# Enhancing Imaging Resolution and Depth with Adaptive Optics Focal Modulation Two-Photon Microscopy

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# Introduction

Photo source: Google Images

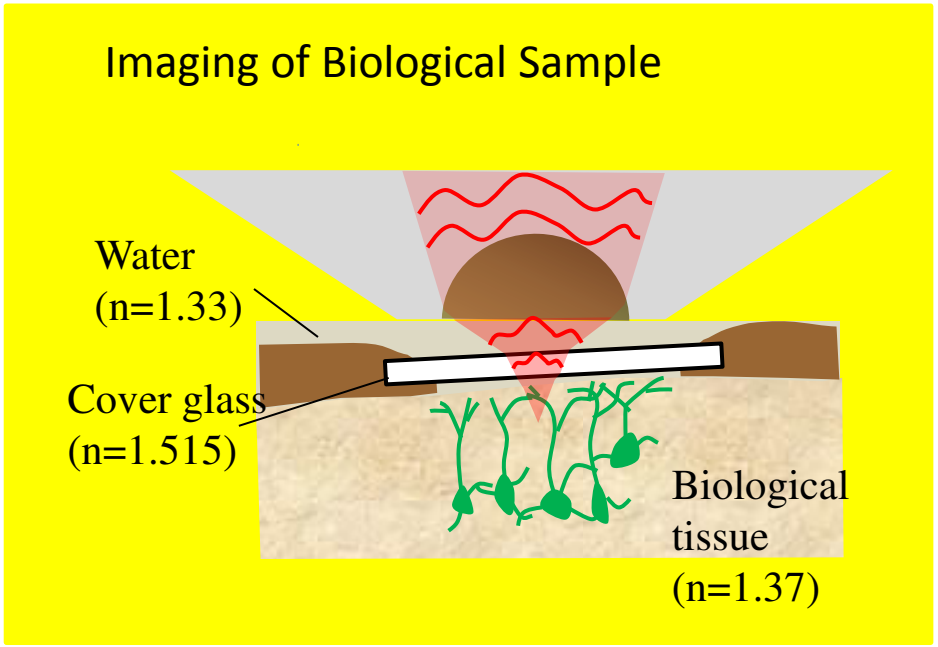
Image is distorted due to aberrations from rain

Photo source: Google Images

Image far away is lost due to absorption and scattering through fog

Image quality is determined by the aberrations and scattering in the optical path

# Issues and Challenges



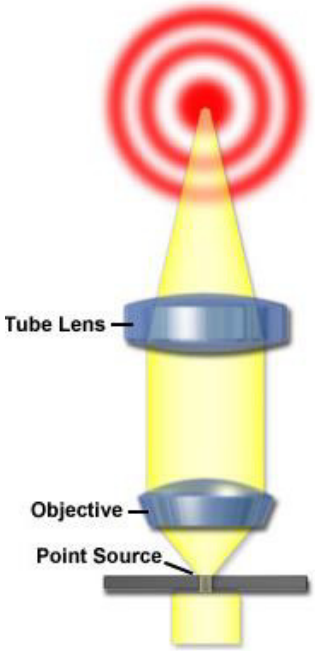

Picture source: UCSC

Deep tissue imaging is critical for scientific discovery and real-time monitoring of drug delivery and medical treatment

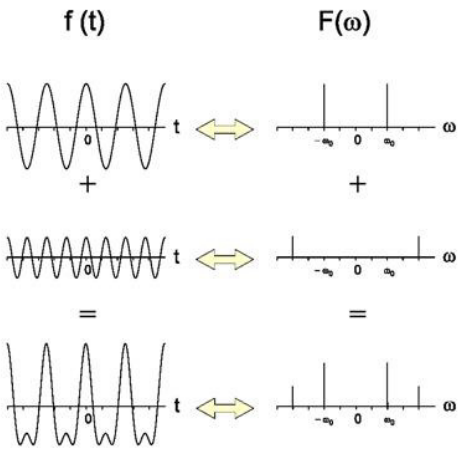
In 2014, Eric Betzig, Stefan Hell, and William Moerner won the Nobel Prize for their pioneer research in optical microscopy for bio-imaging

- Biological tissues introduce both aberrations and scattering
- If background noise from scattering is stronger than the signal of the biological sample itself, no structure is observed.
- Scattering increases with increasing depths

