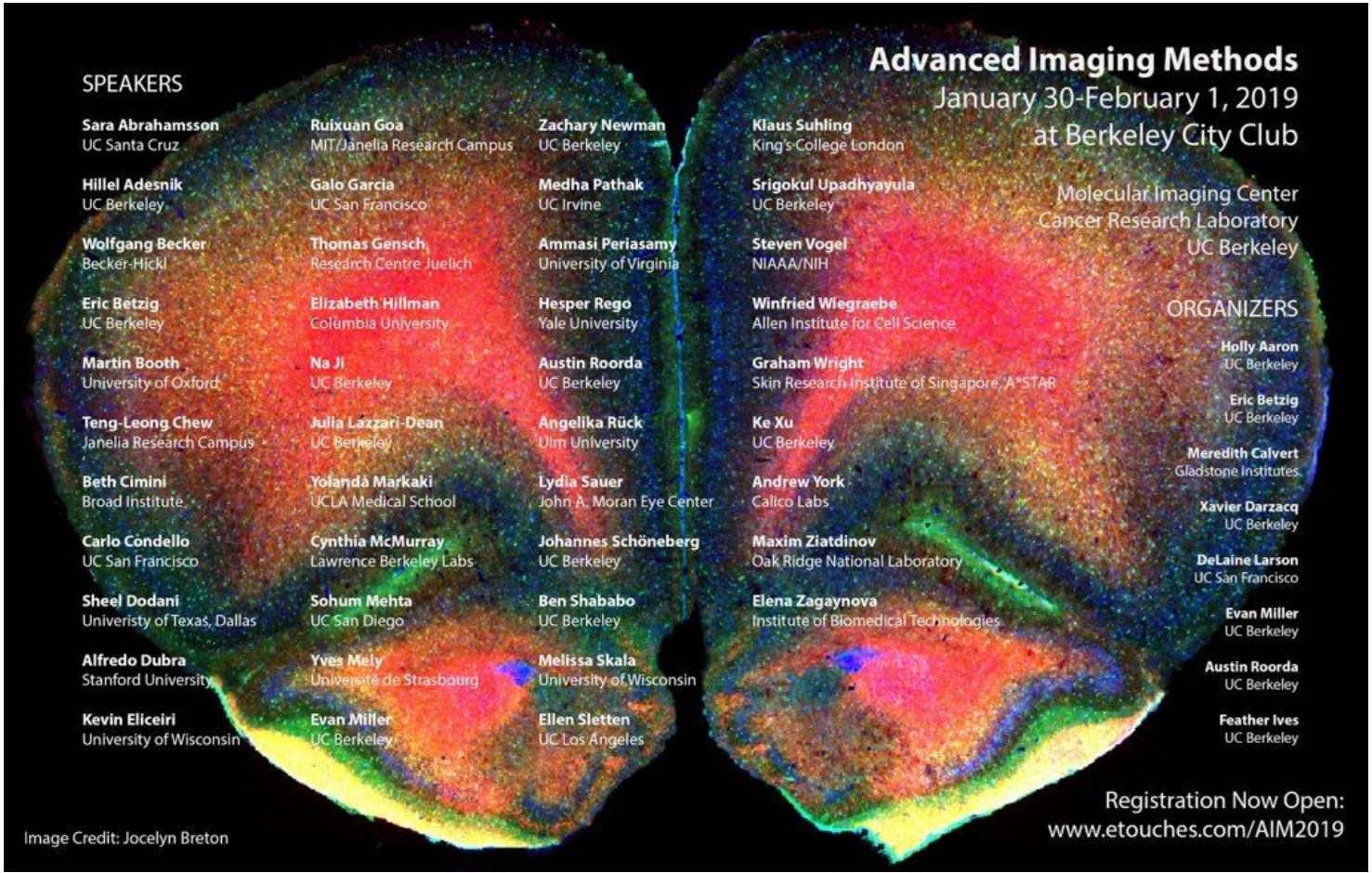
DATABINGE: FEB 8, 2019



OUTLINE

- ~~SCAPE~~
- ~~FEMPTONICS~~
- ~~Image Analysis~~
- Na Ji: Bessel Beam + demo
- Na Ji: AO
- LLSM
- Eric Betzig: Swiss Army Knife microscope
- LLSM demo

DATABINGE THIS SEMESTER

- Polished presentations not required.
- Can discuss: data analysis, new papers, hardware issues, new data etc.

- Any ideas?

