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1. Introduction

iPhys was developed with three goals in mind:

[Acquisition](#) of electrical signals, mainly from a single electrode. Comprehensive online and offline [analysis](#) of extracellular, current and voltage clamp experiments

[Visual stimulation](#) with patterned stimuli, specifically for experiments performed on the visual system of an animal

[Analysis of imaging files](#), with emphasis on repeated measurements of different experimental conditions from a number of different regions of interest ([ROIs](#)).

Optional analysis module performs [Receptive Field](#) estimation, which estimates receptive fields using on radon back projection.

Readable formats include TIFF image files, text data files and iPhys generated data files. The software can be fully controlled from the user interface, and the code is free to modify. Many tasks are automated; for example, acquisition files are automatically saved into the working directory; new traces are added to analysis as they are acquired and basic statistical tests are performed and displayed immediately. The user controls an extensive graphical interface with sticky windows (making dragging multiple windows easy), easy to activate and close plots and single-click generated analysis modules.

Analysis of acquired electrophysiology/imaging can be grouped together. Experiments (data and generated plots) can be saved to memory or hard drive for easy manipulation, batch processing and retrieval. This powerful option makes combining different experiments seamless, while preserving all the information of the individual experiments.

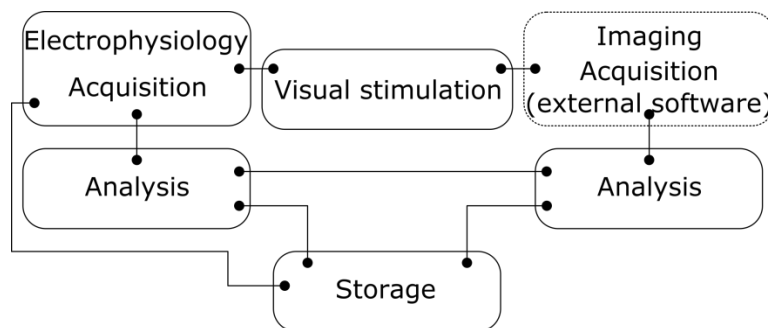


Figure 1-1 iPhys modules

2. Setup

iPhys works best on windows PC. Igor controls electrical data board (currently only ITC18, Instrutech, www.instrutech.com is supported). Igor controls the amplifier (for example Multiclamp 700b, molecular devices, <http://www.moleculardevices.com/systems/conventional-patch-clamp/multiclamp-700b-microelectrode-amplifier>) and can be used to trigger additional devices, such as a 2 photon microscope.

ITC18

Communication with the device is implemented via ITC18 XOP. The user can control basic functions (current/voltage steps) on two analog channels, read/write data from up to 4 analog up to 8 digital channels. **ITC18 needs to be installed on the computer.** See Instrutech website for installation details.

Multicamp

Gain and channel information is read directly from the Multiclamp 700B with axon telegraph XOP. **AxonTelegraph XOP needs to be present in Igor Extensions directory.**

Visual stimulator

The visual stimulator is implemented by a second screen which can be positioned in the animal's field of view or attached to the condenser for retinal experiments. In the latter case the visual stimulator can be composed of an optional LED to project monochromatic light onto a secondary LCD display. The stimulator is positioned beneath the condenser. This arrangement is convenient for the following reasons – (1) monochromatic LED can be controlled independently of the display. Potential usage may be to turn the visual stimulator on during galvo fly-back time. (2) The display can be visualized by a microscope camera. (3) the condenser can be focused on a desired plane independently of the 2p imaging from the objective. (4) minimal cost.

3. Electrophysiology

iPhys can read, store, manipulate and analyze electrophysiological data.

3.1. iPhysiology panel

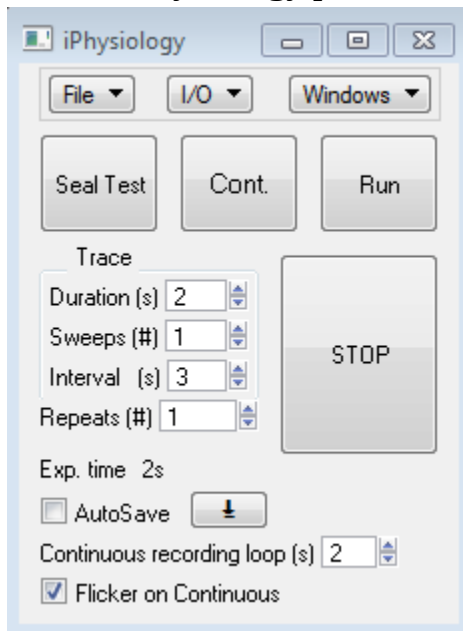


Figure 3-1 iPhysiology, the starting panel for working with electrophysiology

Recording is controlled with the main panel. If a file with the current date doesn't exist in the selected directory, one is created. If a file exists, the current run is appended to the file. In this way information is saved if Igor crashes and only the current run is in memory, which frees resources for analysis. After the run is recorded, it can be appended to the analysis window.

IPhysiology menus:

File->Selection of a new directory for automatic file saving and opening of existing files for viewing/analysis. The panel option opens a [File Info panel](#).


I/O->*Channels*, activates [Channel Info](#) panel for manipulation of recording channels. *Output*, opens **EPhysiol Output** and **Analog Output** windows, both of which are used to control the digital and analog outputs to ITC18. See [Composing Electrophysiology output](#).

Windows->[Visual stimulation](#), a module for controlling visual stimulation. [Electrophysiology](#), the gateway to analyzing electrophysiology. [Image analysis](#), the gateway to analyzing time series imaging files. [Receptive field analysis](#), a module to analyze and reconstruct receptive fields.

IPhysiology Buttons:

Seal Test; injecting brief currents/ voltage steps to assess the resistance of the recording electrode

Cont; continuous acquisition, data is not saved, the duration of the loop is set by **continuous recording loop**. For visual stimulation enabled experiment, continuous acquisition can activate 1Hz Flickers (**Flickers on Continuous**).

Run; acquisition. Data structure is as following – a trace is composed of a variable number of sweeps, where each sweep can have different stimulation parameters. Sweeps are separated by an inter-sweep-interval. Trace recording duration is set by **Duration**, number of sweeps in the trace is determined by **Sweeps (#)** and the time interval between sweeps by **Interval (s)**. Multiple repeats, in which the same stimuli parameters are displayed/injected for each new trace, are set with **Repeats (#)**. Traces are saved to the hard drive sequentially with **AutoSave** (before acquisition) or by pressing the down arrow  following acquisition.

Exp time; the calculated time to finish the recording (calculated as the number of sweeps in a trace, multiplied by the number of traces).

3.2. Data plot

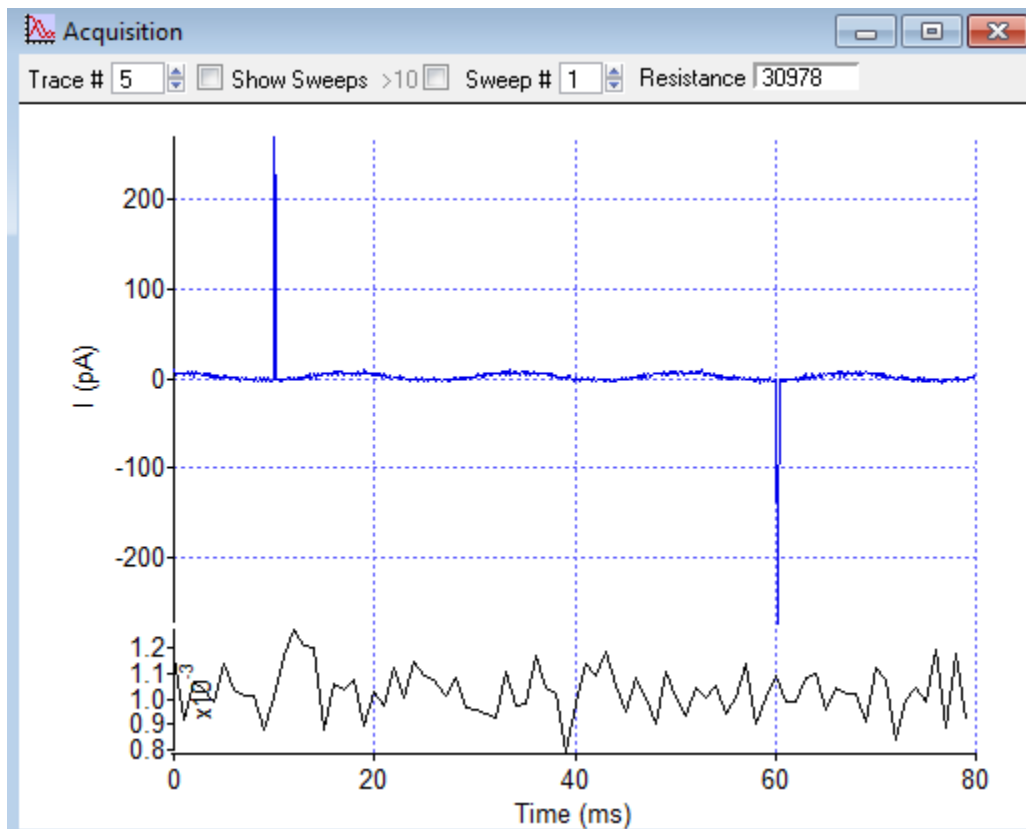


Figure 3-2 Data plot

The plot appears when new acquisition is initiated (either with **seal test**, **cont.** or **run**) buttons on [iPhysiology panel](#), or when electrophysiology file is loaded from the hard drive. Browsing between saved traces is done with the **Trace #** variable. For a large number of sweeps **Show Sweeps** should be deselected, otherwise Igor will stall while it displays a potentially large number of sweeps. **>10** allows to display more than 10 sweeps in this window. **Resistance** shows the calculated series resistance of the recording electrode (relevant for seal test and run modes). All used channels are displayed (in the example, channel 0 in blue, and channel 2 in black).

3.3. File Info panel

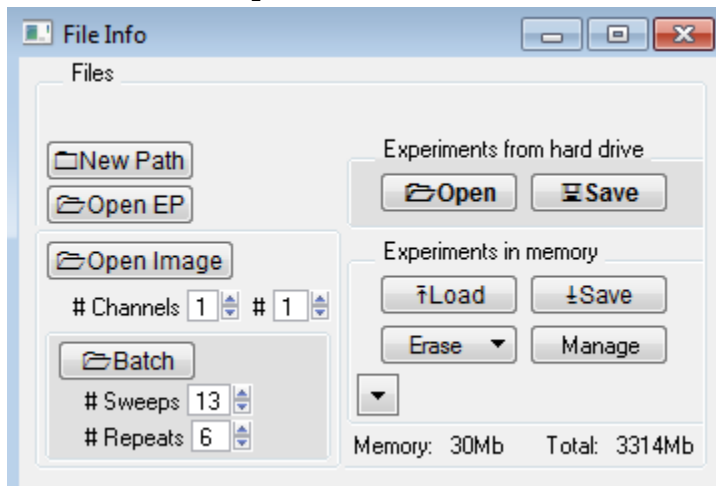


Figure 3-3 File Info enables users to work and manipulate files and experiments in memory and store/retrieve data from the hard drive.

New Path; directory on the hard drive to save and read from files.

Open EP; opens an electrophysiology (.dat) file.

Open Image; opens an image (.TIFF/.TIF) file. It is possible to open individual channel (a subset of images) by specifying the number of channels, and the desired channel (assuming that layers are arranged in the following order: 1,2,3,...n,1,2,3,...,n, with n as the **# Channels**).

Batch; opens a large database that is arranged of # repeats of #sweeps

Experiments from hard drive; Opening a previously saved experiment list appends the list to the current list in memory or saving the current experiments from memory to hard drive. Because the data in memory may be large and exceed the available memory (which can crash Igor), the **Memory** label specifies the memory dedicated to each experiment and how much total memory is left.

Experiments in memory; the current working environment, including analyzed data can be saved into 'experiments' folder, which can be then reloaded, saved and erased from memory. The list of available experiments is revealed by the dropdown menu. **Manage** – opens an [Experiment List panel](#) to view/modify experiments in memory. Experiments can share describing information by **duplicate info** button (see below) and ROIs can be stored and applied for imaging experiments (see below).

3.3.1. Experiment List panel

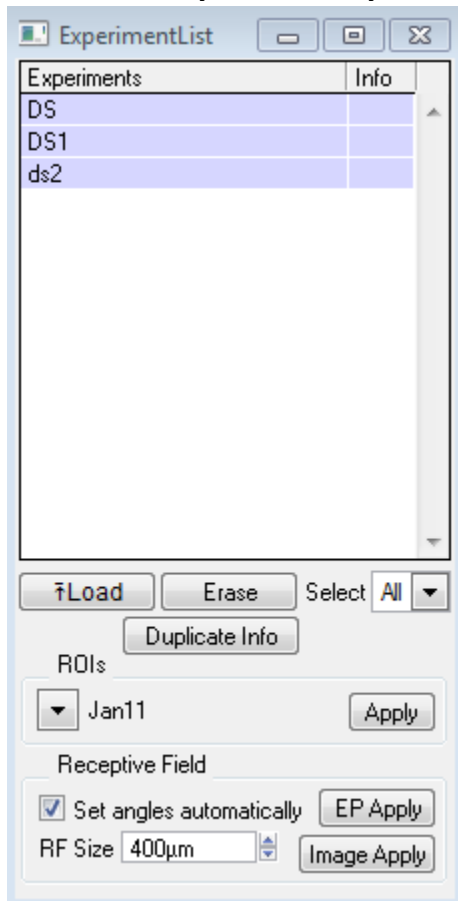


Figure 3-4 Experimental list panel

3.4. Channel Info

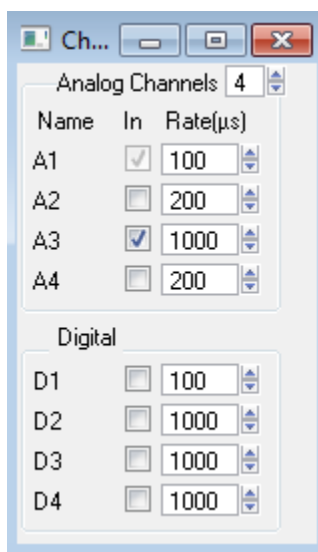


Figure 3-5 channel info panel. Sets active recorded channels and acquisition speed for each channel

This panel shows the available input channels and the corresponding acquisition frequency. The first channel is always selected.

3.5.Composing electrophysiological output

Each of the analog and the digital channels can be precisely manipulated with iPhys. Channels can be filled with arbitrary complex temporal stimulation patterns, which can be stored and retrieved, shared between channels and turned on and off with a click of a mouse.

3.5.1. E output panel

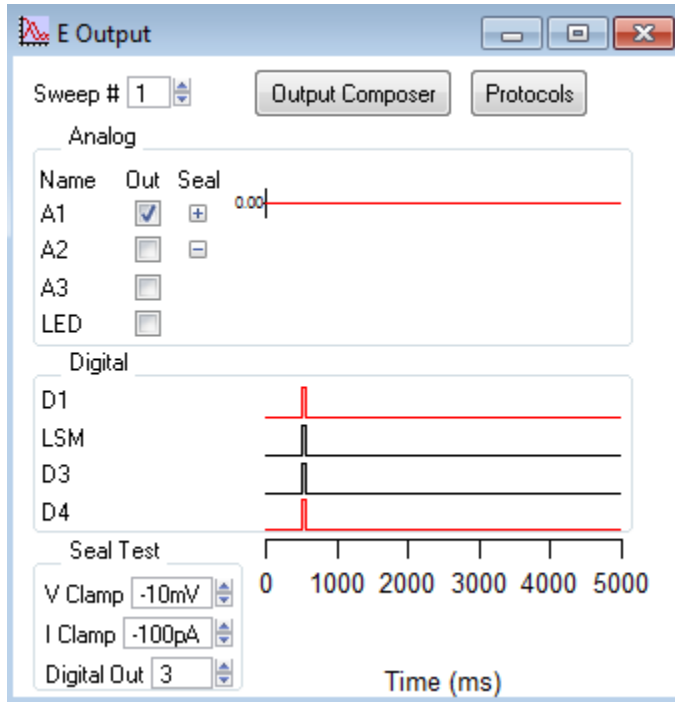


Figure 3-6 E output displays the temporally shaped output on different channels

Display of signals on the available output channels over time for a given sweep number. Channel names can be changed by left clicking on the name ('A1', 'A2', etc). Channels that have an output assigned to them are marked by the 'out' checkbox. Seal test (parameters left bottom part) can be applied to the first two analog channels.

3.5.2. Output composer

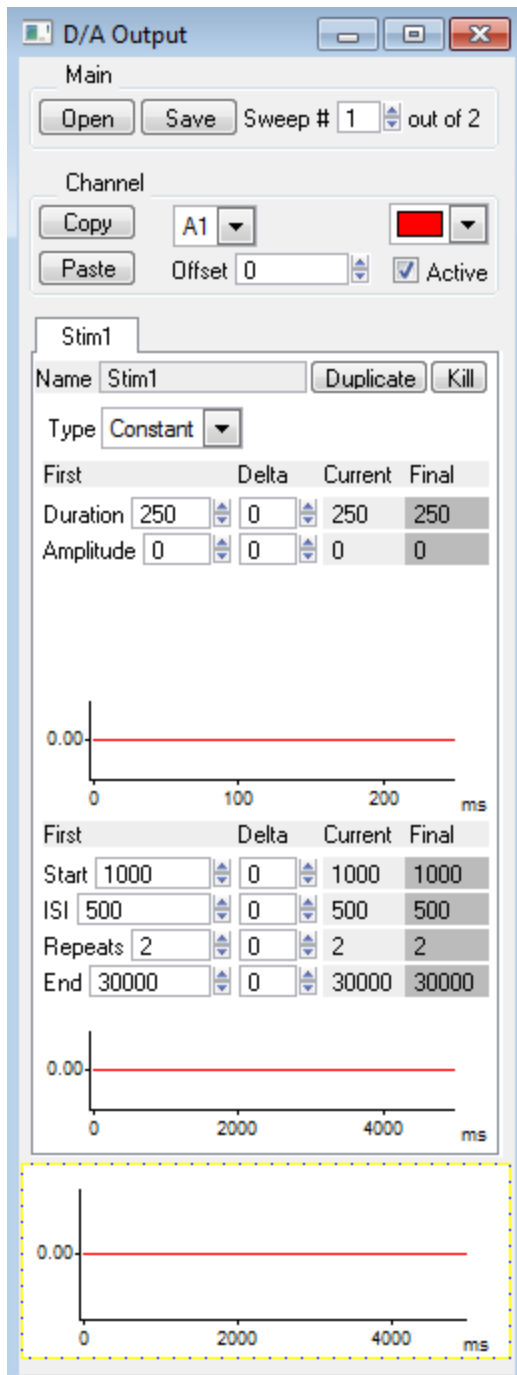


Figure 3-7 The main output composer window, used to create/modify electrical outputs.

Main-> **open** and **save** output protocols, sets the sweep# for viewing.

Channel-> **copy** and **paste** protocols between different channels

Channel popup menu selects the current working channel, which can have a color assigned to ease browsing between channels. **Offset** sets a baseline amplitude applied to the channel at all times. **Active** is used to quickly toggle active/inactive modes.

Multiple stimulation protocols can be assigned to each channel. Each protocol can have a unique name (same names are fine too), as set by the **Name** field. Stimulation protocols can be duplicated or killed.

Each protocol consists of one of the following:

Constant-> applies a constant step set by duration and amplitude fields.

Sine-> applies a sine wave. The user can set the baseline and the peak amplitude values, as well as the frequency and the phase of the sine.

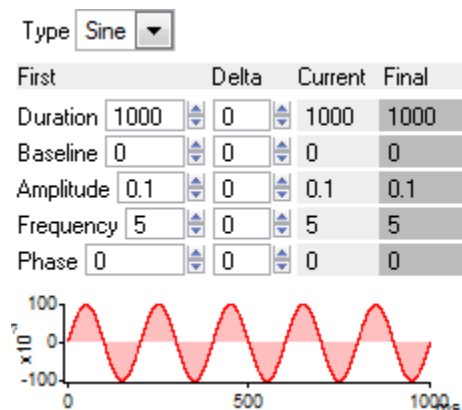


Figure 3-8 Example of a sine wave stimulus

Ramp-> applies a ramp from 'baseline' to 'amplitude' values.

