Autopatcher IG

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Overview

The Autopatcher IG software allows the user to quickly and reliably patch-clamp neurons in acute brain slice preparations. The software, written in LabVIEW (National Instruments) controls several pieces of hardware described below

Hardware

1. Scientifica PatchStar or MicroStar micromanipulators

2. Scientifica XY stage

3. Scientifica motorized objective changer

4. Neuromatic Devices Autopatcher

5. Multiclamp 700b patch-clamp amplifier

6. Data acquisition system (optional)

Software

1. Multiclamp 700b software

2. Data acquisition software (PCLAMP, MIES, etc., optional)

Installation (EXE)

First, install QImaging drivers [32-bit or 64-bit depending on your system] (http://www.qimaging.com/support/downloads/legacy_qcamdriver.php).

Unzip Autopatcher IG EXE.zip. Navigate to Autopatcher IG installer v1.000/Volume/setup.exe.

Follow the instructions on the installation prompt.

The installer will create a folder in your Program Files directory called Autopatcher IG. Navigate to that folder and start Autopatcher IG.exe.

Procedure

The program will auto-start in demo mode. Note: if the program starts with an error about not finding QCam.llb, ensure that you have downloaded and installed the QImaging drivers (previous section).

Follow the prompts in the live slice view (below) to execute the program successfully in demo mode
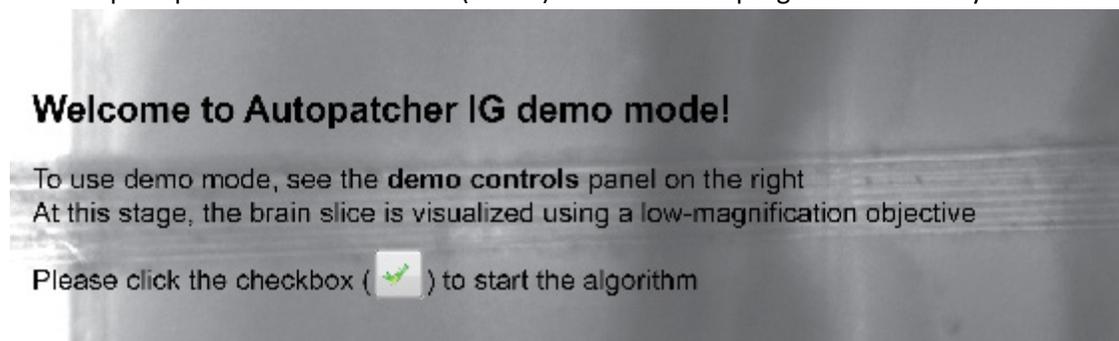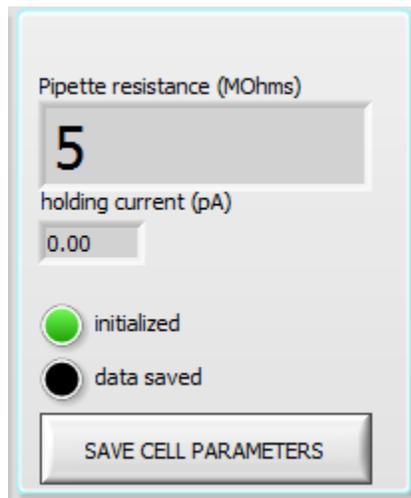

Controls

Demo mode

In demo mode, all device communication with the camera, manipulator, data acquisition [DAQ] device, the pipette pressure box, and Multiclamp is disabled and instead emulated using software. To instead use one or more real devices, see the "demo controls" tab (below) in the tabbed panel on the right side of the screen.

camera type

Demo

Manipulator demo?

DAQ demo?

Pressure box demo?

Multiclamp control?
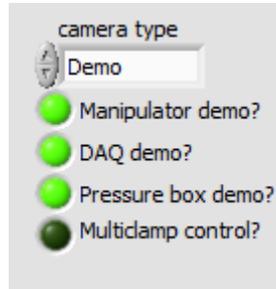
Instructions to the user are shown in the instruction bar below the live view of the brain slice (see below). To move ahead in the program, the user clicks the green check button on the right. At any point, the user may exit the program by pressing the Quit button.

QUIT Make sure pipette is in position

Pipette resistance is read out to the user in the top right panel of the window (below).

Pipette resistance (MOhms)

5

holding current (pA)

0.00

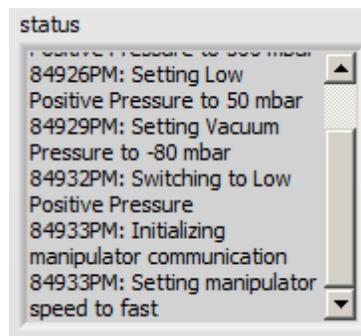initialized

data saved

SAVE CELL PARAMETERS

In experiments with real hardware, the pipette resistance is read out from the DAQ. In demo mode, the user controls the pipette resistance using the Pipette R slider (below).