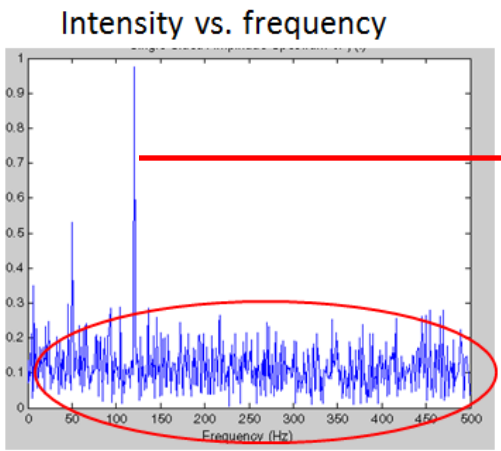
# Theory and Hypothesis




- Optical wavefront can be described by an electromagnetic wave
- When the illumination light is modulated with certain frequency, the resulting image is also modulated with the same frequency
- The random scattered light is not modulated.
- By separating the oscillatory signal from the non-oscillatory component, the background noise is effectively eliminated



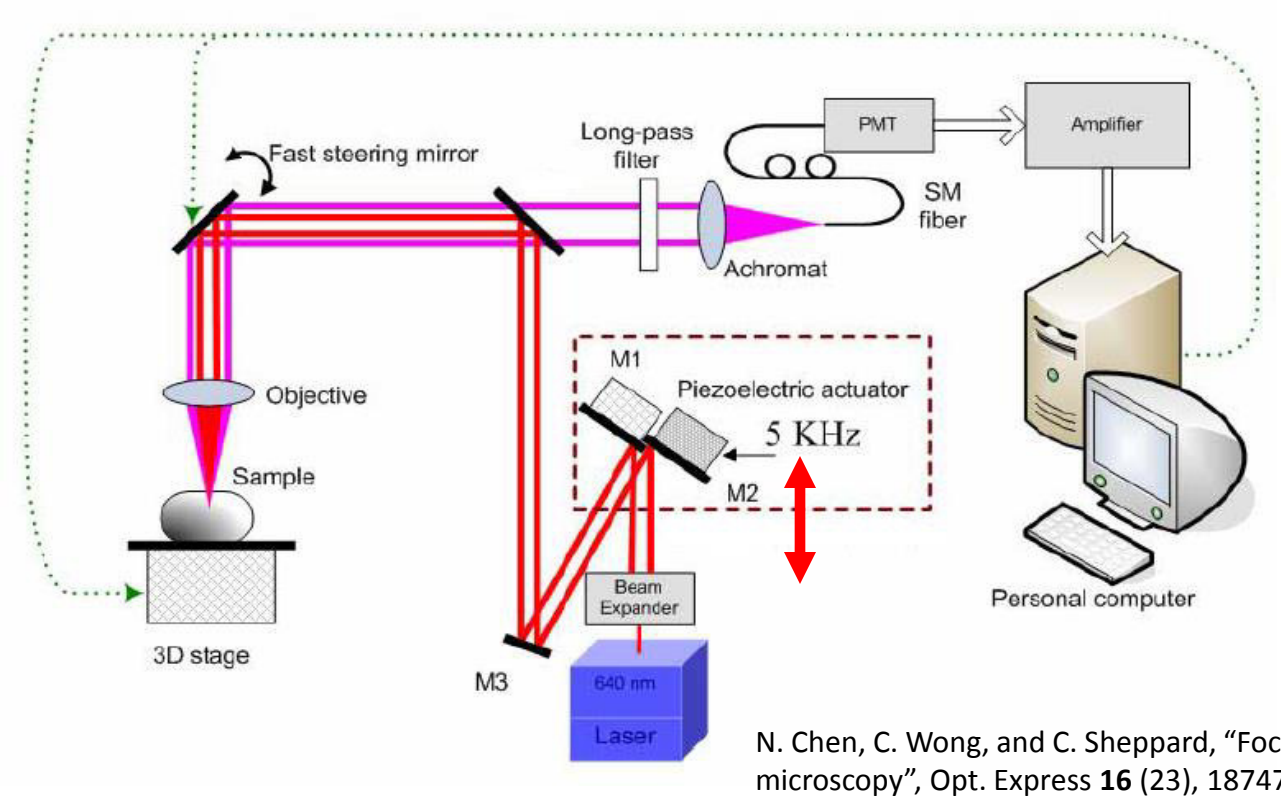

FFT




Keep signal

Remove noise

# Prior Art – Focal Modulation




N. Chen, C. Wong, and C. Sheppard, "Focal modulation microscopy", Opt. Express **16** (23), 18747-18769 (2008)

- Focal modulation microscopy was developed to suppress the background noise
- Piezoelectric actuator was used to modulate the optical path length by moving the mirror

# Adaptive Optics Focal Modulation Two-Photon Microscopy (AOFMTPM)

Proposed method:

