Three Photon Adaptive optics for mouse brain imaging

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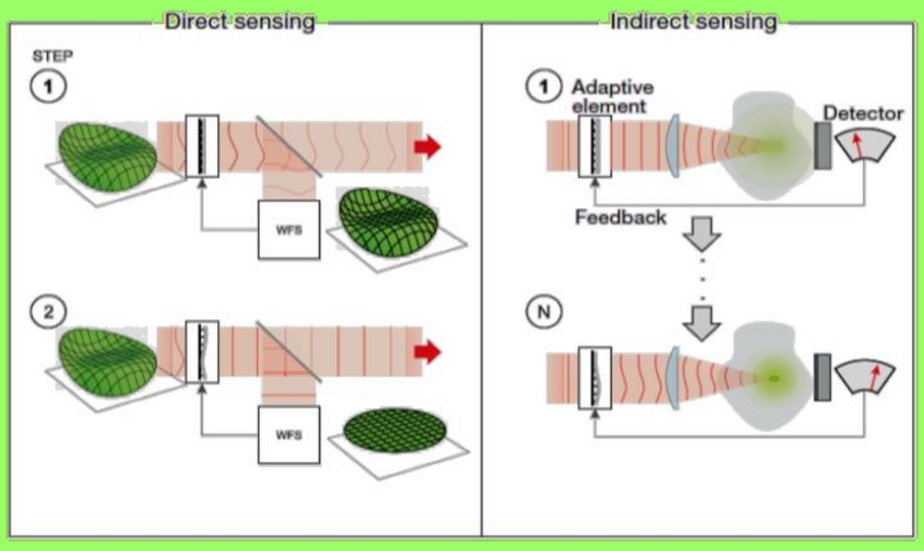
We demonstrate three-photon fluorescence adaptive-optics for *in-vivo* mouse brain imaging based on femtosecond pulses and MEMS spatial-light-modulator. We use the higher nonlinearity of the signal as a feedback to improve resolution and signal. We improve images of YFP labeled neurons in the hippocampus, and manage to resolve dendritic spines in the cortex.

Adaptive optics in 1PM and 2PM



- **Spatial light modulators (SLM) are used to shape the wavefront**
- **Compensating can be done for aberrations / scattering**
- **Feedback is based on image features or on direct wavefront sensing**

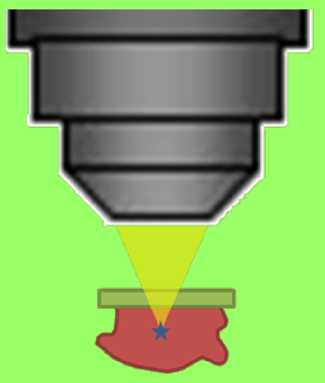
† M. J. Booth, D. De´barre, and A. Jesach, "Adaptive optics for biomedical microscopy." Opt. Photon. News 23, 22–29 (2012).

Nonlinear Adaptive optics: Using the nonlinearity as a guiding star

The expression for nonlinear signal in a thick sample:

$$\left\langle F^{(N)}(t)\right\rangle = \frac{1}{N}\frac{g_P^{(N)}}{(f\tau)^{N-1}}\phi\eta\sigma_N C n_0 \frac{a_N (NA)^{2N-4}}{8\pi^{3-N}}\frac{\left\langle P(t)\right\rangle^N}{\lambda^{2N-3}}$$

