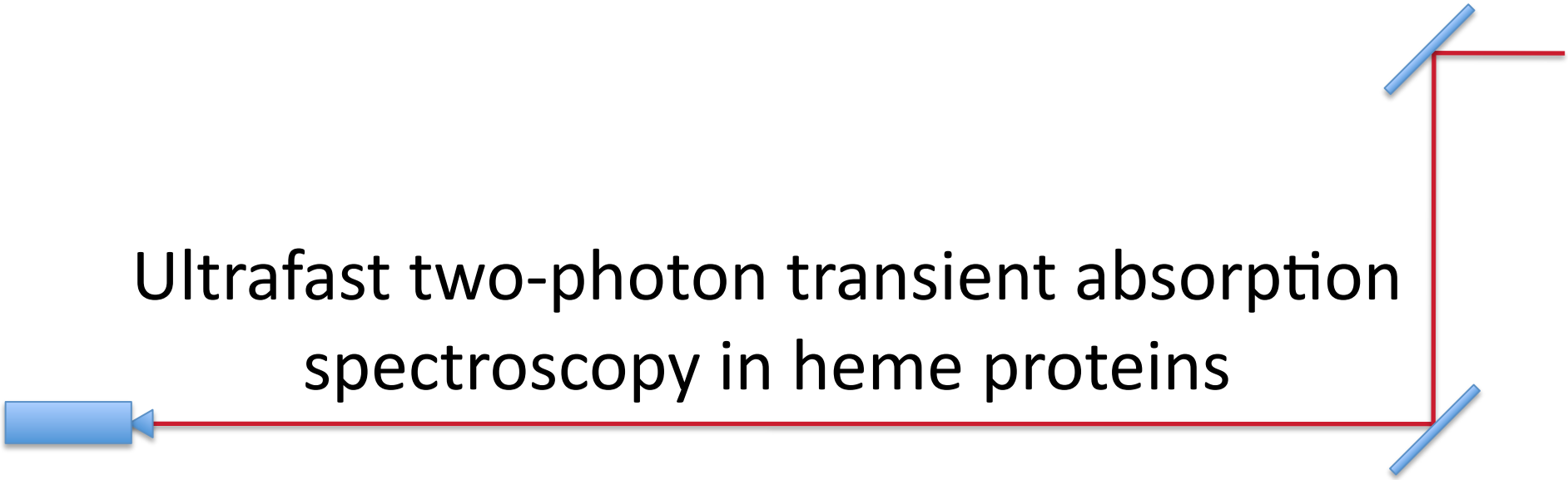
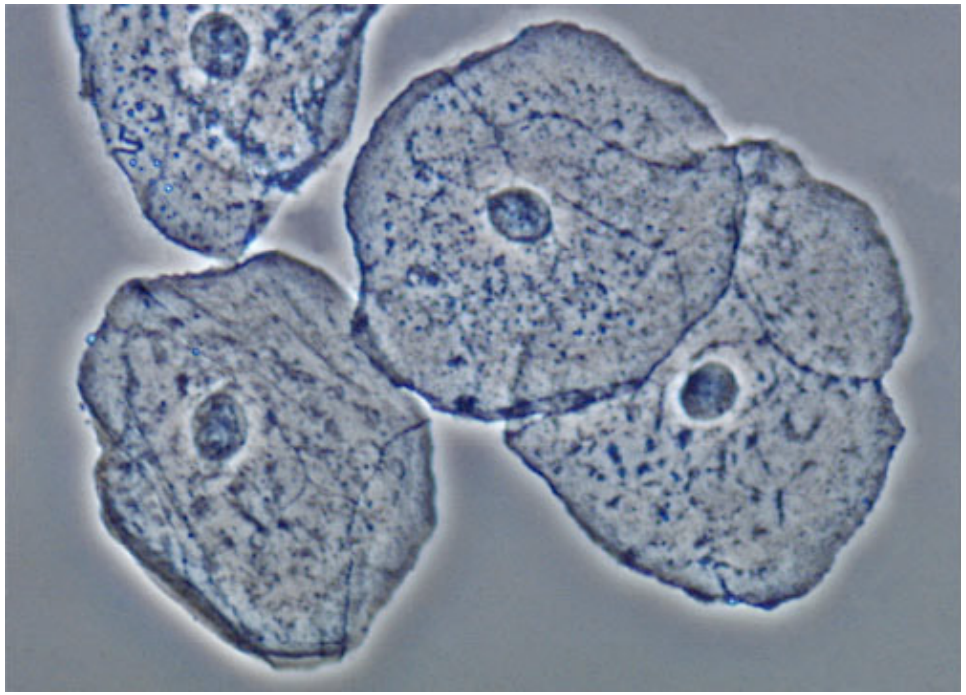
Ultrafast two-photon transient absorption spectroscopy in heme proteins