- Modulate phase at the focal point instead of modulating focal distance
- Apply spatial time-dependent phase modulation with the deformable mirror in additional to aberration correction

$$P_{mod}(x, y, z_0, t) = A(t)\ \frac{2r\theta}{R},$$

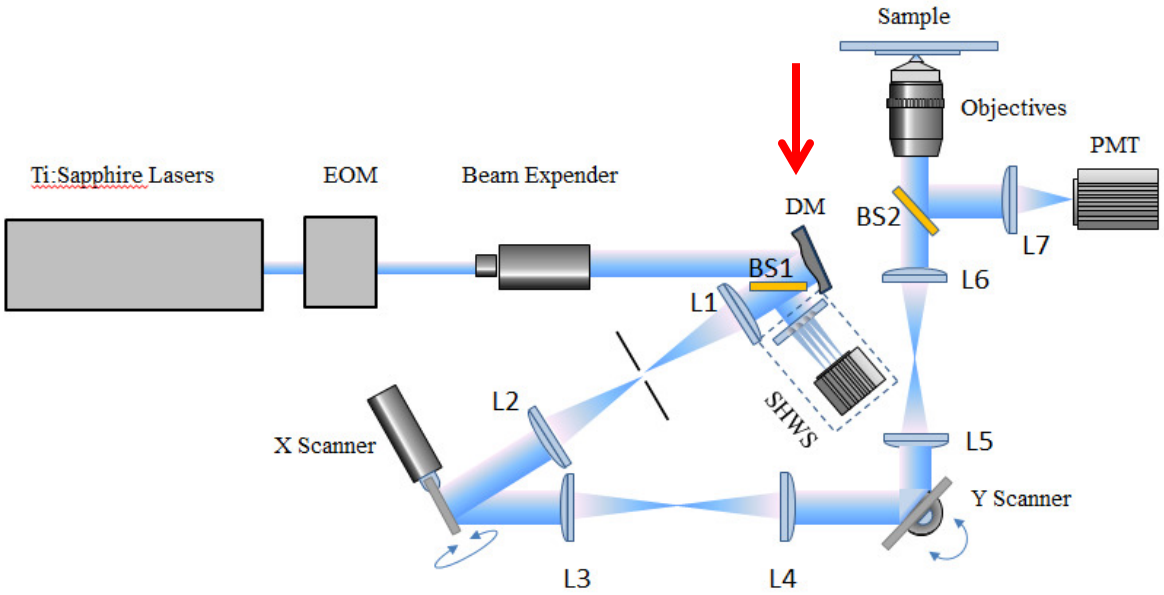
where $R$ is the aperture size of the DM,

$\theta = arctan\left(\frac{y}{x}\right)$, $r = \sqrt{x^2 + y^2}$ and

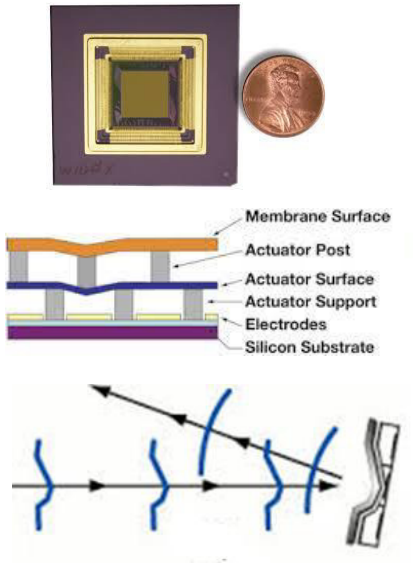
$$A(t) = \begin{cases} t, 0 < t \leq 0.5 \\ 1 - t, 0.5 < t \leq 1 \end{cases}$$

