New techniques in optical microscopy

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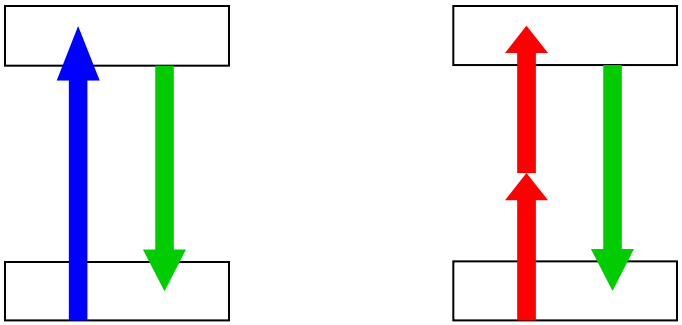
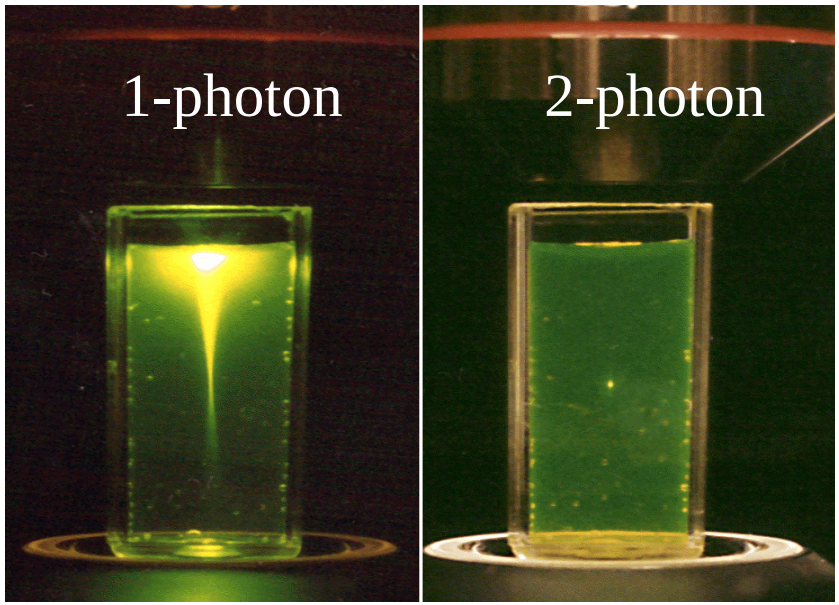
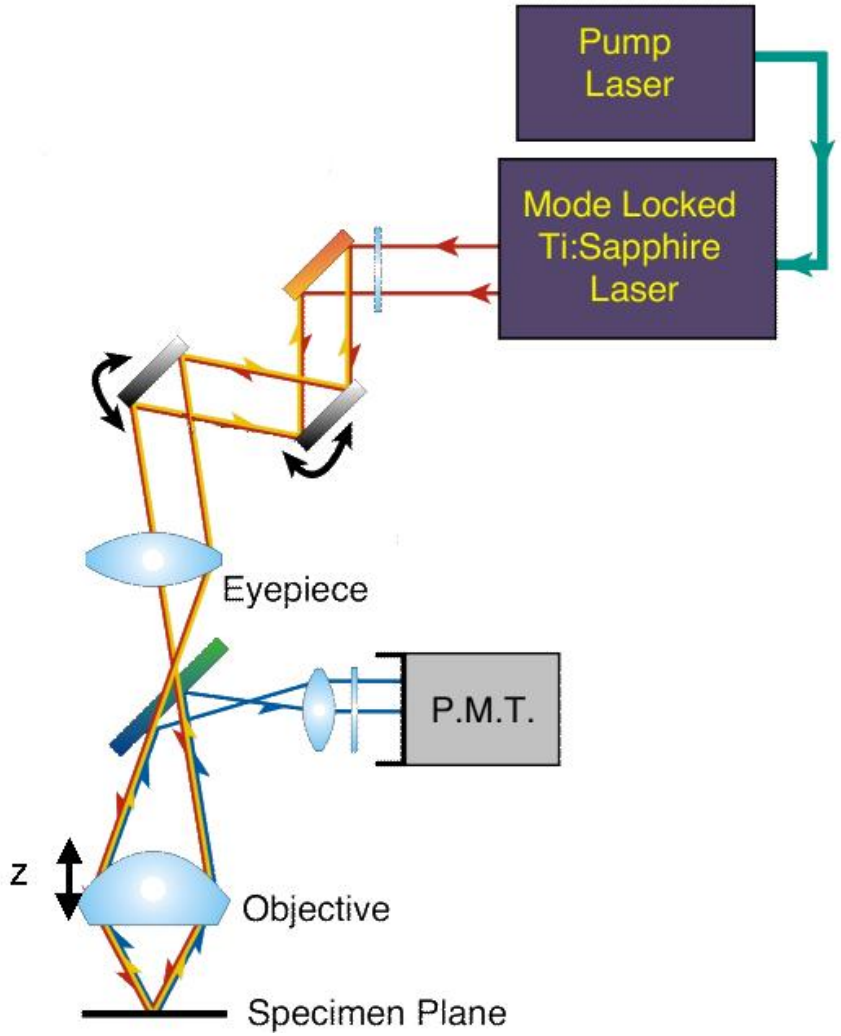
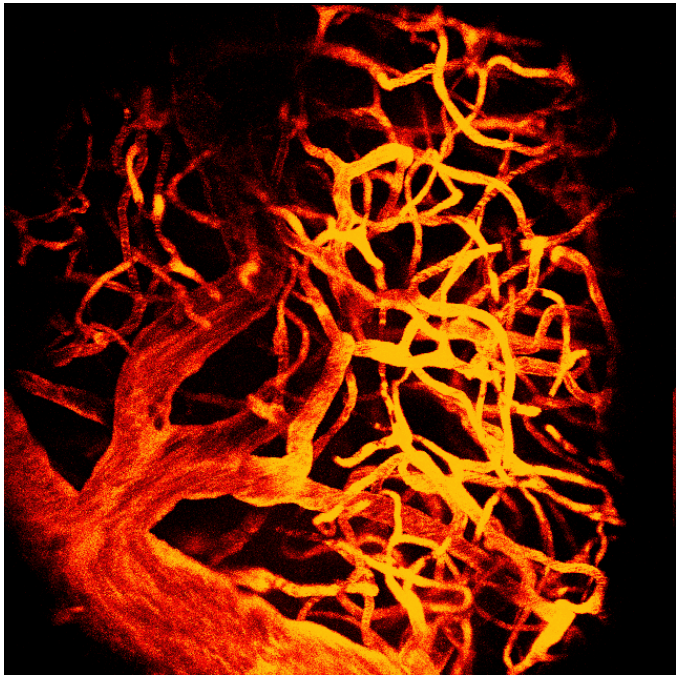
Two-photon fluorescence microscopy



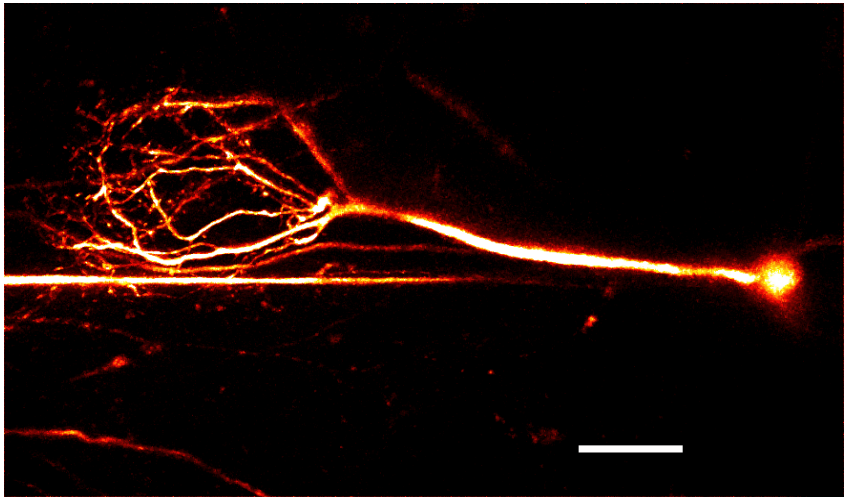


(Webb, Denk 1990)



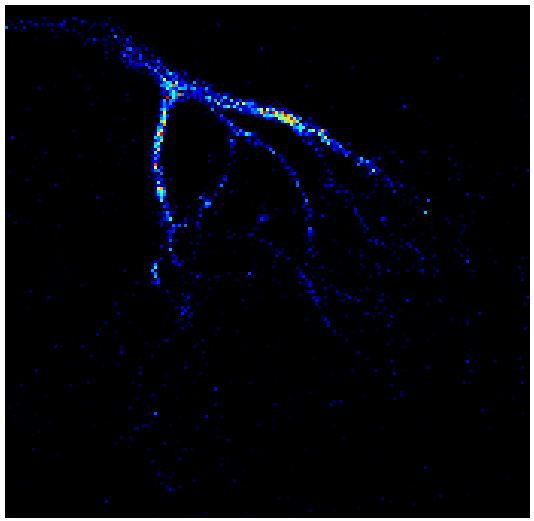


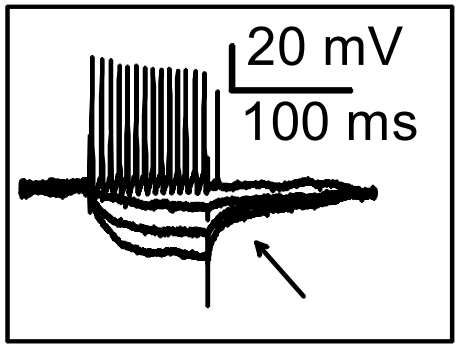
Mitral cell

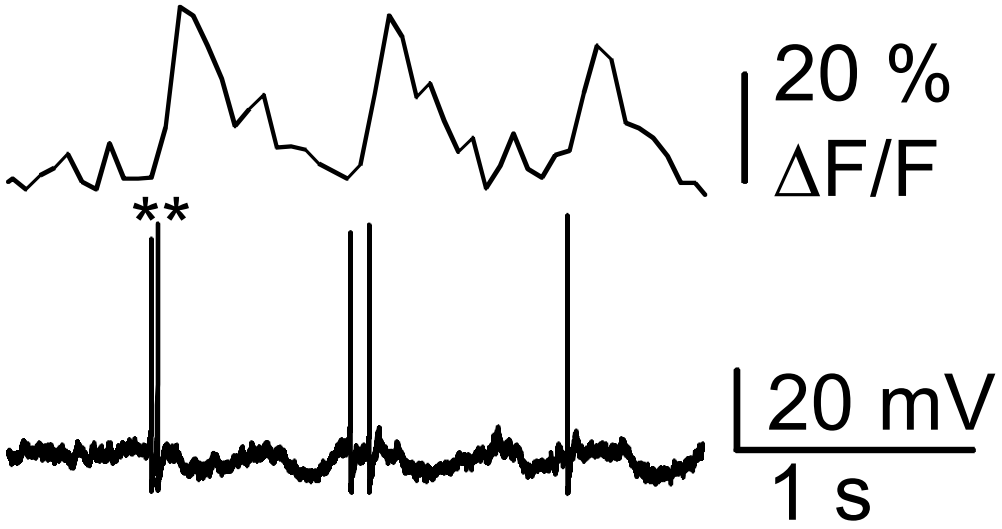


ADVANTAGES:

- (relatively) deep imaging
- less out-of-focus photodamage
- localized photochemistry



