

Calibration

Summary of all calibration experiments associated with
Bruker photostim

(For associated protocols and code to generate figures see
the github: https://github.com/juremaj/photostim_deve)

Calibration protocols

- 1) Microscope (purely optical calibrations):
 - 1) Align and measure PSF in z
 - 2) 'Burn spots' (align imaging and stimulation scanners in x and y)
 - 3) Measure stim timing
 - 4) Measure power
- 2) Response (in vivo calibrations):
 - 1) Optimise parameter (power, time etc.)
 - 2) 'Physiological PSF' in x-y
 - 3) 'Physiological PSF' in z

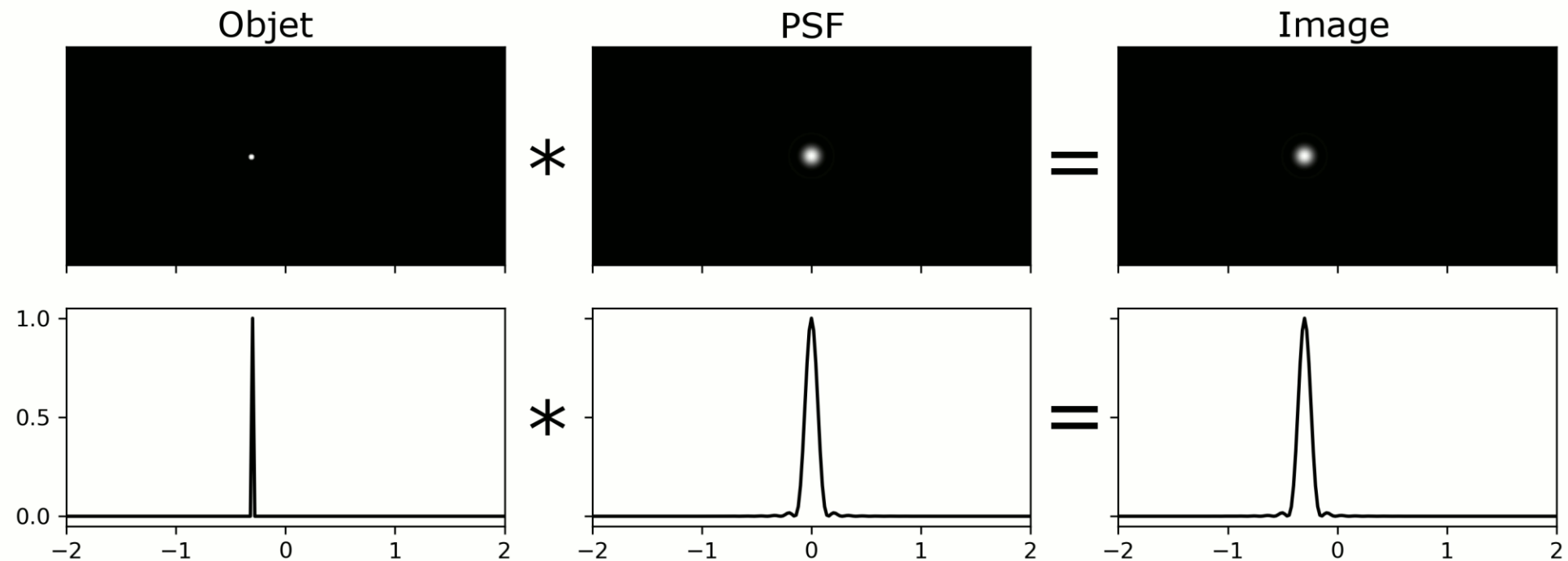
Calibration protocols

- Microscope (purely optical calibration protocols):
 - **z_align_psf.md** - Measure alignment and psf in z for different wavelengths (830nm, 920nm and 1100nm)
 - **burn_spots.md** - Align the scanning between imaging and photostim galvos by burning spots
 - **stim_timing.md** - Empirically measure the duration of stimulation based on the stim artefact
 - **power_measure.md** - Measure out of the objective power for imaging laser (at 830nm, 920nm and 1100nm) and the stim laser
- Response (in vivo calibration protocols):
 - **param_optim.md** - Measure dF/F for different parameter values (e. g. time, laser power)
 - **phys_psf_xy.md** - Measure the 'physiological PSF' - how dF/F depends on distance between a cell and a photostim spot in x and y by doing a grid of spirals
 - **phys_psf_z.md** - Similar as above but by repeating a spiral fixed in x and y, but varying its z position

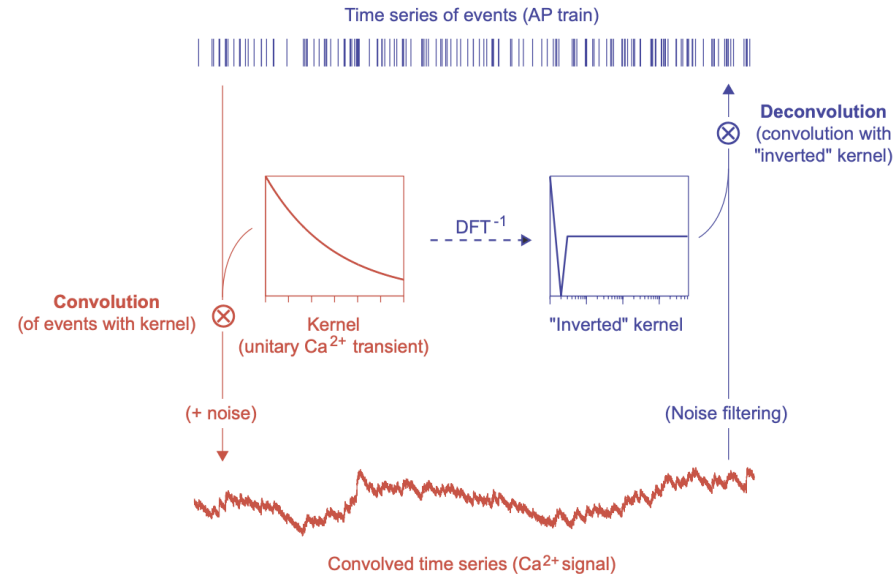
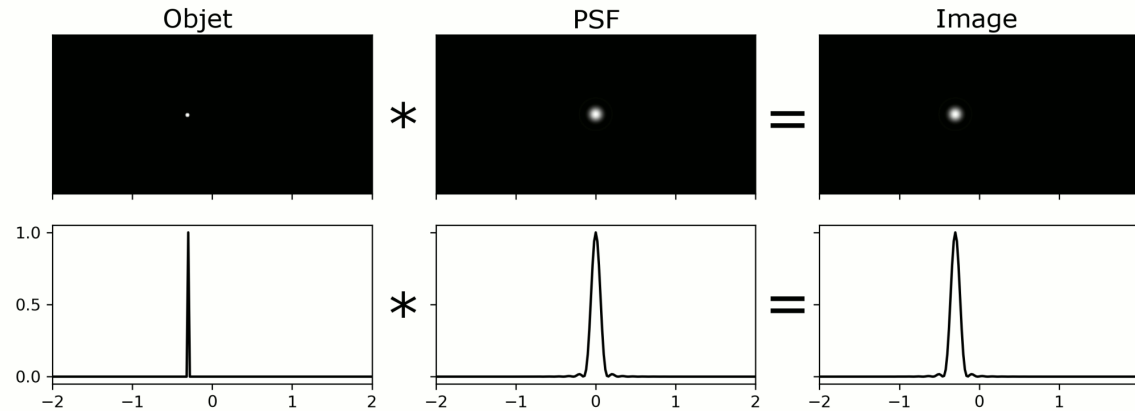
1) Microscope

(purely optical calibrations)

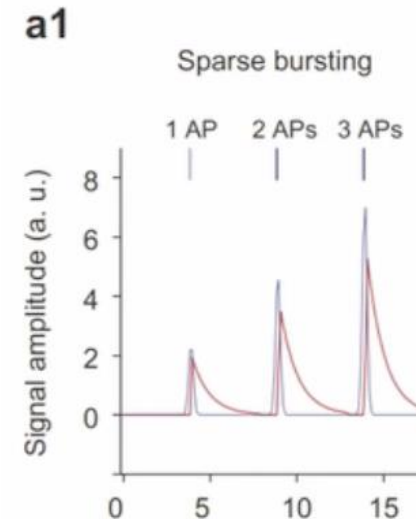
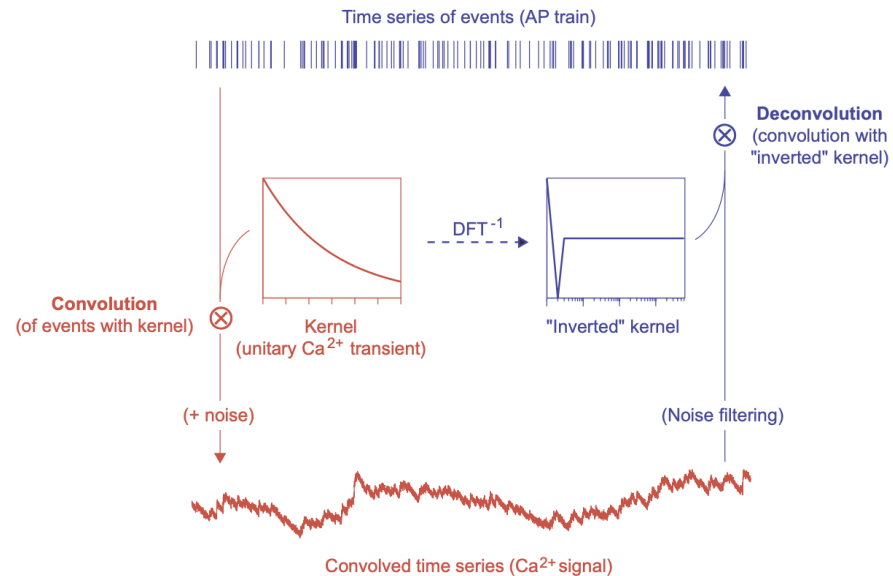
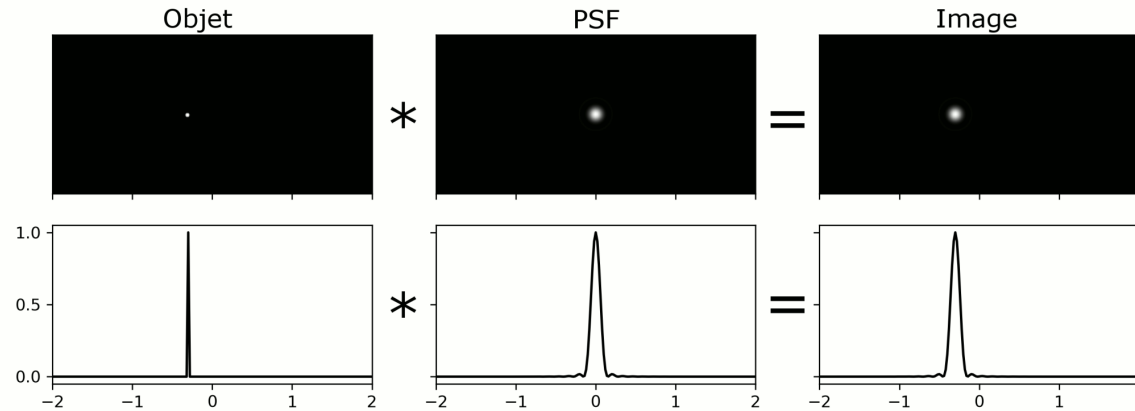
1.1. Microscope: Align and measure PSF in z



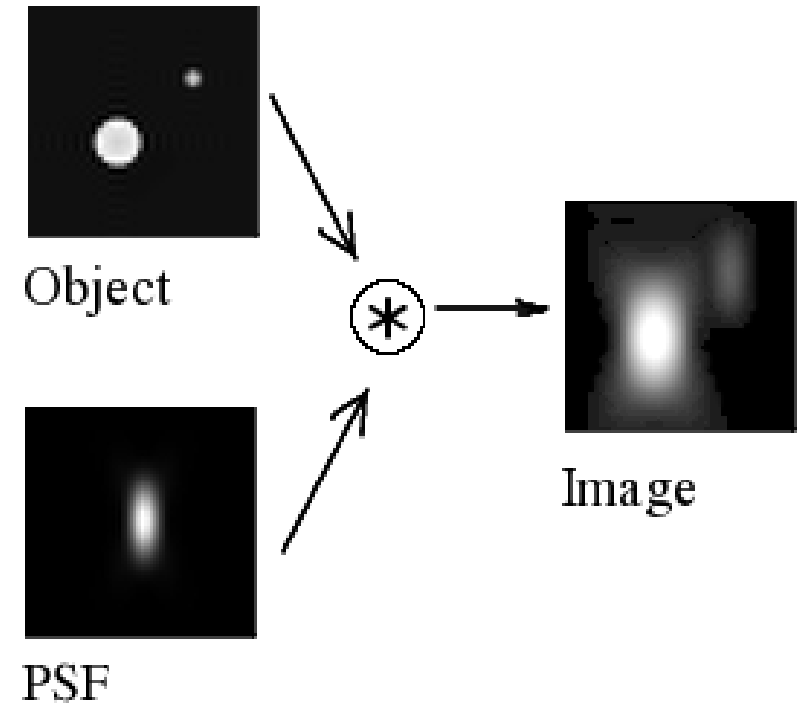
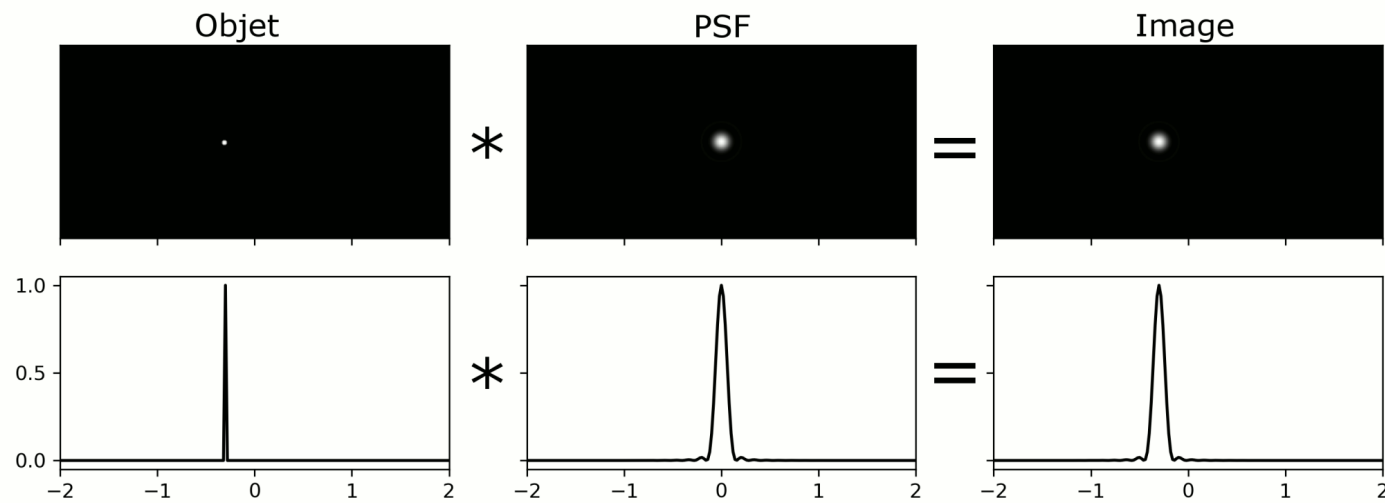
1.1. Microscope: Align and measure PSF in z