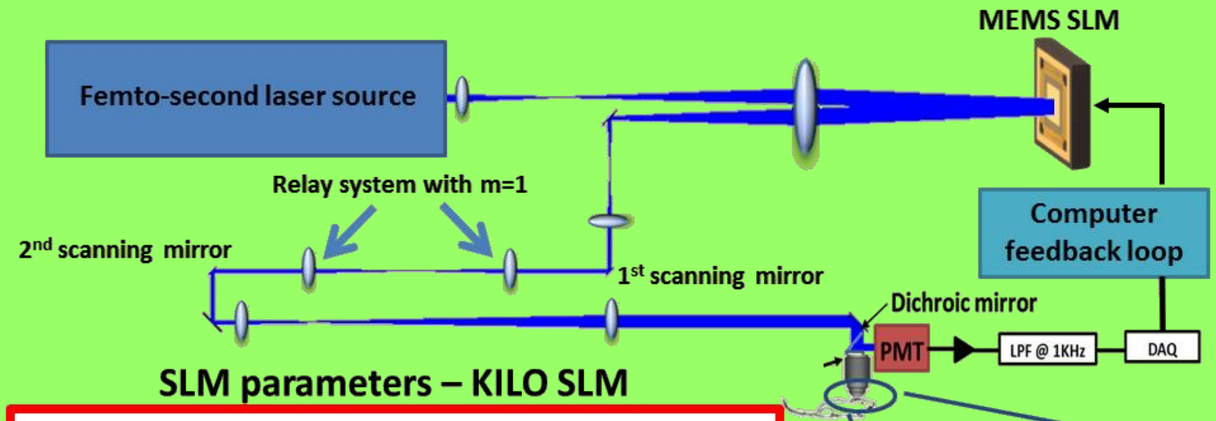
[a_N - volumetric integration factor]



- **Many brain features are larger than the spot size (blood vessels, Neurons) and therefore obey the thick sample Fluorescence equation.**
- **The nonlinearity of the signal serves as a "nonlinear guide star" which allows us to use it as feedback even without direct detection of the wavefront.**
- **For 3PM, the impact of wavefront aberrations will be larger than in 2PM.**

† C. Xu and W. W. Webb, Topics in Fluorescence Spectroscopy, vol. 5 (Springer, 1997).
‡ D. Sinefeld, H. P. Paudel, D. G. Ouzounov, T. G. Bifano, and C. Xu, "Adaptive optics in multiphoton microscopy: comparison of two, three and four photon fluorescence," Opt. Express 23, 31472-31483 (2015) .

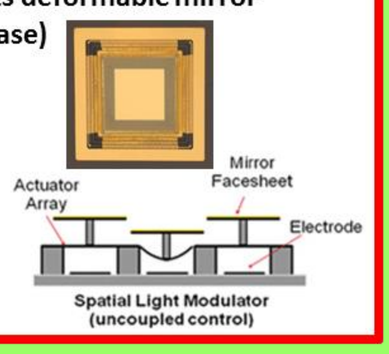
Three Photon Adaptive Optics system

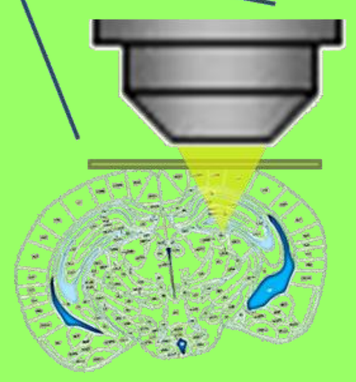


SLM parameters – KILO SLM

- 1020 *MEMS* actuators (32x32 array, without 4 corners)
- 32x32 segmented elements deformable mirror
- Stroke: 1.5 µm (= 3 µm phase)
- Aperture: 9.3 mm
- Fill Factor > 98%
- Pitch: 300 µm
- Max frame rate: 34 KHz

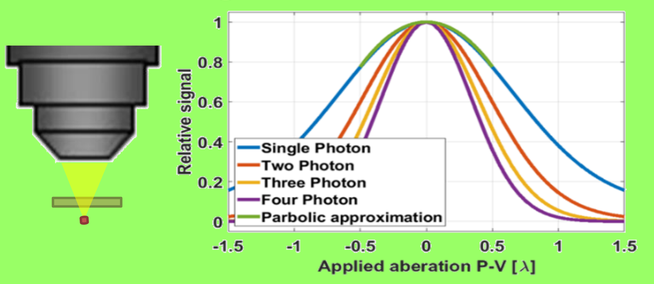
BOSTON MICROMACHINES CORPORATION
SHAPING LIGHT