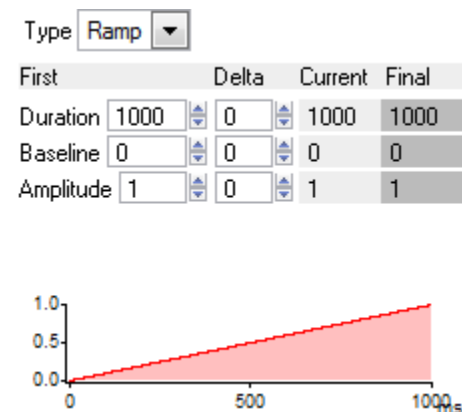


Figure 3-9 Exam,ple of a Ramp output

External-> user selected wave

Each of the fields can be changed between sweeps by 'delta' value. The panel will display the current value, as set by the sweep# (main box).

Each protocols can be repeated a number of times, starting at a 'Start' time, with inter stimulus interval (ISI) and a specific number of repeats. Optionally it is possible to set an 'End' time by which the stimulus will stop.

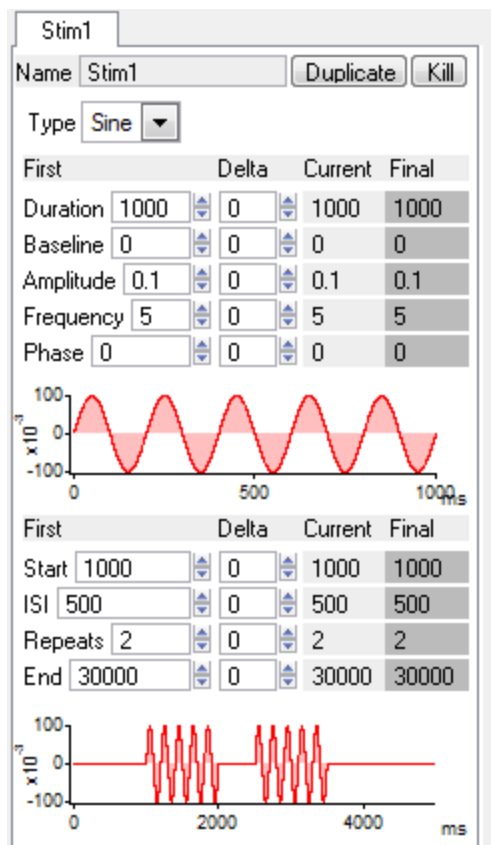


Figure 3-10 Timing and repeated presentation of the stimulus

Example of a repeating sine wave stimulus. The first graph shows the individual stimulus, the second one shows the stimulus as it repeats during an acquisition. Finally, it is possible to combine a number of stimuli to form arbitrary complex stimulation patterns.

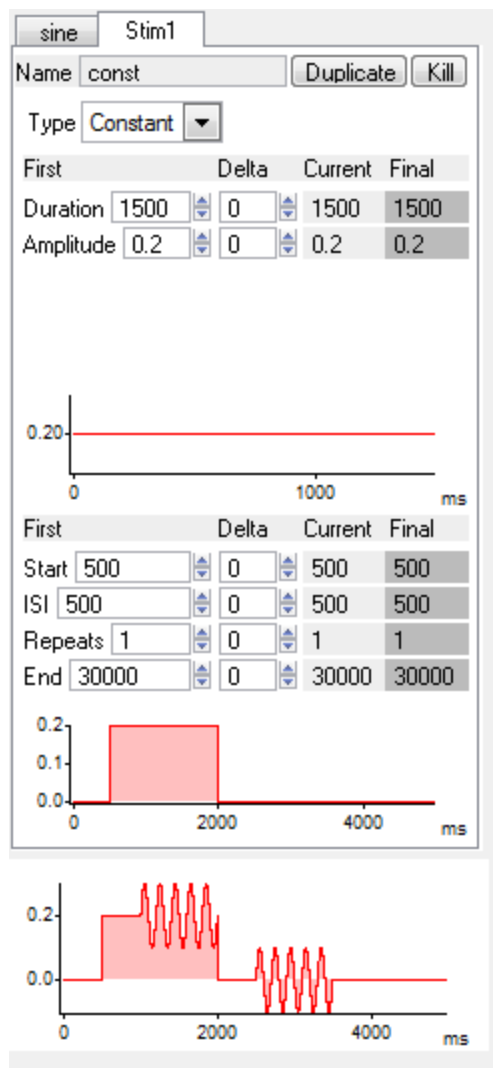


Figure 3-11 Integration of multiple stimuli

Example of a repeating sine wave combined with a constant step stimulus.

3.5.3. Saving and loading protocols

The **Protocols** panel is opened by selecting **E Output->Protocols**.

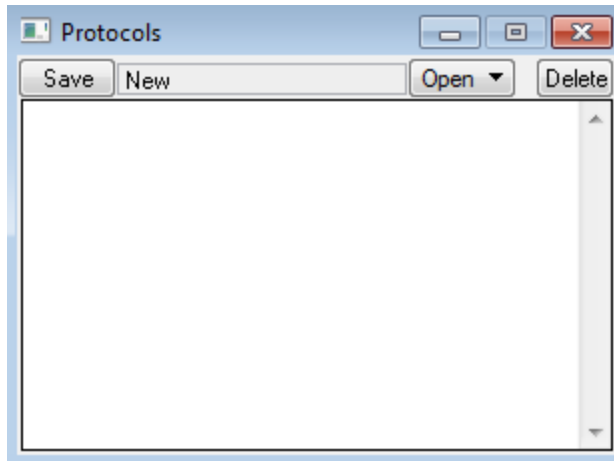
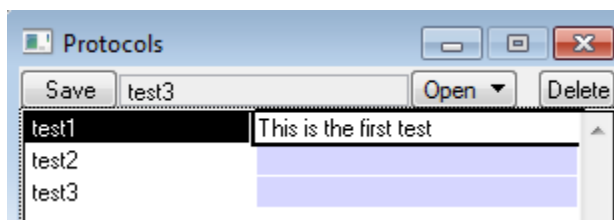


Figure 3-12 Protocols window for storing visual protocols

Current stimulation protocol can be saved by setting a new name under 'New' field and clicking on 'Save'.



The protocol will be saved. The name will appear on the left and an optional comment field on the right. When opening a protocol, the user will get an option to load a visual stimulation or electrophysiology protocol.

4. Analysis of electrophysiological recordings

4.1.EPANALYSIS panel

The main panel to analyze and organize electrophysiological recordings. Online data analysis as well analysis of saved data is possible.

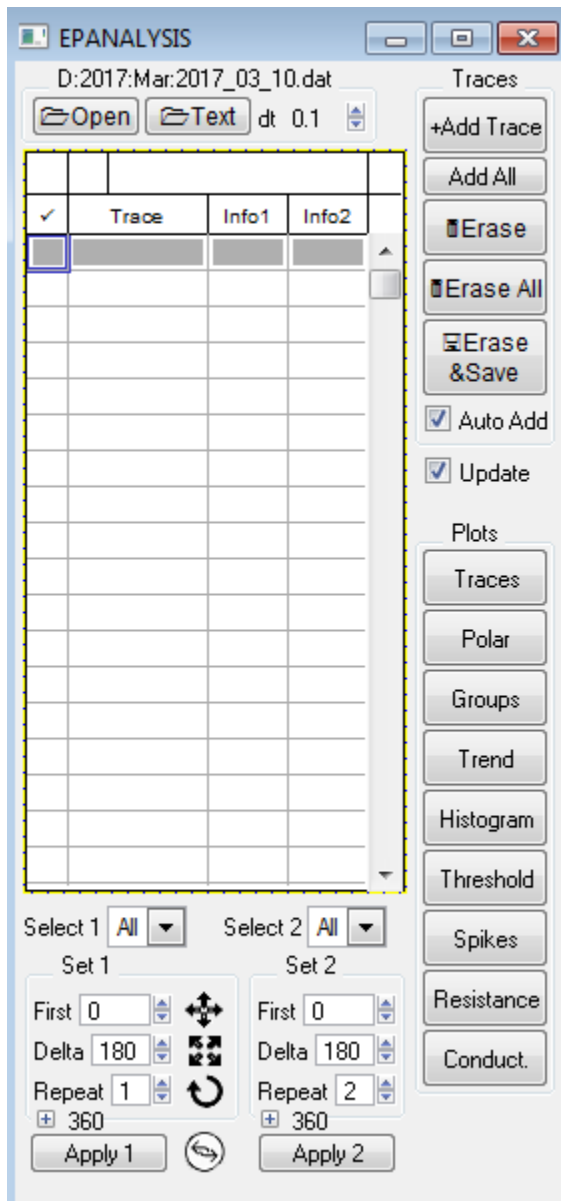


Figure 4-1 EPANALYSIS panel in the absence of traces set for analysis

Traces are added from the [Data plot](#). The number of the trace is followed by the sweep number. Text files can be opened with the **Text** button, with the x time step set by the **dt** variable. **Info1** may indicate groups, degrees or test type. **Info2** can provide additional metadata (see below).

+Add trace appends the current trace from the data plot. **Add all** appends all the traces (may take a while to process).

Erase deletes selected traces. **Erase all** deletes all traces. **Erase and Save** stores the traces in memory (see [File Info panel](#)) and deletes them from the analysis window.

Auto Add option automatically appends traces following an acquisition or when the user browses to a new trace in the data plot.

Update controls whether changes made to selection of traces or trace info update the plots.

Individual traces can be selected/deselected by clicking on them, shift and control buttons can be used as in a regular windows selection scheme.

Selection and metainformation assignment

Information for the traces can be entered manually on the info1 or info2 fields. For automatic population of these fields, several strategies can be used:

Set 1 / Set 2 controls assign numbers to the selected traces. The first '**Repeat**' traces get the '**First**' value. Then this value is incremented by '**Delta**' and the next '**repeat**' traces are assigned. The value is again incremented by '**Delta**' and the process repeats itself. Actual assignment is done with **Apply 1 / Apply 2** buttons. **+360** selection can be used for circular data to keep the values in 0-360 degree range (for example, a 400 value will be translated to 40, -10 to 350 etc).

[illegible]

Figure 4-3 Example selection of traces that had info1 value of 180 and info2 value of 0.

The **plots buttons** are discussed individually below:

4.2. Traces plot

Brings the EPtraces plot. This plot allows to visualize, manipulate and measure recorded signals.

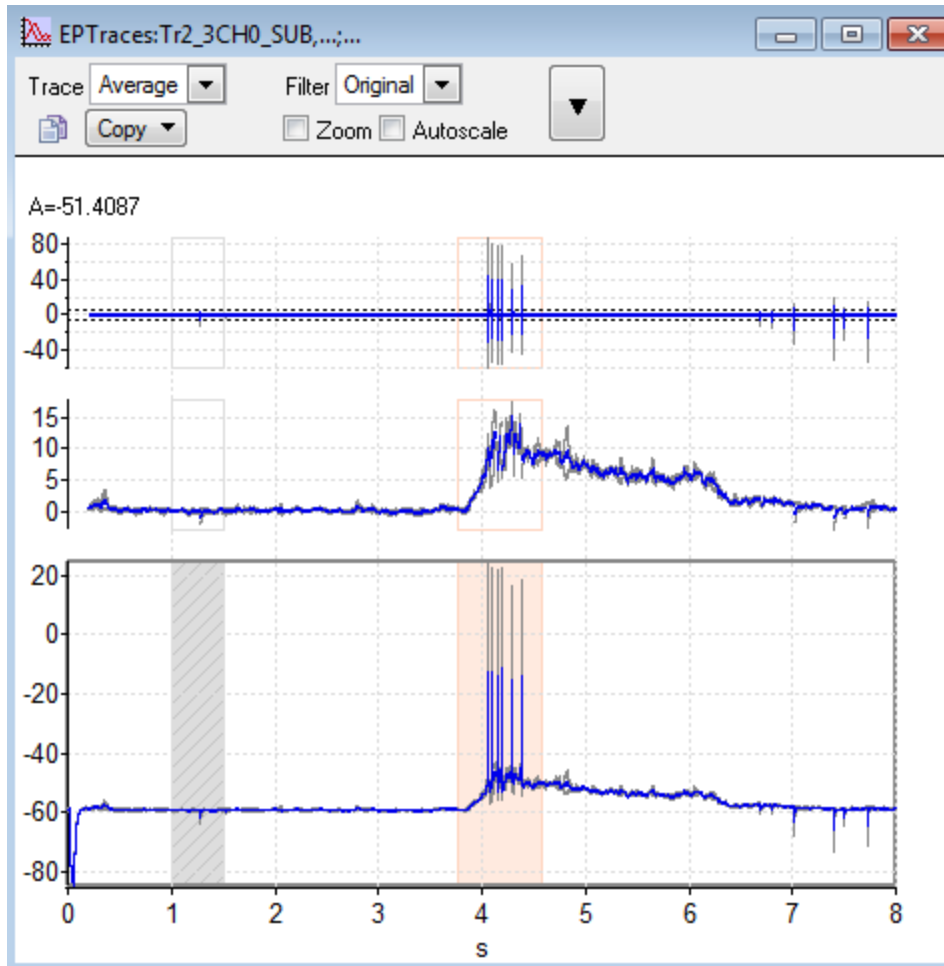




Figure 4-4 Epitraces plot window (collapsed view)

The EPtrace displays the selected traces on three plots. The bottom one is the original recording. The middle plot is optionally base subtracted, filtered, seal test subtracted and action potential subtracted trace. The top plot can be used to detect action potentials. The plot can be copied with the copy button.

The information can be exported in a table with the copy/paste icon . The parameters can be viewed

on the expanded version of the plot (collapsed/expanded view toggled by ).

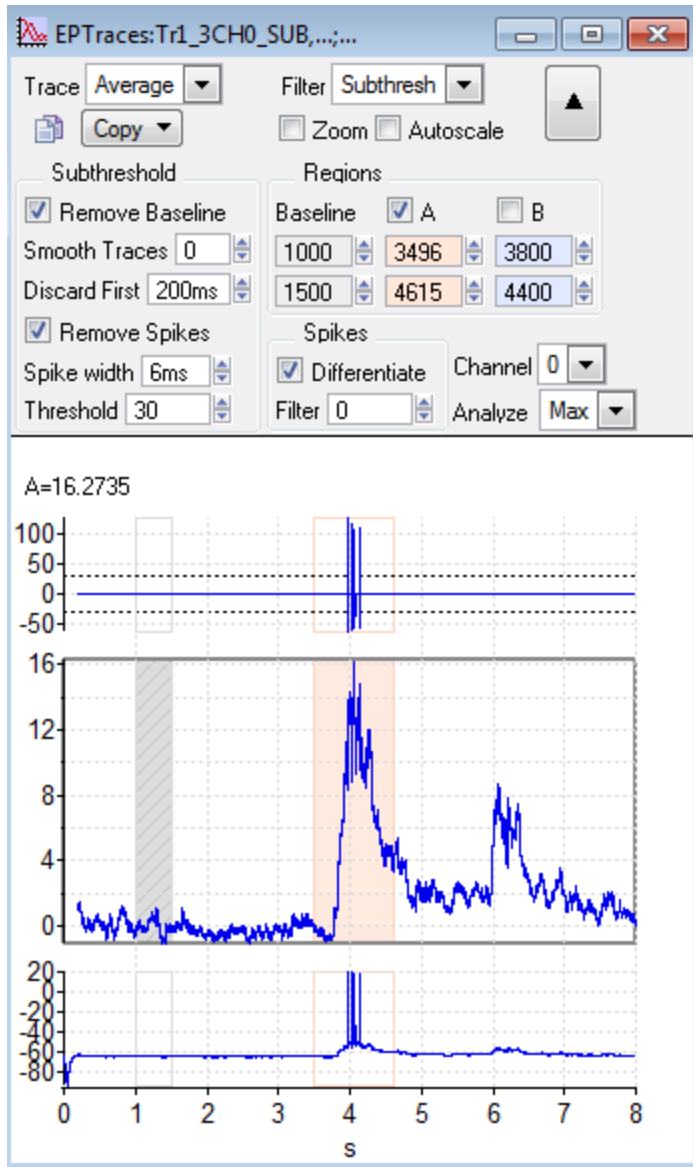


Figure 4-5 Figure 3 15 Eptraces plot window (Expanded view)

Subthreshold->smooth (Igor smooth function). 0 means no smoothing.

Subthreshold->discard first removes the seal test region.

Remove spikes inserts NaNs for '**Spike width**' duration in the subthreshold trace where spikes were detected. Spike detection is done from the top trace, when the signal crosses a threshold defined by a '**SD for AP**' value.

Spike trace can be the differentiated version of the original data (useful for current clamp recordings) or a high pass filtered version (useful for extracellular recordings).

Channel selects the analyzed channel

Analyze popup allows the user to choose from detecting the average, minimum/maximum of the trace or a the largest of the min/max (for spikes trace, only the number of spikes is reported).

The baseline / analysis regions can be set in two ways; first by entering start and end times in **regions** box. There are two potential analysis regions, **A** and **B**. Second, selecting a marquee, right clicking on it and selecting the appropriate name from the list. This will change the start and end field values as well.

Important! The selection (traces/time/spikes vs subthreshold/what to analyze) sets the data used for other analysis windows.

4.3.Polar Plot

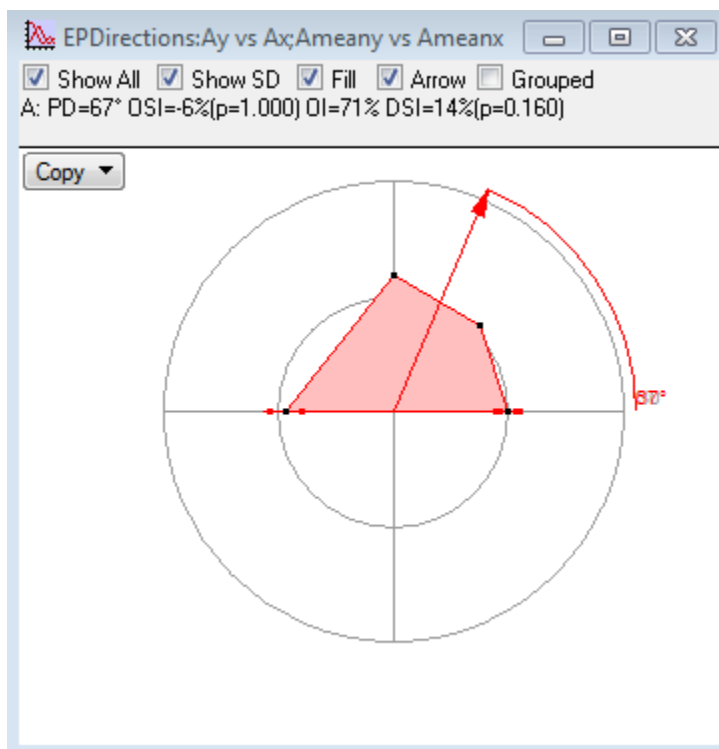


Figure 4-6 Example data displayed on a polar plot

The polar plot is used to display directional data. Angles in degrees are specified by **info1**. Igor will find the vector sum of the directions (here shown as a red arrow, with the angle of 67 degrees for this dataset, also indicated by PD or preferred direction). Individual responses are shown with dots. It is possible to use two types of averages; the first is the average of the calculated responses. The second is to generate an average trace for each direction first, and use these averages to display the directional plot (the second option is selected with '**Grouped**'). This may be useful for noisy data and does not affect the calculated statistics.

The analysis presented on the plot includes orientation selectivity index (OSI) orthogonality index (OI) and direction selectivity index (DSI) with the probability for randomly obtaining the value for each (p value).