where $t$ is the modulation time in seconds

# Experimental Setup



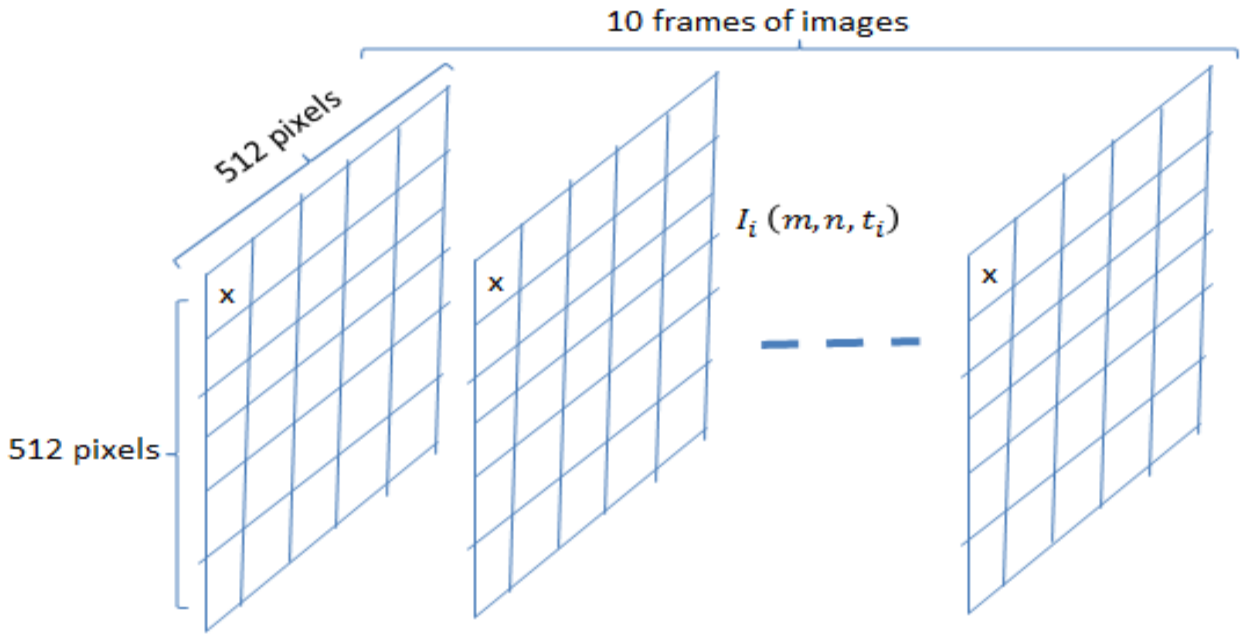

X. Tao, A. Norton, M. Kissel, O. Azucena, and J. Kubby, "Adaptive optical two-photon microscopy using autofluorescent guide stars", Opt. Lett., **38**(23), 5075-5078, (2013)




Voltages were applied to actuators of the deformable mirror to shape the membrane to generate desired phase correction

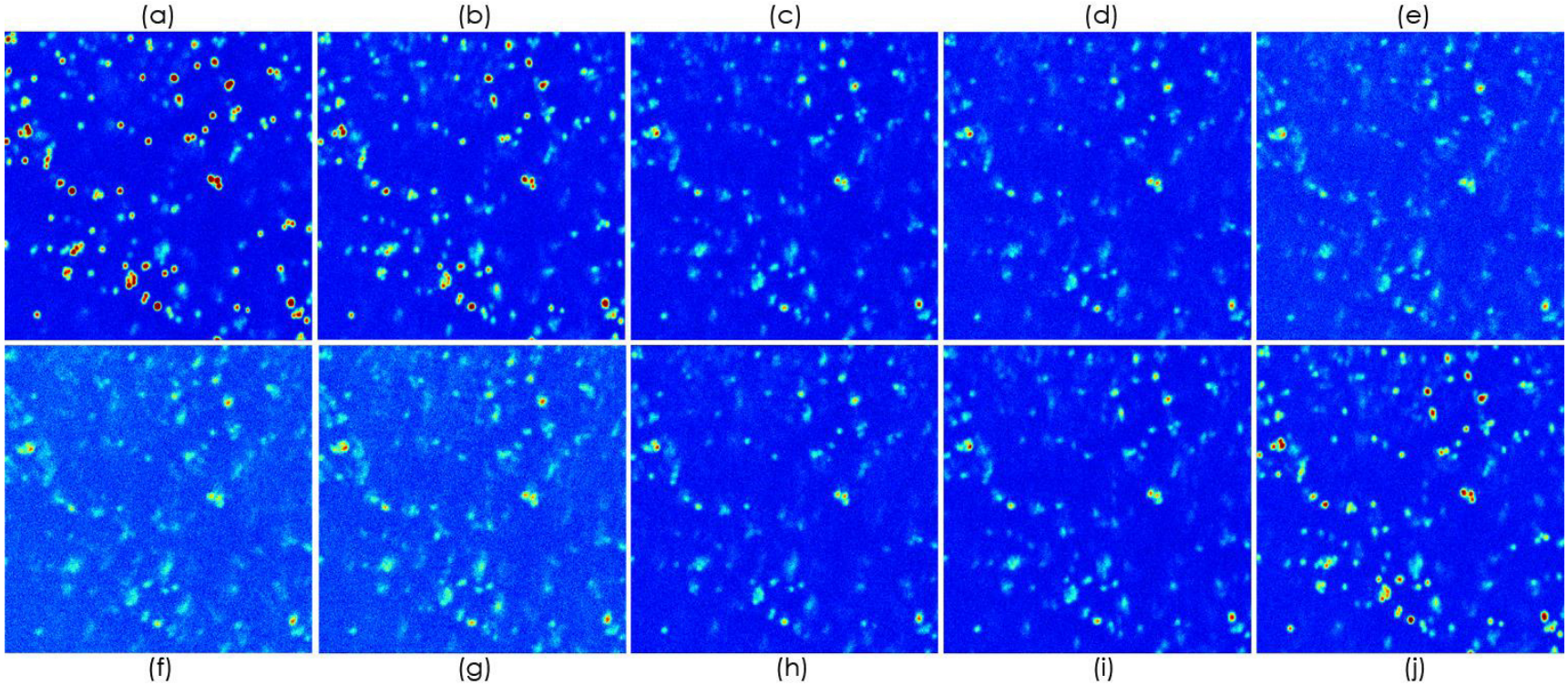
- Proof-of-concept experiments were performed using the Adaptive Optics Two-Photon Microscope developed by Tao and Kubby in 2013
- Tao and Kubby used a deformable mirror to correct the aberrations real-time to improve imaging resolution of live tissues with adaptive optics two-photon microscope
- I used a deformable mirror to provide additional phase modulation in my experiment

