Light and tissue 1

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Outline

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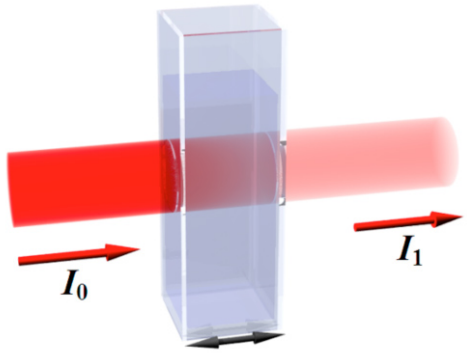
Motivation

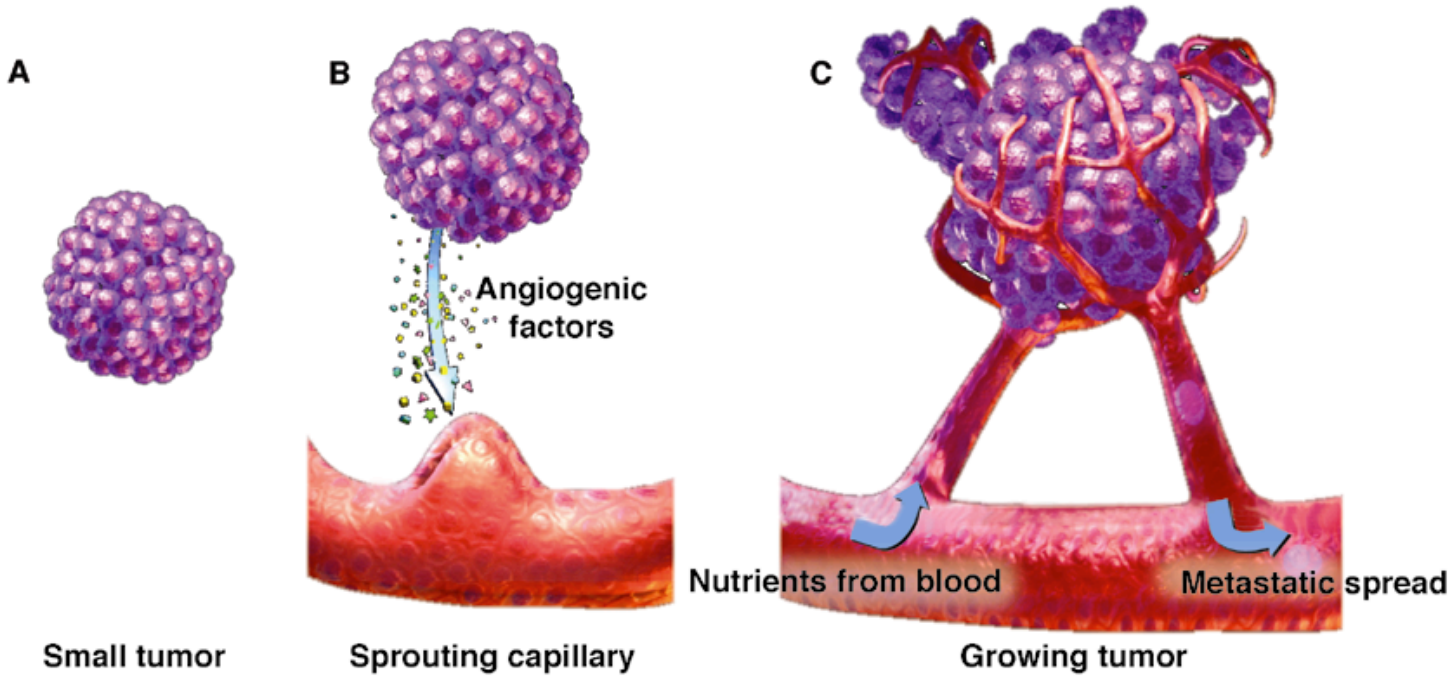


Absorption

Scattering

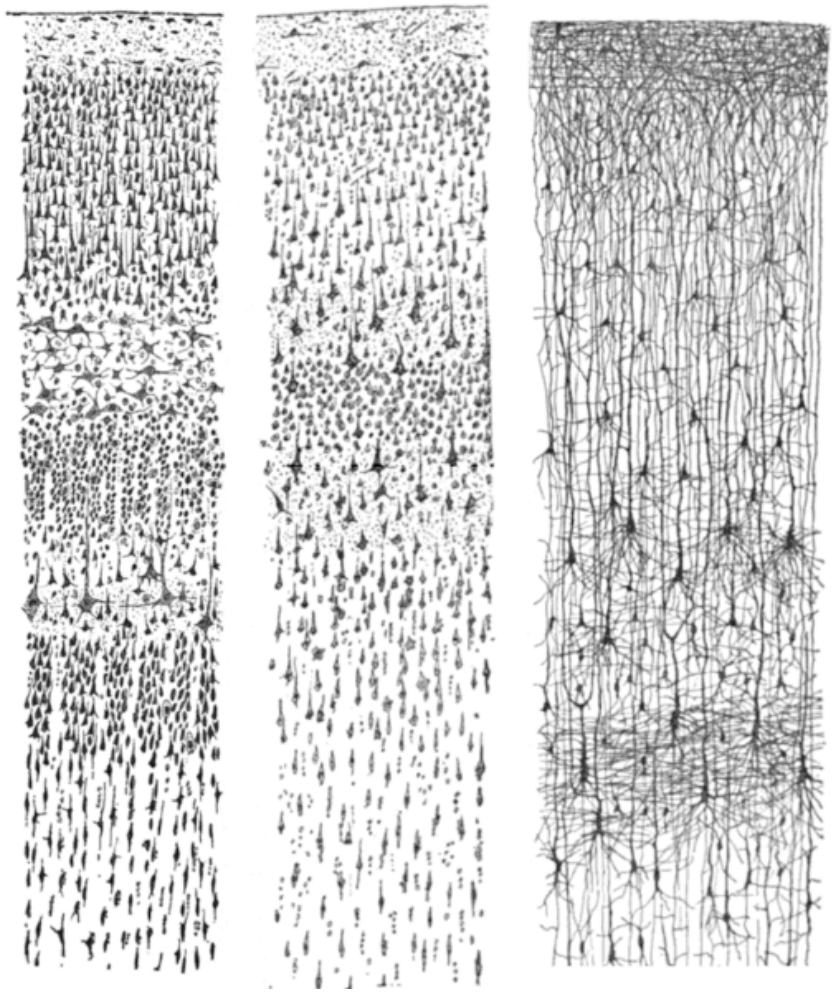
Pushing the limits

Cancer biology



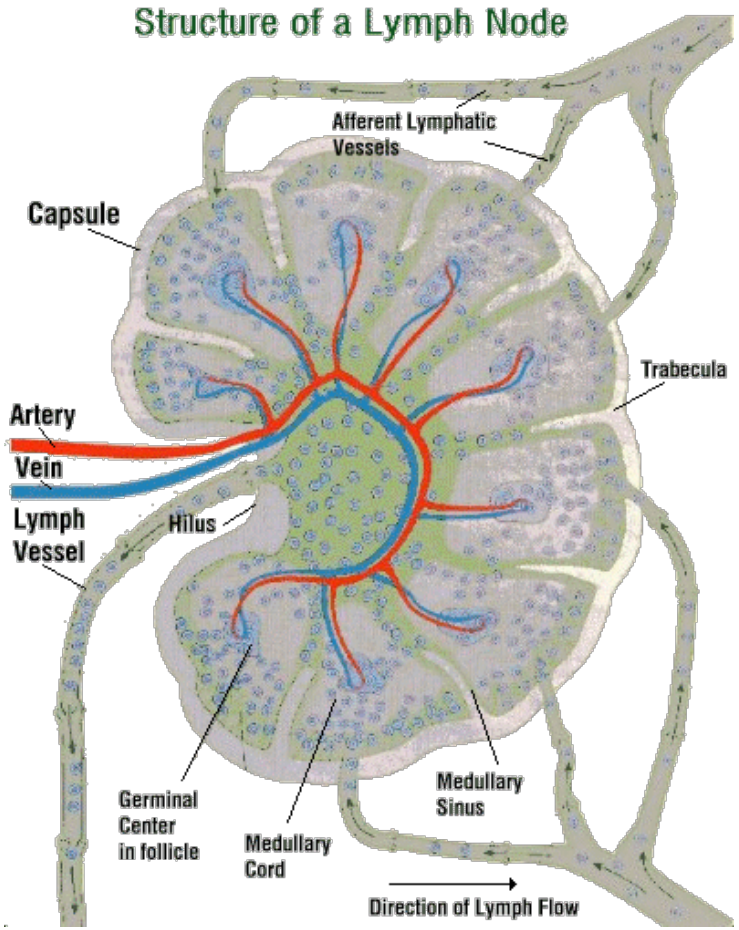
E.g. understanding how tumors form and interact with blood vessels

