Tutorial for VSDI

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Before imaging

Brain slices preparation

Animal subjects: P17-23 preferably use fresh cutting solutions and recording solutions.

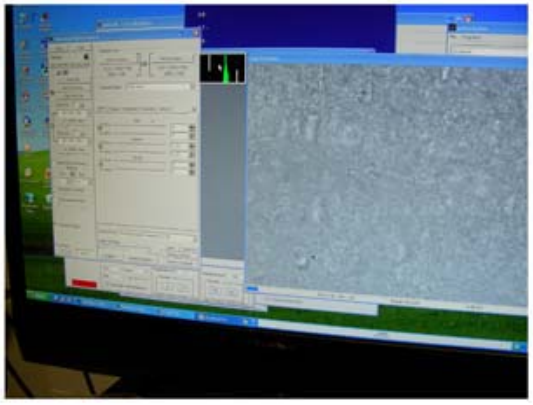
- Cut the brain and incubate the slices in an interface chamber oxygenated (95% O_2 and 5% CO_2) so slices remain healthy for up to 8-10 hours.
- Make sure you have turned on the air table, and the chamber perfusion system is stable.
- Set the perfusion flow (by setting the rate flow regulator to ~150) and remove all air bubbles.
- Set the outflow pump (Watson Marlow) to a relatively slow speed (around the ~ 4 mark). As it is peristaltic pump, it has certain pulsations and may cause small regular recording noises. If the outlet has continuous flow with air bubbles you will likely to have a stable flow.
- Place a healthy brain slice under 4x objective, use a slice anchor to hold it down to the chamber bottom. Make sure you identify the cortical region and cortical layers you intend to record neurons from.

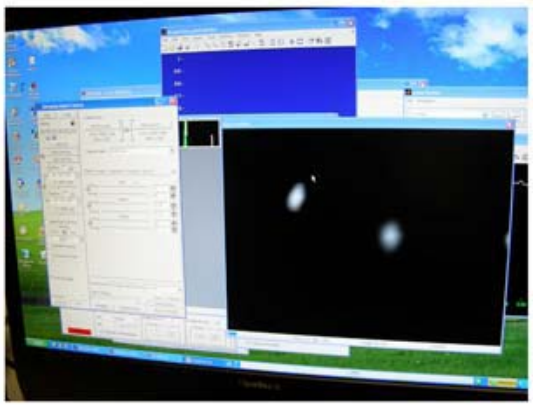
Staining

- Prepare a chamber for staining and wash it with ACSF.
- Weigh 2.5 mg of NK3630 (RH482; available from Nippon Kankoh-Shikiso Kenkyusho Co., Ltd., Japan) and add it to 60 ml of ACSF in room temperature.
- Incubate slices in oxygenated (95% O_2-5% CO_2) solution for about 2 h.
- After incubation move the slices into oxygenated ACSF for 10 min for washing.

The EPHUS software

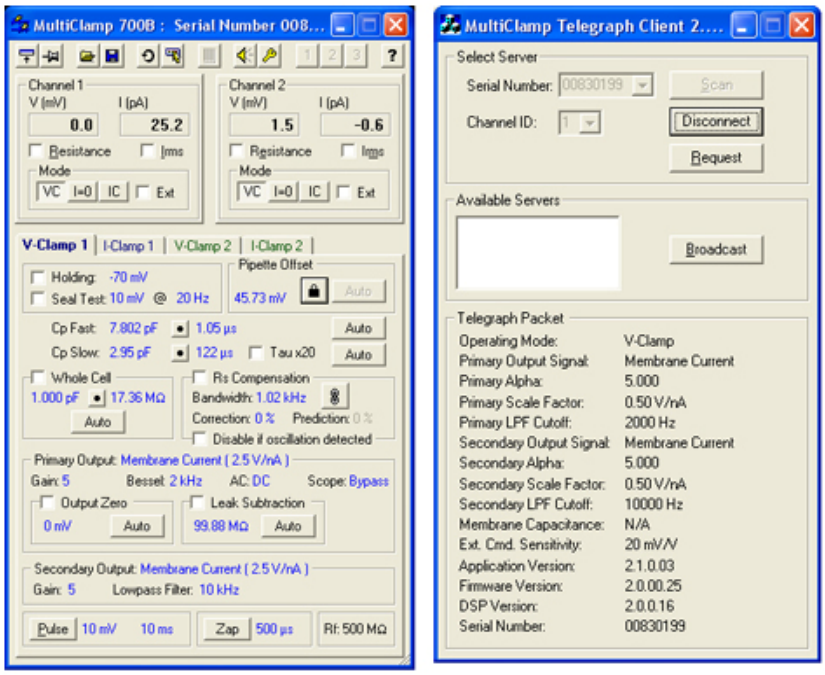
To acquire the physiological data and control the stimulation we use a modified





version of Ephus software (Ephus, available at https://openwiki.janelia.org/).