# Image Acquisition



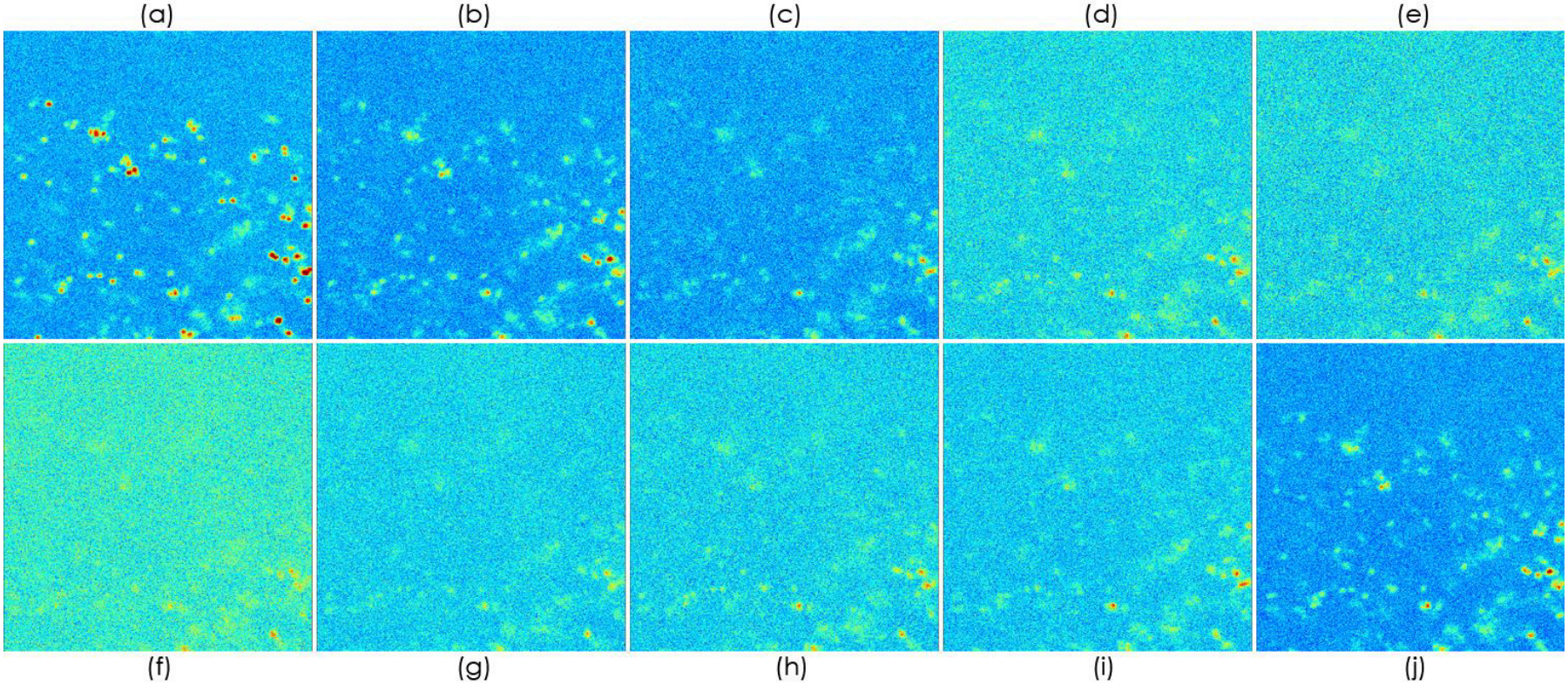

- Artificial tissue sample (1 µm fluorescent microbeads in 5% agarose gel) was imaged from the depth of 200 µm to 600 µm
- 10 image frames were taken in a 1 second period at each depth with phase modulation
- Each image consisted of 512 x 512 pixels

