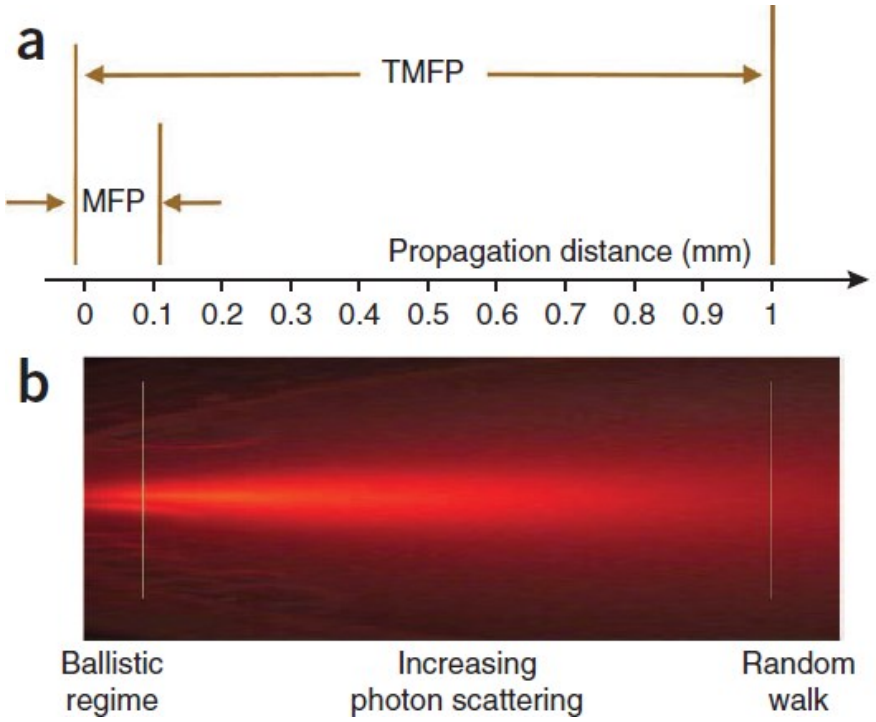
- Conventional microscopy: 10-20 µm
- Confocal and multiphoton microscopy: tens – hundreds of µm

Imaging Limit of Conventional Microscopy 2/2

- TMFP *(Transport Mean Free Path)*
- Depends on the tissue and the wavelength

Table 1 | Optical properties of different tissues

Tissue type	Absorption coefficient (cm^{-1})	Reduced scattering coefficient (cm^{-1})	TMFP (mm)
Muscle	0.20	9	1.1
Brain	0.25	16	0.6
Breast	0.05	12	0.8
Lung	0.10	30	0.3



- Confocal and MP: < 1 TMFP
- Upper limit of the penetration of the microscopic techniques