Pipette R (MOhm)
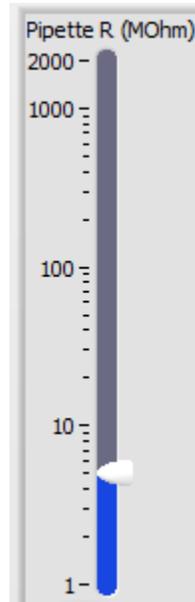
Try adjusting the pipette resistance. You should see the resistance reflected on the pipette resistance indicator.

The status textbox (below) shows the simulated activity of the pipette control box and manipulator, prepended with timestamps.

status

84926PM: Setting Low
Positive Pressure to 50 mbar
84929PM: Setting Vacuum
Pressure to -80 mbar
84932PM: Switching to Low
Positive Pressure
84933PM: Initializing
manipulator communication
84933PM: Setting manipulator
speed to fast

The gigaseal resistance plot (below) is activated once contact with the cell is established. The plot shows the gigaseal resistance as a function of time as the algorithm attempts to form a gigaseal with the cell.
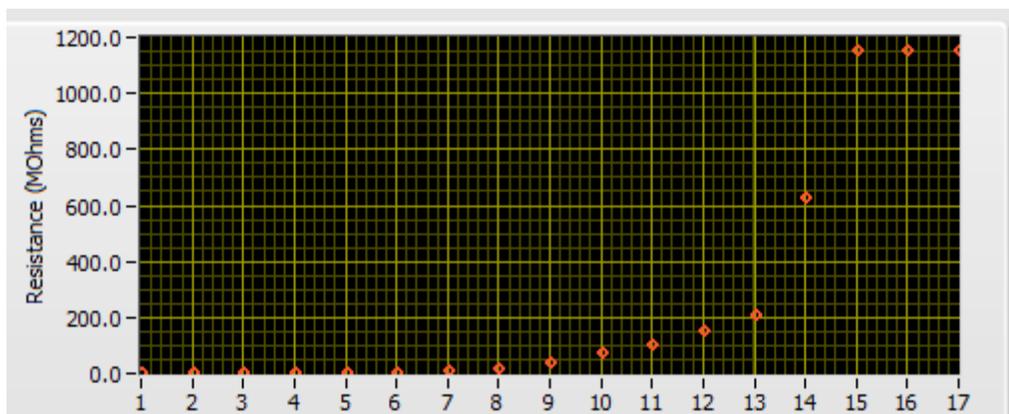
The membrane currents plot (below) shows the instantaneous membrane current in response to a test pulse. This is equivalent to a membrane test pulse in other electrophysiology software packages such as Clampex.
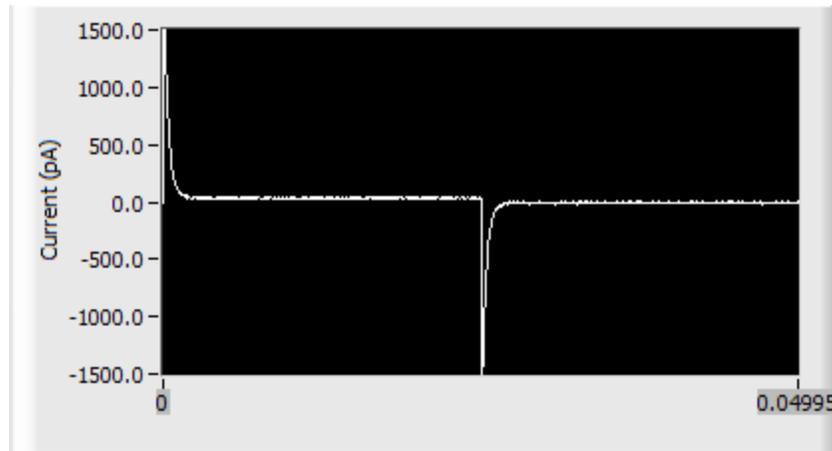
Troubleshooting (hardware + software)

PROBLEM: An error appears at the start of the software saying that a device (manipulator, stage, and/or Autopatcher box) was not connected even though it is.

SOLUTION: This can happen if a device was unplugged from a USB port and then plugged into another USB port.

If the device that cannot be accessed is a Scientifica device (manipulator, stage, objective changer), close slicePatch, open LinLab2, and go to Settings. Go to Devices and look at the top ribbon for tabs like *SliceScope (COMX)* and *PatchStar (COMY)* with X and Y being some number. Note down all the COM port numbers. Go back to slicePatch and scroll to the right menu for more options. Go to the tab "Options". For the device(s) that failed to start, use the drop-down boxes to select the COM port that you noted down earlier. After you select the correct COM port, right-click on the drop-down, go to "Data Operations" > "Use current value as default".