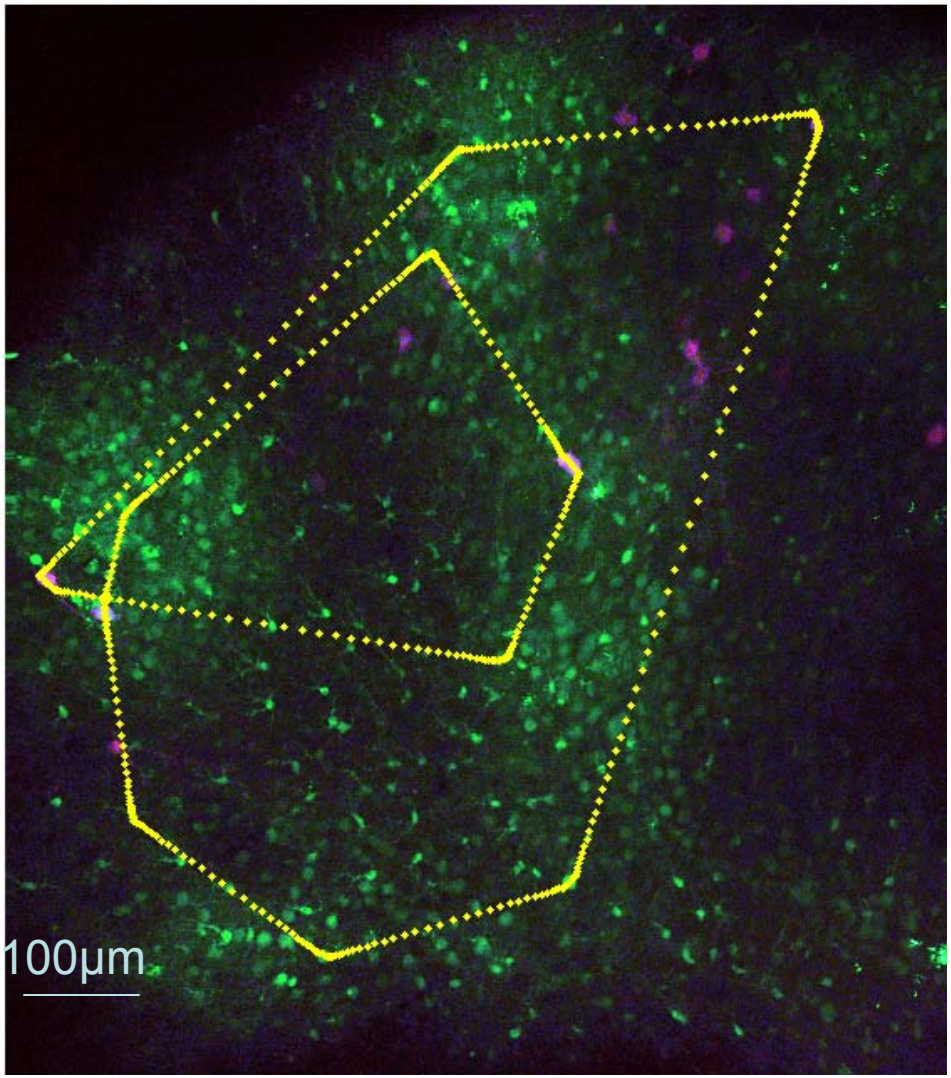

20 mV
100 ms

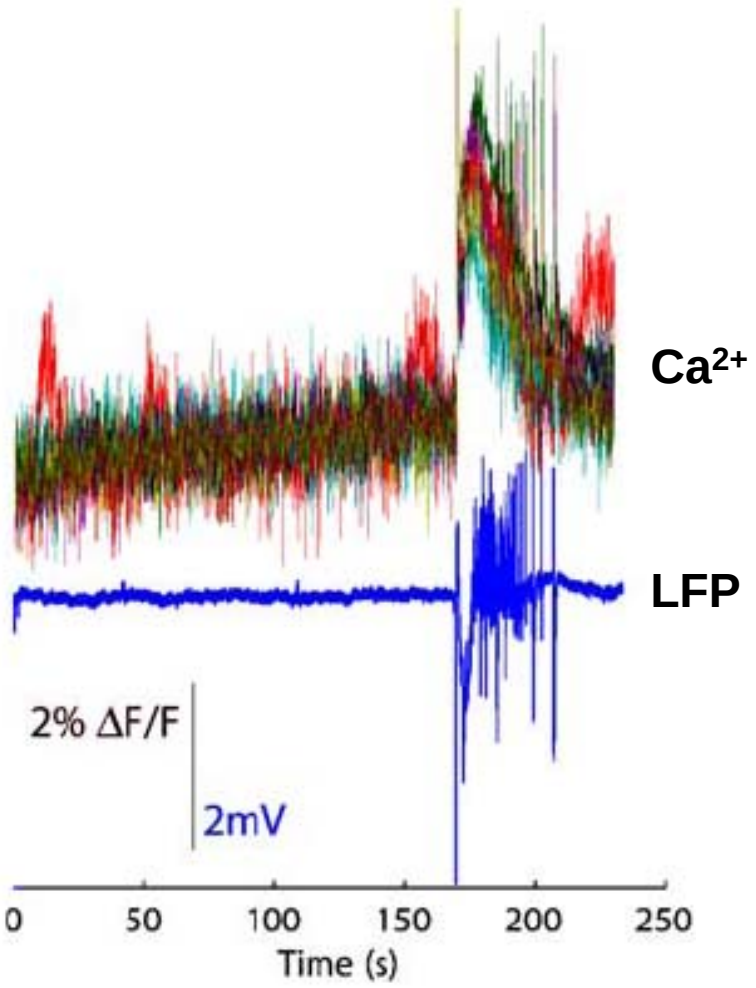

20 %
ΔF/F
**
20 mV
1 s

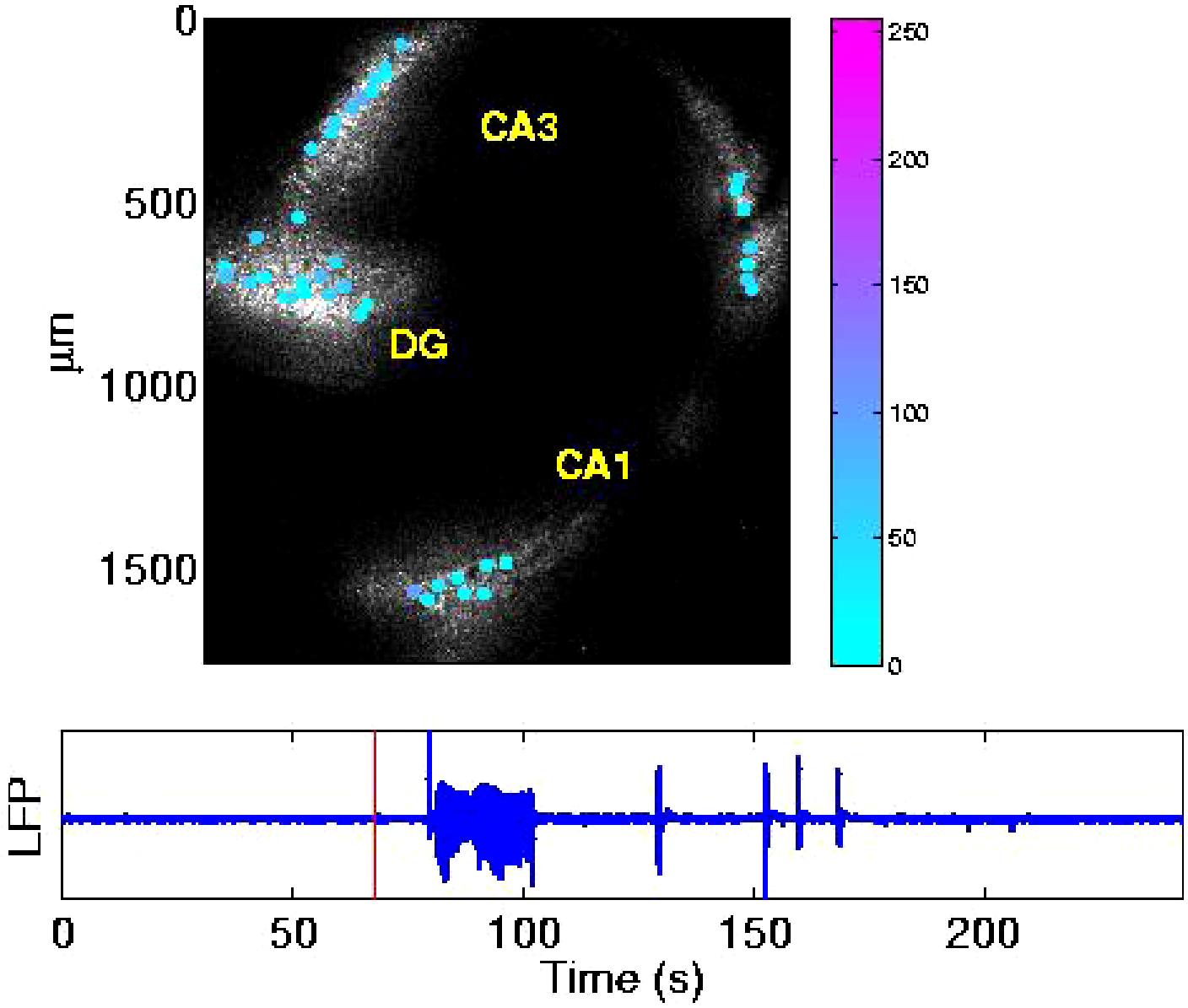
LIMITS

- Speed
- Depth
- Sensitivity

Targeted path scanning (TPS)



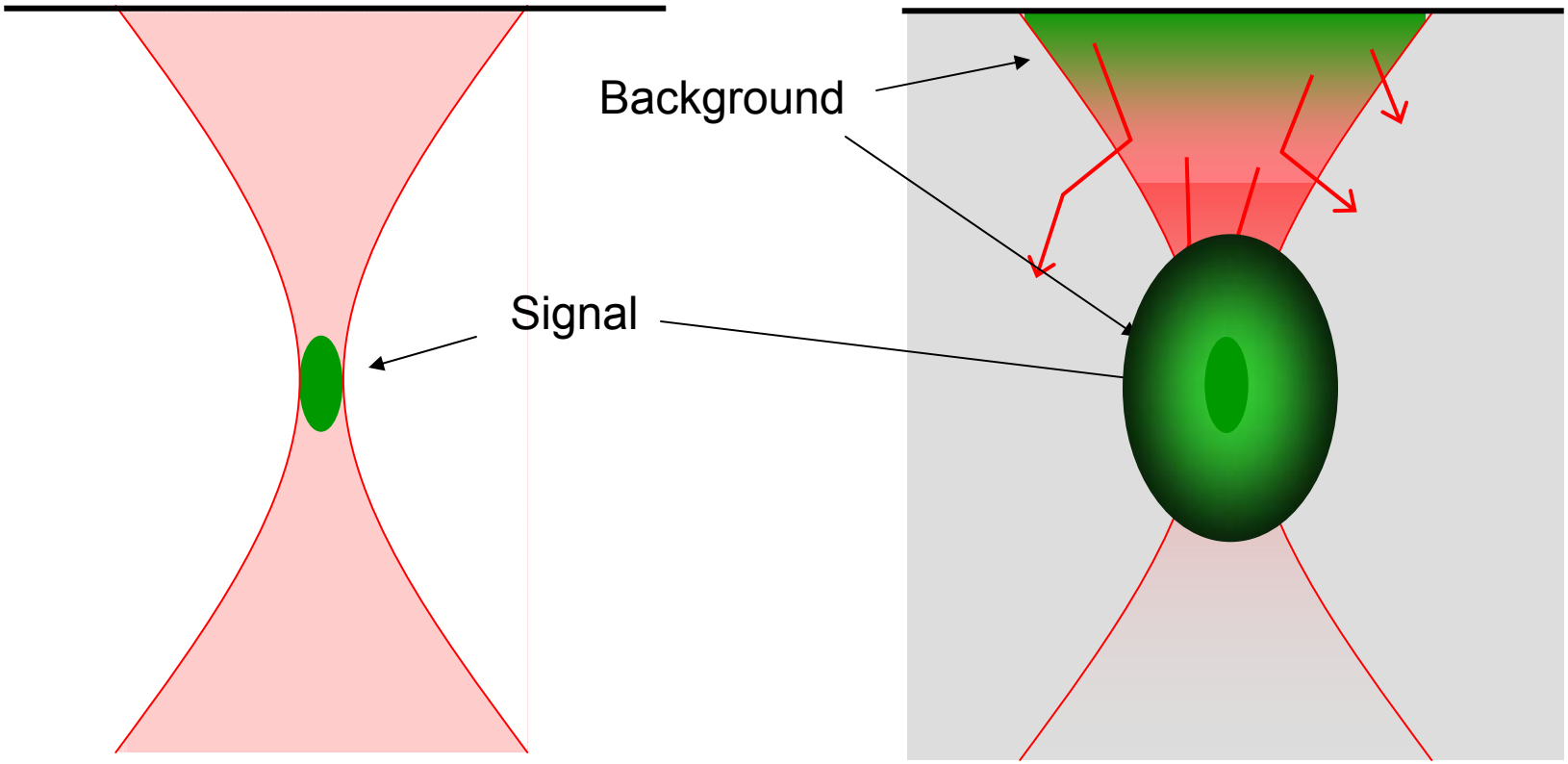



0
500
1000
1500
µm
CA3
DG
CA1
250
200
150
100
50
0
LFP
0
50
100
150
200
Time (s)