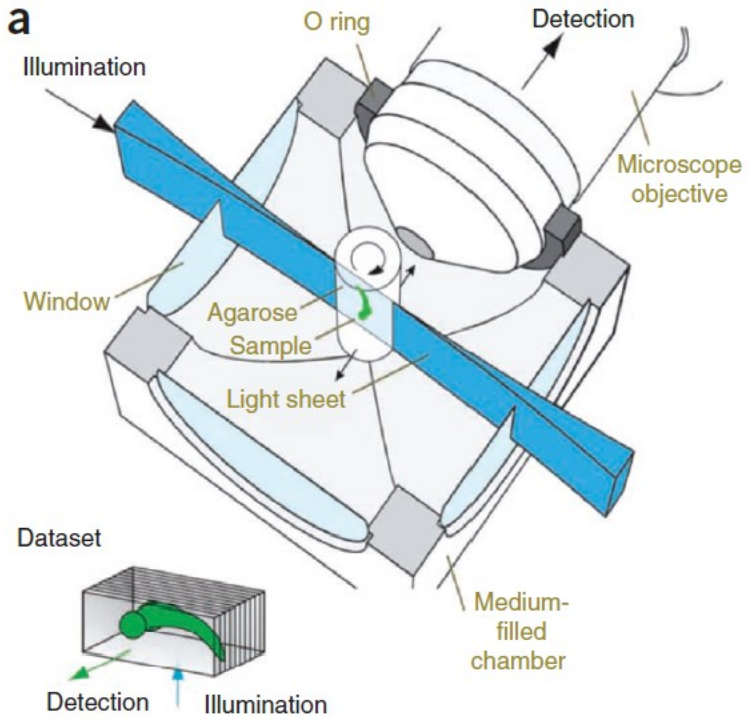
Deep-tissue Microscopic Imaging 1/4

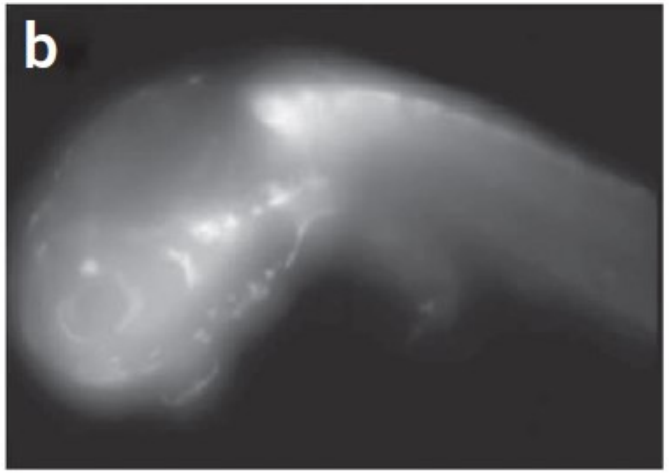
- Confocal and multiphoton microscopy
    - have been used extensively for *in vivo* imaging of fluorescent proteins, probes or dyes to investigate structure, function and molecular events as they occur in unperturbed environments

- Optical projection tomography
    - chemically clean the specimen, then use an approach similar to XCT for acquiring the data and reconstructing it; applicable only post-mortem

Deep-tissue Microscopic Imaging 2/4

- Selective plane illumination microscopy
  - SPIM has been shown to image with 6-µm resolution up to a depth of about 500 µm (~1–2 MFP) in semitransparent medaka fish, attaining resolution improvements over volumetric tissue illumination





Deep-tissue Microscopic Imaging 3/4

- Optoacoustic microscopy
    - powerful approaches for imaging optical absorption in tissues
    - the ultrasound waves are generated by the transient thermoelastic expansion of light absorbing structures, following transient local temperature rise owing to molecules that absorb energy from the photon pulse

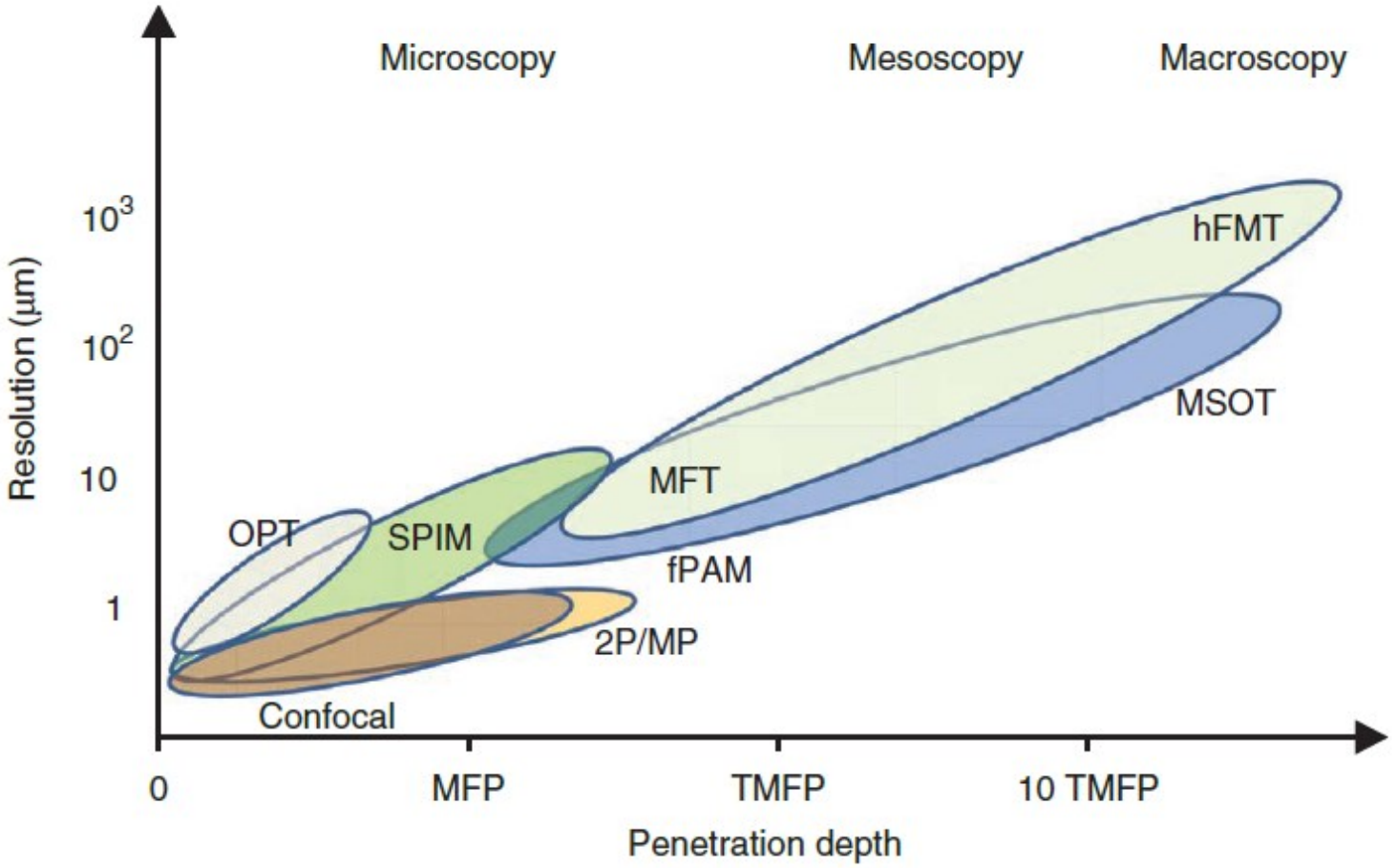
- fPAM (functional photoacoustic microscopy)
    - limitation: ultrasonic attenutation
    - fPAM can offer substantial flexibility in visualizing optical contrast at greater depths than other optical microscopy methods with ~10–20-µm resolution
    - As in confocal or 2P/MP microscopy, fPAM images are generated from raster scans by piecing together information collected from different foci
    - three-dimensional images may be generated with two-dimensional scans