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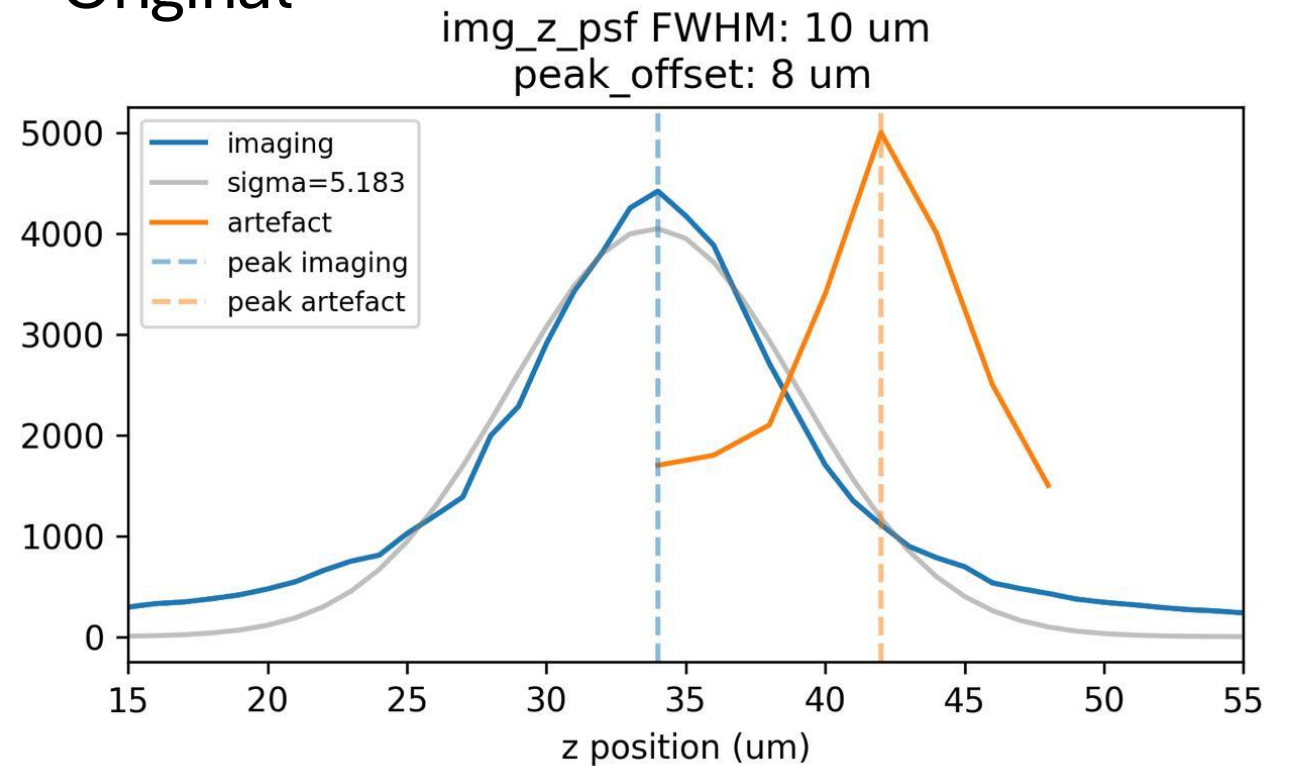


1.1. Microscope: Align and measure PSF in z

Note:

- Can only measure it in z because the optical table is not working (e. g. there is a lot of movement due to vibrations)
- Also, due to that the measurement is an upper bound (PSF would be smaller without movements)

Original

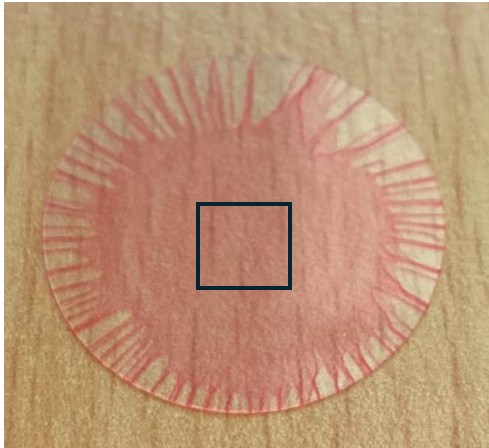


1.1. Microscope: Align and measure PSF in z

‘Optical sheet’

In reality:

- $< 1 \mu\text{m}$ thick
- perfectly flat
- fixed z position (obviously)

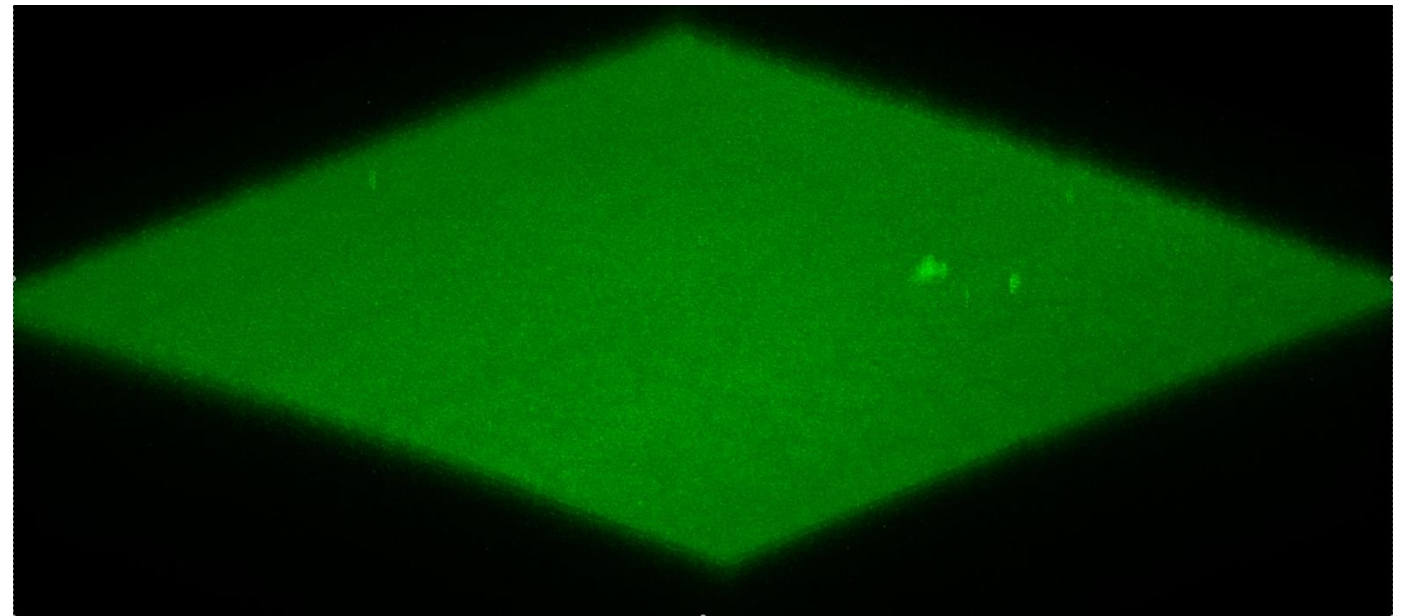
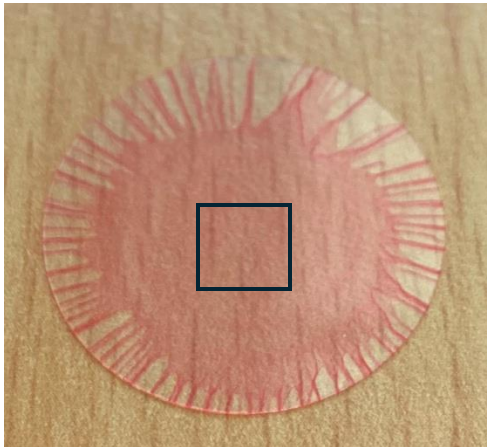


1.1. Microscope: Align and measure PSF in z

‘Optical sheet’

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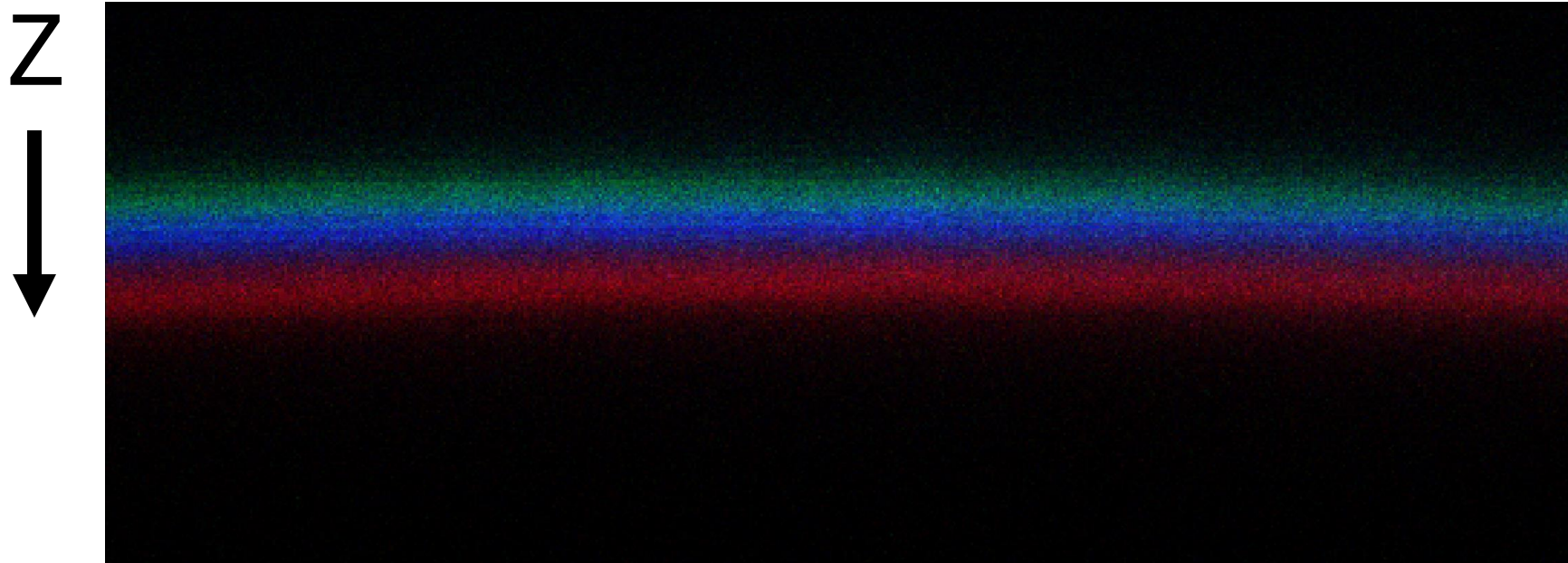
830nm

920nm

1100nm

(sorry the colors don't make good sense)

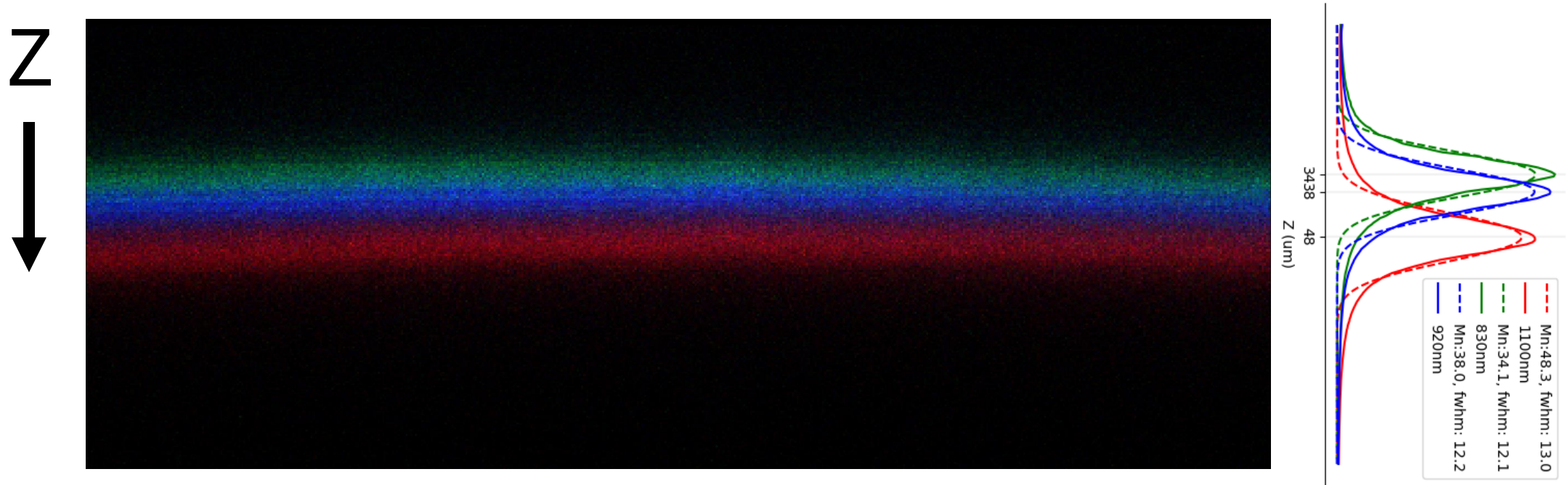
1.1. Microscope: Align and measure PSF in z