Turn on Q-imaging camera and open the Q-Capture software. Make sure your electrophysiology amplifiers are turned on and configured. For the MultiCLamp this includes running MultiClamp Commander and the MultiClamp Telegraph client.

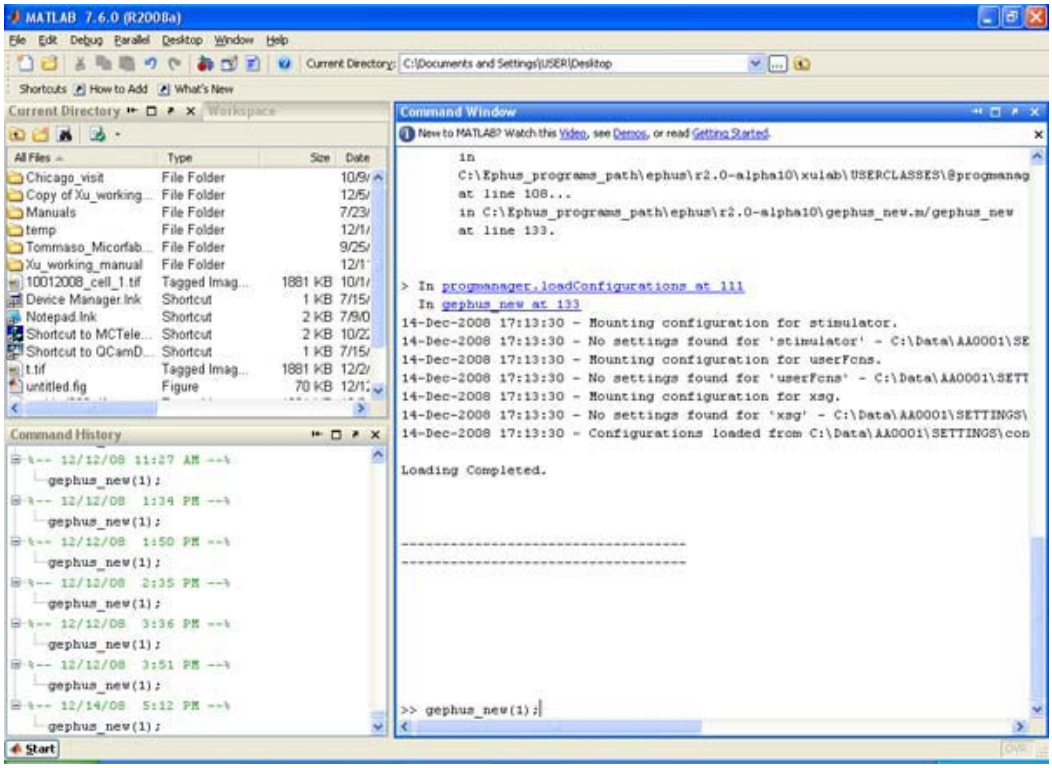


The telegraph client ("Ephus" software interface) has to be scanned and connected to
Multiclamp 700B, as shown above.

