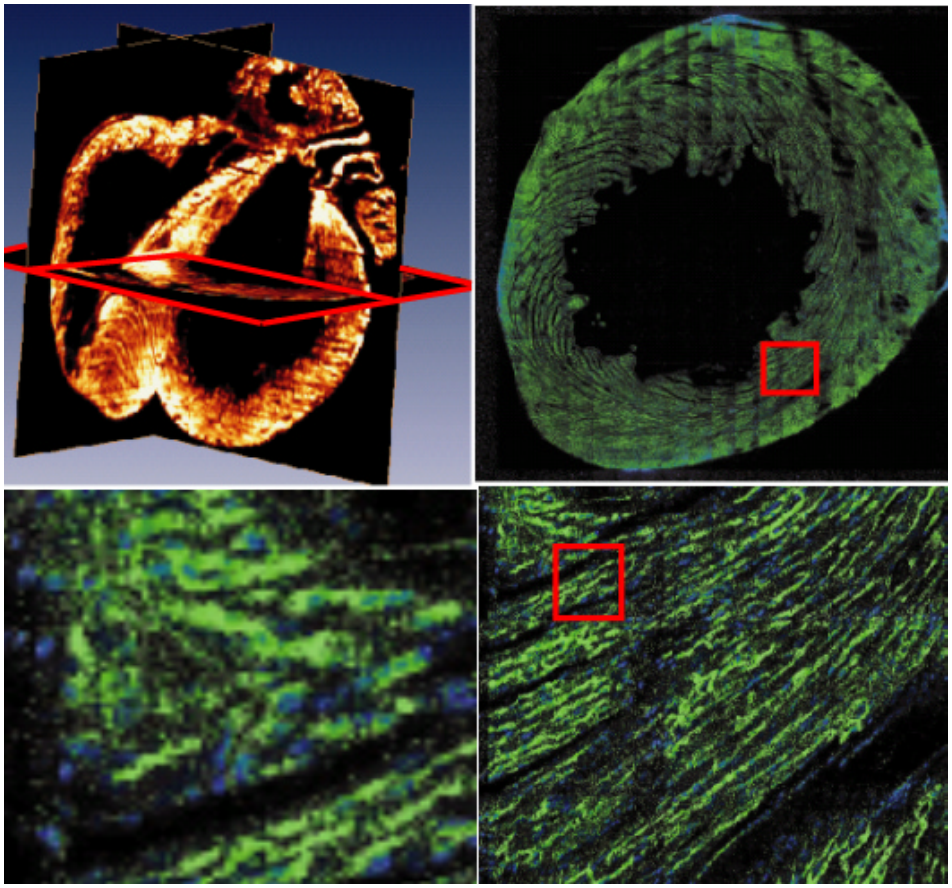
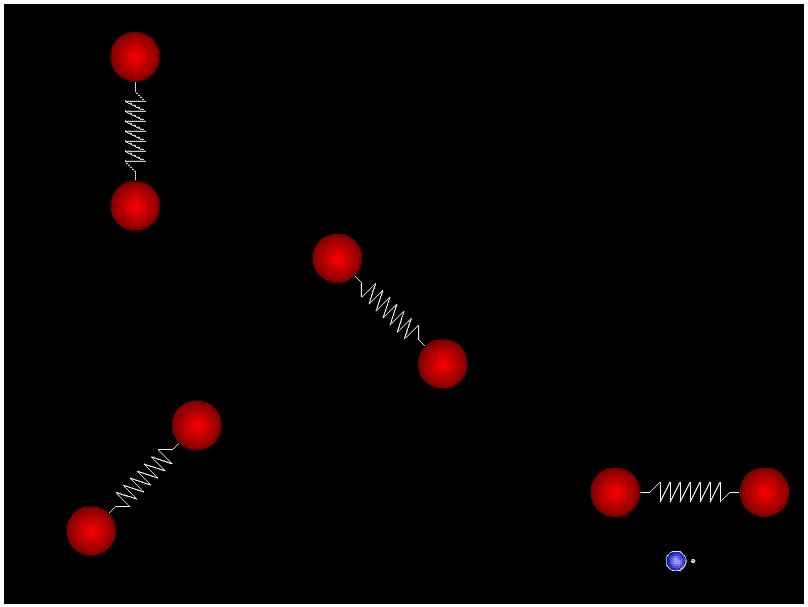
3D Microscopy: Multiphoton Imaging

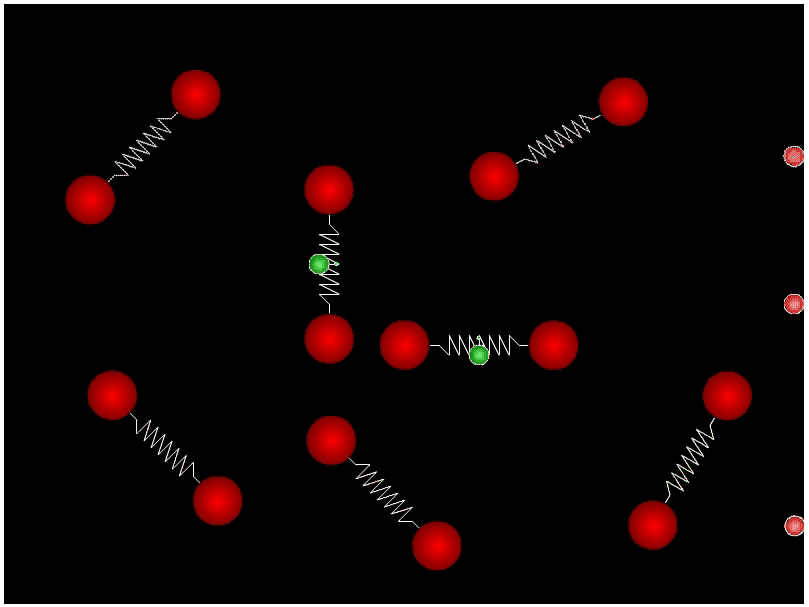


Basic Ideas of two-photon excitation

Two-photon excitation is similar to the one-photon process except the use of two lower energy (infrared) photons.