Neuroscience



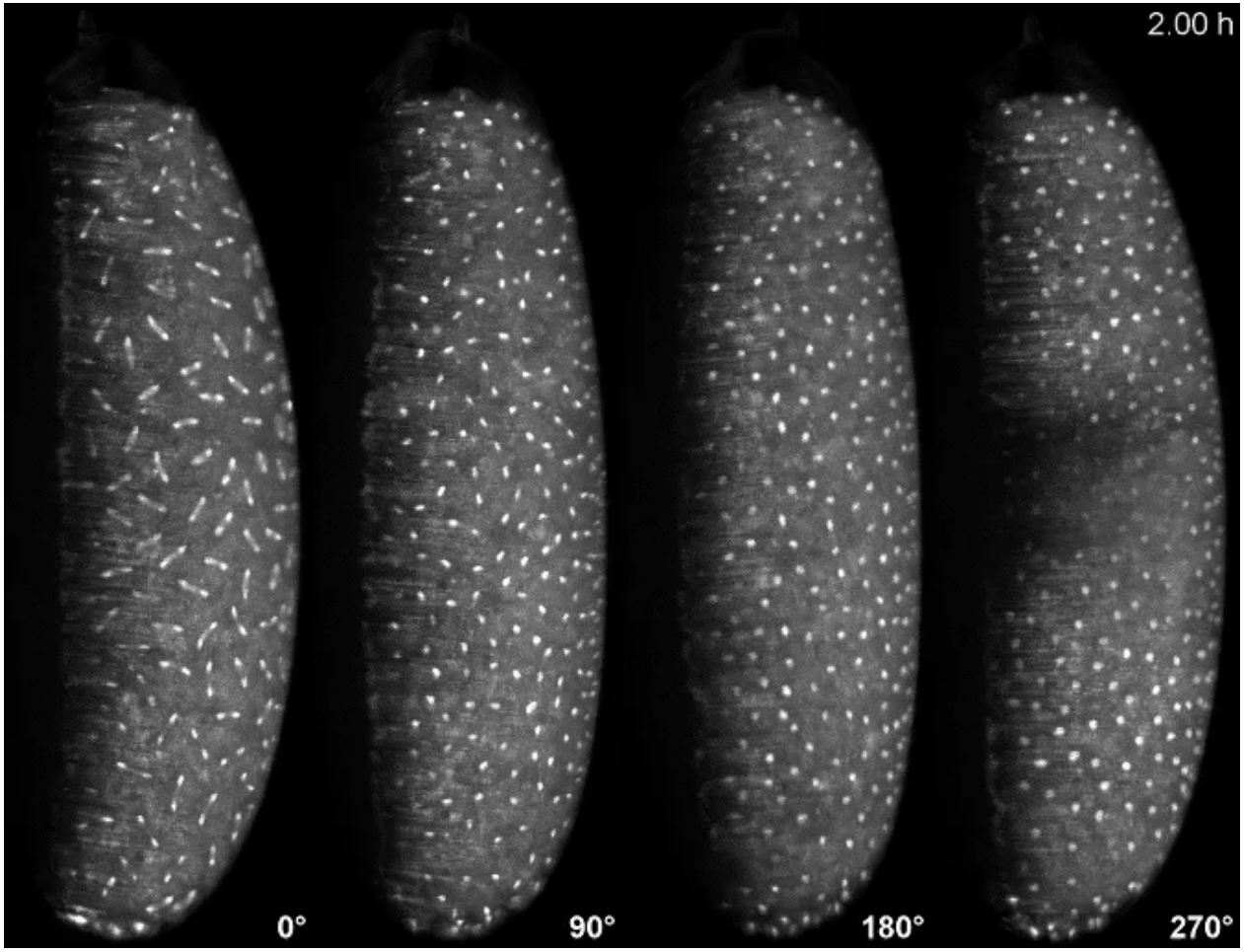
E.g. monitoring activity in deeper layers of the brain

Immunology



E.g. studying the biology of lymph nodes

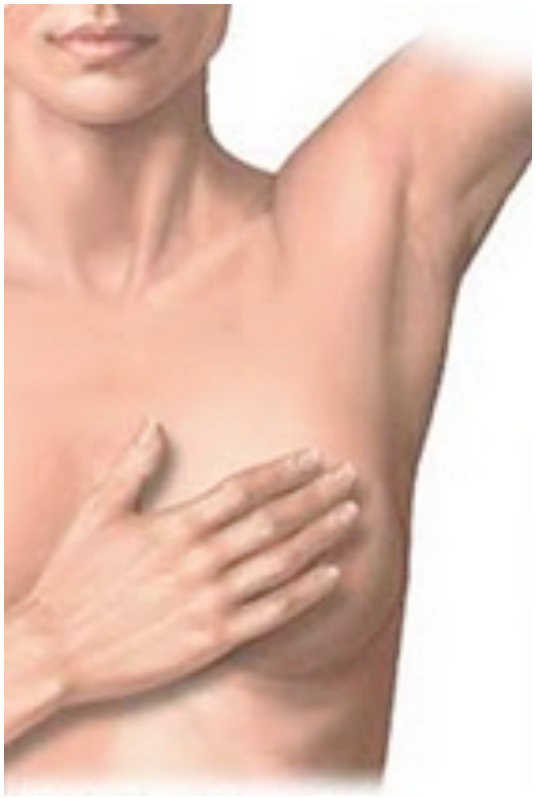
Developmental biology



E.g. monitoring early development in real time

Huisken et al (2004)

Diagnosis



E.g. early detection of tumors in deep tissues

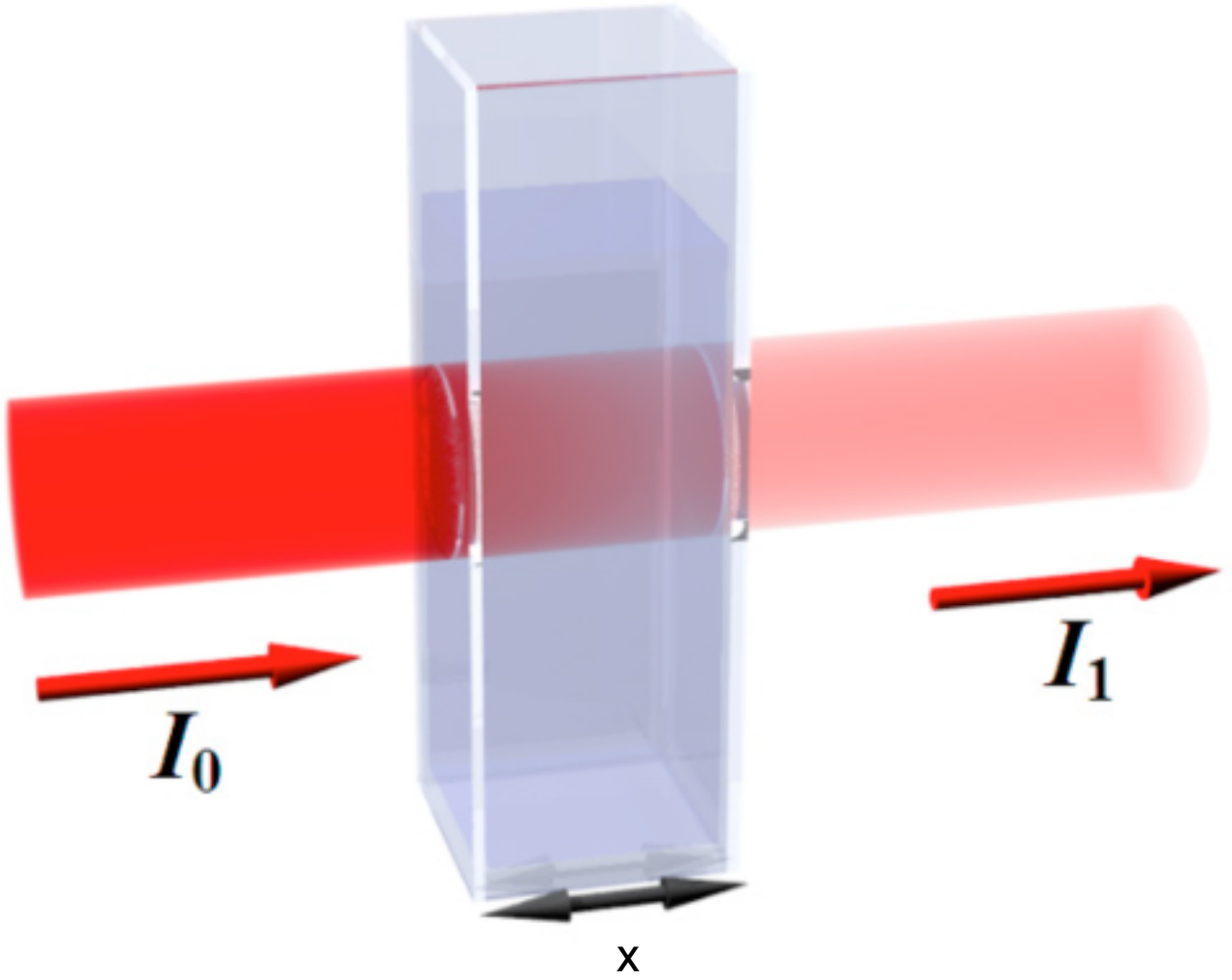
How deep can we see?

With visible light in biological tissues: less than 1 mm, often less than 100 µm

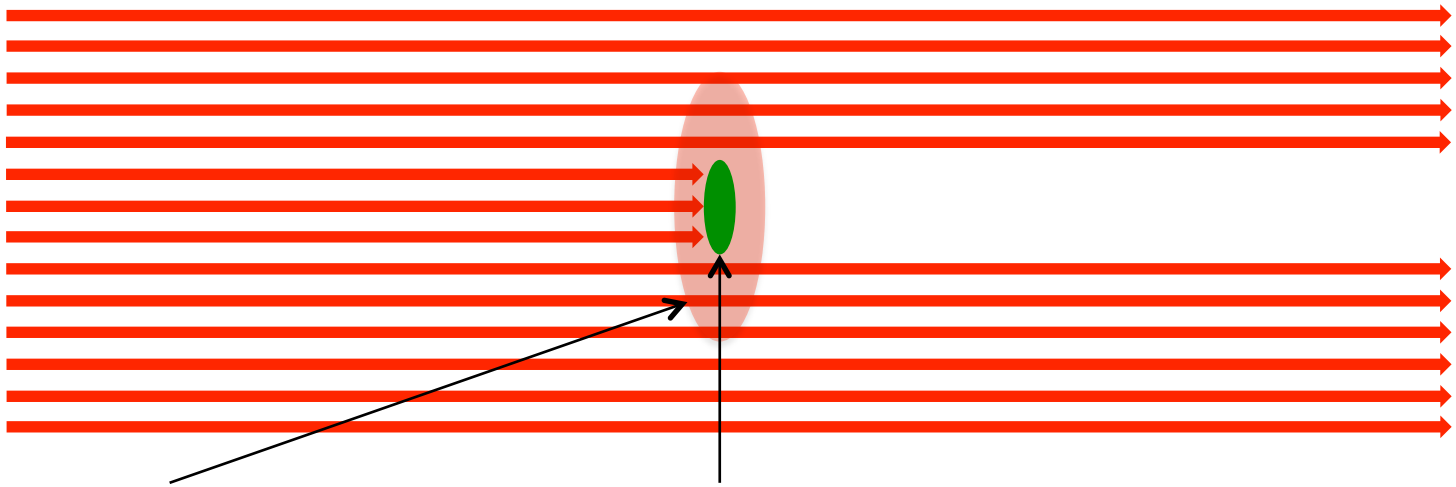
But there are ways to push the limits.