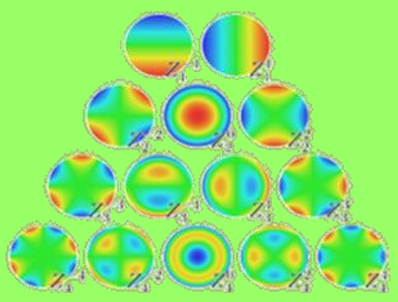


Feedback algorithm: parabolic approximation

Fluorescence signal from a bead:



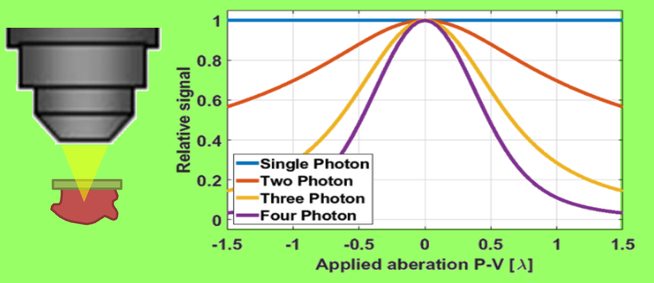
Zernike polynomials

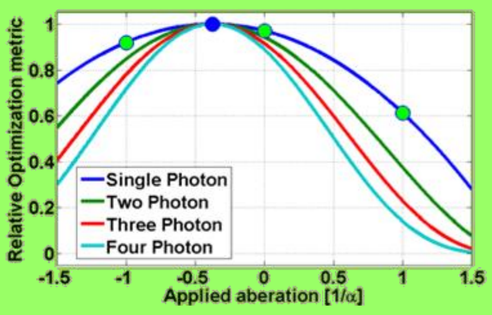


3 point Parabolic approximation

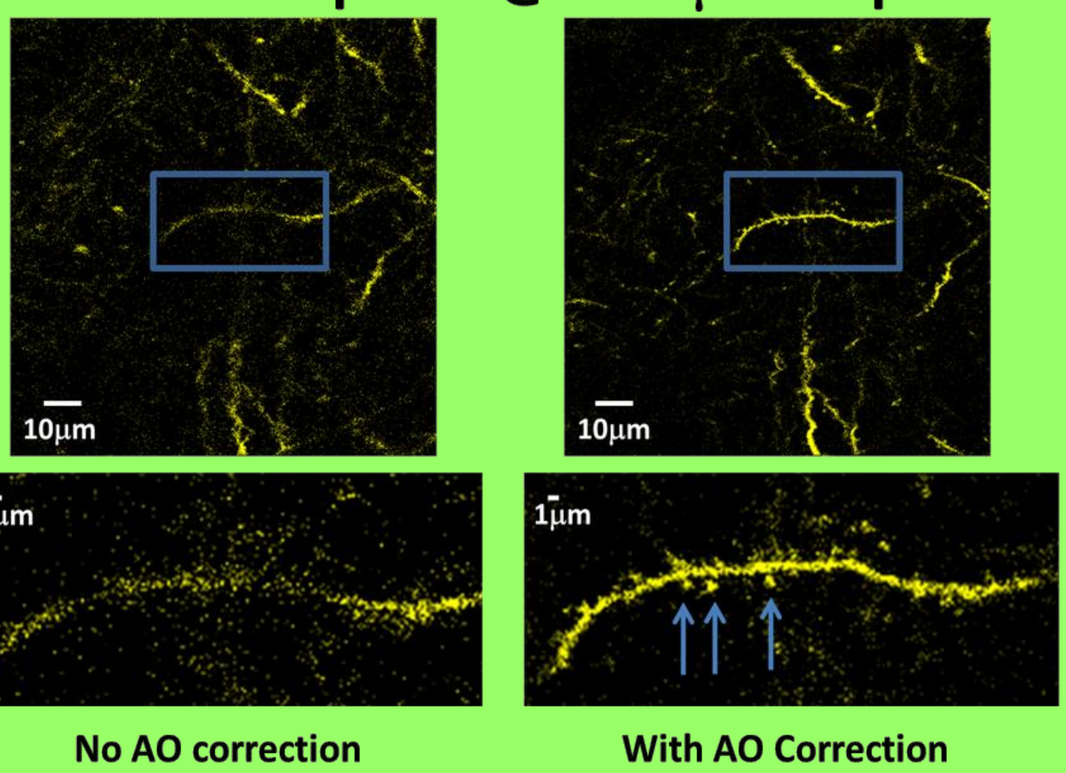
$$C_i = \frac{\sqrt[N]{S_{i+}} - \sqrt[N]{S_{i-}}}{2\sqrt[N]{S_i} - \sqrt[N]{S_{i+}} - \sqrt[N]{S_{i-}}}\frac{\alpha}{2}$$