4.4.Groups plot

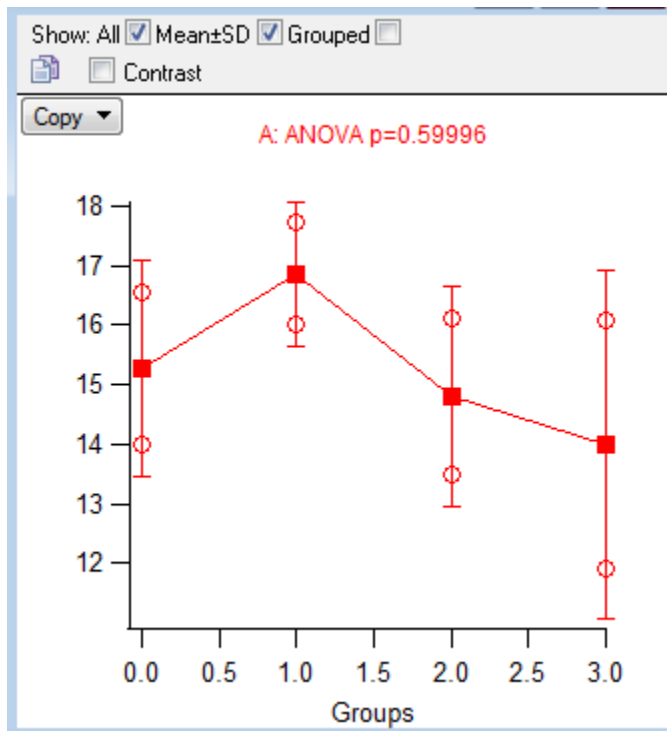


Figure 4-7 Example Groups plot

Presents the data as a function of info1 values. The difference between different groups is computed with ANOVA and the p value is displayed.

4.5.Trend plot

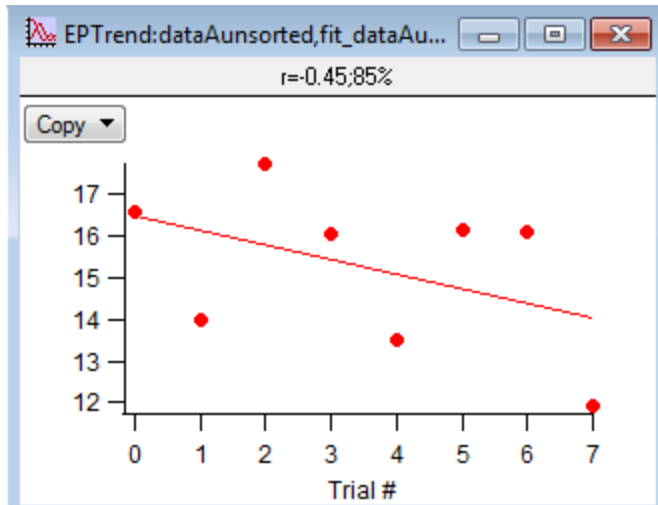


Figure 4-8 Trend plot shows the extracted data values as a function of trace number. Can be used to see if the responses are stable.

4.6.Histogram plot

The histogram plot can analyze the data in several ways; To show the different analysis modes we will use the following dataset, the values for analysis are set by the two colored regions (red and blue) as shown here:

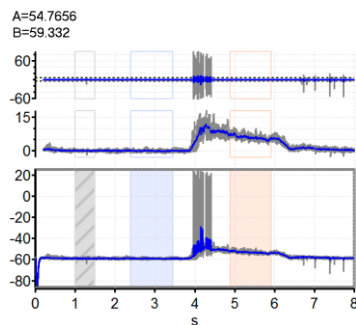


Figure 4-9 Data source for subsequent analysis

4.6.1. **Simple histogram** of each trace (red - A region, blue-B region, black-average trace)

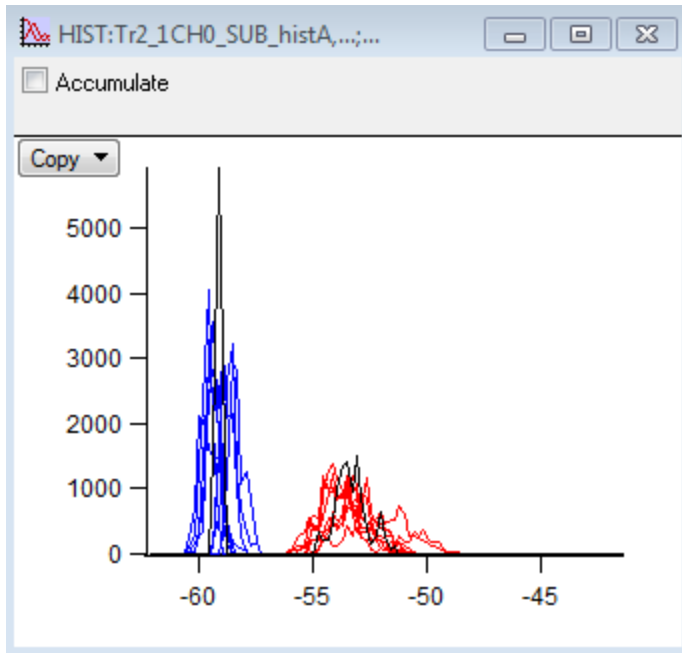


Figure 4-10 Histogram of the data values

4.6.2. When the **Accumulate** option is checked the plot shows a **cumulative histogram**

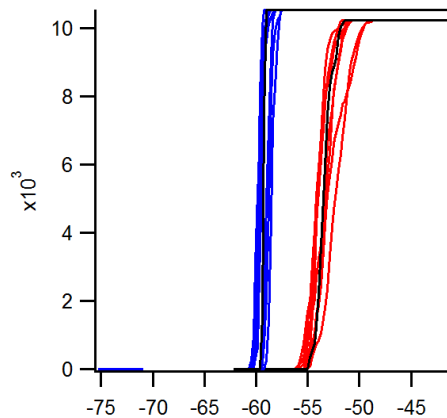


Figure 4-11 Cumulative histogram

4.6.3. **ROC** (reciever operator characteristics) – only when both A and B are selected. The area under the curve varies between 0.5 (identical) to 1 (completely separated).

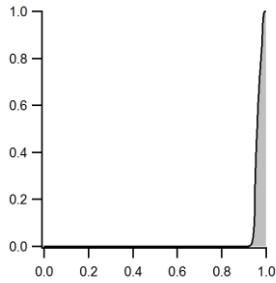


Figure 4-12 Example ROC

4.6.4. ACC Accuracy test - when **accumulate** is unchecked. The test examines how an ideal observer can distinguish between two values depending on the values on the bottom axis. The values range from 50% (identical) 100% (completely separated) according to the following equation:

$$\text{True positive} / (\text{False positive} + \text{True positive}).$$

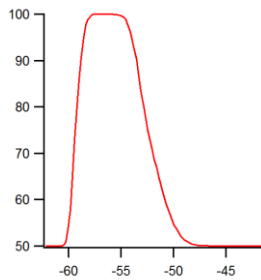


Figure 4-13 Example accuracy test

4.7.Threshold panel

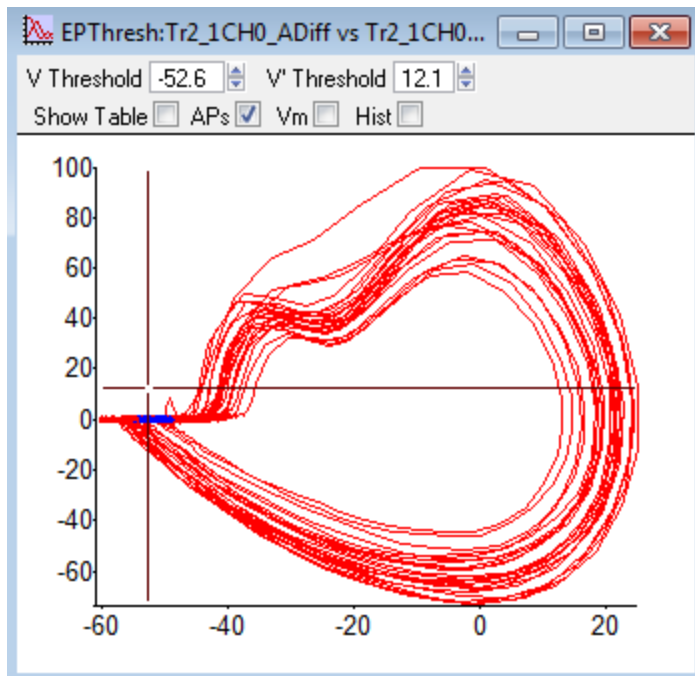


Figure 4-14 Threshold panel – membrane derivate vs. membrane potential

The threshold plot shows the voltage derivative vs. the voltage of the original data. The cursor sets the voltage/derivative threshold from which to detect spike occurrences.

Individual spikes are shown when **APs** is checked

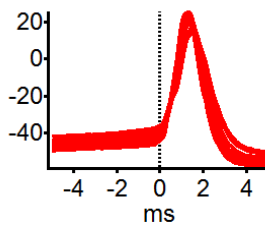


Figure 4-15 Spikes centered around detected threshold

Subthreshold potentials are shown when **Vm** is selected

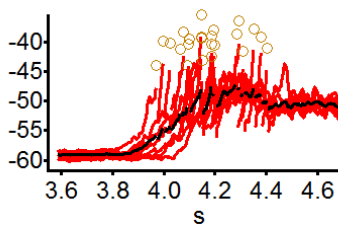


Figure 4-16 Subthreshold membrane potentials

Red- original traces, black -the average trace, circles – AP threshold

Histogram of the subthreshold potentials are shown when **Hist** is selected

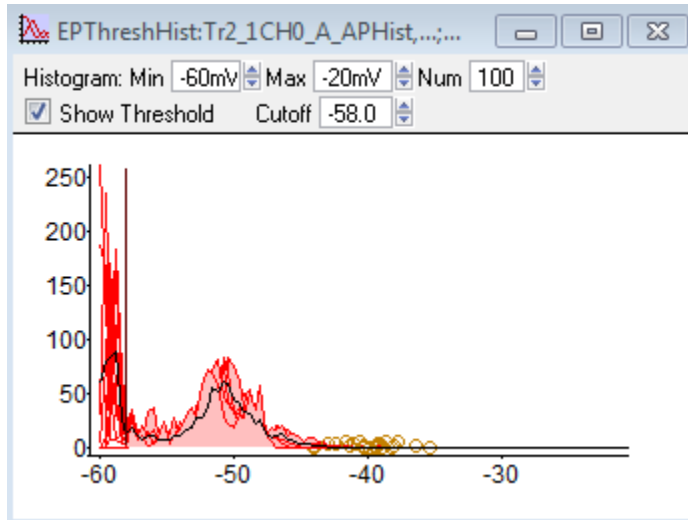


Figure 4-17 Histogram of the membrane potentials

User can select the min and max Vm values to compute the histogram, remove small responses (cutoff value) and show the AP threshold.

4.8.Spikes

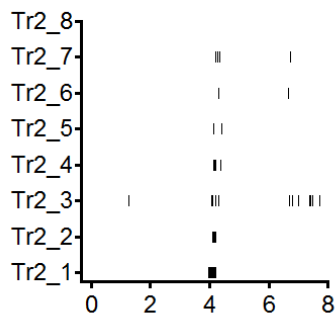


Figure 4-18 Raster plot of spiking activity

Displays the AP (spike) occurrences for different traces as a function of time (raster plot).

4.9.Resistance

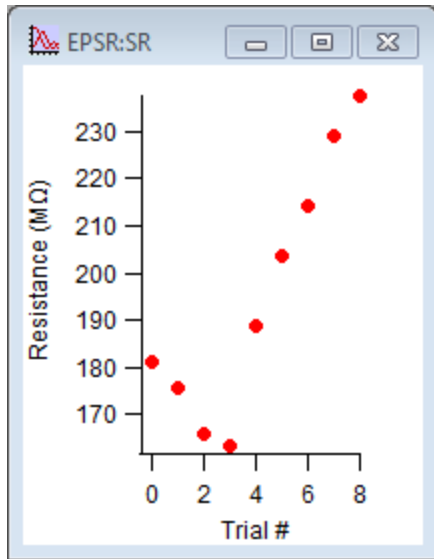


Figure 4-19 Resistance plot shows the series resistance of the traces

5. Visual stimulation

Visual stimuli are presented on a secondary screen. To start visual stimulation, open the **LCD panel** (**iPhysiology**->**Display**).

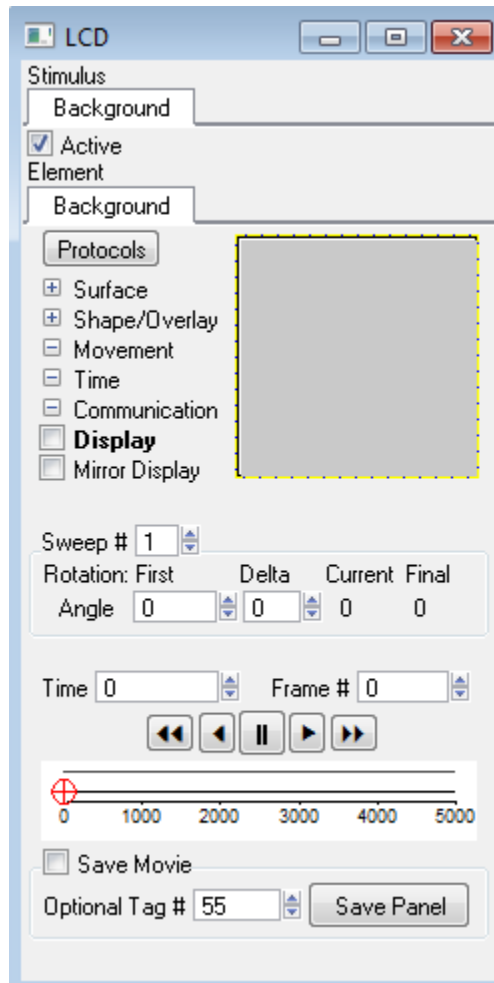


Figure 5-1 The LCD panel

Visual stimulation is generated by different stimuli, each of which can be designed independently and overlaid atop other stimuli to create arbitrary complex stimulation patterns. Each stimulus has the following attributes – surface, shape, movement and timing of appearance.

The panel shows the different stimuli (**Stimulus** tab), which may or not be **active**, each stimulus is composed of one or more **Elements**. As with electrical output, user can change the names of stimuli and elements (see below). To create a protocol the user will open one or more of the following windows ([Surface](#), [Shape](#), [Movement](#), [Time](#)). The [communication](#) panel provides the basic tools to set up the display, **Display** toggles the actual display window. The final stimulus will appear on the embedded plot. Different sweeps can have different stimuli (see below), and the entire display can be rotated by **Angle**, the rotation can be increased between sweeps by **Delta** value. **Time** and **Frame** fields allow the user to

examine the properties of the stimulus at specific time point/frame number. The stimulus can also be previewed as a movie using the buttons. The cursor indicates the shown time point/frame.

Important! The movie will be sent to the display only during acquisition, to view a replica of the actual presented stimulus select Mirror Display.

Save group – saves the movie to be viewed later. The optional tag is updated every sweep, it can be used to sync EP stimulation with imaging file number.

5.1.Communication panel

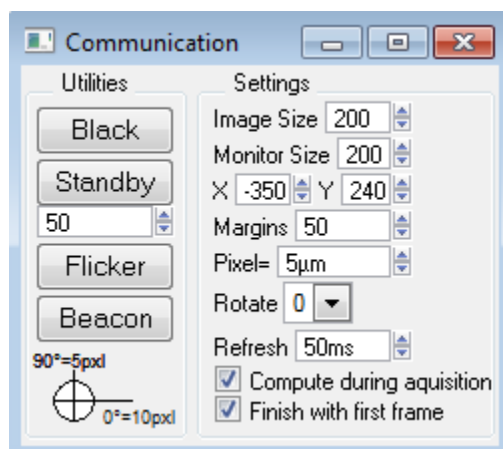


Figure 5-2 The communication panel set the basic parameters of the visual stimulus

This panel introduces some useful visual utilities, such as turning the screen black, or filled with a constant value (**standby**). **Flicker** turns the display from black to white (0 to 255 values) at 1Hz. **Beacon** button displays a central circle with a diameter of 10 pixels with two lines, one going in the 0 deg (right) direction, 10 pixels in length and a 5 pixel long 90 deg (top) line (see illustration on the panel). If the actual image as viewed through the microscope is rotated relative to this convention, rotation values should be adjusted.

Image size – the size in pixels of the movie to be computed. Small images are computed faster, but will produce a low resolution final image. **Monitor size** – the size in screen pixels that the movie is mapped to. Should not be less than image size. **X** and **Y** values set the center of the display window (screen pixel values). **Margins** – number of pixels to keep black around the display region.

1 pixel= and **rotate** are used to properly adjust the magnitude of one pixel on the specimen and what is the direction of the display relative to the microscope axis. **Refresh** sets the refresh rate of the display. Setting the display to a very high refresh rate may result in dropped frames and will take more time to compute.

Computation of next sweep movie can be done during acquisition; this is useful for reducing the time between sweeps. It takes more processing power and is not advisable on slow computers.

Warning – after startup Igor can generate an error message when this field is checked. Disregard it.

5.2.Surface panel

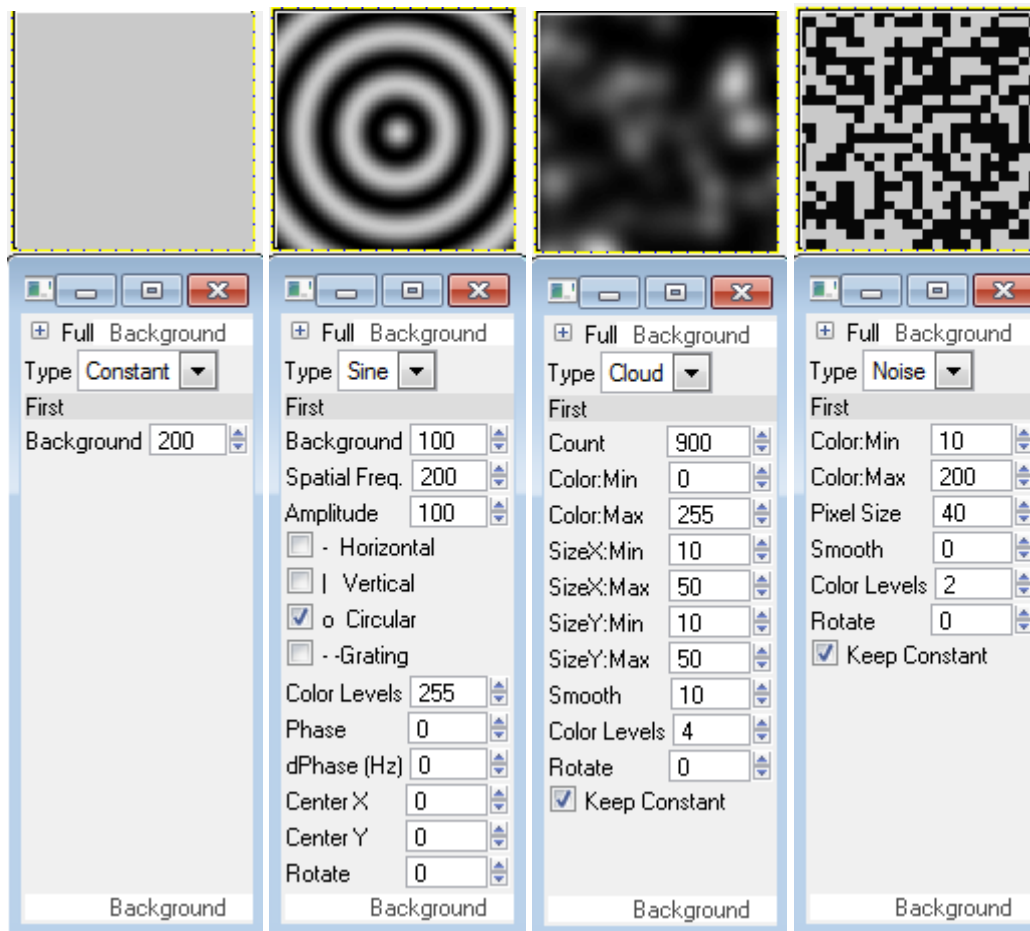


Figure 5-3 Different backgrounds

The surface panel allows the user to select from several options.