The difference between the two can be seen in these movies:





An abbreviated history of two-photon microscopy

(1930) Maria Goeppert-Mayer predicted the existence of two-photon effect

(1961) Franken et al. demonstrated second harmonic generation in ruby

(1961) Kaiser et al. showed two-photon fluorescence in a solid state material

(1964) Singh and Bradley reports three-photon fluorescence

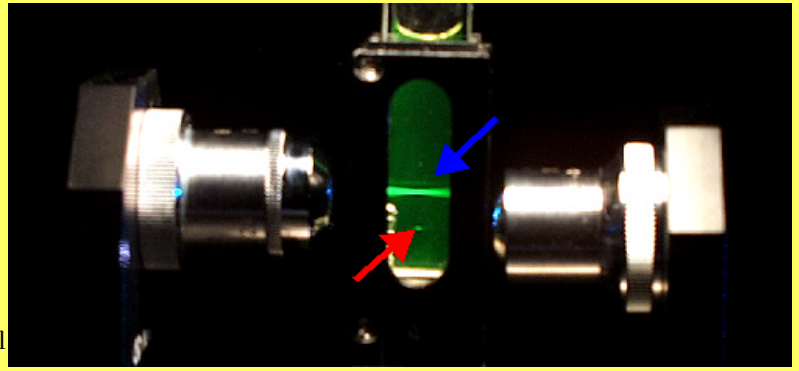
(1970s-1980s) Two-photon effect has been used in biological spectroscopy by researchers such as Birge, Fredrich, and McCain

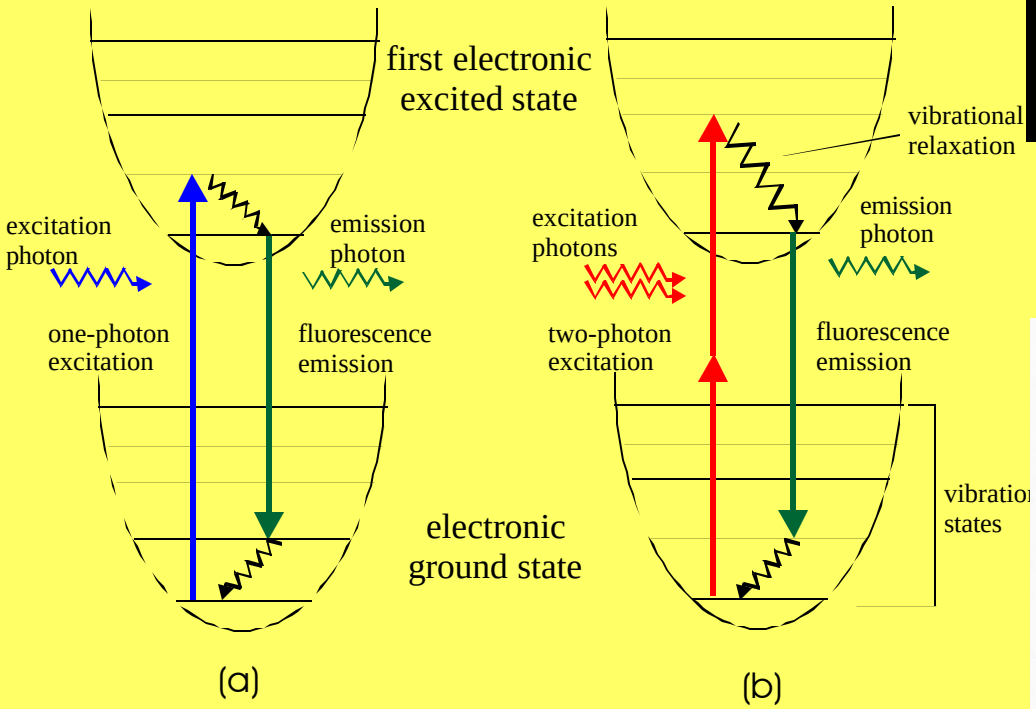
(1970s-1980s) Microanalysis based on non-linear 2nd harmonic generation was developed by researchers such as Freund and Hellwarth

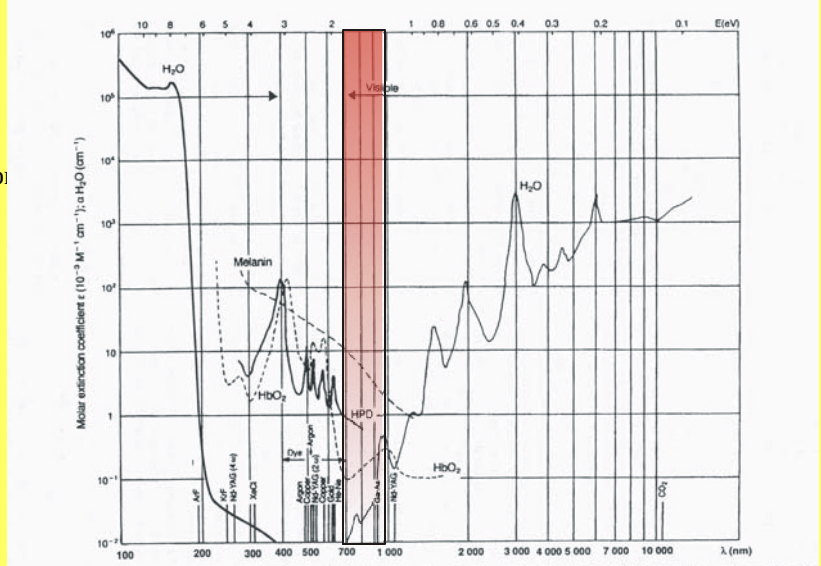
(1976-80s) A number of researchers such as Sheppard, Kompfner, Gannaway and Wilson suggested the possibility of incorporating non-linear excitation \into scanning microscopy

(1989)Denk, Webb and coworkers definitively demonstrated two-photon scanning microscopy and a number of its unique properties such as the triggering of localized chemical reaction