LIMITS

- Speed
- Depth
- Sensitivity

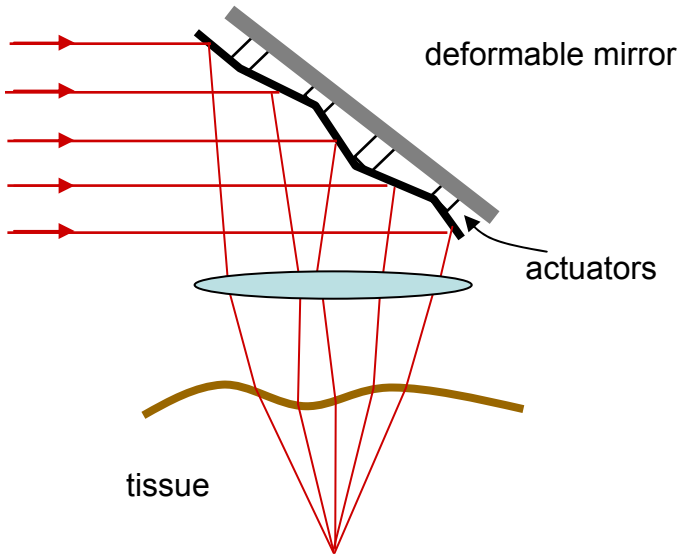
Two-photon fluorescence in volume



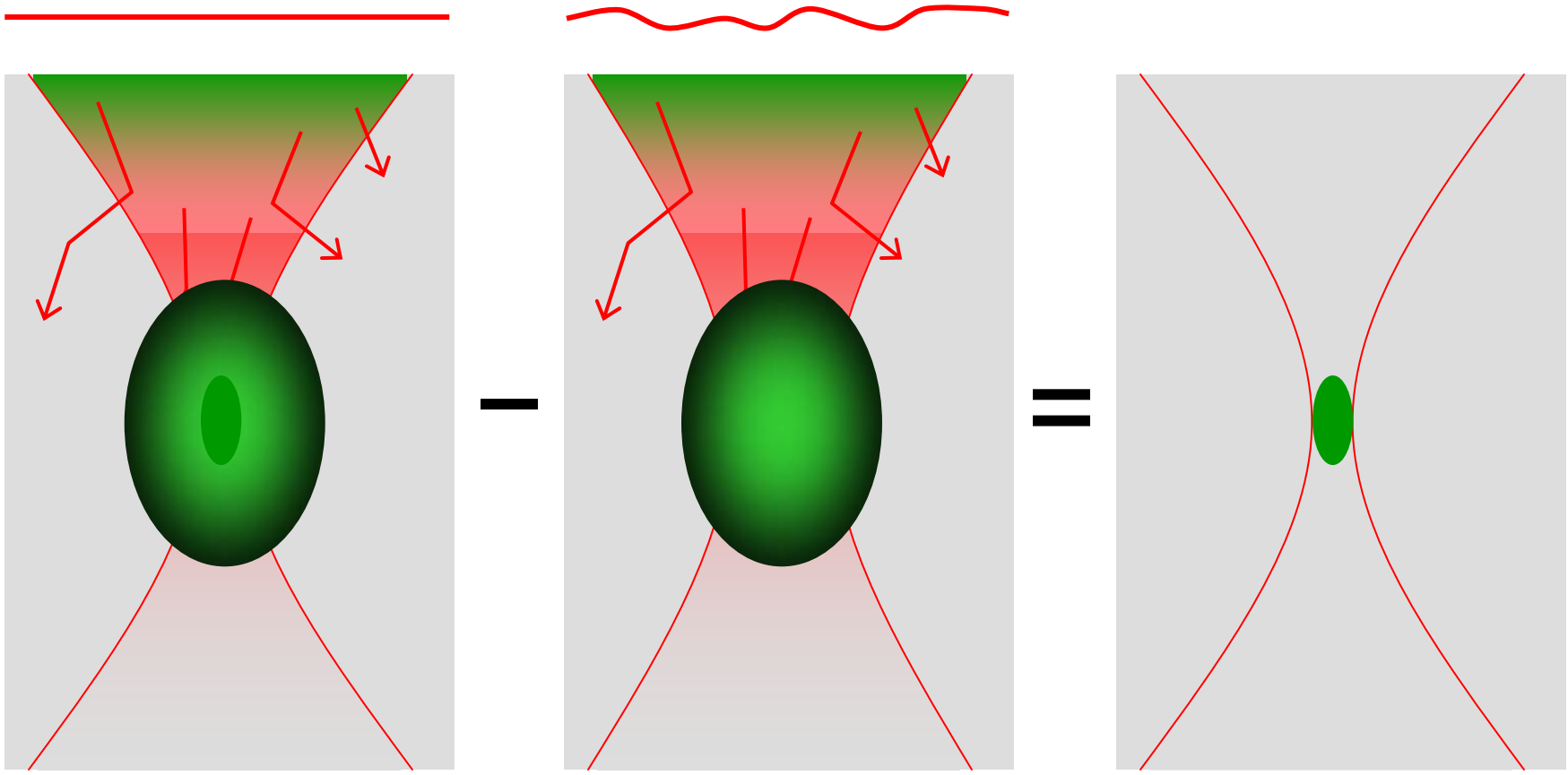
Transparent medium

Scattering medium

Possible solution: Adaptive optics (Hard)

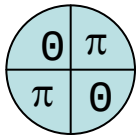


Differential aberration imaging (easy)