# Images at 200 µm



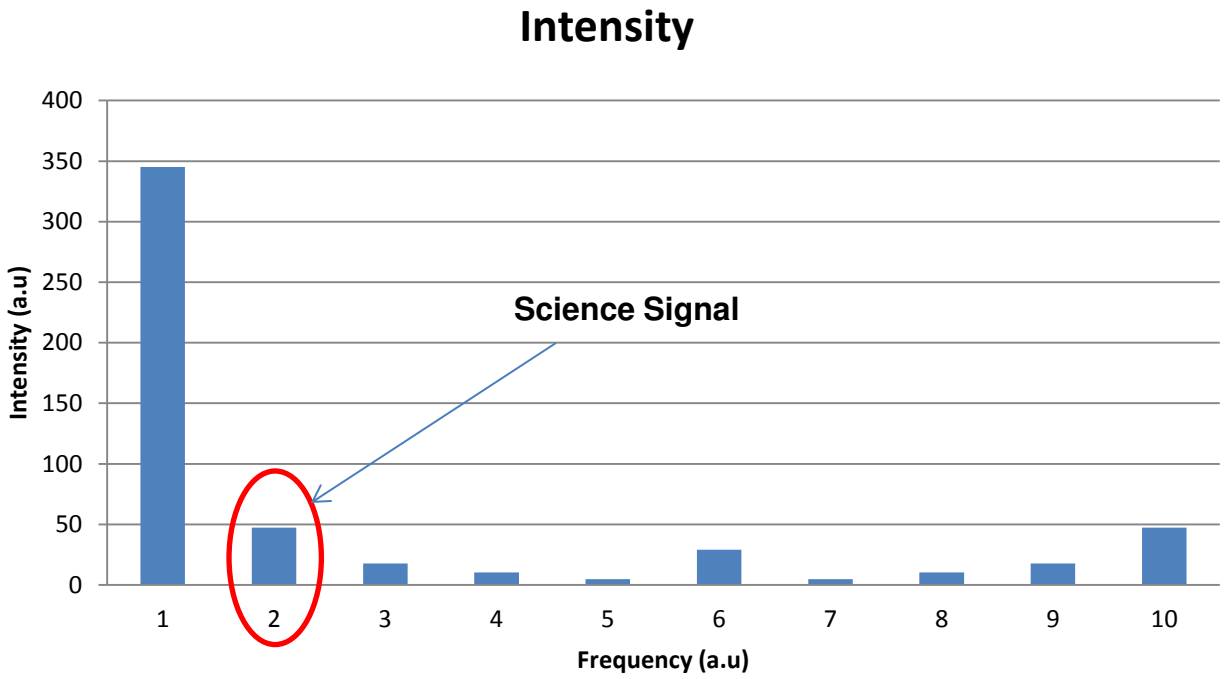

- Ten images were acquired at the time interval of 0.1 seconds from (a) to (j) at the depth of 200 µm with time-dependent phase modulation
- As more aberrations were applied from (a) to (f), images got more blurry
- Applied aberrations were reduced from (f) to (j), and one full cycle of image acquisition was completed

