

Mac instructions for DCProgs

Andrew Plested plested@fmp-berlin.de

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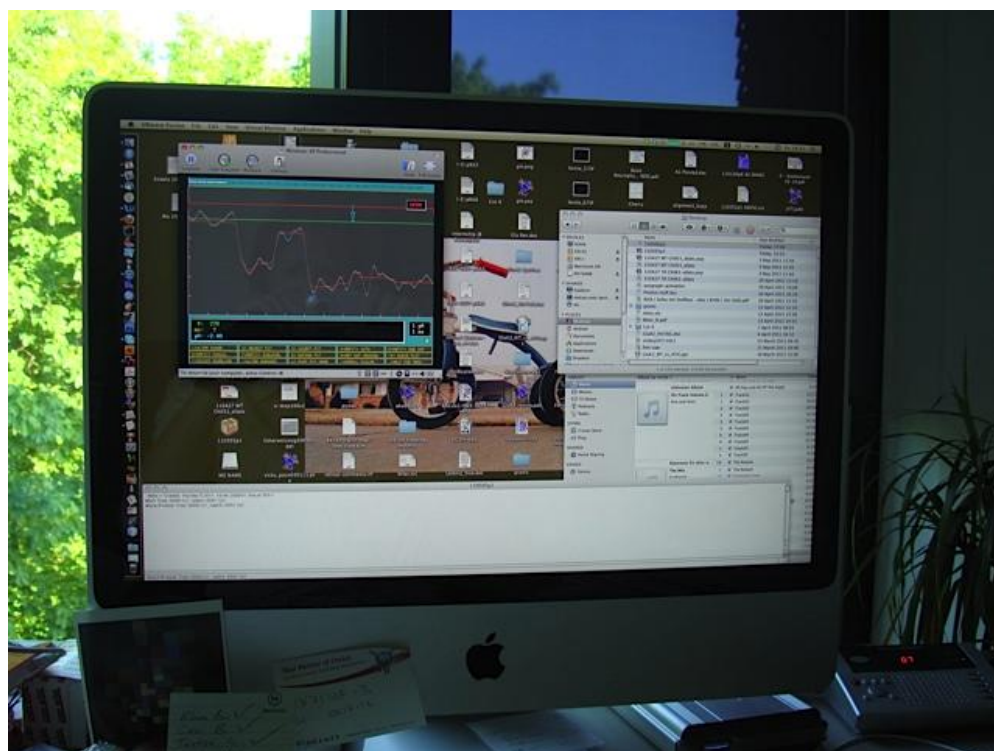
Preparing the analysis environment

First, set up your Mac with DCProgs. DCProgs doesn't run natively under OSX, but on Intel Macs, virtualization is highly evolved and reliable. DCProgs (DOS version) runs fine under a Win XP virtual machine in VMWare Fusion. All of the analysis for Carbone and Plested (2012) was done this way. For machines running OSX Lion (10.7) or later, you will need Fusion 4. Sun/Oracle virtual box also works and is free, but the environment isn't as nice as VMWare. Parallels has not been tried but should be equivalent. DOSBOX is also an option.

Install Windows XP (Open VMWare, select File > New and follow the instructions). Allow some time to install patches to XP. You will need to download some things, so obtain Firefox or equivalent. AVG is a good free antivirus – your Mac will not protect you from rootkits etc! You may opt to turn the network off for the Virtual Machine once you have installed everything. You can still use the network under Mac OS X, and drag files into the VM as needed. XP is creaky now and has for many years been prone to viral infection.

Sun's Free Virtual Host: <http://www.virtualbox.org/>

Once you have a working Windows install, you can proceed to download the DCProgs suite as usual (see http://www.onemol.org.uk/?page_id=8)..



SCAN on AP's 2008 iMac under VMWare Fusion

Whether the DOS programs run in a window or as full screen seems to depend on the type of Mac that you use. On a Macbook Pro, they readily run in full screen, but on a 2008 iMac (24in) they run in a window. On newer 2012 iMacs, it seems that they can run full-screen.



SCAN on AP's 2012 iMac under VMWare Fusion

Preparing VM and Mac keyboard settings for DCProgs

In VMWare, the enter key on the number pad doesn't map to enter on the VM by default, but instead the much less useful 'Alt-Green'. You can alter this by unchecking the 'numpad enter' mapping in the VMWare Fusion preferences.