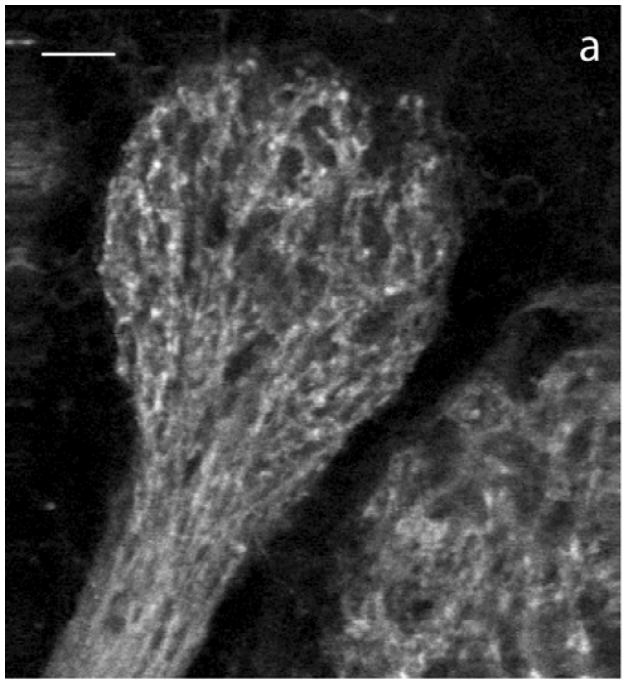


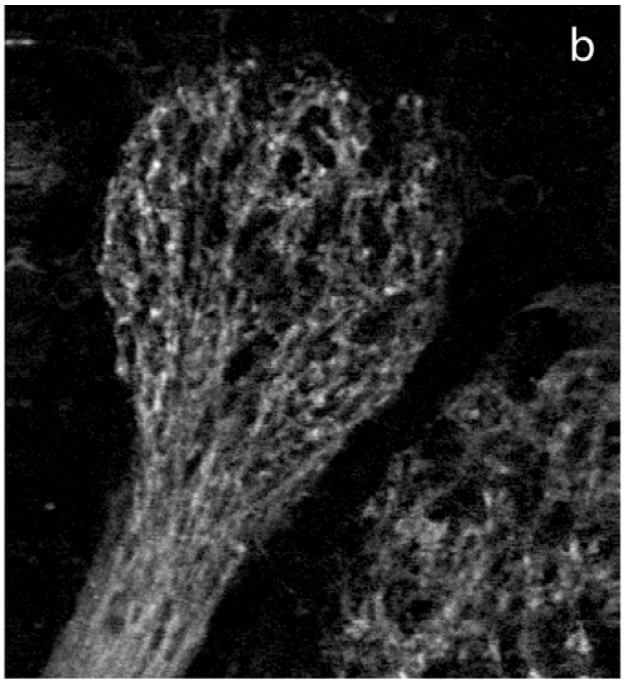
Normal TPEF image

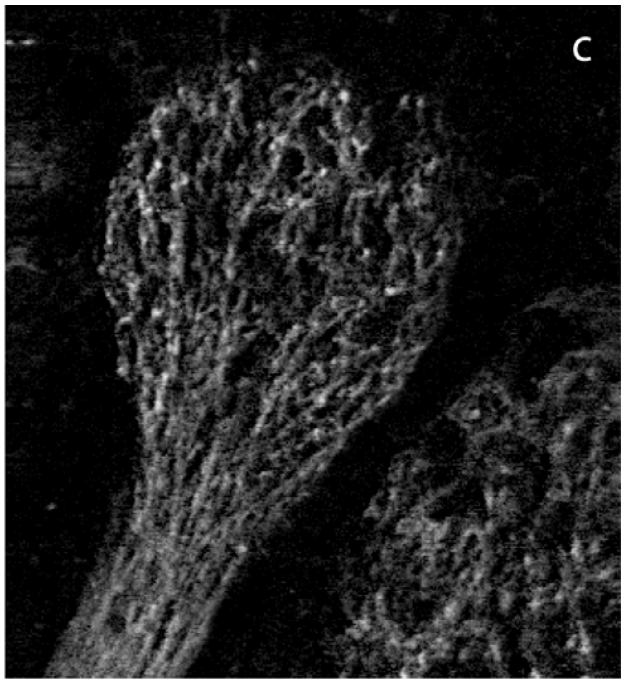
Quadrant phase



Spiral phase

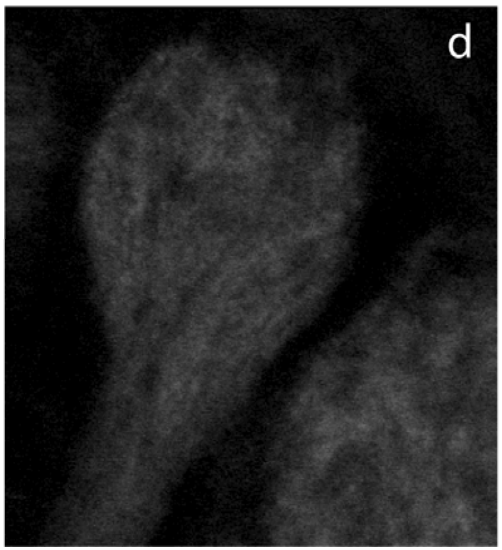


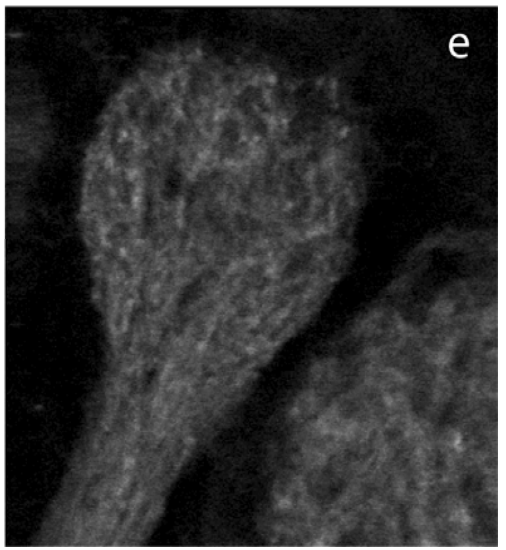




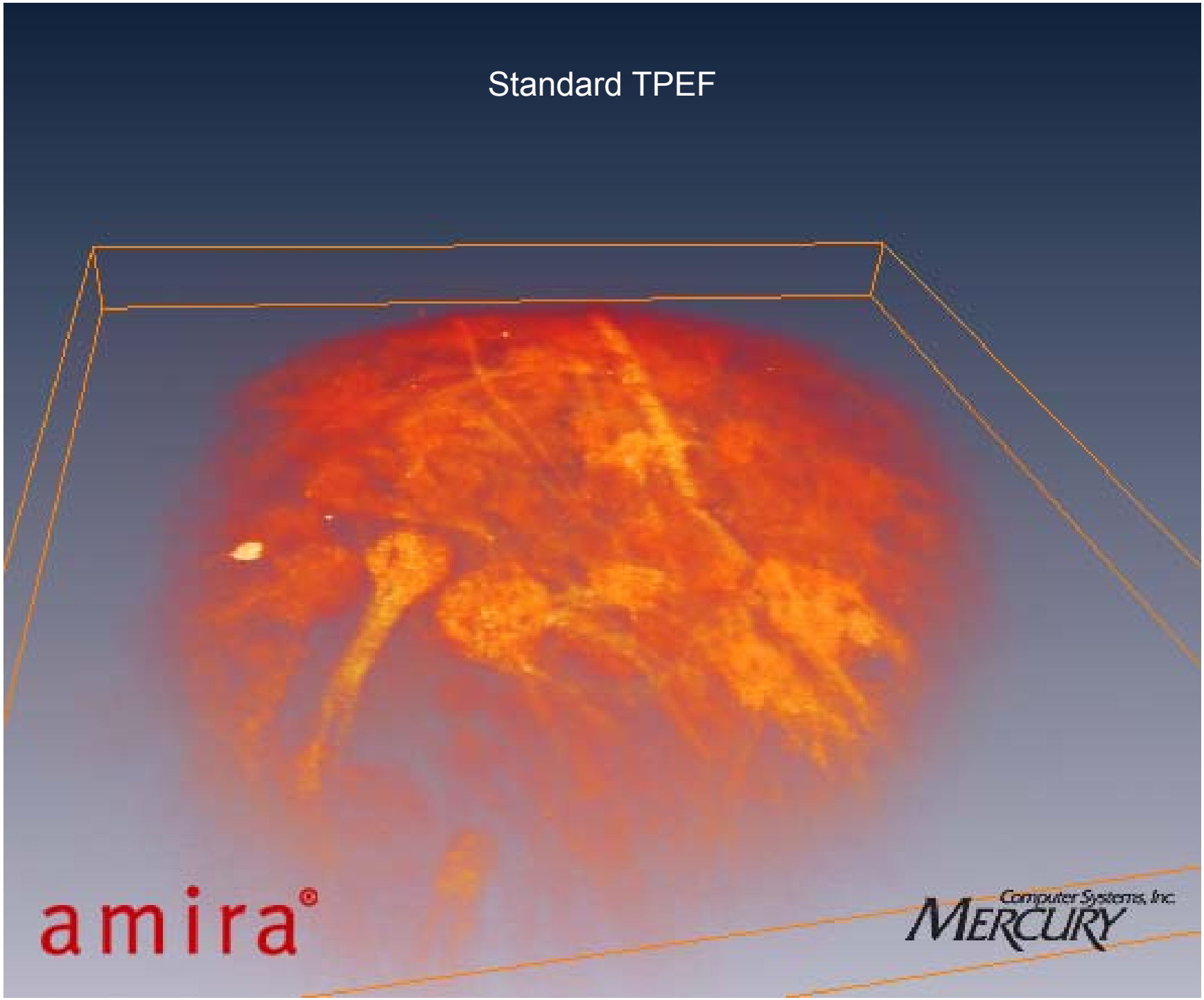
GFP-labeled glomerulus in mouse olfactory bulb

