

Analysis

Analysis routines (run on LINUX):

iman – IMage ANalysis; makes maps out of raw data files saved by the acquisition program (**ContImage**), can make movies, pictures of green, compresses and decompresses raw data files.

mapans – MAP ANalysis Single; map viewer, opens and modifies a map file saved by **iman**.

mapanm2 – MAP ANalysis Multiple 2; combines two maps (adds, subtracts, divides, or other).

binan – BIN ANalysis; opens, manipulates, and animates movies or binned frames made by **iman**.

Flow of data in a typical imaging experiment:

ContImage saves raw frames in a set of files ->

iman (depending on specified command line options) produces a map, binned frames (movies) or condition maps ->

mapans opens and manipulates maps made by **iman** ->

mapanm2 combines 2 maps to remove hemodynamic delay (the two maps are of opposite direction of stimulus flow) or to reduce noise (the two maps are made with identical stimulus)

binan animates and manipulates the binned frames produced by **iman**.

Most common options for optical imaging data analysis:

1. Run **iman** on the raw stream files:

iman -s -D -T1 [-t] t_abc.1x00 where abc is a code for the date and 1 is the experiment number and x is the run number (0-z)

Iman takes -F2 to do orientation out of a 360 direction of movement stimulus.

Iman takes -R1 for episodic stimulus analysis

Iman -w8 to view green files

Running iman with the -t option allows you to look at the power spectrum of any selected point, and at the stimulus phase sawtooths.

All interactive commands except saving the map are given in the interactive window.

In all cases iman produces a mapraw file with a name like t_abc.1x00.mapraw2_46_58_2641dt1, with the numbers telling about the frames and cycles included in the analysis

2. **mapans** can be used to normalize the mapraw files generated by iman

mapans -i -o6 t_abc.1x00.mapraw2_46_58_2641dt1 generates a file of the same name with the suffix _a

To select an area for normalization, hit 'c' (for 'crop') in the interactive window (a grey scale window) and trace a region of interest for normalization (left mouse button to mark points, right to close region), and then hit 'n' (for normalize). If you don't crop first but just hit 'n', then it uses the whole image for normalization. If you like it, hit 'c' and then 'c' again to normalize the whole image according to the values obtained from the region of interest.

All commands are given in the interactive window. 'S' saves the file. 'Q' quits.

With excellent quality images, normalization is not required, but it generally does not hurt if the full range of phases is present in the response, or if there is enough off-brain area with random pixels in the image

3. **mapamn2** can be used to compute the average image from two normalized images that were made from stimuli moving or rotating in the opposite directions (time-reversed stimuli).

mapamn2 -i16 file1 file2 where these files are the output of mapans.

Orientation: file1: CCW (-1), file2: CW (+1).

Topography: file1: 0 deg, file2: 180 deg; or else file1: 90 deg, file2: 270 deg

SF, TF: file 1: ascending, file2: descending

All commands are given in the interactive window. 'S' saves the file. 'Q' quits.

4. To print pictures, **mapans file1** where file1 is the output of iman (ends with dt1) or mapans (ends with dt1_a)

Press <RETURN> in command window. In response to the prompt "Save image?" type y for both the phase and amplitude maps, or Y if you want just the phase map. It will output a string with suggested filename and output method in the form "t_abc.xxxxxxx.ps/VCPS". Copy and paste that string, then press enter to save the PostScript file.

To view the file, **gv file1** where file1 is t_abc.xxxxxxx.ps. To print in color, **lpr -P830c file1** or for black&white use 811 or 830 instead of 830c.

ImAn

iman – IMAge ANalysis. The program generates maps from the raw data files saved by the acquisition program (**ContImage**). Also, it makes movies, pictures of green, compresses and decompresses raw data files.

The program is evoked from the command line of a terminal with one required file name and options. The passed options depend on type of the file to be analyzed as well as on the kind of maps to be produced.

How to make maps

Iman makes maps by finding the phase and amplitude (or equivalently the real and imaginary parts) of the Fourier component at the frequency of stimulation independently for every pixel of the image. The program does not require specification of the reference point and stimulus frequency, it uses the synchronization signal saved in the frame header by **ContImage** instead. The following example shows the simplest use of the **iman**

```
iman -s t_36d.0310
```

The program will open a sequence of files defined by one of its members (`t_36d.0310` in this example). Option `-s` tells **iman** to use internal synchronization. While analyzing the program will display every 100th raw frame on the screen (see Figure 1) and will generate a progress bar (each “.” = 100 frames, each “|” = 1,000 frames).