Constant; the background luminosity is set to 'background' color.

Sine; the background is set to be a one (horizontal/vertical) or two-dimensional (circular) sine wave with mean amplitude of '**background**' and peak amplitude of '**Amplitude**'. '**Spatial Freq**' sets the spatial frequency – the period of the sine wave in microns. '**Color levels**' sets the number of colors to use for the size wave, '**Grating**' is the extreme case of just two colors. '**Phase**' sets the phase of the size wave in degrees, '**dPhase**' is the change in phase in time (used to create expanding or contracting circular stimuli, or linearly moving sine waves). '**Center**' and '**rotation**' set the location (in microns, zero is center of the display) and the rotation (in degrees) of the stimulus.

Cloud; generates fuzzy objects. '**Count**' sets the number of objects. For practical reasons the size of the drawing canvas is larger than the area visible to the user, and therefore some of the objects can be drawn off screen. '**Color**' sets the minimal and maximal colors to use, '**Size**' sets the size of the objects

(rectangles). **'Smooth'** performs an Igor smoothing function. **'Keep constant'** prevents Igor from regenerating new cloud n every frame.

Noise; generates a checkerboard noise, with **'Color Levels'** number of colors ranging from **'Color Min'** to **'Color max'**. the size of each square is set by **'Pixel size'** (in microns).

All panels can be expanded/collapsed to reveal optional changes between sweeps

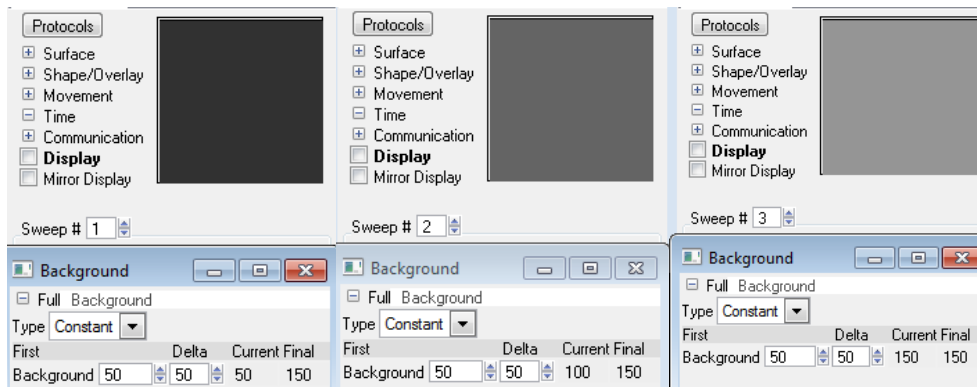


Figure 5-4 Expanded view of the surface panel, in this example the background luminance was increased by 50 between sweeps (set by delta). Left – the first sweep, middle, Sweep #2, Right -last Sweep.

5.3.Shape/Overlay panel

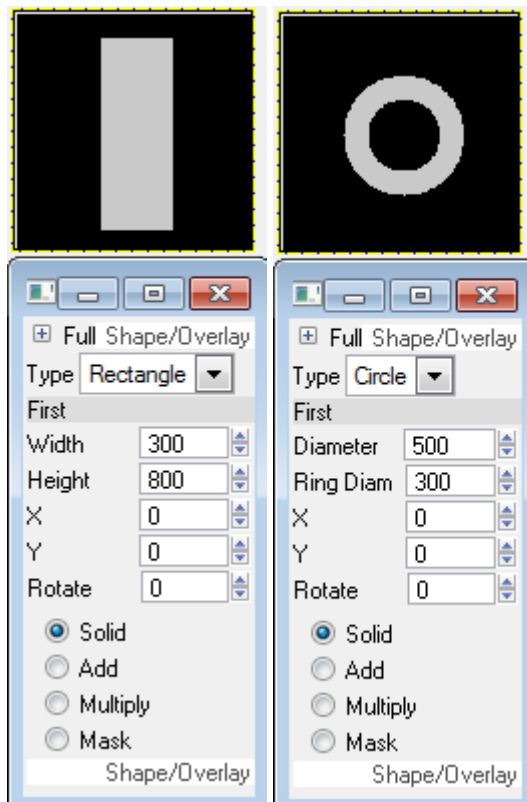


Figure 5-5 Basic Shapes - rectangles and rings

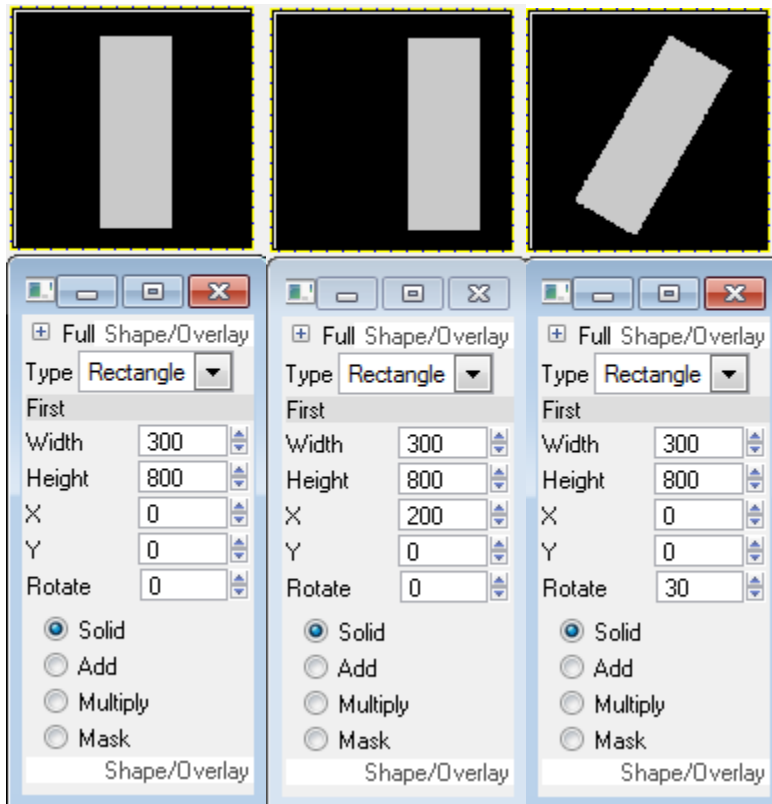


Figure 5-6 Shape offset and rotation

The panel provides the option to create simple shapes – rectangle and rings. All values are in microns (and degrees for ‘rotate’).

5.4.Movement panel

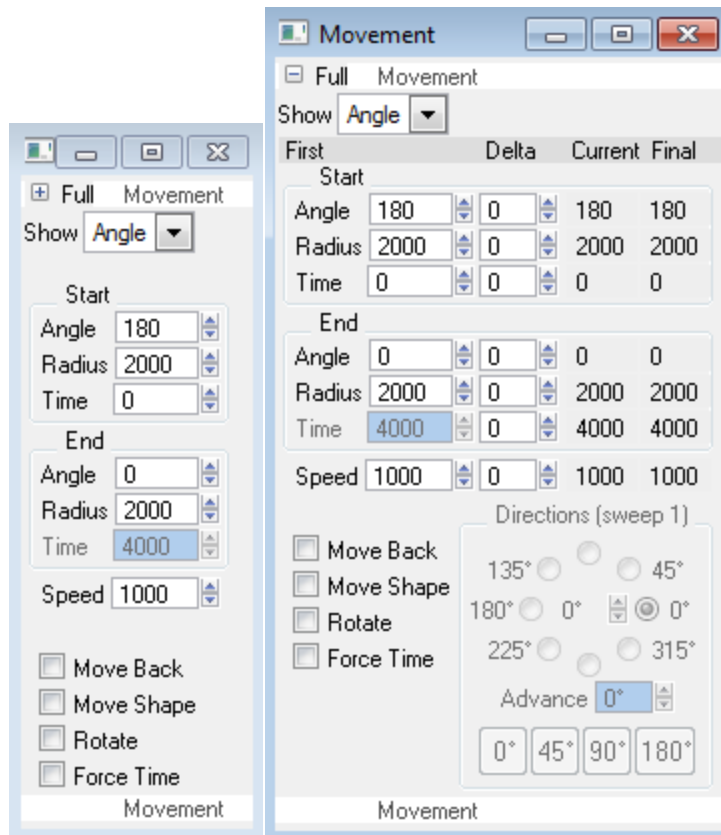


Figure 5-7 The movement panel in collapsed (left) and extended (right) views

The movement is set from a starting position (**'Start'**) to the ending position (**'End'**) in either polar coordinates (**'Show ->Angle'**) or in Cartesian coordinates (**'Show->X,Y'**). **'Time'** indicates the starting time for movement (in ms). These locations can be outside of the viewing area. **'Speed'** is in microns per second.

The user can move the background only (**'move back'**), move the shape only over the background (**'move shape'**) or move both. The shape can maintain its original rotation or can be rotated by the angle of the movement direction (**'Rotate'**). **'Force Time'** makes the shape to be visible during movement only.

Some common movement positions are revealed in **'Directions'** group in extended view. User can select one of 8 directions by clicking on the appropriate radio button. The directions can be increased between sweeps with **'Advance'** field or by pressing the four buttons labeled with the appropriate advance degrees (**'0','45','90','180'**).

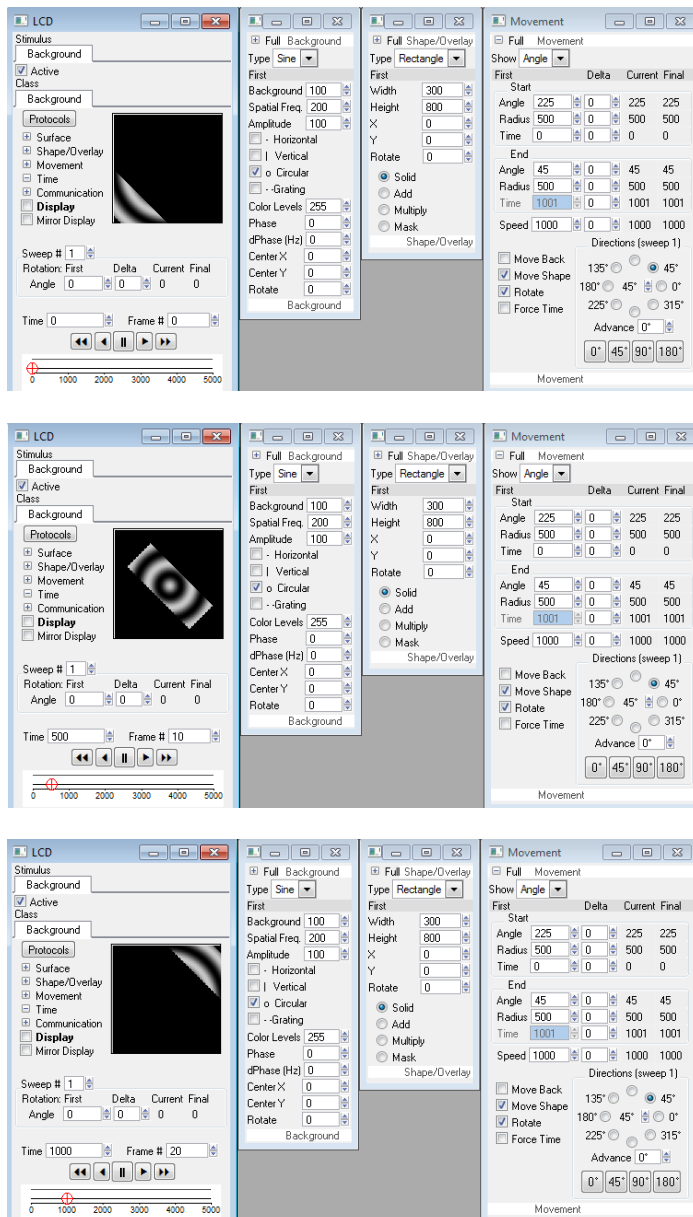


Figure 5-8 Example of stimulus movement, only 'move shape' is checked. Top- 0ms, middle-500ms, bottom-1000ms.

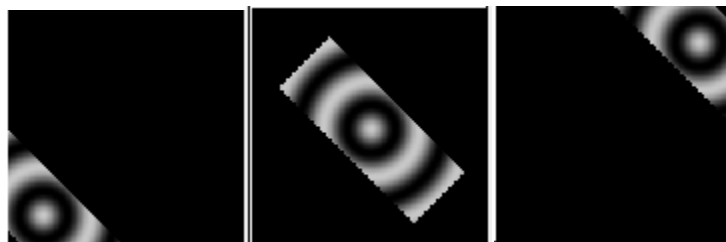


Figure 5-9 Similar example with both shape and background moving

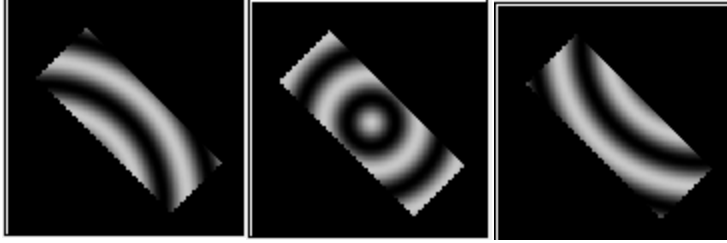


Figure 5-10 Similar example with background only movement

5.5.Time panel

The timing of the stimulus can be set and modulated with the time panel with one of the following options: **none**, **ramp**, **sine**, **noise**. The modulation sets the brightness of the stimulus from 0 to 1, where 0 is invisible. The level of temporal modulation is displayed on the main [LCD](#) panel.

All or none (on-off) modulation of appearance, as given by 'Start' and 'duration' parameters (in ms). Even with this simple modulation method it is possible to set more than a single appearance by increasing the number of 'Repeats'. The inter stimulus interval ('ISI') is the time in ms from one repeat to the next.

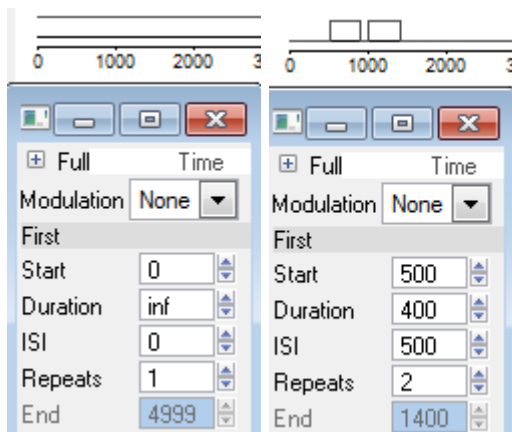


Figure 5-11 Examples of square (on-off) temporal modulation. Left- stimulus always visible. Right -stimulus is visible in two temporal windows.

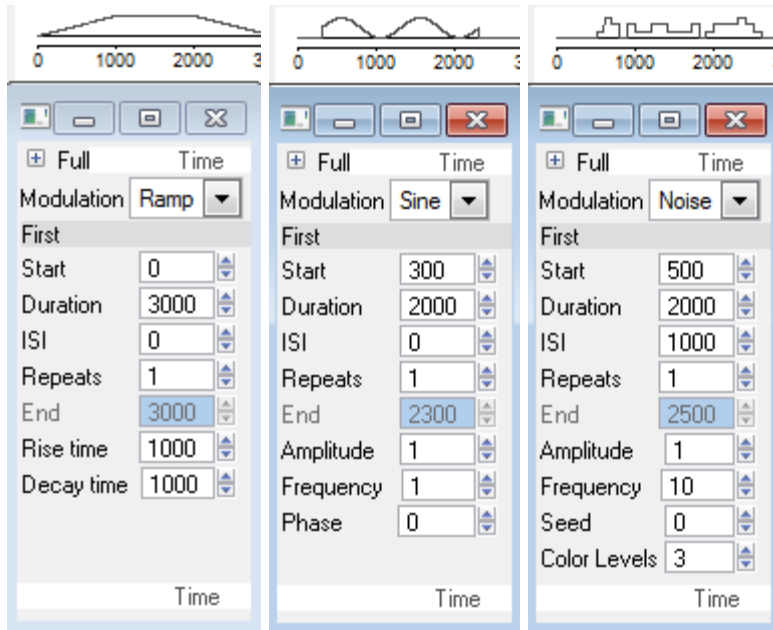


Figure 5-12 Example of ramp (left), sine (middle) and noise (right) temporal modulations

The parameters of the temporal modulations are similar to the parameters of the electrical output.

5.6. Multiple stimuli and elements

Stimuli may be composed of more than one element, or instance. For example, a stimulus may be comprised of two spots on a background, where each of the three elements (spot 1, spot 2 and background) is manipulated independently.

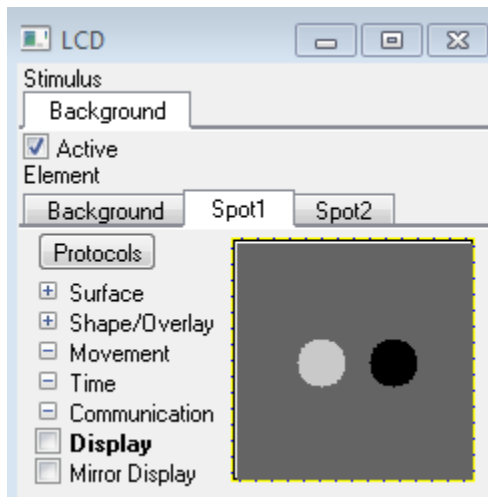


Figure 5-13 Multi-element example, bright and dark spots on a grey background

This multi-element stimulus can be easily composed using iPhys. To introduce a new element or manipulate an existing one, right-click on the Element tab ('background' label in this example).

5.6.1. Elements selection panel

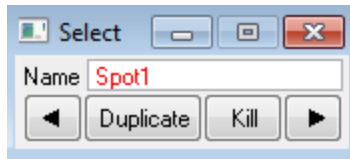


Figure 5-14 Element selection panel

The '**Name**' field can be filled by any string to give more meaningful name for the Element. '**Duplicate**' creates a new element with the same properties as the original. Elements can be deleted with '**Kill**' and moved left or right with the arrow (this corresponds to changing the order by which the elements will be processed to create the final image, see below).

Selection of an element is done by clicking on the appropriate tab. This will update all the open panels. To recreate the example, follow the following steps:

1. Activate Surface panel and set the background to be type->constant with a value of 100.
2. Right click on the LCD->element->background tab, choose 'duplicate'
3. Right click on the new (rightward) 'background' tab and rename it to 'Spot 1' by typing the letter after the name field.
4. Activate Surface panel and set the background to be type->constant with a value of 200.
5. Activate Shape/Overlay panel and set the type to be circle, with Diameter of 200, X of -150.
6. Right click on the LCD->element->'Spot 1' and duplicate it.
7. Right click on the new (rightward) "pot 1' tab and rename it to 'Spot 2'
8. Activate Surface panel and set the background to be type->constant with a value of 0.
9. Activate Shape/Overlay panel and set the type to be circle, with Diameter of 200, X of 150.

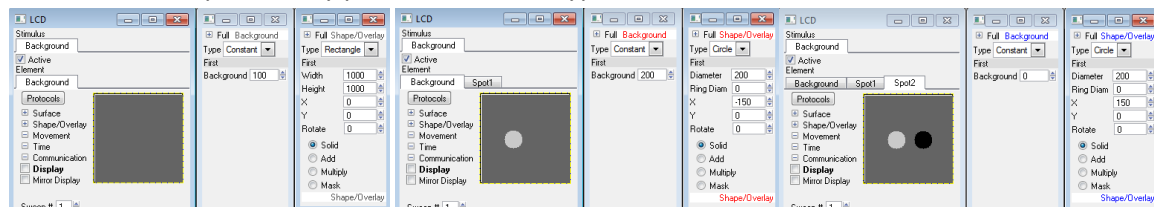


Figure 5-15 Example parameters for multi-element stimulus. Note the different color scheme for each element (left -background, middle -spot1, right -spot 2).

