Kilohertz frame-rate two-photon tomography

Scanned Line Angular Projection Microscopy

Overview

What:

Microscope for super-fast two-photon imaging

Why:

Capture neuron dynamics *in vivo*, fast enough to see

How:

...

Slow 2D Scan: Raster

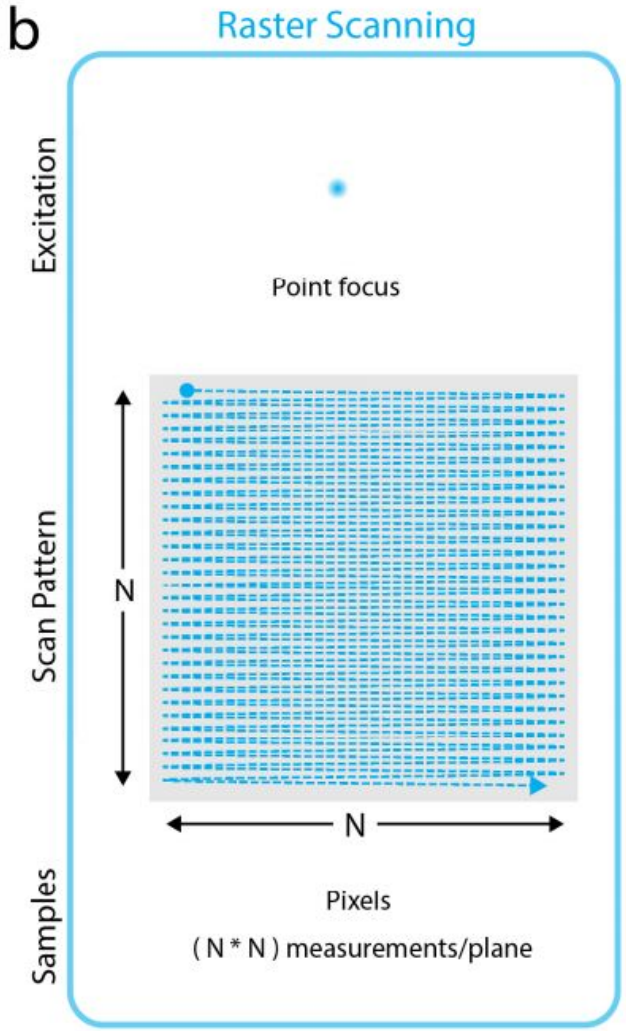
N * N measurements per plane

For 1024 x 1024:

Over 1 million pixels to scan

~10 ns between pixels

=> max ~100hz, slower if you oversample.



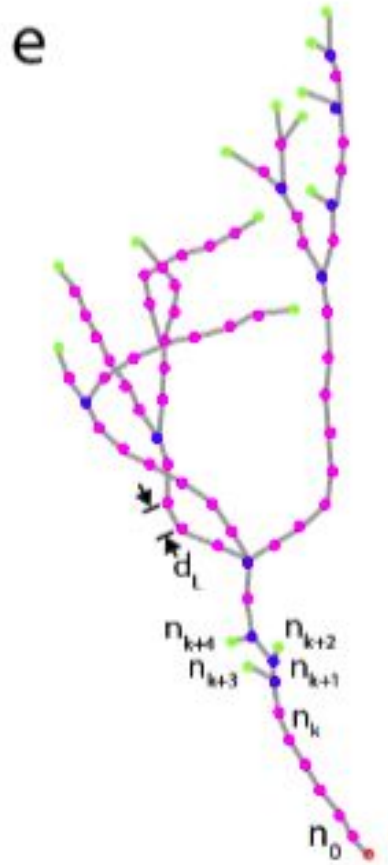
Medium 2D Scan: AOD

Random Access, only scan selected points

Custom number of points per scan

We get ~200Hz for ~100 points on a plane

- Overscan each point to reduce noise
- Sum multiple nearby points to reduce drift