Eric Copenhaver, Daniel Wilcox, Jennifer Ogilvie

2012 University of Michigan REU Symposium

Microscopic Motivation



- Image courtesy of Nikon's Microscopy U: http://www.microscopyu.com/galleries/dicphasecontrast/index.html

- Phase contrast
- Structural, but no chemical information

Microscopic Motivation

- Fluorescence microscopy
- Not all chemical species naturally fluoresce

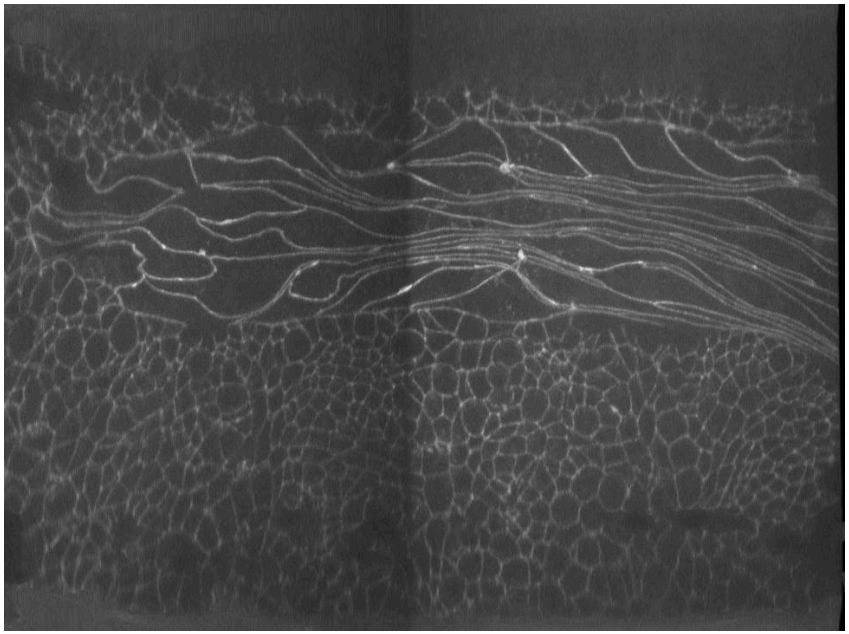
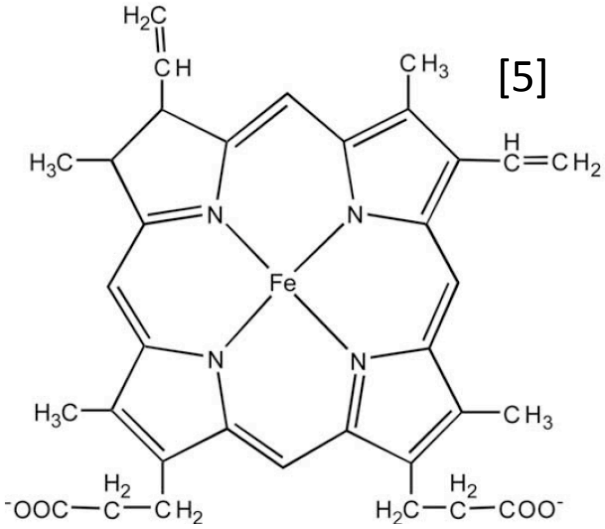


Image printed with permission from Eric Copenhaver. ☺

- Labels
  - Labeling can be difficult
  - Photobleaching
  - Potential effect on function
  - Phototoxicity