Two-Photon Excitation Microscopy



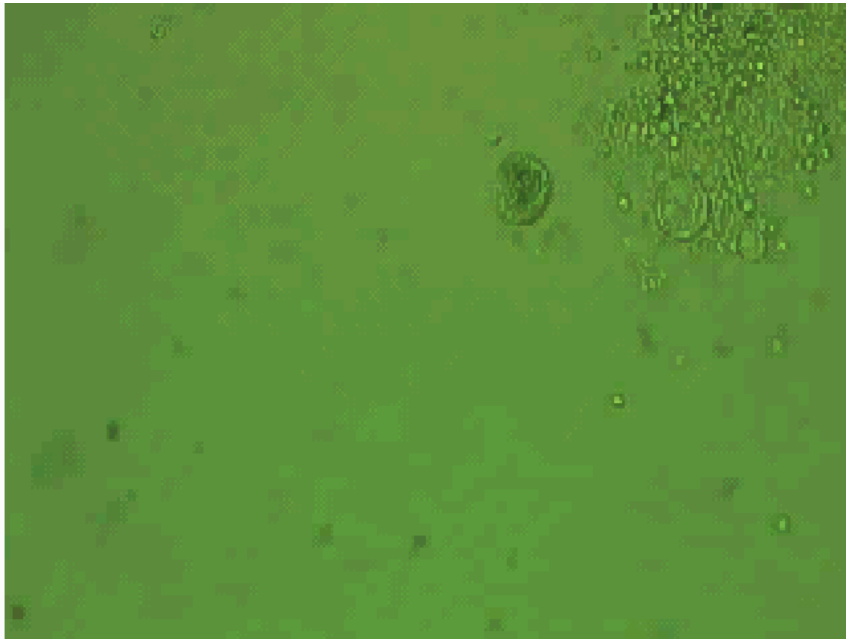


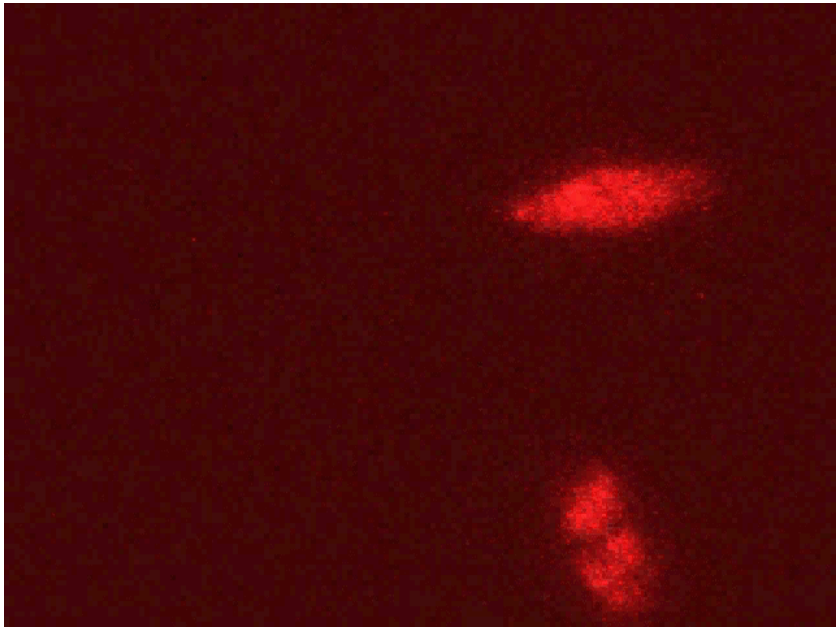


A comparison of two-photon and confocal microscopes

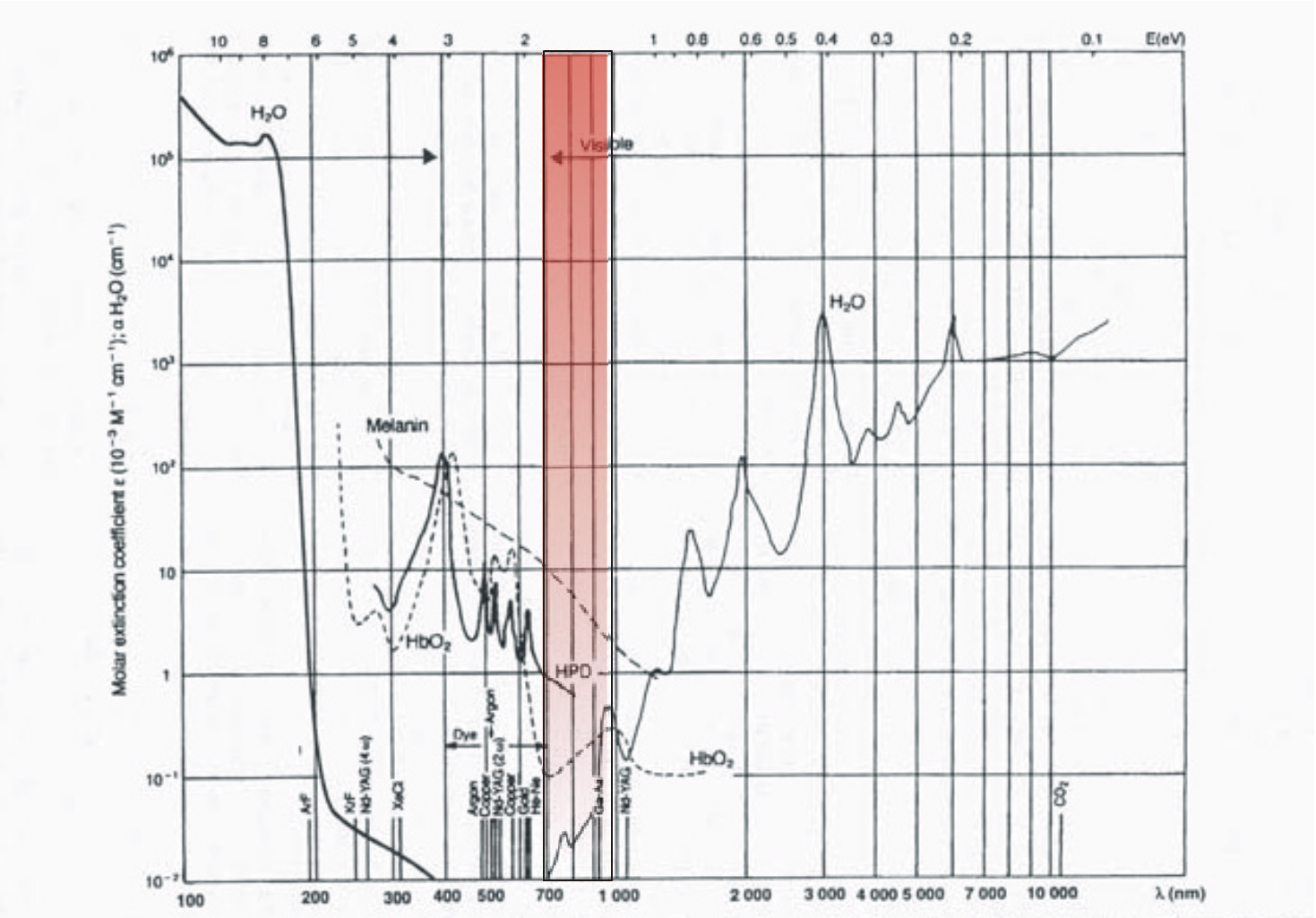
(1) Confocal microscopes have better resolution than two-photon microscopes without confocal detection.

(2) Two-photon microscope results in less photodamage in biological specimens. The seminal work by the White group in U. Wisconsin on the development of *c. elegans* and hamsters provides some of the best demonstration. After embryos have been continuously imaged for over hours, live specimens are born after implantation.