Absorption and Scattering

Absorption in a cuvette



Absorption cross-section



Geometrical cross-section [cm^2]

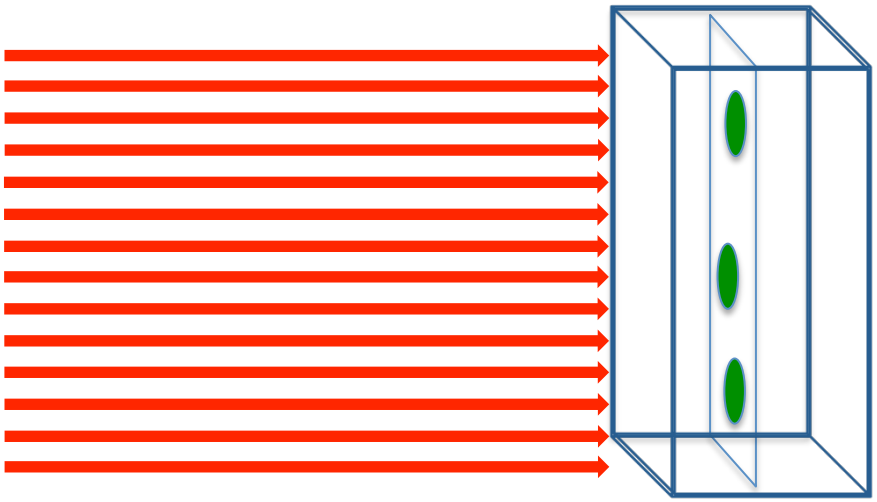
σ_a: Effective absorption cross-section [cm^2] per molecule

N: absorbers per unit volume [$1/cm^3$]

$$\mu_a = \sigma_a \cdot N$$

The absorption coefficient μ_a is the total absorption cross-sectional area per unit volume [cm^2/cm^3]

Deriving the Beer law



Slice area: A

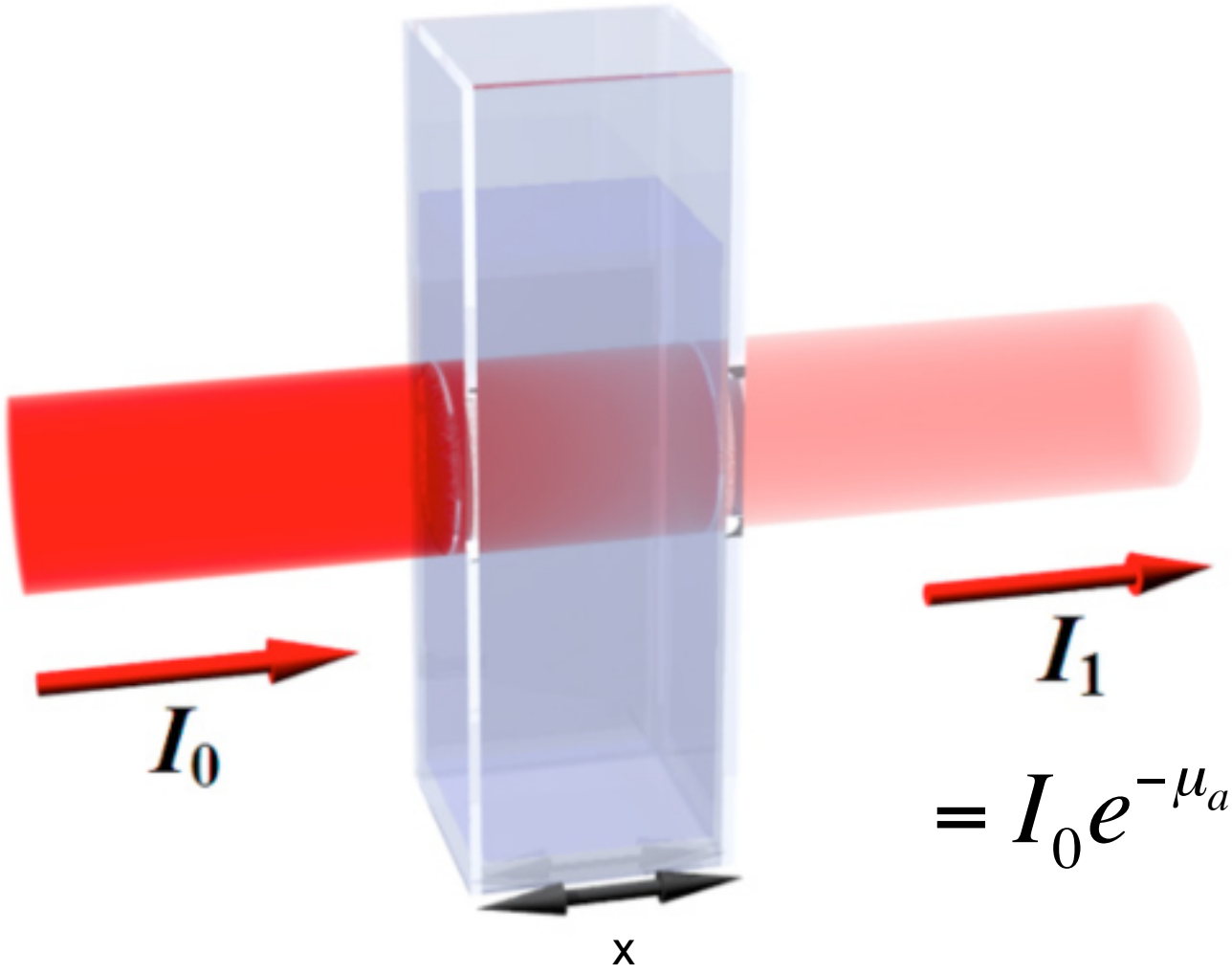
Slice volume: A·dx

Cross-sectional area
in slice volume: $\mu_a \cdot A \cdot dx$

The relative change in intensity equals the total absorption cross-sectional area in the slice divided by the slice area:

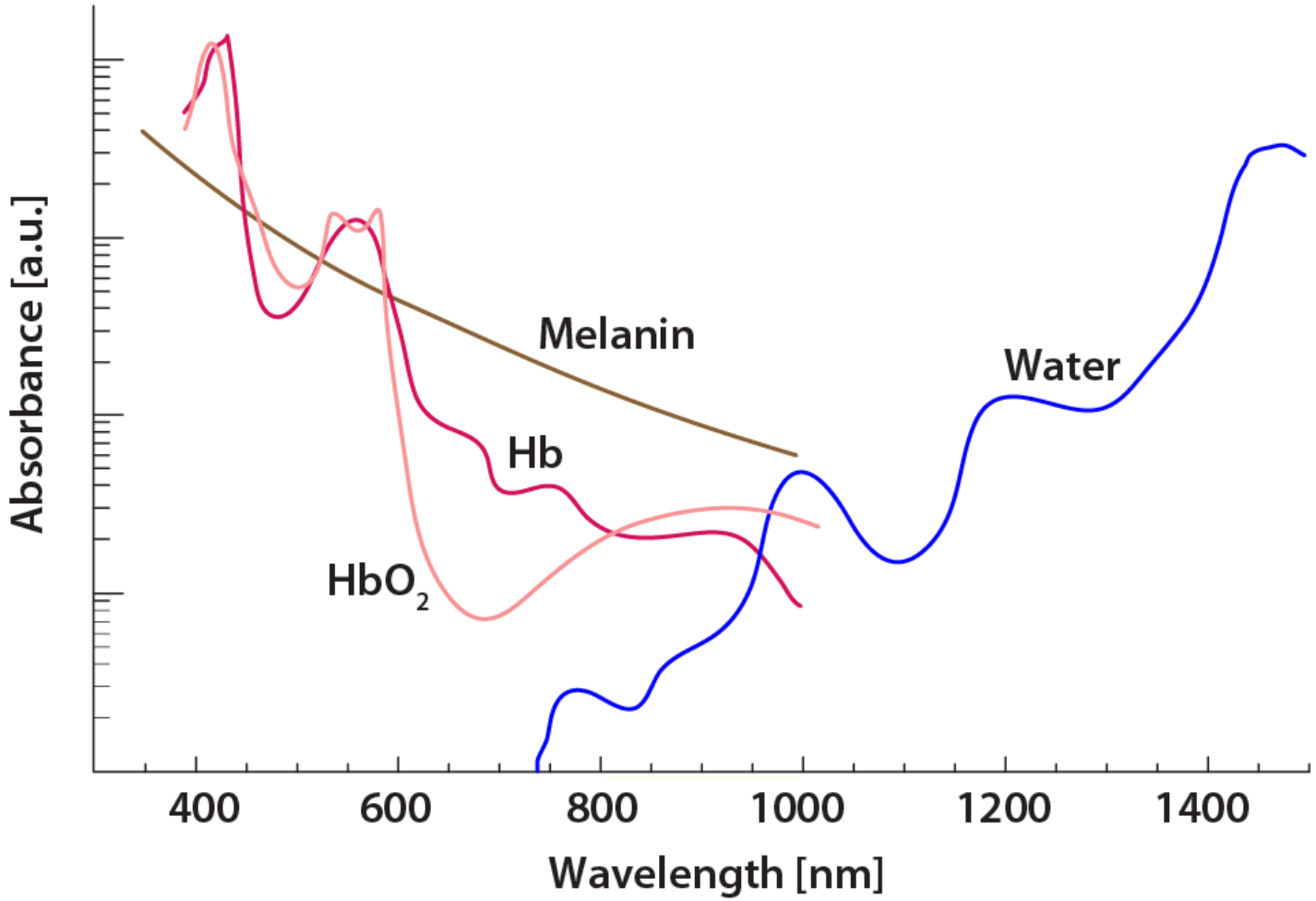
$$\frac{-dI}{I} = \frac{\mu_a \cdot A \cdot dx}{A} = \mu_a \cdot dx \quad \Rightarrow \quad I = I_0 e^{-\mu_a x}$$