The Heme Proteins

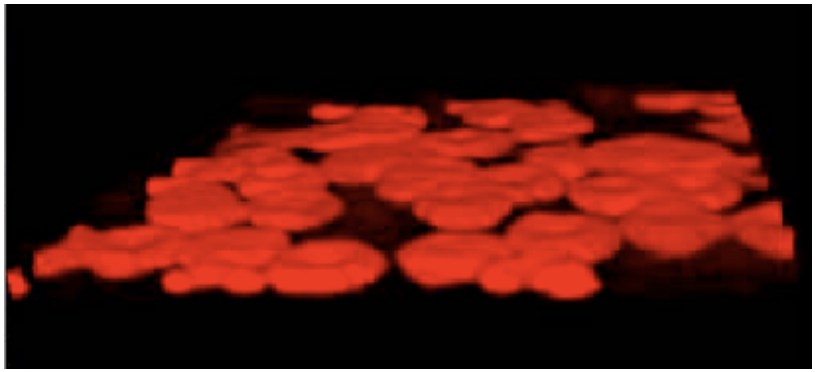
- The heme group
  - Oxidation states of Fe
  - Not fluorescent
  - Absorption near 400nm
- Myoglobin: oxygen storage
- Hemoglobin: oxygen transport
- Cytochrome c: mitochondria, electron transport in ATP synthesis, apoptosis



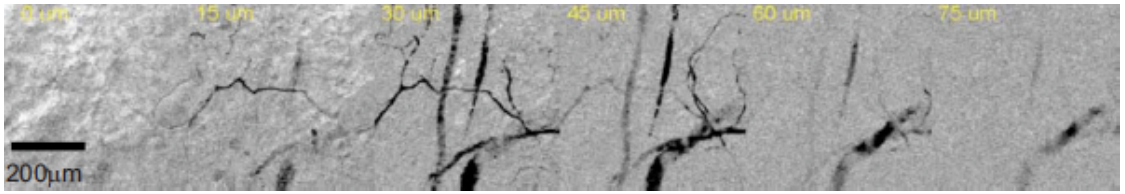
[5]

Macroscopic Rewards

- Hemoglobin as a marker for angiogenesis and tumor growth



[1]



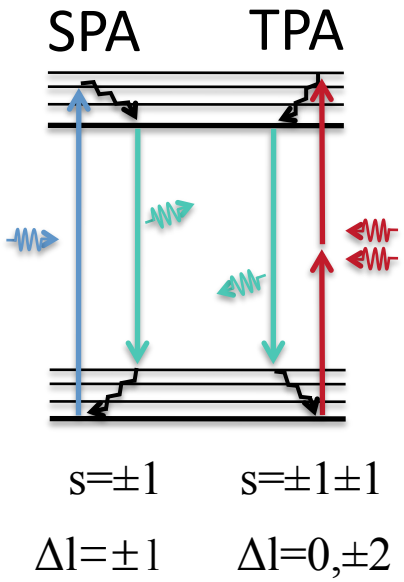
[2]

1 D. Fu, T. Ye, T.E. Matthews, B.J. Chen, G. Yurtserver, and W.S. Warren. High-resolution *in vivo* imaging of blood vessels without labeling. *Opt. Lett.* **32**(18), 2641-2643 (2007).

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Two-Photon Absorption (TPA)