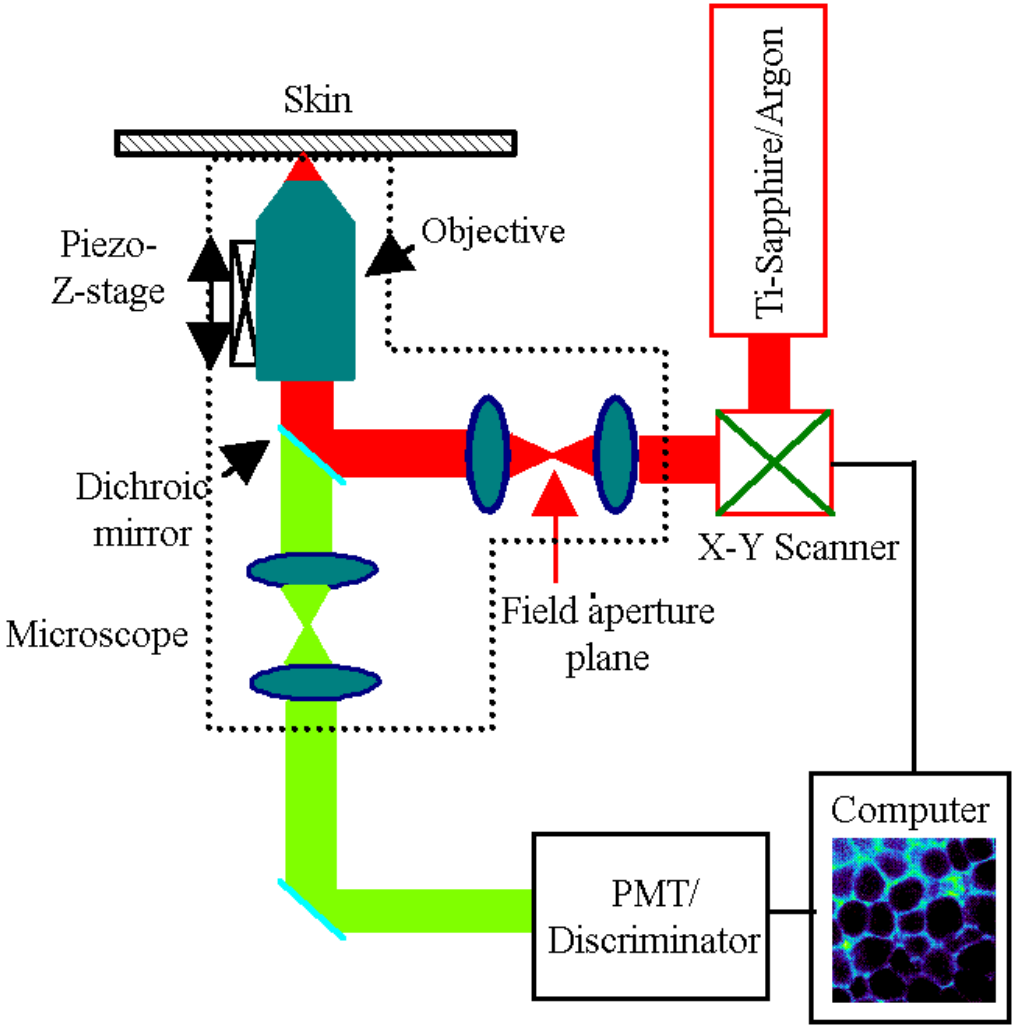


(3) Two-photon microscope provides better penetration into highly scattering tissue specimen. Infrared light has lower absorption and lower scattering in turbid media.

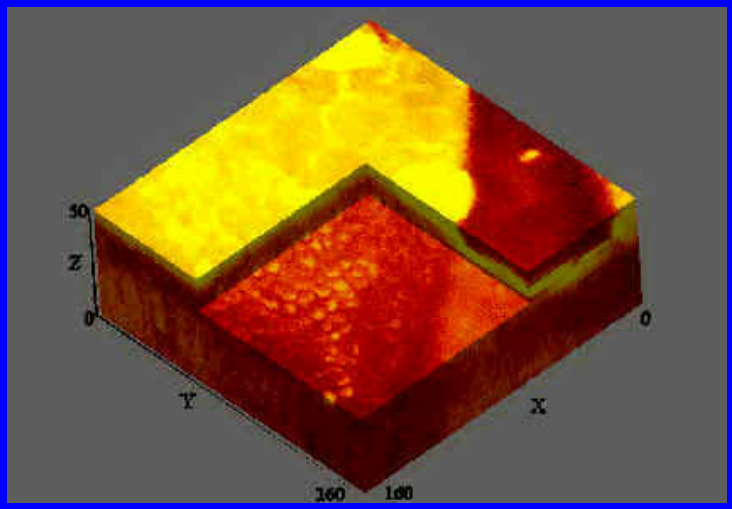


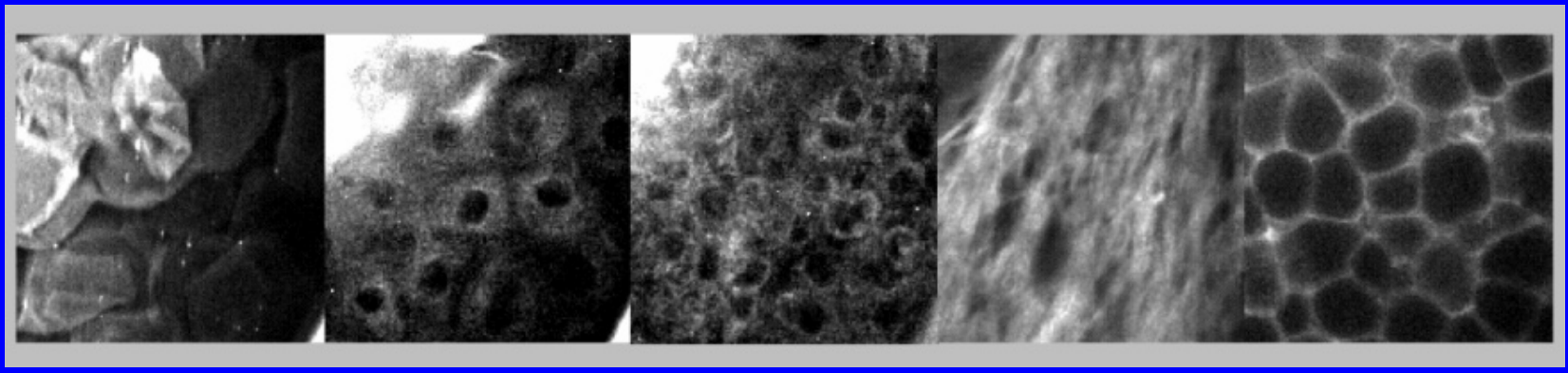
The design of a two-photon microscope

Two-photon microscope design is actually significantly simpler than that of confocal microscope and has much in common.



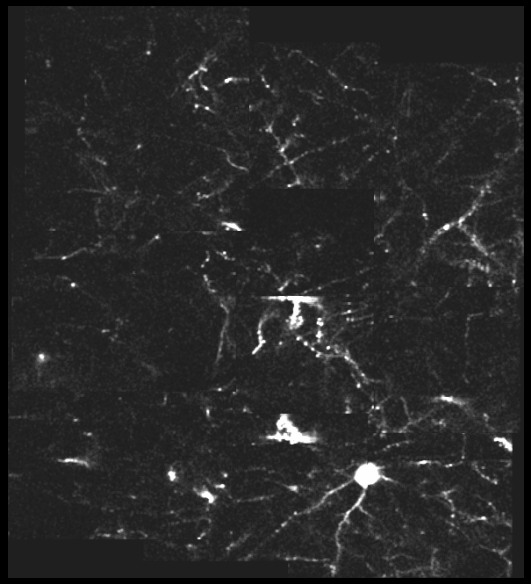
A 3D Reconstructed Movie Of Skin Structures From A Mouse Ear Tissue Punch