Absorption in a cuvette

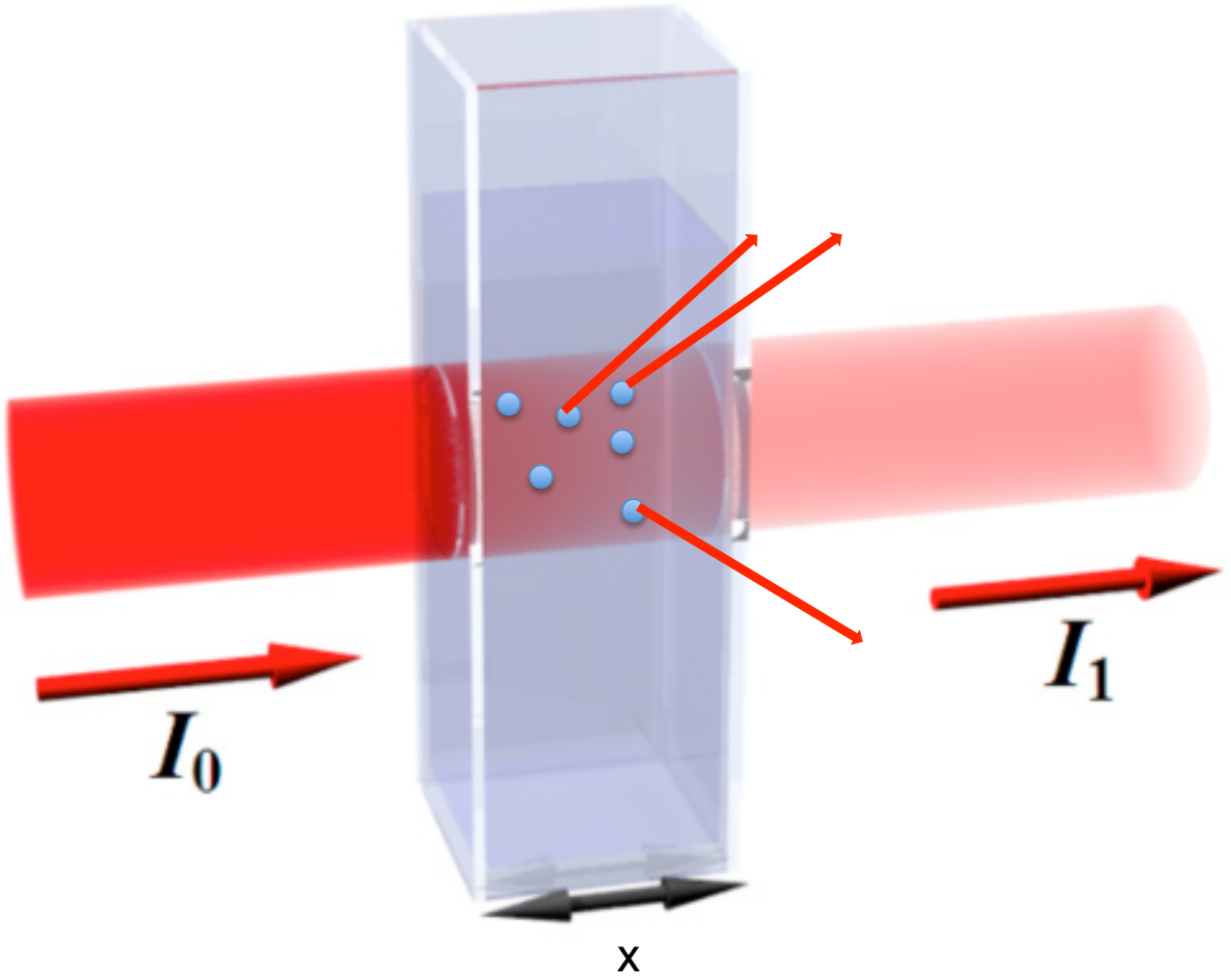


$$= I_0 e^{-\mu_a x}$$

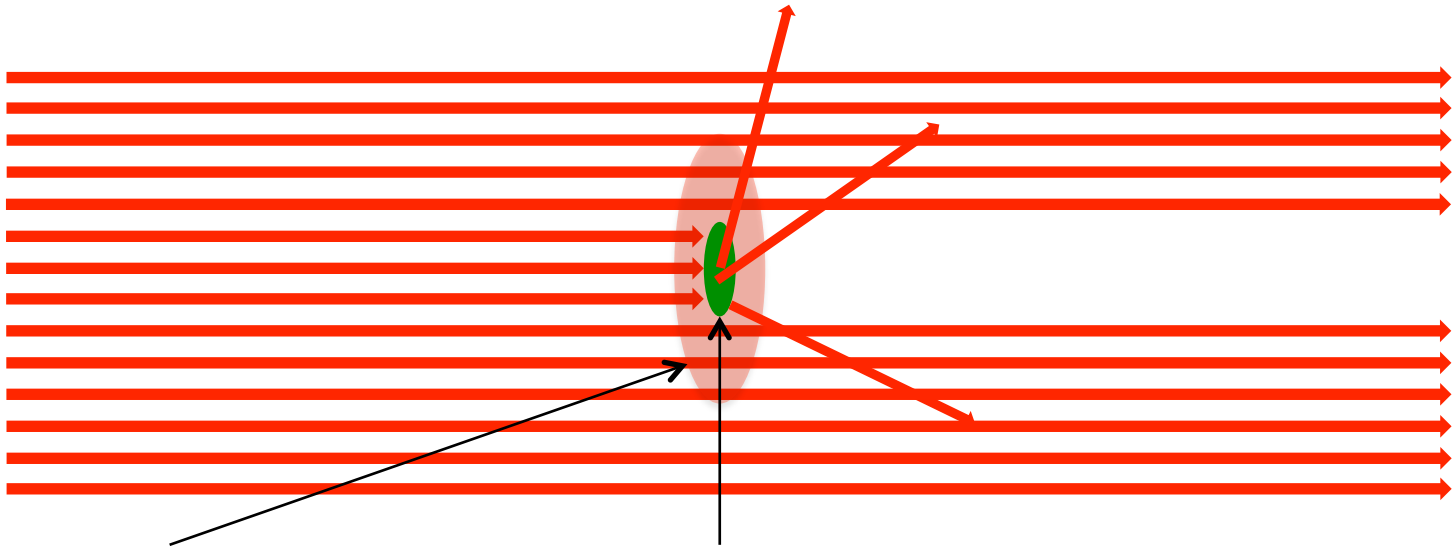
Absorption spectra



Scattering in a cuvette



Scattering cross-section



Geometrical cross-section [cm^2]

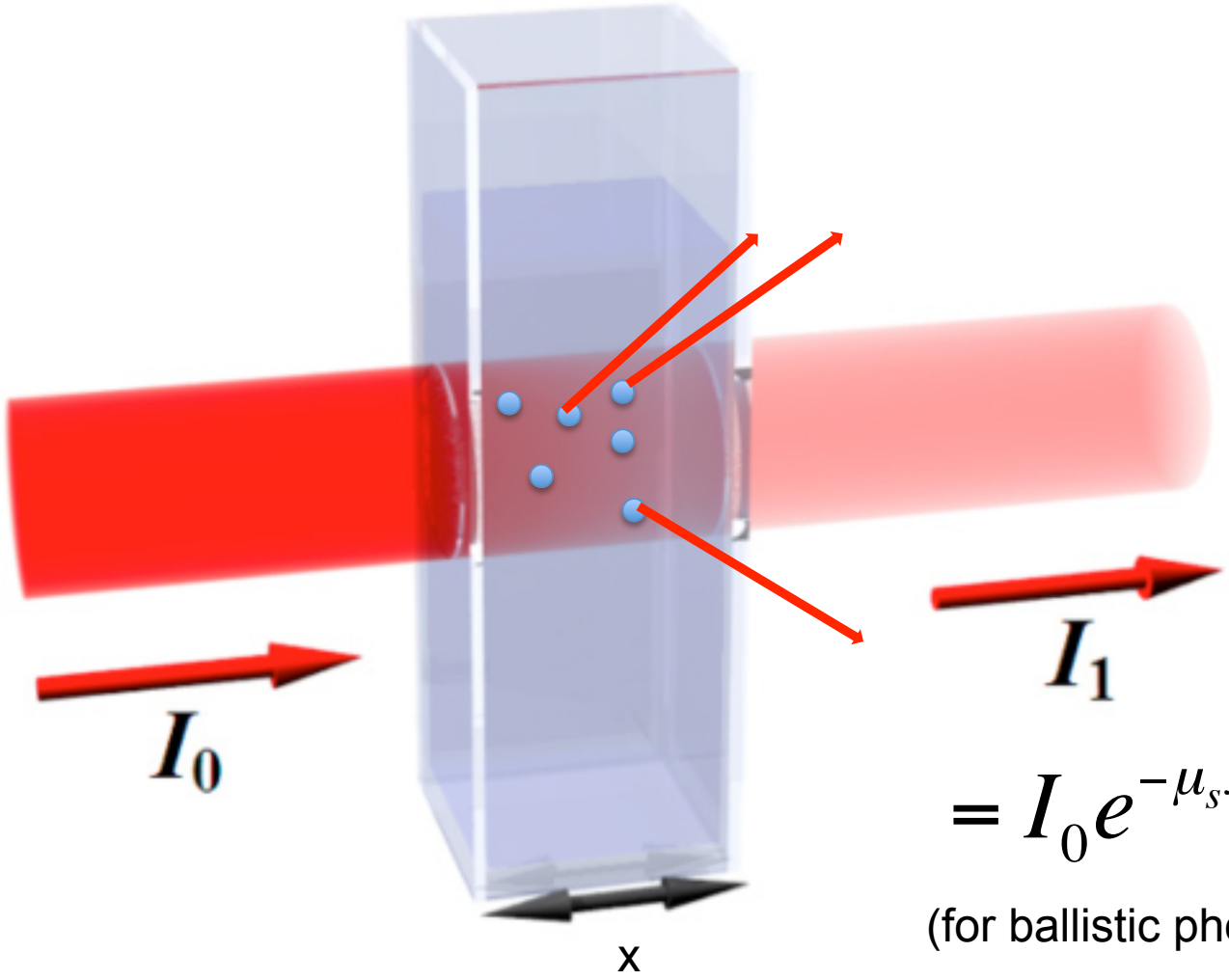
σ_s: Effective scattering cross-section [cm^2]