DCProgs also makes use of the function keys, to save preferences. By default on the Mac these are mapped to hardware volume/brightness controls and dashboard etc. You can change this mapping in OS X under the System Preferences > Keyboard setting ('use F1, F2 etc keys as function keys'). You can still access the hardware controls using the 'fn' key (above 'delete').

<http://rationalpi.wordpress.com/2007/01/31/using-f-keys-in-mac-os-x/>

Download the DCProgs applications

Download DCProgs: <http://www.ucl.ac.uk/Pharmacology/dcprg5.html> (free, but registration needed).

Or, better, download zipped files from the new site http://www.onemol.org.uk/?page_id=8 (no password needed).

Read the notes. You need both the setup and the individual programs. SCAN, EKDIST and AUTPLOT are essential at first. There is also decent Windows version of AUTPLOT. If you do not know the password, you must ask DC. SCAN is available in a windows incarnation from CED, but this is not tried.

Preparing data for export in Axograph Using Axograph 1.3.5

<http://axographx.com/>

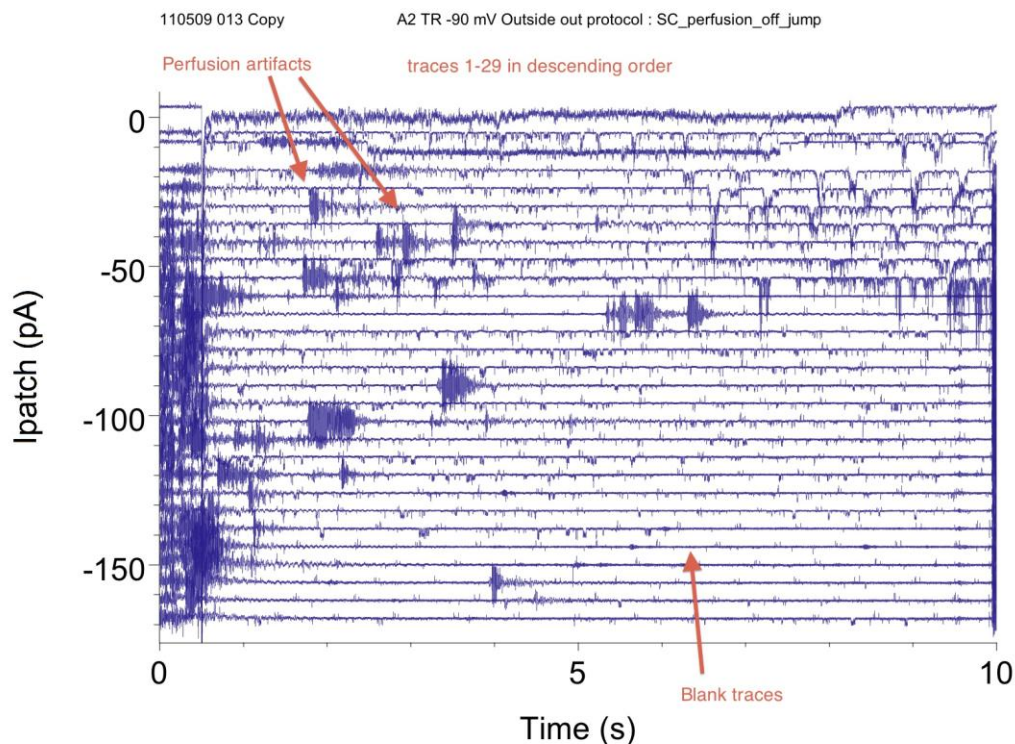
Note: the section below applies to Axograph with episodic data for AMPA receptors but the principles apply to any digital recording method that is not CONSAM or Clampex.

Filter the data (under 'Analyse'). Axograph provides a low-pass Gaussian filter, just what we need. You need to have a decent signal to noise for scanning – for AMPA channels (2pA) this probably means 1kHz filtering unless you have a very quiet record. If in doubt, use a more open (higher valued) filter first, and see how it goes.

It is a nice idea to plot the episodes in a raster format and print it out so you can orient yourself during fitting in SCAN and make notes. You can make annotations to the pdf in Preview on Mac OSX 10.6 and later.

Utilities > Group Manipulations > Offset traces.

Pick an offset about 2 or 3 times the maximum single channel opening.