Interpolated Neuron

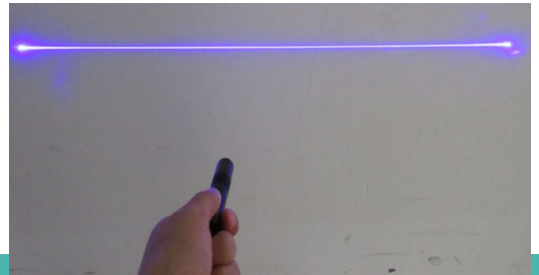
1Khz 2D Scan: SLAPMi

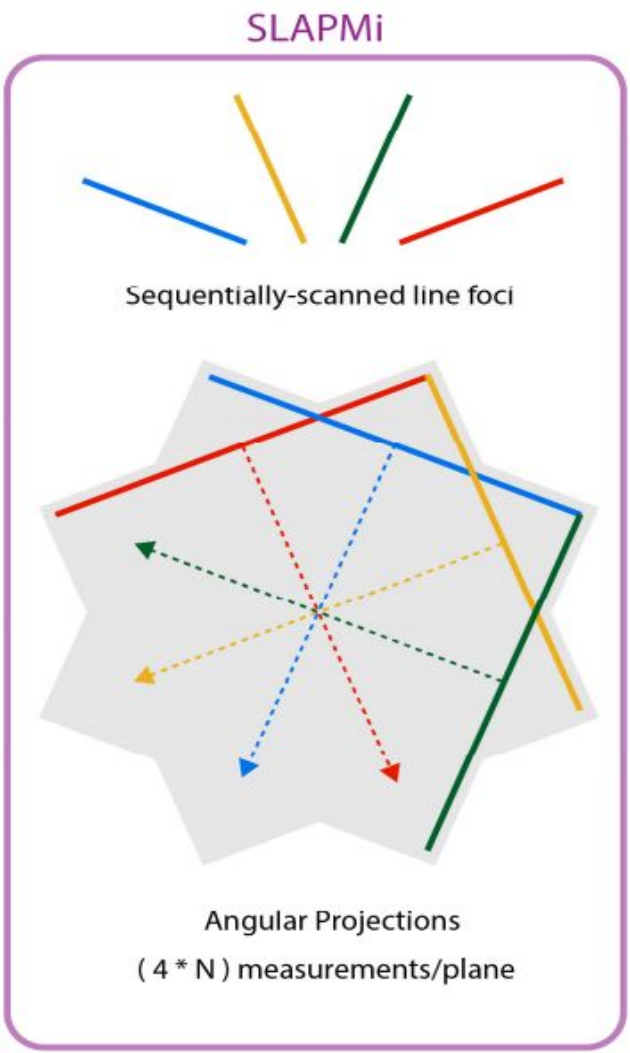
4N measurements per plane:

4 scan directions, offset 45° each

- Combined later to calculate best original image

#photons captured is the sum across the whole line



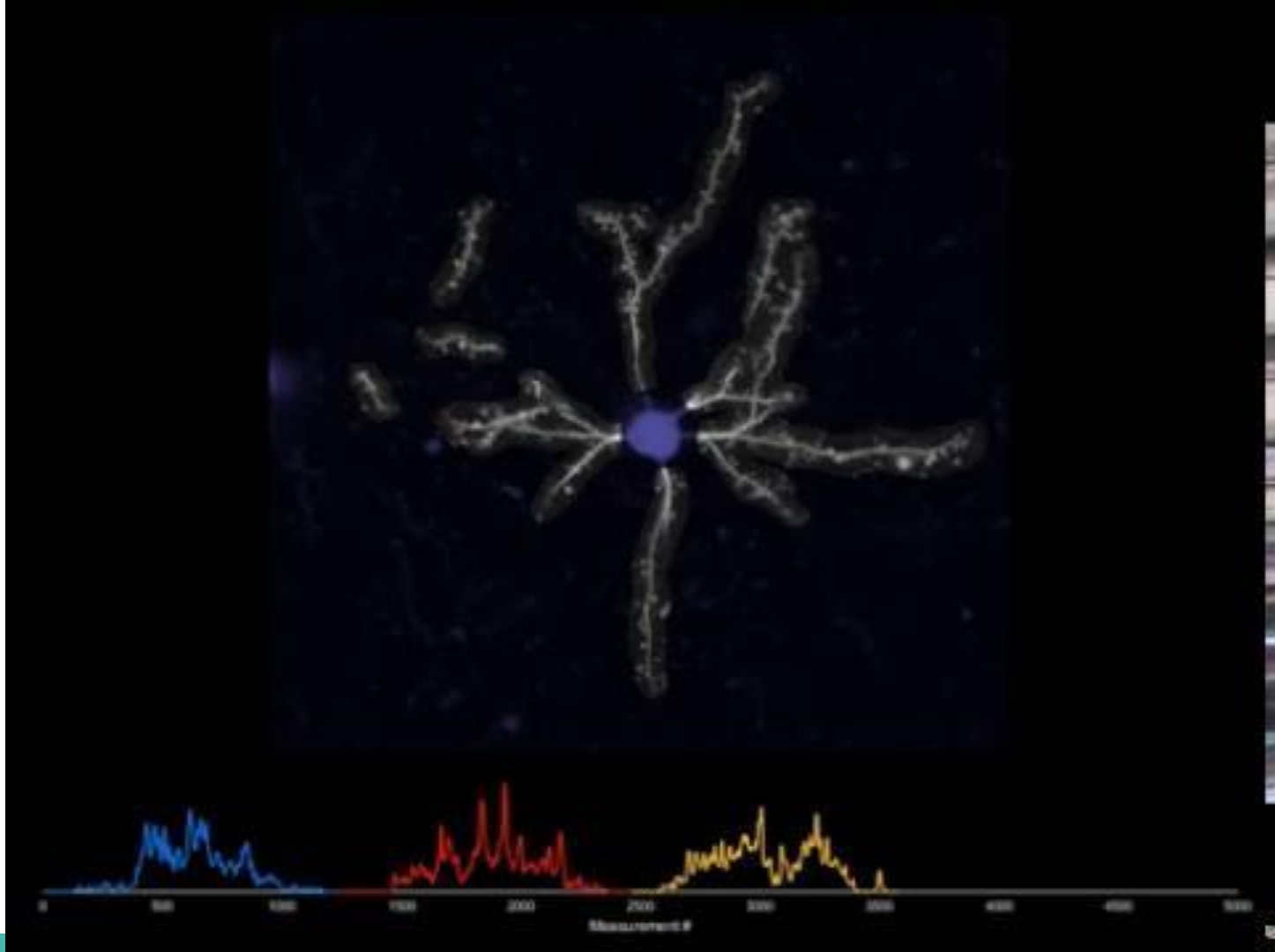


Example
Planar Scan:

4 sweeps
in series

White =
SLM zone

Intensity sum ->

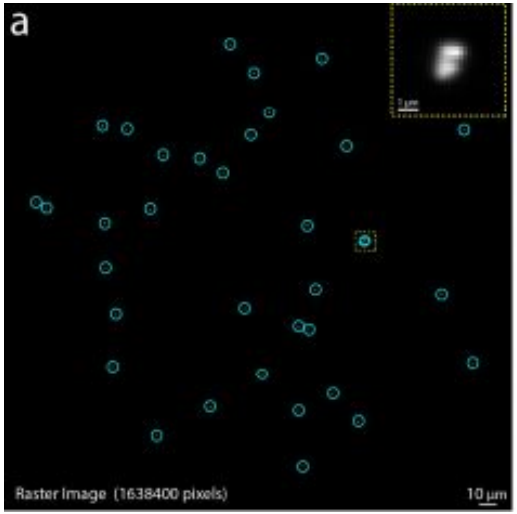


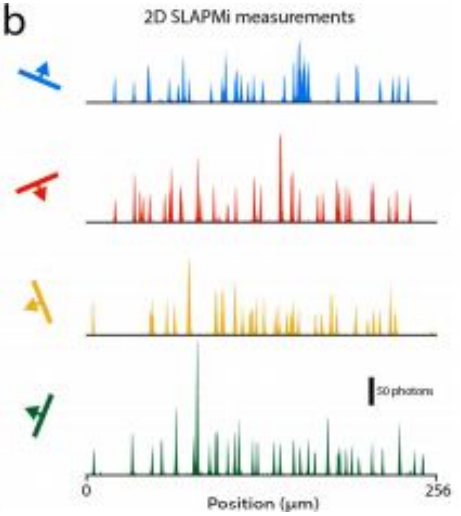
a) Ground truth beads

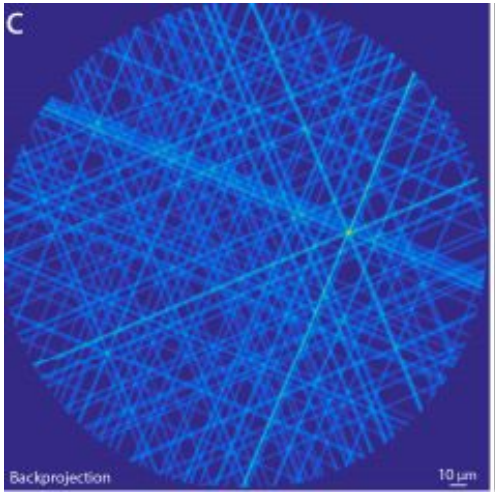
b) Intensity sum along axes

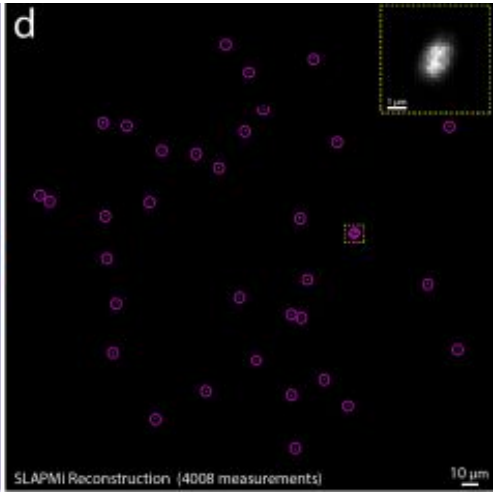
c) Back-projection to 2D

d) Most likely source image







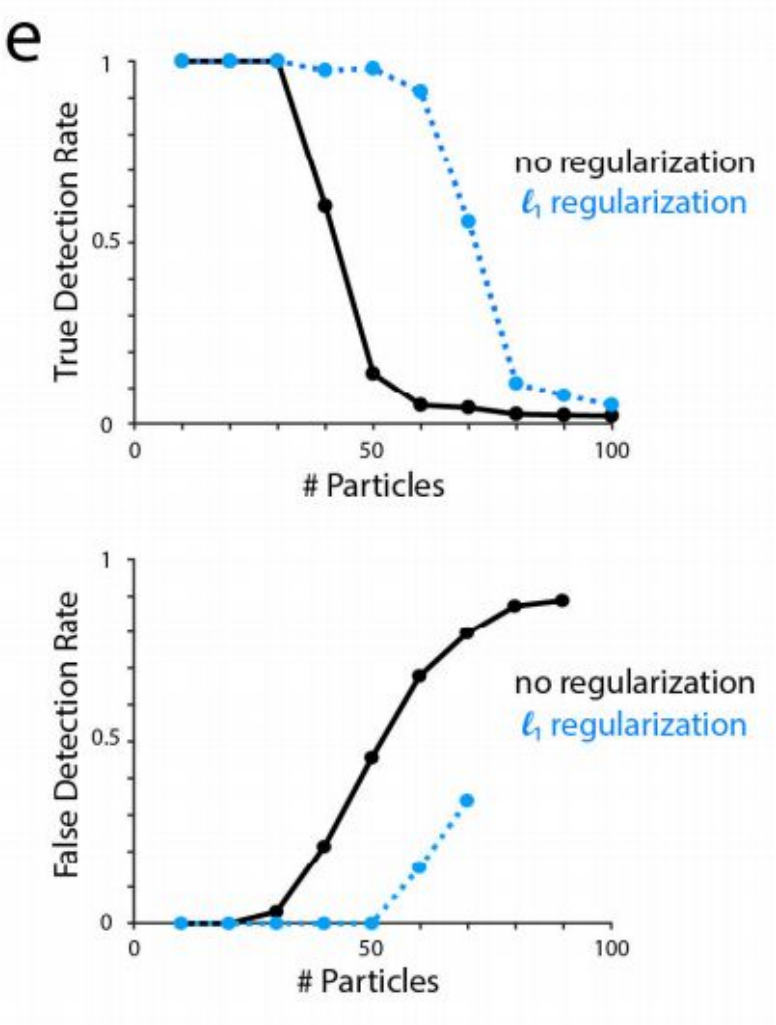


Drawbacks

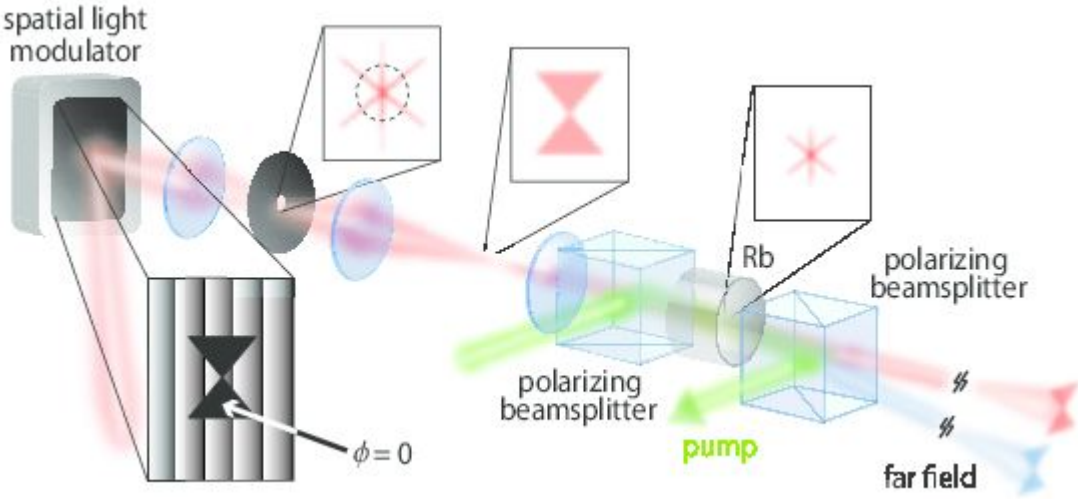
Not unique solution

More intensity sources