5.6.2. Overlay of different elements

So far all the elements were drawn on top of each other, iPhyS however allows more complex forms of overlay. These are given in Shape/Overlay panel:

Solid; is the default setting, the element is drawn over all other elements that are on the left of it (in this example Spot 1 would be on top of Background and Spot 2 would be on top of both).

Add; The background color of the element is added on the values of lower elements.

Multiply; The background color of the element is multiplied by the values of lower elements. The multiplication happens only within the regions of the shape.

Mask; Similar to multiply, but pixels outside of the shape are set to zero.

5.6.3. Examples of interaction between elements

In this set of examples Spot 1 has a sine wave background, as is shown in the following image:

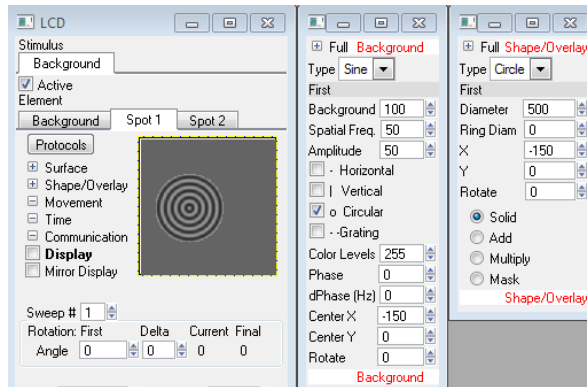


Figure 5-16 Example stimulus

Spot 2 will be overlaid over Spot 1 and the background to show different overlay modes.

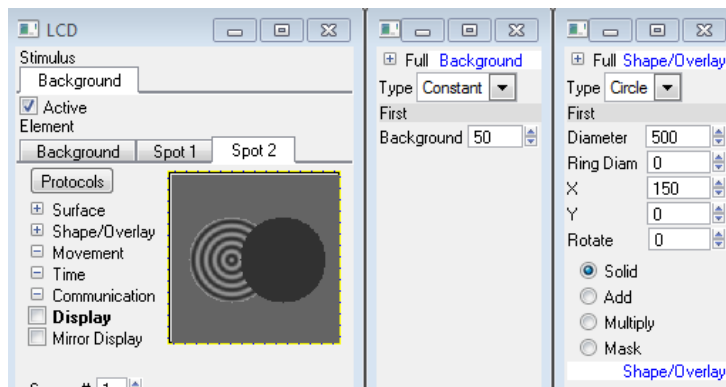


Figure 5-17 **Solid.** Spot 2 (with background of 50) will completely obscure Spot 1 whenever they overlap

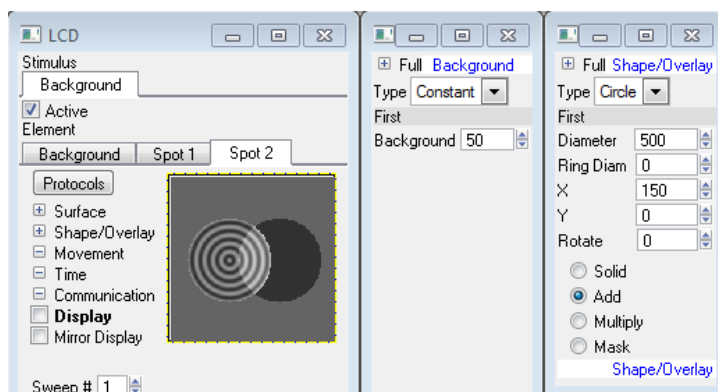


Figure 5-18 **Add.** Spot 2 is added to the background luminosity

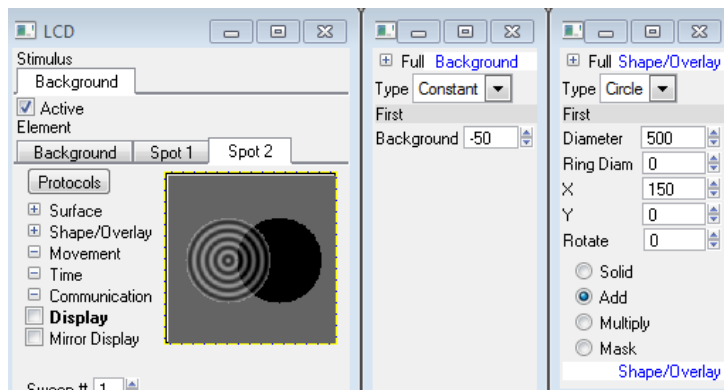


Figure 5-19 **Add**. Background value can be set to negative (-50 in this example) to reduce pixel values (darker image)

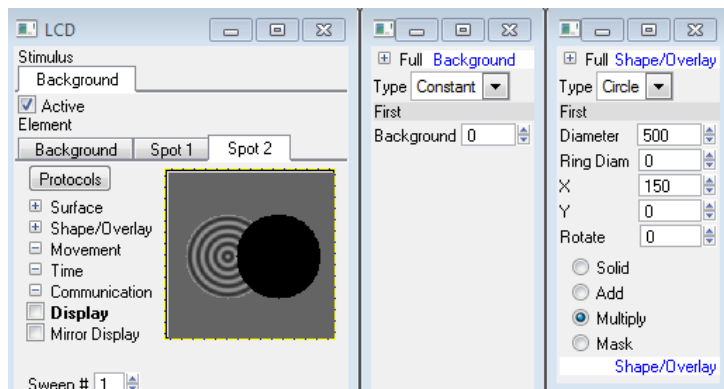


Figure 5-20 **Multiply**. With background of 0 Spot 2 sets all pixels within its shape to zero.

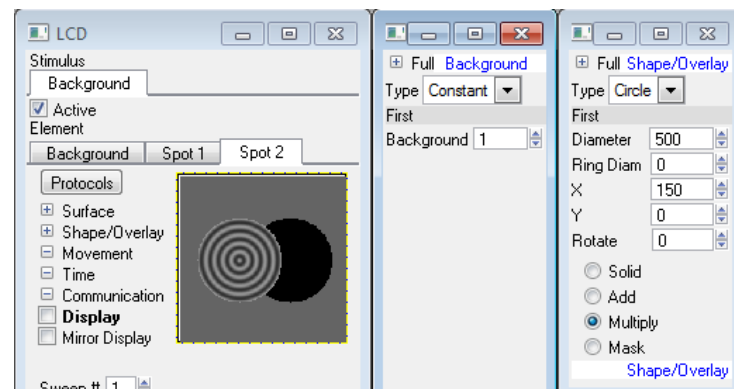


Figure 5-21 **Multiply**. Background of 1 for Spot 2 sets the pixels of Spot 1 to their original values, but interactions with lower elements ('background') results in black (zero) pixels. This behavior is discussed below.

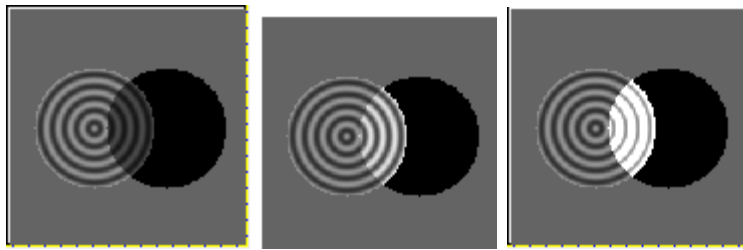


Figure 5-22 Examples of multiplication with 0.5, 1.5 and 3.

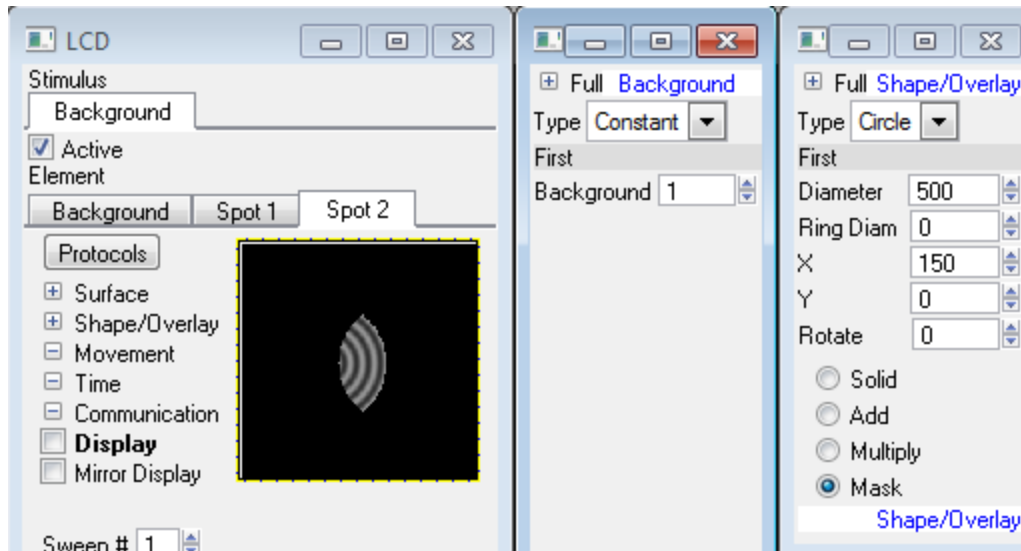


Figure 5-23 **Mask**. Only pixels that are not zero are shown.

When more than two elements are present, there would be multiple overlay options (number of overlays is the number of elements-1). Elements are stacked on top of each other sequentially, with the overlay parameter for the corresponding element. In the case of 3 different elements, first element 3 is combined (overlaid) with element 2 based on overlay parameter of element 3, the resulting image is stored temporarily in element 2. This image is then combined with element 1 based on the overlay parameter of element 2.

To continue previous example, with Spot 2 is added to Spot 1, when Spot 1 is solid, we will get the following final image:

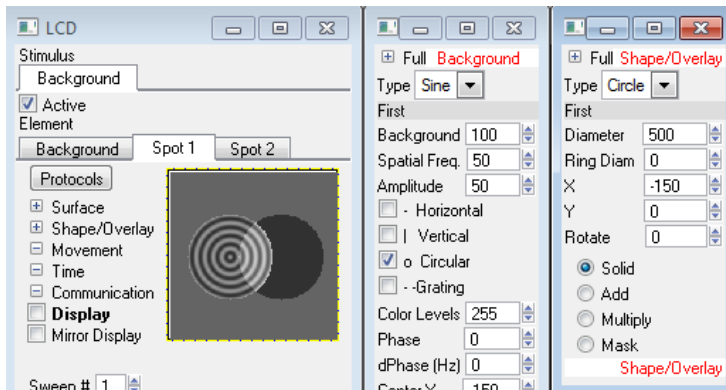


Figure 5-24 Overlay of multiple elements; solid

When spot 1 is added to background, both spot 1 and spot 2 regions will increase in intensity:

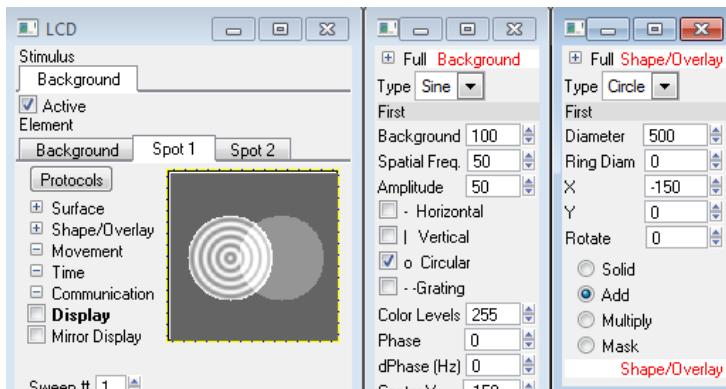


Figure 5-25 Overlay of multiple elements; add

Tip - it is generally a good idea to put the mask as the top element

Each element can be turned on and off, modulated in space and time independently, allowing for a wide variety of possible generated stimuli. However, under some conditions, a single stimulus may be not sufficient. For instance, when one element masks another, the mask would propagate across all subsequent elements. Generation of more than one shape/mask becomes difficult in this case.

iPhys includes a possibility to create more than one stimulus. Creation and removal of stimuli is similar to creation and destruction of elements. To create a new stimulus right click on the Stimulus tab, select 'Duplicate' from the stimulus selection panel that appears. This panel is similar to the [Elements selection panel](#) discussed above.

5.6.4. The difference between elements and stimuli

Multiple stimuli are comparable but not exactly similar to multiple elements. The differences between elements and stimuli are as following:

1. Stimuli are composed of elements. Stimuli are processed sequentially, and overlaid on each other. Overlay options apply for all elements as before, but most overlays propagate till the very

bottom element in each stimulus but not to other stimuli. For example, mask operation in the top element in the top stimulus would affect all the elements in that stimulus only, and not elements in other stimuli.

2. How are stimuli overlaid on top of each other? The only overlay command that is not in use in computing the image with multiple elements is the overlay command of the very bottom element in the stack. This overlay option is reserved for interaction between different stimuli.
3. Stimuli can be turned on and off by toggling the '**Active**' checkbox below the stimulus tab.

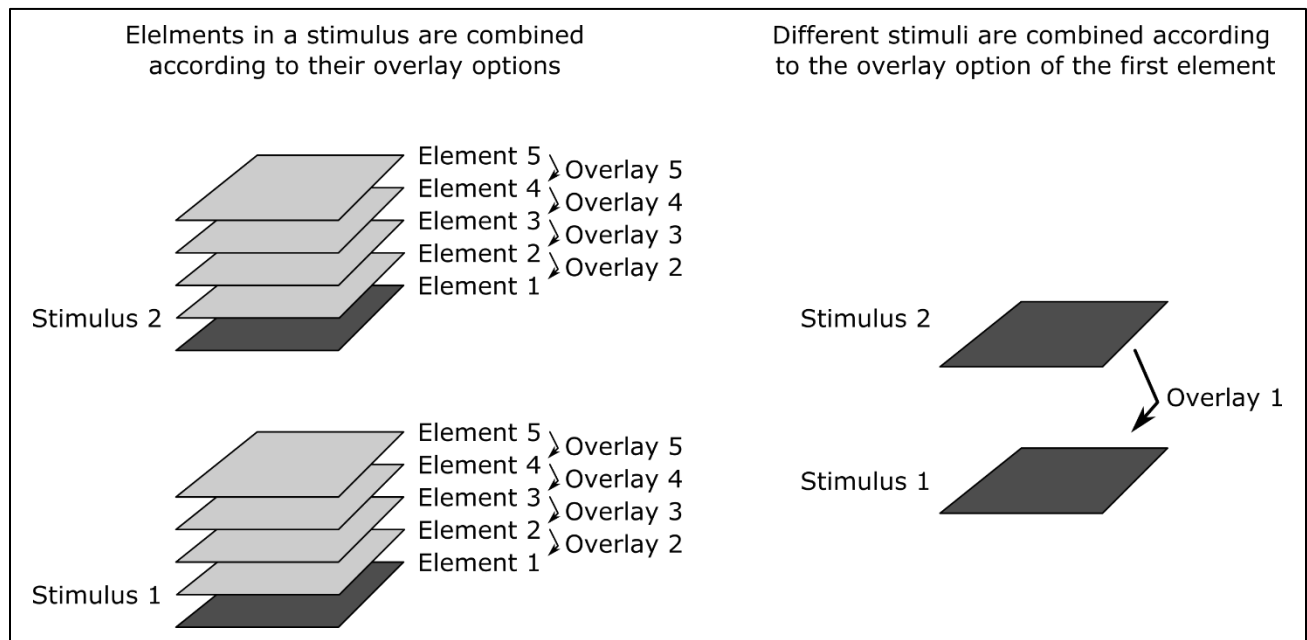


Figure 5-26 Overlay rules of multiple stimuli

5.7.Viewing saved movies

iPhys allows to [store and retrieve protocols](#) and view projected movies.

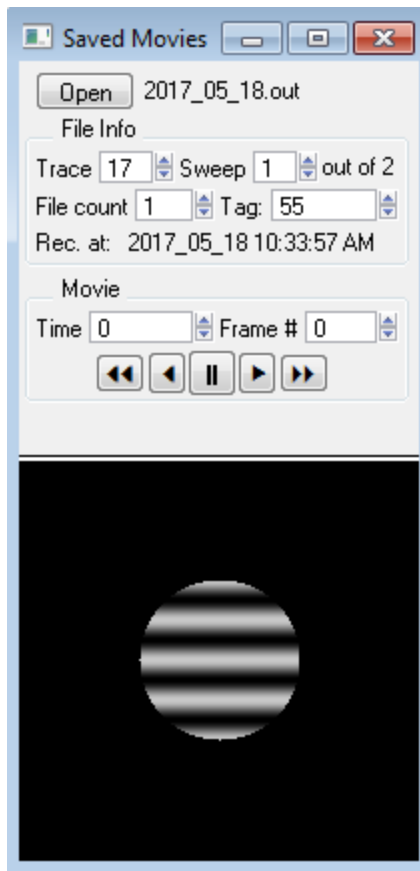


Figure 5-27 Saved Movies panel is used to view saved stimuli

Movies are saved in the working directory. When the LCD->**Save Movie** is enabled, the visual stimulator output for each sweep will be saved to the hard drive as a 'XXX.out' file, where XXX is the date. Movies are opened with the '**Open**' button. Note that if new movies are appended to the file, they will be immediately available for viewing.

Movies can be selected based on the EP '**Trace**' and '**Sweep**' combination, the user defined '**Tag**' and by their location in the '.out' file ('**File count**'). The movie will be loaded, recording time will be displayed under file info and individual frames can be accessed using the '**Movie**' controls.

6. Image analysis

iPhysiology->Windows->Image analysis

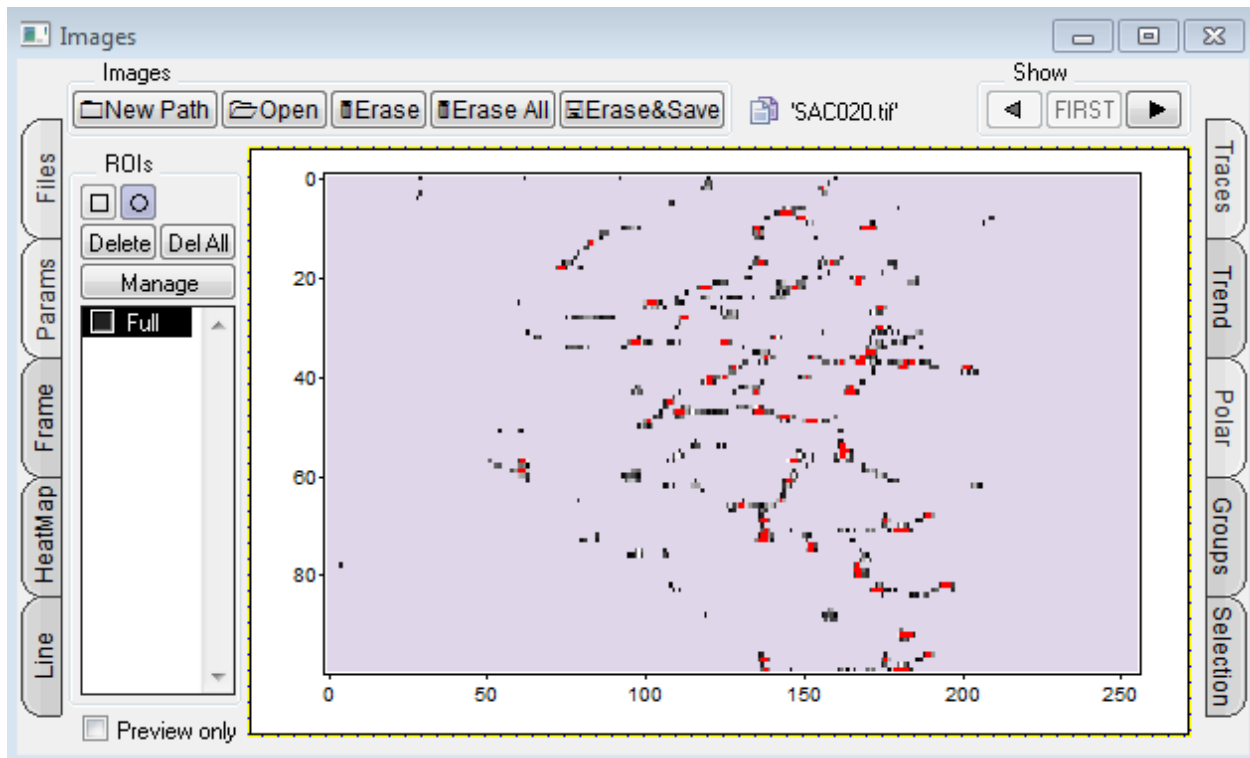


Figure 6-1 Images panel

The panel is the starting point for working with images. It allows to load and erase TIFF image files, select regions of interests (ROIs) and access various plots and accessory panels. Note that similar to the electrophysiology panels, windows that are evoked by different commands can be made to stick and follow the Images panel simply by bringing the edges of the windows together.

