Nonlinear Microscopy with Shaped Laser Pulses – Shedding New Light on Tissue

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Outline

- Linear and nonlinear optical microscopy
- Novel contrast mechanisms
  - ➢ *Two-photon absorption (loss modulation / modulation transfer)*
  - ➢ *Self-phase modulation (spectral hole refilling)*
- Applications / Progress
  - ➢ *Melanin imaging*
    - Melanoma
  - ➢ *Hemoglobin imaging*
    - Angiogenesis
    - Tissue oxygenation
  - ➢ *Functional neuroimaging*

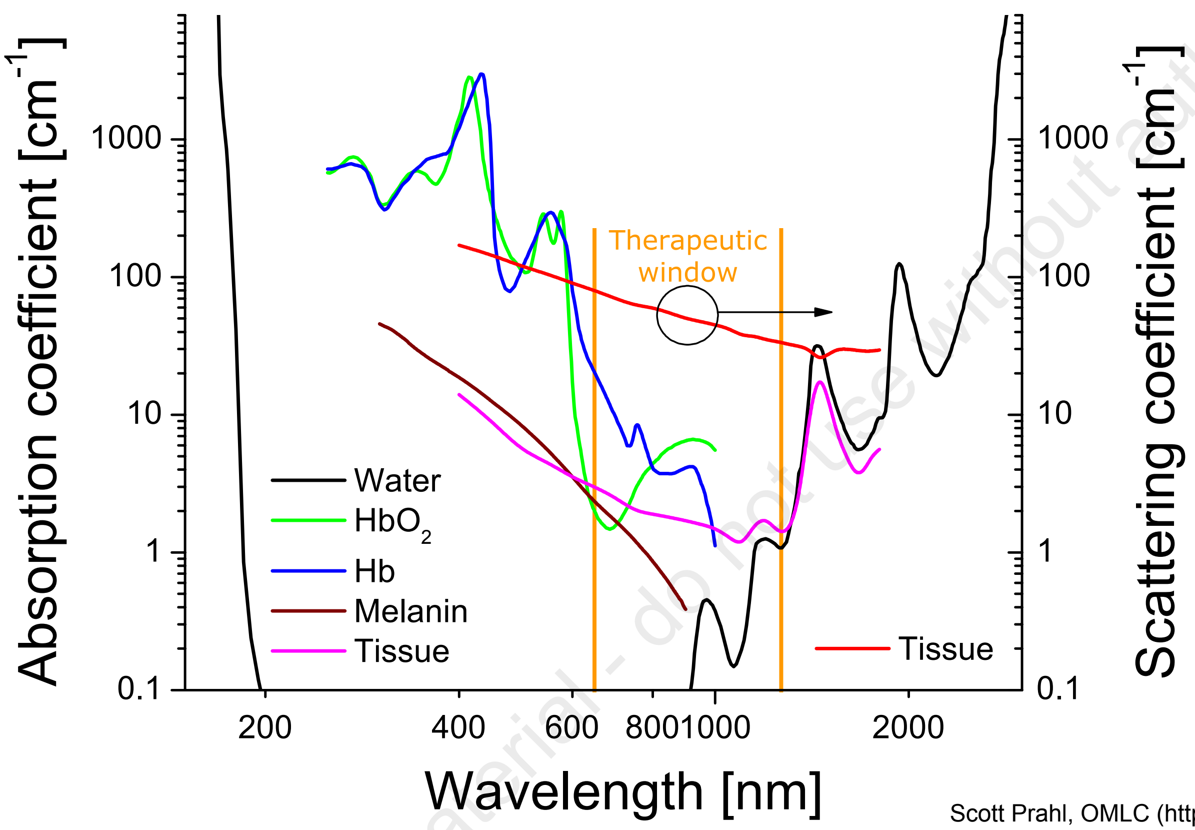
Linear Optical Microscopy

Requirement:	Optical Microscopy:
• High spatial resolution	✓
• High temporal resolution	✓
• Non-invasive	✓
• Deep penetration	?
• Structural contrast	✓
• Functional contrast	?

?

Contrast in deep tissue ???

Penetration Limitation in Tissue



- Absorption length:

λ	l_a
650 nm	3 mm
1300 nm	7 mm

- Scattering length:

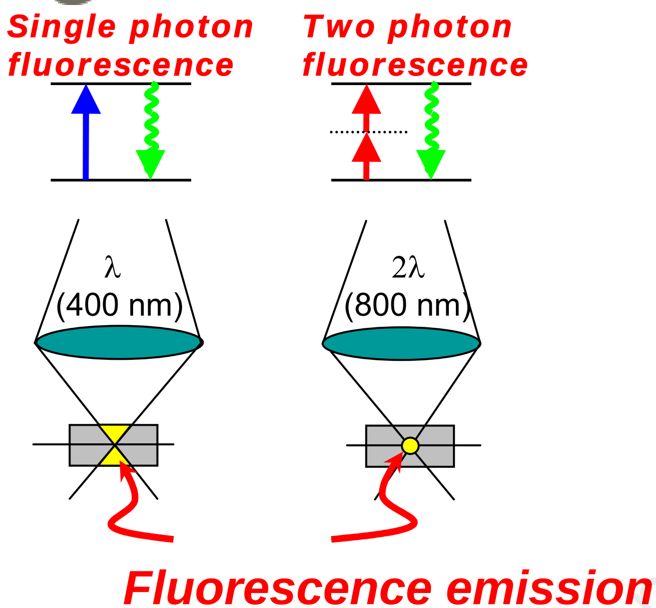
λ	l_s
650 nm	100 µm
1300 nm	250 µm

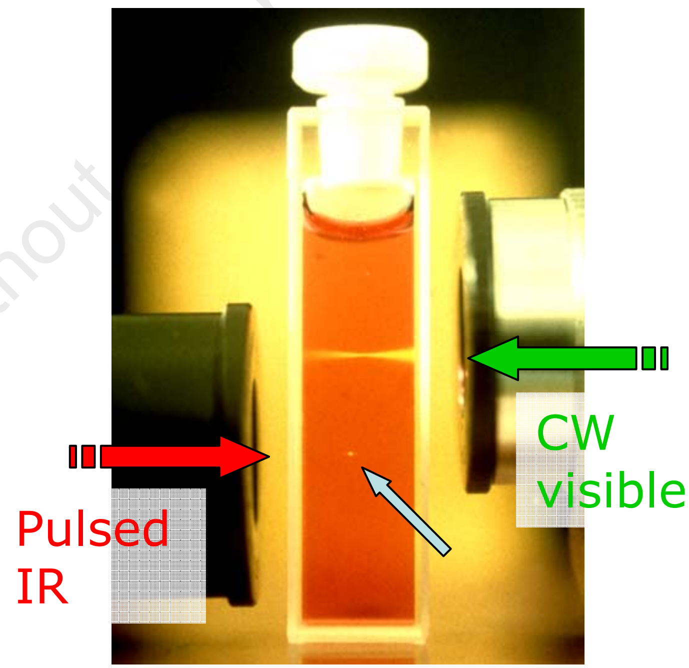
Scott Prahl, OMLC (http://omlc.ogi.edu/) , 943 (1996)
Tong Ye, Duke

- Scattering is major limitation
- Large penetration ⇔ Good contrast

Nonlinear Microscopy: Two-Photon Fluorescence

Brad Amos, Cambridge, UK





- Localized excitation (signal $\propto I^2$)
- High-efficiency collection
- Contribution to background from scattered light is small
- Long wavelength "indirect" excitation → small extinction
- Good penetration (~ 1 mm)

⇒ Contrast other than fluorescence ?

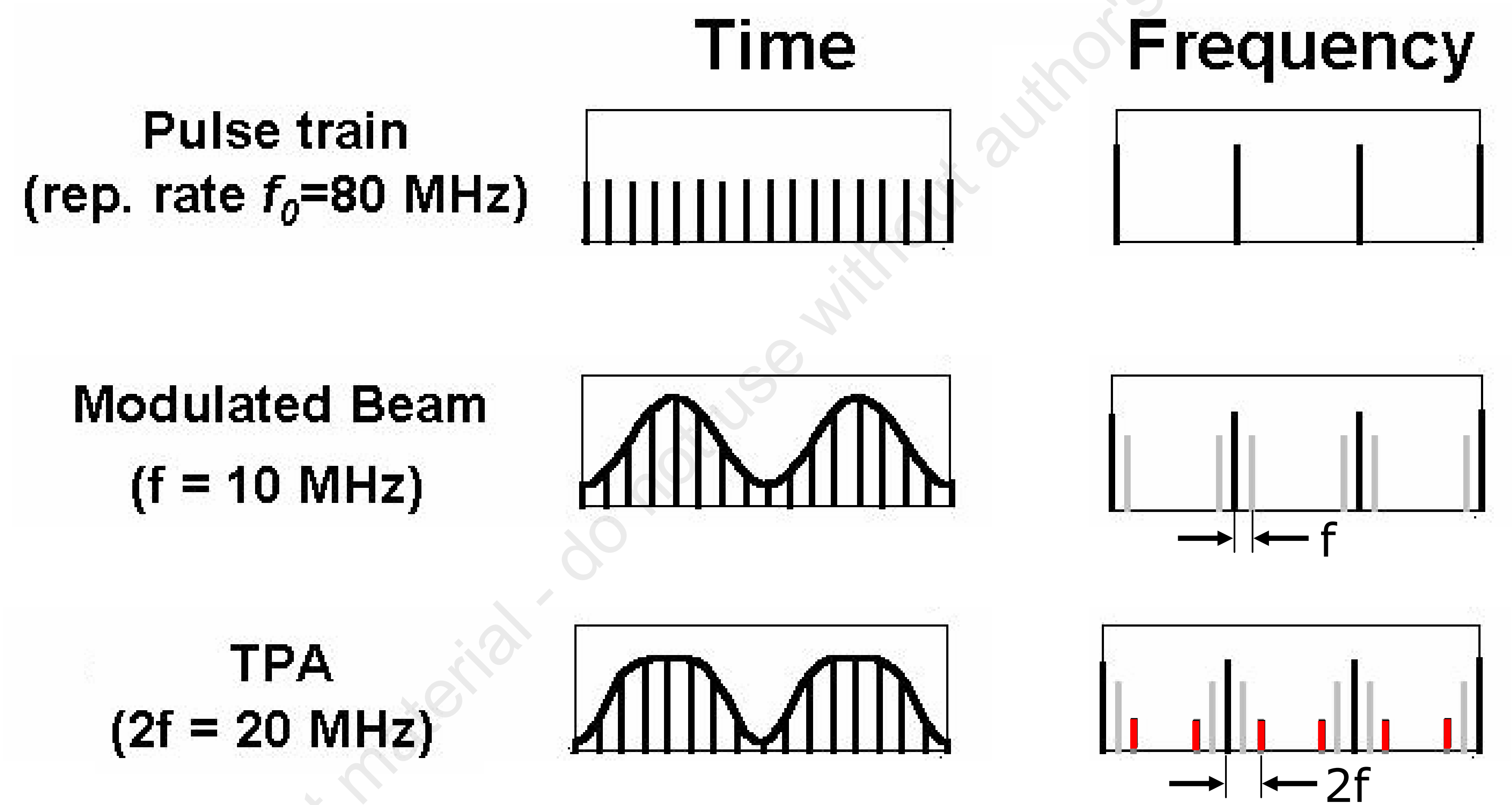
Two-Photon Absorption (TPA)

