Intrinsic Optical Signal Image Acquisition, User's Guide

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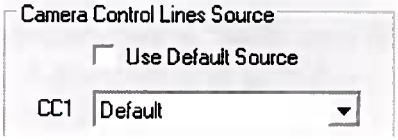
1) Log into the IOS acquisition computer as "**labview_2011**", password: **pw4dklab.**
2) Check all of the connections: DAC0 should be connected to the Trig_0 input on the blue IMAQ breakout box. DAC1 should be connected to input 3 (row 2) on the 32x amplifier inputs. Both DAC0 and DAC1 go to the oscilloscope for monitoring. Amplifier input 3 drives channel 3, the red (left) mini-banana output on amp 2. The high voltage lines from channel 3 go into the piezo holder; the red output on amp goes to brown on piezo, controlling the X position; white is ground. Vector rack is turned on. High voltage power sources are *both* turned on.
3) Open the link to "**ISI_recordframes_piezo.vi**" on the desktop. This file is the main entry point for data acquisition, initializing both the image acquisition (IMAQ) card and the data acquisition (DAQ) card. The Labview VIs are located in Desktop \IOS imaging labview.
4) Turn on the power source. LED control switch should be in the middle, and the course voltage adjustment knob (far right) should be turned down. The power supply should be displaying the output current.
5) **Open the NI Measurement and Automation Explorer (MAX).** This program will give you access to the raw video output from the camera, and let you take single frame images. In the configuration, navigate to:
   Devices and Interfaces > NI-IMAQ devices > img_2011 : NI PCIe-1427 > Channel 0: img_2011
   "img_2011 : NI PCIe-1427" sets the camera definition file (img_2011.icd) for what is plugged into the IMAQ 1427 card and allows Labview to communicate with the camera. "Channel 0: img_2011" sets the camera interface file (img_2011.iid). Neither of these files should be overwritten! When closing MAX, **if prompted to save camera interface file, select NO** (default is yes)**.**

6) To set the camera to "free run" in MAX, turn off the external trigger on CC1. **Switch CC1 from "External Trigger 0" to "Default".** Note that running the Labview VI will automatically switch this back to external, since the data acquisition VI is using this setting.

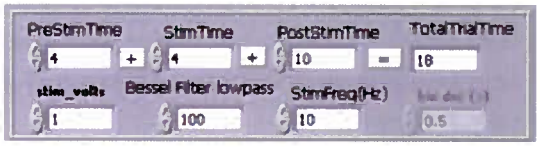


7) **Press "Grab" to view frames**, allowing the camera to "free-run".
8) Position the head-fixed animal under the lenses. Use the extension hoses to run isoflurane to the animal from one of the surgical tables. **NOTE: light isoflurane (1.25-1.75%) gives better signal in rat.**
9) Adjust the focus and animal position until the desired ROI is positioned in the field of view. Lock down the animal plate.
10) **Turn the LED switch** to the **left**, activating the **blue** (475 nm) LEDs. **Adjust the illumination** by turning up the power supply voltage to obtain maximum contrast without saturation. The maximum pixel value is 4095; the Histogram button toggles a real-time histogram. There may be some saturating pixels on the margins, adjust the intensity so there is no saturation along the cortical surface. **Do not exceed 1 A.**

11) You should now see a clear high contrast, unsaturated image of the surface vasculature. **Click "Save Image" to save the current image as a PNG.** Save the image to "E:\IOS data\", or your desired

folder. **Do NOT click "Save"**, as this overwrites the camera interface file. If the animal moves under the camera, you will need to repeat steps 10-12 to have an accurate reference image.
For greater fidelity of the surface vasculature map of a convex cortical surface, take multiple images at different focal depths, and create a frame stack in Photoshop. Further details are in the Technical Overview.

12) Turn down the voltage on the power source. Turn the LED switch to the right to activate the red (625 nm) LEDs. Adjust the illumination with the power supply voltage and avoid pixel saturation. Typical values are around 0.10-0.2 A. Use the histogram. Again, the margins may saturate, which will result in a ratio value of 0. Avoid that on the cortical surface.
13) Click "Grab" to stop collecting frames. Click on "NI-IMAQ devices" in Configuration to allow Labview to access the camera. Leave MAX open in case you need to take more stills.
14) Switch to Labview and the opened **ISI_recordframes_piezo.vi**.
15) Type in the desired filename into the **filetag** field. All filenames will have a time stamp (XXX_hhmmss.dat) following the tag you define. Check that all stimulus parameters are correct. Typical values : **PreStimTime=4 sec**, **StimTime=4** (although 0.5 can be used), **PostStimTime=10** (13.5 if 0.5 stim is used). TotalTrialTime will update automatically. **stim_volts = 1V** directly controls piezo deflection amplitude. 1V typically gives a ±.8 mm deflection. **Bessel Filter lowpass=100 Hz** is the cutoff frequency to reduce ringing from piezo output. Values of 100-300 should be adequate, but visually check to see if the tip rings with a slower stimulus frequency.



StimFreq=10 Hz gives a square wave with a period of 100 ms, or 5 deflections per 0.5 sec frame. Changing these values once the VI is running will have no effect. Stop and restart to change.
16) Place the rodent's whisker in the capillary tube attached to the piezo (or stimulate in desired fashion, depending on target area). Position the piezo mount such that the capillary tube won't affect surrounding whiskers. It is often good to trim the whiskers to ~10-20 mm length, making this easier.
17) **Click the hollow right arrow at the top left to start the VI.** This sill start a free-run mode in the left frame, allowing you to adjust focus one last time. The deflection protocol will also have started, so check to make sure the capillary tube isn't tapping any other whiskers. If necessary, reposition the piezo mount. If an error comes up saying the camera is already in use, check step 14.
18) Press "**Start**" to begin recording. The "RUNNING" light will eventually turn on, indicating that it is collecting data, the trial #=1, and the buffer counter will run, indicating frame within the trial at the 20 fps rate. The "DAQ WAIT" light will turn on when the VI is dumping the buffers to disk. Files are written to the SSD at **E:\IOS data**.
19) **Adjust the sliders** below each frame to select which frames to visually compare. Default baseline frame is 3 sec, or bin frame 6. **Adjust the image contrast** to optimally match the ratio to pixel display such that the img min and max are around 0 and 4095, respectively.
20) Press "**Stop**" to finish the current trial and complete the header. Pressing the stop sign will kill the running VI and render the mostly written data file unusable, missing the correct number of trials.

The raw frames are written to the *.dat file; off-line analysis must be done for accurate mapping. In Matlab, run "**ISI_analysisGUI.m**" for the easy-to-use interface, or "**ISI_analysis_multi.m**" for greater flexibility.