Important to take into account when choosing cells based on opsin expression (imaged at 1100nm)

830nm
920nm
1100nm

1.1. Microscope: Align and measure PSF in z



Important to take into account when choosing cells based on opsin expression (imaged at 1100nm)

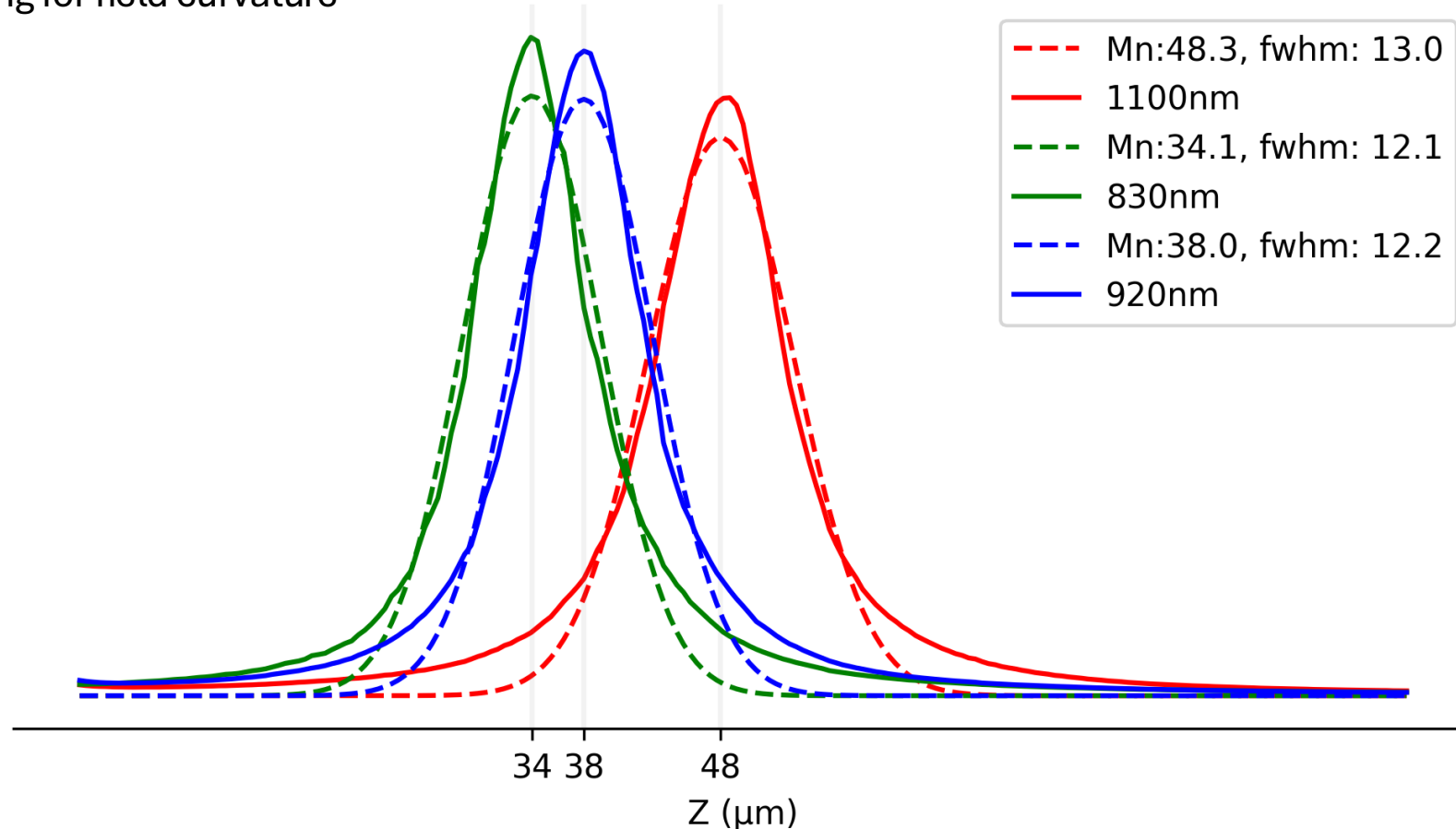
830nm
920nm
1100nm

1.1. Microscope: Align and measure PSF in z

Note: not accounting for field curvature
(see next slides)

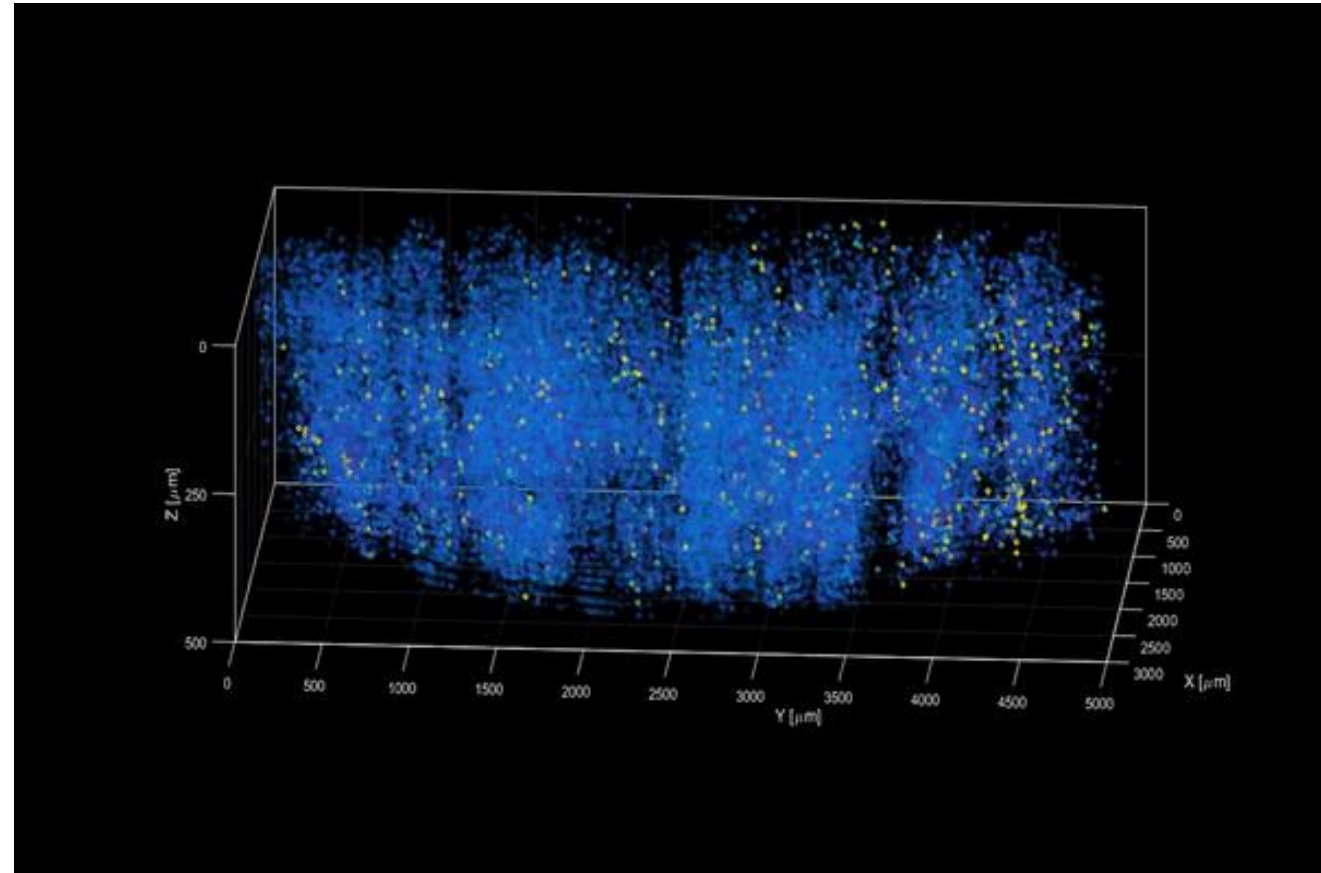
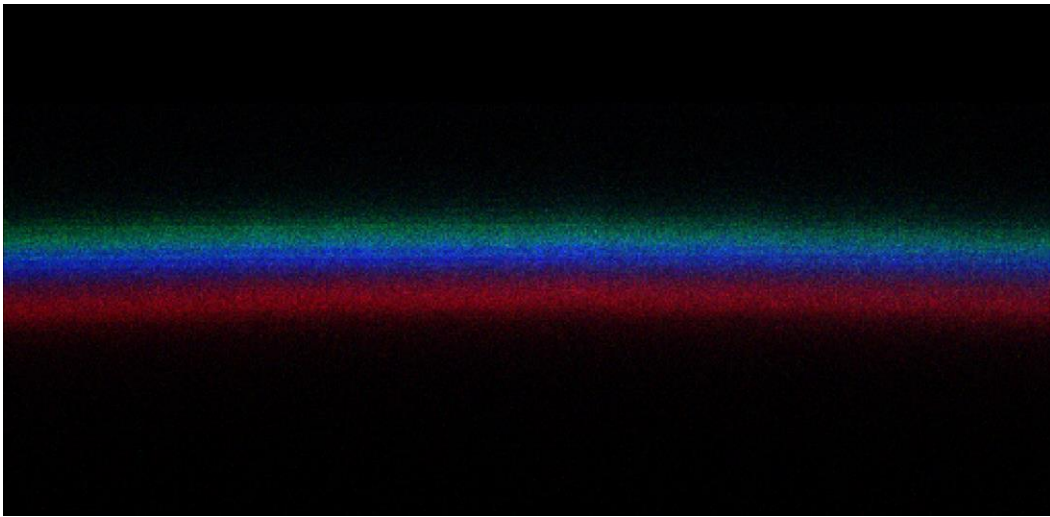
Distances:

- b-g = 3.9 μm
- b-stim = 8 μm
- r-b = 10.3 μm



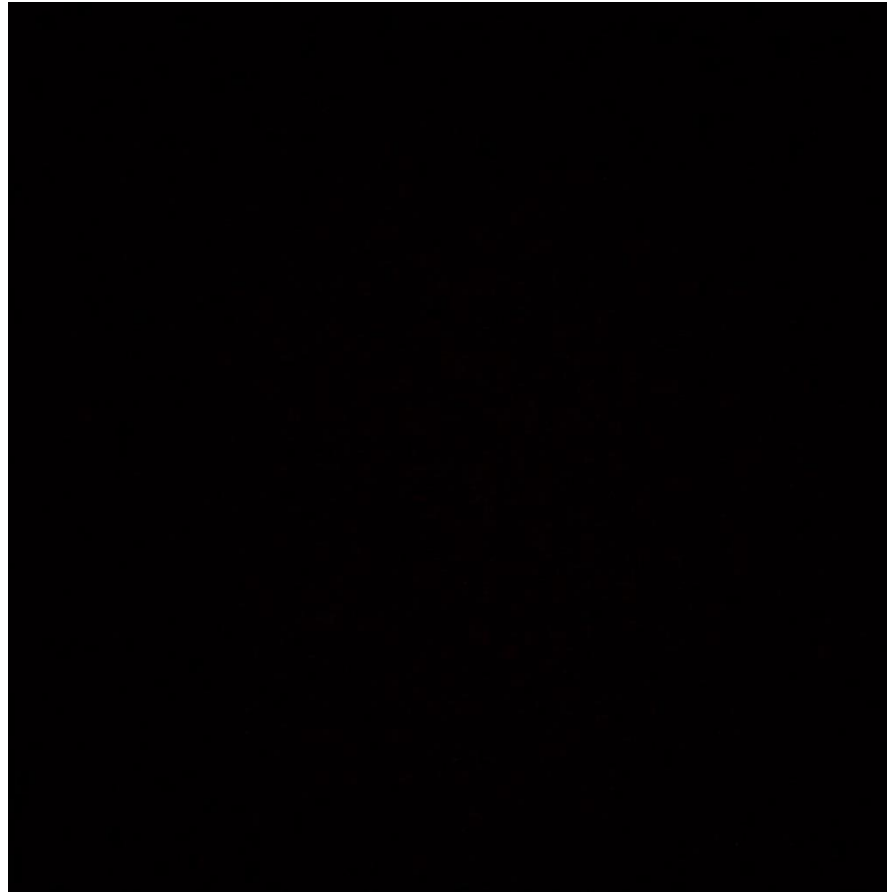
1.1. Microscope: Align and measure PSF in z