- Two-photon fluorescence without fluorescence
- Intensity-dependent absorption:
  $\alpha = \alpha_0 + \alpha_2 I$
- Measures resonant interaction
  - *Melanin (distribution, type)*
  - *HbO/Hb (Oxygenation level)*

But: Small effect on large background

$\Rightarrow$ Move small nonlinear signal away from large background

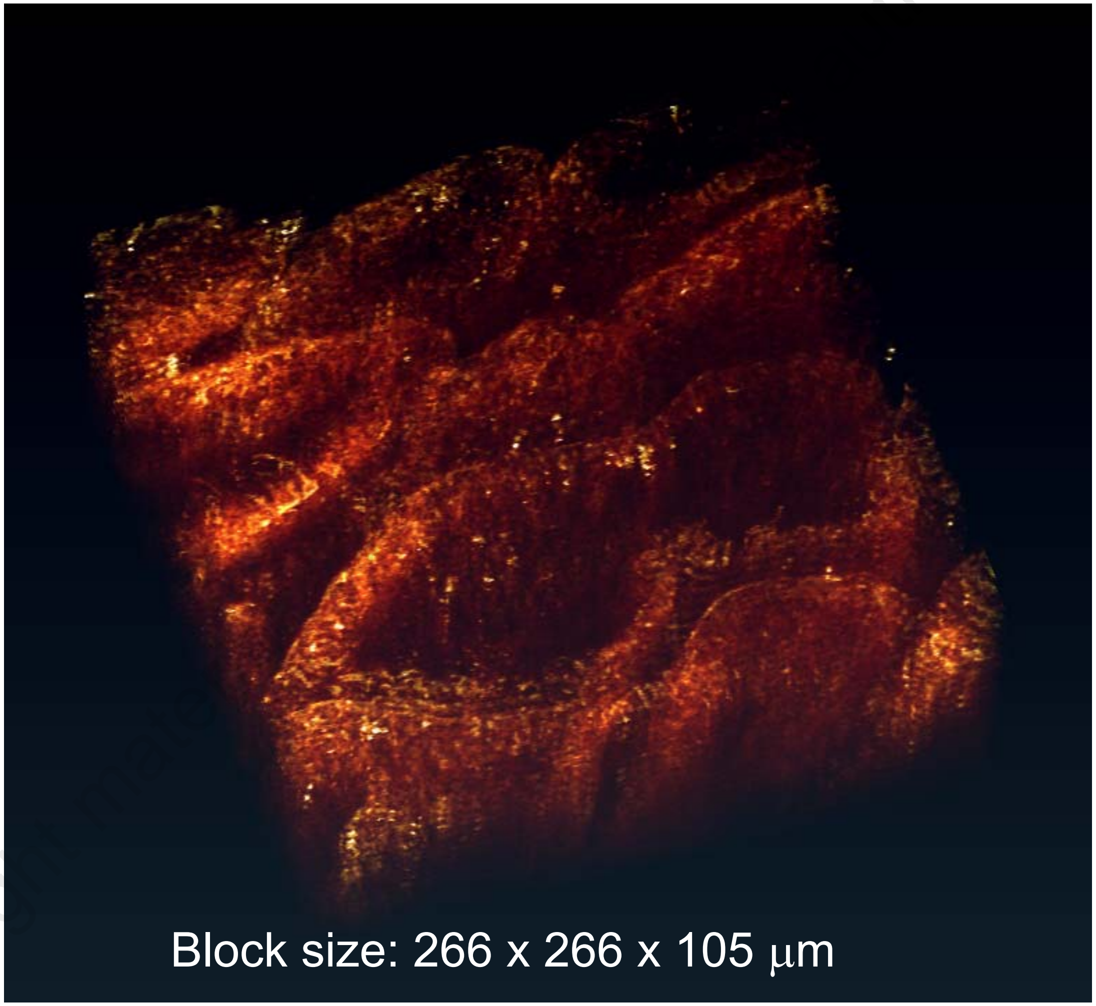
Loss Modulation



P. Tian and W.S. Warren, Opt. Lett. 27, 1634-1636 (2002)

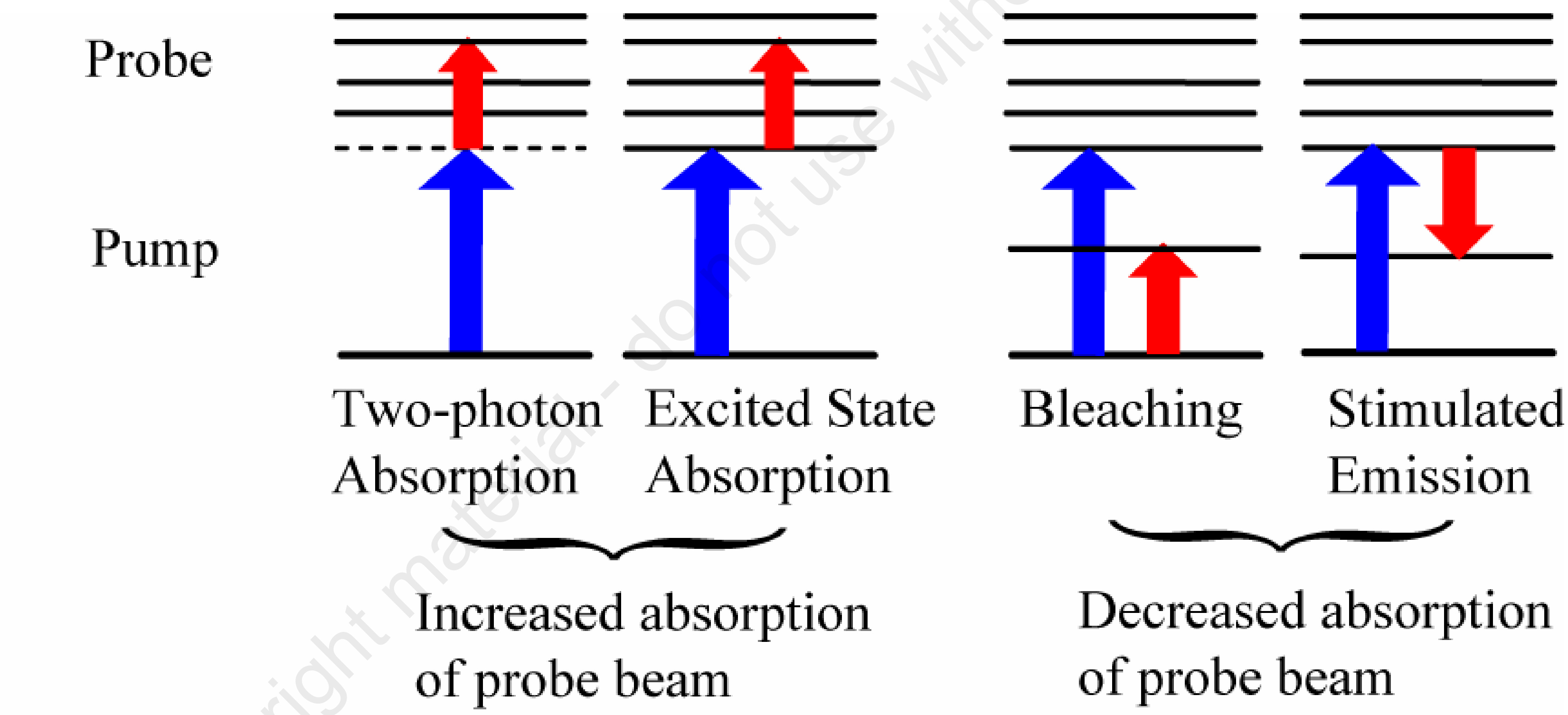
TPA in Human Melanoma Lesion

- Human Melanoma Lesion (grafted on mouse)