Fluorescence signal from a thick sample :

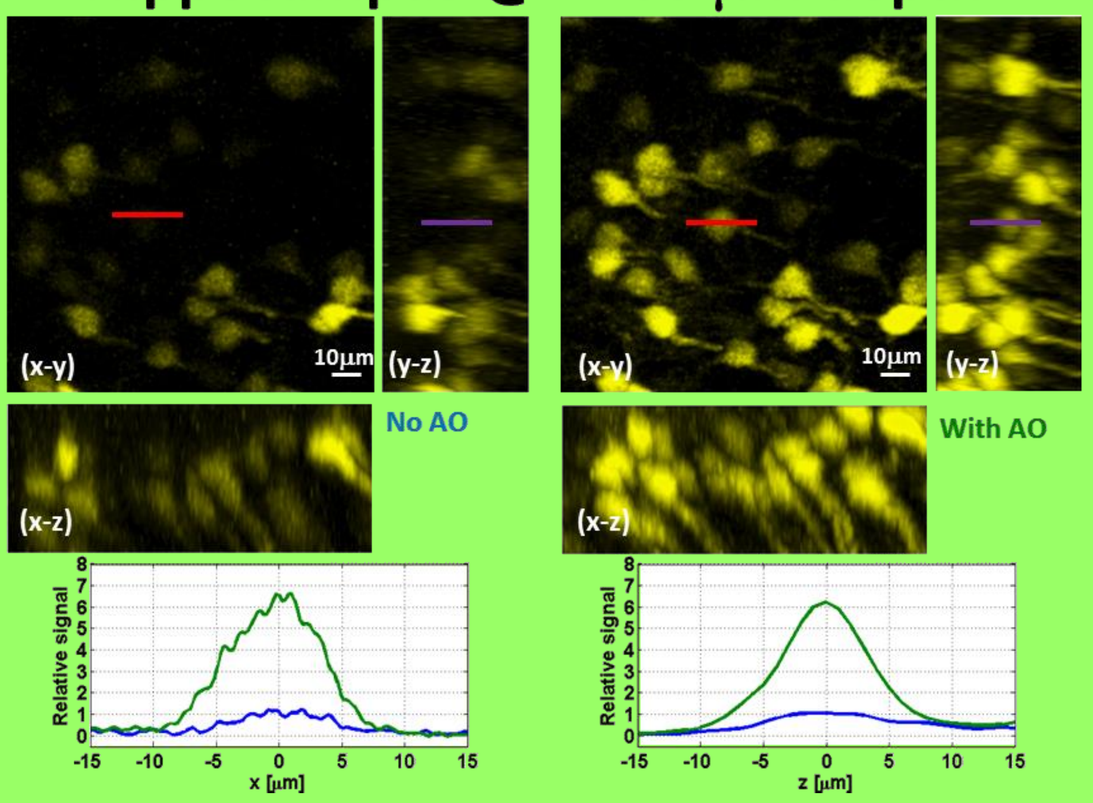




Resolution improvement, YFP mouse: Dendritic spines @ 570 µm depth



Signal improvement, YFP mouse: Hippocampus @ 1100 µm depth



Correction dependency on Field of view: Hippocampus Neurons @ 1120 µm depth.

SLM phase maps at different imaging depths:

D. Sinefeld et al. "Three Photon Adaptive Optics for Mouse Brain Imaging", Frontiers in neuroscience, 16, 880859 (2022).