= more errors



Mitigation #1: SLM

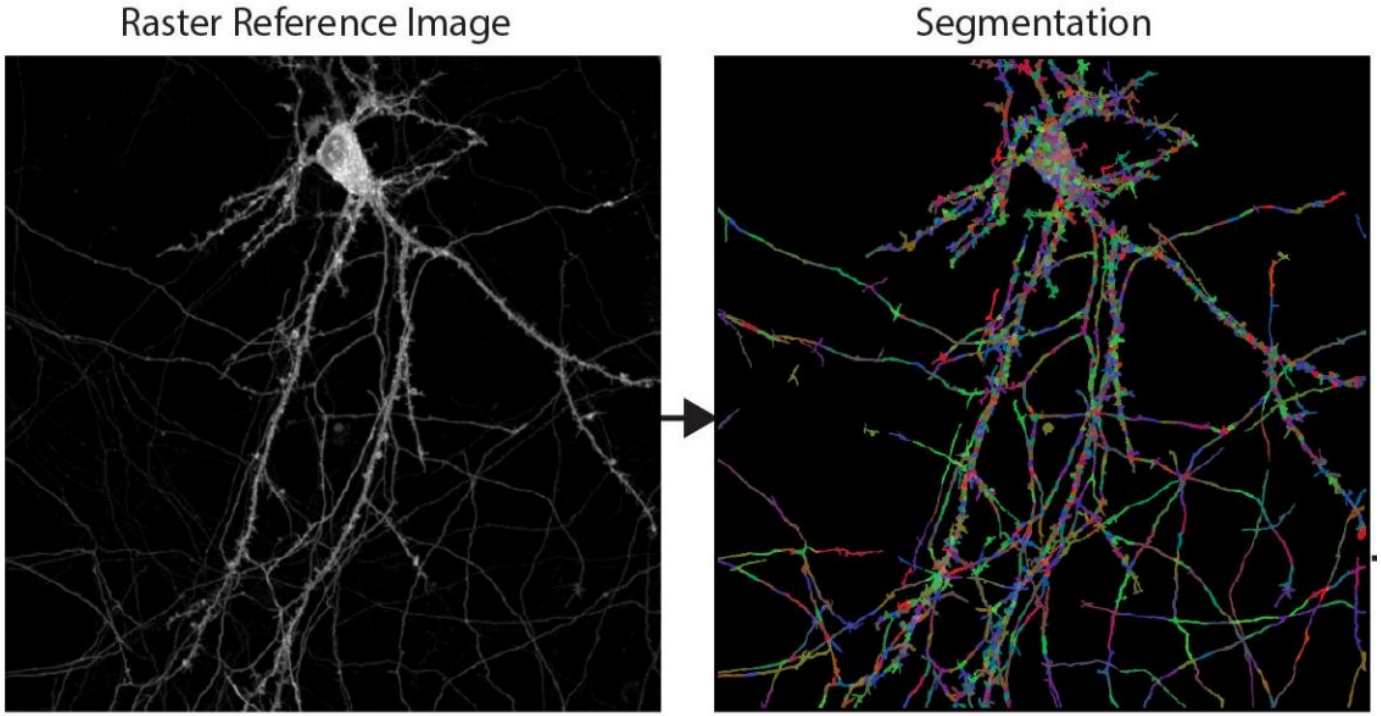


Reflect light off SLM

Control where the reflection happens in 2D

- Blank out areas not of interest
- Fewer sources, better fit for where you do care about.

Mitigation #2: Segmented reference



1) Segment raster image with Ilastik, ~1000 segments per plane

Segmented Fit

$$\mathbf{y}_t \sim \text{Poisson}(\mathbf{PSx}_t + \mathbf{b}_t),$$

$$\mathbf{x}_t = \theta \mathbf{x}_{t-1} + \mathbf{w}_t,$$