NA JI: EXTENDED DEPTH OF FOCUS IMAGING WITH BESSEL BEAMS

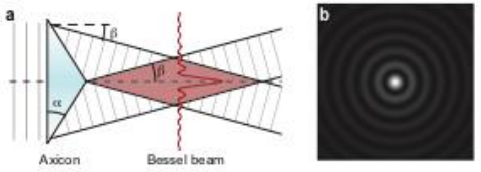


Fig. 1. **An axicon produces a nondiffractive beam.** (a) Schematic representation of an axicon (conical lens) of angle α illuminated by a plane wave. After the axicon, the interference pattern generates a Bessel beam, characterized by the deviation angle, β. The radial profile of a Bessel beam is traced in red. (b) Transverse intensity profile of a Bessel beam.

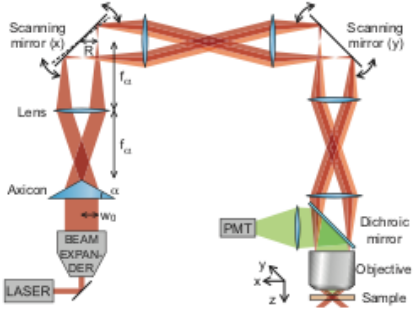


Fig. 2. **Schematic of the set-up.** A Ti:Sapphire laser illuminates an axicon of angle α followed by a lens (focal length f_α), which transform the laser beam into an annulus of light of radius R. This annulus is imaged into the back focal plane of the objective lens, which creates a tightly focused Bessel-Gauss beam in the sample. The scanning system (in our case a pair of galvanometric mirrors and relay lenses) enables a beam tilt in the back focal plane of the objective, leading to an x-y scan of the beam in the sample. Fluorescence light is retro-collected with the objective and directed to a photomultiplier tube (PMT) with a dichroic mirror.

- Allows "volumetric" recordings at a 2d frame rate.
- Janelia paper with SLM: Lu et al. Nat. Neuroscience 20 (2017) 620.
- Earlier papers from Quebec City with Axicons: Theriault et al. Optics Express 21 (2013) 10095 & Theriault et al. Fron. Cell. Neur. 8 (2014) 1.

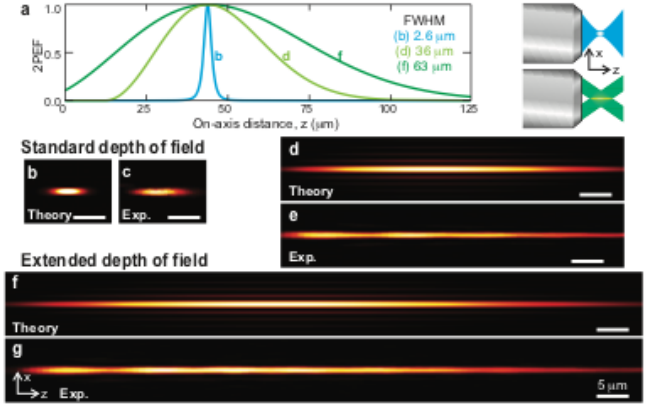


Fig. 3. **Longitudinal point-spread function.** (a) Two-photon excitation fluorescence (2PEF) signal along the z axis for an extended depth of field set-up (green) compared to a standard set-up (blue) with the same transverse resolution. (b), (c) Calculated and experimental PSF in the x-z plane with standard depth of field set-up ($w_0 = 2.7$ mm). (d) to (g) Calculated and experimental PSF in the x-z plane with extended depth of field set-ups ($w_0 = 0.27$ mm for d-e and $w_0 = 0.47$ mm for f-g). Experimental PSFs were measured with