New Path and **Open** buttons are the same as the corresponding commands in [File Info](#). **Erase** deletes the selected TIFF file(s), while **Erase all** erases all files the Images window and cleans the directory. **Erase and save** saves the TIFF files and ROIs to an experiment in memory and erases all image files from the Images window. The name for the new experiment can be specified in the [File Info](#) panel.

Show allows simple browsing between opened images (left arrow – previous file, right arrow – next file, 'First' shows the first file in the list).

6.1.ROIs

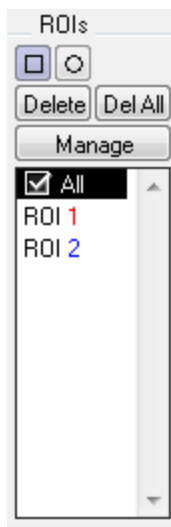


Figure 6-2 ROIs box

Regions Of Interest (ROIs) define area on the image to choose for the analysis. iPhy allows to create an arbitrary number of ROIs with arbitrary shapes, and change ROI location between different imaging files. When no ROI are selected, the analysis is performed on the entire image ('All').

6.1.1. Creating a new ROI

Left-click and drag on the area of the image that you want to assign to a new ROI. Igor will generate a marquee selection. Right click on the marquee and select 'New ROI'. The newly created ROI will be either rectangular or circular in shape, based on the shape selector on the top of the ROIs box. Different ROIs cannot overlap. For this reason, if new selection overlaps with an existing ROI, the newly created ROI will not include the pixels occupied by other ROIs.

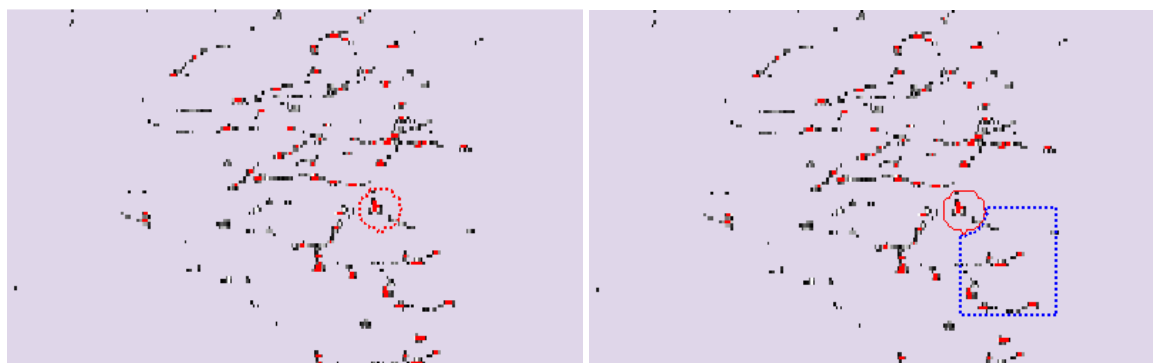


Figure 6-3 ROI creation rules do not allow for overlap. Left – Single ROI (ROI1). Right- Newly created ROI (ROI2, blue) doesn't include the area marked as a part of ROI1.

6.1.2. Changing the shape of an ROI

Area can be added or subtracted from an existing ROI by selecting a new marquee and holding the shift (to add) or ctrl (to subtract) keyboard buttons during selection of the desired ROI from the popup menu (as before, the menu appears by right-clicking on the marquee).

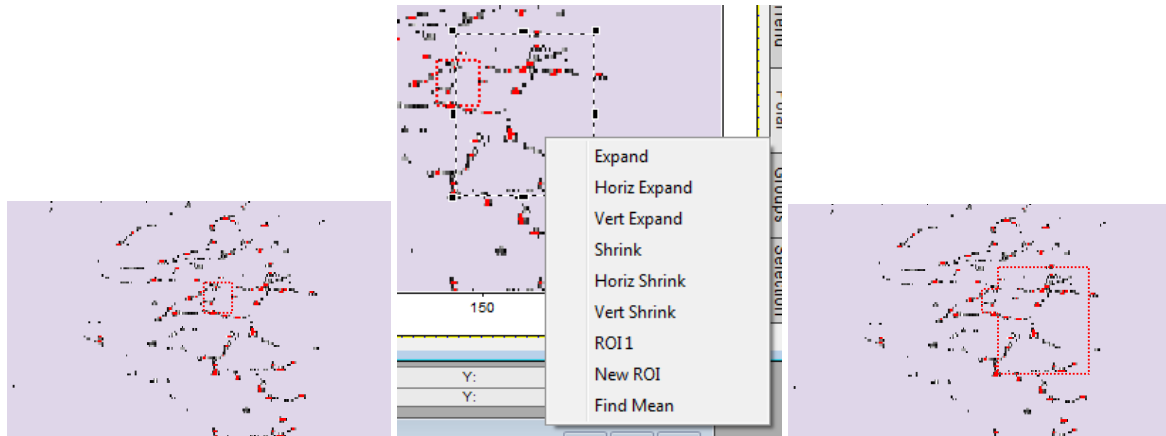


Figure 6-4 Changing the shape of the ROI

Because recorded images can shift in between trials, individual ROIs can be moved from image to image. This can be done by navigating to the desired image and selecting a new location for one of the ROIs. Subsequent images are updated automatically to have the new ROI boundaries.

6.1.3. Managing ROIs

Individual ROIs can be deleted with 'Delete' button. All ROIs can be deleted with 'Del All' button.

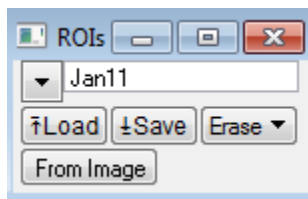


Figure 6-5 ROIs panel

ROI position can be shared between experiments by selecting the "Manage" option on the Images panel. This will open a ROIs panel. The panel allows to specify a unique name for the ROI set, save and load previously created ROIs and erase saved ROIs from memory.

Tip! Preview only option prevents the update of plots and calculation of ROI values, it can be used to quickly position the ROIs w/o waiting for all the plots to update.

6.2.Files panel

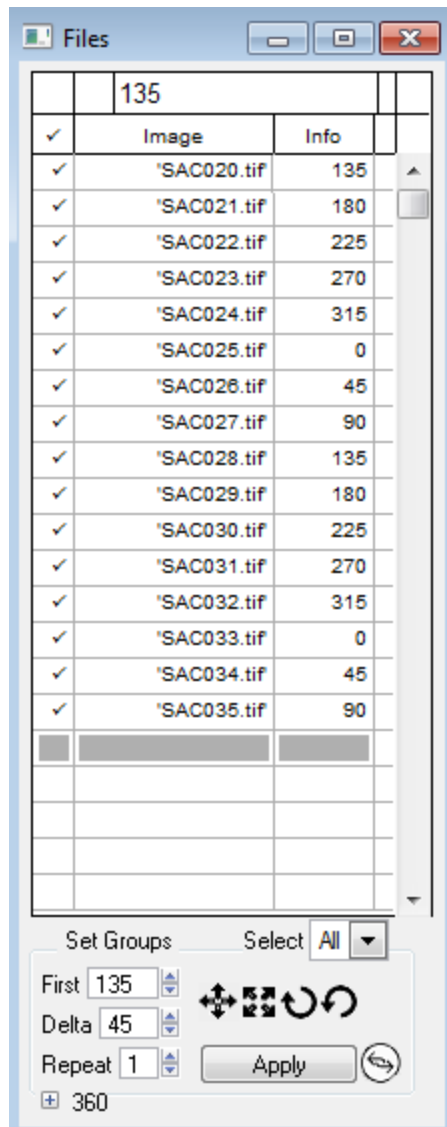


Figure 6-6 File panel manage images

The panel displays the list of images in the current experiment, metadata for each image (such as stimulation intensity, direction of stimulation or another experimental condition) and the delay (in ms) to the beginning of the image.

Selected images are marked. Images can be selected by clicking, making a table selection or selecting one of the groups with a **select** pulldown menu. Shift and Control keys can be used to expand or change the selection according to the standard Windows convention. The info information can be entered manually, automatically with the present buttons (arrows and curve at the bottom), with a set group variables (which set the value for the first group, difference between subsequent groups and the number of items in each group) or from electrophysiology (right down arrows in a circle button). For more information, see similar parameters for [electrophysiology](#).

6.3.Params panel

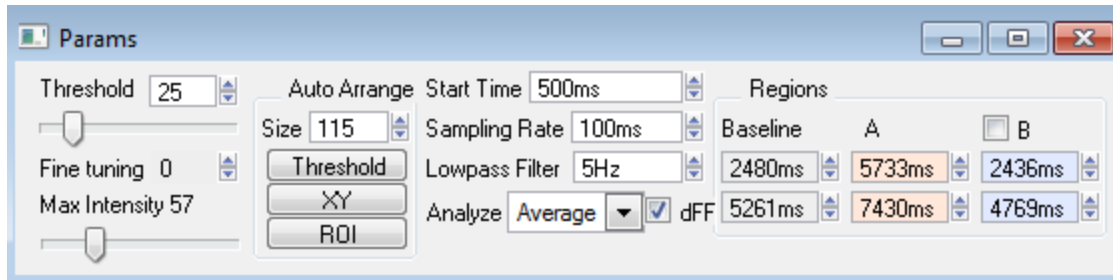


Figure 6-7 Params panel sets the parameters of the image analysis

The **Threshold** sets the background pixel level from which dF/F is calculated. As light intensity can change between images (due to shifting focus or bleaching), the **Fine tuning** variable can be used to change the threshold for each image independently. Max intensity is mainly used to control the **Heat plot** display.

Auto Arrange activates a set of command which attempt to automatically place ROIs. The **Threshold** button attempts to fine tune threshold so that all images will end up with similar intensity values. **XY** button moves the images in XY to reduce XY shift. The conversion may not be perfect and images scan become shifted erratically. **ROI** generates new ROIs with pixel area specified by the **Size** variable.

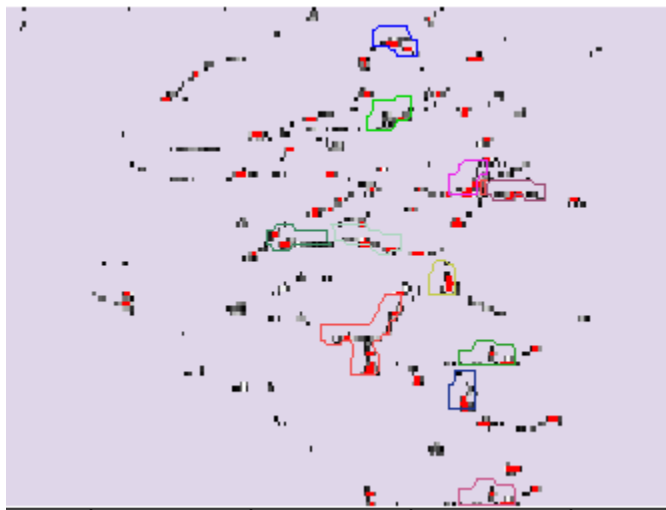


Figure 6-8 Example of automatic ROI creation

The **start time** is the delay between electrophysiology and start of imaging, if present. The **sampling rate** is determined by the interval between frames. A **Lowpass Filter** can be applied to reduce noise. dF/F displays the change in Fluorescence in time over the baseline Fluorescence.

The **regions** box define the baseline period (where the baseline F is calculated) A and optionally B are two areas of analysis.

6.4.Traces plot

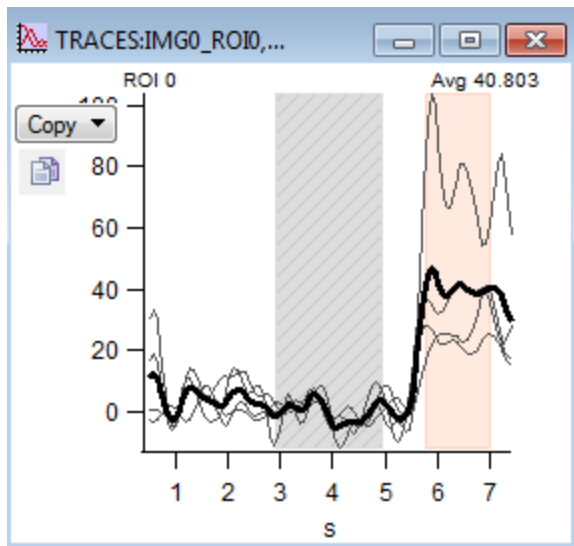


Figure 6-9 Traces plot

As its name implies, the graph shows the selected ROI from the selected images. The baseline, A and B regions are shown in striped gray, pink and light blue. These regions can be changed with the marquee menu. The bold trace is the average of all the traces on plot. The copy button duplicates the plot and saves it in the selected experiment.

6.5.Polar plot

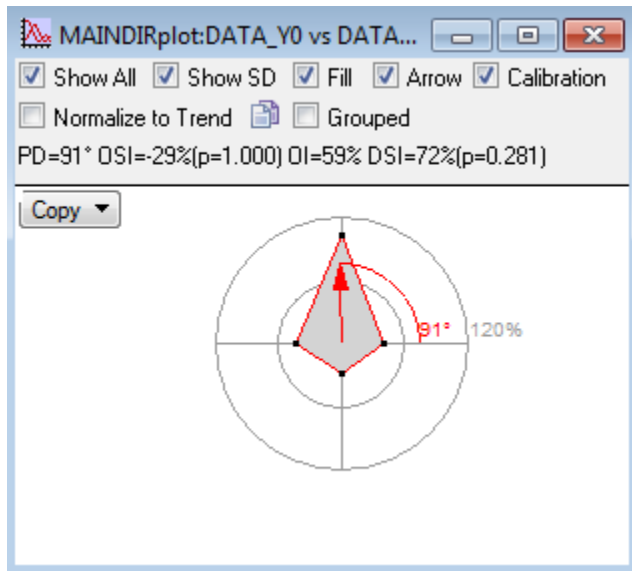


Figure 6-10 Polar plot

This plot is used to display directional data. Angles in degrees are specified by the info on Groups panel. Igor will find the summed vector of the directions (here shown as a red arrow, with the angle of 91 degrees). Individual responses are shown with dots. Lines connect the averages. It is possible to use two types of averages; the first is the average of the calculated responses. The second is to generate an

average trace first, and then calculate the response in it (the second option is selected with 'Grouped'). This may be useful for noisy data. However statistics are calculated based on the first option. Similar to the electrophysiology [polar plot](#).

6.6.Groups plot

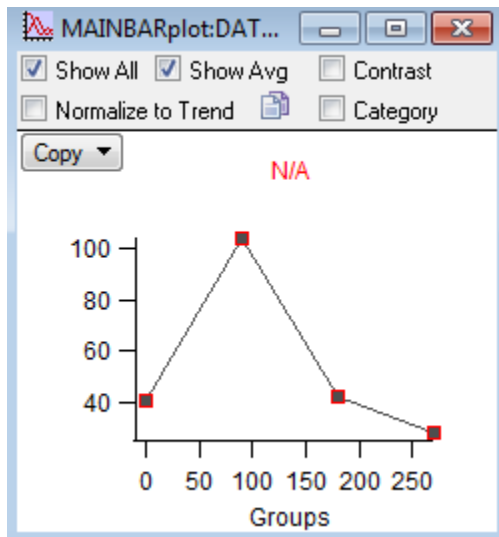


Figure 6-11 Groups plot

A useful plot to display the different groups (circles are individual data points, squares are the averages). The contrast option treats the data points as display pixel values, starting at the selected base value. The annotation shows the result of an ANOVA test on the data. Similar to the electrophysiology [Groups plot](#).

6.7.Trend plot

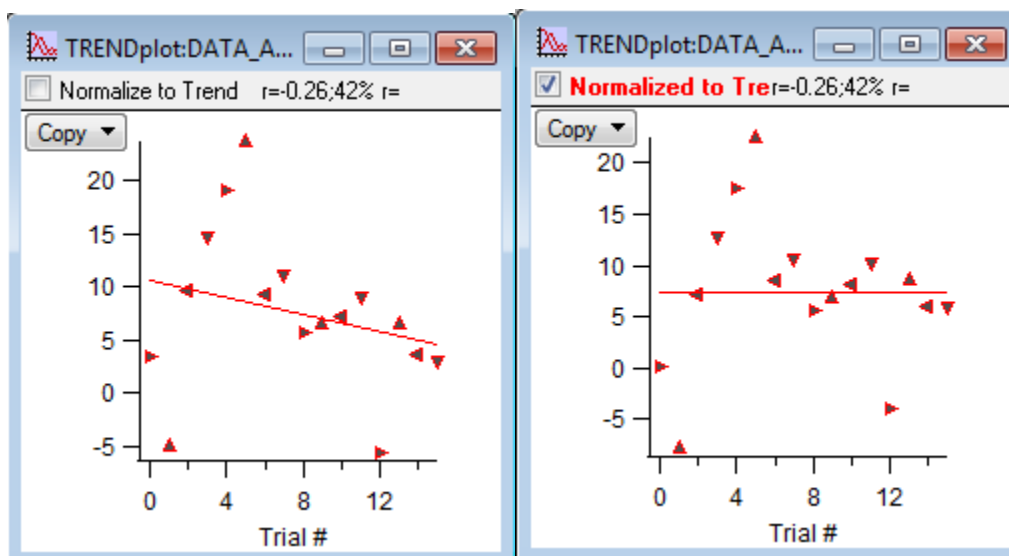


Figure 6-12 Trend plot. Left - original data. Right - normalized data

As sometimes the baseline of the images changes (perhaps due to bleaching), data can be normalized to a linear trend. This changes the statistical analysis and the directions plot, but not the actual traces. Compare to [Trend plot](#).

6.8. Frame plot

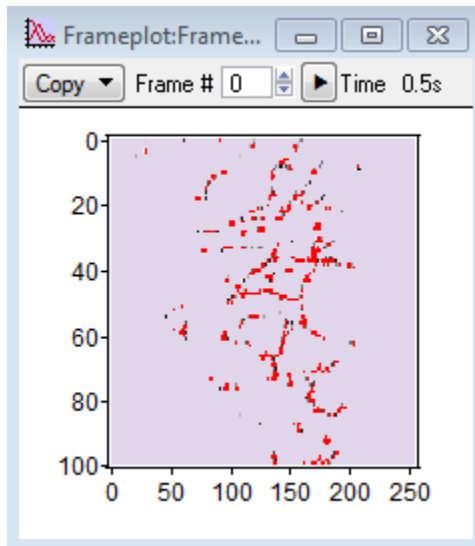


Figure 6-13 Frame plot shows the frame-by-frame image data

This plot visualizes specific frame, representing one time point, across all selected images. The **play** button generates a movie from the different frames. Color coding is set by **threshold** and **max intensity** parameters found in [params panel](#).