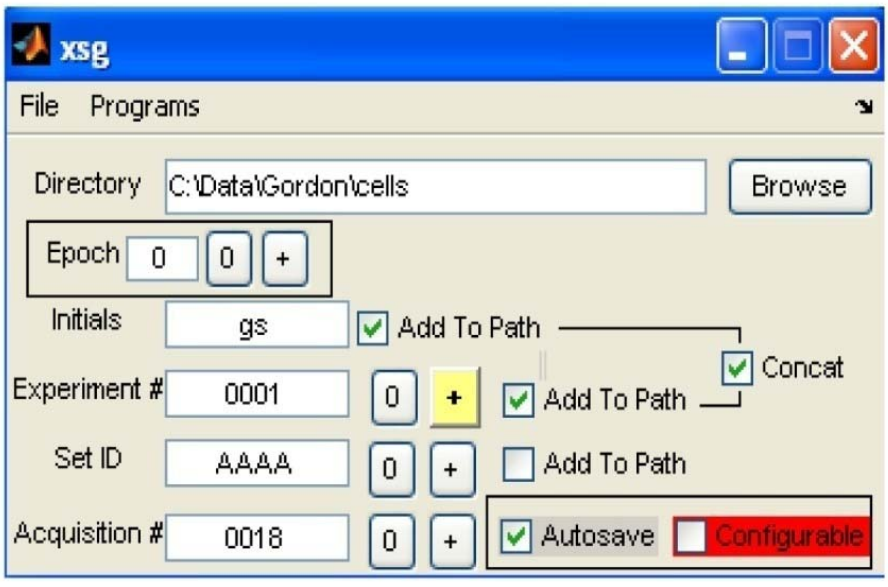
Starting EPHUS

- Start MATLAB
- From the command line type Gephus_new(1);.
- When promped for configuration file, select the recent one.

You should see a number of messages, concluding with 'loading competed'.



We assume that the physiological recording of either whole-cell, loose-patch or LFP is already established. Please refer to the relevant recording guide.



- To start VSDI find the XSG window and set up file saving locations to a directory with the current date

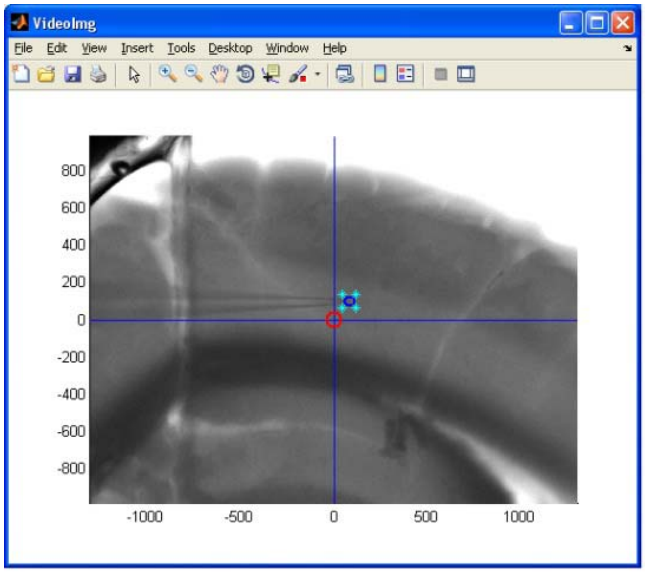
in your own directory and put your initials.

The following part should be done only after the VSDI system is ready for imaging (see below).

- The image should be centered to the field of view of the VSDI camera.
- Pull the lever marked 'VSDI' and snap a frame with the Q-imaging software (see instructions) and save it to your experiment directory.
- In the 'Hotswitch' window, press 'VSDI 8 s'. This should set all parameters to recording VSDI to map stimulation at intevals of 8 seconds.

Running a mapping experiment

Specify the mapPattern and the spacing between positions.



- Use "Grab video" to load the snap picture via the Q-imaging software (It is saved at the
-
- corresponding cell folder).
- You can label the cell soma by clicking "[1]"
- Use the '=' tick to set the X and Y spacing to the same value. The mapping grid should appear overlaid on the videoImg window. Position the map, both offset and rotation , using 'dX' 'dY' and 'deg'

You can click "Use as offset" to make the pattern centering around the cell soma.

For VSDI use 4x4 or 3x4 and cover the area of interest.

Once the map is ready you can follow the instructions for BV and hit 'Map' when the BV is ready.