N: scatterers per unit volume [$1/cm^3$]

$$\mu_a = \sigma_a \cdot N$$

The scattering coefficient μ_a is the total scattering cross-sectional area per unit volume [cm^2/cm^3]

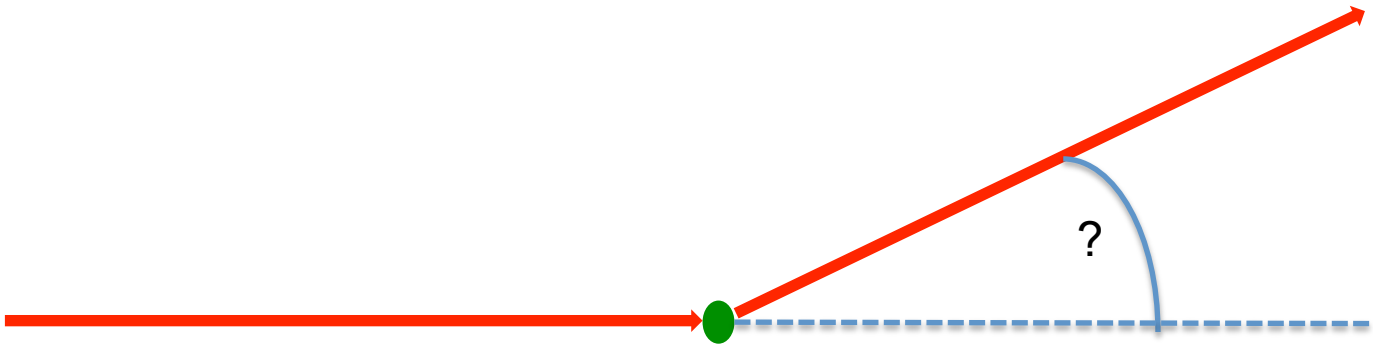
Beer law for scattering



$$= I_0 e^{-\mu_s x}$$

(for ballistic photons)

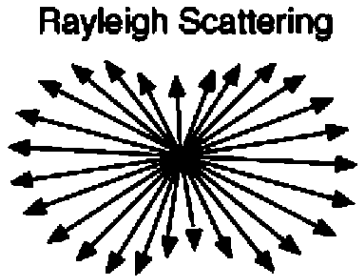
What happens to the scattered photons?



Scattering Regimes

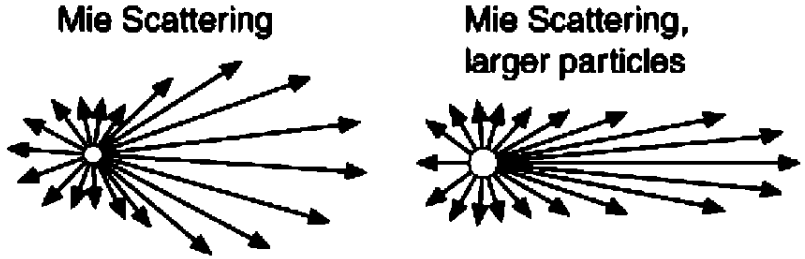
$< \lambda$ Rayleigh Regime

- E.g. particles in the sky
- Strongly wavelength dependent
- Mostly isotropic



$\geq \lambda$ Mie Regime

- Cells, water droplets (fog)
- Anisotropic: mostly forward scattering

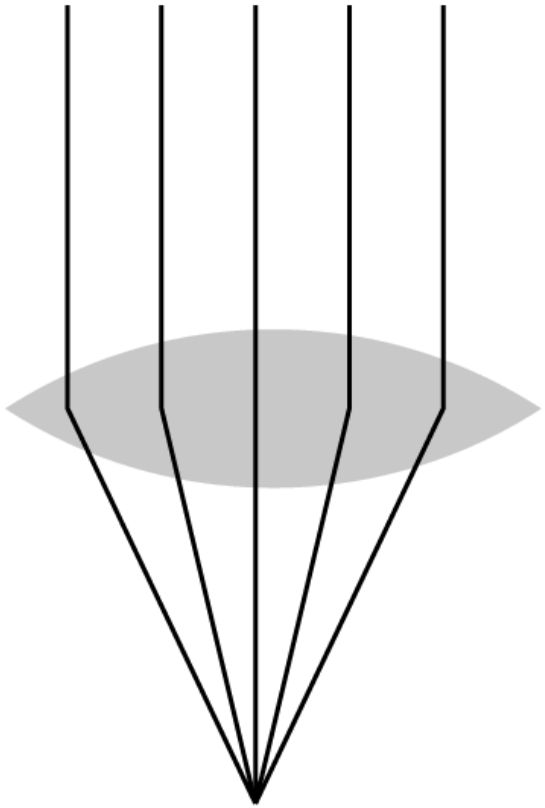


Anisotropy factor: g

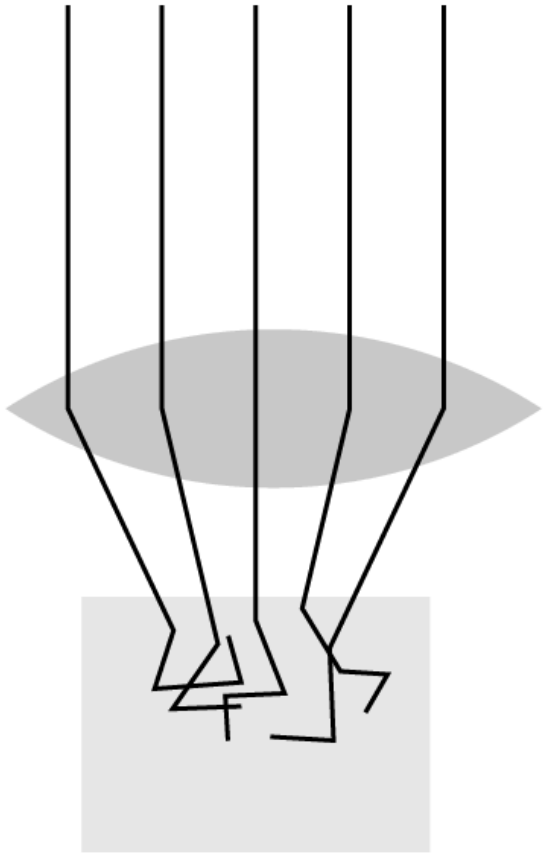
$$g = \begin{cases} -1 \ldots 0 & \text{Backward scattering (anisotropic)} \\ 0 & \text{Unidirectional scattering (isotropic)} \\ 0 \ldots 1 & \text{Forward scattering (anisotropic)} \end{cases}$$

g for most biological tissues: ~ 0.9
(highly forward scattering)