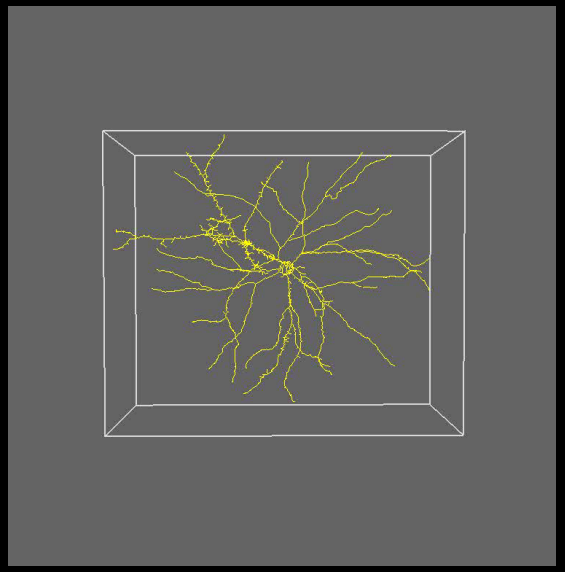


In collaboration with I. Kochevar, Wellman Labs, MGH and B. Masters

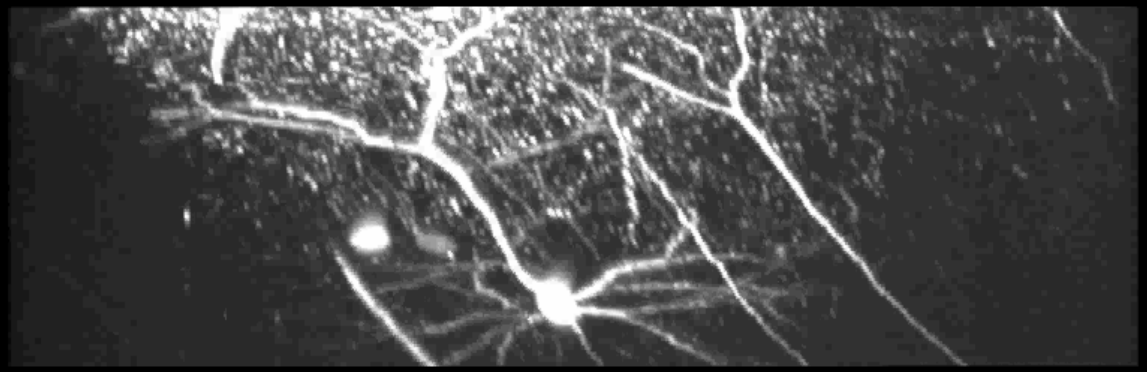
IN VIVO IMAGING OF NEURONAL DEVELOPMENT



Z-Stack, Individual Slices



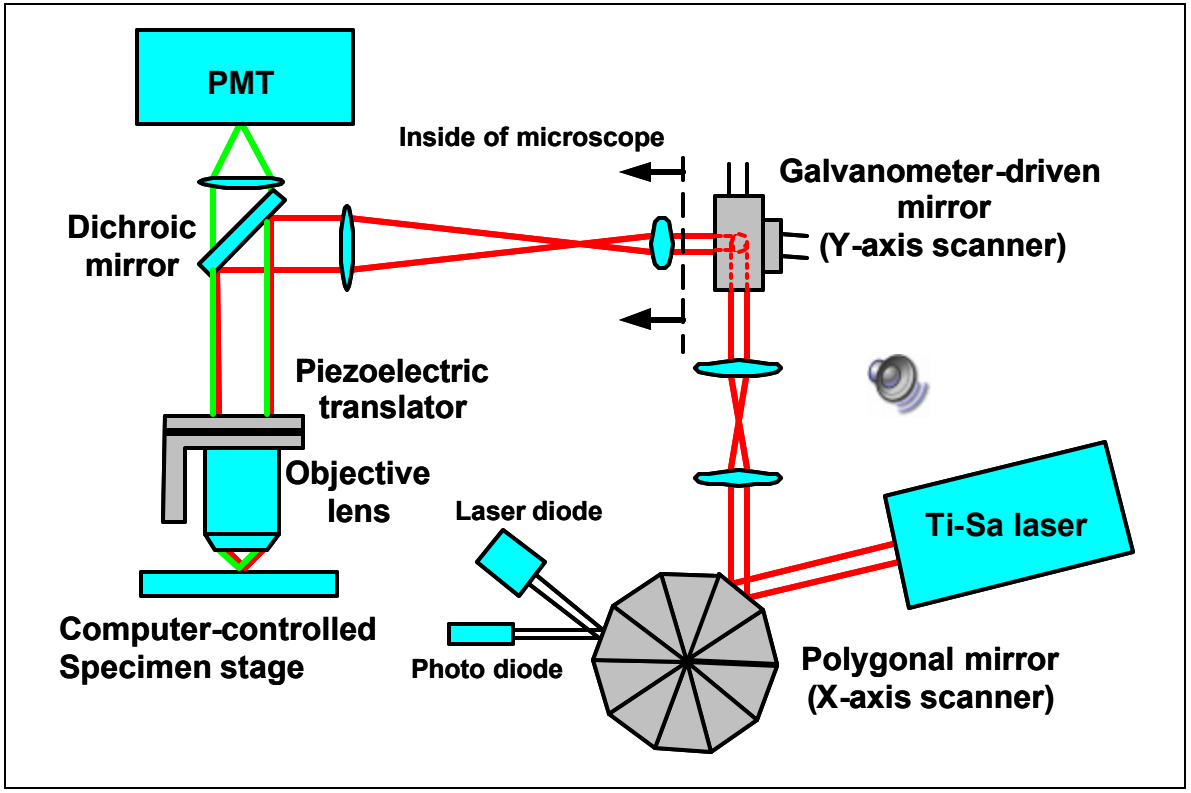
Computational Model of Dendrite Branches



Reconstructed 3-D View

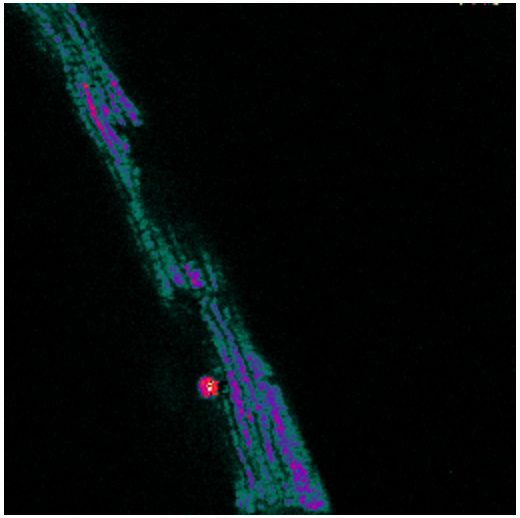
In collaboration with Wei Lee & Elle Nevidi, MIT