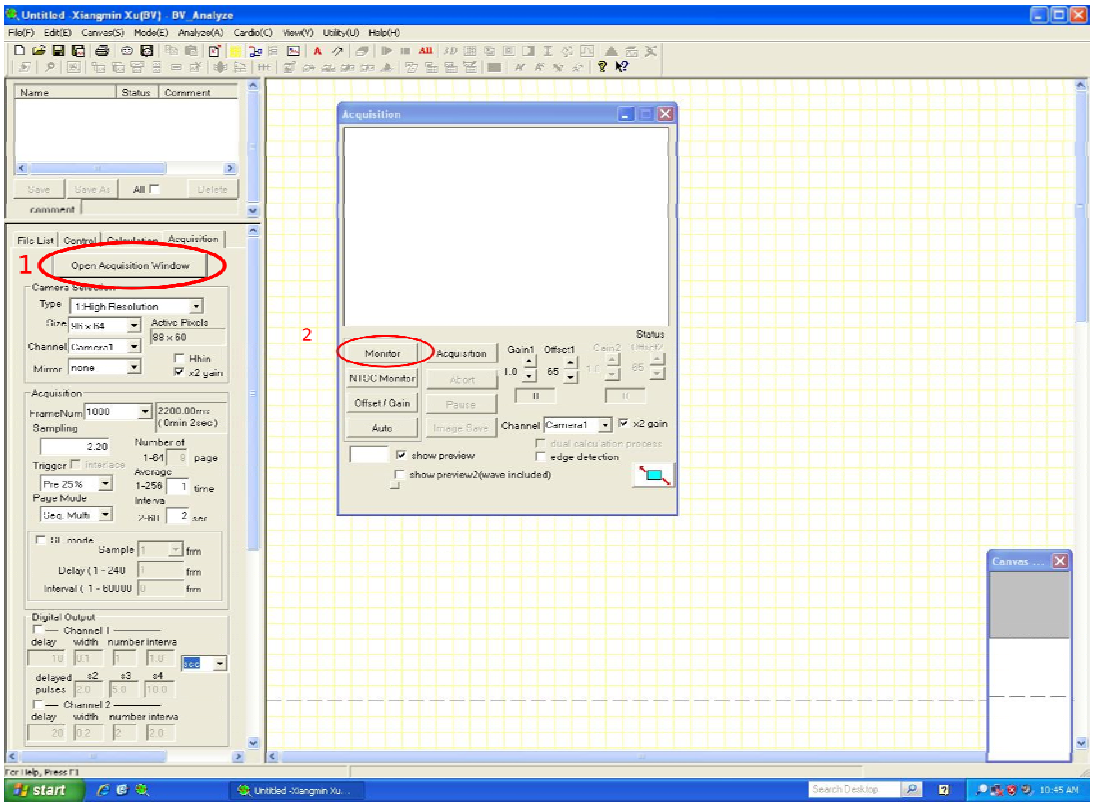
The BV software

Setting the microscope for VSDI

- Verify the filter position on the bottom to VSDI-filter (red arrow).
- Remove the IR filter - pull low lever out.
- Push lever on camera port in (marked VSDI)

Acquisition

Open the BrainVision software.
On the left select the acquisition tab and open 'acquisition window' (1).



It will open a new acquisition window (Figure1) . Pressing 'monitor' (2) will display your image. Turn on the light, adjust intensity and position as well as the focus on the slice.

Move down and press 'AutoExec' (see figure 2 #1) . This will open a new window with settings specific for your experiment. Here we will give some important command but you should see the full documentation for detailed script language.

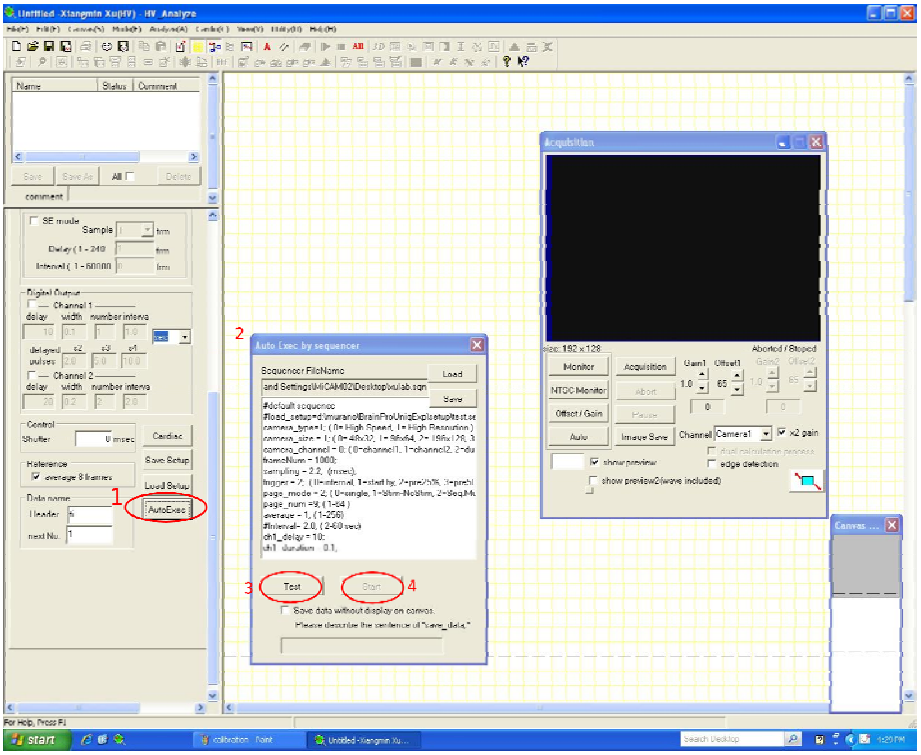
- Set number of pages - corresponds to the number of laser grid points.