If you have episodic data you can convert it to continuous in Axograph using the menu command

Utilities > Graph Utilities > Episodic to Continuous

You may choose to remove the "dirty ears" of the record if you are using automated perfusion. By automated perfusion, we mean turning off the suction line with a valve during the recording, but opening the valve in between episodes to ensure that the bath is drained. The noisy sections at the beginning and end of each episode can be excised, using the command 'duplicate selected range of front group'. Save from Axograph as pClamp binary file ('pClamp ABF' in the dropdown menu). Use a convenient location on your Mac. For DOS Scanning, it is necessary to use an '8+3' filename of the following format:

yymmddPn.abf

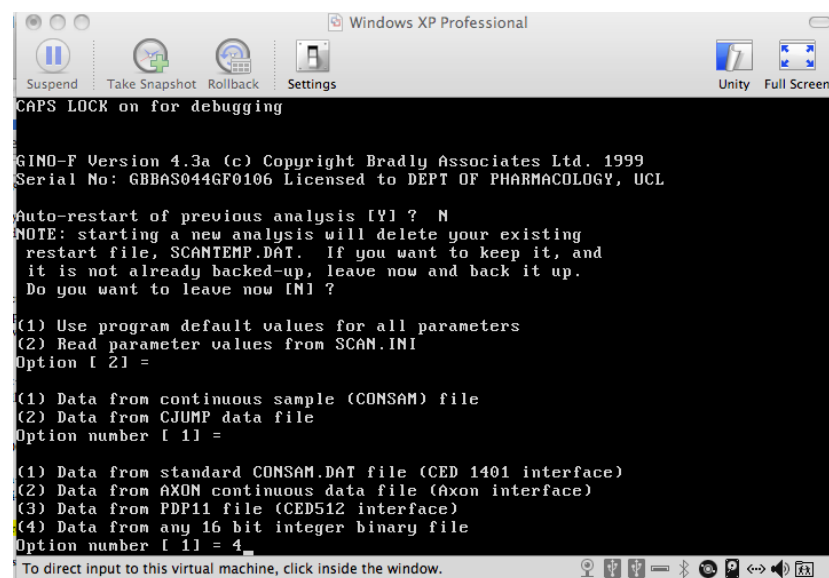
where: yy is the year, mm the month and dd the day, and n the patch number from that day. If you have more than 10 patches in a day, you are in trouble. DC says that this doesn't happen. You can use hex!

Critical Select option 'Integer' after choosing filename, otherwise import to SCAN will fail

Drag the file into a directory on the virtual machine that is easy to get to (eg C:\) you can move it later when you are done. You can simply drag onto the desktop and then move the file within XP if you prefer. You will not be able to enter complex directory trees in DCPROGS – you could modify autoexec.bat to include a PATH to your data directory, but this is for experts only. If this is all Greek to you, I wouldn't bother.

Time course fitting

Start SCAN from the shortcut in the DCPROGS folder. You will need to set up the machine on the first run. Options like 'colour screen' give an impression of the age of the program. The first time, it will crash no matter what. Rerun.



Initial input to SCAN defines the type of file that will be analysed

Auto-restart is a useful option later, but not now.

Read parameters from Scan.ini – we can change settings in a moment (but not much can be changed after analysis begins!). We have a continuous file (CJUMP data not tried).

So far, we use ITC-18 digitizers. This device is an 18-bit A-D converter. All the same, select option 4 ('Any 16-bit integer binary file') even though there is an ABF option – this will fail. Even though your file was originally converted as 18-bit, it is not 18-bit any more.

Choose any odd number around 2000 for the header length.

```
(2) Read parameter values from SCAN.INI
Option [ 2 ] =

(1) Data from continuous sample (CONSAM) file
(2) Data from CJUMP data file
Option number [ 1 ] =

(1) Data from standard CONSAM.DAT file (CED 1401 interface)
(2) Data from AXON continuous data file (Axon interface)
(3) Data from PDP11 file (CED512 interface)
(4) Data from any 16 bit integer binary file
Option number [ 1 ] = 4

Name and path for data file (use home/end/arrows/bs/ins/del)
c:/110509p1.abf.....
Byte # for start of data (=header length+1) [ 1 ] = 2049

Calibration factor (pA per ADC unit) = 0.00076
Sample rate (Hz) = 5e4

Low pass filter (Hz, -3dB) = 1e3

Generic data file has 27342878 bytes: name = c:/110509p1.abf
gain (pA per ADC unit) = 0.760000E-03: sample rate (Hz) = 50000.0
Display only (no step-response function) [N] ?
To direct input to this virtual machine, click inside the window.
```

What is the calibration factor? On the Axopatch 200B, full range at 100x gain is 200 pA (–100 pA to +100 pA). At 200x it is 100 pA and so on.