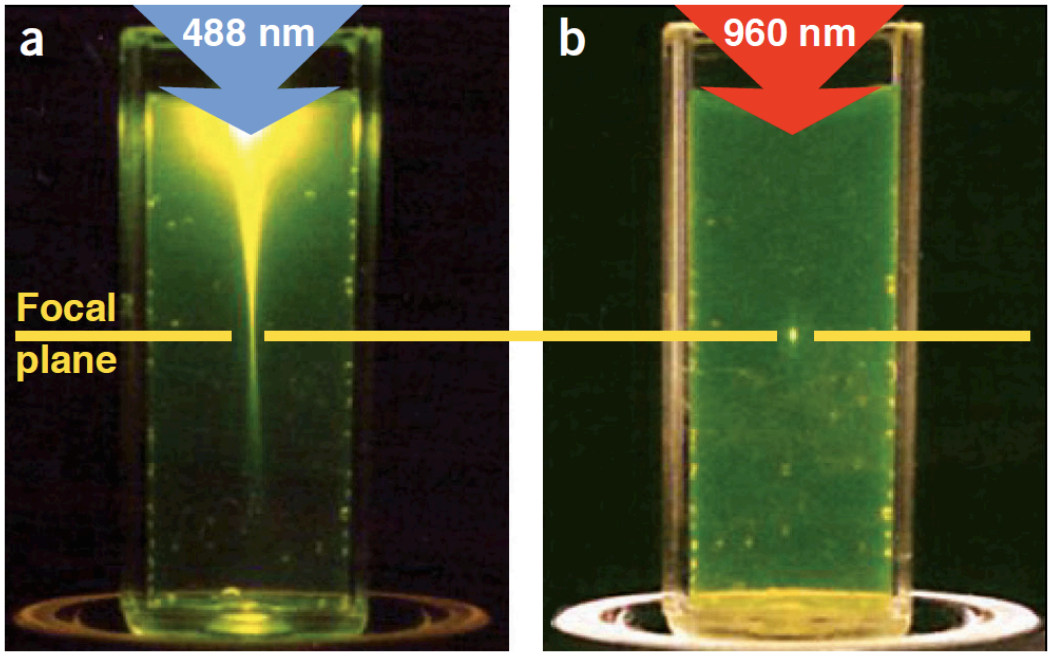
- Single-photon Absorption (SPA): photon energy matches transition
- TPA: Two photons' energies add to the transition energy
- Different selection rules
- Different states are accessed



Two-Photon Absorption (TPA)

TPA is a nonlinear process

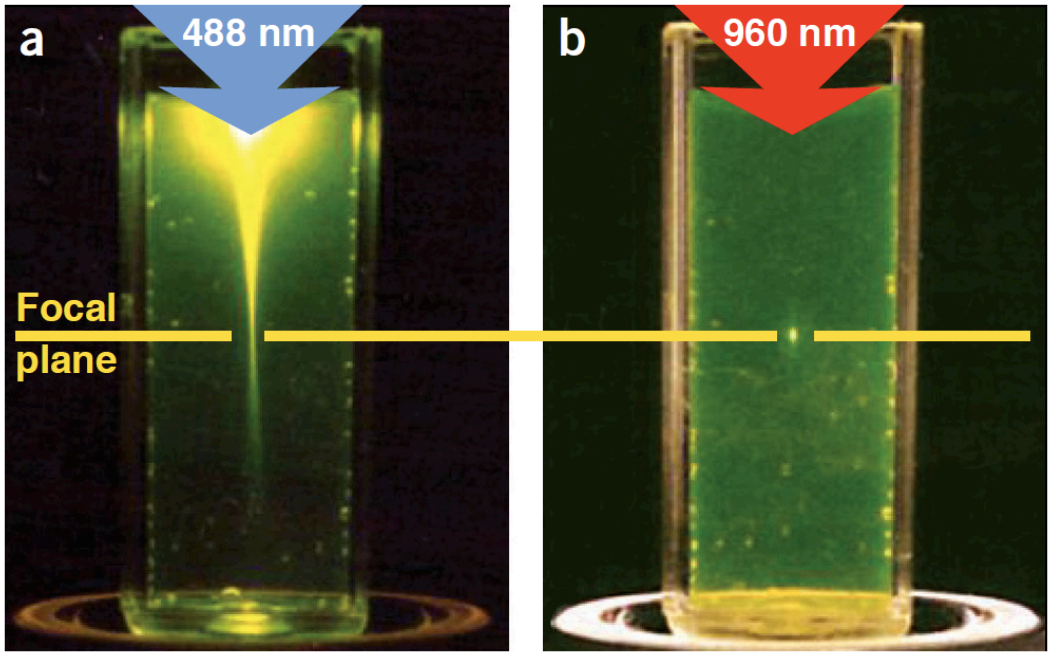
$$Pr \sim I^2$$



16 W.R. Zipfel, R.M. Williams, and W.W. Webb. Nonlinear magic: multiphoton microscopy in the biosciences. *Nat. Biotechnol.* **21**(11), 1369-1377 (2003).