- Create a directory in C:/Data with the current date.
- Set 'save file'- to the current date

Note that for file naming you should put your initials followed by the date of the experiment. After the date put "_" (underscore) and the cell number corresponding to the XSG directory in the EPUS program.

- Set 'save dir'- to the current date
- Press 'test' button (3).

If the test is completed successfully you are ready to run the experiment.

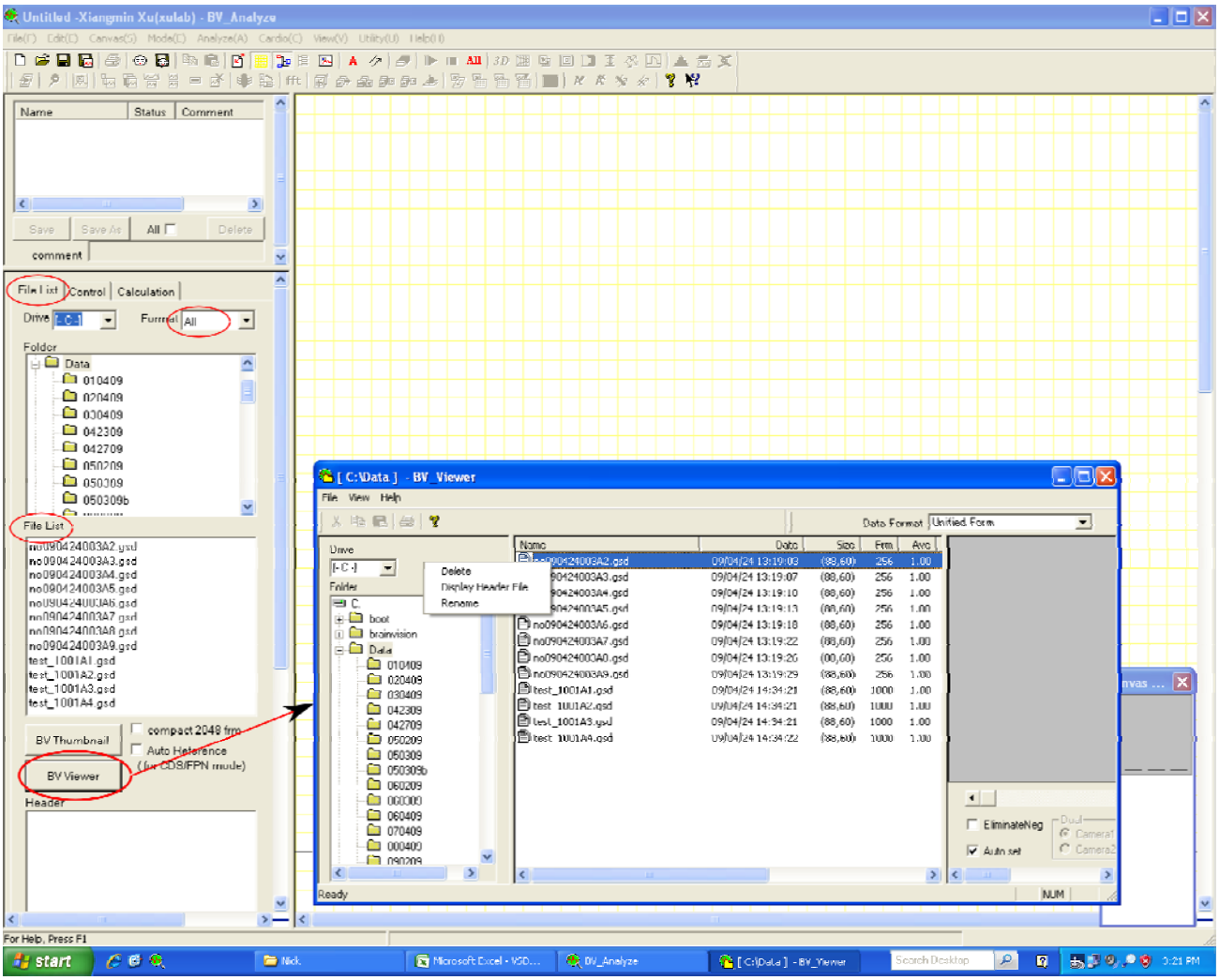


You can now:

- stop the monitor
- stop perfusion
- press start (4)

Analysis

Open the brainvision program and press the 'File List' tab.

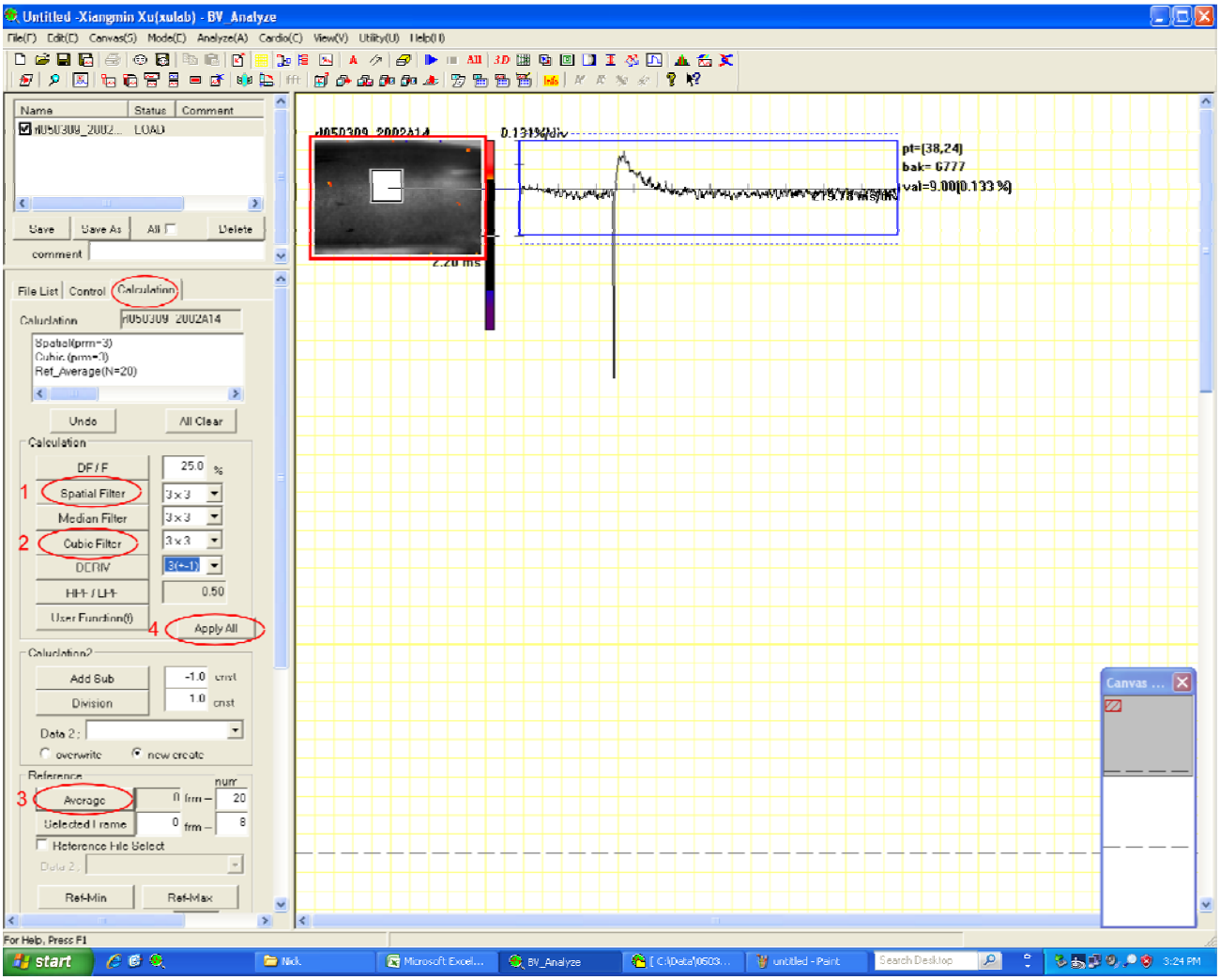


Loading movies

You can load the movies by dragging them directly to the software window from the explorer.