Field curvature



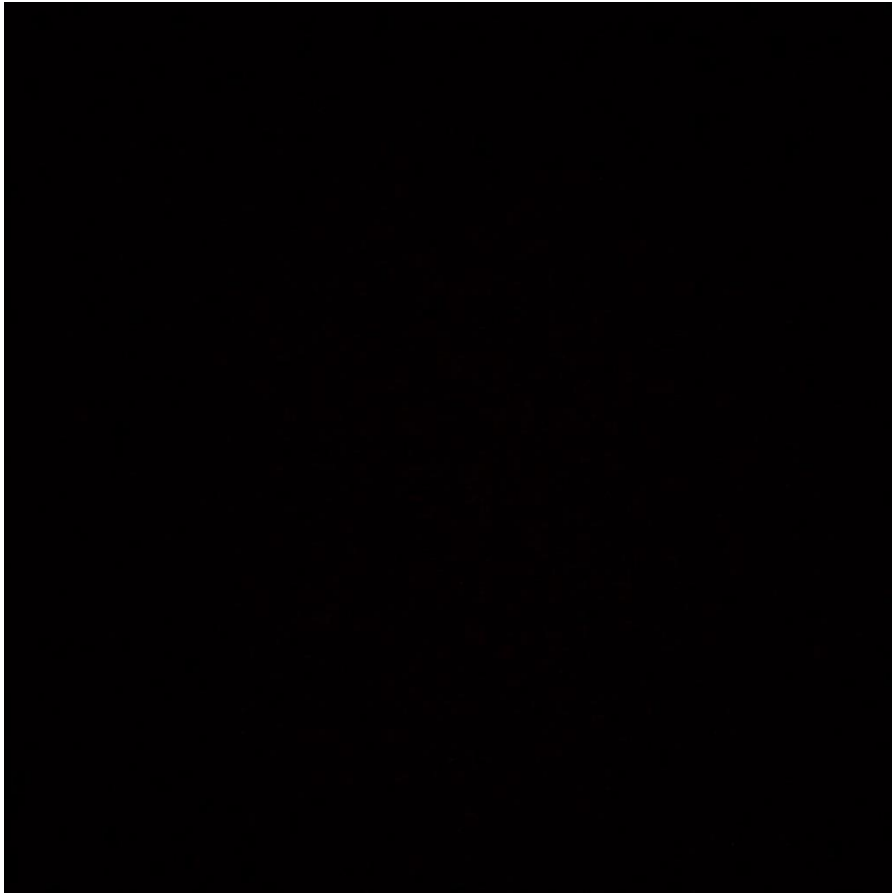
1.1. Microscope: Align and measure PSF in z

Field curvature
(single channel, moving in z)

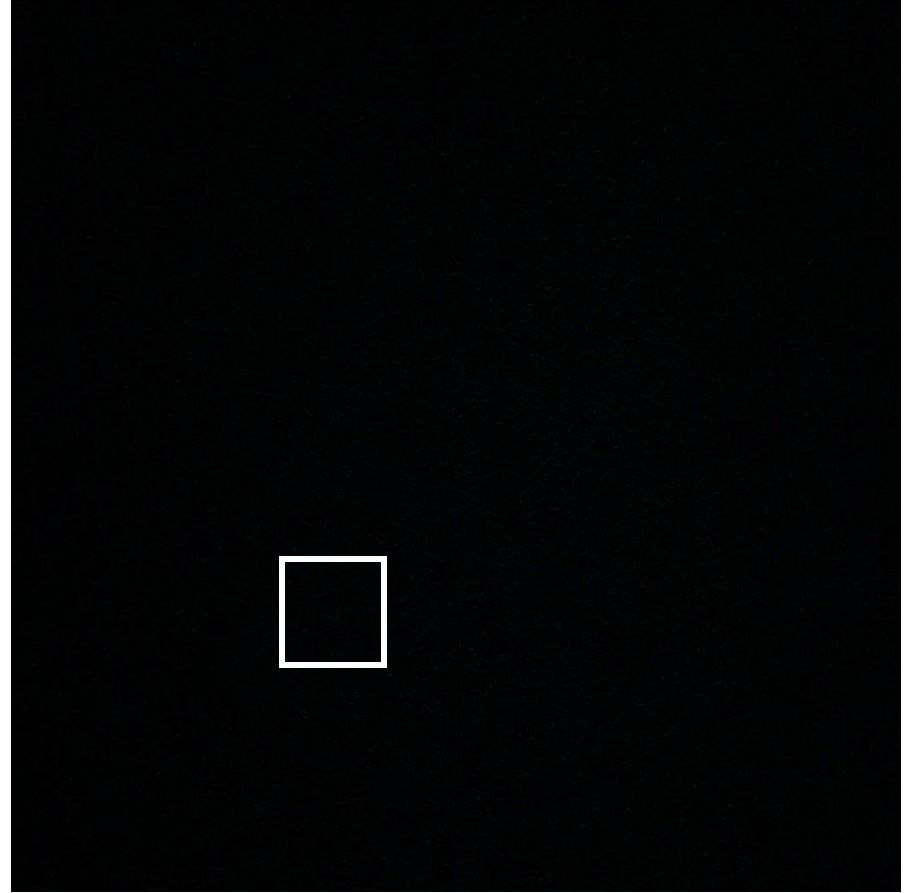


1.1. Microscope: Align and measure PSF in z

Field curvature
(single channel, moving in z)



Seem to be aligned in x-y (see the object
a bit left and down of center)
(multi-channel, moving in z)

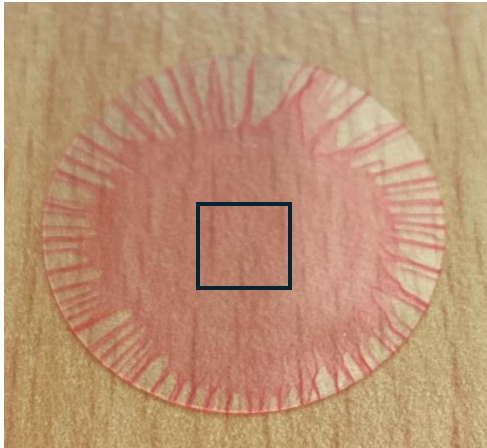


1.1. Microscope: Align and measure PSF in z

‘Optical sheet’

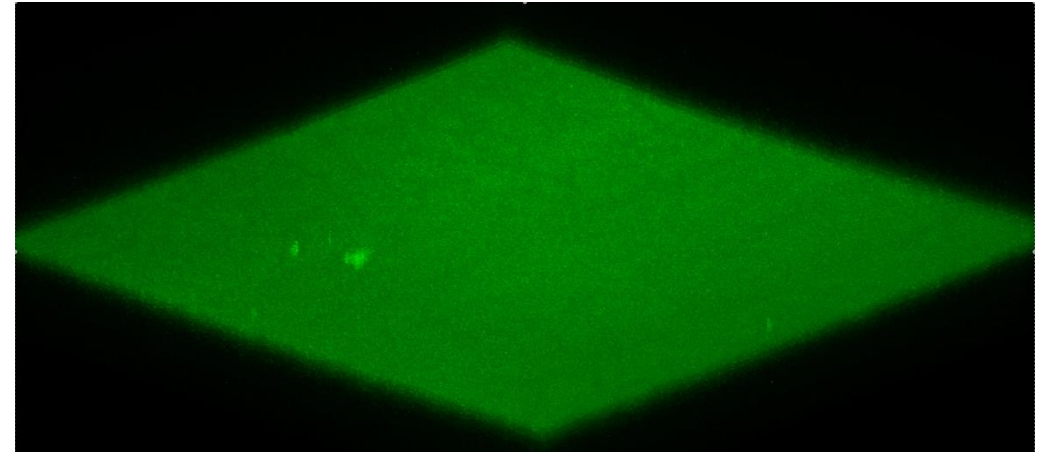
In reality:

- <1 μm thick
- perfectly flat
- fixed z position (obviously)



In the image:

- ~10 μm thick
- curved (concave)
- z position depends on stimulation wavelength



1.1. Microscope: Align and measure PSF in z

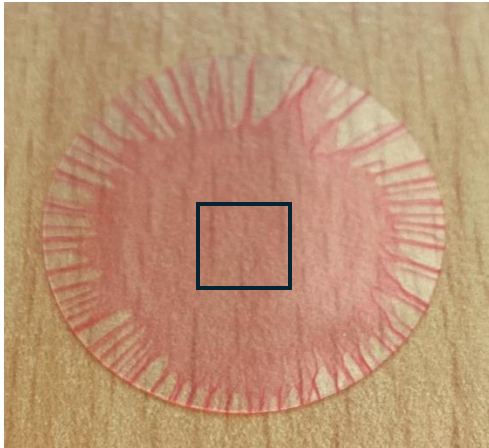
‘Optical sheet’

In reality:

- <1 μm thick
- perfectly flat
- fixed z position (obviously)

Measure z for stimulation laser:

- repeatedly scan a 64 μm spiral throughout a zstack acquisition
- No image though because light isn’t ‘descanned’ correctly
- Less straightforward to measure field curvature (didn’t do it)

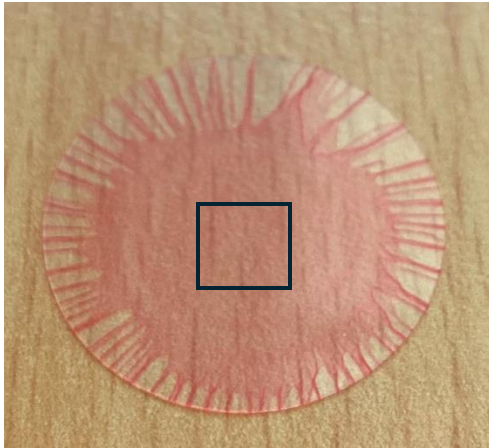


1.1. Microscope: Align and measure PSF in z

‘Optical sheet’

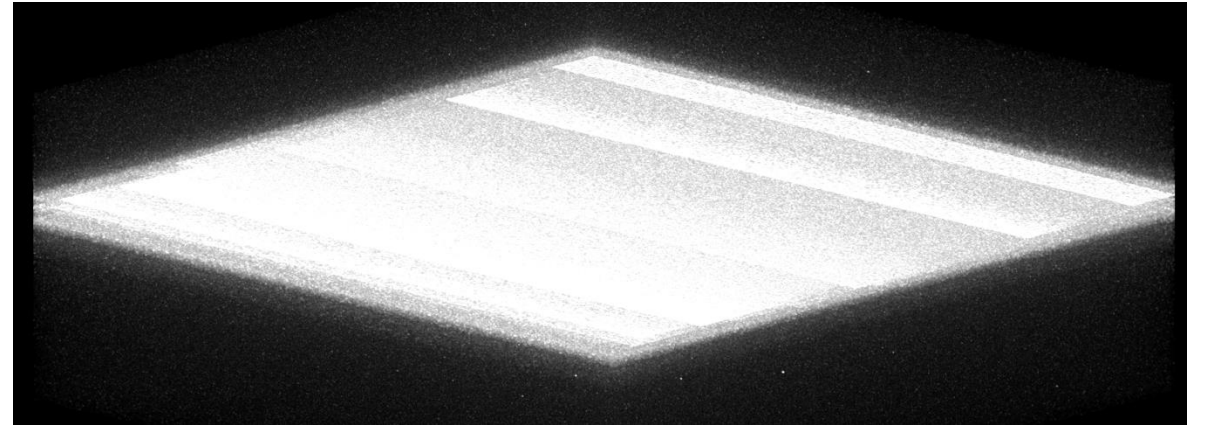
In reality:

- <1 μm thick
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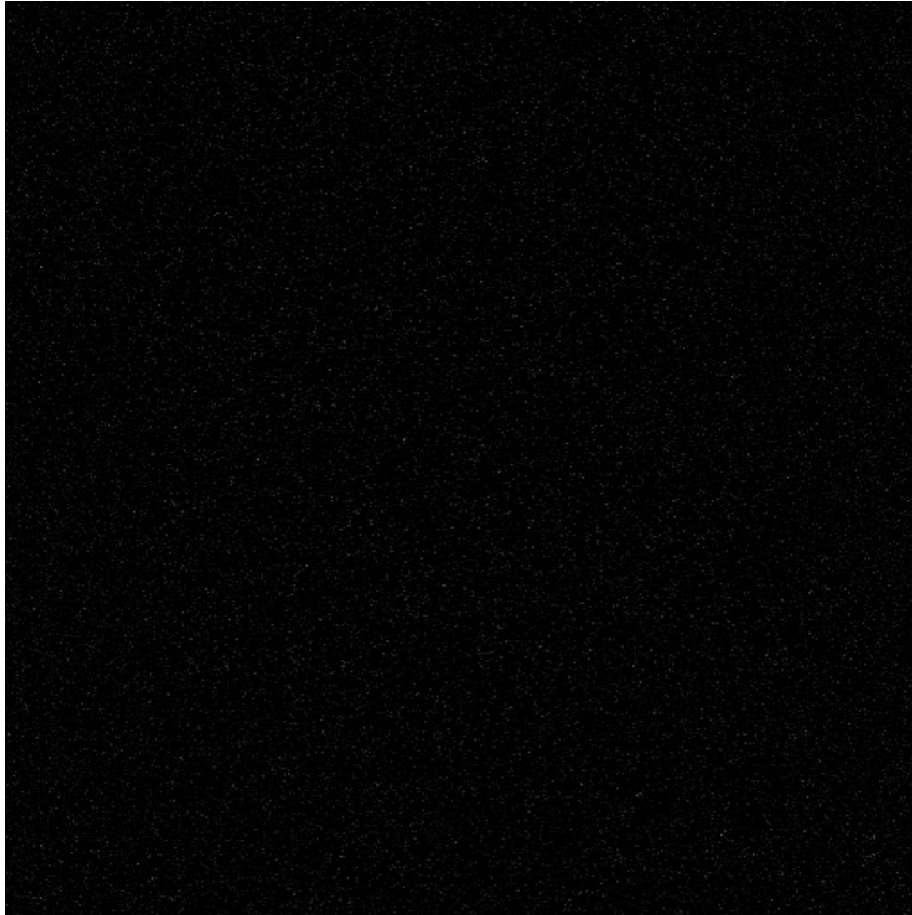
Measure z for stimulation laser:

- repeatedly scan a 64 μm spiral throughout a zstack acquisition
- No image though because light isn't ‘descanned’ correctly
- Less straightforward to measure field curvature (didn't do it)