Two-Photon Absorption (TPA)

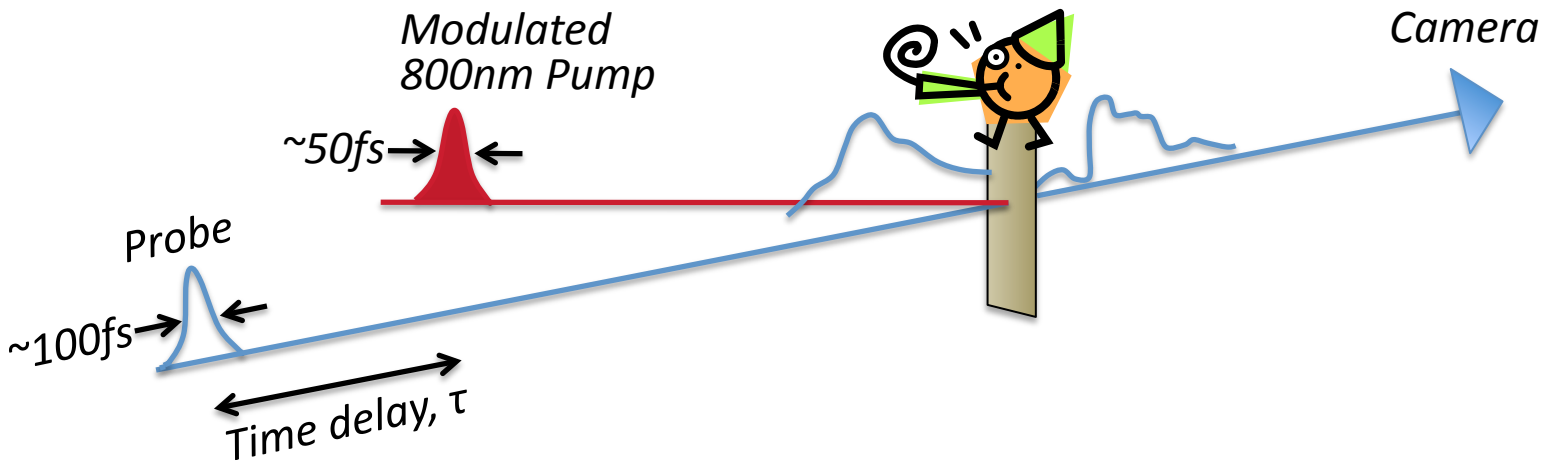
Differences in TPA characteristics provide chemical contrast in heme proteins



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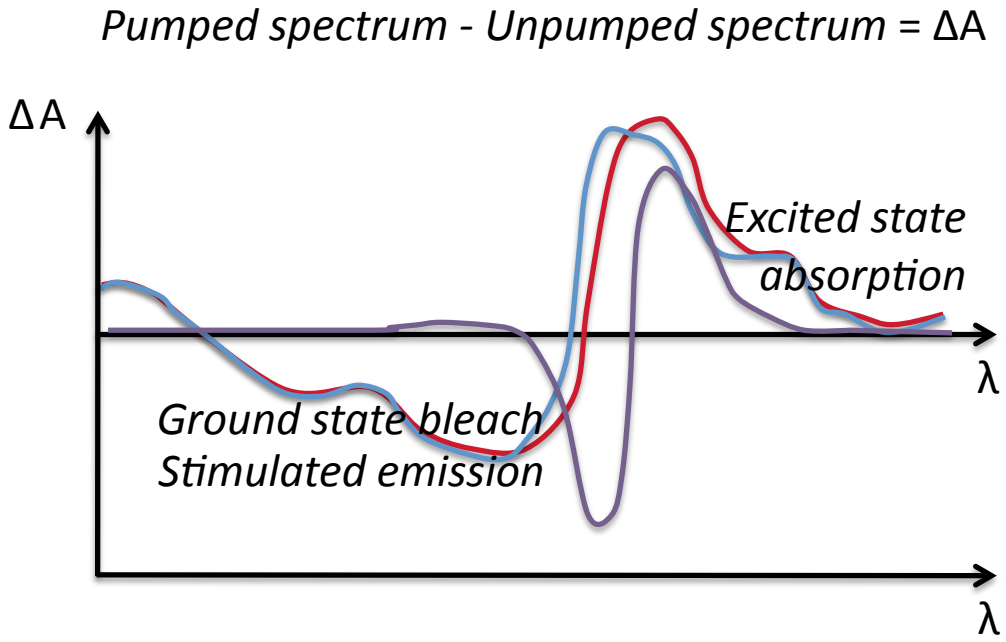
Transient Pump-Probe Spectroscopy