NA JI: EXTENDED DEPTH OF FOCUS IMAGING WITH BESSEL BEAMS

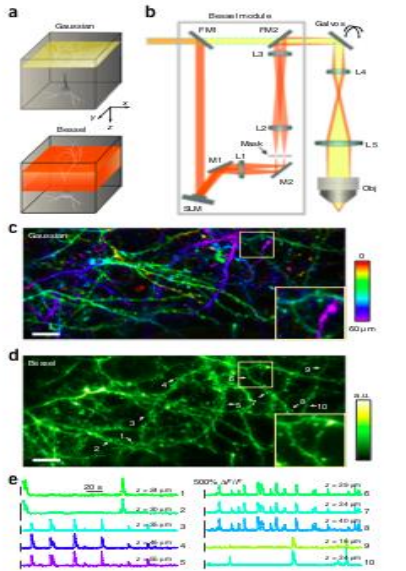


Figure 1 Concept, design and performance of Bessel module for *in vivo* volume imaging. (**a**) Scanning a Gaussian focus in the *x*–*y* plane images a thin optical section (yellow-shaded region), whereas scanning a Bessel focus in *x*–*y* images the structures in a 3D volume (red-shaded region). (**b**) Schematics of Bessel module, composed of an SLM, three lenses (L1, L2, L3) and an annular mask. The module generates an annular illumination pattern on the galvanometers (galvo) and, after conjugation by lenses L4 and L5, at the back pupil of the objective (Obj), resulting in a focus approximating a Bessel beam. M1 and M2, folding mirrors; FM1 and FM2, flip mirrors to switch between Gaussian (yellow with dashed blue outline) and Bessel (red) paths. (**c**–**e**) Example application of Bessel focus scanning to a volume of GCaMP6s+ neurites in awake mouse cortex at 30 Hz. (**c**) Mean intensity projection of a 60-µm-thick image stack collected with Gaussian focus scanning at 1-µm *z* steps, with structures color-coded by depth. (**d**) Image of the same volume of neurites collected by scanning a Bessel focus with 0.4 NA and 53-µm axial FWHM. Arrows and numbers label individual axonal varicosities (putative boutons, 1, 2) and dendritic spines (3–10). Insets in **c** and **d** show zoomed-in views of dendritic spines. Scale bars, 20 µm. (**e**) Representative calcium transients measured in boutons and spines from four neurons (color-coded by *z* depth). Objective, Olympus 25× with 1.05 NA; wavelength, 960 nm; postobjective power, 30 mW for Gaussian and 118 mW for Bessel scanning. Representative images from three mice are shown.

- Janelia system uses a spatial light modulator to create the bessel beam, since it is not a fixed optics like the axicon, with some engineering, the extended depth of focus could be made adjustable.
- Thorlabs has picked this up and is developing a product. Demoed in Na Ji's lab during the Berkeley meeting.
- It works!

NA JI: EXTENDED DEPTH OF FOCUS IMAGING WITH BESSEL BEAMS

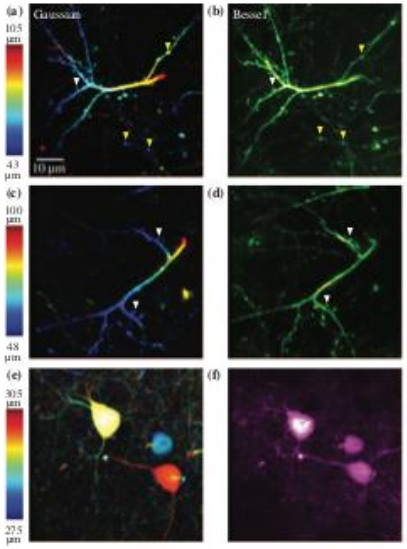


Fig. 3. *In vivo* volumetric three-photon fluorescence microscopy of mice brains. Maximum intensity projection of a (a) 62-µm and (c) 52-µm thick volume at ~80 µm below dura, color-coded by depth, imaged with a Gaussian focus, at a 2-µm axial step size, under 1300 nm excitation. Post-objective powers: 3 mW and 4 mW, respectively. (b, d) Image of the same volume as (a, c), respectively, obtained with a Bessel focus of NA 0.6 and axial FWHM of 38 µm. White arrowheads label dendritic spines, and yellow arrowheads label axonal boutons. Post-objective powers: 30 mW and 40 mW, respectively. (e) Maximum intensity projection of a 30-µm thick volume at ~290 µm below dura, color-coded by depth, imaged with a Gaussian focus, at a 2-µm axial step size, under 1700 nm excitation. Post-objective power: 15 mW. (f) Image of the same volume as (e) obtained with a Bessel focus of NA 0.6 and axial FWHM of 27 µm. Post-objective power: 73 mW. A Thy1-GFP line M mouse was used for (a–d), and a Gad2-IRES-Cre × Ai14 for (e, f). Imaging frame rate: 1 Hz (256 × 256 pixels per frame).

- Recently extended to 3p.
- Rodriguez et al. Optics Letters 43 (2018) 1914.
- Uses an axicon.
- Paper demos the bessel beam with 3p, but not at depths unreachable by 2p.
- For this, the authors state that Adaptive Optics is necessary to compensate for aberrations in the sample.