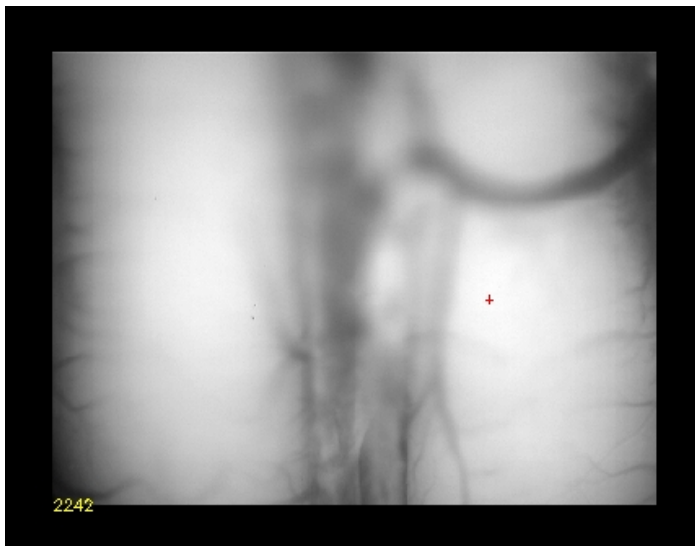


Figure 1. Screen shot of the main display window of **iman** (cat cortex). Yellow numbers in the left bottom corner show number of the frame being currently processed. Every 100th frame displayed. Red cross shows maximum, green cross minimum (not seen, hidden beneath the frame number).

Upon completion the maps are displayed in a four-panel window (top-left – phase map, bottom-left –

amplitude map, top-right – real part, and bottom-right – imaginary part) (see Figure 2) and the user is prompted (in the terminal window) to save the maps. The file name is automatically generated from the raw filename, the passed options, and from other information used to generate the map such as number of cycles, initial frame and final frame used for analysis.