Deep-tissue Microscopic Imaging 4/4

- Optical coherent tomography
    - illuminates tissue with light of low coherence and detects back-reflected light based on coherence matching between the incident and reflected beams using an interferometric approach

- Contrast enhancement methods
    - capturing contrast associated with different biochemical parameters of the molecules involved in image generation

Comparison


Microscopy
Mesoscopy
Macroscopy
Resolution (µm)
10^3
10^2
10
1
hFMT
MSOT
MFT
OPT
SPIM
fPAM
2P/MP
Confocal
0
MFP
TMFP
10 TMFP
Penetration depth

Macroscopy 1/2

- Optical system + (MRI or XCT) = hybrid implementations

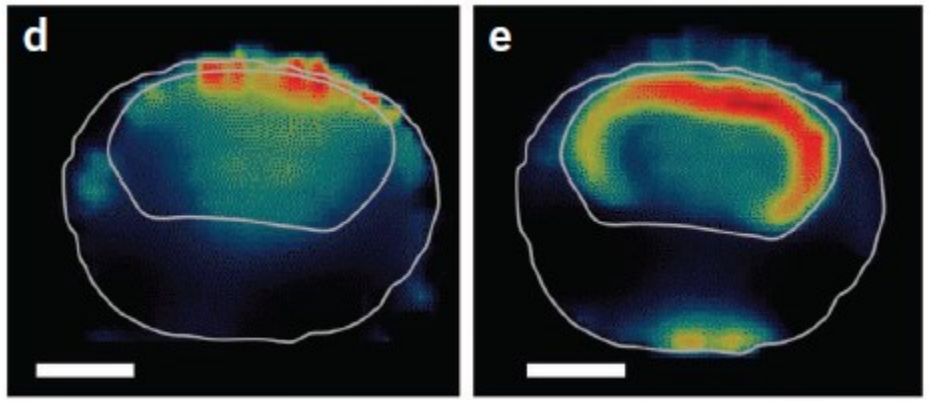
- Macroscopic applications can be considered for small animal imaging (mouse and rat), imaging of certain organs or in endoscopic and intraoperative applications