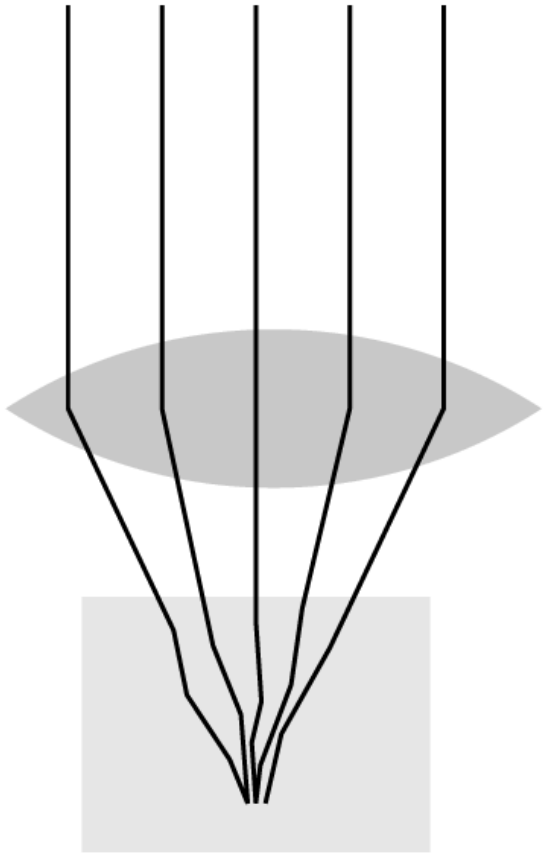
Imaging and anisotropy



Focusing without scattering

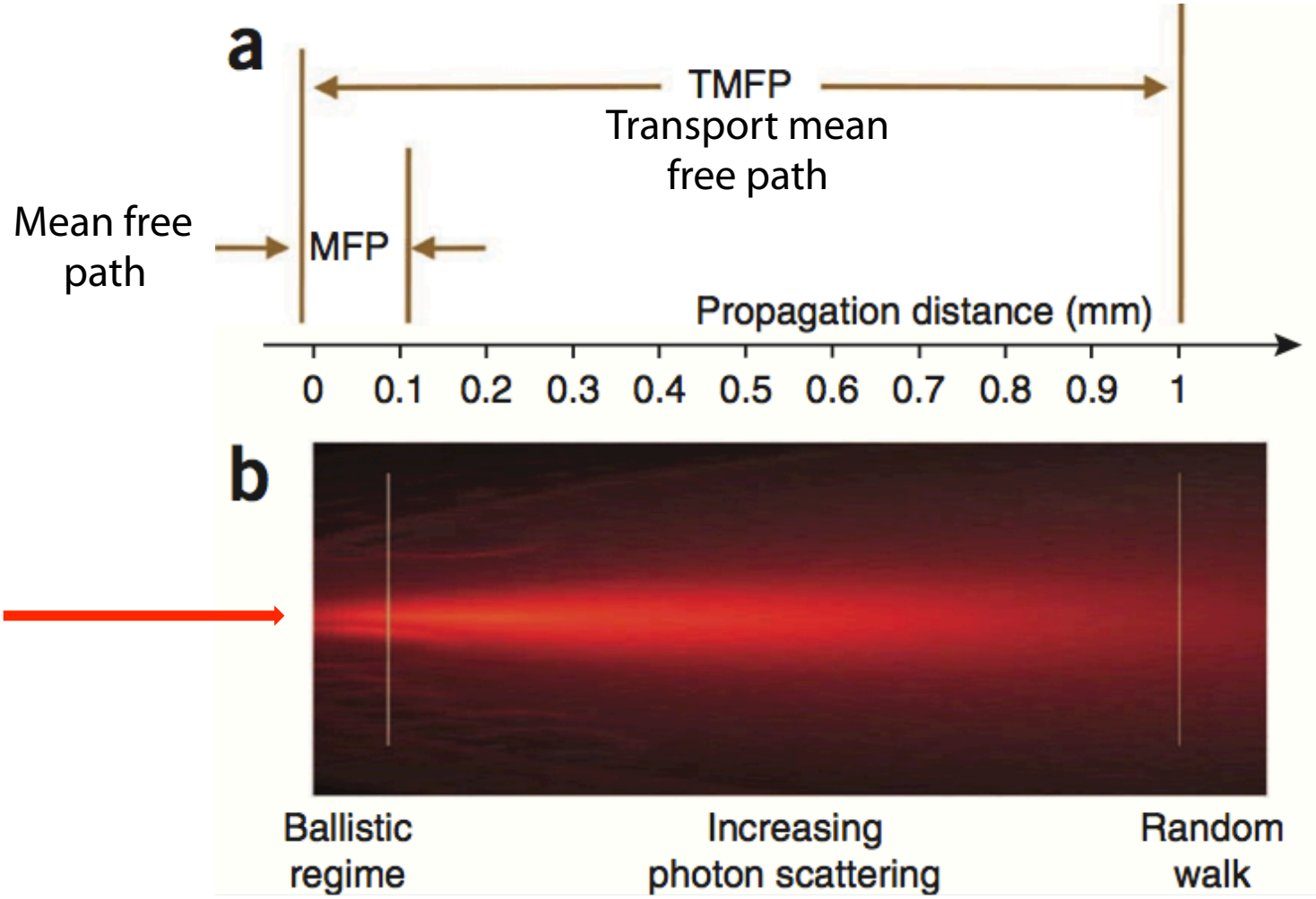


Focusing through isotropic scatterer (g = 0)



Focusing through forward scattering medium (high g)

Mean free path



Ntziachristos (2010)

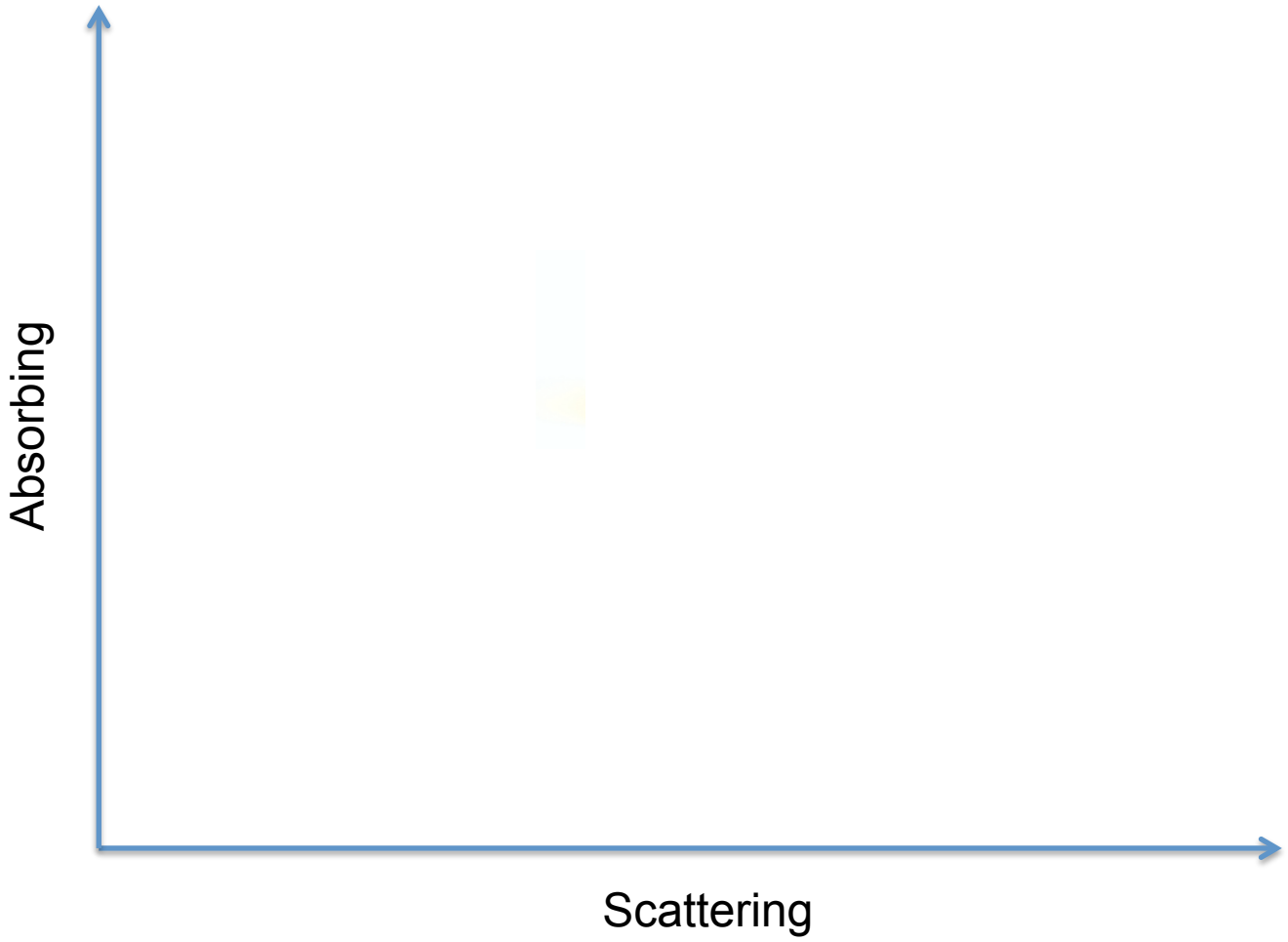
Mean free path

Mean free path: $\frac{1}{\mu_s}$

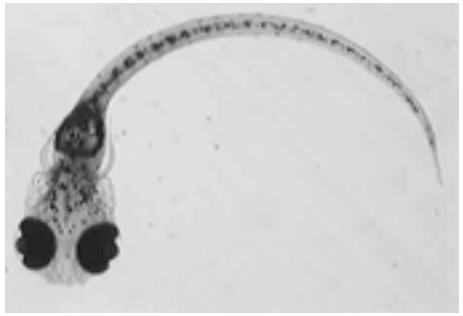
Transport mean free path: $\frac{1}{\mu_s \cdot (1-g)}$

- The scattering mean free path is the average distance between scattering events (in biological tissues around 100 μm)
- The transport mean free path can be thought of as the mean distance after which a photon's direction becomes random (in biological tissues around 1 mm)

Absorbing or scattering?

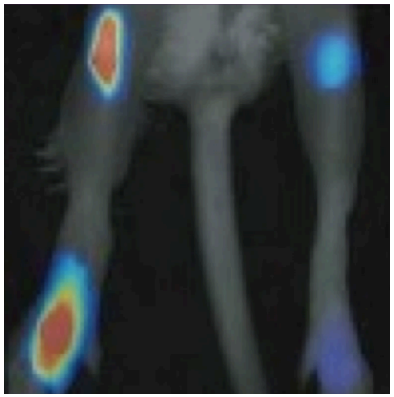


How do we image deep?