NA JI: ADAPTIVE OPTICS

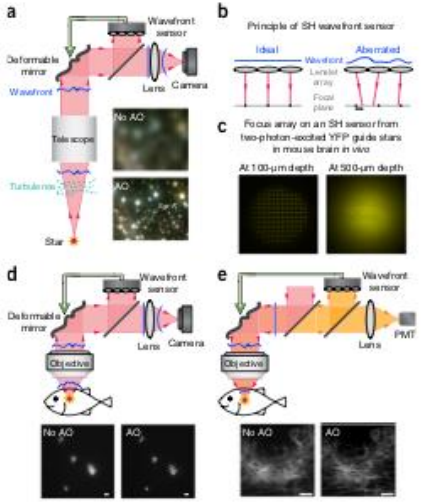


Figure 3 | Adaptive optics using direct wavefront sensing. (**a**) Distortion of wavefront (blue lines) is directly measured with a wavefront sensor and minimized by a wavefront modulator (e.g., a deformable mirror) to improve image quality for a telescope. Sgr A*, Sagittarius A*. (**b**) Principle of SH wavefront sensor. (**c**) Images on a SH sensor of two-photon-excited YFP guide stars at 100- and 500-µm depth inside a mouse brain *in vivo*. (**d**,**e**) Direct-wavefront-sensing-based adaptive optical widefield fluorescence microscope (**d**) and two-photon fluorescence microscope (**e**). Insets represent images of (**a**) stars near Sagittarius A*, (**d**) beads inside a *Drosophila* embryo[17], (**e**) neurons in zebrafish larval brain[22] obtained without and with AO correction. Scale bars, 2 µm (**d**) and 10 µm (**e**).

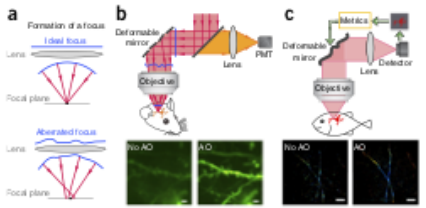
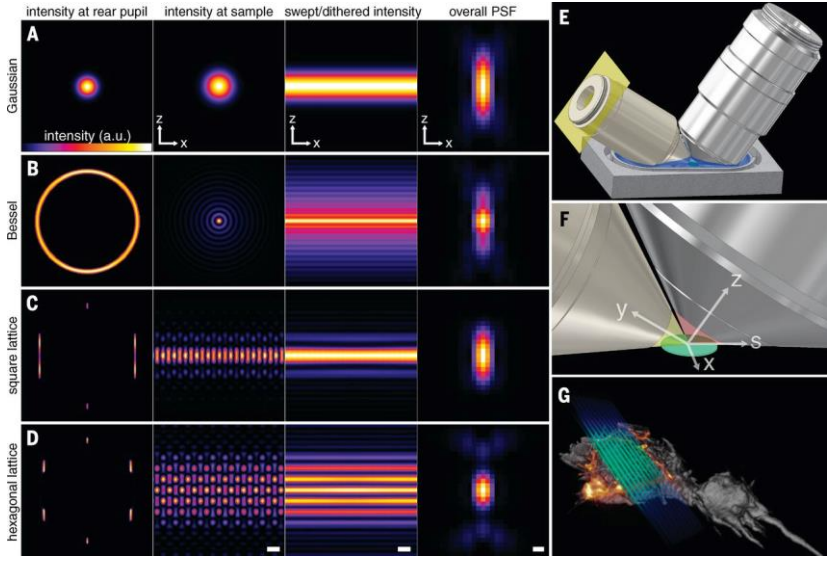