1.1. Microscope: Align and measure PSF in z

Z stack for laser stim

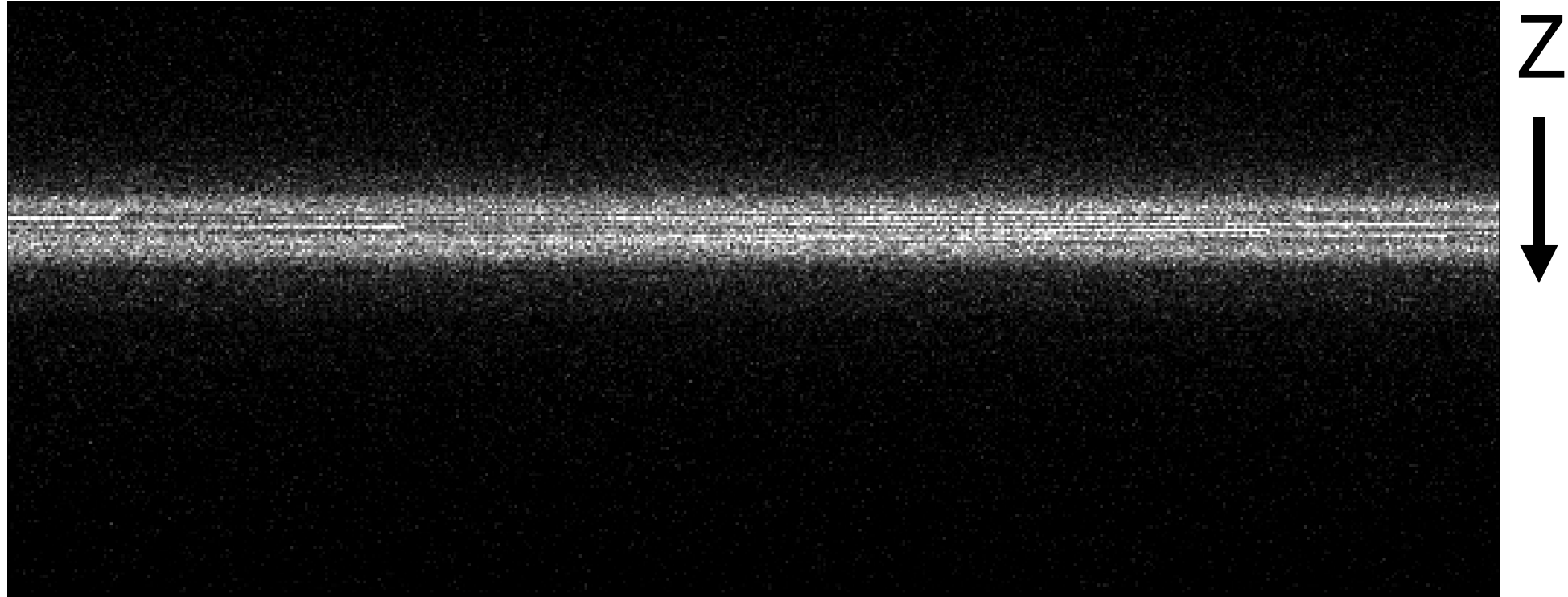


Notes:

- Don't see field curvature
(no 'descanning')
- Artefacts due to spiral scanning
(not 100% why)

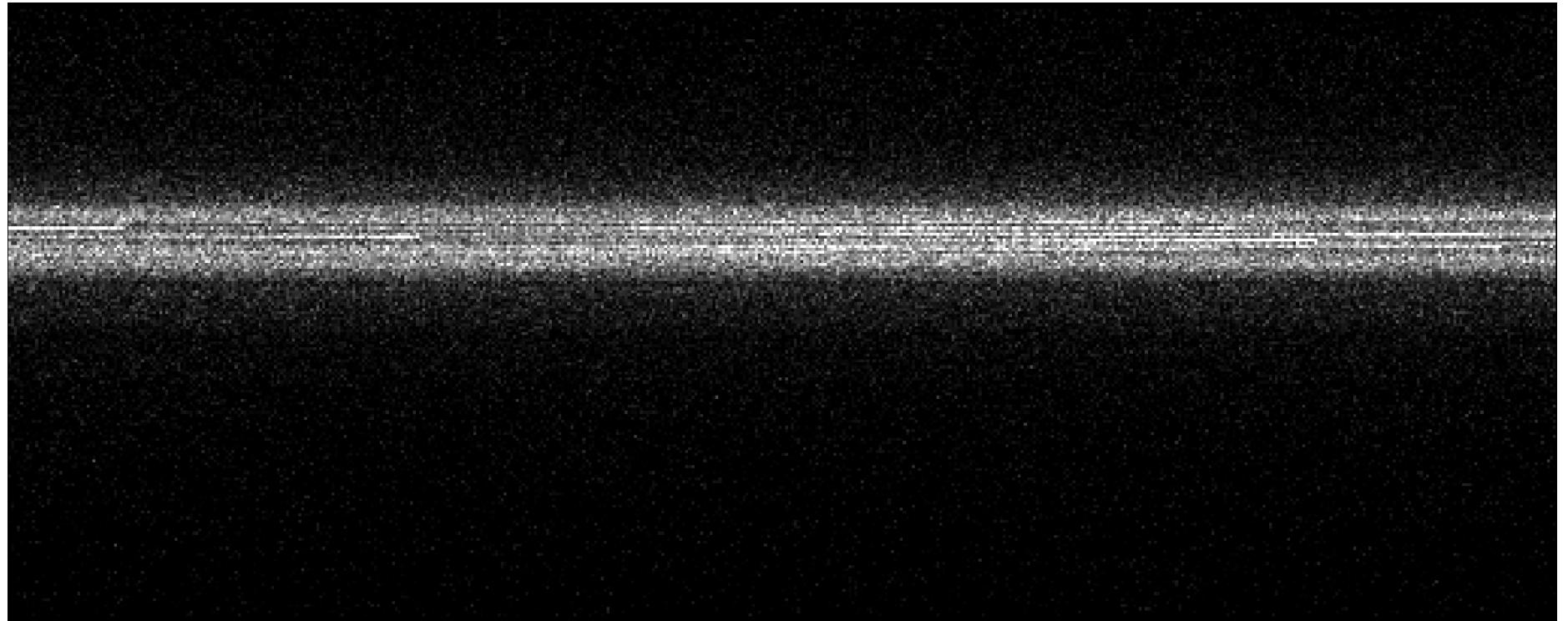
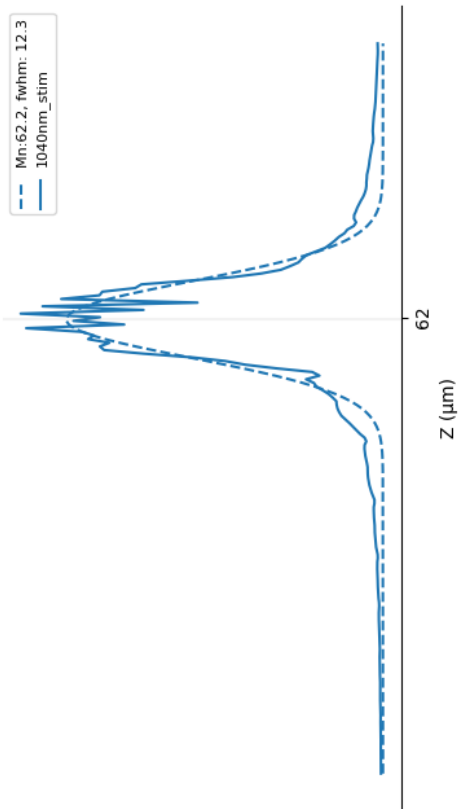
1.1. Microscope: Align and measure PSF in z

Z stim laserstack for



1.1. Microscope: Align and measure PSF in z

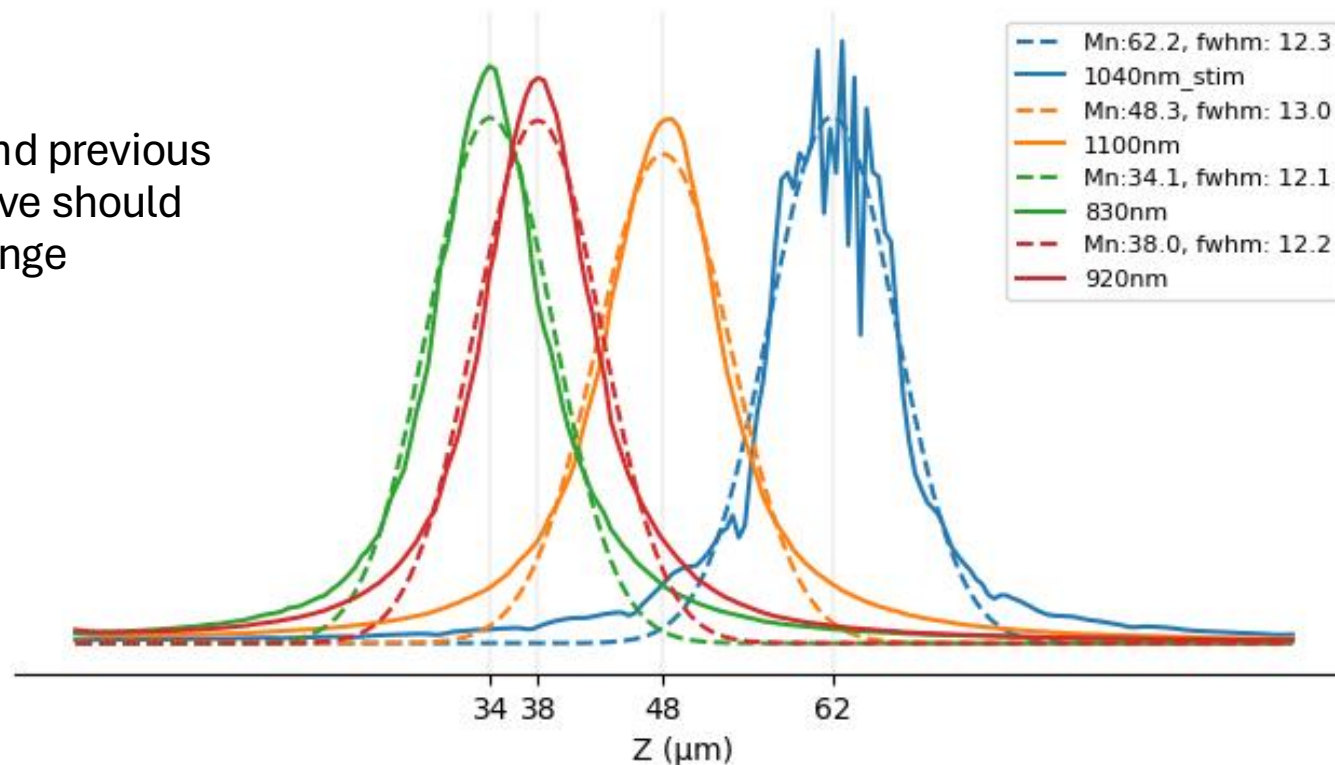
Z stim laserstack for



1.1. Microscope: Align and measure PSF in z

TODO: during recording I didn't correctly align the imaging and stim lasers so need to measure the shift between these still:

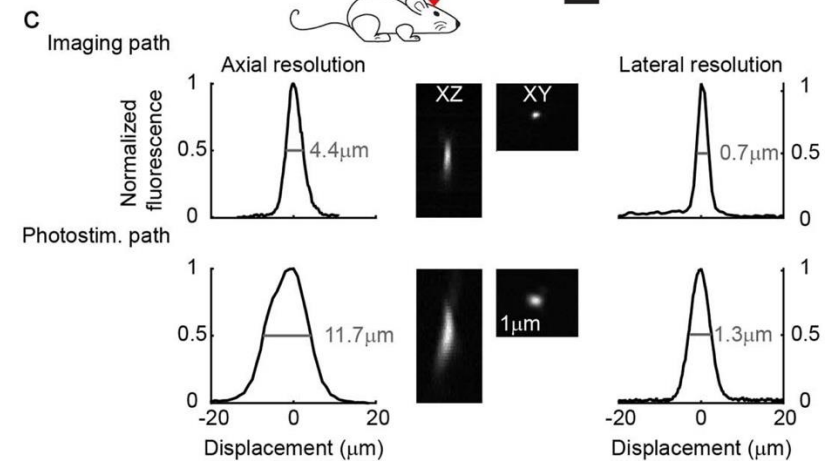
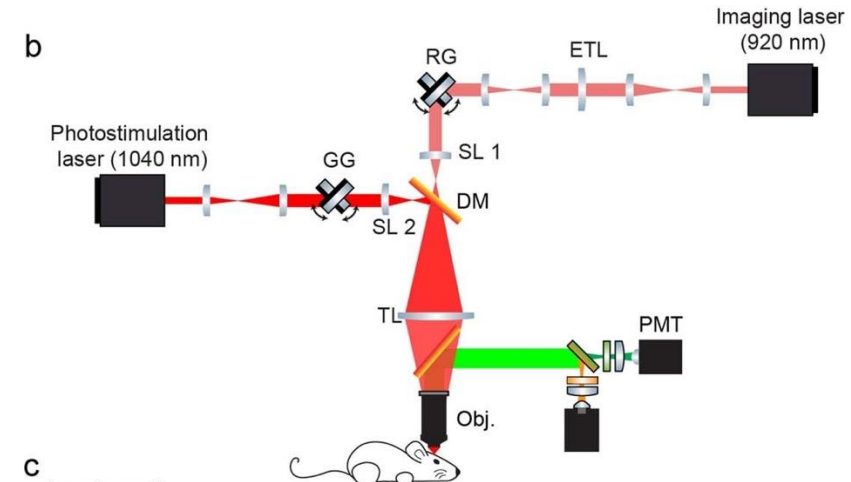
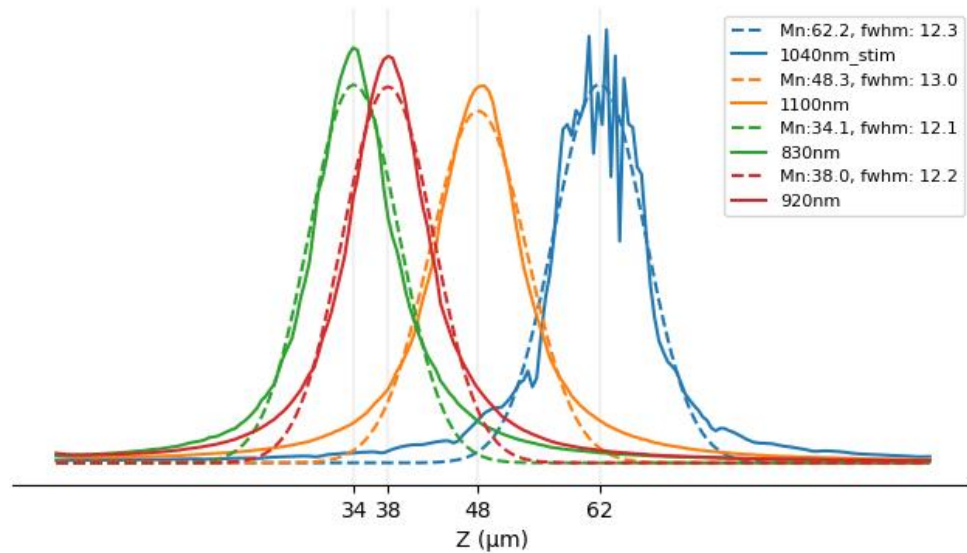
Here based on optics and previous experience the blue curve should be between red and orange