- Light attenuation depends both on tissue scattering and tissue absorption

- 3-6 cm for muscle or brain, 10-12 cm for breast tissue

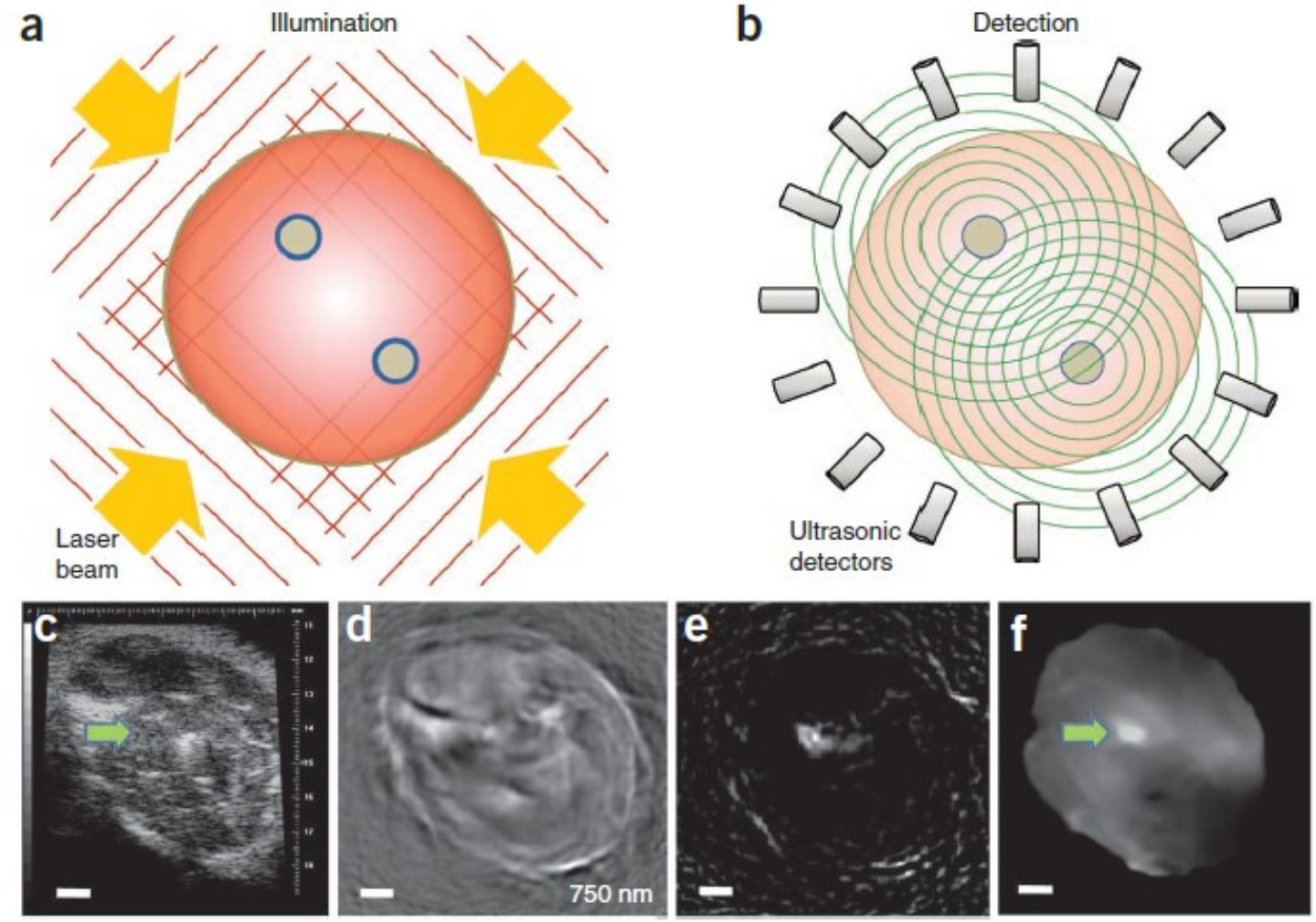
Macroscopy 2/2

- Hybrid fluorescence molecular tomography
  - different methods combined
  - image prior