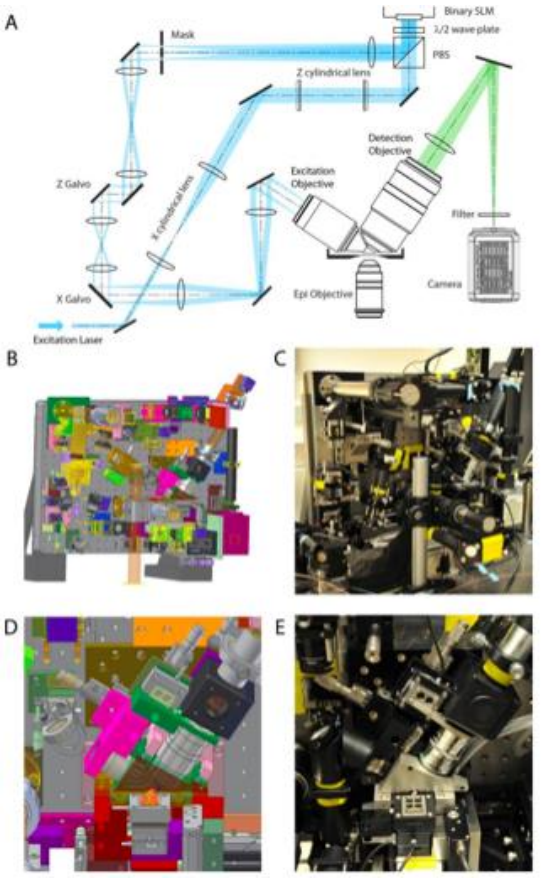
Figure 4 | Adaptive optical fluorescence microscopy with indirect wavefront sensing. (**a**) Formation of a focus without and with aberration. Black dots indicate focal positions in ideal aberration-free systems. (**b**) In a two-photon fluorescence microscope, the wavefront is separated into segments, and individual light rays representing each segment are measured and corrected to recover optimal imaging performance. Insets, dendritic spines in mouse brains imaged without and with AO correction[34]. (**c**) Metric-based optimization methods collect images with known wavefront distortions introduced, from which an optimal corrective wavefront is obtained. Insets, super-resolution single-molecule localization microscopic images of microtubules measured without and with AO correction[34]. Scale bars, 2 µm (**b**) and 1 µm (**c**).

- Good review: Ji Nat Methods 14 (2017) 374.
- Direct VS Indirect wavefront sensing (WFS)
- For direct WFS see Wang et al. N Comms 6 (2015) 7276.
- For indirect WFS see Wang et al. Nat Meth 10 (2014) 1037.
- Current state of the art is AO with 3p imaging. Showed spines over 700 um deep in vivo.
- Manuscripts are in prep.
- Also developing a stand alone box to do AO with any 2p scope.