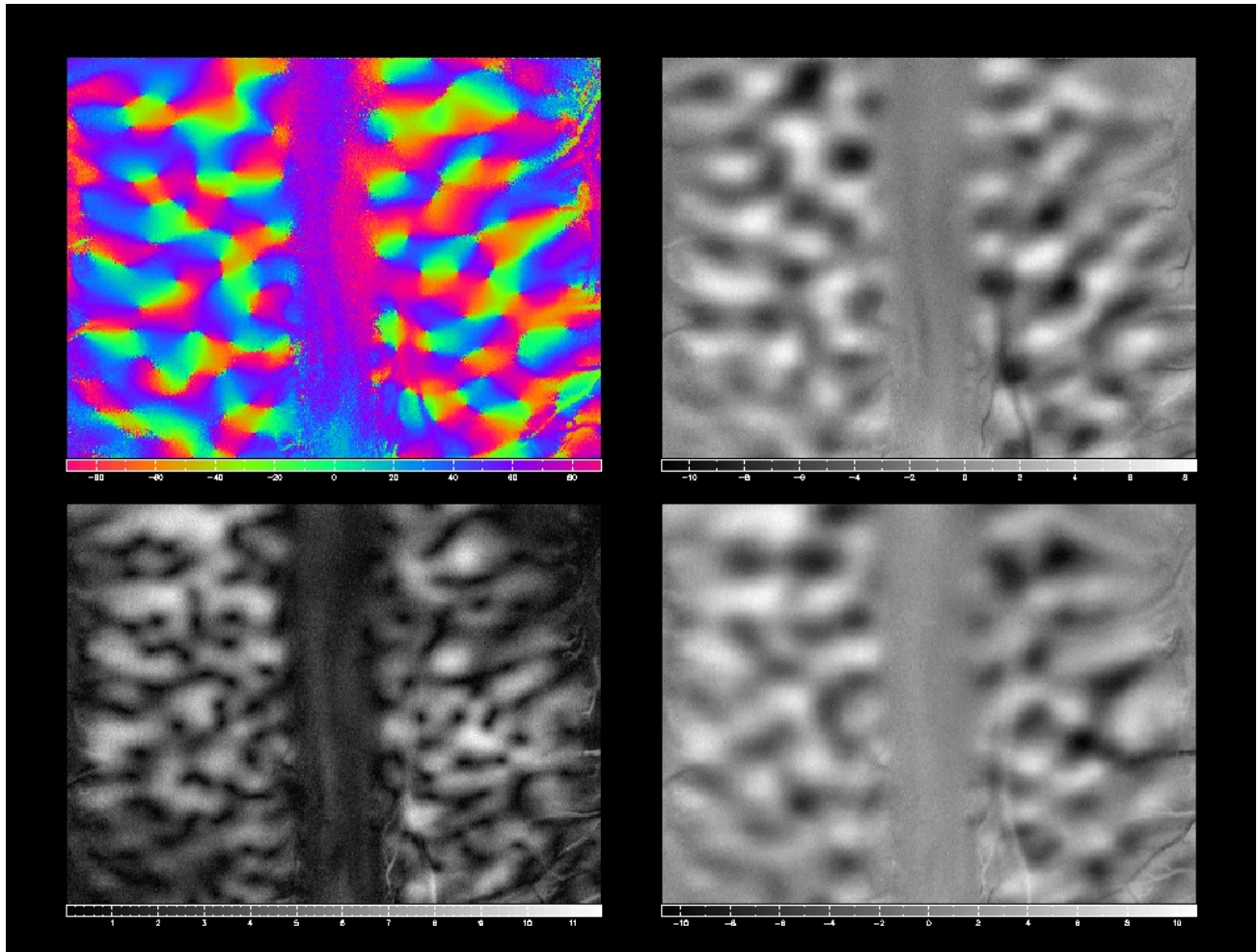


Figure 2. Screen shot of the map display window of **iman** (cat cortex). Displayed maps are phase map (top-left panel), amplitude map (bottom-left panel), real part (top-right panel), and imaginary part (bottom-right panel). These maps are generated with a command line `iman -s -T1 -D -F2 t_36d.0310` (see below).

The frame headers of the raw data files may contain up to four synchronization channels. **Iman** uses channel 0 by default (`-s` is equivalent to `-s0`). To specify channels other than 0 use appropriate channel number after `-s` option. Only one channel is used usually.

The simplest application of **iman** does not remove effects of the slow artifacts of hemodynamics. Time averaging is used to remove these artifacts. Option `-Tx`, where x is a half-width of the sliding window measured in cycles of the stimulus, tells **iman** to run the time series through a sliding window filter before the Fourier analysis. Typical value for the half-width is 1. The smaller values may attenuate the evoked response, and the larger ones may not remove the slow hemodynamics artifacts effectively. The following demonstrates the use of **iman** with both Fourier and sliding window filtering

```
iman -s -T1 t_36d.0310
```

Maps generated by the invocation line shown above will have the amplitude of response calculated as absolute values, where strongly illuminated regions (usually the center of the map) will have stronger response than the periphery, that is the strength of the response will depend on the level of illumination. To eliminate this dependence of amplitude of the response on uniformity of illumination the maps should be divided by average of all frames used to make maps, thus producing fractional response. This average is somewhat equivalent to the cocktail-blank average used in the conventional imaging method. To normalize maps by the average option `-D` should be used:

```
iman -s -T1 -D t_36d.0310
```

Typical value of the fractional response is about 0.0001-0.001. To translate it to a human friendly range the response values are multiplied by 10,000.

Iman generates maps at the frequency of stimulation by default. This is correct setting for maps of retinotopy or direction maps (drifting-rotating grating stimulus). Maps of orientation selectivity require doubling of the frequency of stimulation since every orientation is stimulated twice by any rotating stimulus. Option `-Fx` is used to specify harmonic of the frequency of stimulation, where x is the harmonic. Default execution assumes $x=1$. To generate orientation maps the following line should be used:

```
iman -s -T1 -D -F2 t_36d.0310
```

Another useful option (`-t`) does not effect map generation it allows the user to evaluate quality of the intrinsic signal (trace analysis). If this option is specified **iman** will spawn two extra windows for interactive analysis. One of the windows

(interactive window, see Figure 3) will display first raw frame from the series; this window has a cross cursor. By clicking on the image the user can plot the intrinsic signal at the clicked on pixel as a function of time or frame. The plot will be made in the other window (plot window, see Figure 4). The following line will allow interactive evaluation of the intrinsic signal:

```
iman -s -T1 -D -F2 -t t_36d.0310
```