Background images

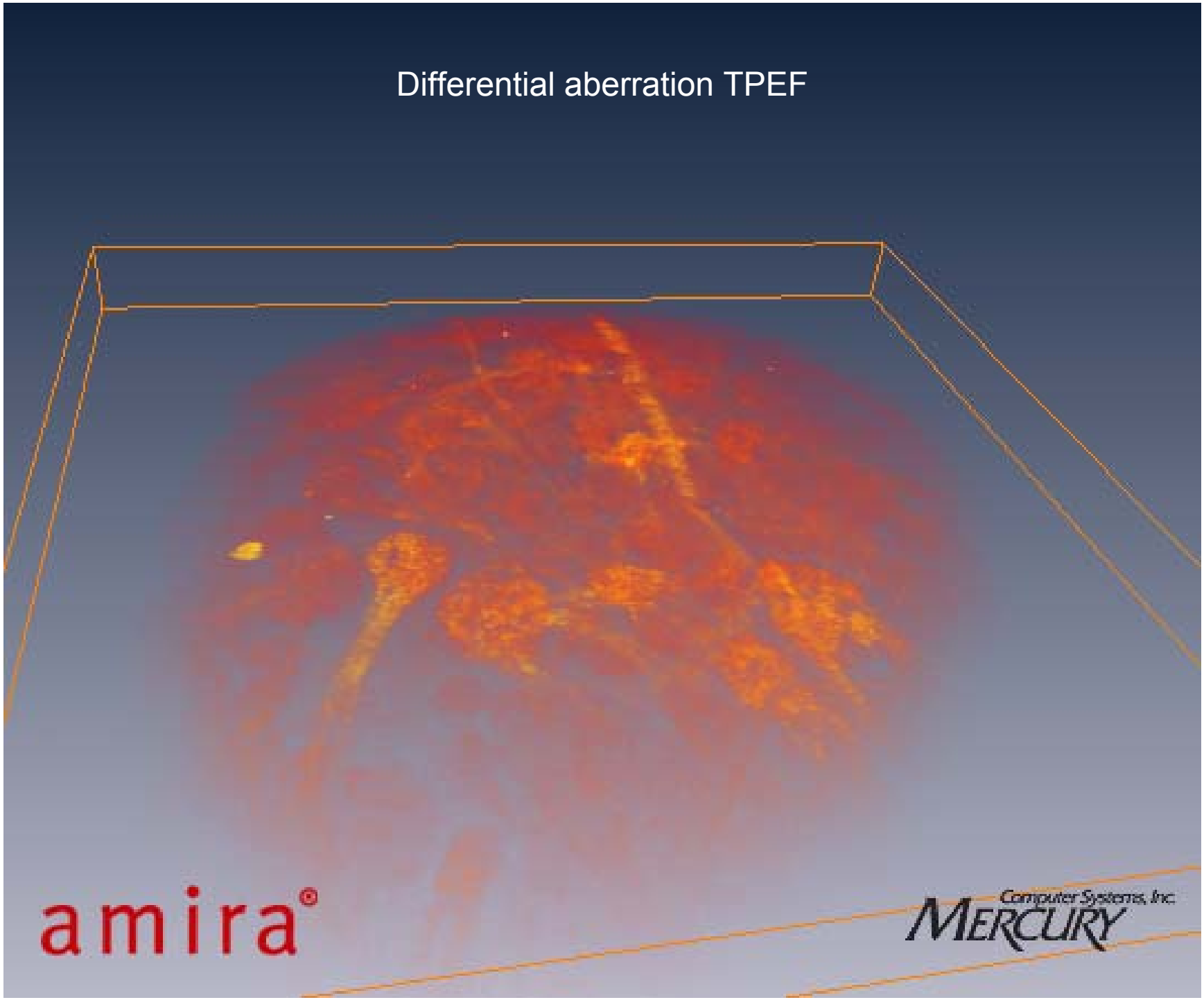




Standard TPEF



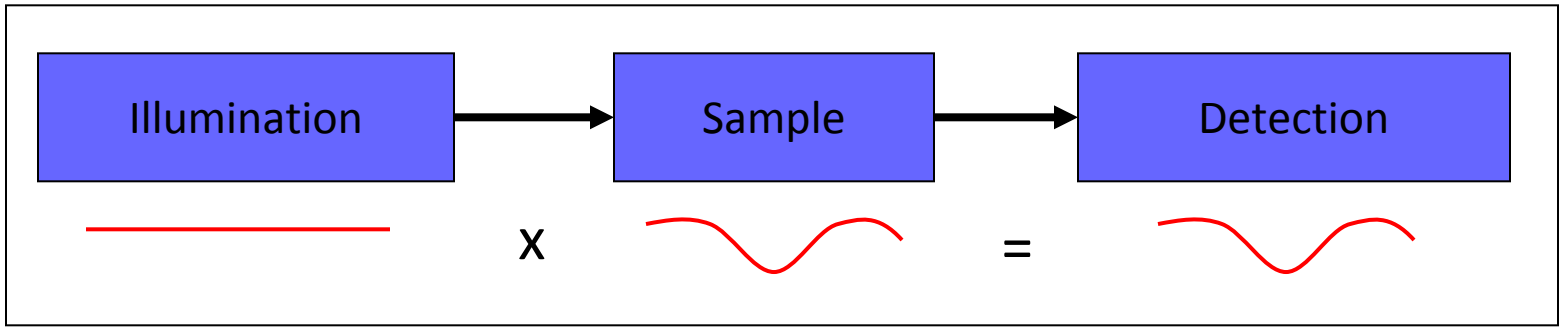
Differential aberration TPEF



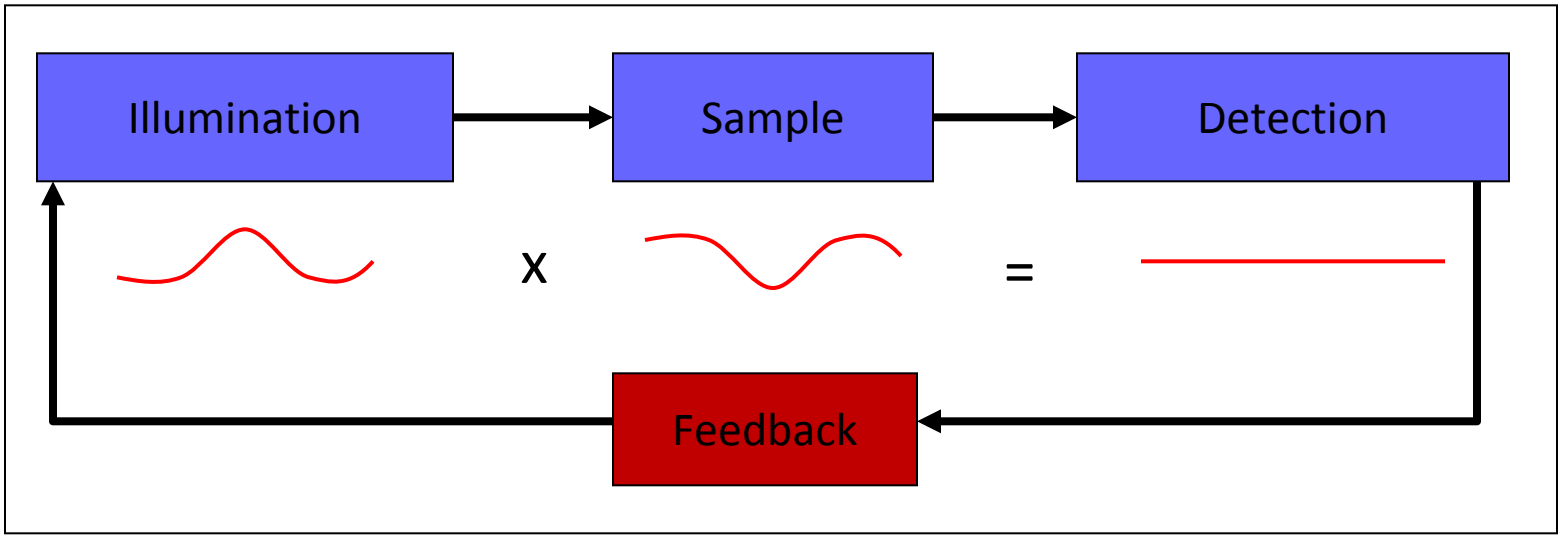
LIMITS

- Speed
- Depth
- Sensitivity

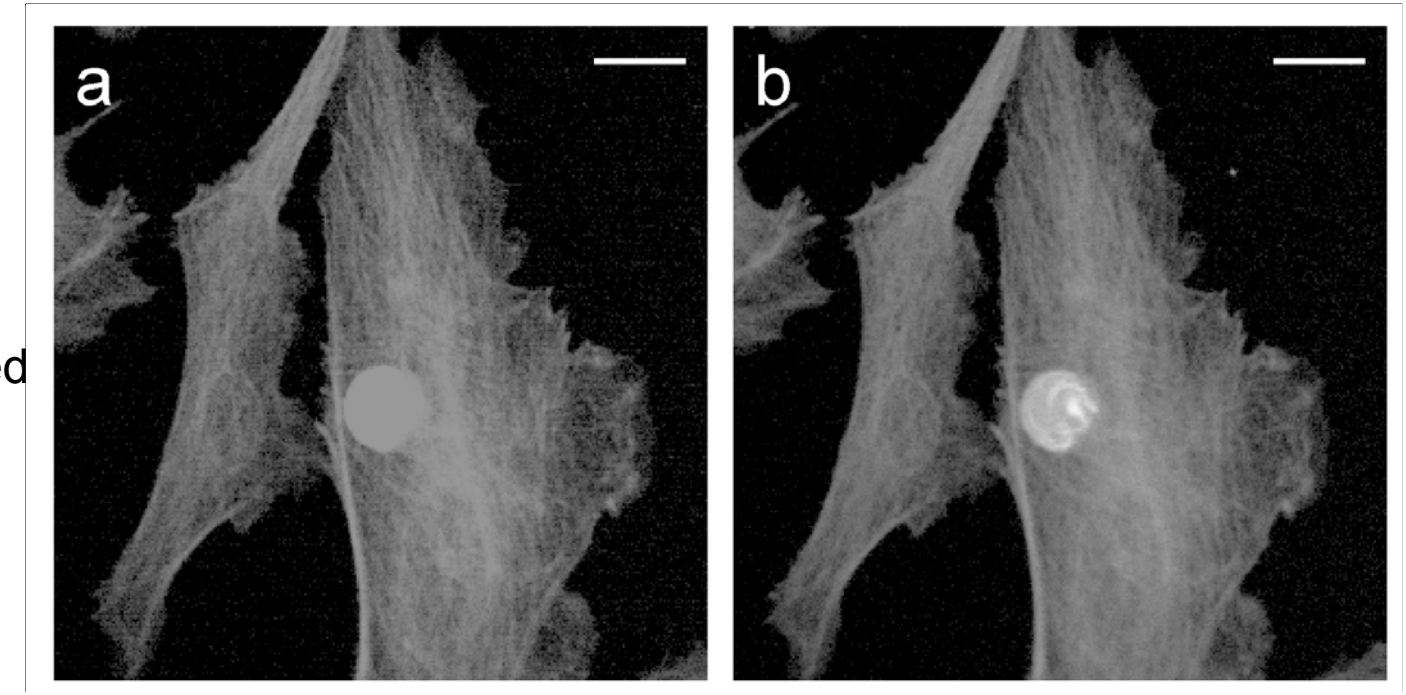
Conventional



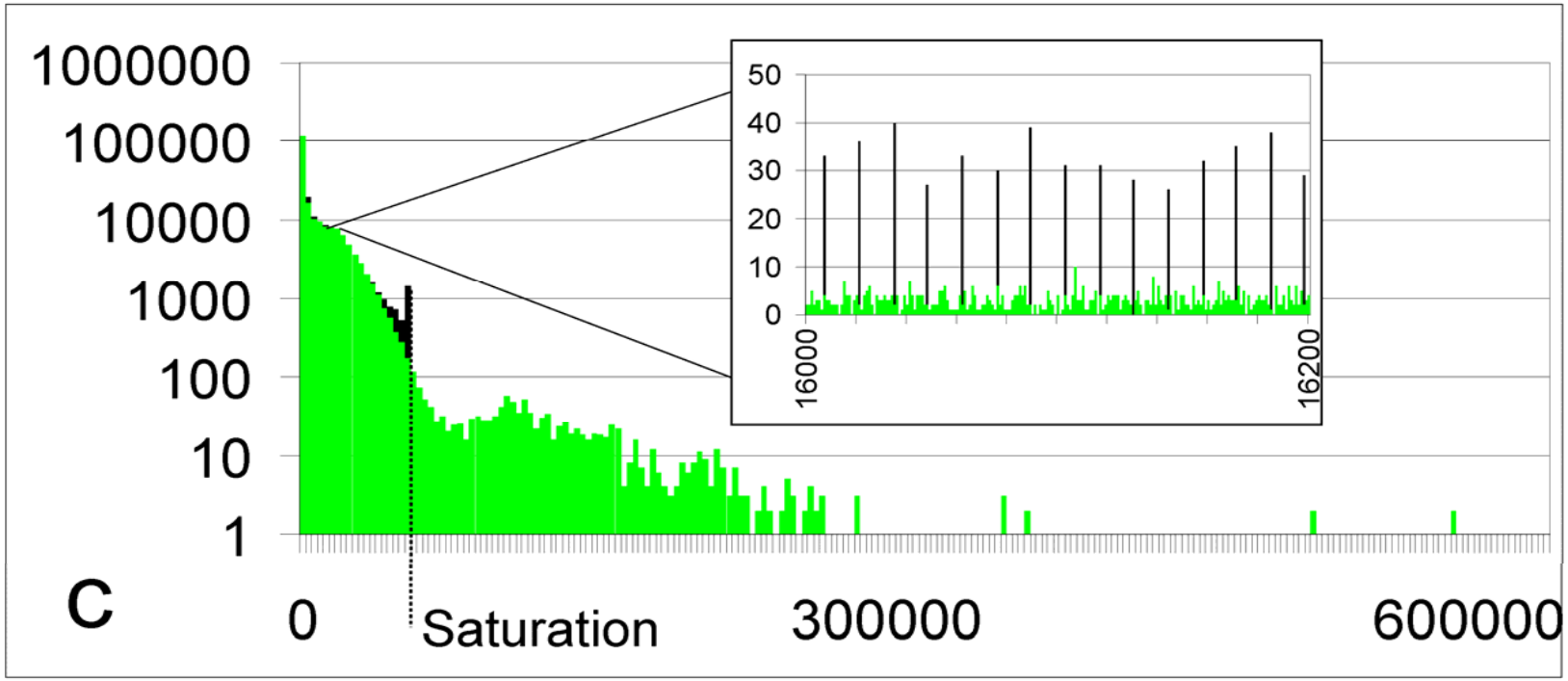
Adaptive Illumination



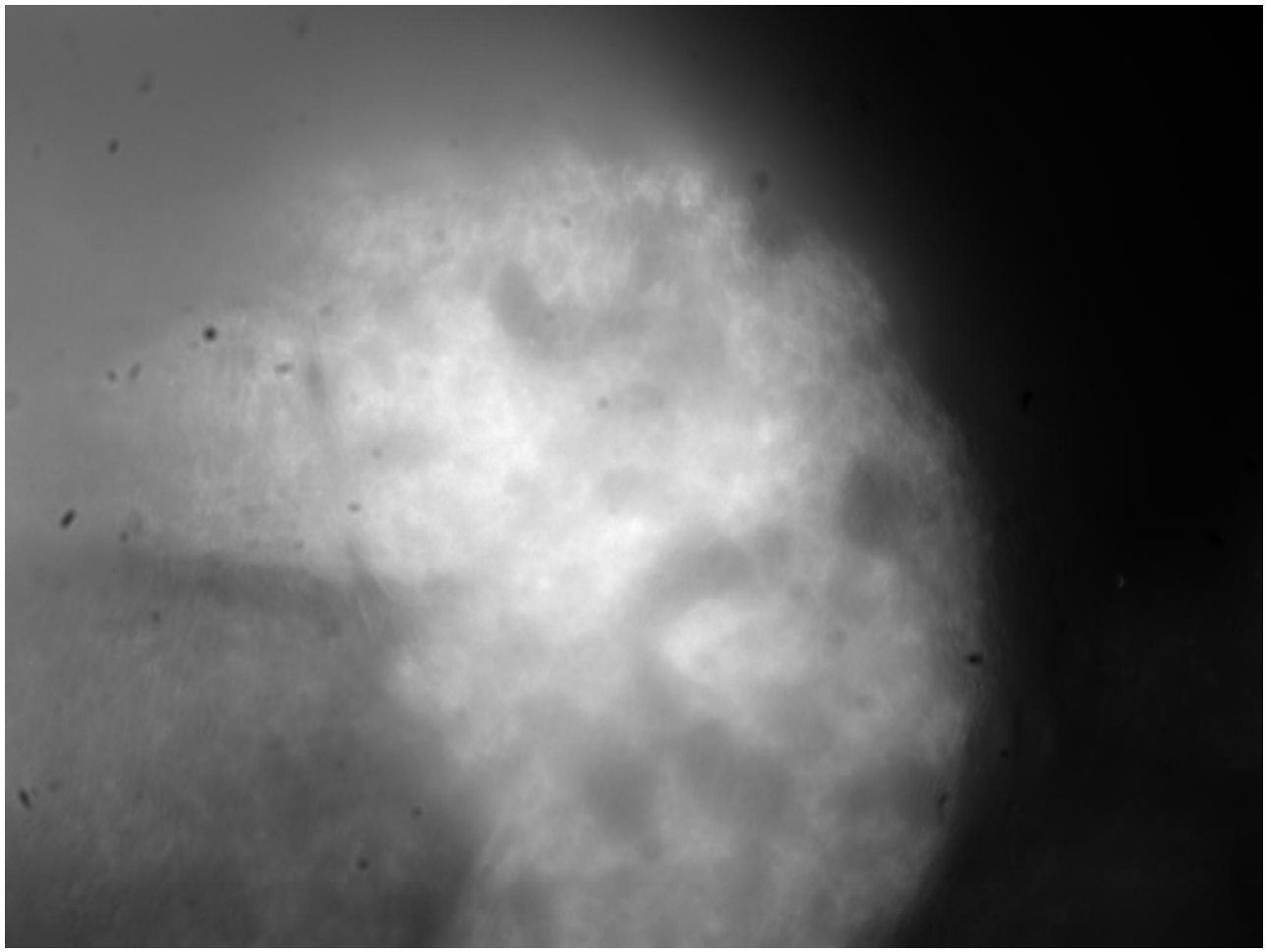
Saturated



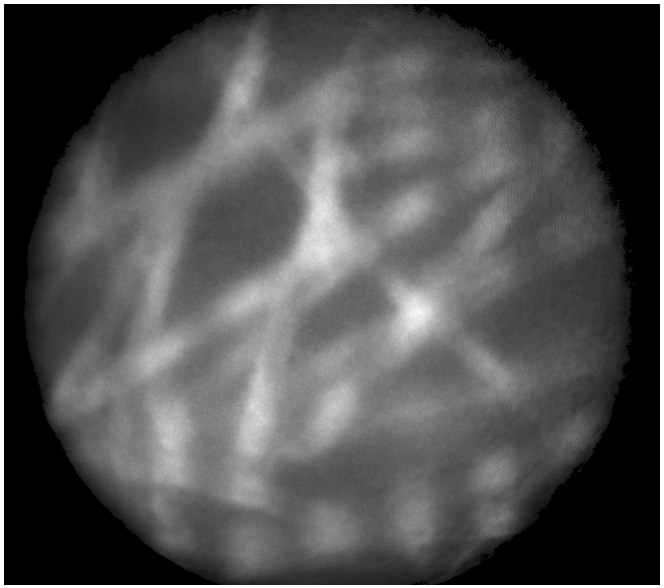