$$\boldsymbol{P} \geq 0, \mathbf{S} \geq 0, 1 > \theta \geq 0, \mathbf{w}_t \geq 0, \mathbf{b}_t \geq 0,$$

X	Segment intensity	Calculate from θ and w
S	Map Segment to 2D space	Known before
P	Map 2D space to microscope lines	Known before
b	Baseline fluorescence	Estimate before
Y	Measured line intensities	Measured
θ	Indicator decay rate	Known before
w	Fluorescence increase (‘estimated spikes’).	Fit for this

Result

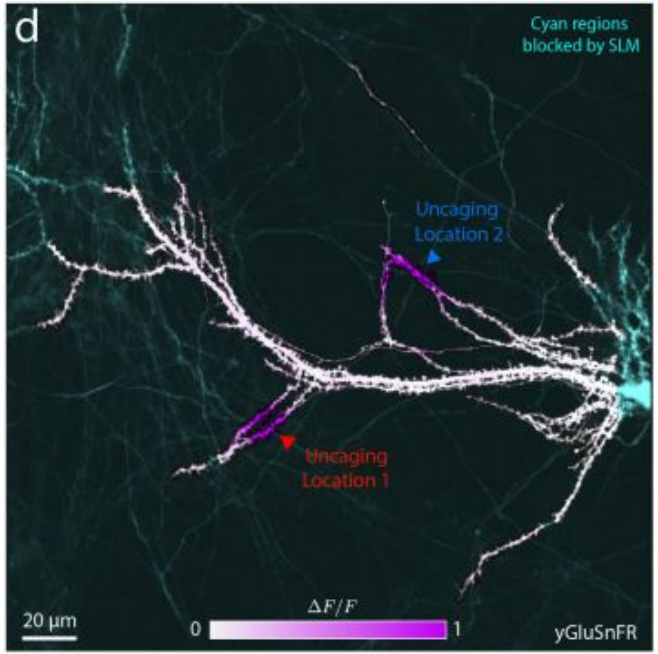
Solution gives you:

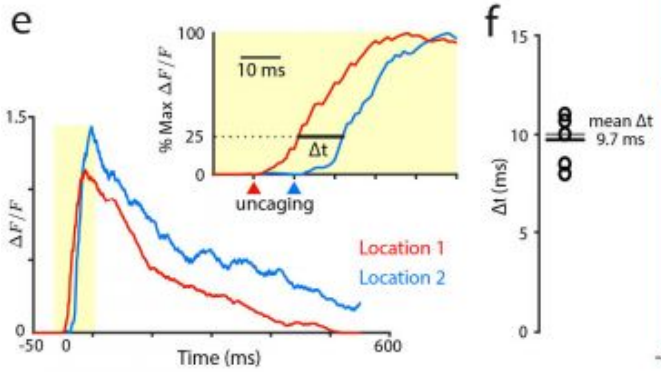
w = increases
per segment

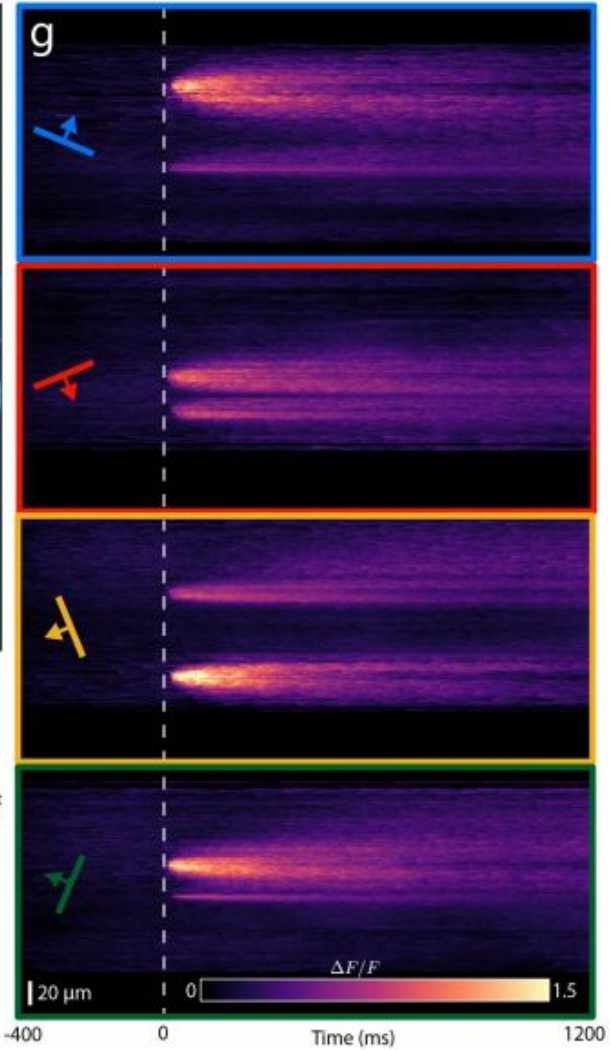
(both time and size)

Convert into:

x = fitted ΔF/F0 traces







More options #1: Source Triangulation

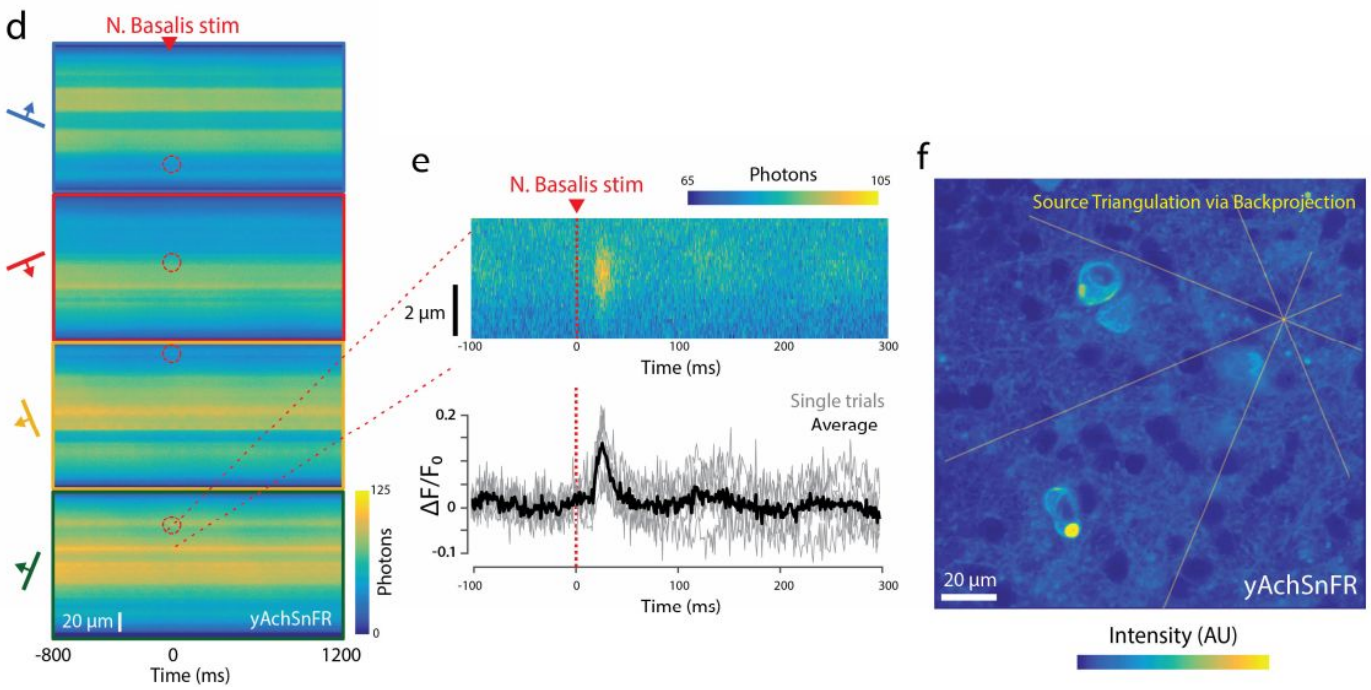
Measure all

(no segmentation)