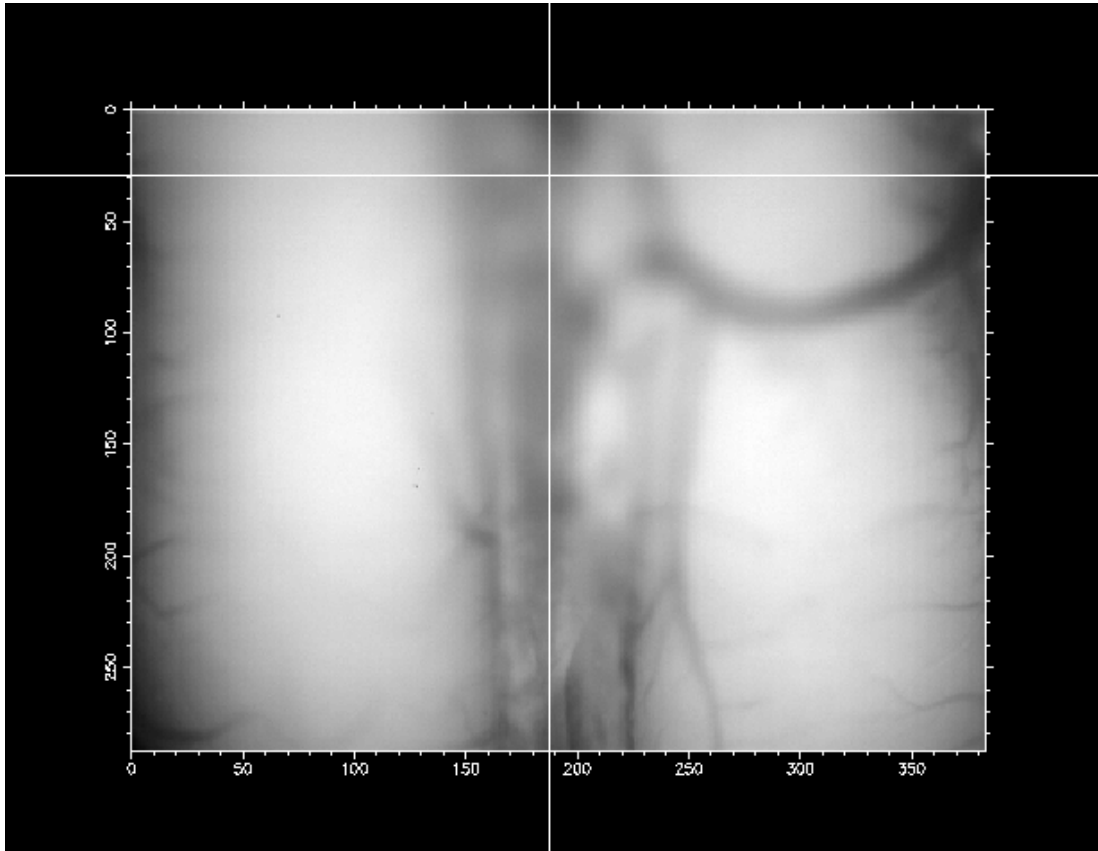


Figure 3. Screen shot of the trace interactive window of **iman** (cat cortex). User can select pixels of interest by clicking on the image. The plot window (Figure 4) will display intrinsic signal from selected pixels.

The plot window is divided into three panels. The top panel shows the signal from the last selected pixel (green trace). Also, it shows the averaged signal (white trace). A label on top of the panel shows coordinates of the selected pixel. The middle panel plots signals from last 4 selected pixel, thus allowing comparison of the intrinsic signal from different parts of the image (Figures 4 and 5). The traces are plotted in different colors. Labels on top of the panel show coordinates of the selected pixels. The same panel also plots the synchronization signal (white saw-tooth trace).