3D Multiple Particle Tracking with Video Rate Two-Photon Microscopy



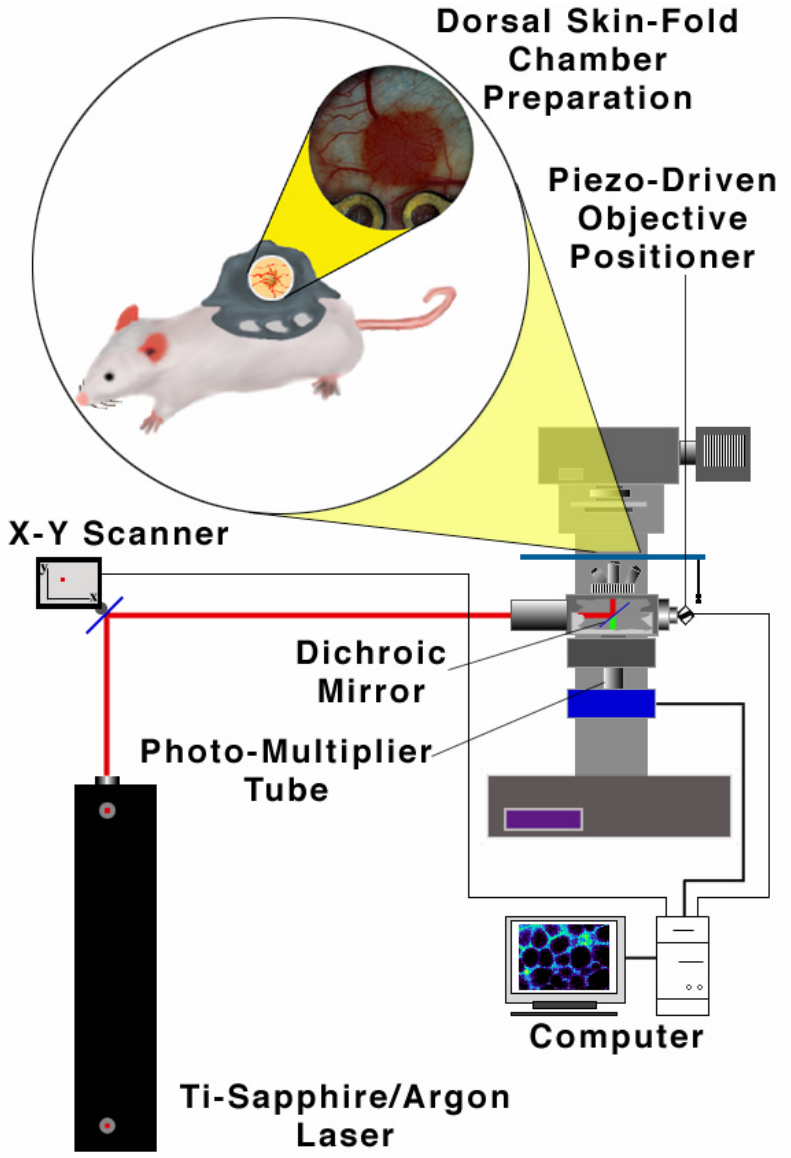
In collaboration with Ki Hean Kim (MIT)

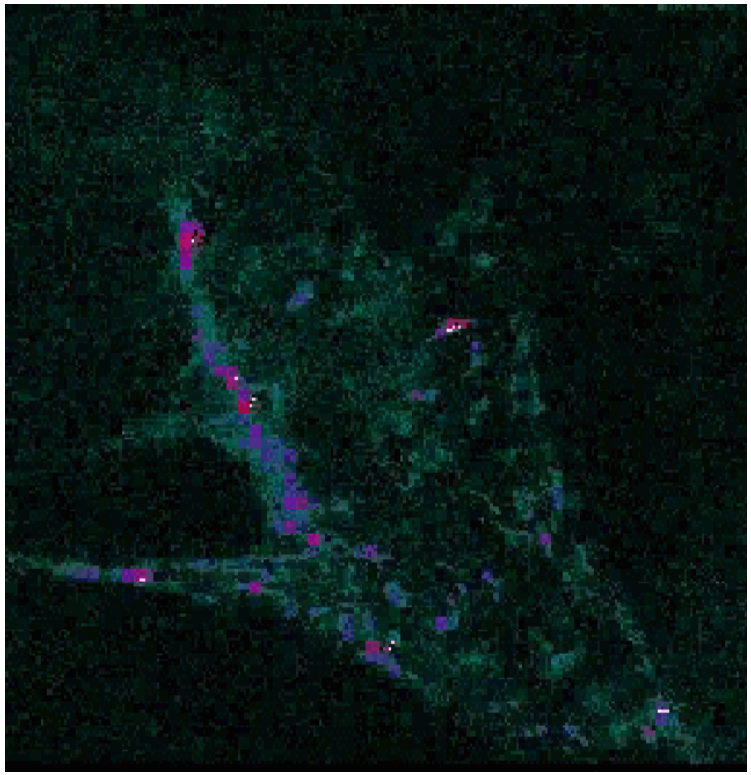
Imaging of myocyte contraction -- R6G labeled mitochondria



In collaboration with J. Lammerding, H. Huang, K. Kim, R. Kamm, R. Lee (MIT and Brigham & Women's Hospital)

3D Quantification of Blood Flow in Solid Tumors

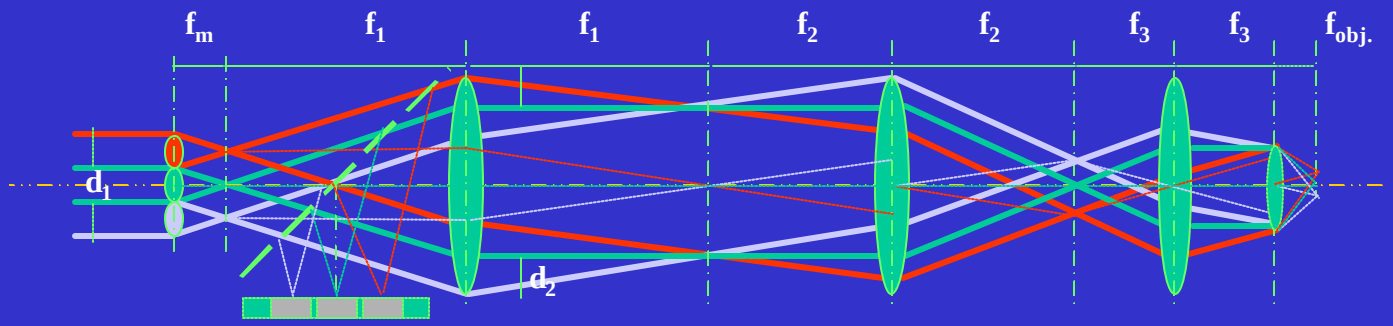


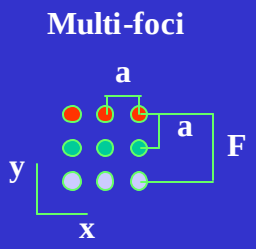


In collaboration with Rakesh Jain, MGH

MMM Ray Tracing

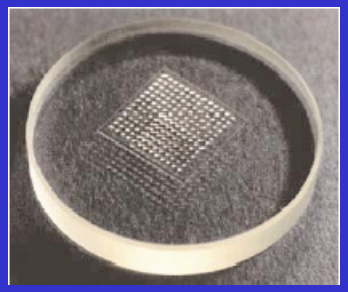
2-Photon Microscope – multi foci scanning