Adaptive illumination



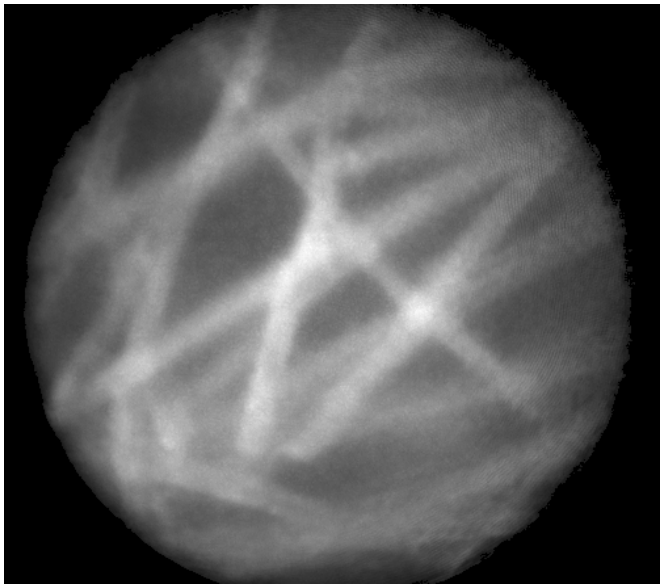
Problem: no optical sectioning



HiLo microscopy

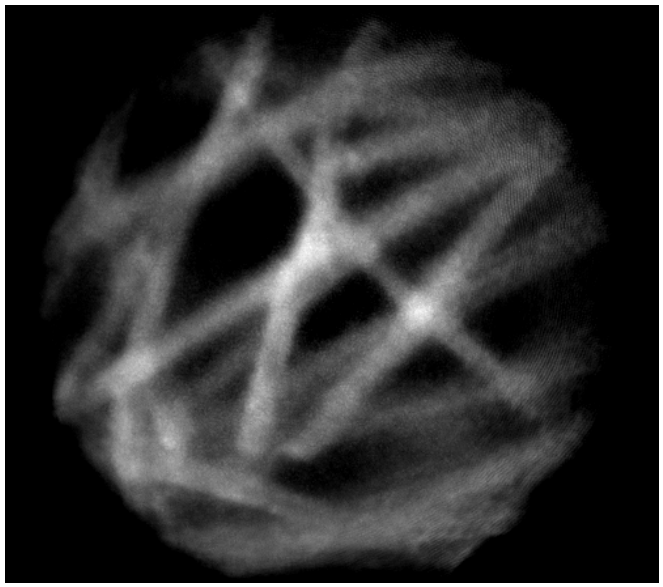


Structured illumination

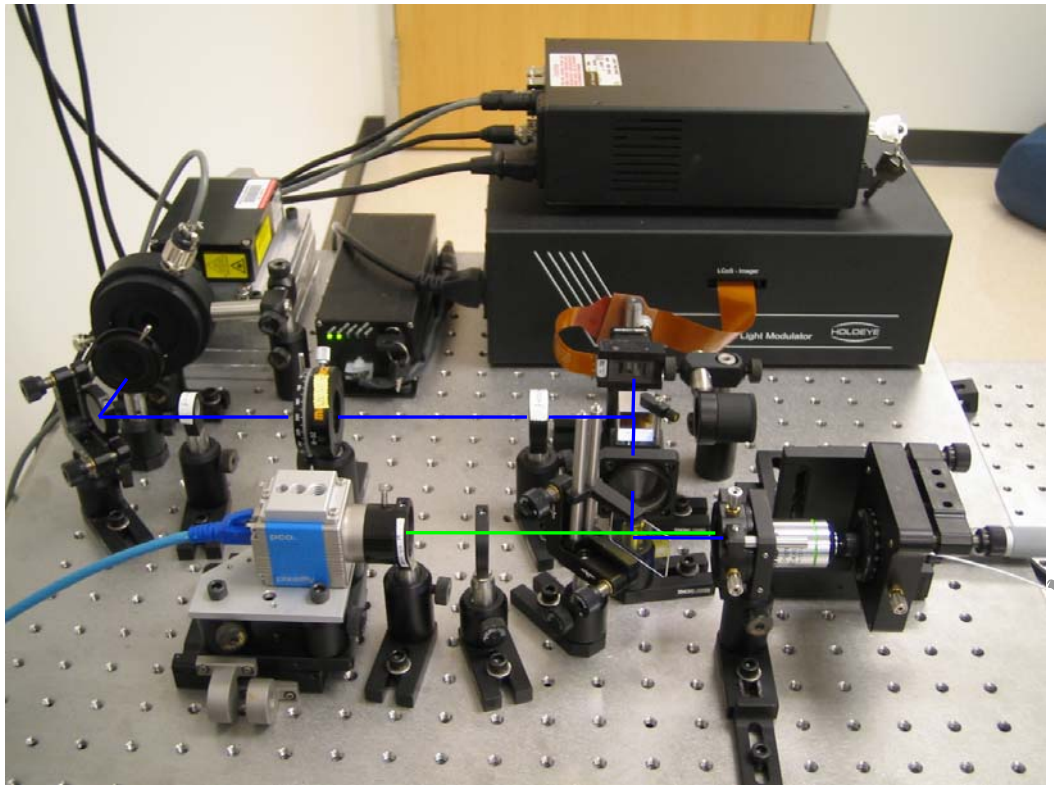


Uniform illumination

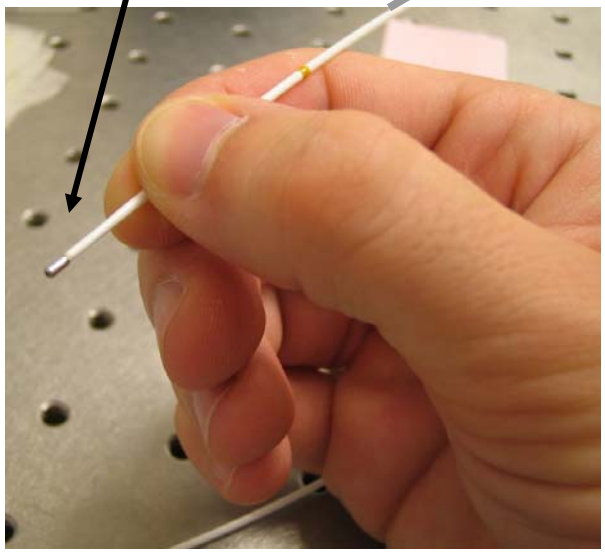
HiLo image



Fluorescence endomicroscopy

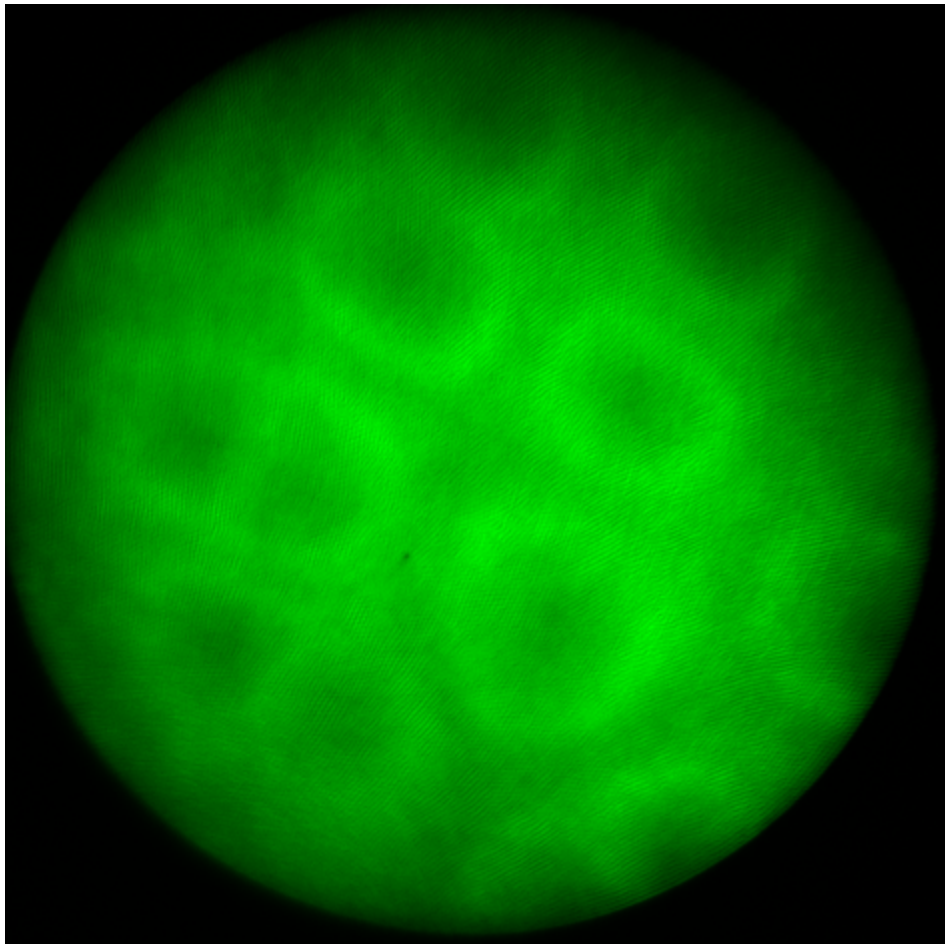


Mauna Kea Technologies

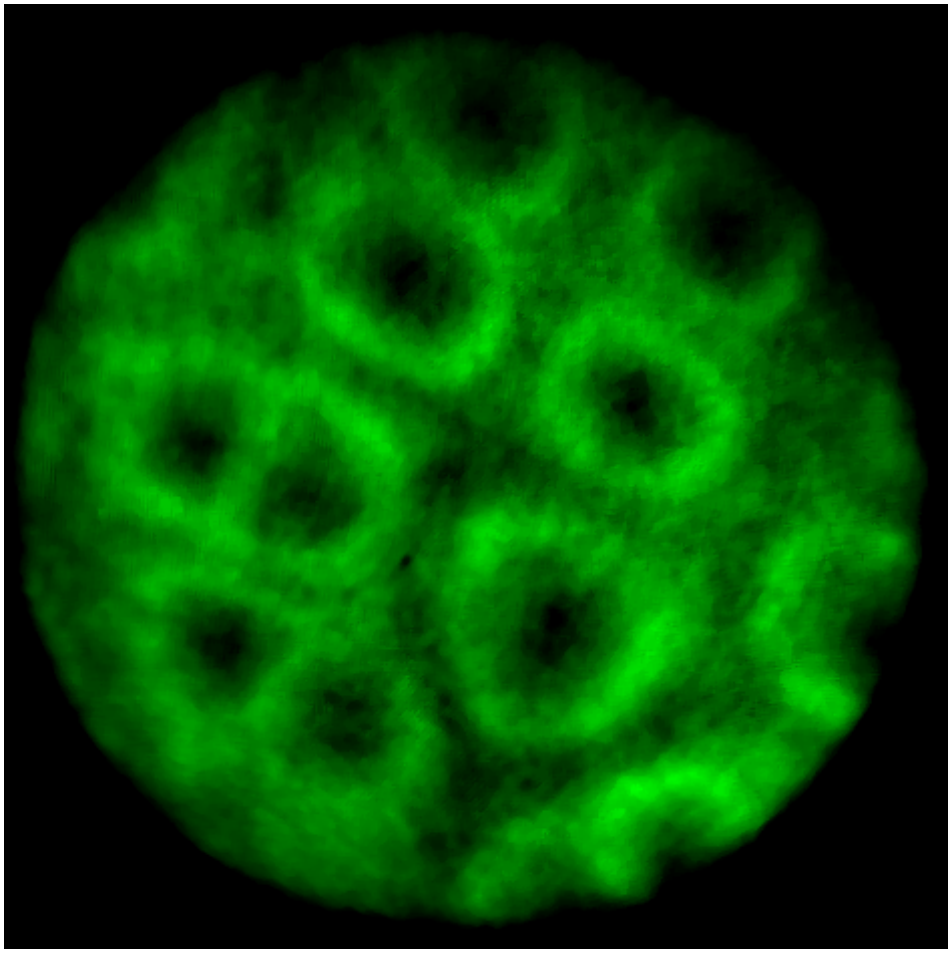


30,000 fibers!