6.9. Line plot

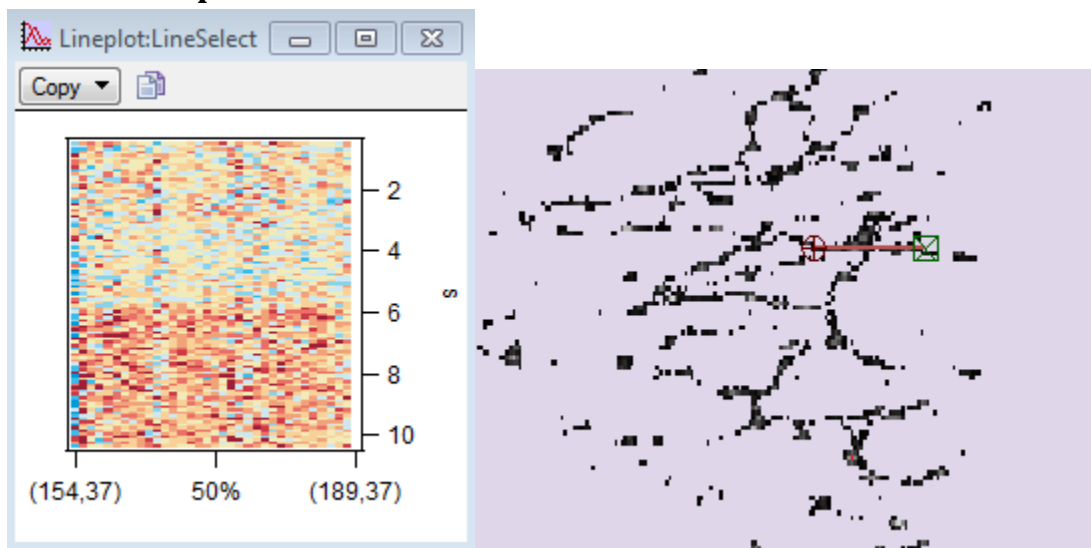


Figure 6-14 Line plot shows the pixel values over a specific line (right – the location on the image) as a function of time.

This plot visualizes change in pixel intensity over a line that is selected by placing and dragging cursors on the [main imaging window](#).

6.10. Heat map plot

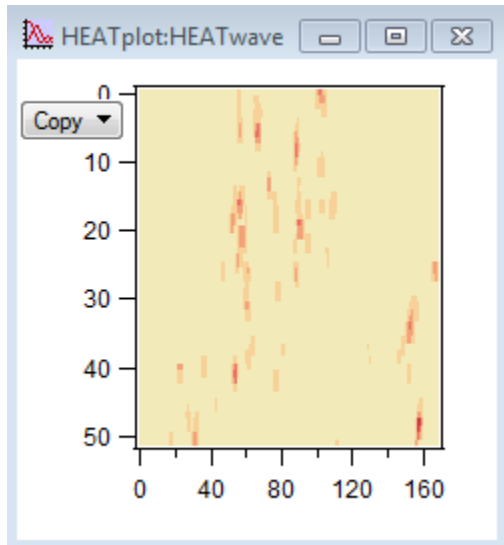


Figure 6-15 Heat plot shows the peak intensity of the pixels of selected images

6.11. Selection panel

The selection panel is a versatile analysis platform which allows for precise selection of images/ROIs, and conducting more in depth investigation of the statistical properties of the dataset.

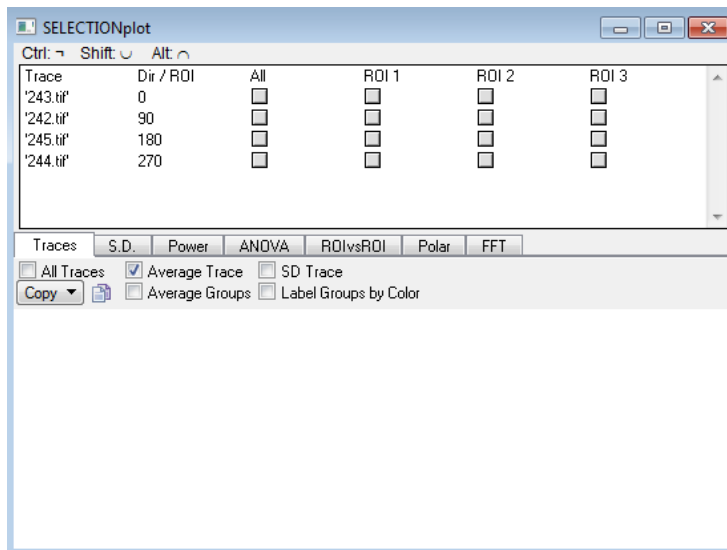


Figure 6-16 Selection plot

Selecting data

Selection is done by checking the desired ROI and image combination. Note that the images are grouped by their metadata information. Clicking on the name of one of the following items will select all the data that belongs to the item (items are ROI #, trace names and groups). Clicking on 'Trace' will select all data points. Elaborate selections are possible with manually checking/unchecking data points. Control key makes a 'not' selection, i.e. unchecks the selected data, Shift will add to selection while clicking with an Alt key will select the common data between the checked data and the newly selected data. Right mouse key unchecks data points.

The selection panel is fully resizable, which helps to visualize large data sets.

6.11.1. Traces tab

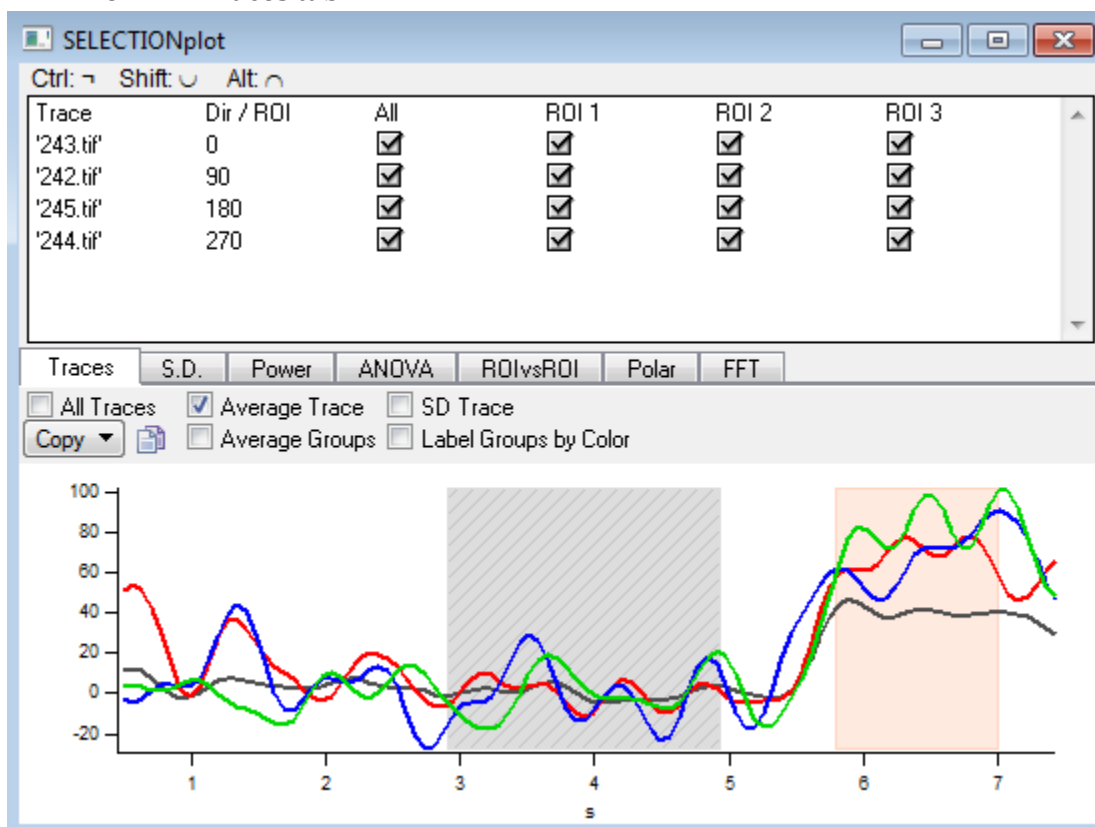


Figure 6-17 The traces tab displays traces, general averages or group averages

The display options include showing all traces, just the average traces for each ROI, average trace for each condition (metadata info) and adding the standard deviation on top of the average traces. The copy icon will put all the traces/information in a table and automatically appends the data to clipboard.

6.11.2. S.D. tab

The standard deviation tab shows the variability of the traces. It allows to show the variability of each trace within the analysis window (i.e. the variability of the data points within that window). Each ROI is analyzed independently.

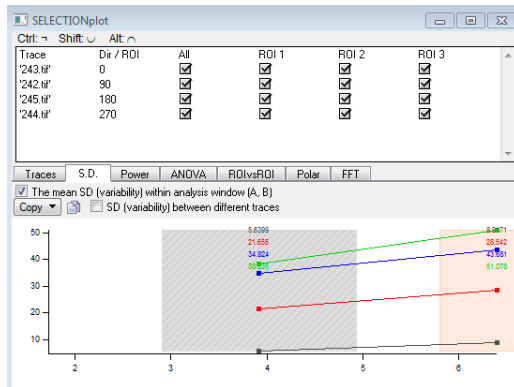


Figure 6-18 SD within window

The second option is to calculate the difference between traces for each time point. Each ROI is analyzed independently.

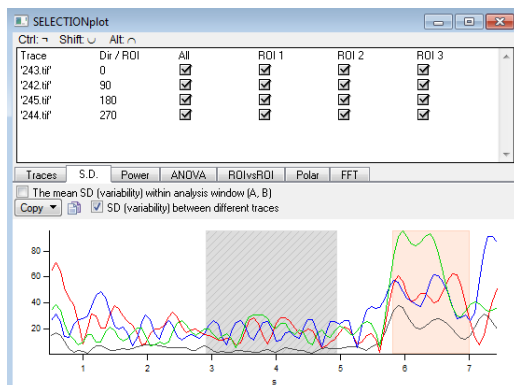
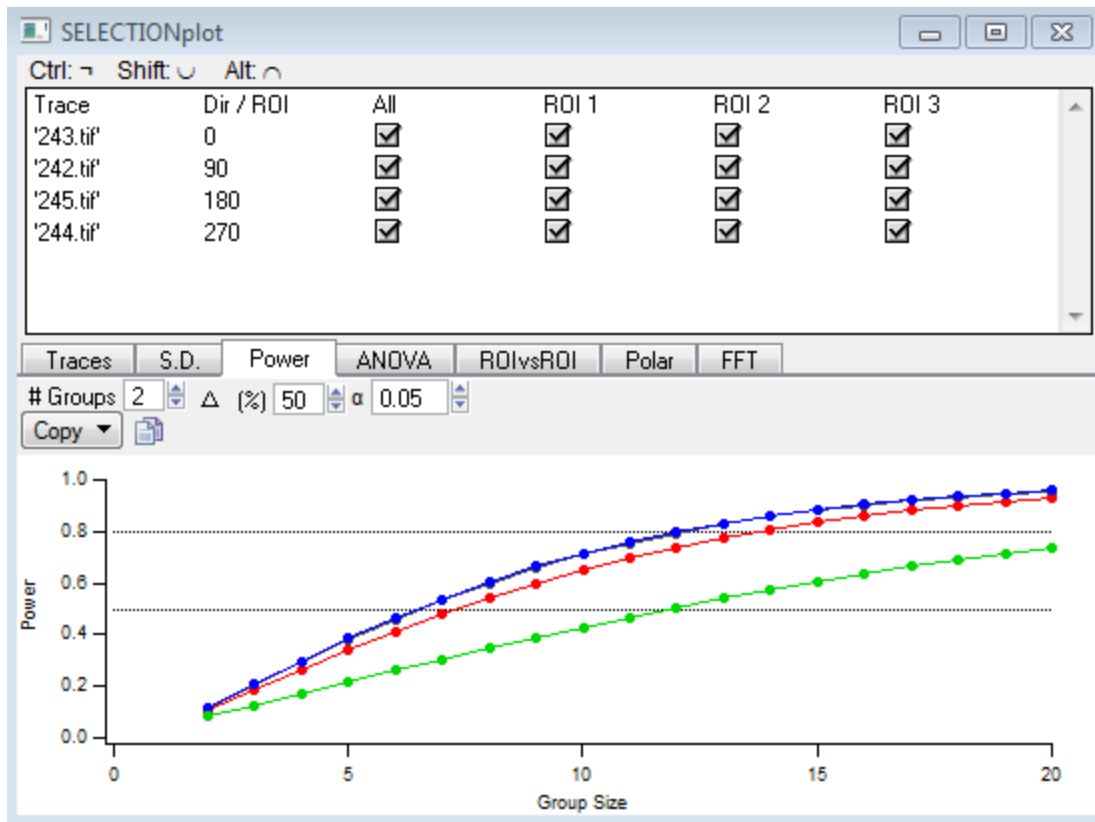


Figure 6-19 SD between traces

To understand the differences between these two calculations, consider the following examples:

1. All traces are repeats of the same fast sine wave. Within window SD will be high, between SD will be zero as all traces are the same.
2. All traces are flat, constant values with different intensities between traces. Within window SD will be zero as all the data points for each trace are the same, but between traces SD will be high.

6.11.3. Power tab



The power of the data in each ROI to detect changes between groups. Usually this is done in a pilot run for one group, and extrapolated to the number of groups used, the desired difference (delta) and the significance level (alpha). The output is a sample size (for each group) required to reach a specific power level.

6.11.4. ANOVA tab

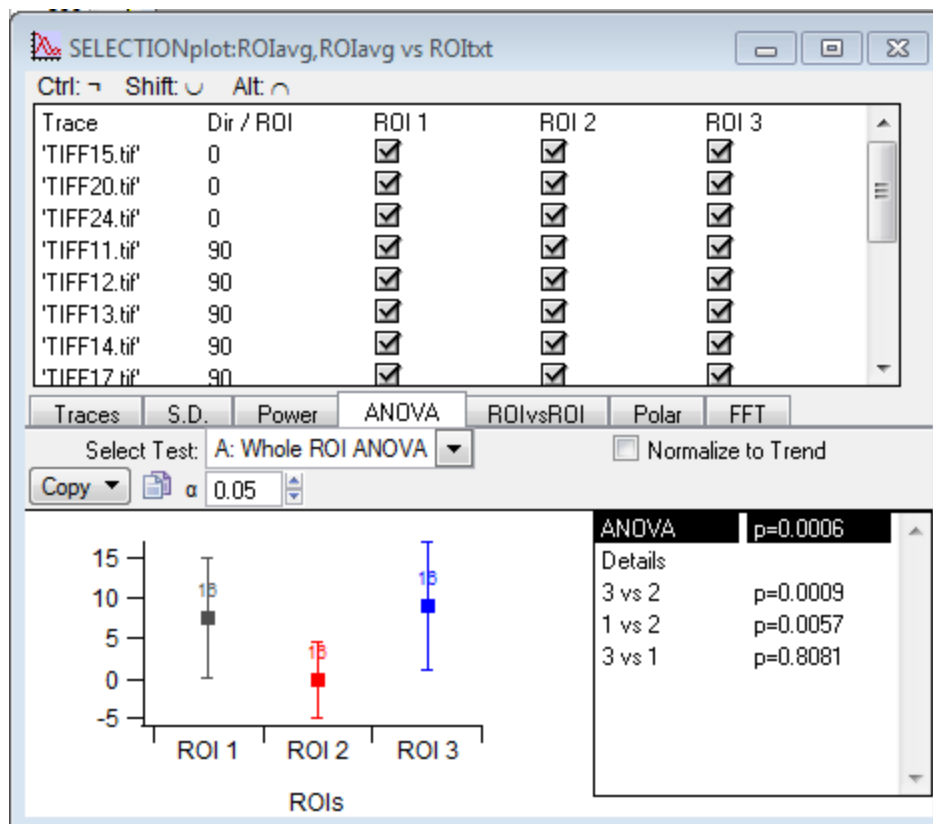


Figure 6-20 Whole ROI ANOVA

The ANOVA tab can perform a number of statistical tests on the data, plot the information and save the values in a table. The tests are:

Whole ROI ANOVA performs a comparison between different ROIs (in this example ROI 1,2,3). The ROIs may have a different number of points (the number is shown on the plot). The resulting p value is displayed in the table on the right. When the p value is below the alpha level, Turkey test is done to find the statistical significance between the examined ROIs.

Group ANOVA examines all the possible combination of groups and ROIs. In this example, there are 4 groups and 3 ROIs, which makes 12 comparison groups in total. These tests can be performed for both A and B regions.

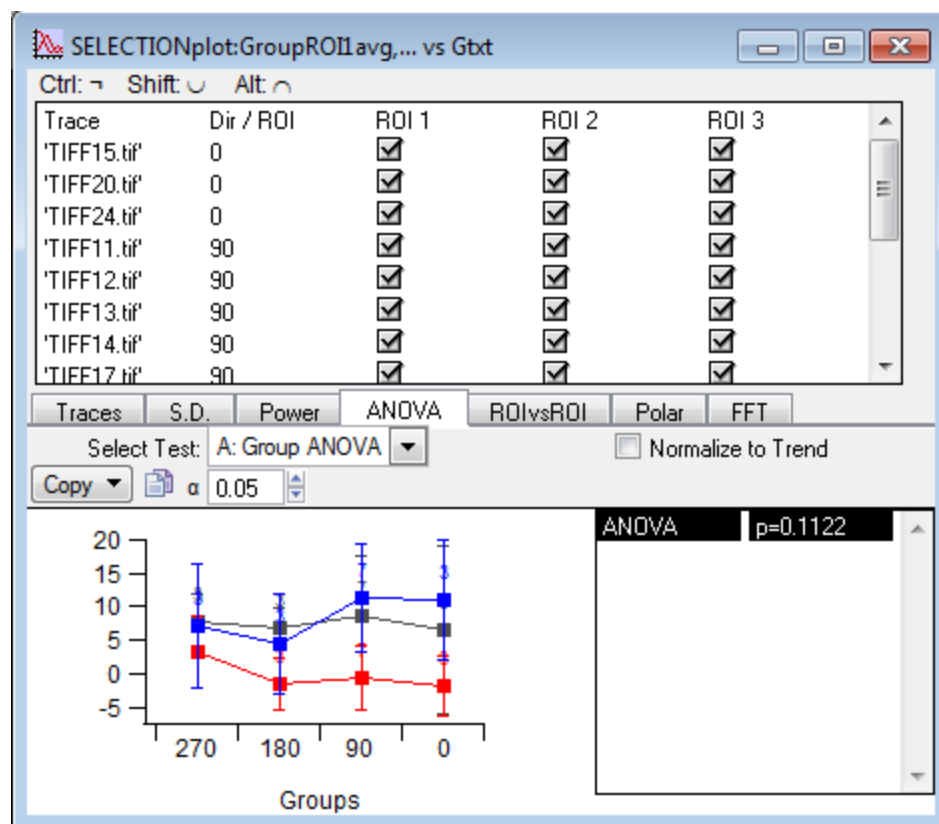
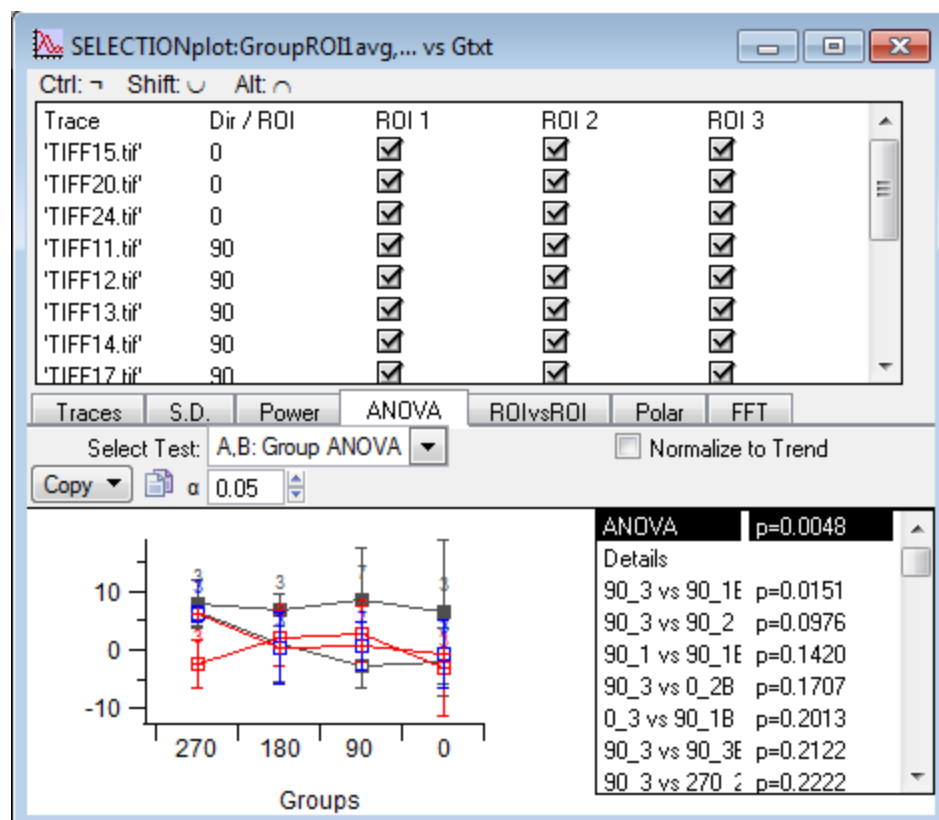


Figure 6-21 Group ANOVA

Paired performs a paired t-test between regions A and B.