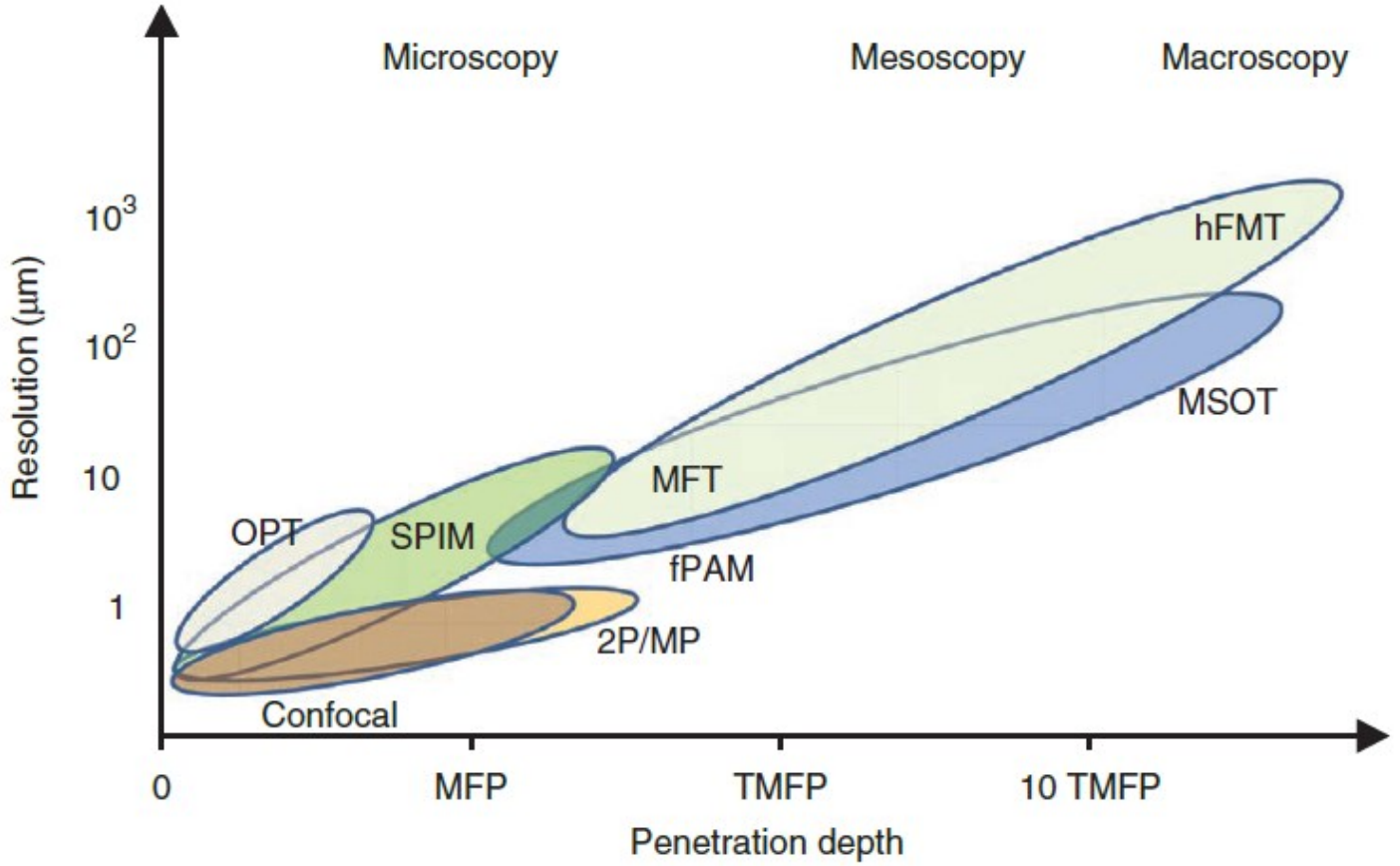


- Multispectral optoacoustic tomography
  - tomographic reconstruction in this case is based on mathematical inversion methods, using a model of acoustic and possibly of photon propagation in tissue
  - challenge: accurate quantification

Multispectral Optoacoustic Tomography (Spectral Photoacoustic Tomography)



Comparison 1/2



Comparison 2/2

Table 2 | Summary of performance characteristics

	Resolution (μm)	Penetration depth	Sensitivity	Phototoxicity	Cost/ complexity[a]	Inversion (versus scan)
Confocal	<1	2–3 MFP	<nM	+++	++	No
2P/MP	<1	3–4 MFP	<nM	+++	+++	No
OPT	~1–10	~0.2 MFP[b]	<nM	++	+	Yes
SPIM	0.5–10	<1 MFP	<nM	++	+	No
fPAM	5–20	~1 MFP to few TMFP	nM	++	+++	No
MFT	>20	~1 TMFP	nM	++	+	Yes
MSOT[c]	>20	>1 TMFP	nM–μM	++	+++	Yes
hFMT	>500	>1 TMFP	nM	+	++	Yes

[a]More plus signs indicate greater cost and/or greater complexity. [b]OPT can generate images at greater depths than 0.2 MFP but at strongly deteriorating image resolution and fidelity. [c]Metrics listed for mesoscopic and macroscopic MSOT.

Differences between methods

- Resolution and propagation depth

- Detection sensitivity: depends on the imaging depth, the time allowed for image acquisition and on the particular technology used

- Scanning techniques vs. reconstructing methods

Thank you.

references:

- Going deeper than microscopy: the optical imaging frontier in biology, V. Ntziachristos, Nature Methods 7:603-614, 2010