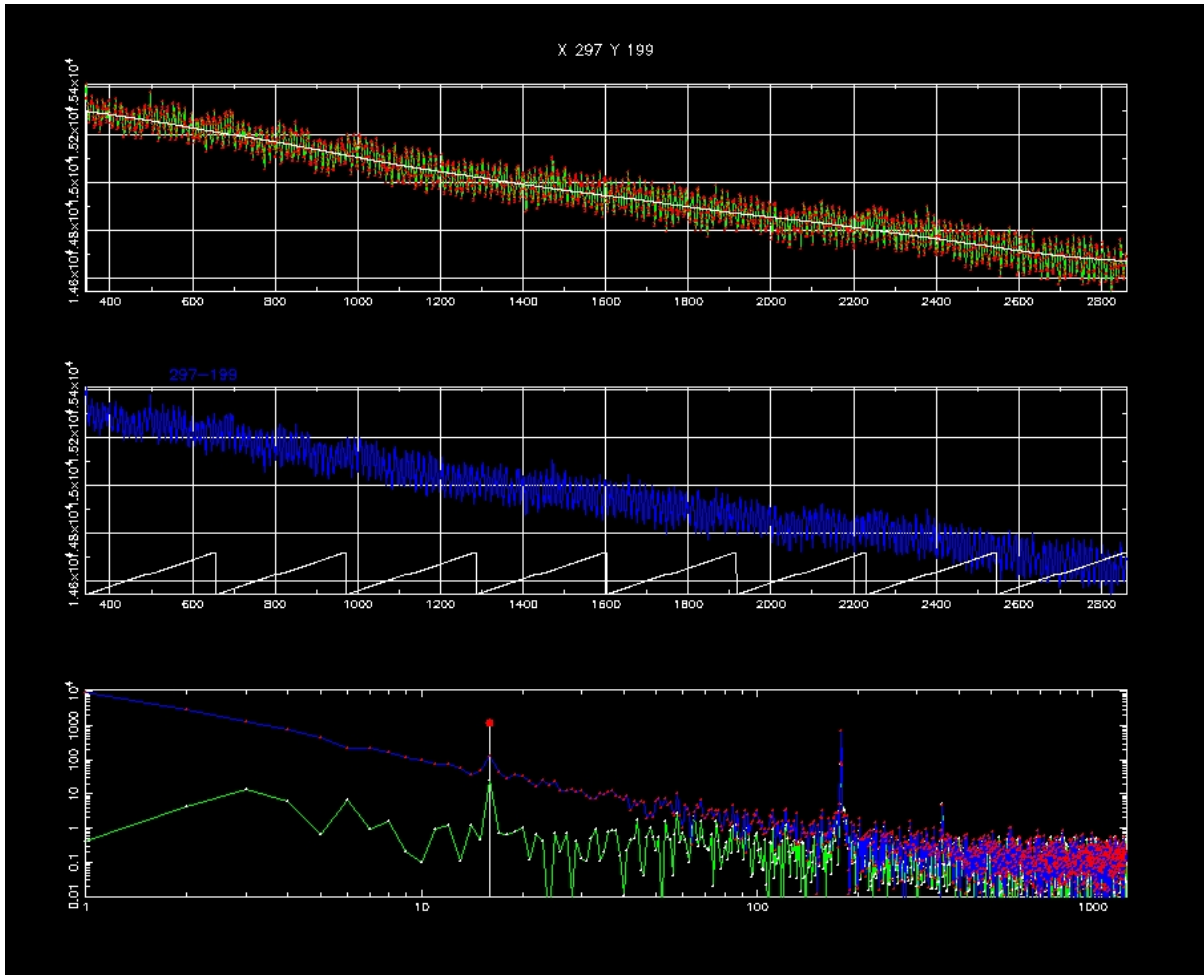


Figure 4. Screen shot of the trace plot window of **iman** (cat cortex). (Top panel) Intrinsic signal dynamics from select pixels (see Figure 3) is plotted in the top panel (green trace with red dots showing actual values). Abscissa – frame number, ordinate – intensity of reflected light. The white trace is plot of the averaged signal. The averaged signal is used to compensate for slow artifacts (drift). Label on top shows coordinates of the selected pixel. (Middle panel) This panel accumulates traces from up to 4 selected pixel for comparative analysis (only one trace is shown for clarity, see Figure 5 for another example). The traces are plotted in different color (same color is used to show coordinates of the pixels, see left-top corner of the panel). The white tooth-like plot is the synchronization signal (8 cycles here). (Bottom panel) Power spectrum of the accumulated pixels. Abscissa – number of cycles, ordinate – power of intensity of reflected light, both in log-scale. The color used to plot power spectrum traces is the same as that used for temporal series in the middle panel. White vertical bar with red circular cap identifies the frequency of stimulation (16 cycles here, 8 cycles of direction stimulus were analyzed at second harmonic of the frequency of stimulation to generate orientation map). The green trace is the power spectrum from the last selected pixel with removed slow component, that is the white trace (see top panel) was subtracted from the green one and power spectrum of the result was computed. Note strong spike at the frequency of stimulation (at 16 cycles). Even stronger spike is present at about 180 cycles. This is respiration artifact (this animal was artificially ventilated). Both the strong linear drop and ventilation artifacts are effectively removed by **iman**.