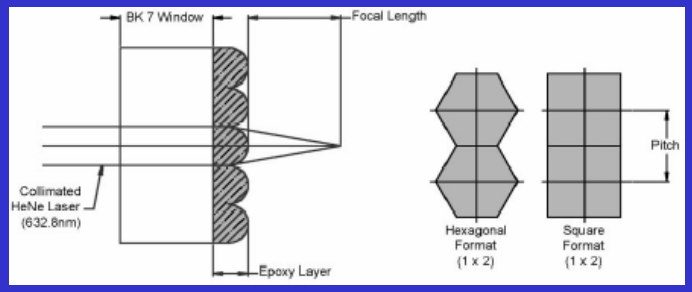




CCD

Resonant-mirror-position

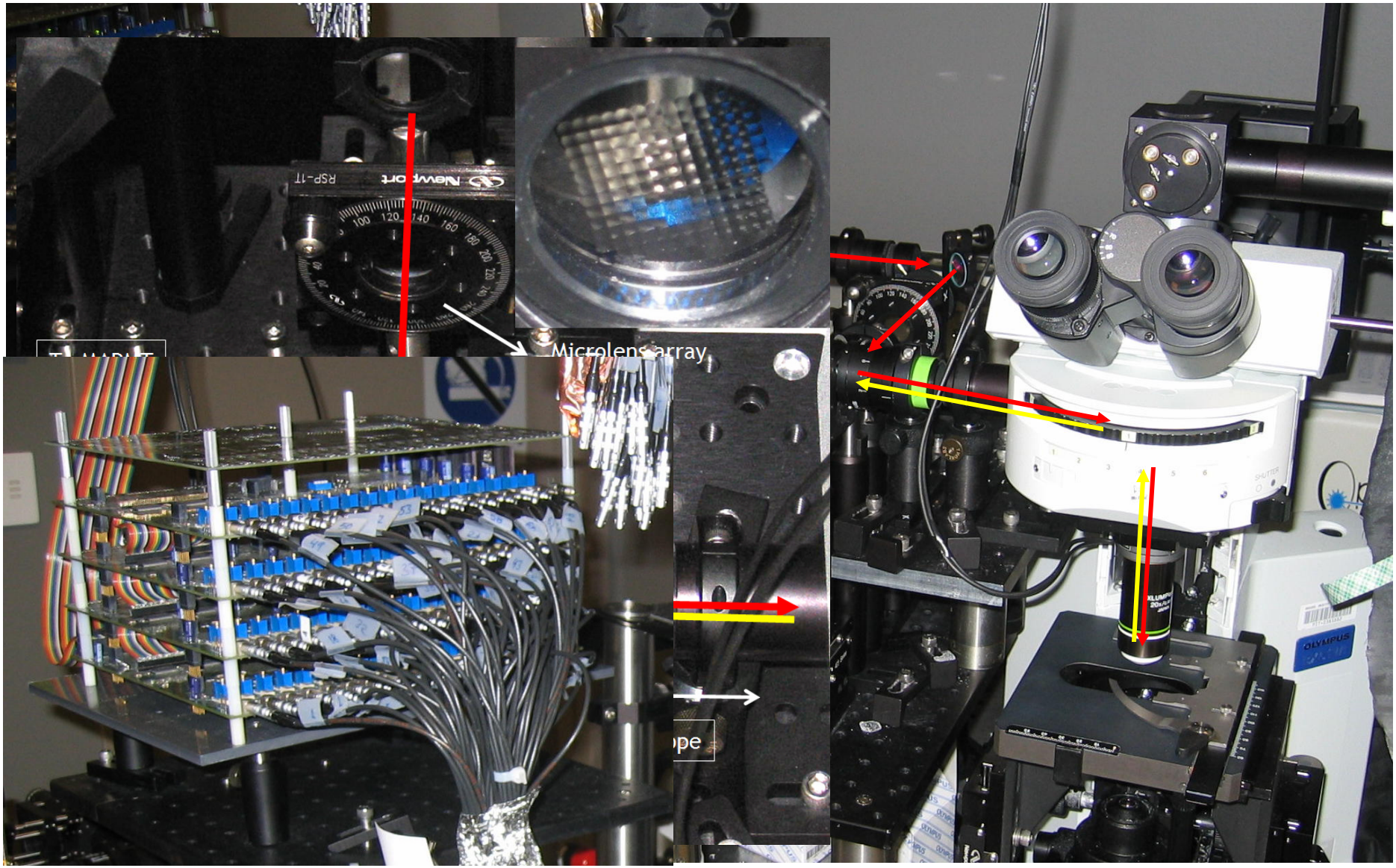




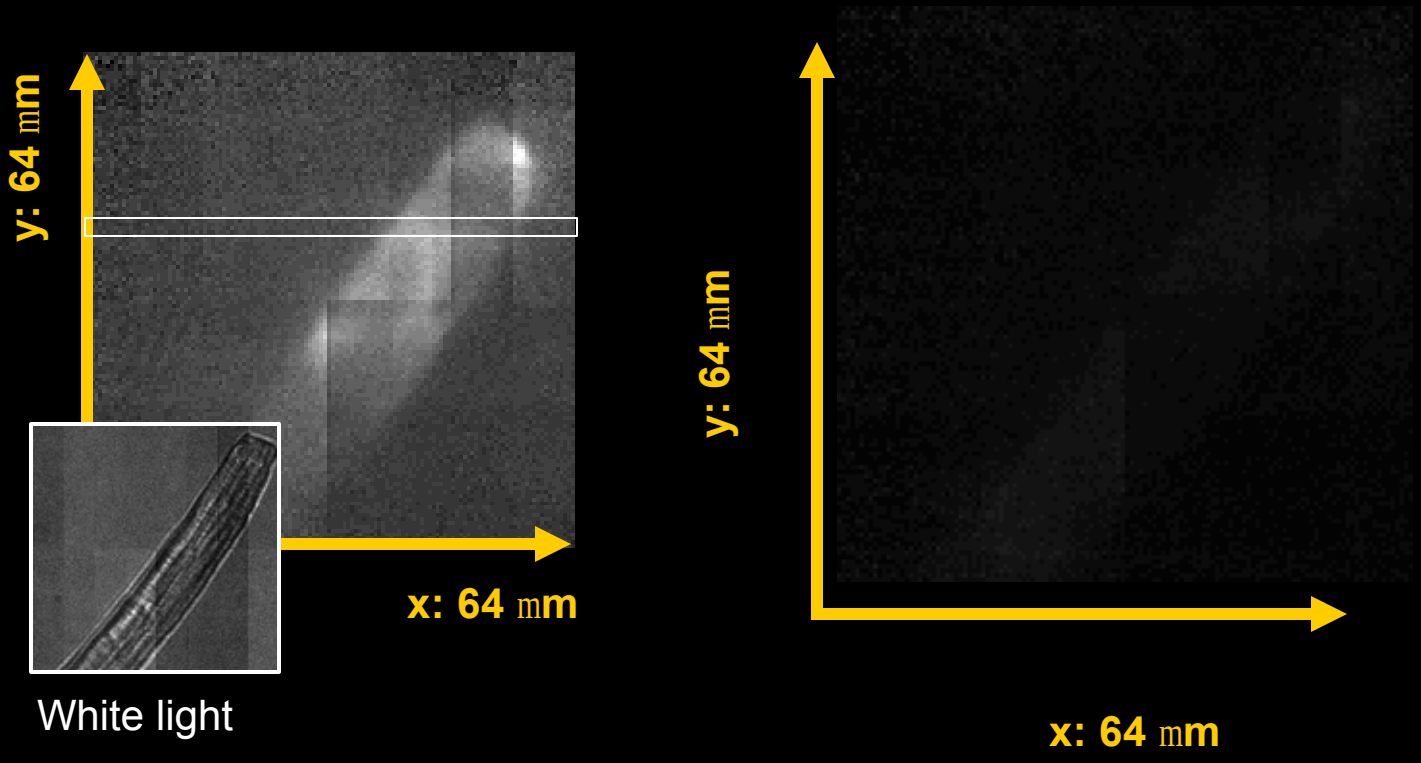
Fixed micro-lens array for Resonant-mirror setup