Find aberrations

Triangulate to

find source

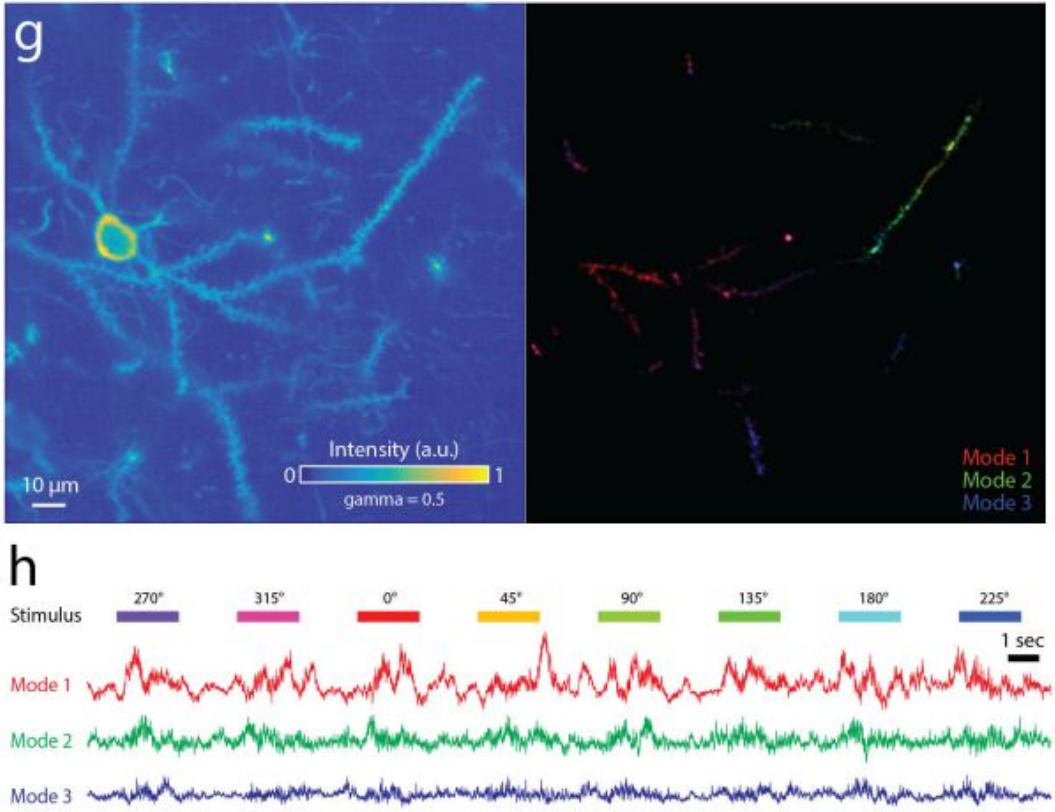


More options #2: Component detection

Measure 4 directions

Apply PCA to extract temporal ‘modes’