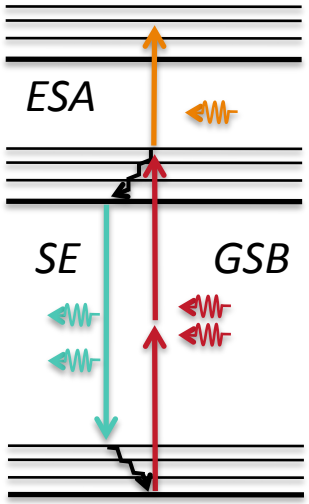
- Single photon pump-probe in hemes (400nm) is well-studied [6-15]
- Ultrafast (~ps) dynamics require short pulses



Transient Pump-Probe Spectroscopy

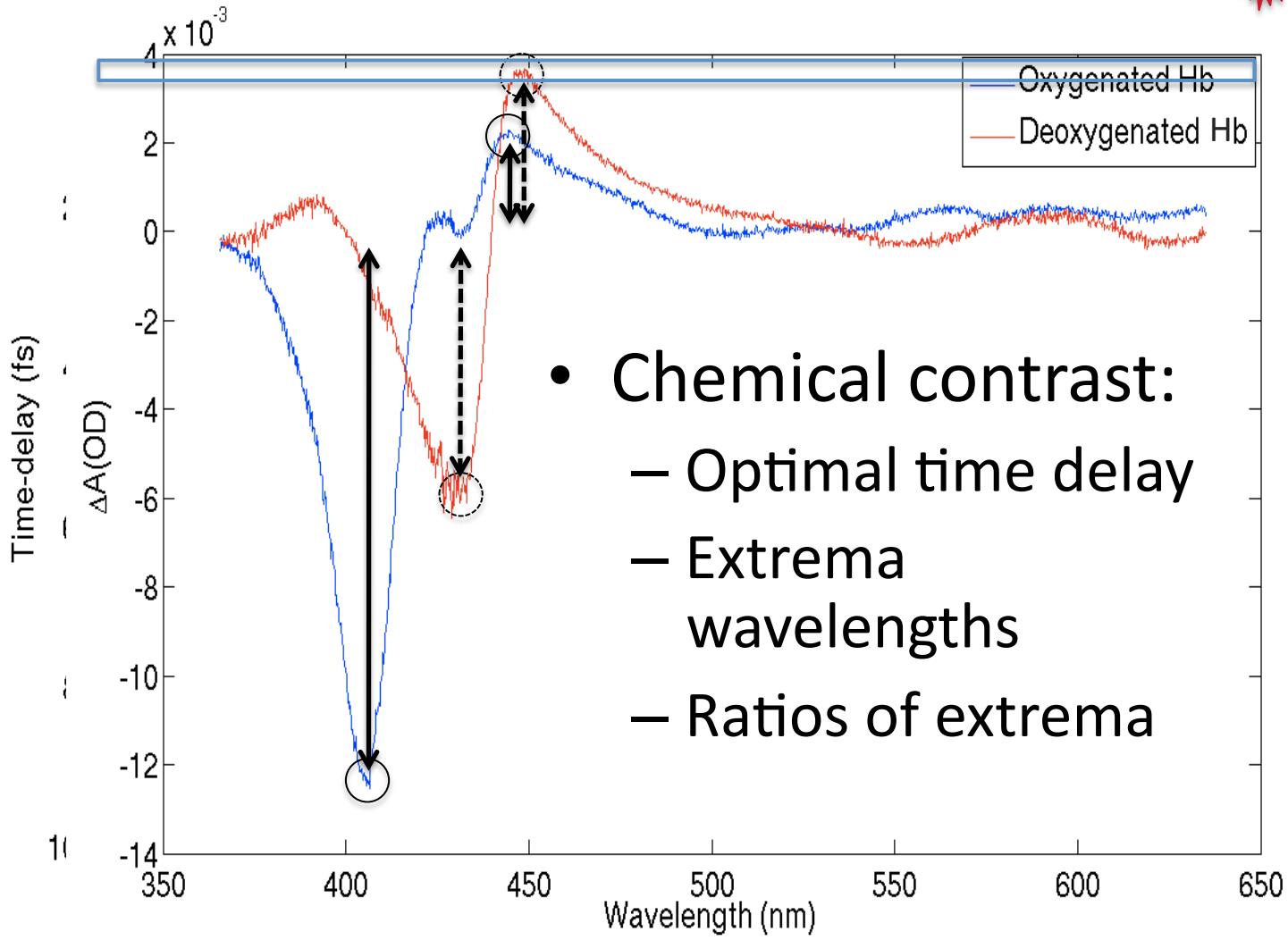
- The difference spectrum at each delay τ:





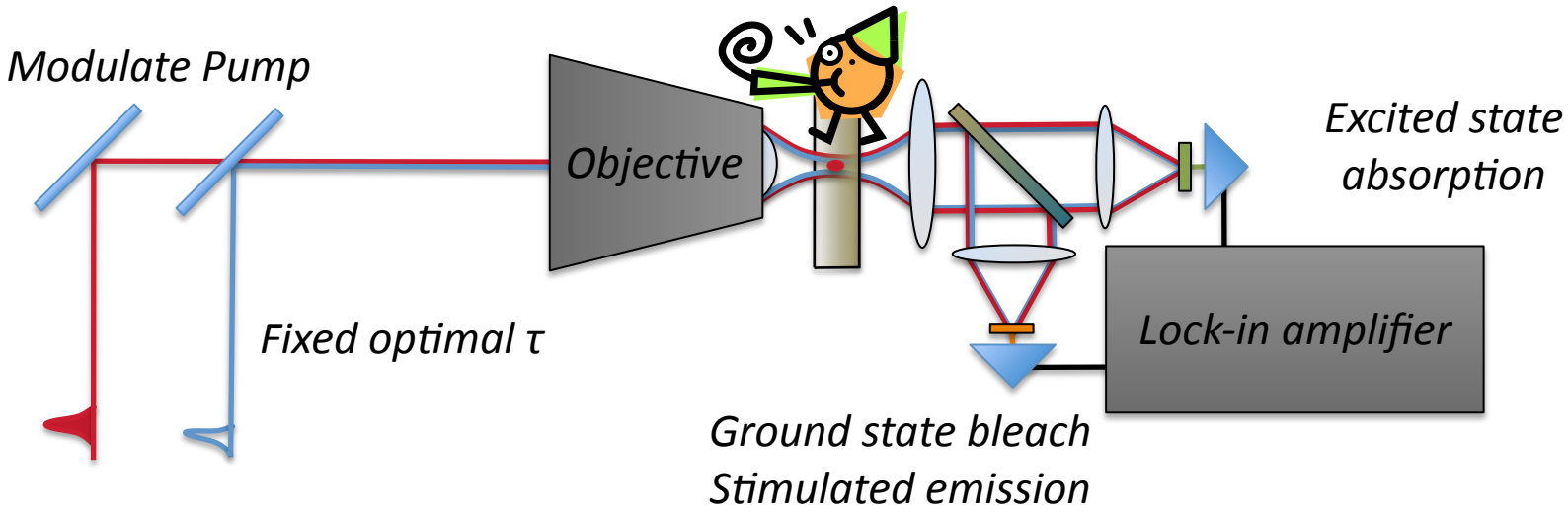
Spectroscopy Results

OxyHb
OxyMb
DeoxyHb
DeoxyMb
HbCO
MbCO
MetHb
MetMb
Ferric cytochrome c
Ferrous cytochrome c



- Chemical contrast:
  - Optimal time delay
  - Extrema wavelengths
  - Ratios of extrema

TPA Microscopy Setup



- 3D sectioning, endogenous (label-free) imaging [1-3]
- Increased penetration depth [4]
- Reduced photodamage of biological samples

Summary

- Achieved endogenous chemical contrast in heme proteins using two-photon spectroscopy
- Two-photon absorption microscopy provides:
  - 3D sectioning, endogenous (label-free) imaging [1-3]
  - Increased penetration depth [4]
  - Reduced photodamage of biological samples

References

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