The bottom panel plots the power spectrum of the selected traces (up to 4). The power spectrum is handy for evaluation of the response and artifacts. The white vertical bar identifies the frequency of stimulation. Pressing “q” in the interactive window will quit the trace analysis and **iman** will proceed to map generation. Pressing “Q” will end program’s execution.

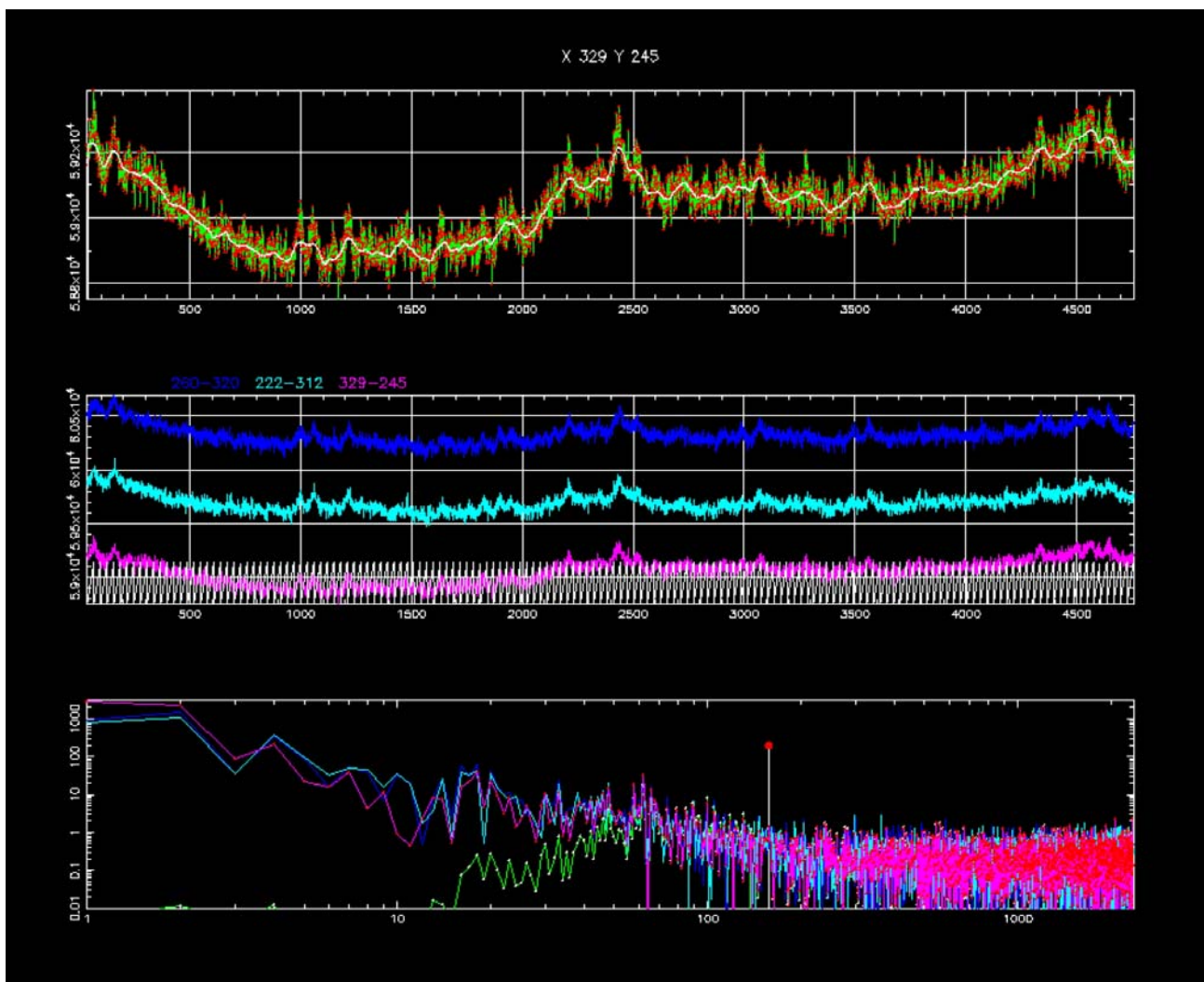


Figure 5. Screen shot of the trace plot window of **iman** (mouse cortex). This example shows multiple (3) traces. The signal is quite contaminated by vasomotion. There is no visible response at the frequency of stimulation (bottom panel).

The trace analysis allows identifying hemodynamic artifacts in both temporal (top panel) and spectral (bottom panel) domains. If the signal has sharp jumps (see Figure 6) or spikes, and not dominated by them, it is recommended to remove the

unstable part of the signal by first finding the range of “stable” frames (abscissa of this panel shows frame numbers), quitting the program by pressing “Q”, and restarting it with two extra options which specify beginning (-B) and end (-E) of the sequence of interest. For example, the