The other way to is to use BV_viewer. It will open an explorer window. Change the data format, on the top right to 'All' form. Highlight your files and drag them into the window. A right click on the file can give you its header information such as the number of frames etc.

Double click on a frame and a rectangle will appear with a trace representing the

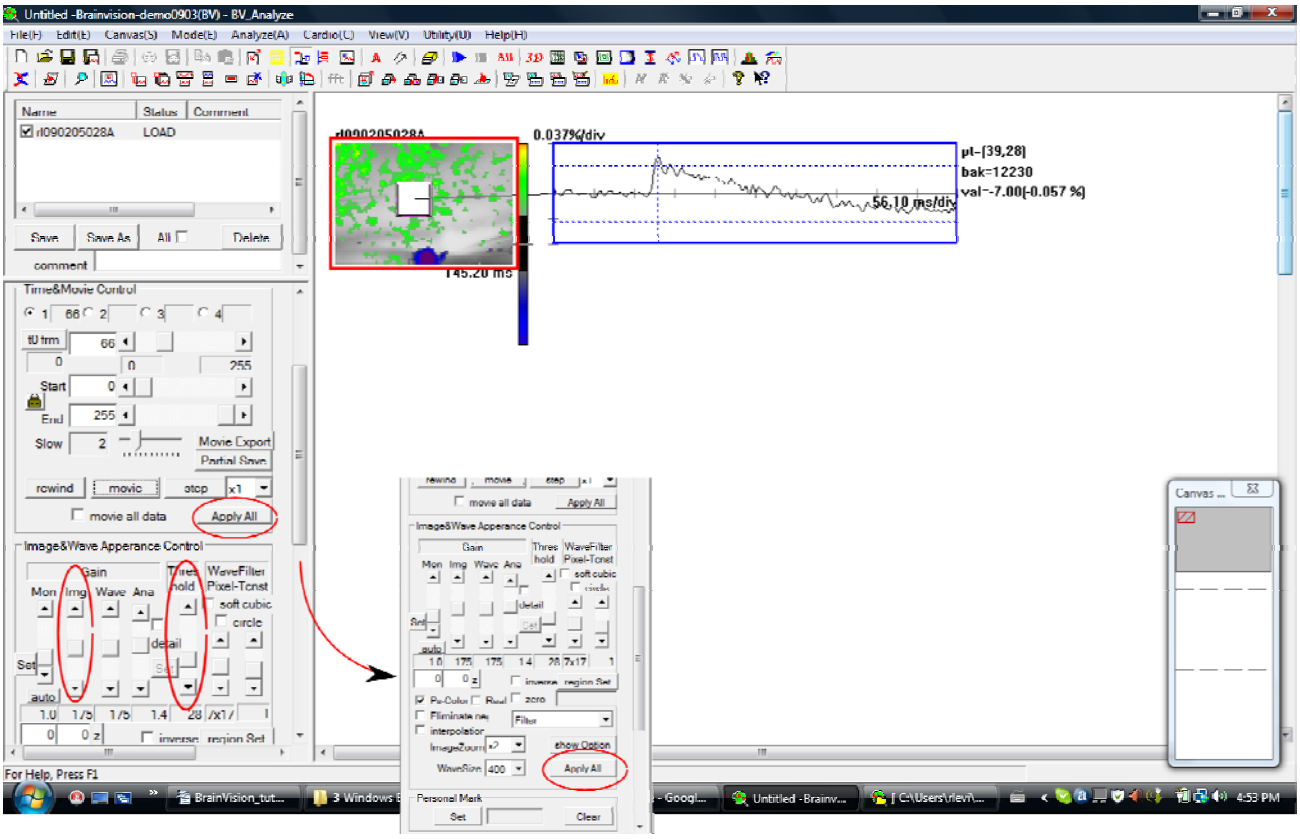


VSDI change in the region over time.

Press the calculation tab. While one frame is selected press 'Spatial Filter' (1) and 'Cubic Filter' (2). For a quantitative measure it is necessary to select reference frames. One way is to use the first 10 or 20 frames (3).
If you have multiple files loaded - as you normally would- after applying all these changes to one file you should press 'Apply All' (4) to apply them to the rest of the files.