Rat colon labeled with acridine orange

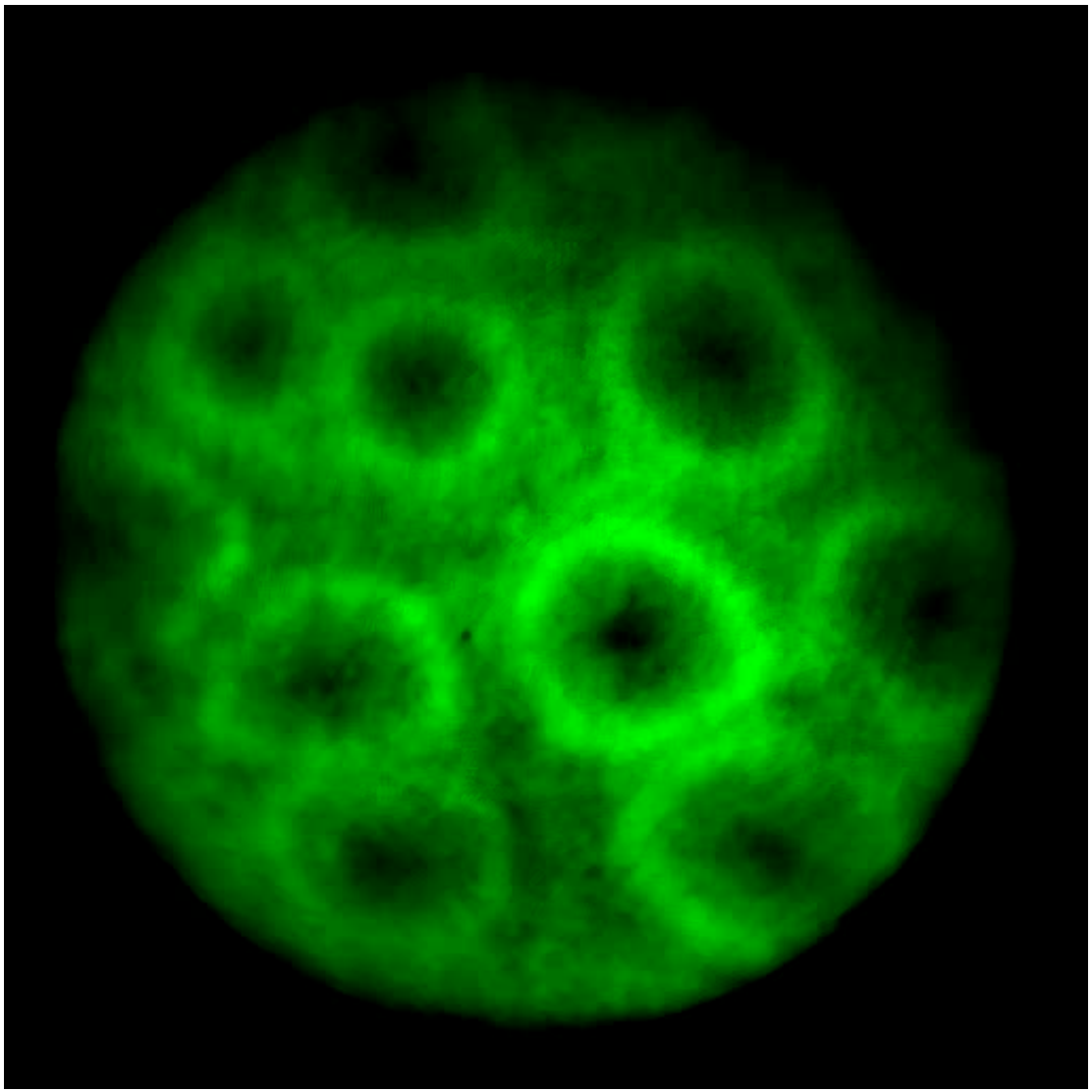


Widefield endomicroscopy



HiLo endomicroscopy

Rat colon labeled with acridine orange

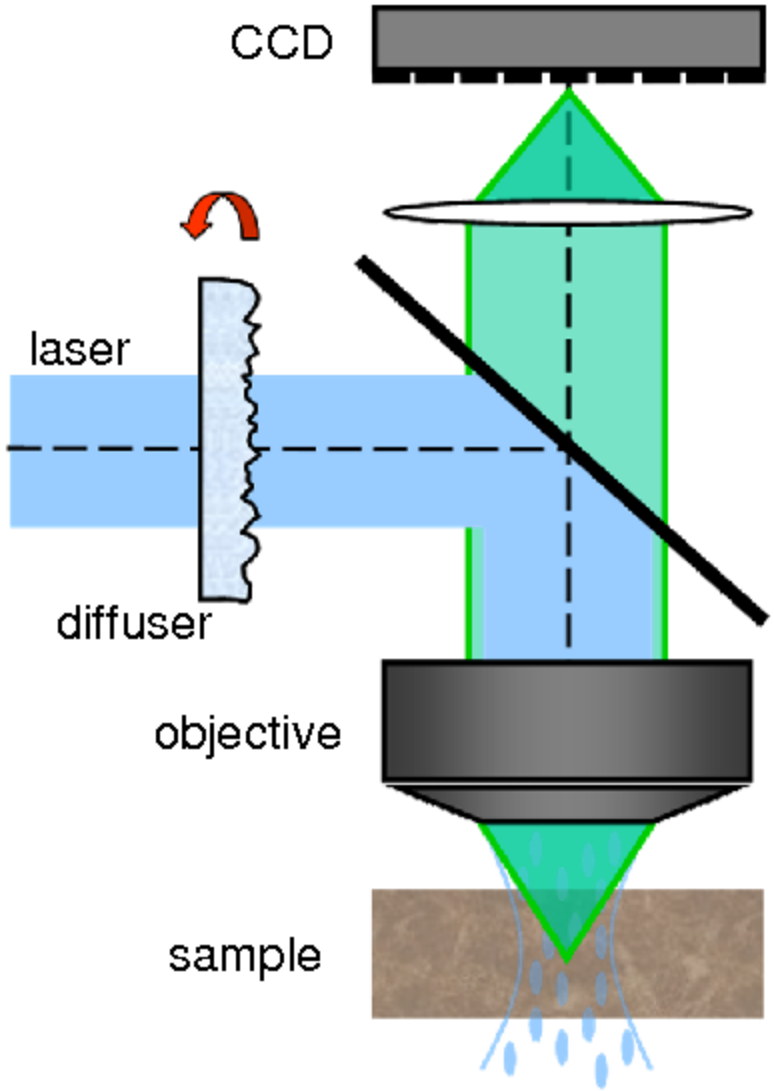


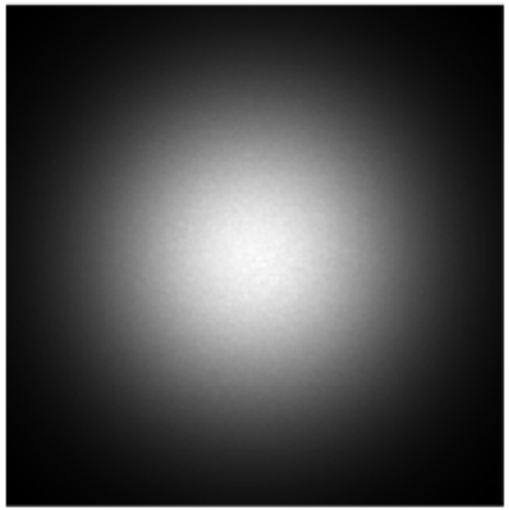
HiLo Advantages

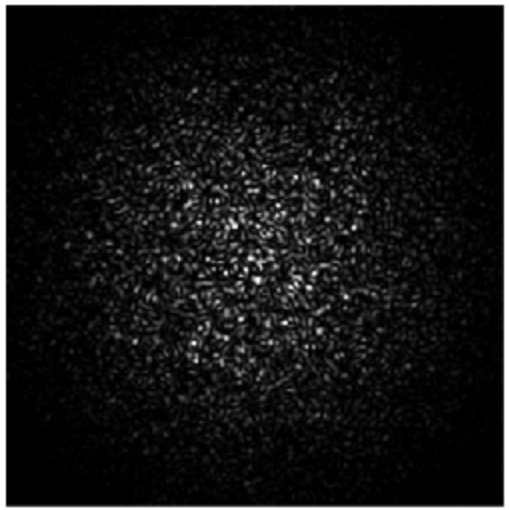
- Simple (no moving parts)
- Fast (only two shots required)
- No motion artifacts
- Robust (insensitive to defects in structured illumination)

