2p calcium imaging protocol

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Protocol to image hippocampal neural activity during behavior (virtual reality, etc.) on the Han lab behavior rig. We will be mostly imaging pyramidal cells? in CA1 of the hippocampus with GCamp8f. This protocol may have added instructions for SST-cre optogenetics experiments.

1. Get mice to image from satellite room and bring them across the hall from the main lab area to the imaging room
   1. Put cages on the cart in the back to the right side of the behavior rig
   2. Make sure the cart is not against the wall (mice may feel reverberations), and order from first to last mouse to be imaged, left to right
2. Switch on pockel cell (box above the computer to the right of the behavior rig)
   1. The pockel cell modulates passing of polarized light
      1. It works to modulate the resonant galvo
   2. Make sure it is at –114
   3. May change over time so just readjust if necessary
3. Switch on all 4 CPUs
   1. Check to make sure HD has space
4. Switch on IR lights
   1. Power strip behind the behavior rig on the left, underneath the screen switch
5. Switch on air
   1. Blue knob below the water tower on the right of the rig near the mouse cart
   2. Only switch on halfway
6. Check water levels to the right of the rig
7. On the computer in the middle of the computer stack to the left of the behavior rig, turn on ‘Clampex’
   1. In File > Set datafile…, make a new folder based on date/animal number and save the binary file
   2. The naming starts with 000 for the day
8. On the same computer, turn on MaiTai
   1. Press and hold the ‘On’ button until the EMISSION text pops up
   2. Press and hold until ‘Shutter’ is on
9. Log in to imaging computer (to the right of the behavior rig); password = han\_lab
   1. Start MATLAB
   2. Run ‘scanbox’ command
10. Turn on projections using remote
    1. Projectors are on the back to the left side of the behavior rig
    2. To turn off, press twice
11. Run ‘virmen’ command after opening MATLAB on the VR computer (top computer to the left side of rig)
12. Start camera software (Spinview’) in imaging computer and eye computer (bottom computer to the left side of rig)
    1. Pull the window towards the right side
    2. Click play
    3. Start Bonsai
       1. Select ‘chunkdata’ program
       2. Select string
       3. Enter date and name of mouse
    4. Trigger mode (in settings in Flip) to off if not acquiring images / training, on if otherwise.
    5. Note: the exposure may need to be adjusted based on your laser settings right before the recording
13. Select experiment for virmen (open file…)
14. On the imaging computer, set scanbox settings
    1. Start with ~10% laser power
    2. SST experiment: 40,000 frames
    3. Lines/frames: 512 (do not need to change)
    4. Magnification: start with 3.4/2.8 depending on mouse
    5. Unilateral for finding planes
       1. Bidirectional: need to realign again in suite2p
    6. Create folder structure
       1. Left bottom panel
    7. PMT  A for GCamp
    8. Select camera path
15. Get DI water in transfer pipette from lab
    1. Fill out calibrated pipette (tips in imaging room, bevel should point downward towards mouse) with DI water
16. Flip up (regular water delivery) switch of solenoid (box above pockel cell) to get water out of lick port for animal
17. To turn on front camera (looking into the animal’s head), switch on camera, take picture, and click mini camera button
    1. Turn off after setup, before putting the screen back up
18. Bring mouse from the back of the room and mount headplate
    1. Hold the mouse gently with the headplate
    2. Loosely screw in arm on the further side
    3. Adjust arm towards you to and screw it on
    4. Tight small screws and big screws on lever
19. Put DI from pipette to the side of cannula at an angle
    1. Make sure to put on the side of the cannula
20. Put 2 more drops of DI water from transfer pipette
    1. Water at this point should have some surface tension
21. Bring objective down with Z motor until you touch the water, then pull back up to see the edge of the cannula
    1. Use flashlight to see
    2. Scroll down to see striations/surface of the brain
    3. Zero XYZ on objective controller at this point
22. Bring objective back up to 20,000 um, put in light shield (making sure the pointy end is facing towards the animal’s head), turn front camera off, and bring objective back down to 0
23. Adjust VR screens if needed
    1. Align screen to the further side to the tape
    2. On the side towards you, align so that the projector window meets in the middle (remember that you can put the MATLAB window in the VR computer slightly off screen to do this)
24. Put front cover/screen back on to cover the behavior rig
25. In the imaging computer/scanbox software
    1. Turn off camera path
    2. Switch on the PMT to 0.4-0.6
    3. Make sure laser power is 10-40%
    4. Use ‘focus’ to view image
    5. Find surface of the brain
    6. Scroll down to find moderately bright cells
    7. Make multiple reference planes
       1. Switch to ‘accumulate’ in PMT
       2. Acquire 100 frames after hitting focus
          1. Can use this to look at an FOV better too
       3. Then click ‘reference’ button
       4. Drag the ROIs from the window around your cells
       5. Deselect accumulate  none
       6. Hit stabilize
       7. Go back to accumulate
       8. Get a snapshot of the frame
          1. Can save in xx0 or reference\_frames folder
       9. Move the FOV slightly, and check plugin and focus to algin the FOV automatically
    8. Align to an already made reference frame
       1. Scroll to an area that is approximately your reference frame
       2. Shut off focus, and select ‘Plugin’ and ‘Focus’ on the right of scanbox
       3. Select ‘searchref’ from the dropdown menu below
       4. Switch on focus, and wait for new window to pop up
          1. Select reference frame from data folder
          2. Wait for it to align to your FOV
          3. Adjust and X Y if needed after alignment
       5. If needed, align to extra reference frames near the surface of the brain
       6. IN NEW MICROSCOPE SOFTWARE
          1. When initially aligning to a reference plane, instead of ‘searchref’ in the dropdown menu, first select ‘rolling’
          2. Wait for software to collect the image (????)
          3. After that, you can primarily use ‘searchref’ for reference plane alignment
26. Switch on Clampex, all cameras, VR, and imaging (‘grab’) in that order
    1. 20230227 – check to see in both eye and tail camera (Spinview) that trigger mode is on before pressing start
       1. If bonsai / Spinview crashes (image of mouse does not show up when you press start and are acquiring images), ESPECIALLY on the eye camera computer, restart computer
27. Shut down rig if not in use for the rest of the day
    1. Enable camera path in scanbox & shut down scanbox window and camera windows
    2. Shut down imaging computer
    3. Turn off laser and shutter in MaiTai and close MaiTai and Clampex; shut off Clampex computer
    4. Turn off pockel cell box
       1. IMPORTANT because it has a shelf life
    5. Close MATLAB and shut off VR computer
    6. Close camera windows and shut off eye camera computer
       1. If stuck on shutting down screen for > 2-3 minutes, do a hard shut down
    7. Close air valve and IR lights
    8. Close projections by double clicking remote
    9. Discard of pipettes and tips and cover up rig
    10. (If needed) vacuum around rig and clean with ethanol?
    11. Shut off all lights