# Images at 500 µm



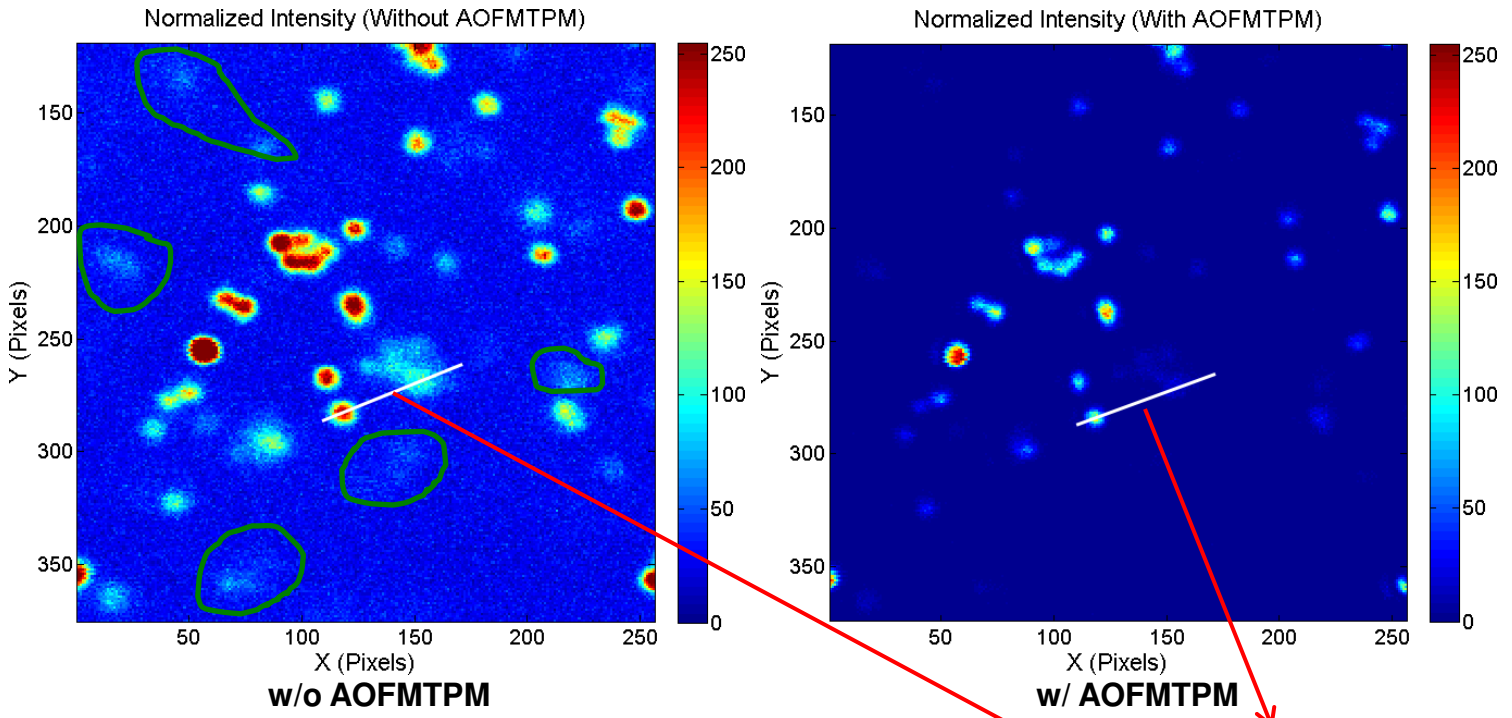

- Images of the microbeads are blurrier compared to those at 200 µm
- Background and random noises were more severe when imaging deeper into the tissue

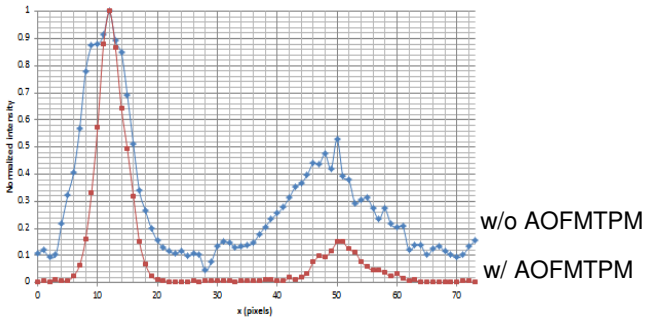
# Image Processing




- Fast Fourier Transforms were performed on the images
- The first AC component, associated with the sampling frequency, was saved. The DC component and high-order AC components were eliminated
- This process removed the undesirable out-of-focus fluorescent background and scattering noises
- Several processing algorithms were developed in MATLAB, C++, and finally in CUDA , which enabled parallel processing to increase image analysis speed