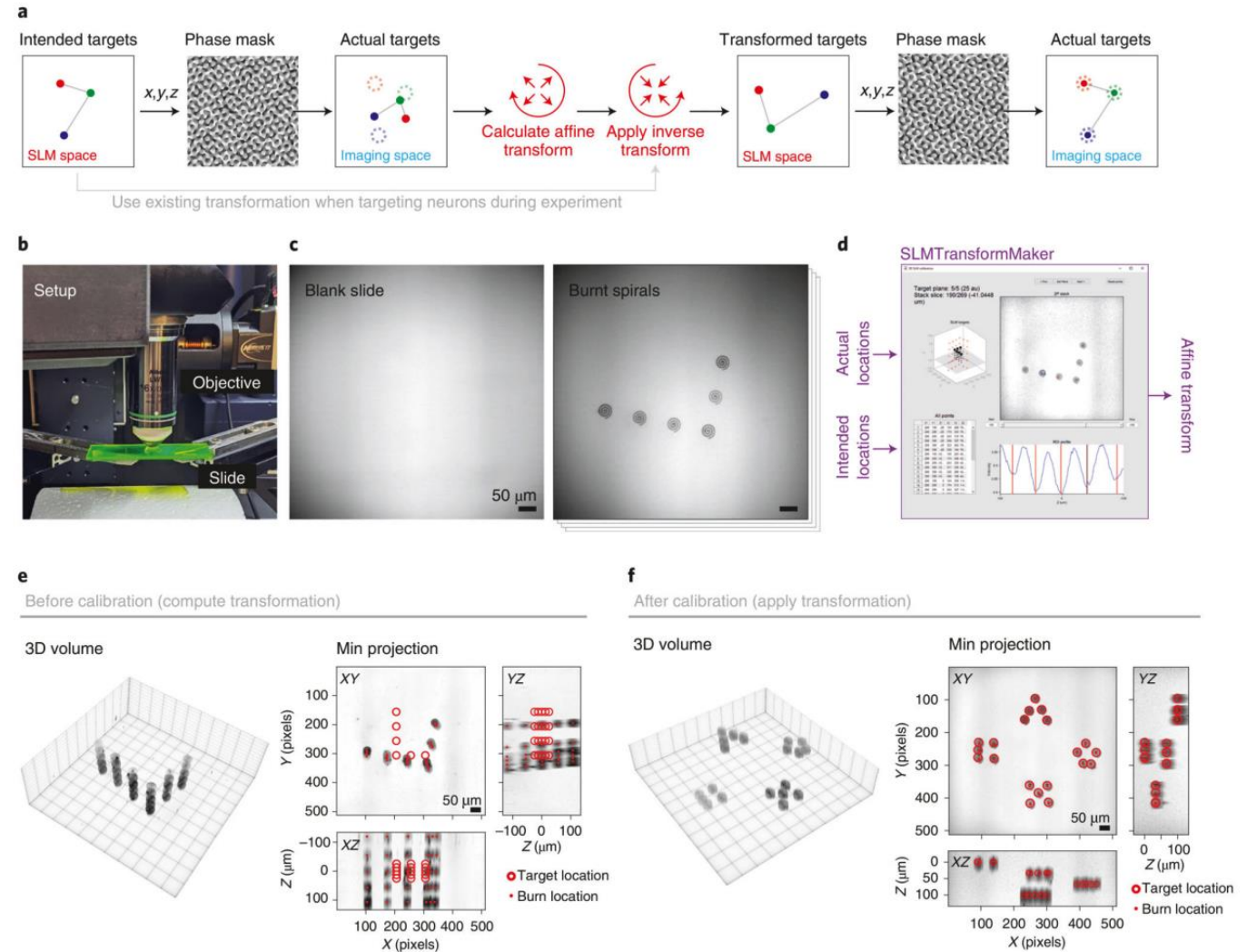
1.1. Microscope: Align and measure PSF in z

Comparison to Daie et al. 2021 for Z PSF:

- Imaging is 3x more extended in Z
- Photostim is approximately the same



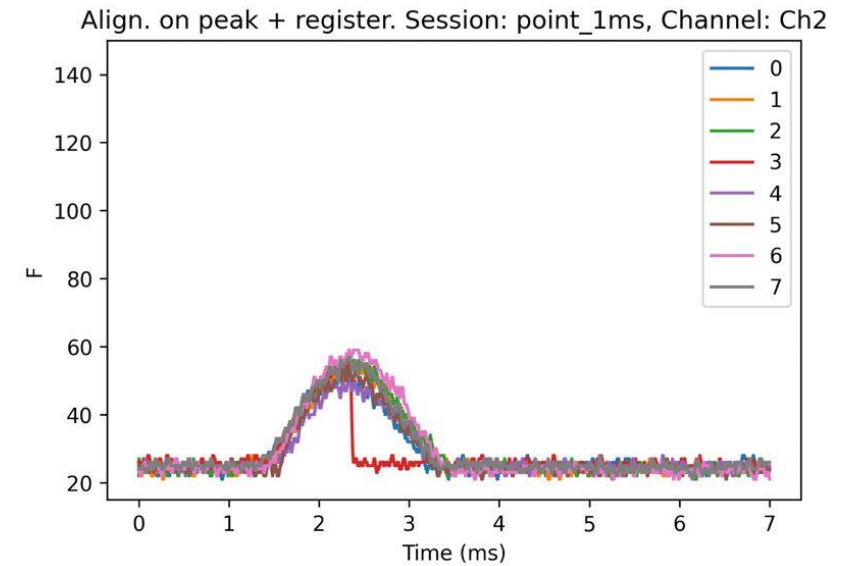
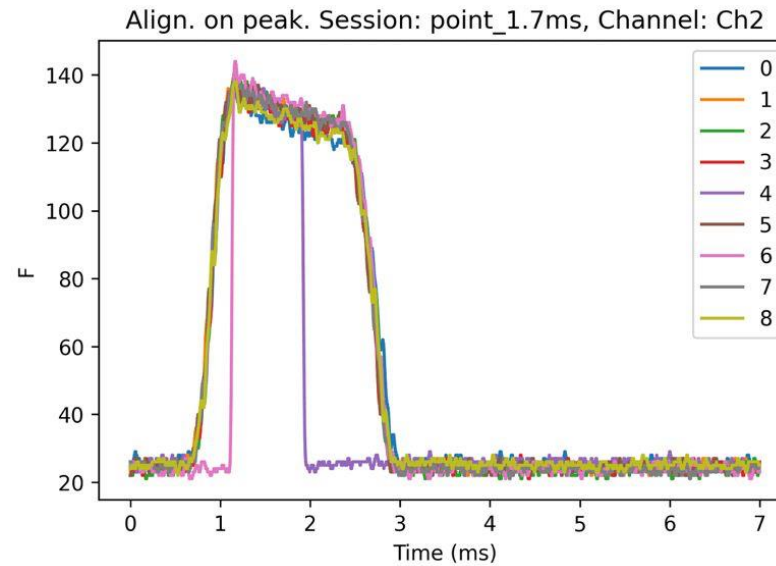
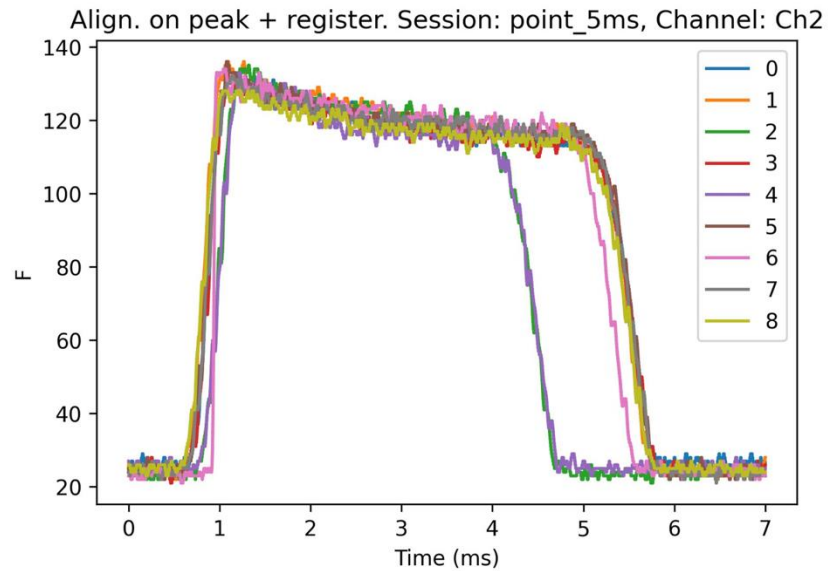
1.2. Microscope: ‘Burn spots’



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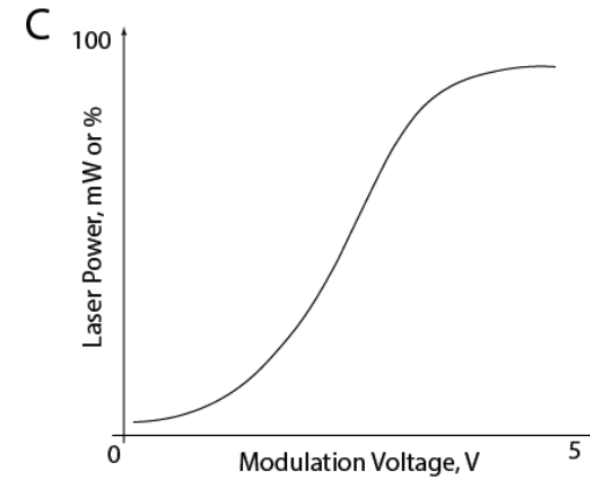
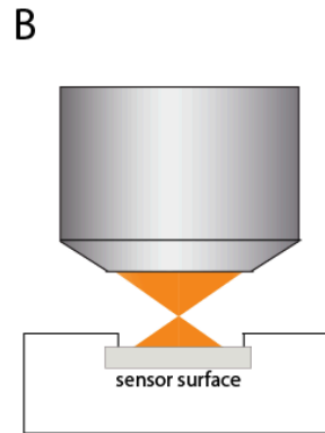
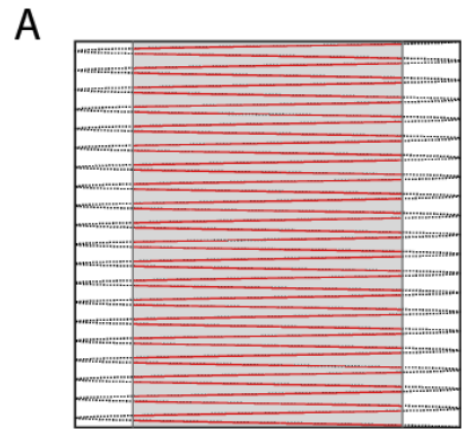


1.3. Microscope: Measure stim timing



1.4. Microscope: Measure power

...