If the device that cannot be accessed is the Autopatcher, go to Control Panel > Device Manager. Click on the "+" next to "Ports (COM & LPT)". A menu of connected devices should pop up. One of the devices should be an Arduino. Note down the corresponding COM port. Go back to slicePatch and scroll to the right menu for more options. Go to the tab "Options". Find the COM port control corresponding to the Autopatcher. Use the drop-down boxes to select the COM port that you noted down earlier. After you select the correct COM port, right-click on the drop-down, go to "Data Operations" > "Use current value as default".

PROBLEM: Camera image looks too washed out and low-contrast.

SOLUTION: This may happen if the CameraViewer software was run prior to running slicePatch. Close slicePatch, unplug the USB and power from the camera and plug it back in. Re-start slicePatch and the image quality should improve.

PROBLEM: The pressure on the pipette is not being set properly when checked by the manometer.

SOLUTION: Ensure that house air is connected to the port in the back of the Autopatcher. Ensure that as soon as the box is turned on, the LCD screen lights up and a hissing noise comes out of the autopatcher. Ensure that the box pressure is set on "Auto" mode, as indicated by an "A" icon on the right on the LCD screen. If the box is turned to "Manual" (or "M" on the LCD screen), turn it to "Automatic" by pushing the knob on the front of the Autopatcher box. If the pressure reading is still not correct when checked by the manometer, it could be because downstream flow is not restricted enough. Place a pipette into the pipette holder or close off the air flow using a Luer valve.

PROBLEM: Finding pipette tips under the 40x objective is difficult because pipettes do not show up onscreen immediately.

SOLUTION: This is likely because of variability in lengths of the left and right pipette. Pull a set of pipettes and inspect them by eye or using a microscope. Make sure their lengths are identical by aligning their non-sharp ends. If one pipette is longer than the other, adjust the location of the pipette stopper on the Sutter puller. It may take a few attempts to get the pipettes to be exactly the same length.

PROBLEM: Upon descending the pipette towards the cell of interest, the pipette is way off.

SOLUTION: The manipulator/stage relationship may need to be recalibrated. Contact Ilya Kolb.

Troubleshooting (patch-clamping)

This section will address problems with the process of patch-clamping, not necessarily specific to slicePatch software. These are some less well-known tricks of the trade. In general, you should achieve a gigaohm seal with your target neuron in ~80% of attempts and successfully break in ~80% of those attempts. If your success rate is significantly lower, there may be fundamental problems with your setup. A good reference is the troubleshooting section of [1].

Here are some random pointers in no specific order:

- Use a low-resistance pipette but not too low. 4-6 MOhms is perfect.
- When approaching cells, make sure you use the approach axis of the pipette (approach function on Scientifica manipulators). This makes a huge difference because it improves the mechanical stability of the patch.
- Here are some things to look for when approaching a cell. That is, at what point do you release positive pressure?
    - In some preparations, you can see a "dimple" form on the cell as the pipette approaches it. In some cases, you can actually see the dimple grow. If you see this, this is the *best* indicator that you will have a good cell. Do not linger, as soon as you see the dimple, release the positive pressure. A gigaseal should form within a few seconds.

- o When you cannot see a dimple, sometimes you may see a quick "snap" of the membrane when you release positive pressure and as the pipette starts adhering to the cell membrane. This is often a good indicator that you will get a gigaohm seal.
- Ensure that the pipette spends the *minimum possible amount of time* in the tissue. As soon as the pipette is in, form the seal as quickly as you can.
- Do not move the pipette inside the tissue too much. If you see major deformation of the tissue around the shaft of the pipette, this is bad and could mean that you will not get a stable seal.
- If you are filling the cell with a dye, apply small positive pressure (5 mbar) to increase the flow of dye. The autopatcher algorithm already does this.
- Take a picture of your patched cell 5, 10, and 20 minutes after you've patched on and compare those images to the patchedCell.png image that is generated by the software. Does the pipette seem to have drifted from its original position at all? If so, this is a major problem that could manifest itself in shortened recordings due to cells sealing back up or leaky cells. To fix this, make sure all bolts on the rig are fastened tight, the air table is floating, and the cables are not taut and are not tugging at any part of the rig.
- If you see your image under 40x "jitter" back and forth at a high frequency when you stomp your foot on the ground, there is a problem with the mechanical isolation of the air table. If you see the same jitter in your pipette, this could ruin your seals.
- Spend a lot of time looking for the *best possible* cell to patch. It is better to spend 3 minutes looking for a good cell and have it yield great data than to spend 30 seconds per trial going for less optimal cells and having to do many more attempts.

References

1. Castañeda-Castellanos DR, Flint AC, Kriegstein AR. Blind patch clamp recordings in embryonic and adult mammalian brain slices. Nat Protocols. 2006 Jul;1(2):532–42.