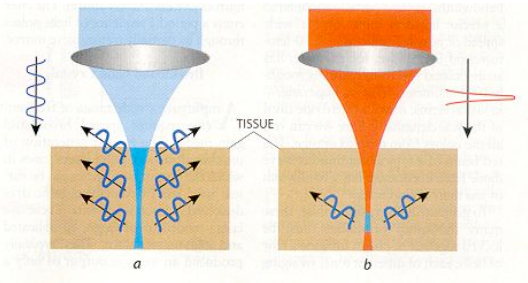


Choose transparent sample, long wavelength

Make sample transparent



Give up on resolution



Push the limits with modern microscopy

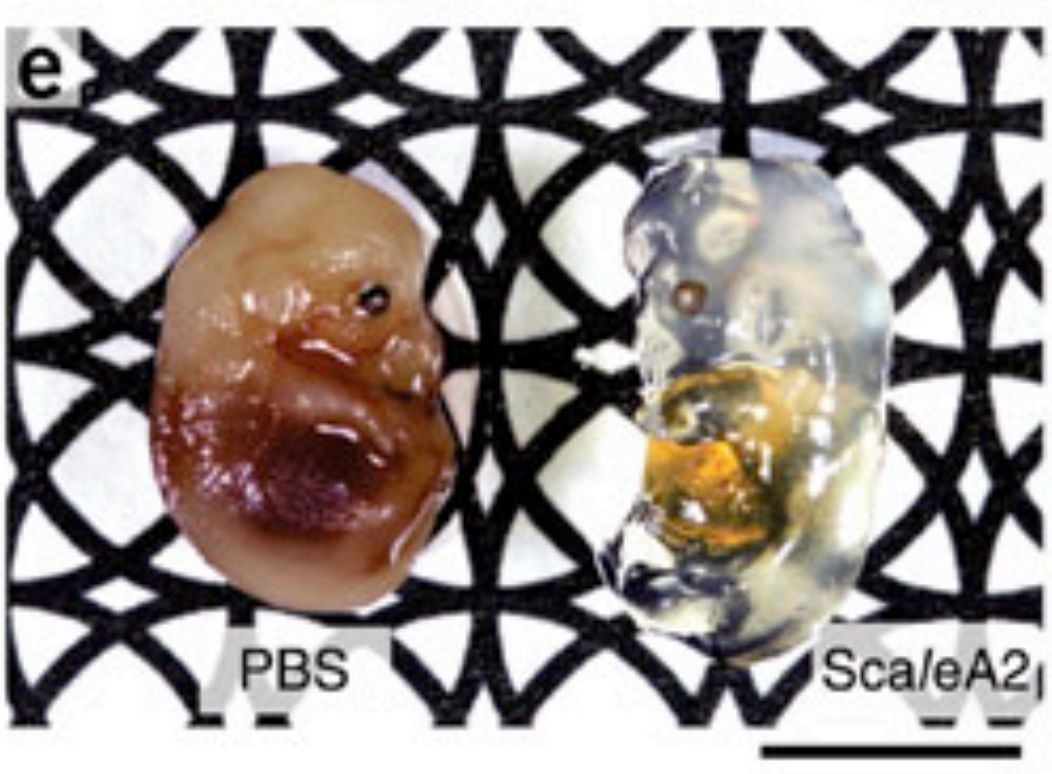
Choose transparent samples

Xenopus laevis tadpole

Make samples transparent

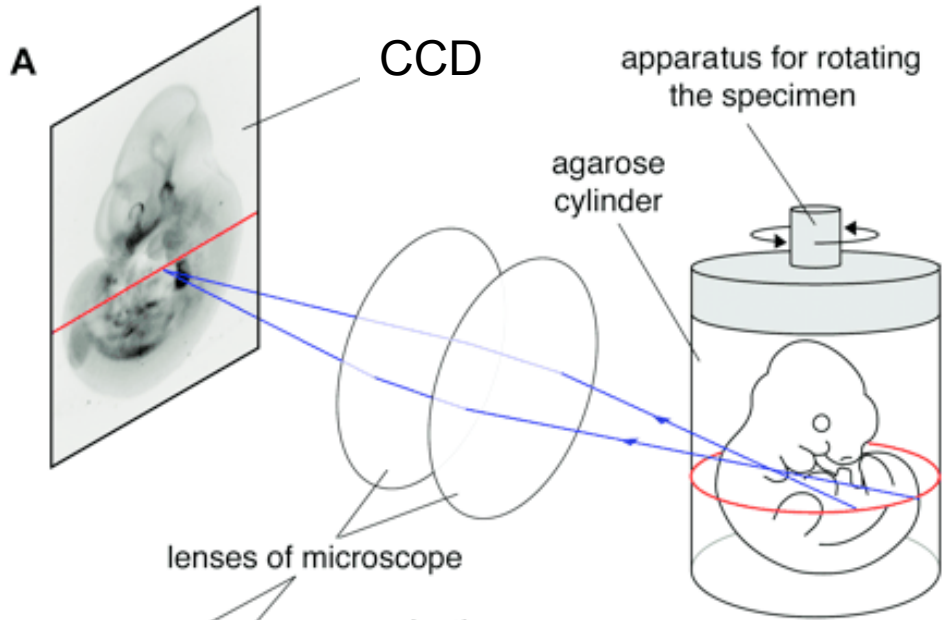
Jährling et al (2010)

Make samples transparent



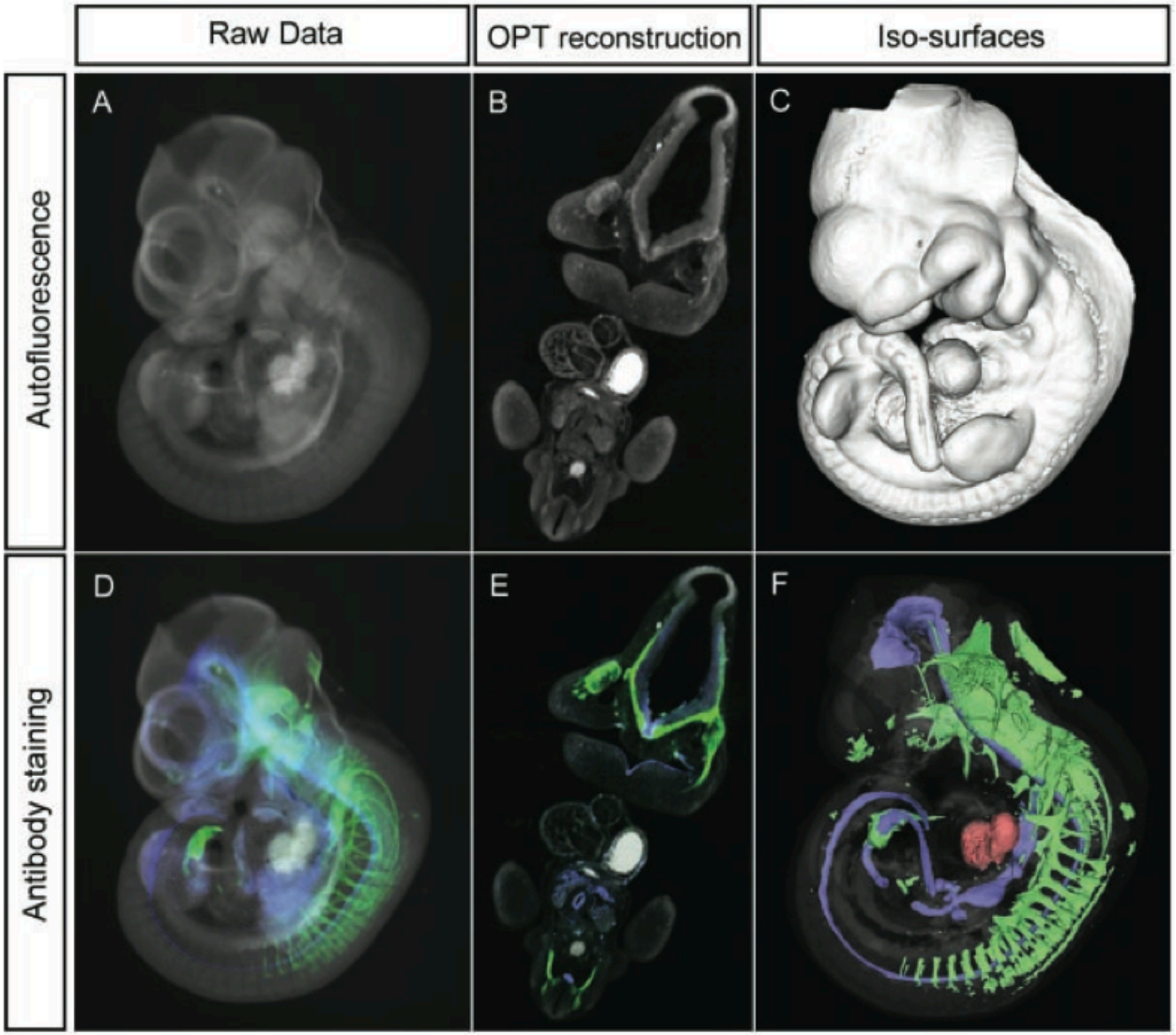
Hama et al (2011)