In collaboration with Karsten Bahlmann (MIT), Mike Kirber (BU Medical Center)

MAPMT-based MMM

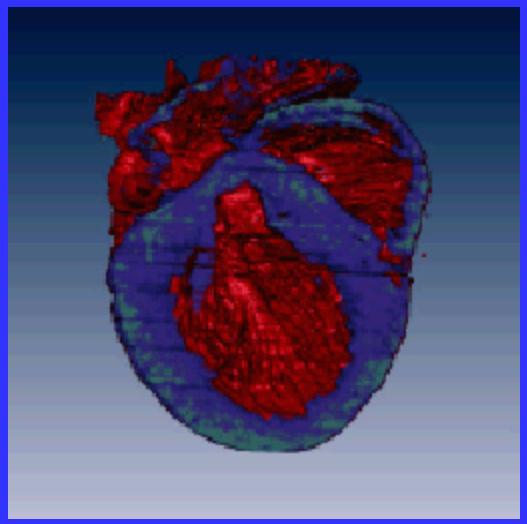


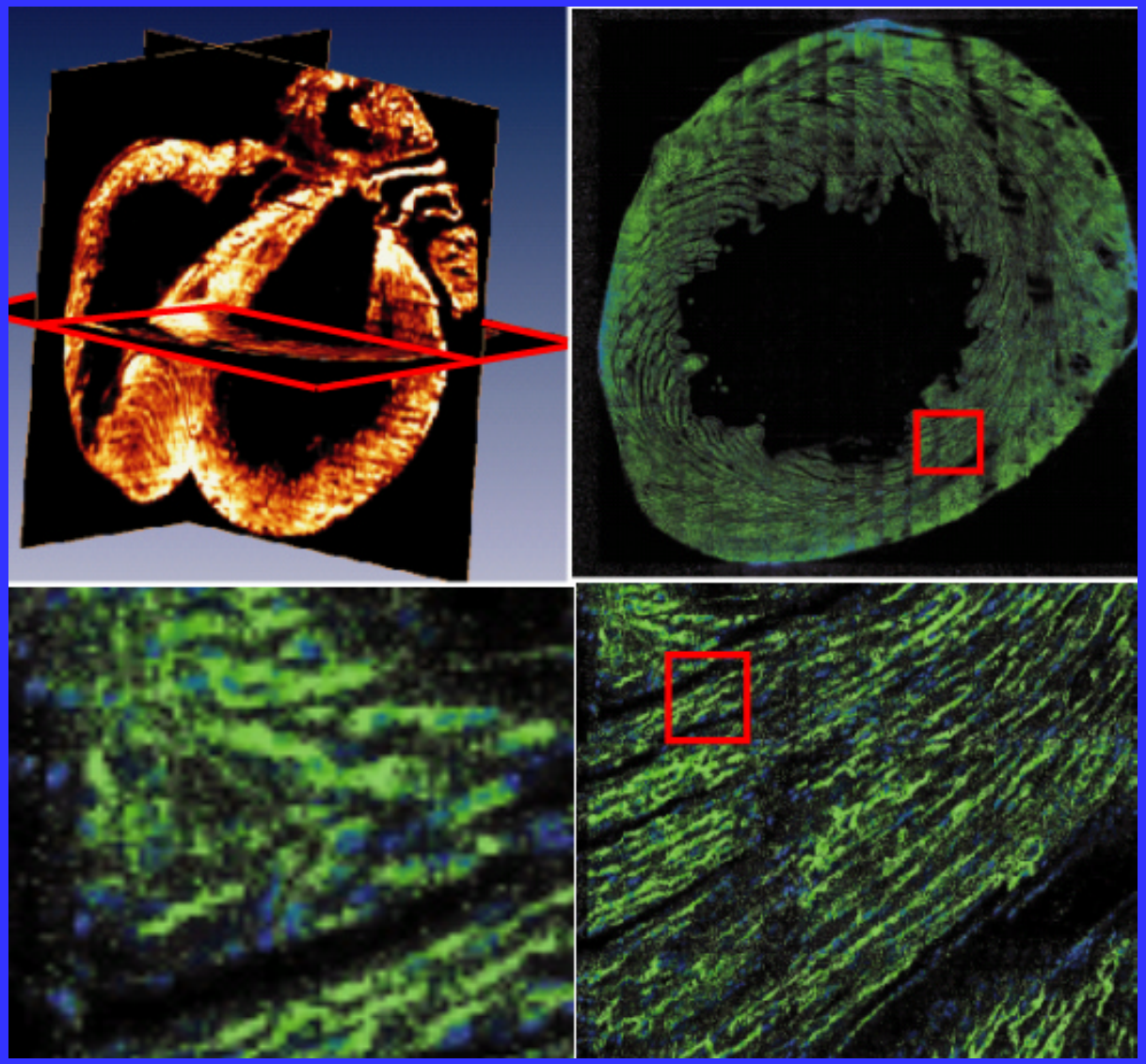
Spontaneous contraction of cardiac myocyte cells labeled with fluo3



800 images at 640 frames/sec : 1,25 sec sequence

QUANTIFYING AND UNDERSTANDING GENETICALLY INDUCED CARDIAC HYPERTROPY





Macroscopic View of Whole Mouse Heart with Microscopic Subcellular Image Resolution