1P AOSLO Maxwellian View Position Confirmation

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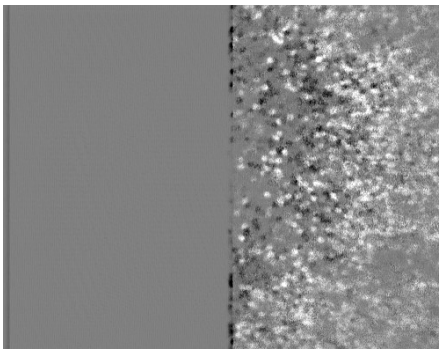
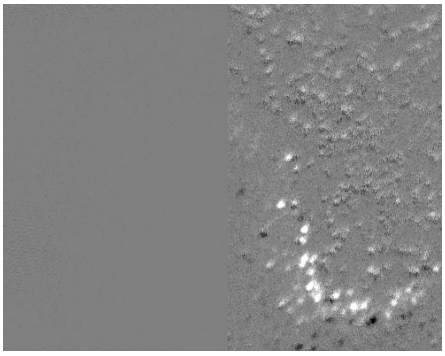
Centering the Maxwellian View on RGC receptive fields: The Maxwellian view spot should be fully covering the receptive fields of the RGCs being imaged. After alignments or in new imaging areas this sometimes doesn't happen and requires some extra work to get right. The problem with having it off-center isn't just that some ROIs don't get the stimulus – it's that the edge of the Maxwellian view spot moves across the retina with the residual eye movements and that's a good stimulus for RGCs – you will need to omit all ROIs anywhere near the edge so mitigating this is good to get the most data.

1. **Coarse alignment and stimulus validation.** Run the intensity increment. You should see a huge increase in fluorescence after frame 500. Register the fluorescence video and drag it into ImageJ. Open up "*Plugins>New>Macro*", then go to "*File>Open Recent*" and click on the most recent one (named something like stim response map). Click "*Run*" on the macro window (see ImageJ step above), set stim frame window to 500-750 (might be default, can't remember) to make a change in fluorescence (dF) map. You should clearly see the responsive cells (almost all respond to intensity increment). You're looking for regions where you know there are cells from the fluorescence images, but you're not seeing them in the dF map; this could mean the Maxwellian View isn't hitting their receptive fields. This will give you an idea of coarse alignment.



2. **Fine alignment to fully center stimulus and remove edges.** Run LightsOn and then Baseline. LightsOn contains 80 seconds of adaptation time to the new light level and is important so that the response due to a change in mean light level doesn't confound your centering measurements. Drag the unregistered Baseline video into ImageJ and press "*Run*" on the macro again. Set stimulus frames to something like 500 to 1500. In the baseline trial, the only stimulus possible would be the edge of the Maxwellian view spot as it moves with the animal's respiration – if you aren't centered, it will make an edge as in the image below. This one left ~half the ROIs unusable. Once the cells are adapted to the background LED light, you should see the edge in the brightness of the GCaMP6 fluorescence too, but it's more subtle. Use this for fine-tuning the rotation/pitch to get the RFs better aligned with the Maxwellian view.



If you do move around to better center the stimulus, make sure to take another baseline trial afterwards so you can confirm it's centered (or at least know where the edge is so you can omit those ROIs when analyzing offline). The extra effort will be worth it because, as long as the alignment/pellicle don't change between experiments, you can be confident in this location next time. I still always check with the steps above at the beginning of an experiment.