Note that the reference frame is not applied and you will have to calculate is separately for each file.

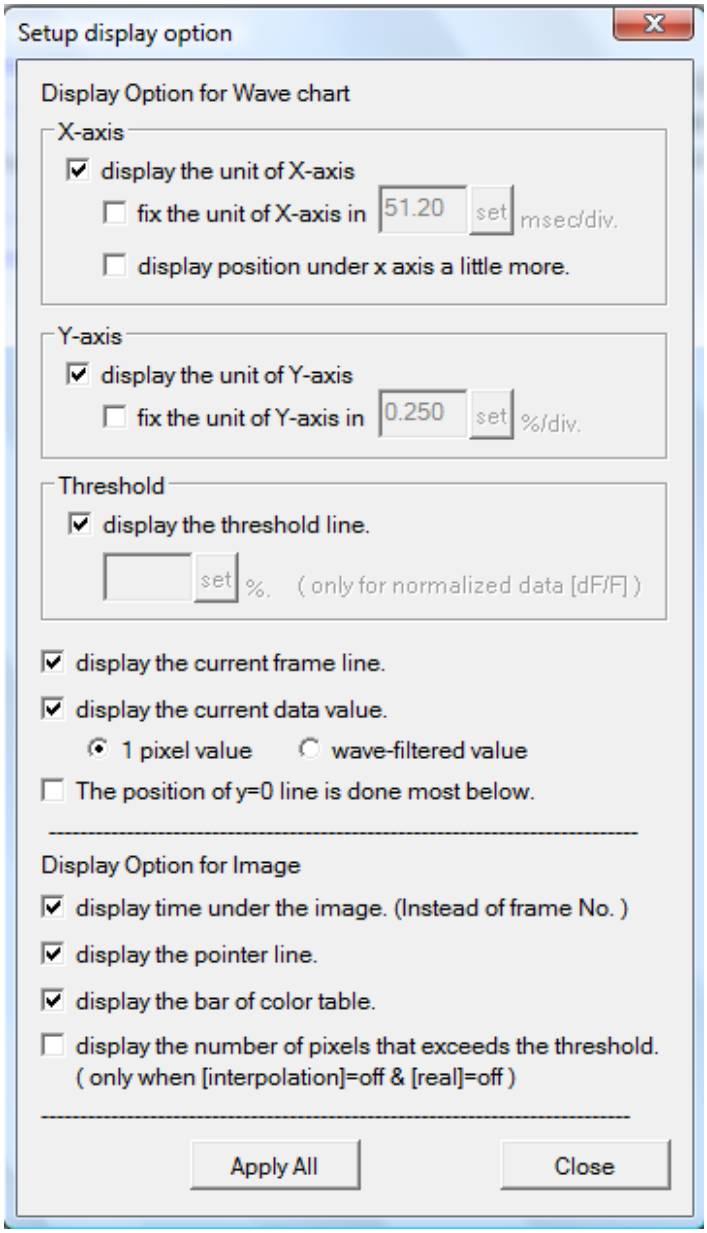
Press the 'Control' tab and scroll down.



At the trace, move the rectangle until a clear signal is seen. Then click on the peak of the signal - a dashad line should mark your position on the trace.
When at the peak adjust 'Img' and 'Threshold' until a region is green or somewhat redish (but not too much red). Make sure that when you click on the trace at the pre-stimulus region it is mostly gray. If it is green or red redus 'Img' and increase 'Threshold'.

To apply the settings to all files press 'Apply all' below 'step' then scroll down and apply all again below 'show option'.

It is usfull to set identical scale for all traces. To do so you need to press 'show Option'. In the window that opens up you can check 'fix the units of Y axis' and select a value.



Saving results

Saving the canvas in *.rmg format will also save all modification done. From the menu select 'File' and 'Save'.

To export the images you need to select 'File' and then 'Canvas image Save as'. It will promp you to save all pages or a selected page.
You can increase the size of your canvas by slecting from the menu 'Canvas' and then 'Canvas size'.

Calibration

- Take the camera off the microscope.
- Put the camera cap on.
- Change settings to 192x128 @ 5msec/frame.
- Click the offset arrow down until you see blue on the display window.
- When you start to see blue, click offset arrow up until the blue disappears.
- When the blue disappears, click offset arrow up 5 additional times.

The system should be calibrated properly.