user acquired 5000 frames. The trace analysis reveals a strong change of the signal around frame 4050. To remove the “bad” frames from the analysis the user should restart the program with the following command line

```
iman -s -T1 -D -F2 -t -E4050 t_36d.0310
```

-E4050 tells **iman** to use frames up to 4050 only. Similarly, option -B limits the used frames from the beginning of the frame sequence (see Figure 6).

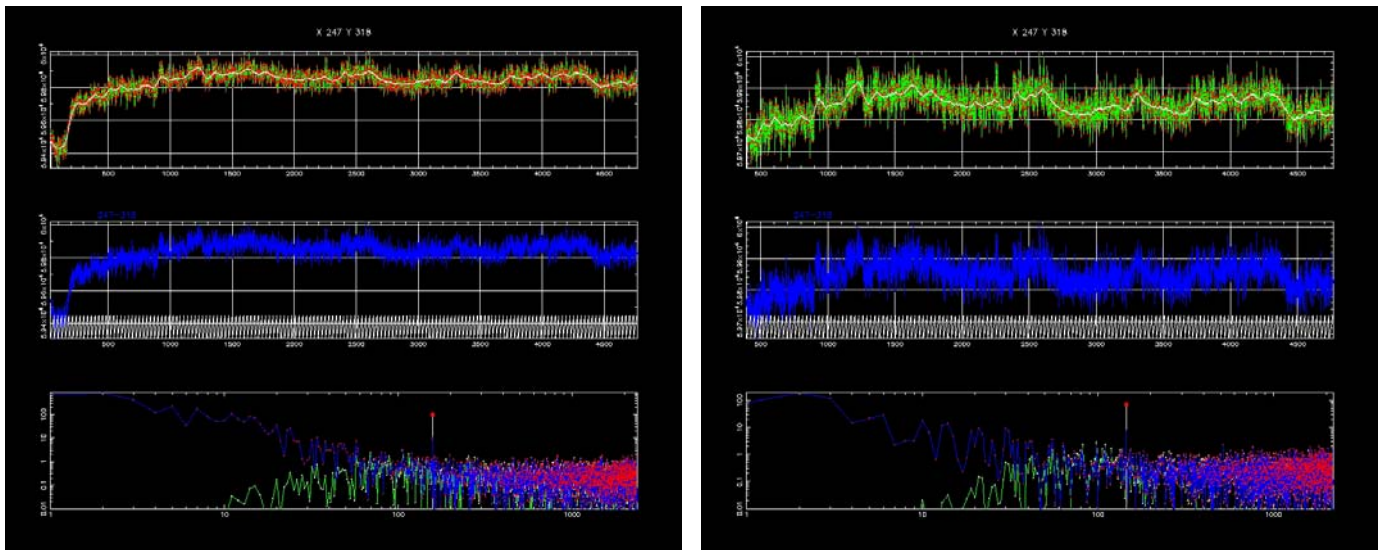


Figure 6. Screen shot of the trace plot window of **iman** (mouse cortex). (Left panel) This example shows a trace with a relatively strong jump in the beginning of recording (approximately between frames 200-350). The program was started with the following command line: `iman -s -T1 -D -t t_3a5.0e00`. (Right panel) Trace from the same pixel as in left panel. **Iman** was restarted with the following command line: `iman -s -T1 -D -t -B350 t_3a5.0e00`. This time the first 350 “bad” frames were removed from the analysis. Note that the power spectrum of slow part of the spectrum went down by at least half an order of magnitude. There is a strong response at the frequency of stimulation in both cases.