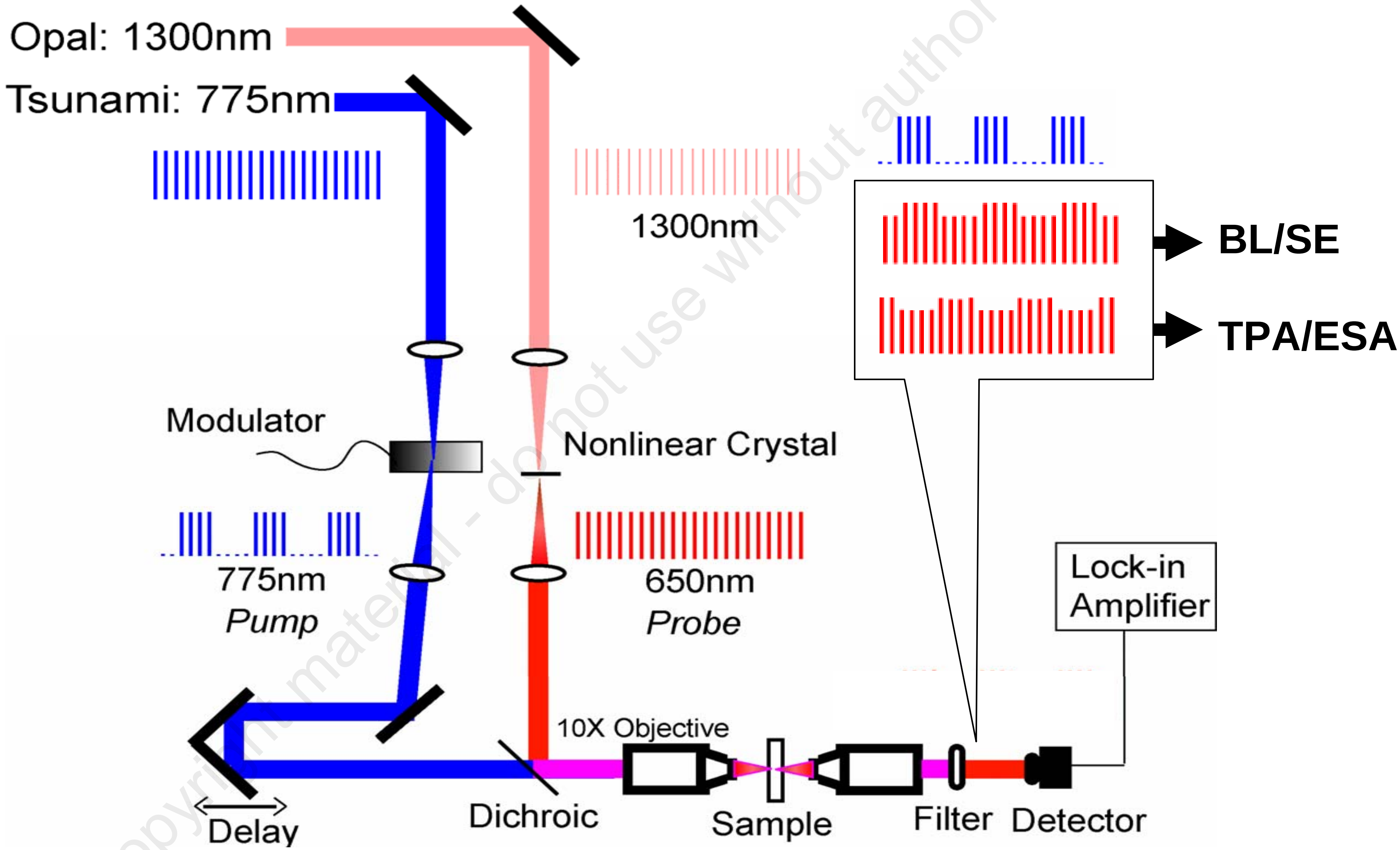


Multi-Color Absorption

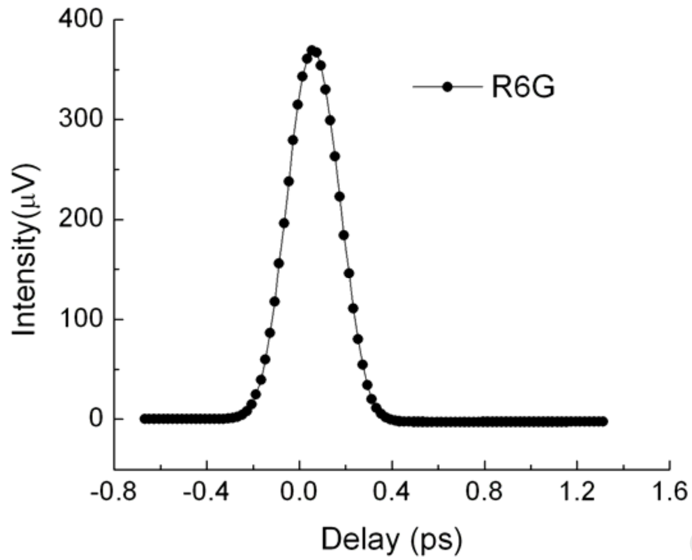
- Different nonlinear absorption processes contribute to the signal with different phases