Optical projection tomography



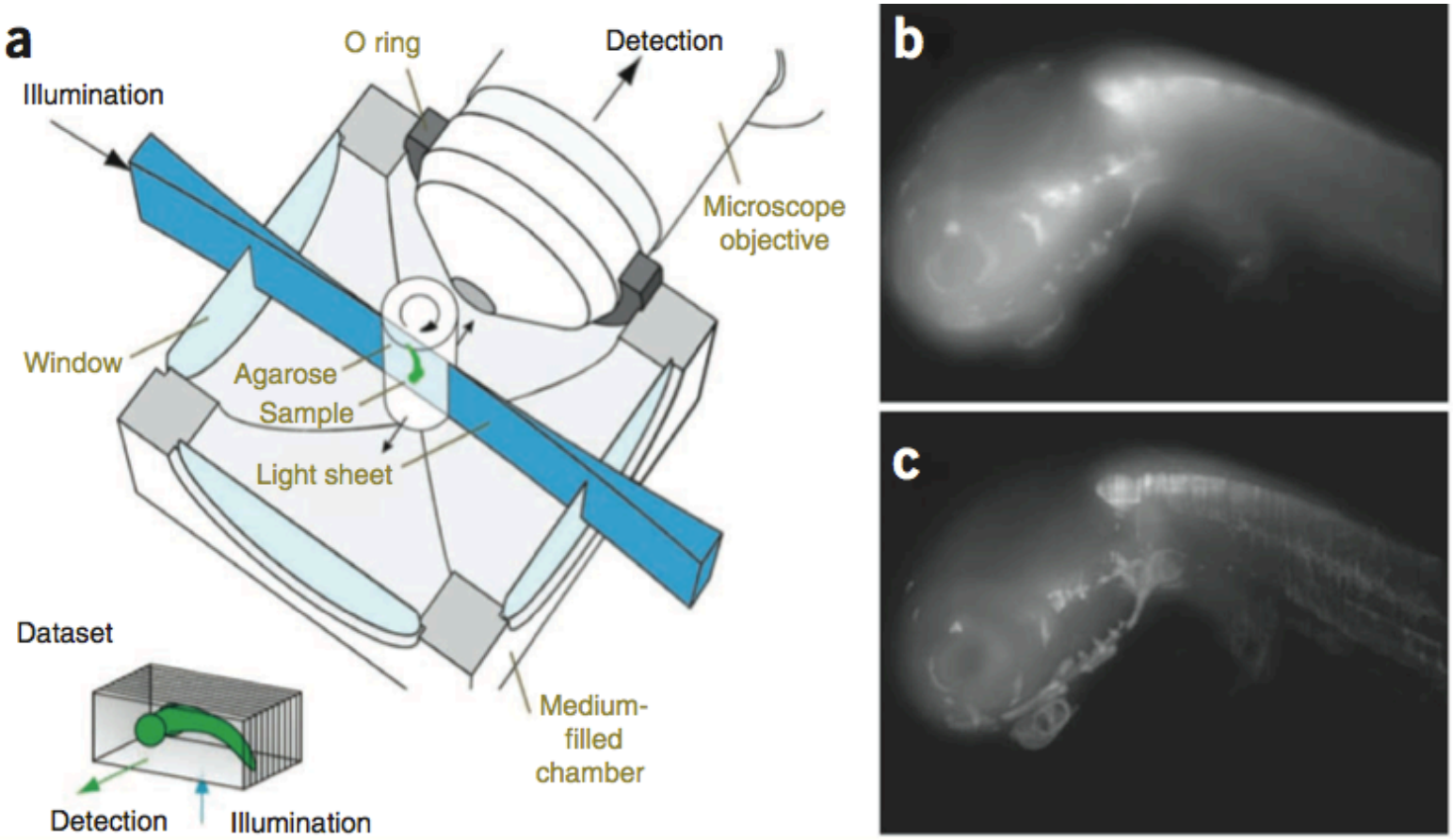
Sharpe et al (2002)

Optical projection tomography



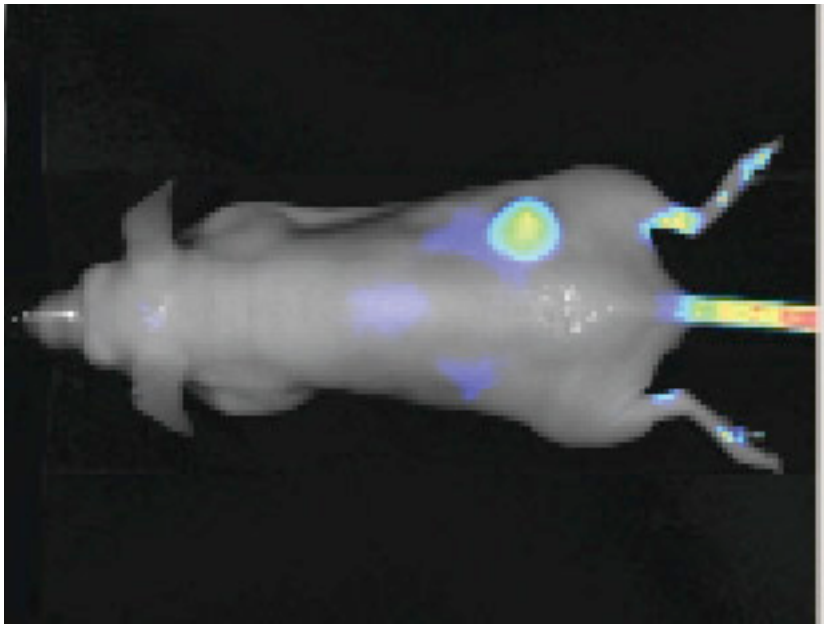
Sharpe et al (2002)

Sheet illumination



Ntziachristos (2010)

NIR fluorescence imaging

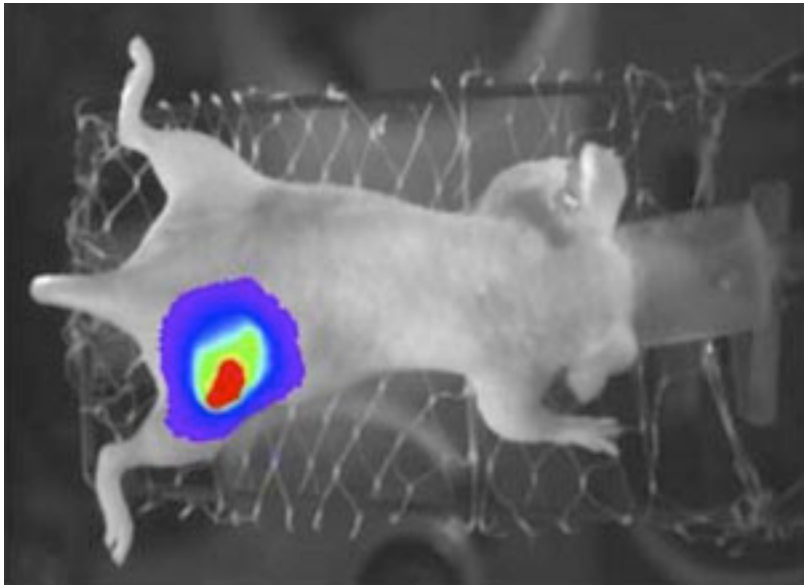


IR Dye emission at ~ 800 nm

Bioluminescence imaging

$$Luciferin + O_2 \xrightarrow{Luciferase} Oxyluciferin + Light\ (590nm)$$

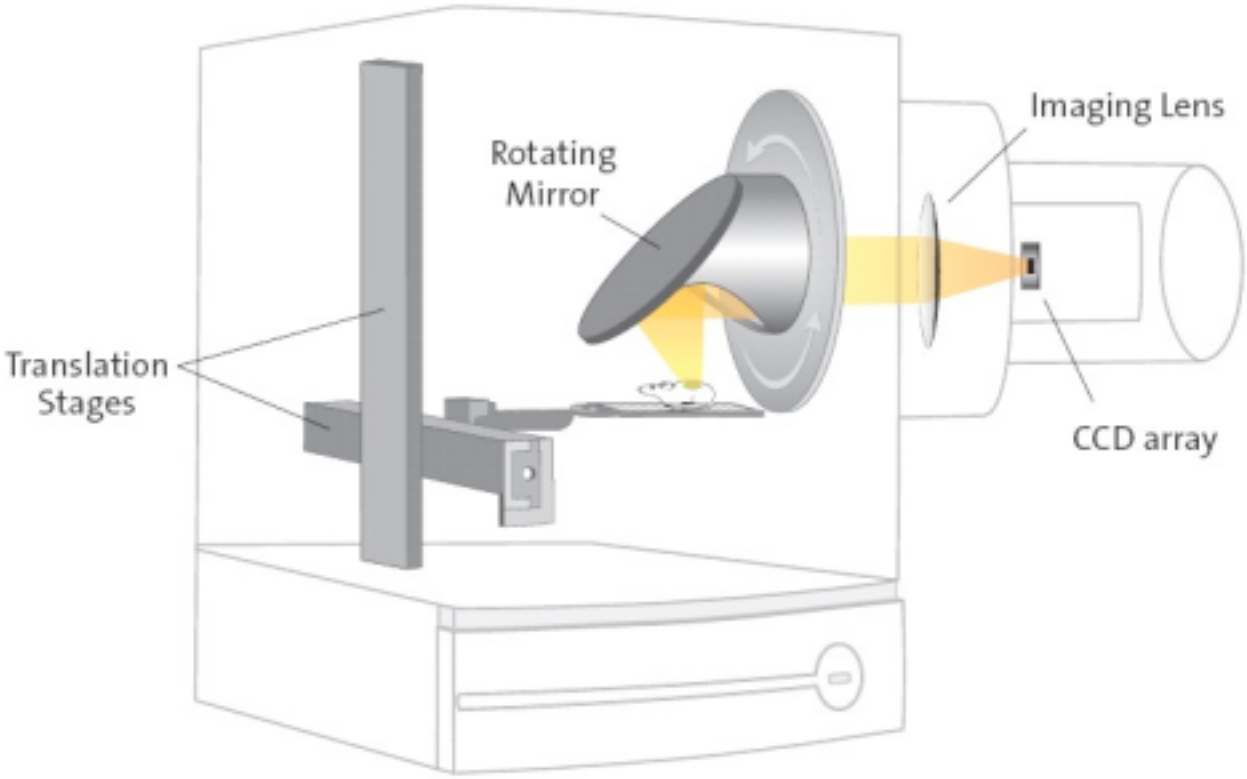
Bioluminescence imaging



Mouse grafted with cells genetically encoding luciferase
Imaging following injection of luciferin

No background autofluorescence.

Bioluminescence imaging



Summary

- In biological tissues, scattering dominates over absorption
- Scattering in most samples is anisotropic (high g)
- Scattering mean free path and transport mean free path are a measure of the penetration depth limit.
- Scattering and absorption are reduced at longer wavelengths

- To image deeper, the simplest solution is to use transparent samples
- Other samples can be cleared optically (but they need to be fixed)
- Optical projection tomography and sheet imaging can be used to image large transparent samples
- NIR fluorescence imaging and bioluminescence have a penetration depth of several mm, but sacrifice resolution.