The power spectrum panel (bottom panel) allows identification of hemodynamic artifacts since they are usually of cyclic nature. Large humps in the plot reveal spectral band contaminated by the hemodynamics (vasomotion). If the vasomotion is very strong it can be identified on the temporal plot (top panel) as well. The

frequency of stimulation (shown on the plot as a vertical white line) should always be chosen away from these artifacts.

How to make “green” images (pattern of vasculature)

A simple invocation of **iman** with a “green” file name will display the cortical vasculature

```
iman g_36d.0310
```

The user will be prompted: “Save green (y/n): ”. If “y” is entered the user will be prompted for file name and device information. **Iman** can save images in various formats. Preferred format, however, is PostScript. This format offers highest image quality and it always can be converted to other formats, for example by Photoshop. To save the green in file “green.ps” in PostScript format the following line should be entered

```
green.ps/vcps
```

where /vcps specifies device or file format (vcps stands for Vertical Color PostScript). Iman will save the file and exit. The saved image will have coordinate system with label drawn around it. To save green image only (without any labels) option -V should be used. Option -wx, where x defines size in inches, controls size of the green image. It is 5 inches by default. UNIX programs gv and ghostscript can be used to view PostScript files.

Appendix

Frequently used options.

- h – print list of options and exit.
- s[integer] – use specified synchronization channel (from the frame header of the file produced by **ContImage**). If no integer is specified (-s) channel 0 is used. Most of the experiments use only one synchronization channel (data passed by **ContStim** to **ContImage**). The synchronization is required for identification of the state of the stimulus at the moment when the frame was acquired.
- T<float> – specify half-width of the sliding time average window in terms of number of full cycles/conditions. The first and last cycles are excluded from the map assembly and used only for averaging by default. To retain the boundaries use -U option.
- D – divide (normalize) the map by average of all frames. Similar to cocktail blank normalization. The final pixel values are multiplied by 10000.
- F<integer> – make map of an <integer> Fourier component. -F1 is assumed by default.
- t – perform full Fourier analysis of individual pixels.
- B<integer> – start analysis from frame <integer>.
- E<integer> – end analysis on frame <integer>.

Full list of options

- l - do linear fit, (default off, -r and -R supersede this option)
- A <int> - additional frames (default 0)
- B <int> - initial frame (default EPST=0 COST=1)
- C <int> - number of cycles (default 1)
- D - divide by average image (evens illumination non-uniformities) (default off)
- E <int> - final frame (default 1+1*NFRAMES/CYCLE)
- F <float> - harmonic (default 1.00)
- H <float> - high-pass filter domain (default 0.0) (size of green)
- I <float> - period of stimulus in seconds, uses interframe times (default off)
- L <float> - low-pass filter domain (default 0.0)
- N [int(default 1)] - number of bins for temporal frame binning, requires -T option (default off)
- P - do precise analysis (default off)
- Q - decompress file

-R [int(default 0)] - rule number for binning
-S <int> - number of bins, do statistics (default off)
-T <float> - 1/2 of time averaging window (cycles/condition blocks)
(default off, trace default 100 frames)
-U - sacrifice boundaries off, works with -T (default on) (format
green output)
-a - do averaging (default off)
-b - find best cycle (default off)
-c - draw contour lines (default off)
-f <filename> - needs 1 files
-h - print this help
-i - show interframe timing (default off)
-k - keep original (compressed/decompressed) files (default 0)
-l <int> - Fourie series lower cut-off (default 1)
-m - multiple frame mode (default single)
-p <float> - period of stimulus (frames) (default frames/cycle, can be
bogus)
-q - compress file
-r [int(default Ncycles*0.10+2)] - degree of fitting polynomial,
(default off)
-s [int(default 0)] - use synchronization channel (default off)
-t - do trace analysis (default off)
-u <int> - Fourie series upper cut-off (default 1000)
-v - verbose mode, prints out a lot of crap (default off)
-w - size of green save image (default 6)