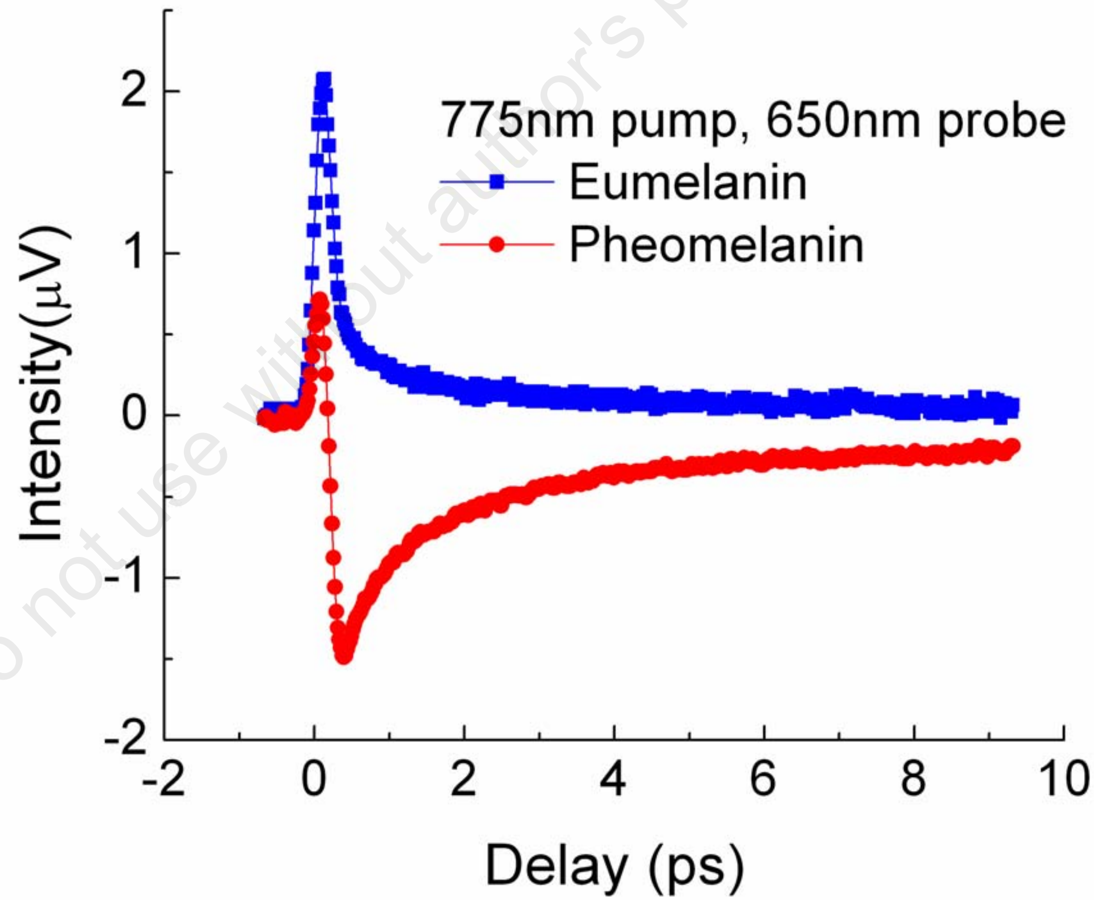


Experimental Setup



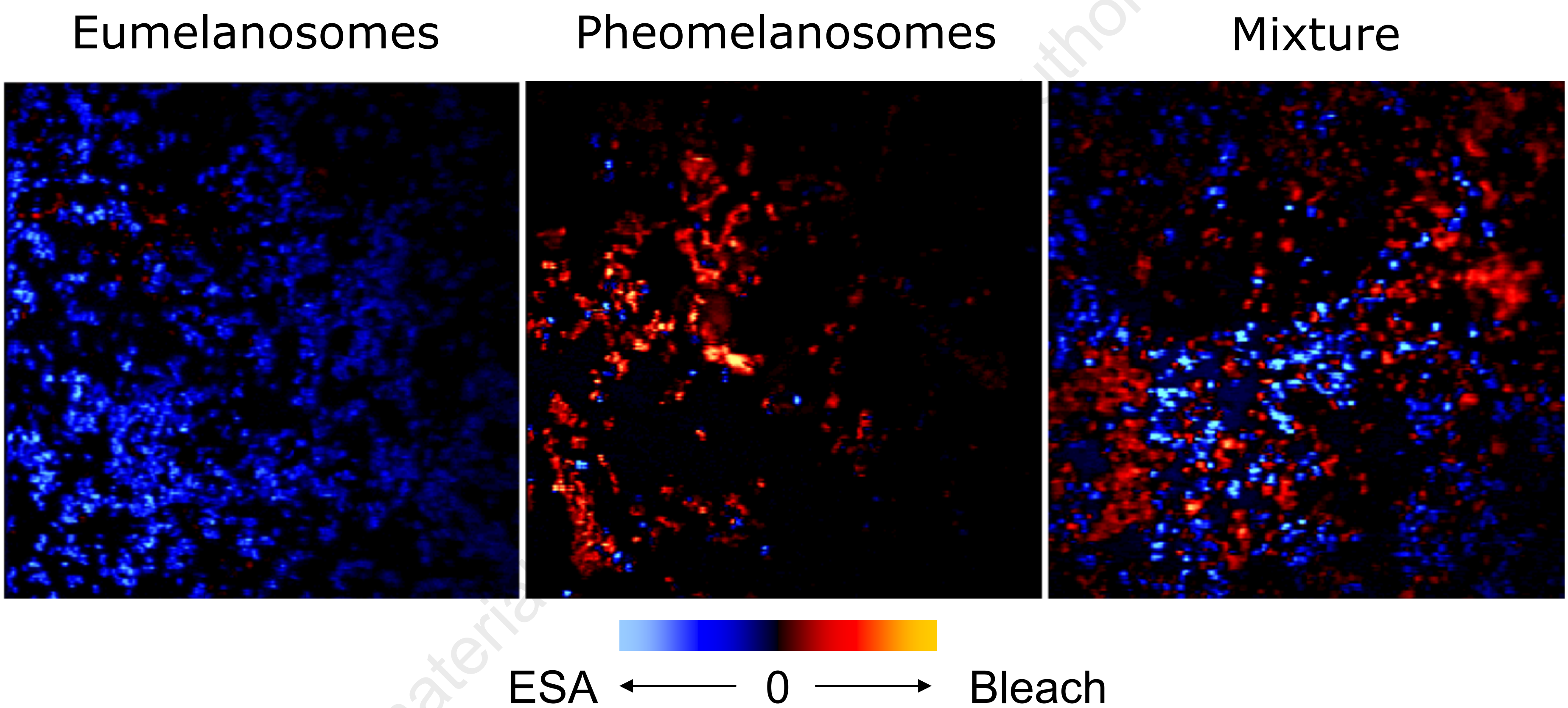
Two-Color TPA in Melanin





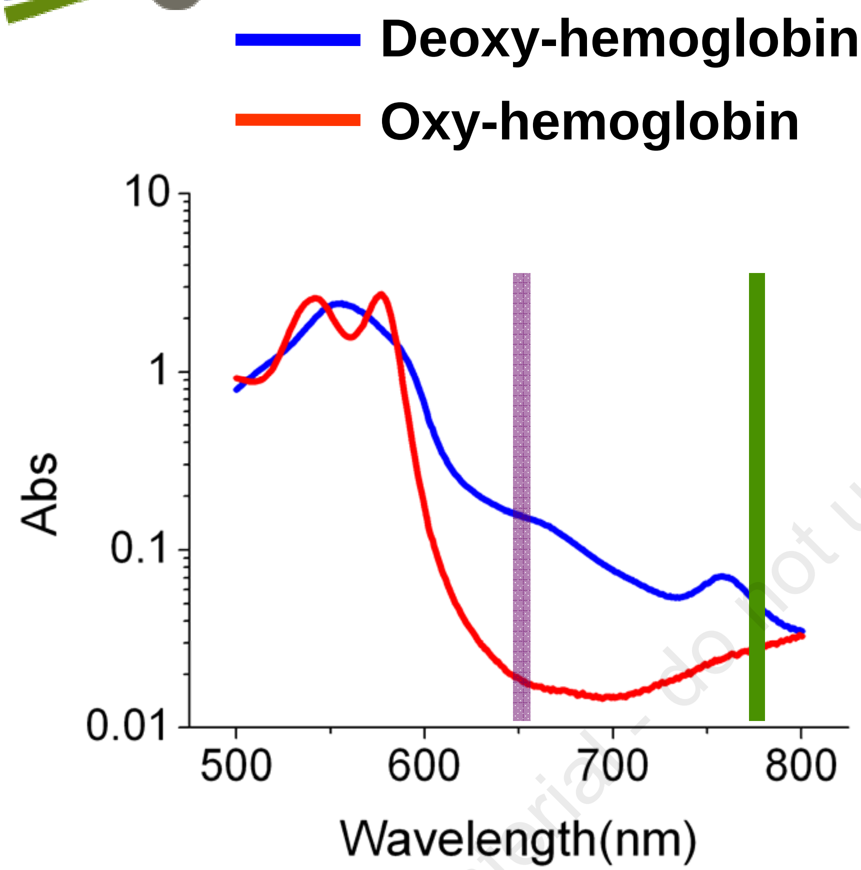
- Melanins show different absorption dynamics
  - *Excited state absorption / bleaching*
  - *Opposite phase*