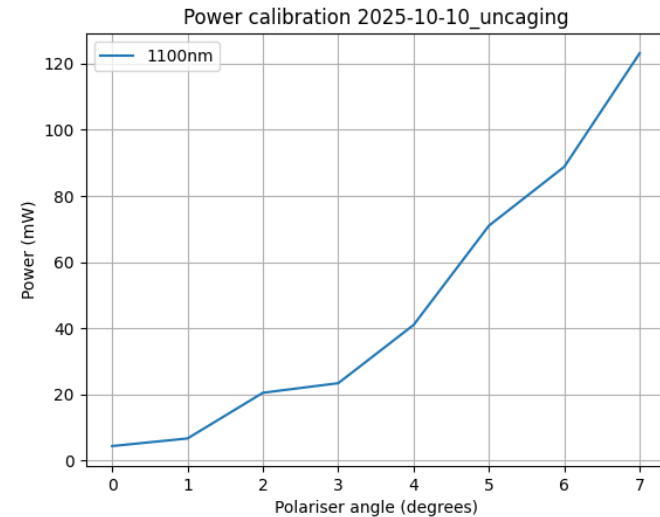
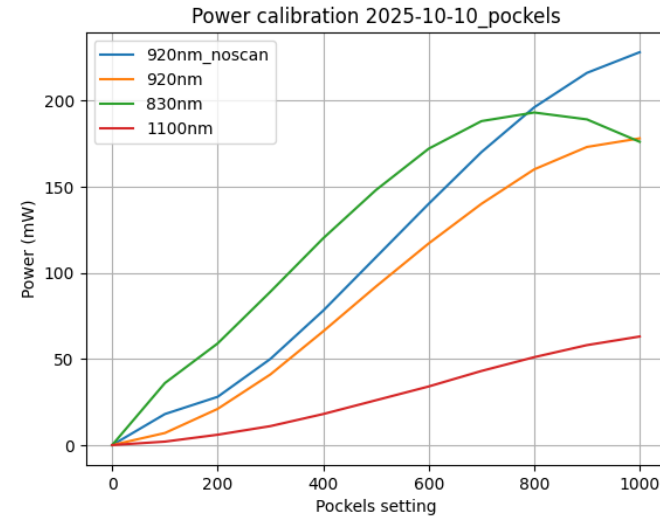


Lees et al. 2025 Nature Protocol

1.4. Microscope: Measure power

pockels	920nm_noscan	920nm	830nm	1100nm
0	0	0	0	0
100	18	7	36	2
200	28	21	59	6
300	50	41	89	11
400	78	66	120	18
500	109	92	148	26
600	140	117	172	34
700	170	140	188	43
800	196	160	193	51
900	216	173	189	58
1000	228	178	176	63

uncaging	1100 nm
0	4.4
1	6.7
2	20.5
3	33.4
4	41.0
5	71.0
6	88.8
7	123.1

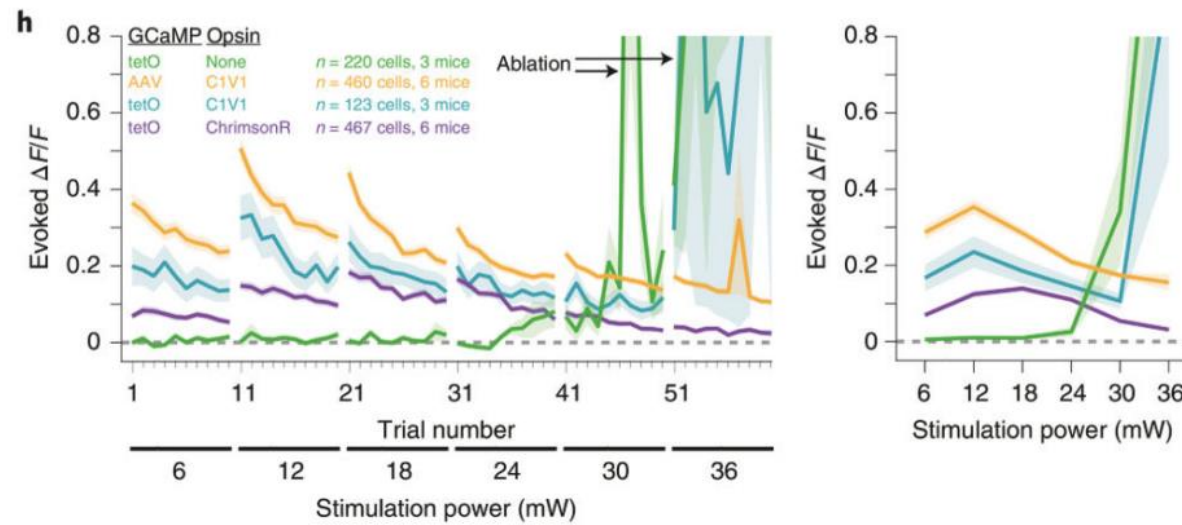


2) Response

(in vivo calibrations)

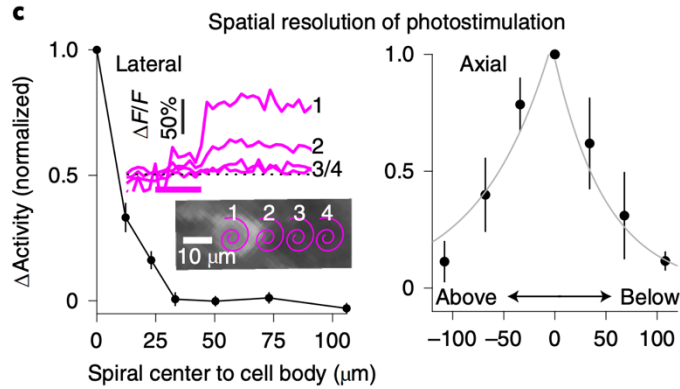
2.1. Response: Optimise parameter

Russell et al. 2022 Nature Protocols

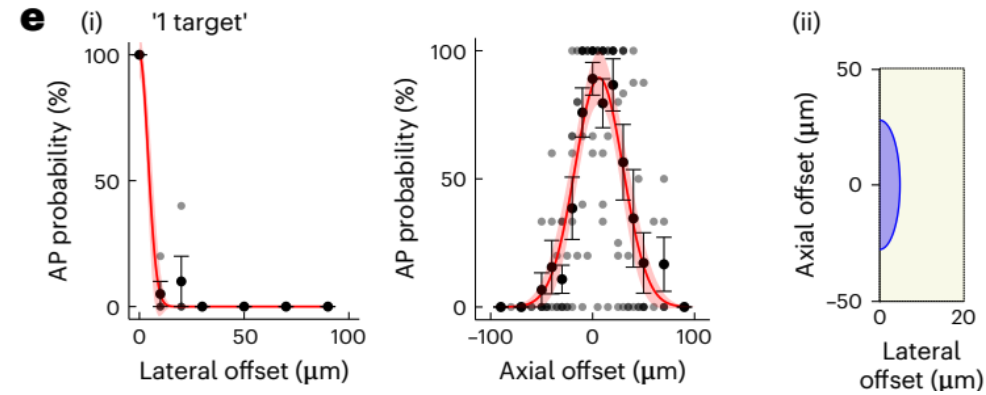


2.2. Response: ‘Physiological PSF’ in x-y

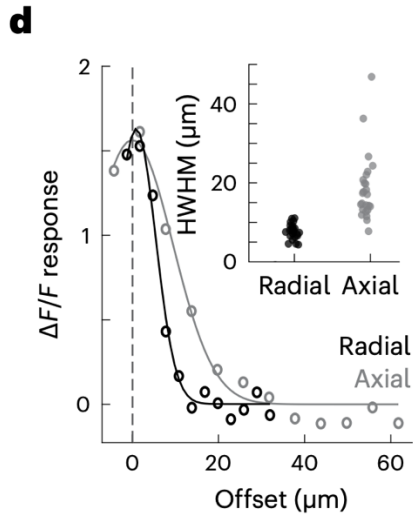
Daie et al. 2021 Nature Neuro



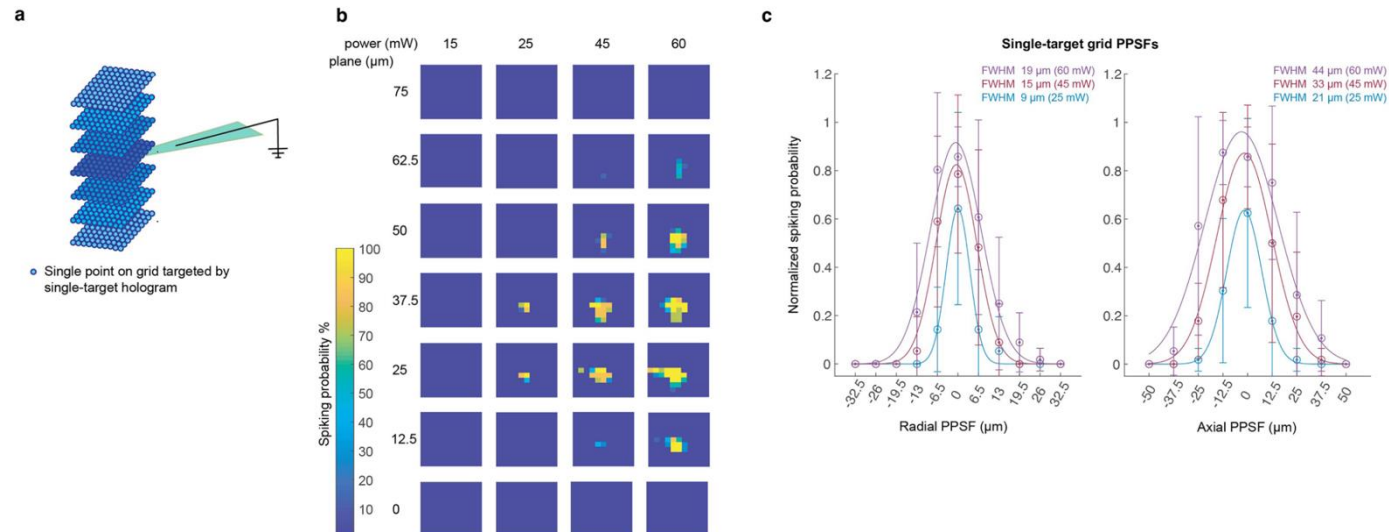
Chen et al. 2025 Nature Neuro



Oldenburg et al. 2024 Nature Neuro



Triplett et al. 2025 Nature Neuro



2.3. Response: 'Physiological PSF' in z

- For now see previous slide

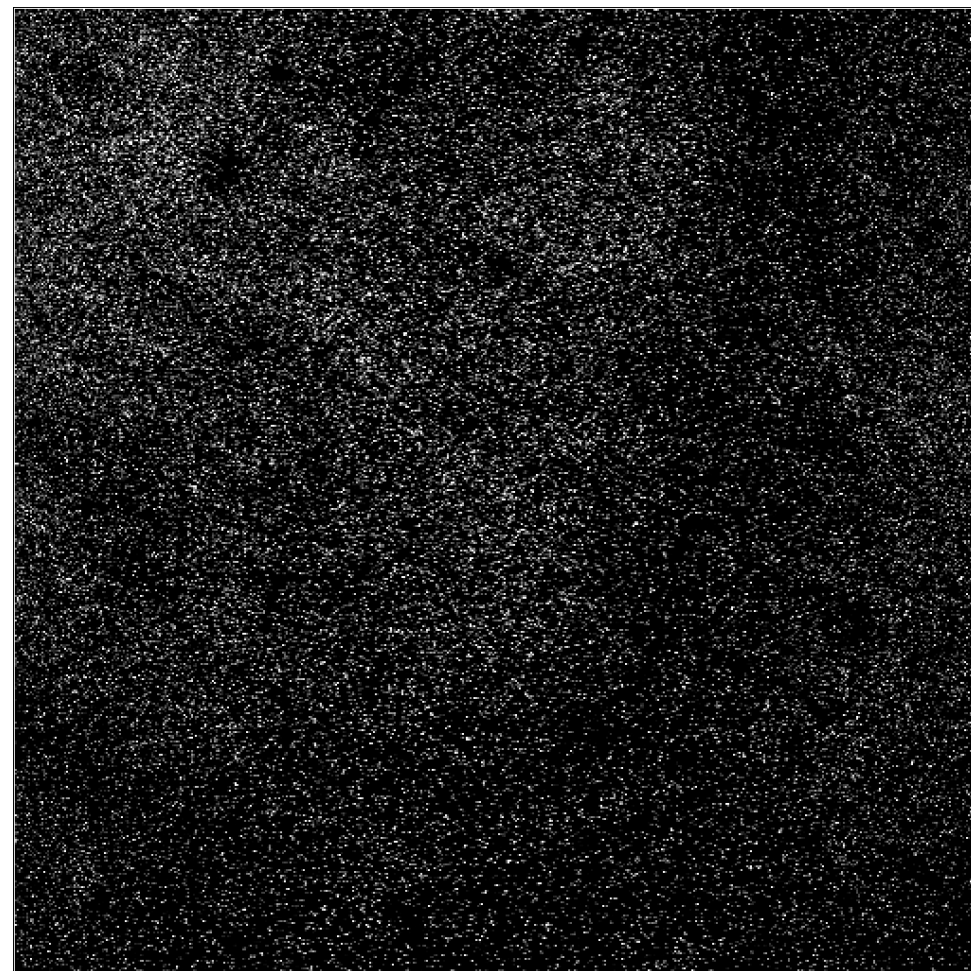
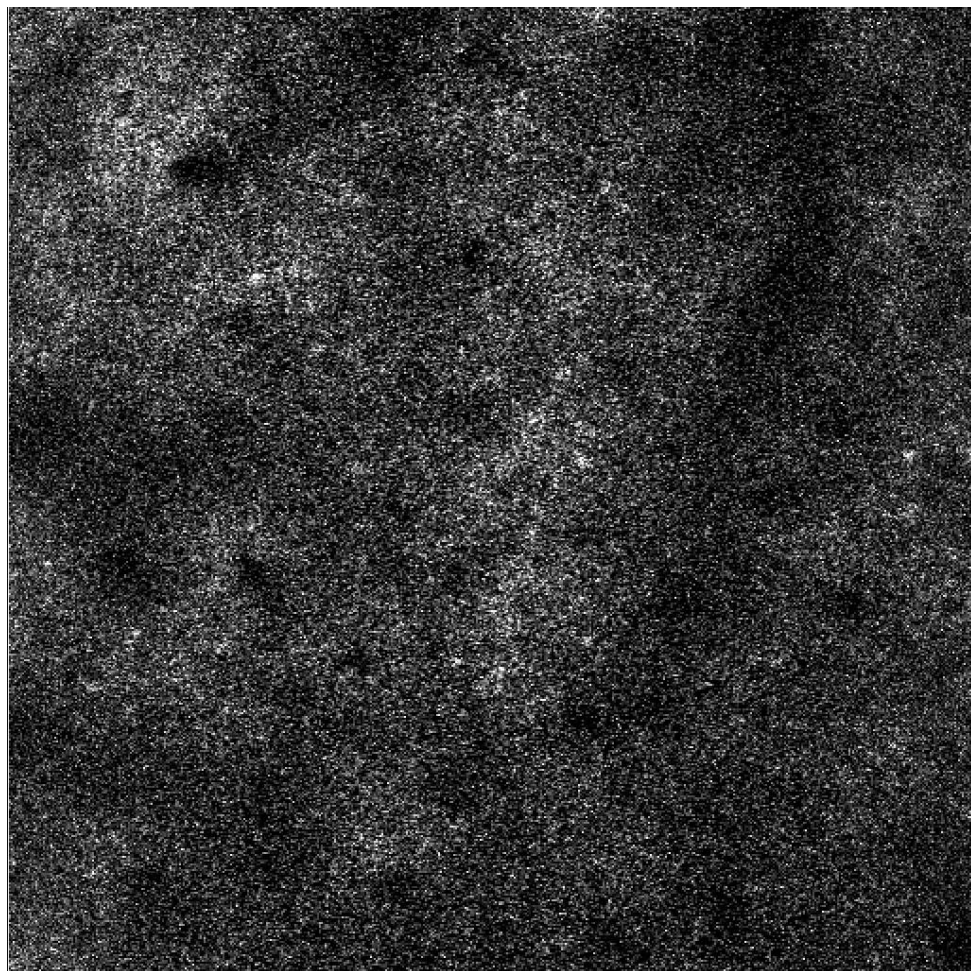
Comparison of imaging upstairs and downstairs