SCAN is works according to 16-bits, therefore ADC units = $2^{16} = 65\,536$

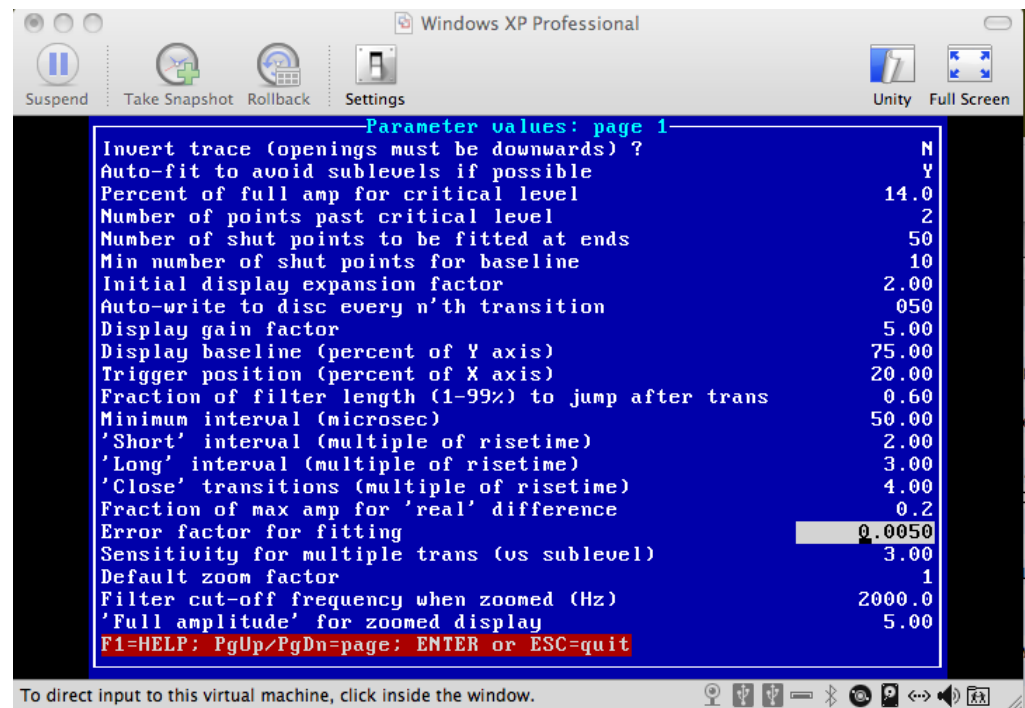
At 100x gain, pA per ADC unit: $200/65536 = 0.0030518....$

Enter the first few significant figures *e.g.* 0.00305

Input the sampling rate. Here I put 50 kHz (5e4). It must match your data file, or the timing will be messed up.

Input the filter you used in Axograph (1 kHz = 1000 or 1e3). SCAN relies on the properties of the filter in oversampled data so this combination is fine.

You may have to adjust SCAN.ini settings to get an appropriate gain, in order to see things on the screen. Fraction of full for real difference is a question of taste. Probably 0.1 is a good setting. Depending on how many sublevels you have. You can check that you put the right scaling factor by looking back to a good full opening in your Axograph file. Frequent auto-writing to disc is a good idea and doesn't hinder performance these days.



Begin scanning!

You'll likely need to read the DC progs manuals
dcmanuals.pdf

Printing

From DOS Autplot, print as "postscript" – the large file warning is a thing of the past. You will obtain a file (in the DCProgs home directory) that can be opened in 'Preview', 'Illustrator' or 'Photoshop' on the Mac. It seems unlikely that any printing could occur (especially if no printer is installed on the VM).

From Windows Autplot, I have usually printed as pdf and dragged to Mac. Switchers with good memories will know that printing as pdf isn't available Windows XP. You can install Bullzip or another pdf printing application to do that.