Two-Color TPA in Melanosomes



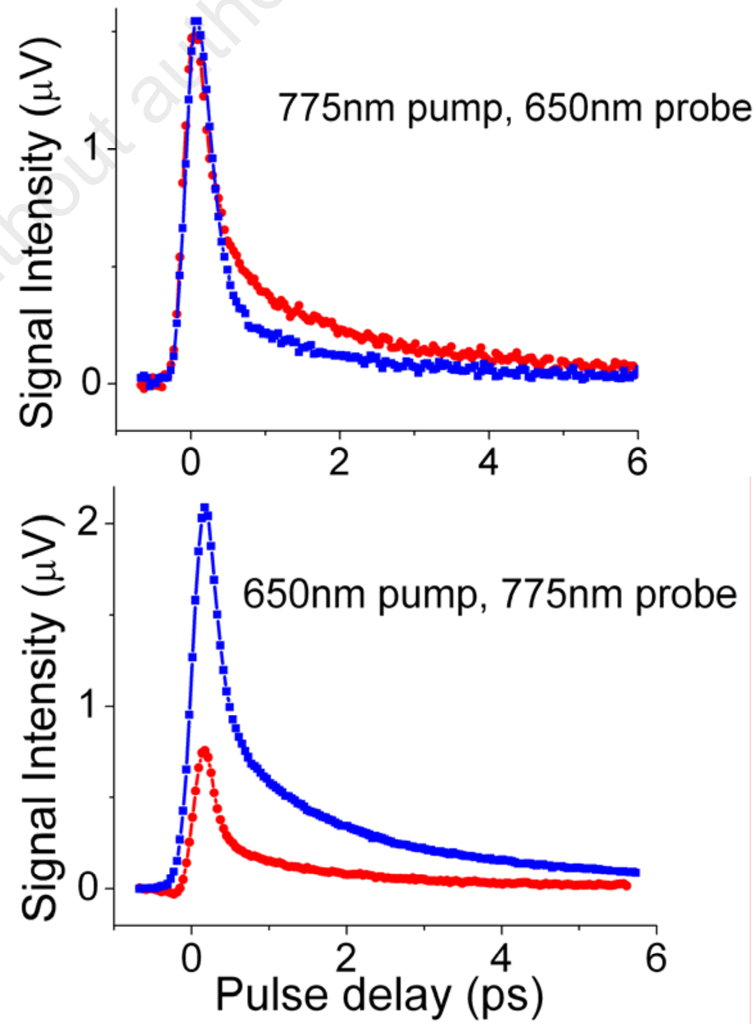
Differentiation of melanin type

Oxy- vs. Deoxy-Hemoglobin Nonlinear Absorption Dynamics

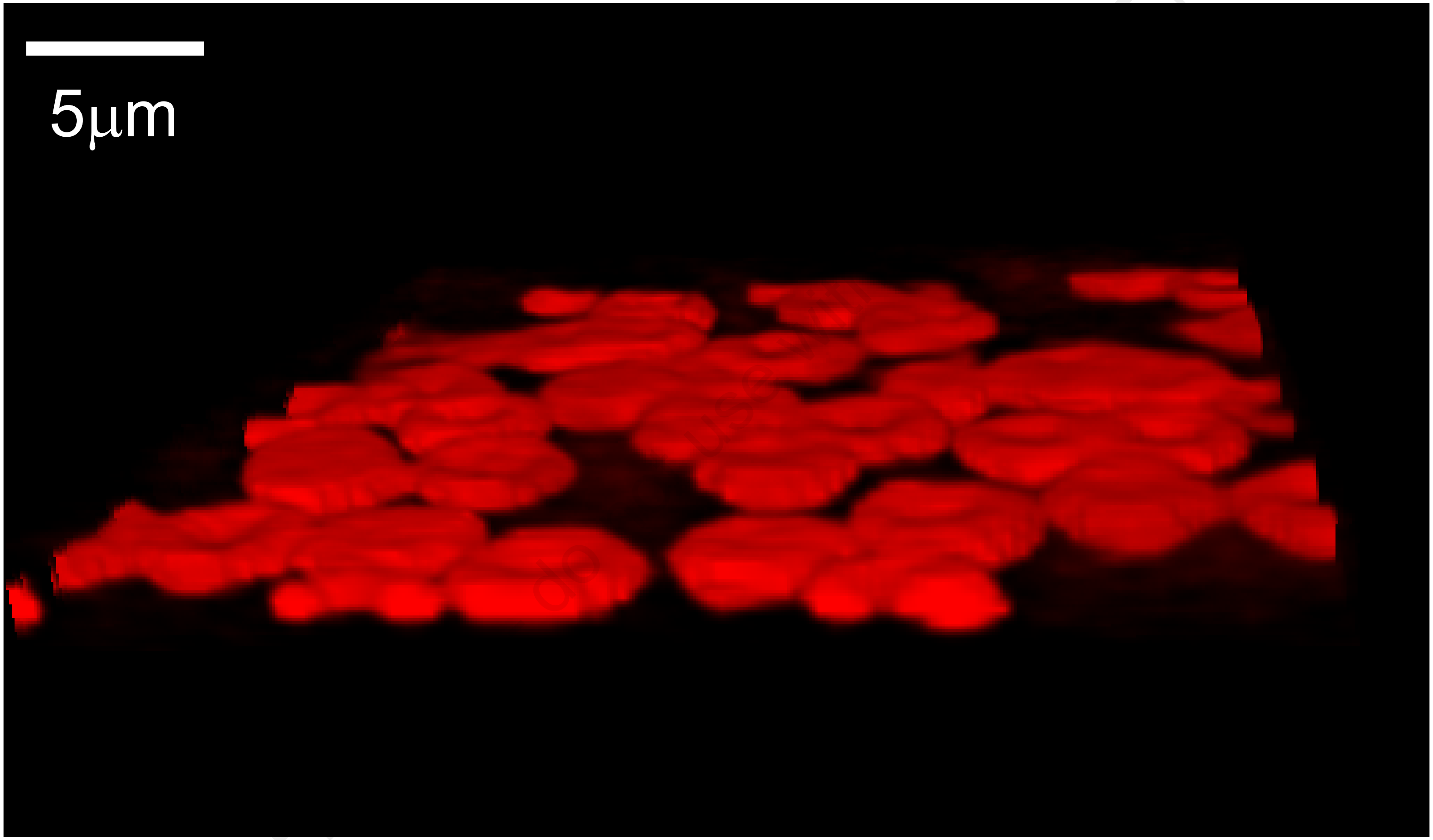


0.46mM / 1mm glass cuvette

Excited state absorption signal

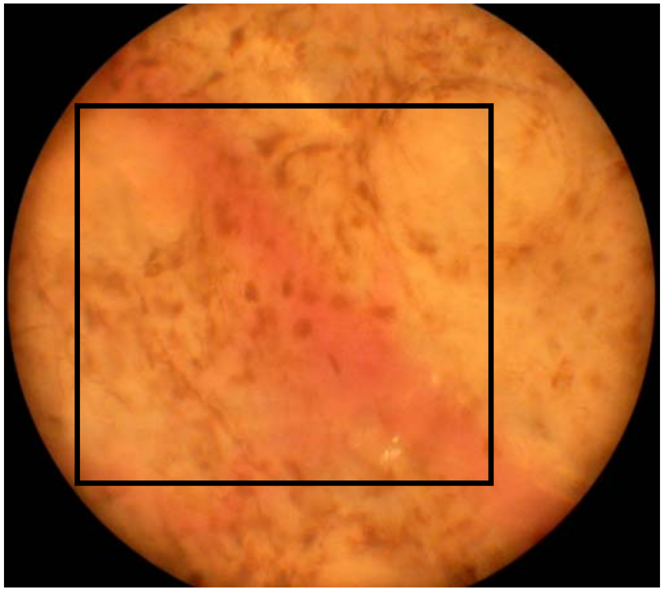


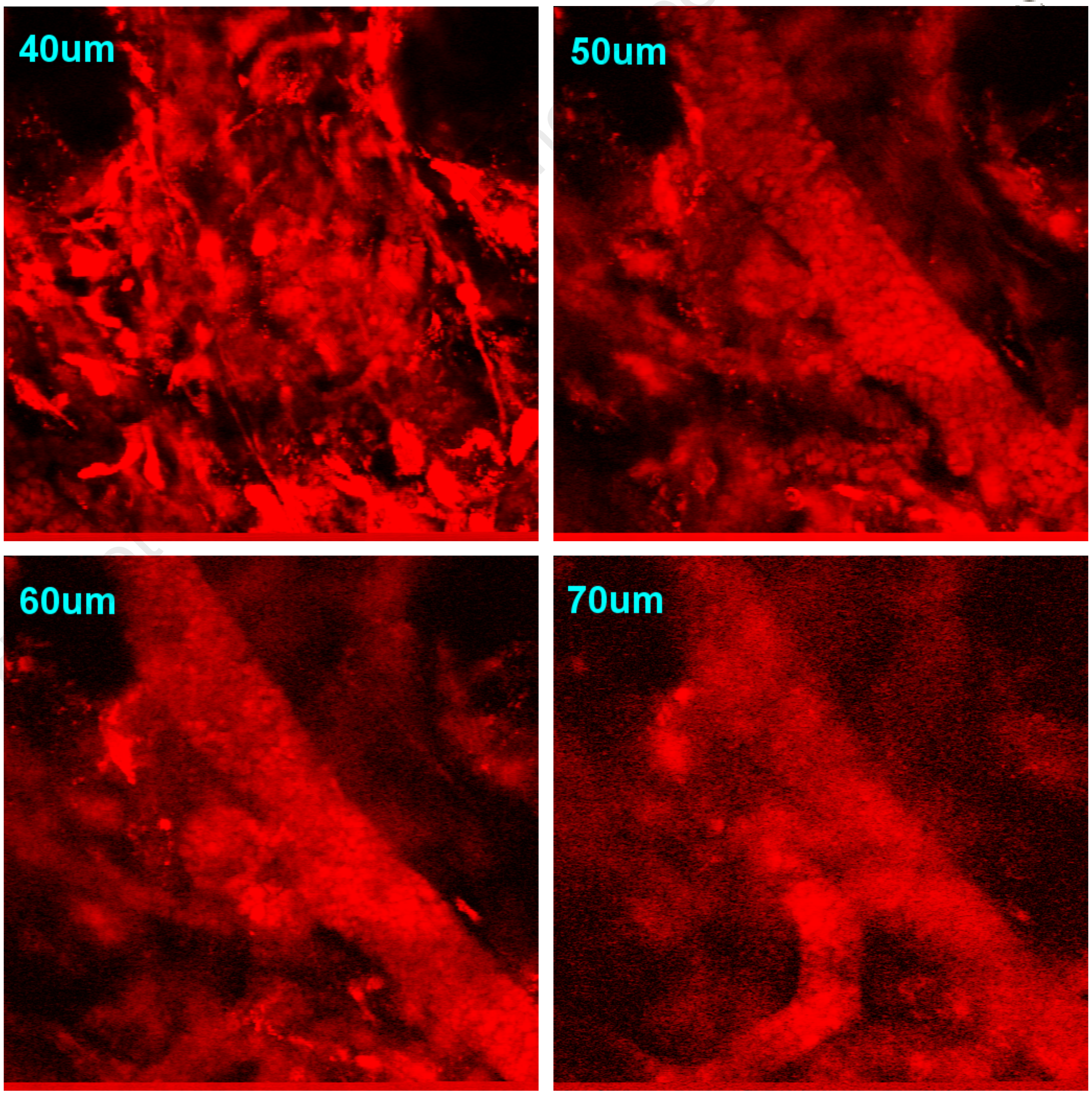
Imaging of Fixed Red Blood Cells



Reconstructed from 10 layers with 1μm step size

Ex Vivo Imaging of Blood Vessels in a Black Mouse Ear





650 nm pump (2.4 mW)
775 nm probe (1.4 mW)
210 × 210 μm

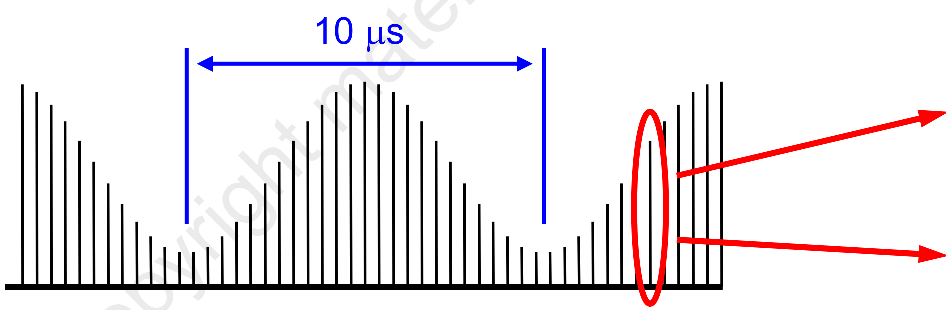
Other Contrast Mechanism?

- Self phase modulation (SPM)
  - *Nonlinear phase contrast*
  - *Intensity-dependent refractive index $n = n_0 + n_2 I$*
  - *Resonant and non-resonant interaction*
  - *Structural component*

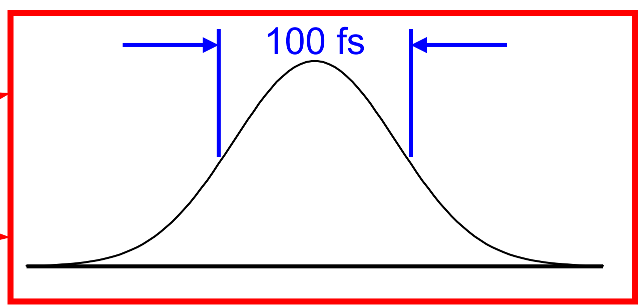
But: Loss Modulation insensitive to SPM

Solution:

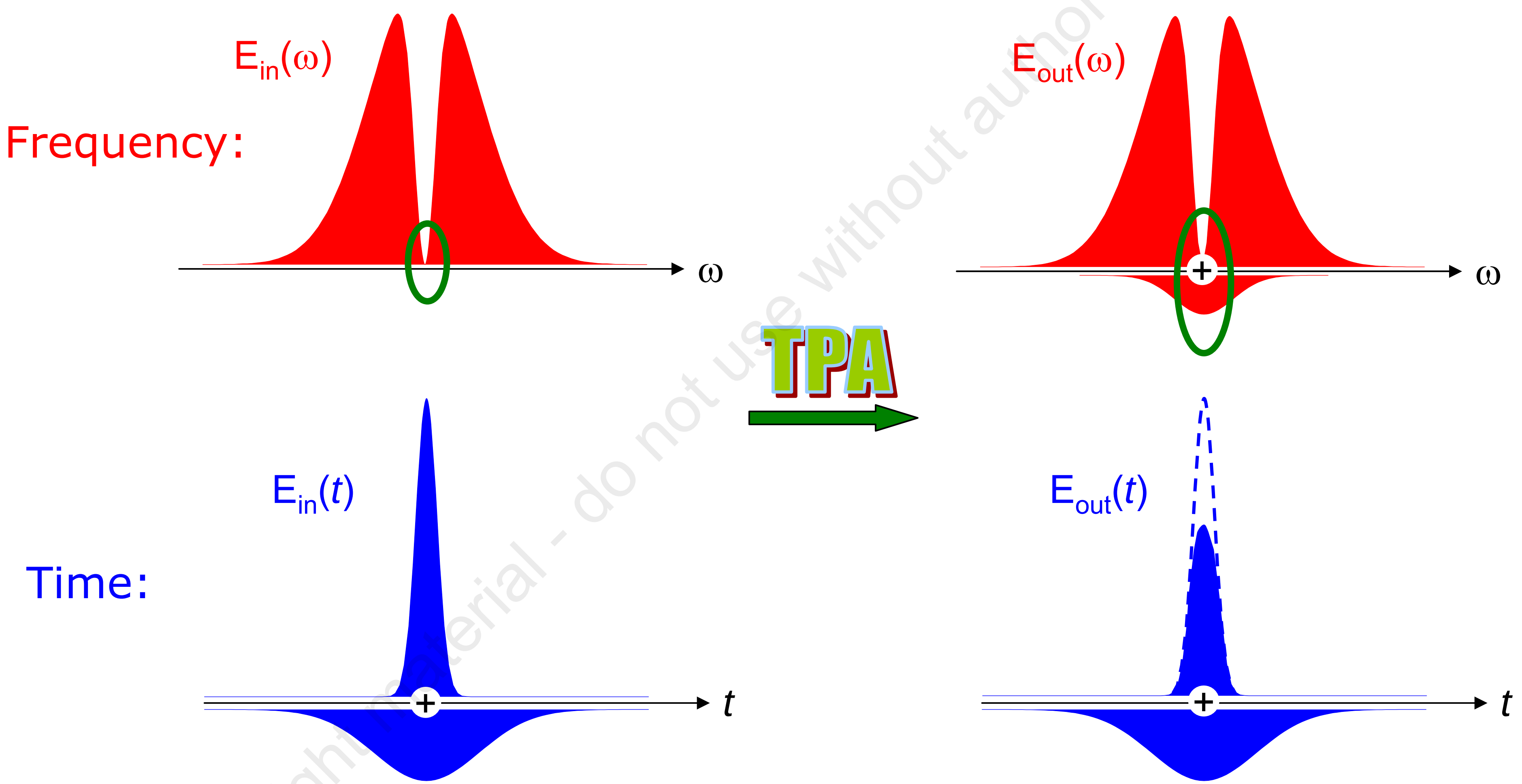
Pulse train shaping



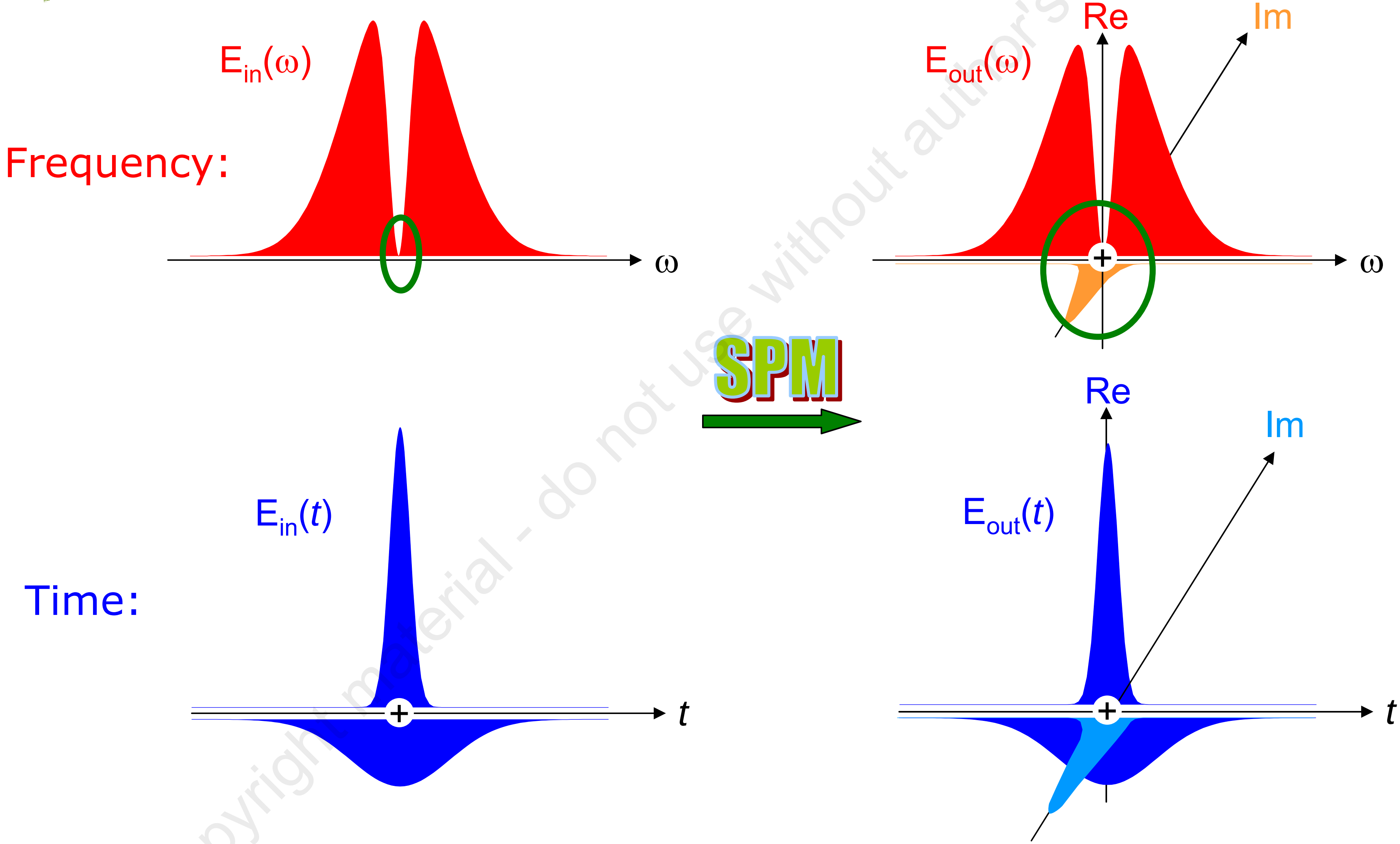
Pulse shaping



TPA Can Refill "Spectral Hole"



SPM Can Refill "Spectral Hole"



Phase Measurement

TPA:

- Absorptive
- Out of phase with main parts of pulse

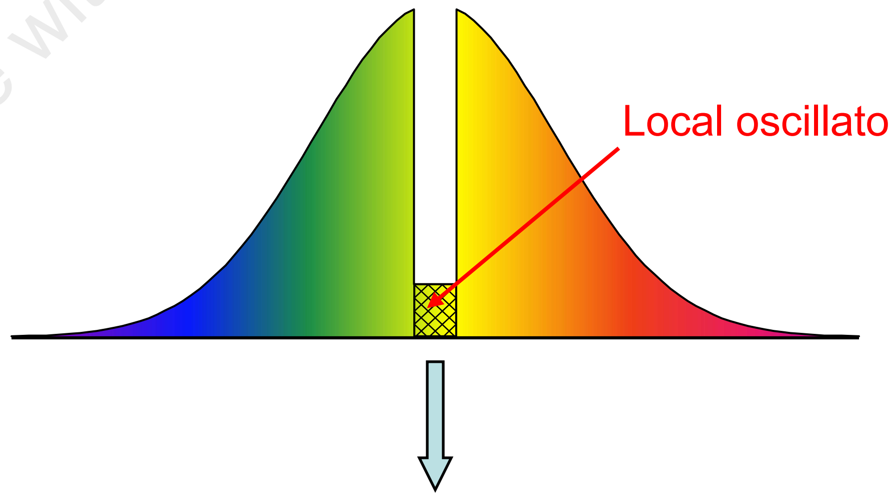
SPM:

- Dispersive
- In quadrature with main parts of pulse

TPA+SPM:

- Measure phase to distinguish

Homodyne:

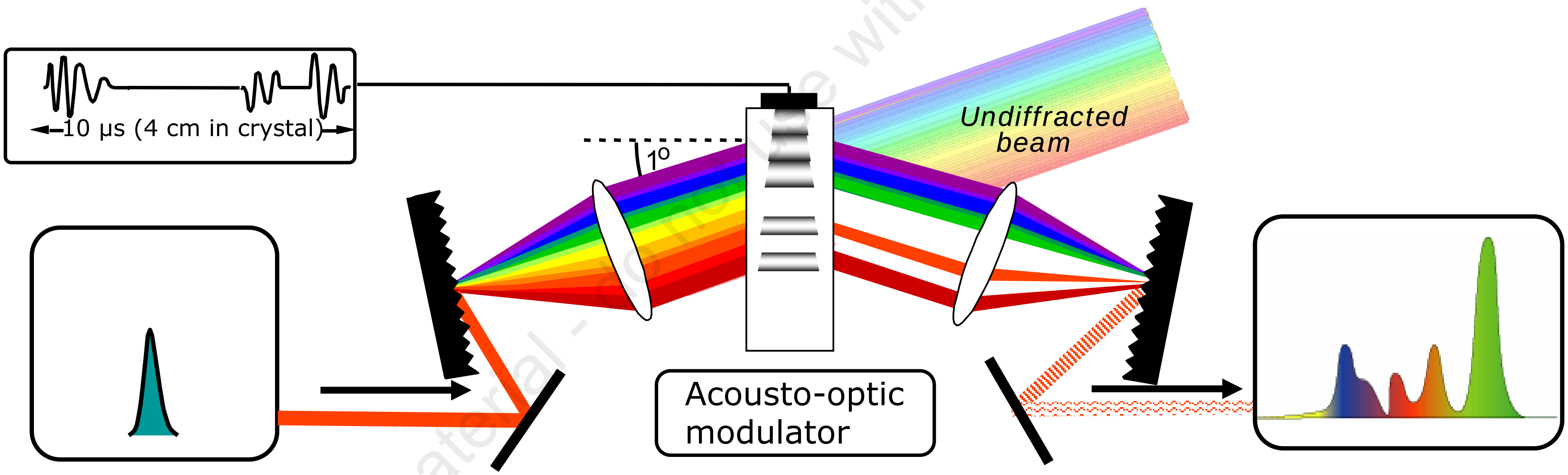


Interference between LO and hole refilling

Fischer, Warren, *et al.*, *Opt. Lett.* **30**, 1551 (2005)

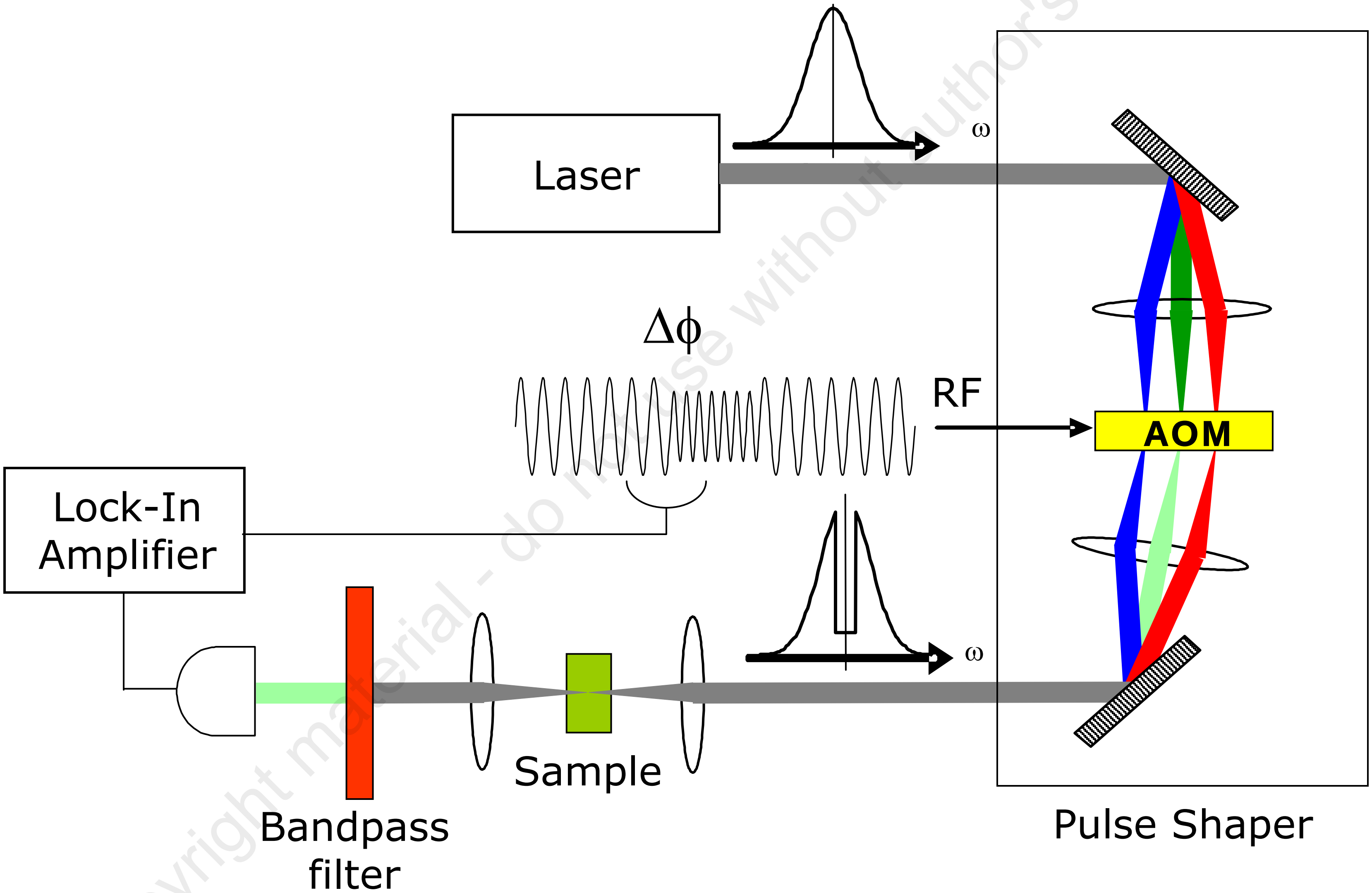
Pulse Shaping

- Pulse widths ≈ 100 fs
  ⇒ too fast for "direct" shaping
- Shaping in the frequency domain:

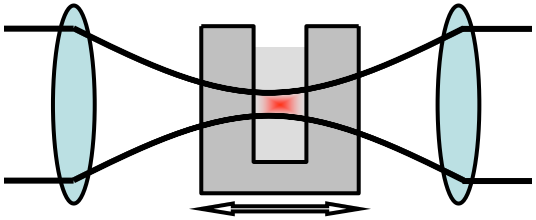


⇒ Arbitrary "fast" laser pulse shape by shaping "slow" RF waves

Experimental Setup

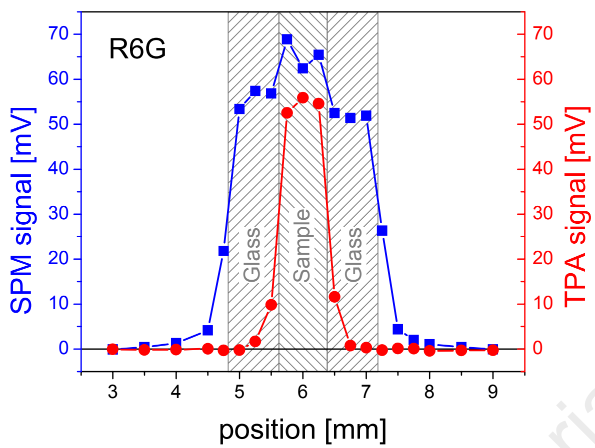


TPA / SPM Measurements

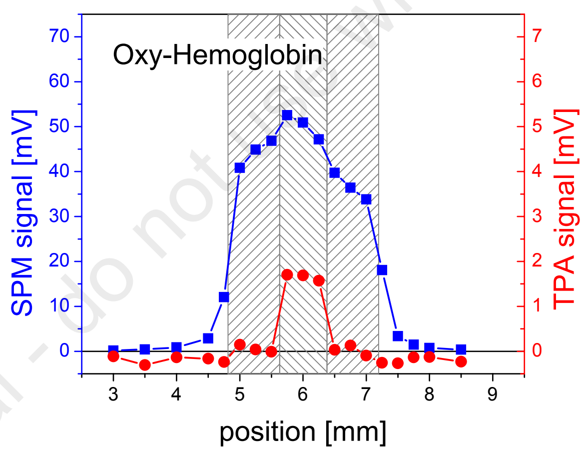


- 100 fs pulses at 20 kHz
- 400 μW (20 nJ/pulse)
- Phase rotation at 1 kHz

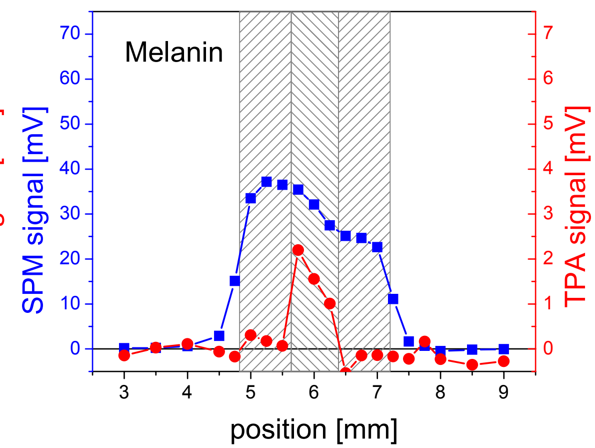
Rhodamine 6G:



Hemoglobin:



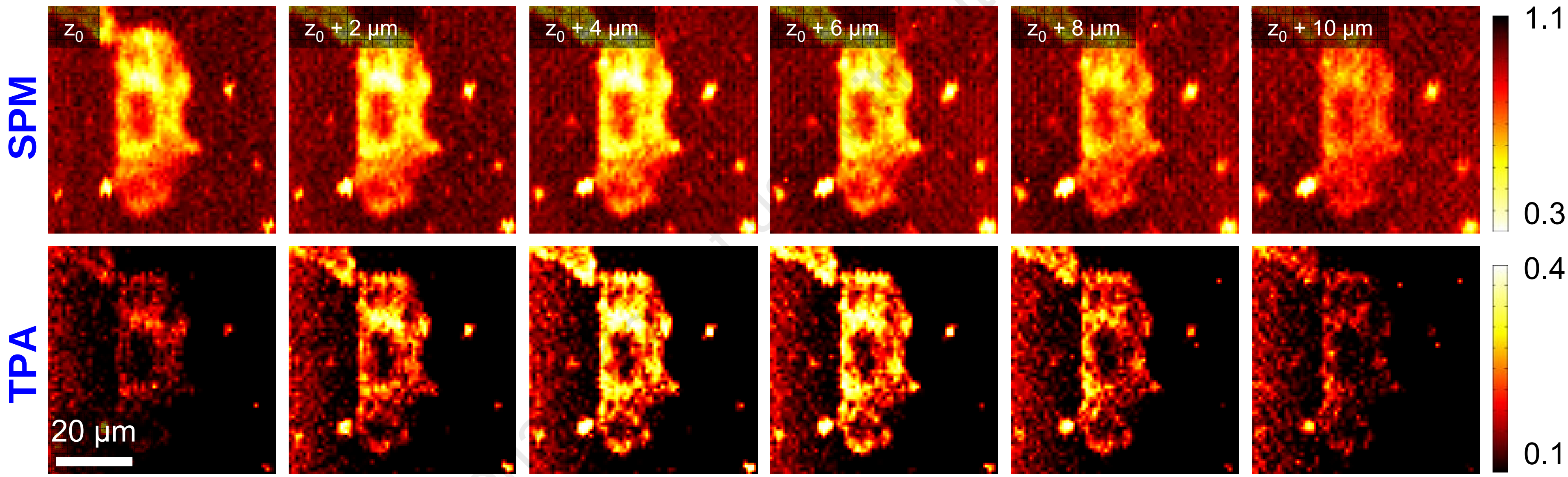
Melanin:



- Simultaneous SPM/TPA measurements
- Signals in biomarkers

TPA / SPM Cell Imaging

- Cultured B16 melanoma cell
- 100 fs pulses at 20 kHz
- 100 μW (5 nJ/pulse)



- High resolution measurements
- TPA dominated by melanin; mounting medium shows SPM
- SPM/TPA contrast difference

Functional Contrast

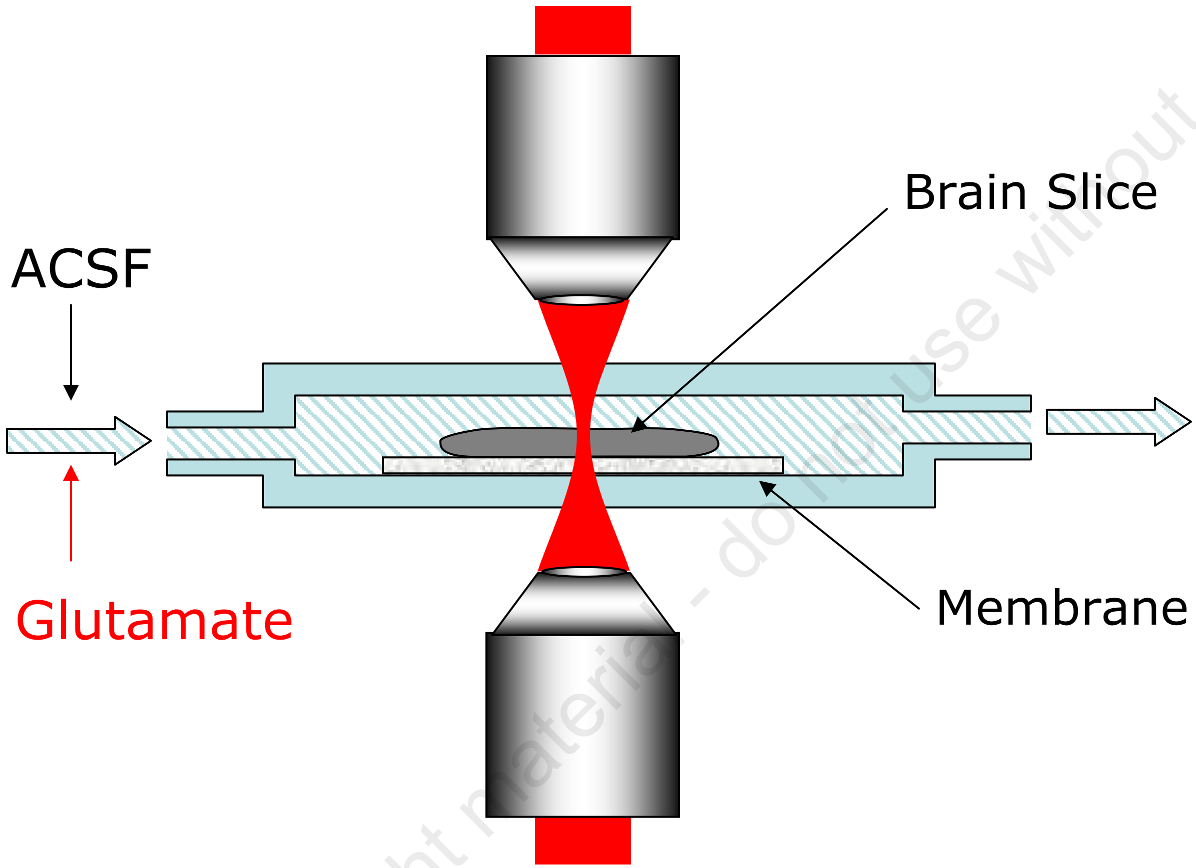
- Imaging of neuronal activity
  - *3-dimensional images with good penetration*
  - *High spatial and temporal resolution*
  - *Non-invasive, intrinsic contrast*
- Current measurement methods:
  - *Electrodes*
    - Localized, invasive
  - *EEG/MEG (electro/magneto-encephalography)*
    - Low spatial resolution
  - *Functional MRI*
    - Low resolution, slow
  - *Optical diffusion tomography*
    - Low resolution, slow
  - *Voltage/Calcium-sensitive dyes*
    - Absorption, TPF, SHG measurements
    - Invasive (exogenous contrast)
  - *Scattering / absorption / birefringence*
    - Low contrast

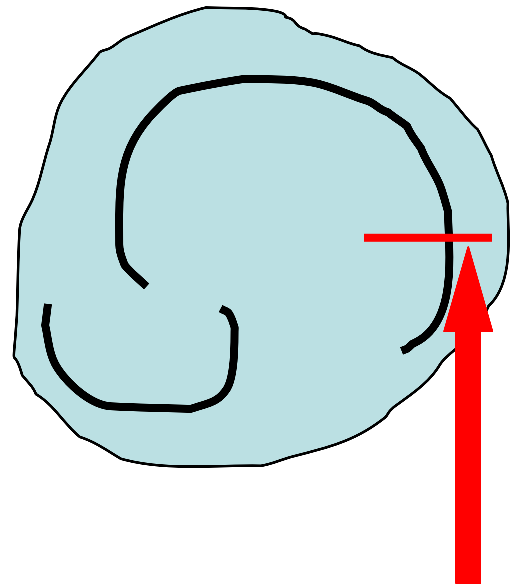
SPM

- Combine intrinsic optical signatures with nonlinear imaging ?