LATTICE LIGHT SHEET MICROSCOPE

- Published Science 2014, With AO: Science 2018, With ExM: Science 2019

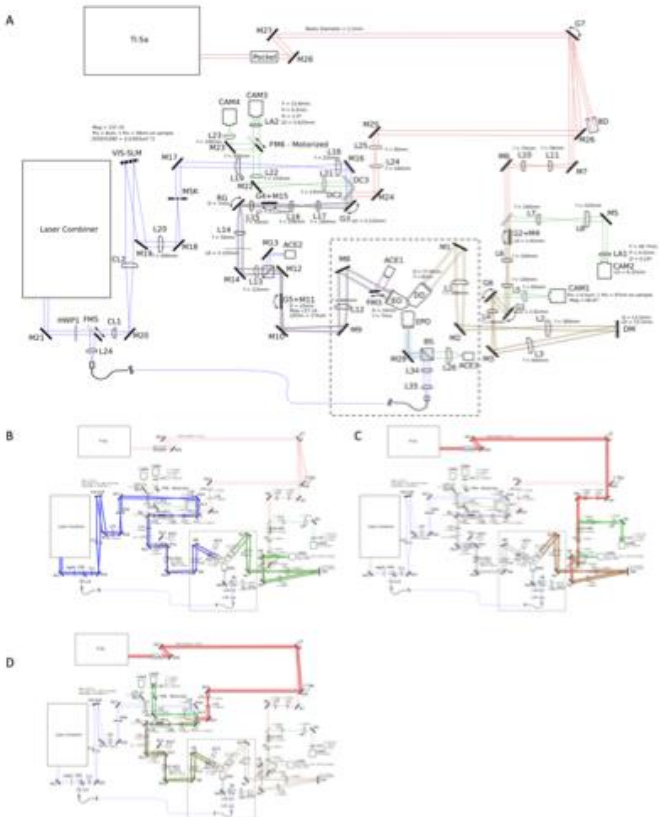




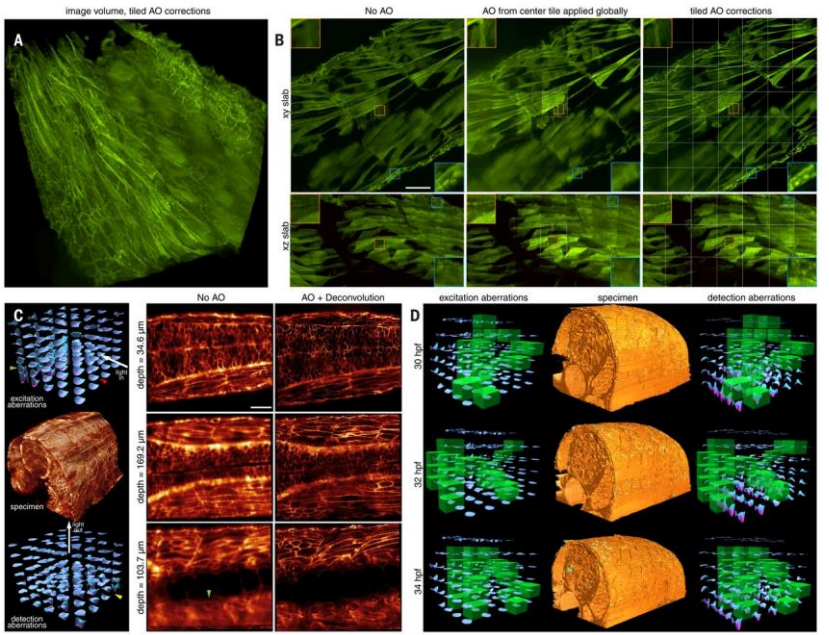
LLSM DATA EXAMPLE

- Show movie 1 from 2014 Science paper,

LLSM WITH AO (LUI ET AL. SCIENCE 2018)



- Why is this necessary? To maintain image quality in a complex specimen.

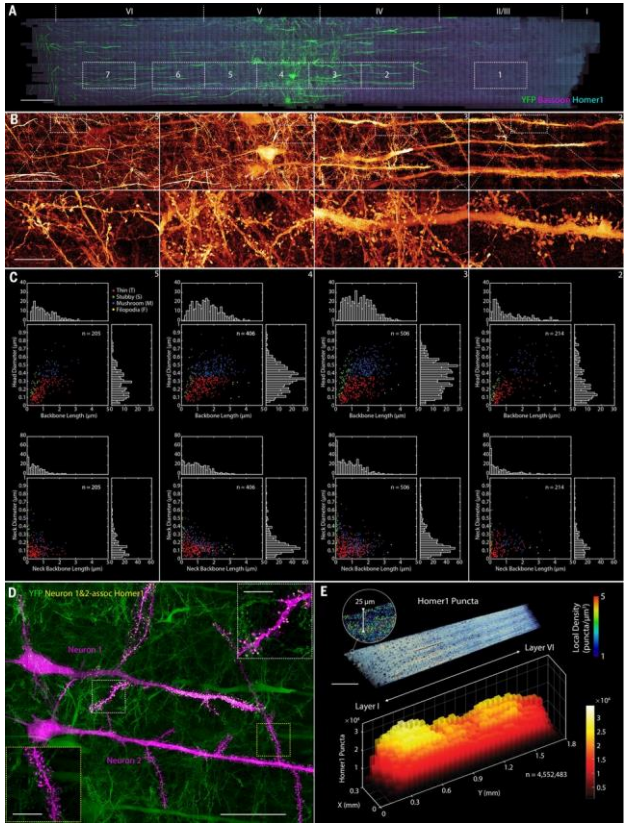


LLSM WITH AO - EXAMPLES

- Show supp movie 1

- Show example movie 9

EXM-LLSM (GAO ET AL. SCIENCE 2019)



- Another application of the lattice light sheet: Synapse scale info, over mm scale of tissue, imaged quickly enough to make comparisons possible.
- Same Rig, but with the AO off
- Resolution 60nmx60nmx90nm
- "~20 trillion voxels/mm3/color. This in turn necessitates imaging at speeds on the order of 100 million voxels/s to complete the acquisition in days rather than weeks or more"
- 1900x280x70um slice Thy1-YFP mouse
- Morphological characterization 1500 spines to compare with EM and ensure similar results

SWISS ARMY KNIFE MICROSCOPE

- Janelia to release plans and hold workshops on how to build an AO-LLSM, but not this design.
- Hardware to build LLSM includes: cameras, detectors, objectives, galvos, SLM, visible lasers, ultrafast laser, etc
- has high overlap with many imaging techniques: Gaussian and lattice light sheets, 3d SIM/PALM, 3d single molecule tracking, 2-photon point or bessel beam scanning.
- Build one microscope with a reconfigurable light path to enable use of the same components to do multiple techniques at the flip of a switch.

LLSM DEMO FROM 3I

- Lattice light sheet was licensed to Zeiss. Still in development...
- Available through the Janelia AIC proposal system
- Also, sub-licensed to 3i.
- Can buy an updated version of the 2014 Science paper system.
- Demo was on-going during the meeting...