**Two Photon Rig Visual Stimulation Experiment Master Protocol**

1. Turn on both Bruker and stimulus computers, login required for stimulus computer (on bottom left of screen).
2. Start MATLAB on stimulus computer.

**NB. At this stage it is assumed that the animal is headfixed in the system and ready to image.**

**Bruker Computer**

1. Start PrairieView Software.
2. Create data folder for the experiment in Raw Data Drive- Should be split into animal ID (subdivided into date [YYYYMMDD format] for chronic animals).
3. Create subfolder in experiment folder for the first imaging run, usually named for prestimulus time, stimulus time and repeat (i.e “5off\_5on\_01”).
4. In PrairieView, in 2P laser menu, turn on laser, open shutter and select excitation wavelength.
5. Ensure that laser compensation is set to an appropriate value.
6. Depending on objective, make sure there is or is not water in the imaging chamber.
7. Use oculars to make sure the microscope is focused onto brain surface with brightfield or epifluorescence.
8. Use 2P live imaging function to find first imaging location and make sure that the animal is expressing calcium indicator.
9. Place light shielding around animal, if using mouse be sure to use moldable clay to cover seam between objective cover and implant.

**Stimulus Computer**

1. Plug in and place secondary stimulus monitor in front of animal.
2. Choose visual stimulus script from “C:\All Docs\code\matlab\Two\_photon\_imaging\PTB\_code”.
3. Set up experiment call with appropriate parameters, see help for each experiment.
4. Start experiment script to get estimate of experiment runtime.

**Bruker Computer**

1. Adjust T Series tab # Reps field to modify duration to the experiment run time (+ 10 seconds) and match that time for the Time in Voltage Recording Window.
2. In T Series tab ensure Max Speed, BOT and start with input trigger are checked.
3. Set synchronize to voltage recording to CURRENT.
4. Set save path to the created folder for this recording folder ( step 5) and set iterator to 00.
5. Use live view to find good imaging area, zero XY to location and set Z to 0 for brain surface.
6. Stop imaging at appropriate imaging depth and using BOT window set ROIs for cells of interest.
7. Check on animal to make sure proper anesthesia and that eyes are moist.
8. In T Series tab select the appropriate save path and select Start T Series.

**Stimulus Computer**

1. Continue experiment to start stimulus presentation, image recording should start simultaneously.

**Bruker Computer**

1. Let experiment run its course.
2. Cancel processing images at this time.
3. Create new recording folder (ie.“5off\_5on\_02”).
4. Repeat step 21-25 as much as needed.
5. At end of experiment use Image Block Ripping Utility to batch convert all raw files for the day.

**Stimulus Computer**

1. Copy .mat files from “C:\PostDoc Docs\Ca Imaging Project\PTB\_Timing\_Files” for the particular day’s experiments, there should be one stimParams and another events file for each run. Make sure all the .mats for the particular day are in a single folder. These files should be the only files in that folder.

**Bruker Computer**

1. Start the *TransferEvents2DataFolders* app which should be on the screen bottom panel (insert icon here).
2. Follow instructions in app and it will copy the .mat files into the appropriate recording folders
3. SHUTDOWN
   * 1. Stimulus Computer
     2. Use 2P laser tab on Bruker Computer to close shutter and shutdown laser
     3. Close PraireView and shutdown computer
     4. Turn off power bank on Buker Computer Rig