Apply NMF to convert back to spatial responses

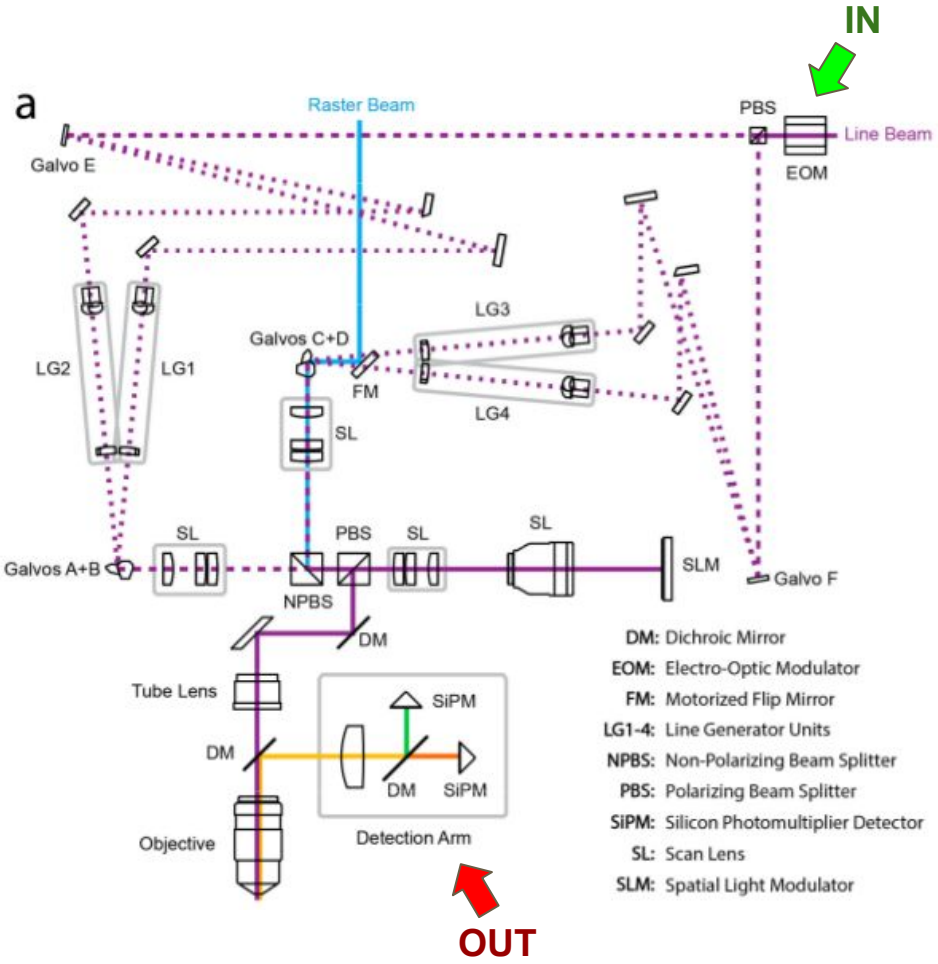


Thanks! (End of slides)

Extra backup slides following if anyone’s interested…

Optic Path overview

- Four paths, one at a time
- Line Generator units:
  - 3 cylindrical lenses
  - Convert beam into line segment
- Spatial Light modulator
  - Reflect off custom 2D shape
  - Allows setting a mask
- Silicon Photomultiplier Detector
  - Better PMT

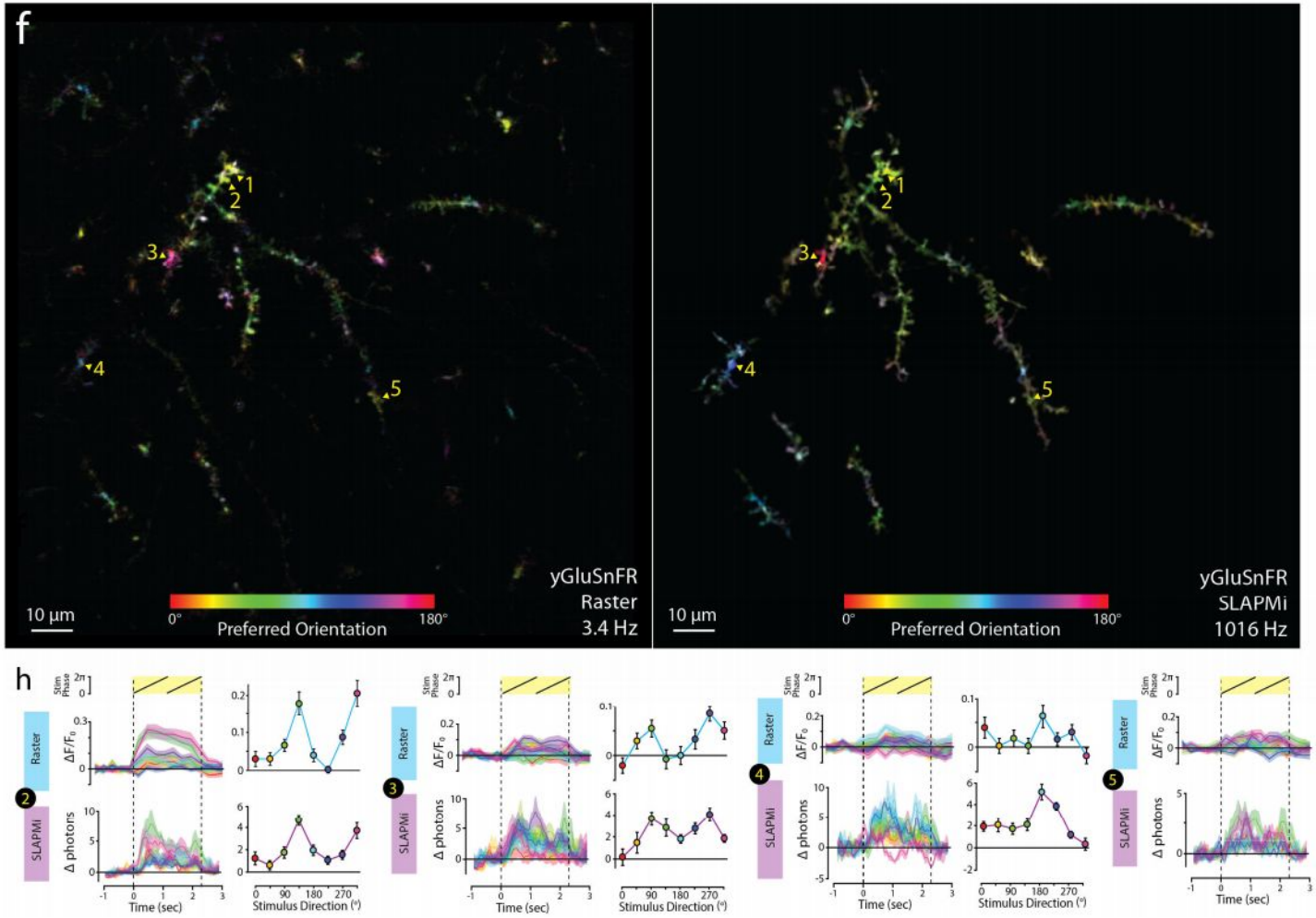


Extra

Orientation tuning

Compare

SLAPMi & Raster



Extra

Second example for

converting scans

into spatial

components