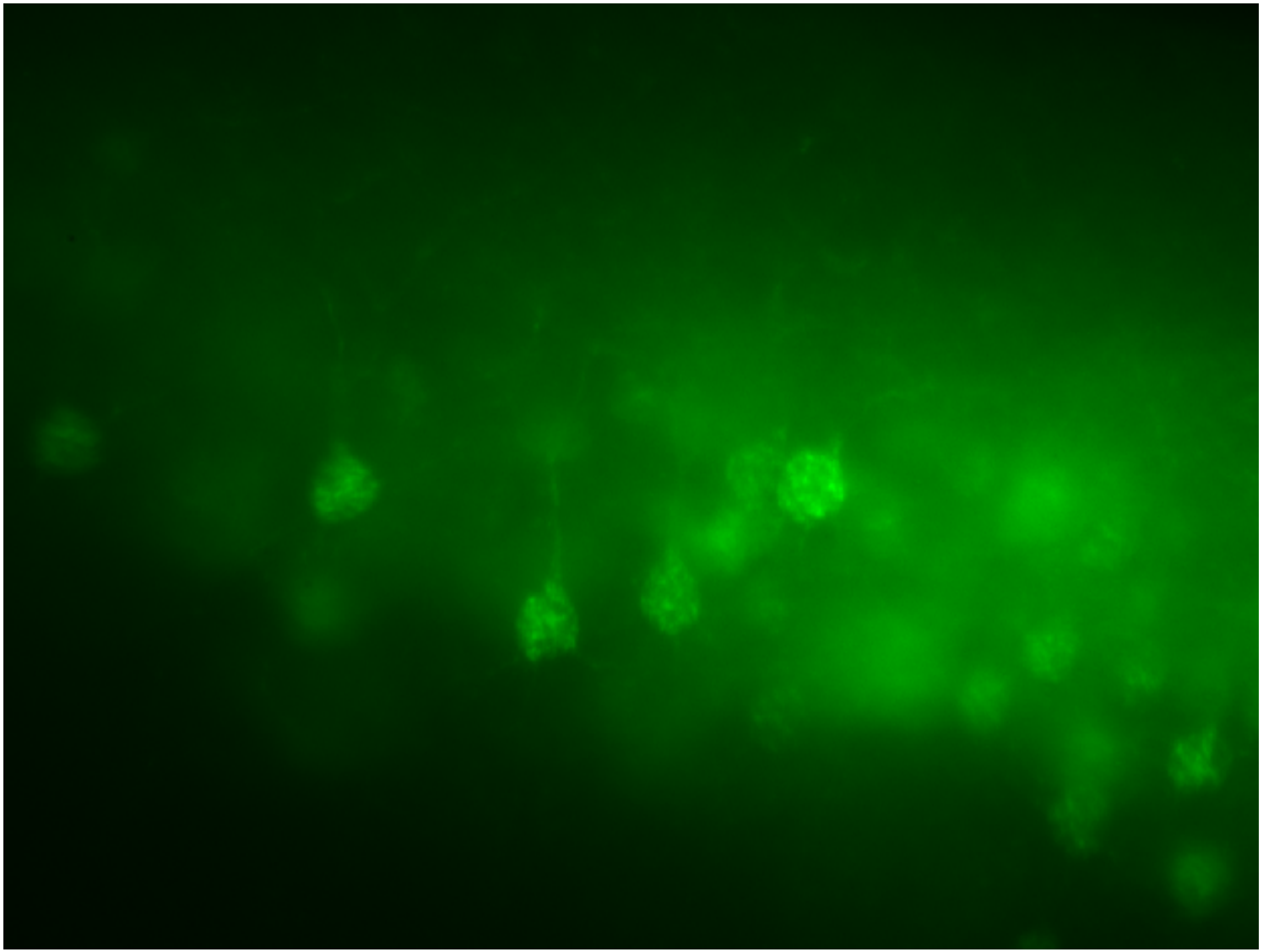
HiLo microscopy with speckle

HiLo microscopy with speckle

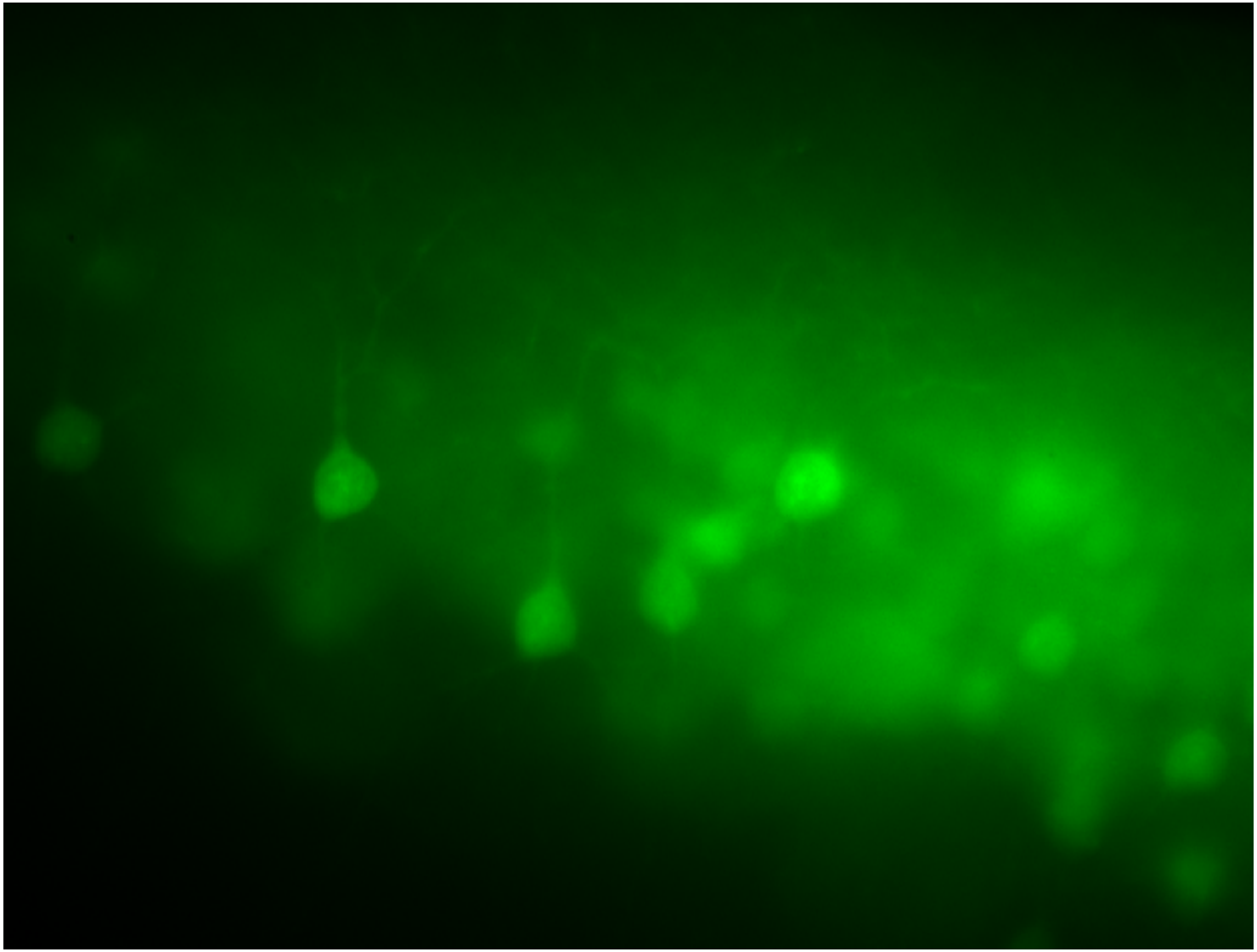




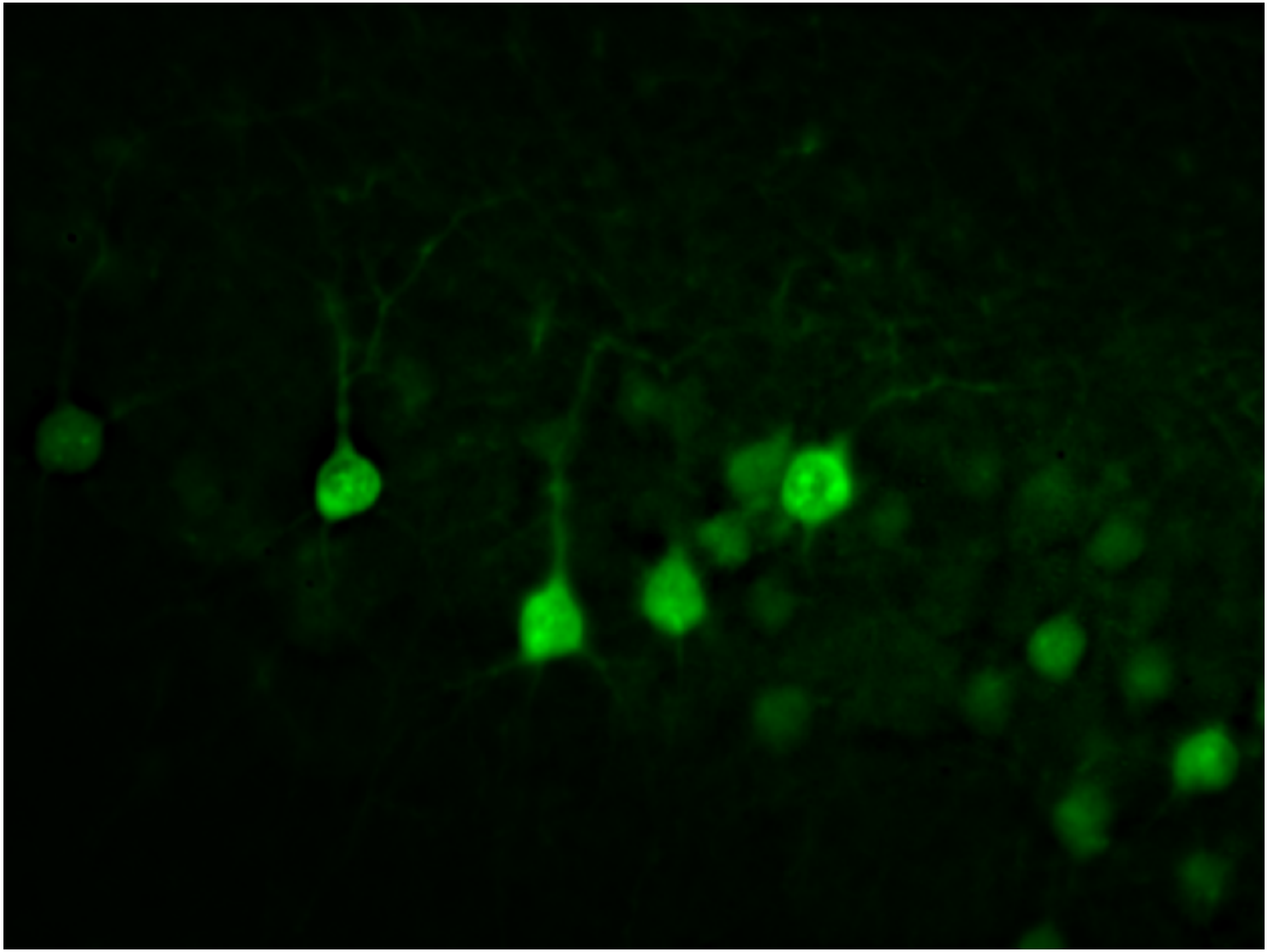




Speckle illumination



Uniform illumination

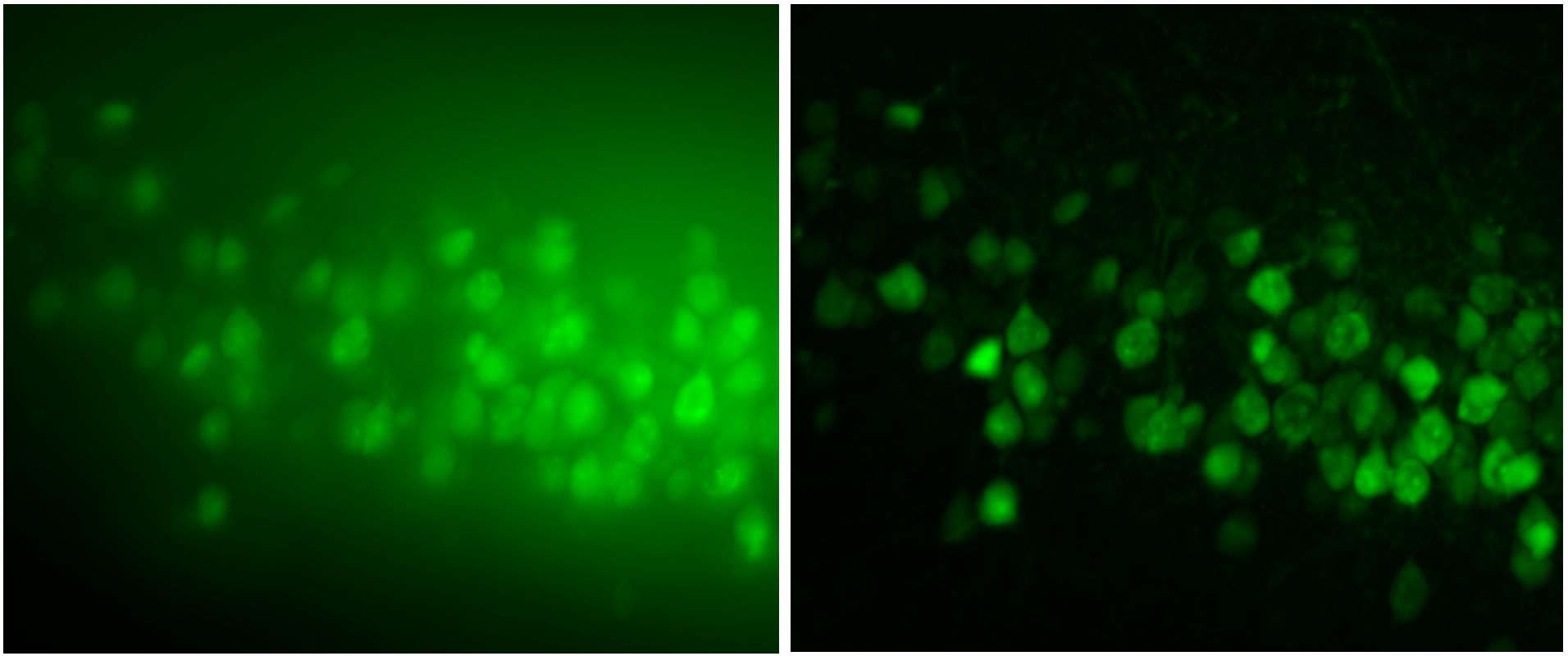


HiLo

Neurons in cleared mouse brain

Standard widefield microscopy

HiLo microscopy

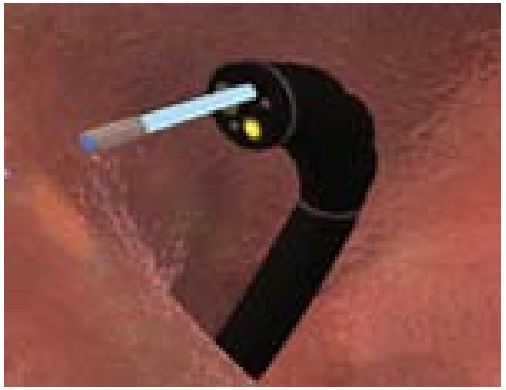


30 µm

Extended focus images of cortex slice

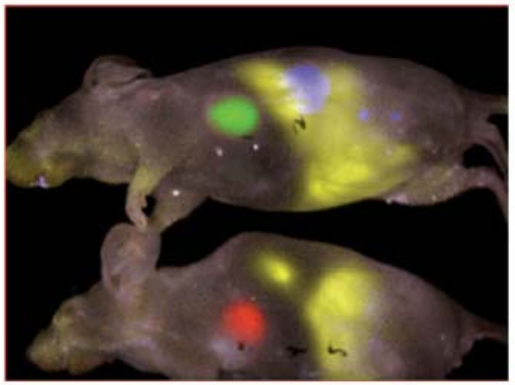
(max. intensity projection)

HiLo



Endoscopy

Microscopy



Macroscopy