jm055

hSyn-gcamp8m

1:40 diluted cre virus

Cre-dependent Chrmine



Comparison of imaging upstairs and downstairs

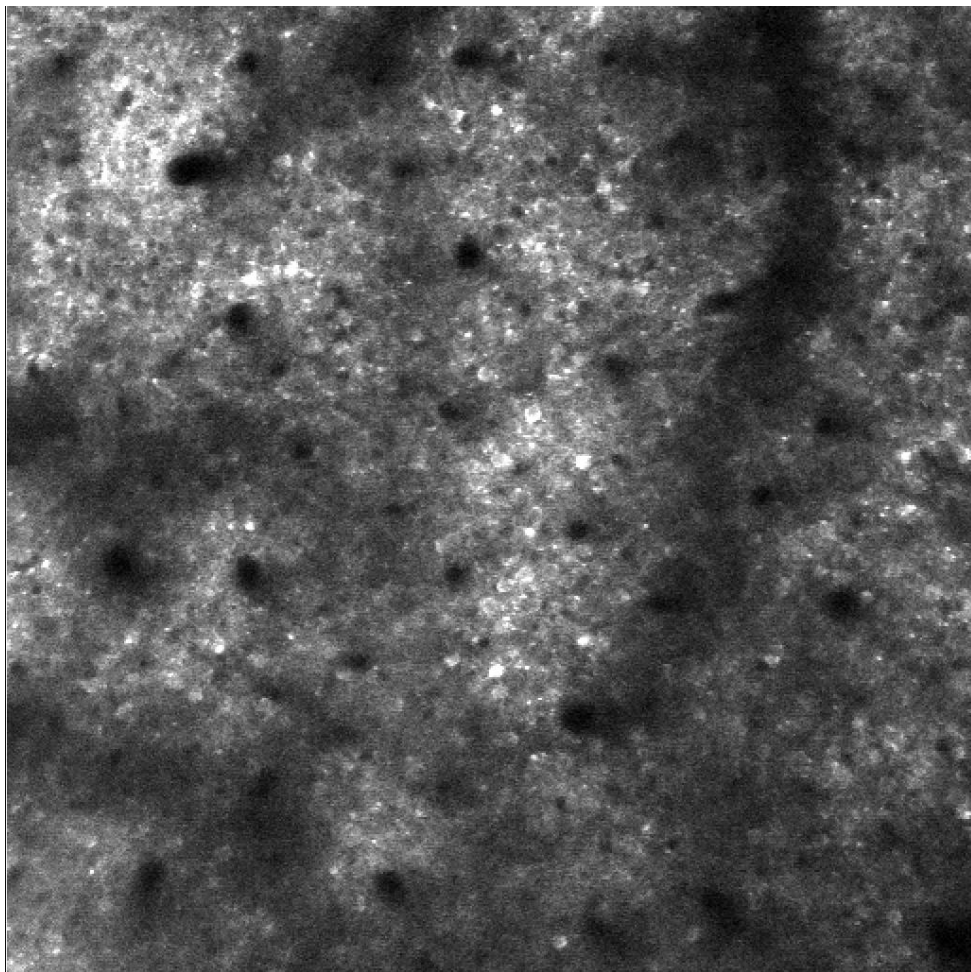
jm055

hSyn-gcamp8m

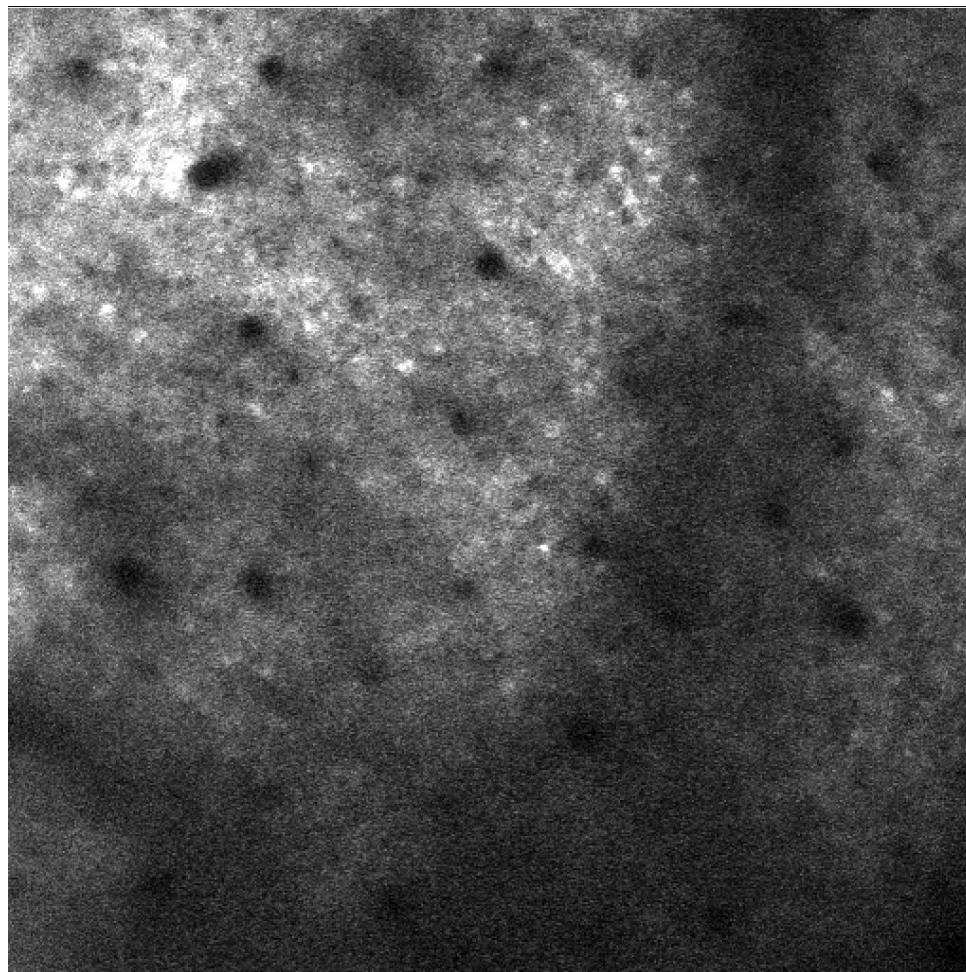
1:40 diluted cre virus

Cre-dependent Chrmine

up



down



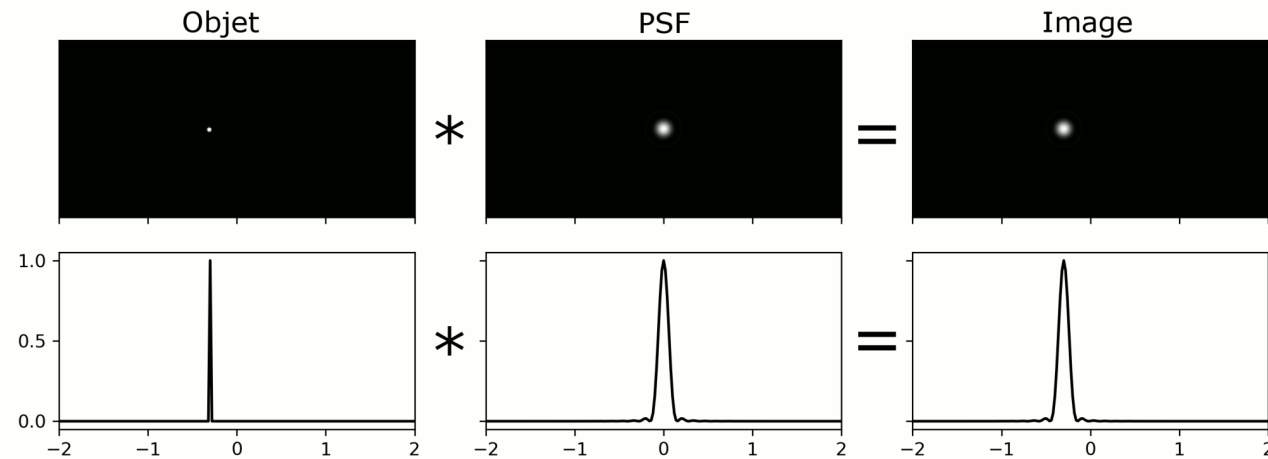
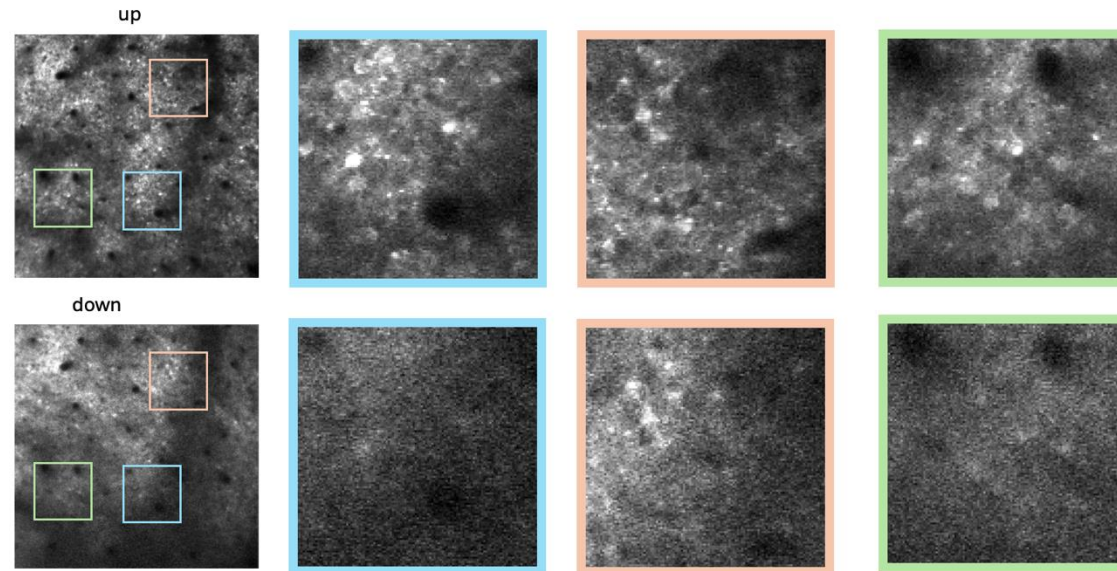
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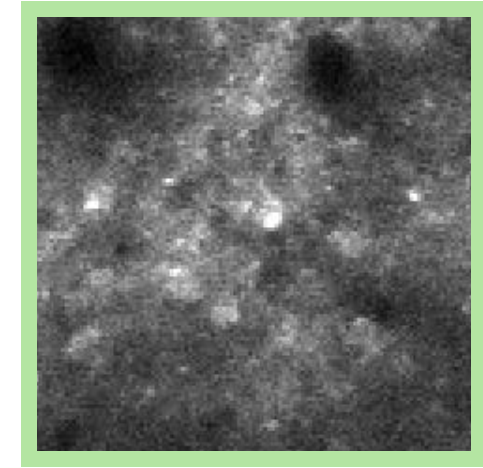
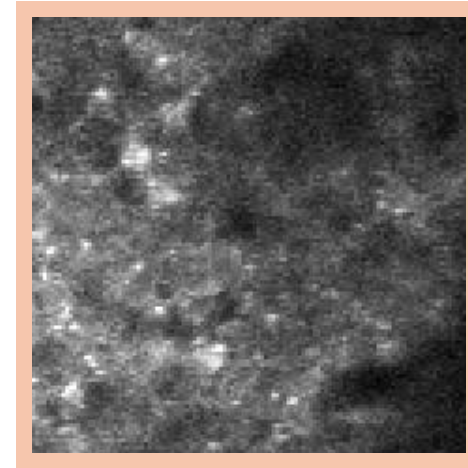
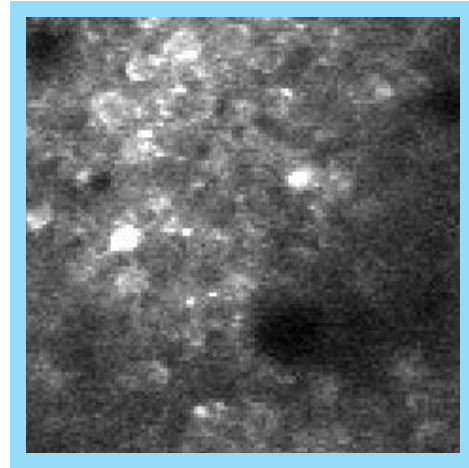
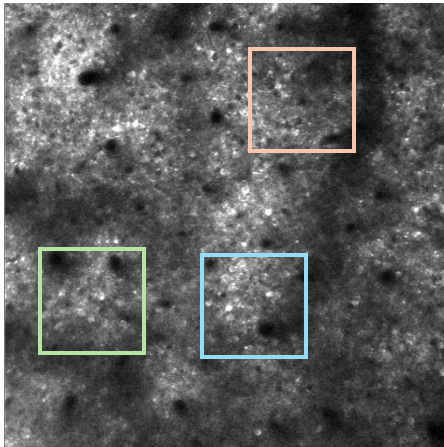
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down