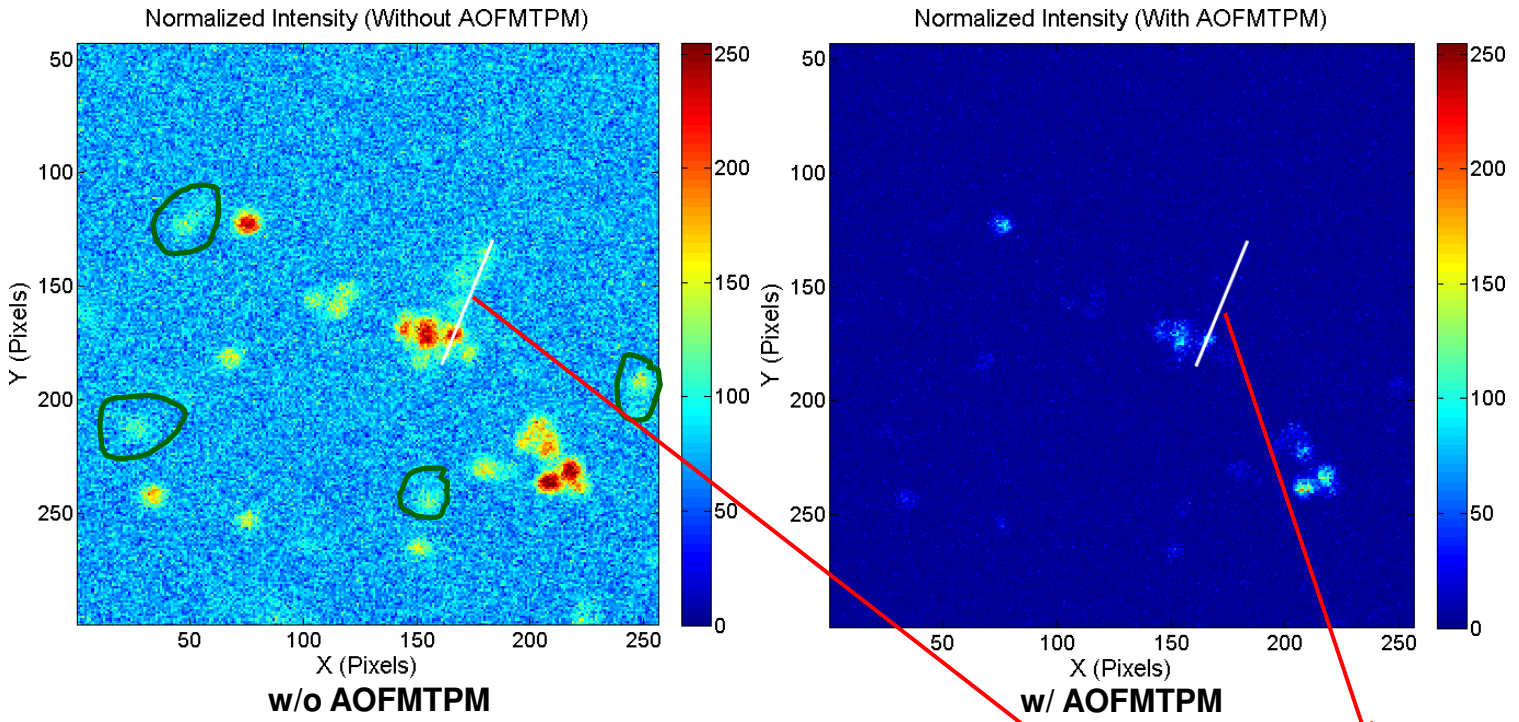
# Imaging results at 200 µm



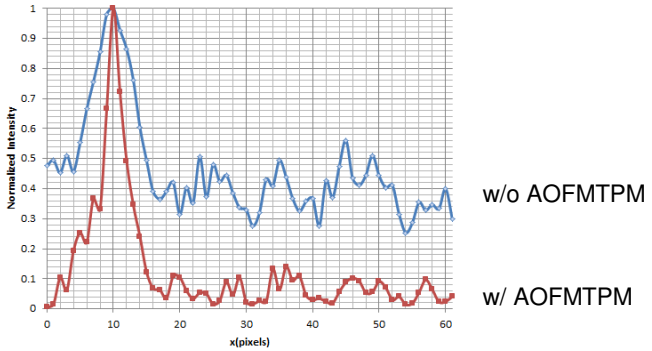




- The out-of-focus fluorescent noise in the highlighted areas was almost completely removed after applying AOFMTPM
- Random background noise was suppressed
- Final image was much sharper
- The full width at half maximum (FWHM) for imaged beads was reduced to about half

# Imaging results at 500 µm




- Random background was effectively suppressed with AOFMTPM at 500 µm
- The signal-to-noise ratio was improved by 7 dB
- The ratio of the FWHM without and with AOFMTPM is about 2.1 for ten beads
- Resolution is effectively doubled



# Conclusions

- Successfully developed and experimentally demonstrated a novel microscopy method to enhance imaging resolution and imaging depth through a highly scattering medium.
- By introducing spatial time-variant phase modulation at the focal plane of a two-photon microscope with adaptive optics, the background fluorescence and scattering noises were effectively suppressed.
- Developed a fast algorithm with CUDA to separate the desired science signal from background noise. Imaging process time was reduced from 15 seconds to 0.5 seconds.
- Lateral resolution was doubled and signal-to-noise ratio was improved by 7dB at the depth of 500 μm.
- Fluorescent microbeads up to a depth of 600 μm were successfully measured in an artificial tissue sample.

# Future works

- Investigate the phase modulation functions and find the function which optimizes the signal-to-noise ratio and provides the best image
- Explore the depth limitation of this method with biological tissues
- Further improve the speed of data acquisition and data analysis for real-time imaging of a live tissue, possibility for video
- Develop a 3D reconstruction tool for a 3D illustration of the tissue sample

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# Acknowledgements


- National Science Foundation Center for Adaptive Optics
- W.M. Keck Center for Adaptive Optical Microscopy
- Prof. Joel Kubby and Dr. Xiaodong Tao for technical guidance and support
- My high school teacher Dr. Lazar and high school principal and counselor for their encouragement


*Thank you!*