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Holographic calibration

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We use this protocol and it's working

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Abstract

This is the standard protocol for 2-photon holographic calibration with In vivo Ultima Bruker microscope through GRIN lens used for Rodrigues-Vaz and Athalye et al, 2025.

Materials

- Fluorescent slide
- Distilled water
- 2-photon laser scanning microscope with spatial light modulator module (Ultima In Vivo, Bruker)
- Femtosecond Ti:saphire laser (Chameleon Vision-S, Coherent)
- Low repetition photostimulation laser (Monaco 1035-40, Coherent)

Safety warnings

-Wear appropriate PPE as required by your institution.

Before start

-2-photon lasers (imaging and stimulation) and Pockels cell controller were turned on.

General calibration

1 Confirm imaging laser and photostimulation laser are aligned with target at objective output.

2 Place fluorescent sample under 16X water-immersion Nikon objective.

3 Focus on top of the sample.

4 Follow PrairieView software's calibration routine to ensure alignment between photostimulation and imaging lasers: burn spots

5 Burn single spot and confirm calibration worked. If not, repeat step 4 as many times as necessary.

Power calibration

6 Measure power of stimulation laser at the 16X water-immersion Nikon objective output. This was done at least once at beginning of each cohort.