### General notes:

* The flashlight is typically near the imaging computer
* Bidirectional scanning with scanbox is 2x faster
* When we select ‘Camera path’ in scanbox, a mirror is inserted in the camera path
* For saving data, **copy** to external hard drive, then **copy** to workstation, process data, **then** delete off the imaging/camera computers
* Make sure bidirectional setting is on before recording
* For camera software, make sure you click ‘start recording’ and then press ‘play’ right before you start imaging
  + When closing, stop recording with red button and in recording window
* Turn off the shutter if not imaging right after
* Note the z-depth you are imaging at from the surface of the brain (can also find out from z-stack)

To image/view in the red channel

1. Switch MaiTai to 1040nm and allow power to drop to ~0.65
2. Switch on PMT B and turn up the red channel
3. Note that red lasers typically need higher power, so when switching between 1040 to 920, you must lower the power.

### To take multiple imaging planes

1. In the ‘Optotune and Volumetric Imaging’ box, set power and depth of first plane (deepest plane) and click ‘Add’
2. Use the slider to navigate to the next (more superficial) plane, adjust power again, and click ‘Add’
3. Keep going until all planes are added
4. Click ‘Enable’ and ‘Slice view’ to look at all 3 planes at once in Scanbox
5. NOTE: click ‘Focus’ after setting planes to view planes at once and check image quality, then hit ‘Bidirectional’, then ‘Focus’ again to warm up the bidirectional mirror, and get ‘Grab’ to acquire
6. After imaging, bring slide down to first (deepest) plane
7. While setting up scanbox again after closing, move slide up and down to reset ETL/mirror value

### To take a z-stack

1. On the right side of scanbox, switch the settings in the drop down menu from ‘real time’ to ‘knobby scheduler’
2. Go –30um to –70um below the lowest plane of imaging
3. Scroll up to the surface of the brain and note the range you need (e.g. 30 + 190)
4. Set the um you want to image (the stack starts from the bottom and moves up)
   1. Typically 200um+
   2. This is your range
5. Set the step to 3um
6. Collect 40 frames
7. Select Arm and Return
8. Set imaging to unidirectional
9. Change/note the name of the zstack in the spreadsheet
10. Hit ‘grab’ and check to see if the step and z-depth changes on the motor control
    1. Reduce power manually as you go up!
11. Note that for the red channel, you need a) a higher power b) the screens off to avoid artifact
    1. Can reduce both power and PMT manually as you go up

### Troubleshooting tips

1. If there is an excess of bubbles on the surface of the cannula that does not seem to go away after resetting the DI water configuration
   1. Use 70% ethanol in the pipette and drop a small amount in the middle of the cannula, keeping in mind to avoid the dental cement
   2. Use a kimwipe to absorb that ASAP and then rinse the cannula and dental cement with DI water 3-5 times
   3. Apply DI water on cannula and refocus