Sample

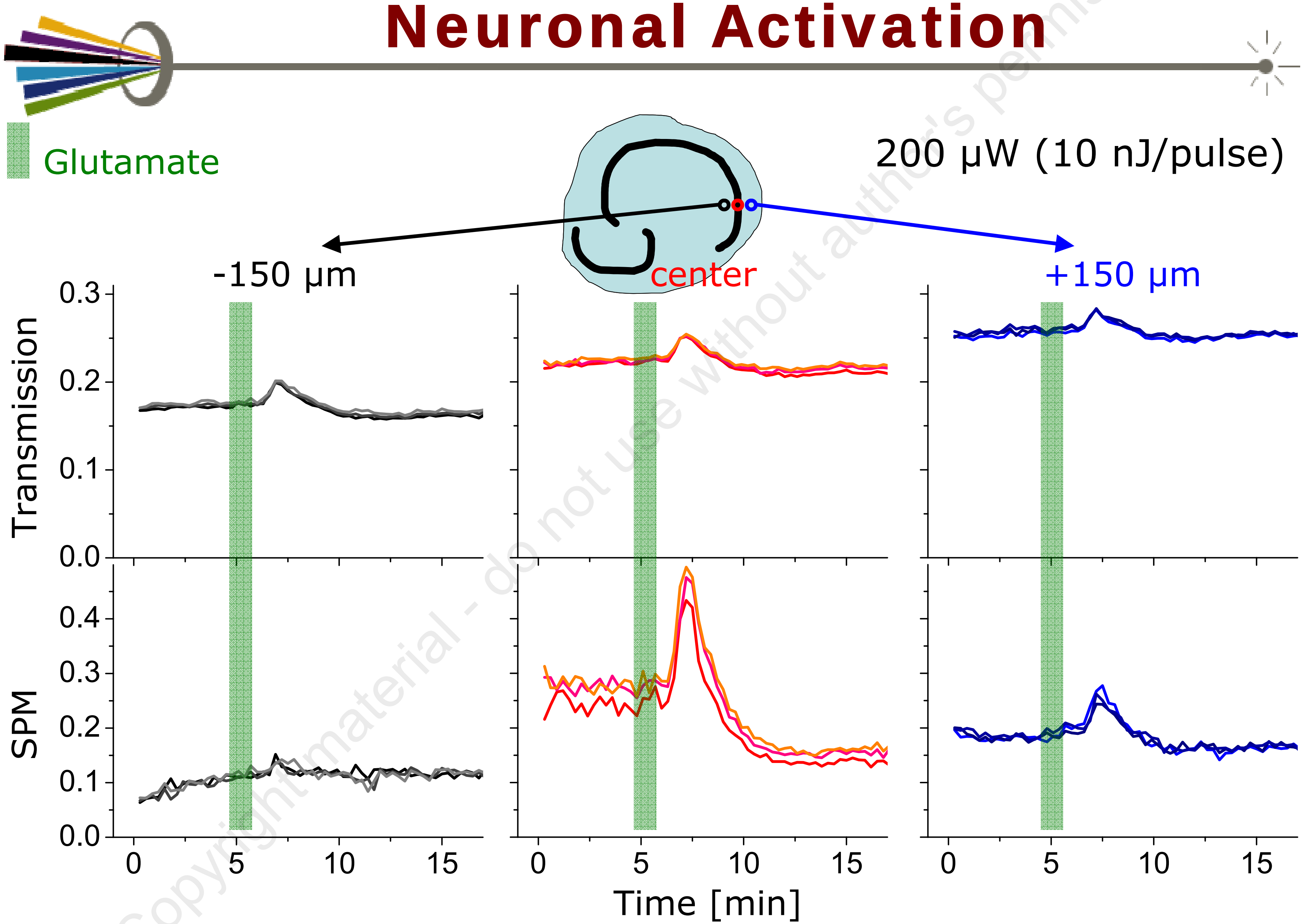
- Hippocampal brain slice from rat



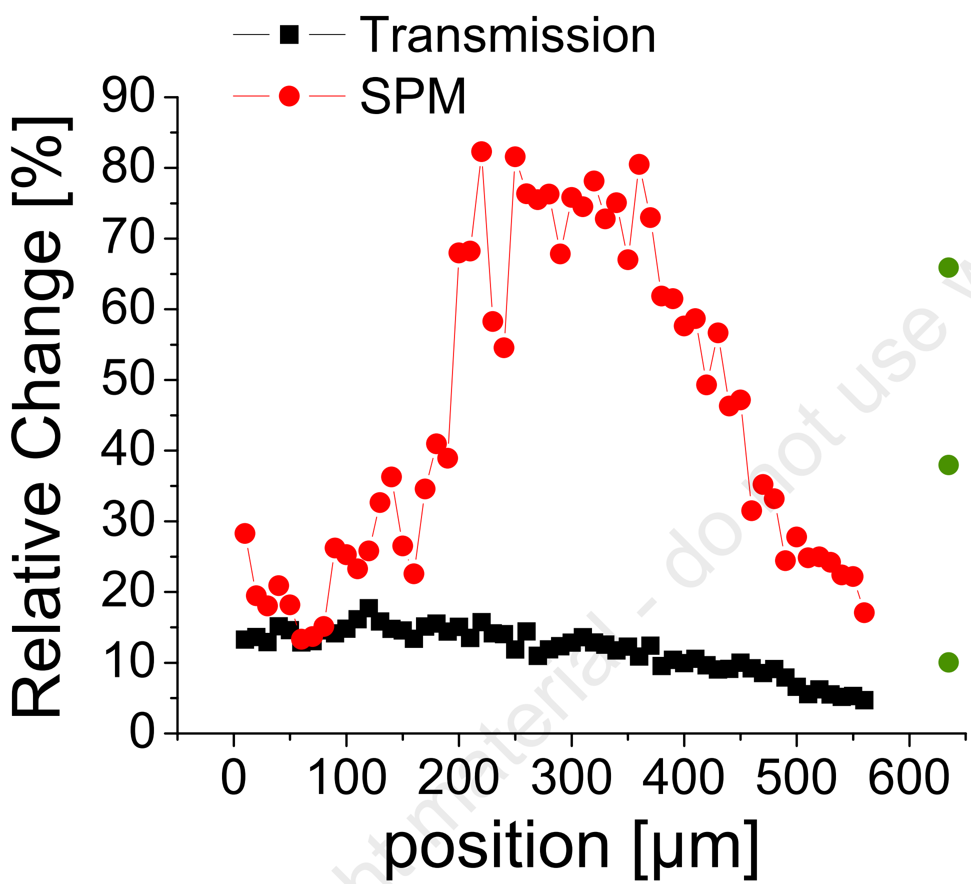


⇒ Activation of neurons with Glutamate

SPM Signatures of Neuronal Activation



Localization of Signal Change



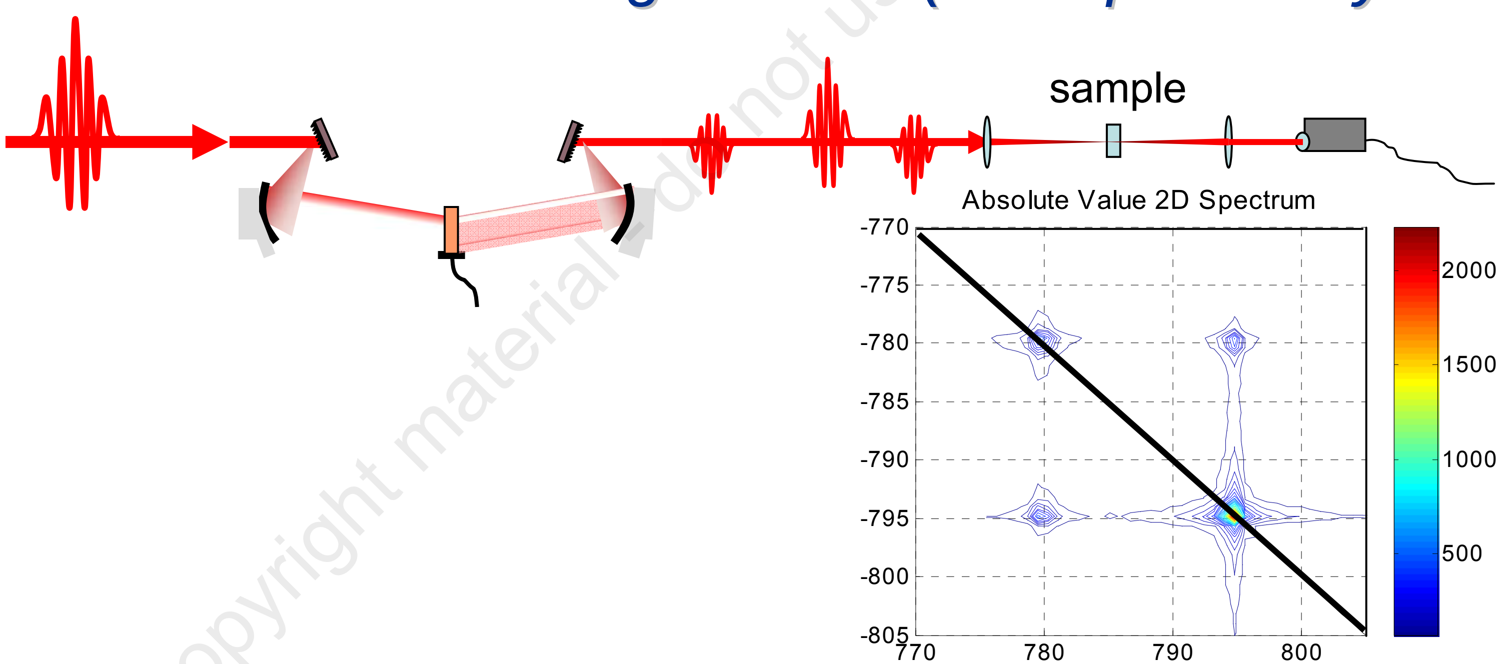
- Strong nonlinear signal change during activation
- Small transmission (scattering) change
- Localized around cell body layer
- Suppress activation with tetrodotoxin (TTX)

Coming Soon ...

- Investigate signal origin / optimize
  - *SPM in neurons*
    - Electrophysiology, compare to exogenous contrast
  - *TPA in Hb/melanin*
    - Wavelengths, delay
- Refine technique
  - *Epi-mode*
  - *Faster acquisition*
- Move towards clinically relevant samples

Future directions

- Pulse-shaped Raman
  - *Optimize generation of coherence / detection using shaped pulses*
- 2D optical spectroscopy
  - *Collinear configuration (with phase cycling)*



Conclusion

- Femtosecond pulse shaping offers new nonlinear contrast for tissue imaging
  - *Structural*
  - *Metabolic*
  - *Functional*
- Imaging technique
  - *Fast*
  - *High resolution (µm scale)*
  - *3D capability (optical biopsy)*
  - *Non-invasive*
  - *Intrinsic contrast*

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