Both the group and ROI ANOVA tests can be performed between regions A and B, resulting in double number of comparisons. The details are shown in the table, where the first number is the group, the second number is the ROI and B designated region B.



6.11.5. ROIvsROI tab

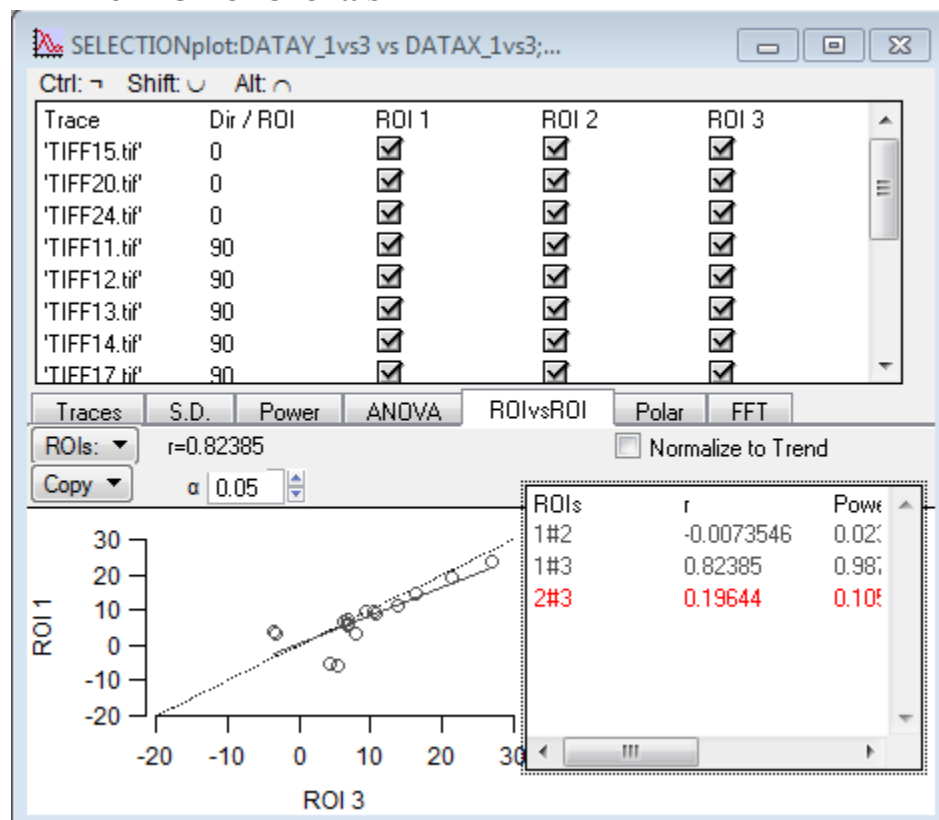


Figure 6-22 ROI vs ROI tab correlates between ROIs

A tab for performing simple linear regression analysis between pairs of ROIs (the pair can be selected from the ROIs pull down menu). The analysis provides with a r and p values, as well as the power to obtain the result.

6.11.6. Polar tab

Draws a polar plot of the selected ROIs and computes the variables described in [Polar plot](#).

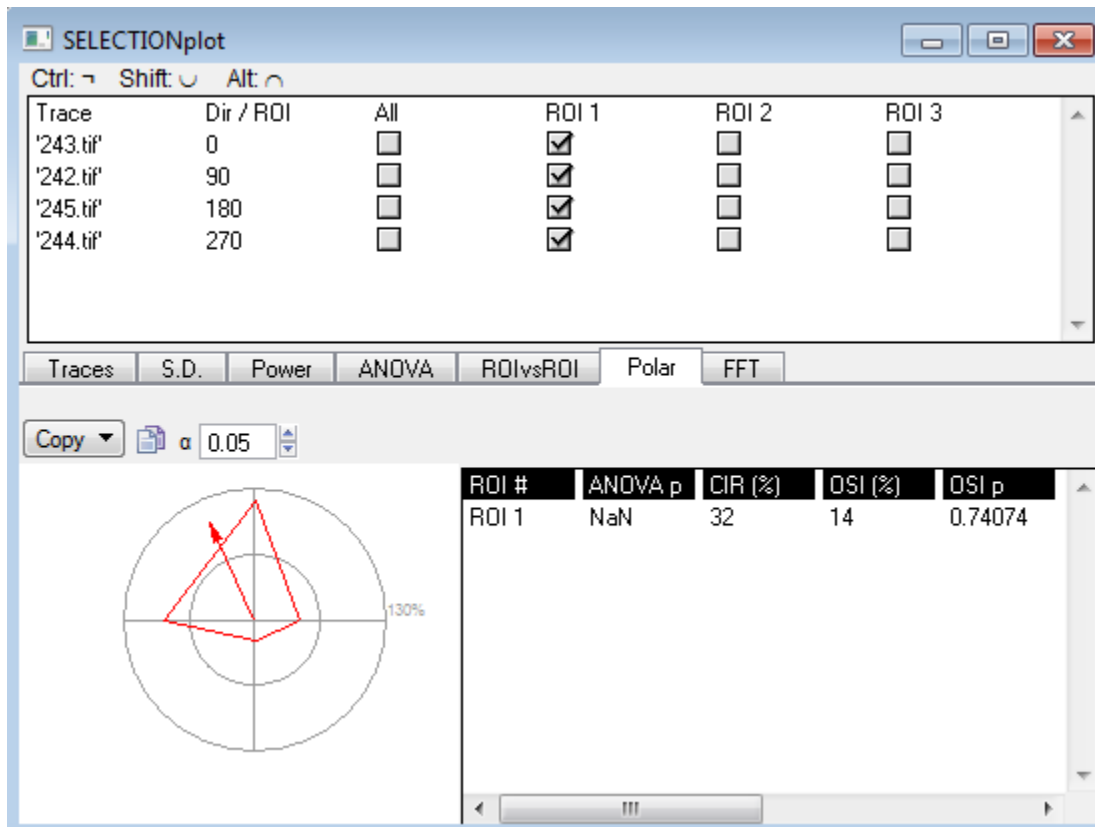


Figure 6-23 Polar plot based on selected traces

6.11.7. FFT tab

Performs a fast Fourier transformation on selected ROIs.

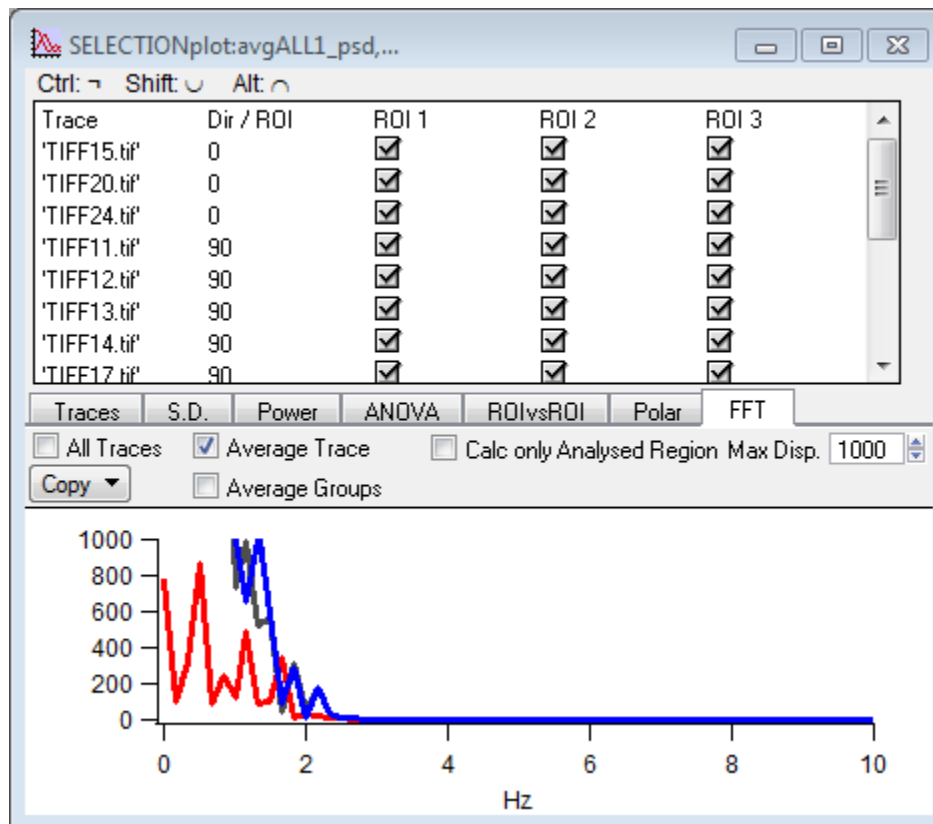


Figure 6-24 FFT plot

7. Receptive field mapping

Receptive field (RF) mapping module adds an extension to the basic iPhys package. The package was developed to perform filtered back projection (FBP; Johnston et al 2014, J Physiol 592-22 4839-54). RF mapping with FBP is performed by stimulating the eye with bars that appear on different locations and at different orientations within the expected RF. RF is reconstructed by overlaying the magnitude of the responses for each stimulus with the stimulus shape. The process can be performed on EP and imaging data as described below.

7.1. Receptive Field panel

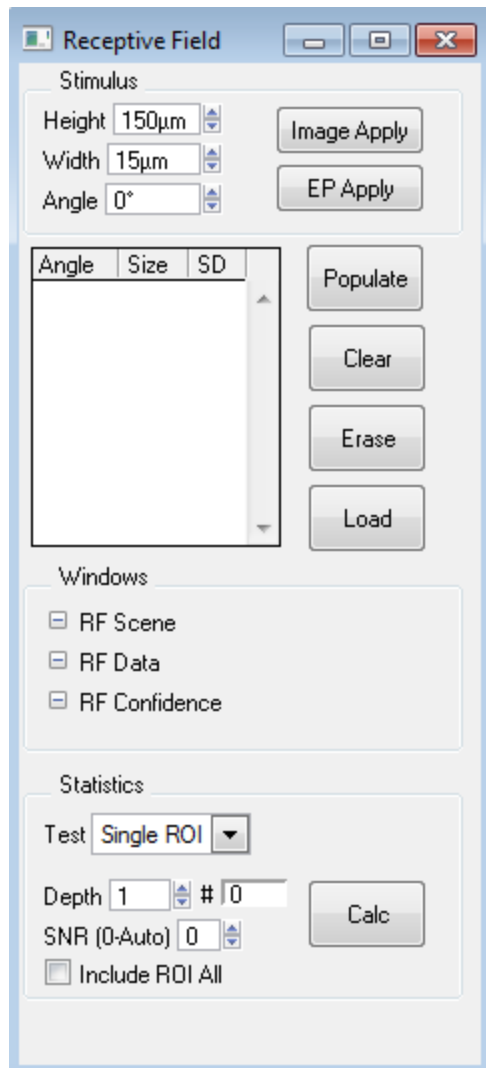
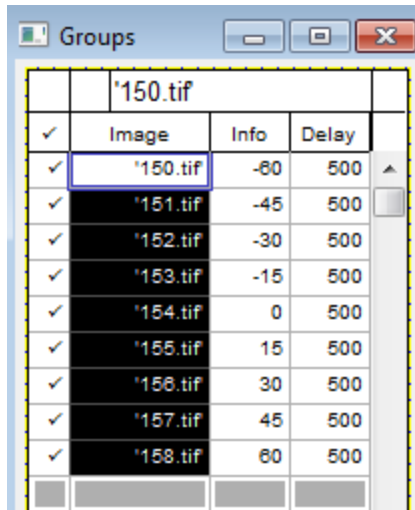


Figure 7-1 Receptive Field panel

The panel is opened from the iPhysiology->Windows popup menu. Stimulus box describes the shape (width and height) and the orientation of the stimulus. The two '**Apply**' buttons add the current Image or EP analysis data to the RF.



Groups			
	Image	Info	Delay
✓	'150.tif'	-60	500
✓	'151.tif'	-45	500
✓	'152.tif'	-30	500
✓	'153.tif'	-15	500
✓	'154.tif'	0	500
✓	'155.tif'	15	500
✓	'156.tif'	30	500
✓	'157.tif'	45	500
✓	'158.tif'	60	500

Figure 7-2 Example imaging stimulus set to be added to the RF analysis. The stimulus consisted of 9 bars projected at the locations specified by the Info field.

When data is applied to RF, it would appear on the RF table. Clicking on the table or on the 'Populate' button will open two new windows -> 'Field' and 'RF data' that show the calculated RF and the data amplitude at each location respectively.

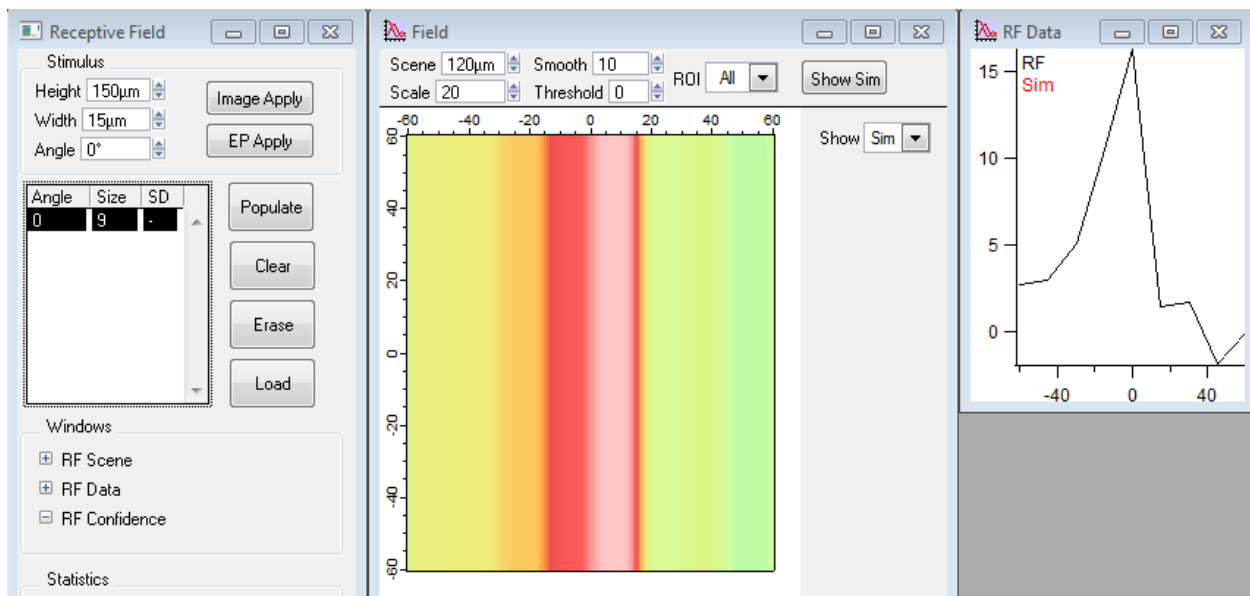


Figure 7-3 RF after addition of stimulus data for a single orientation

7.2. RF data plot

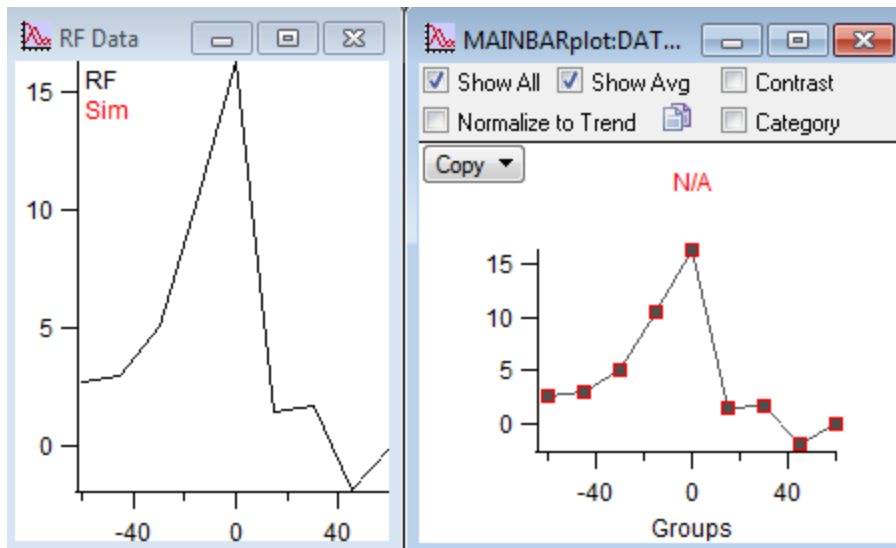


Figure 7-4 Similarities between RF data and Bar/contrast plot

The amplitude of the data is similar and is derived from to the [Bar/contrast plot](#) for images or [Groups plot](#) for EP.

Stimulus orientations can be added to the RF sequentially by opening the corresponding data and adding it to RF using the apply buttons (be careful to set the correct stimulation angle).

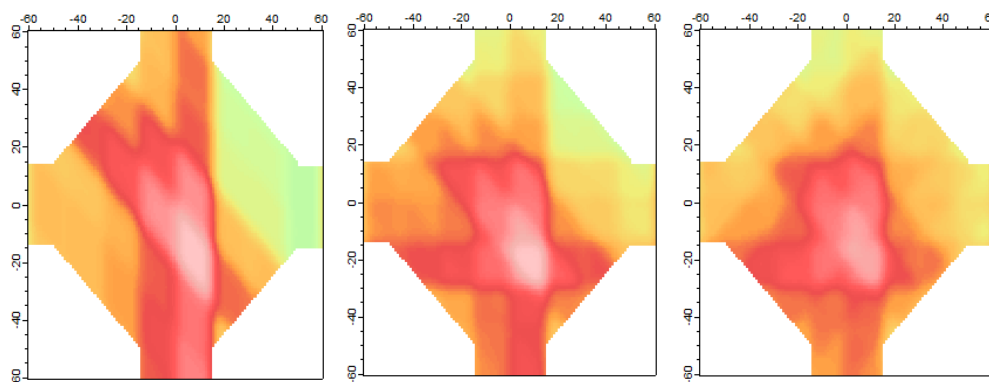


Figure 7-5 Enhancement of RF estimation with 2 (left), 3 (middle) and 4 (right) stimuli orientations

The following strategies can be used to improve RF estimation:

- a) Larger number of stimulation directions. generally, 5 or 6 directions are preferable. The directions should go up to 180 degrees (not including 180°, as it is equivalent to 0°). The following table shows stimulation angles that should be used for different number of directions

Number of directions	4	5	6	8
----------------------	---	---	---	---

Preferable stimulation angles	0° 45° 90° 135°	0° 36° 72° 108° 144°	0° 30° 60° 90° 120° 150°	0° 22.5° 45° 67.5° 90° 112.5° 135° 157.5°
-------------------------------------	--------------------------	----------------------------------	---	--

- b) Narrower bar widths. Bar width is one way to set the resolution of RF reconstruction. The width of the stimulus can in many cases also affect the amplitude of the signal, with narrower bars typically evoking lower responses. In this case the width of the stimulus should be optimized by trial and error to have the best SNR and RF resolution combination.
- c) Numerous bars. A different strategy to increase RF resolution is to have overlapping stimuli. This is a major advantage of the FBP approach, as it allows to probe RF properties below the resolution of an individual stimulus.

7.3. The field plot

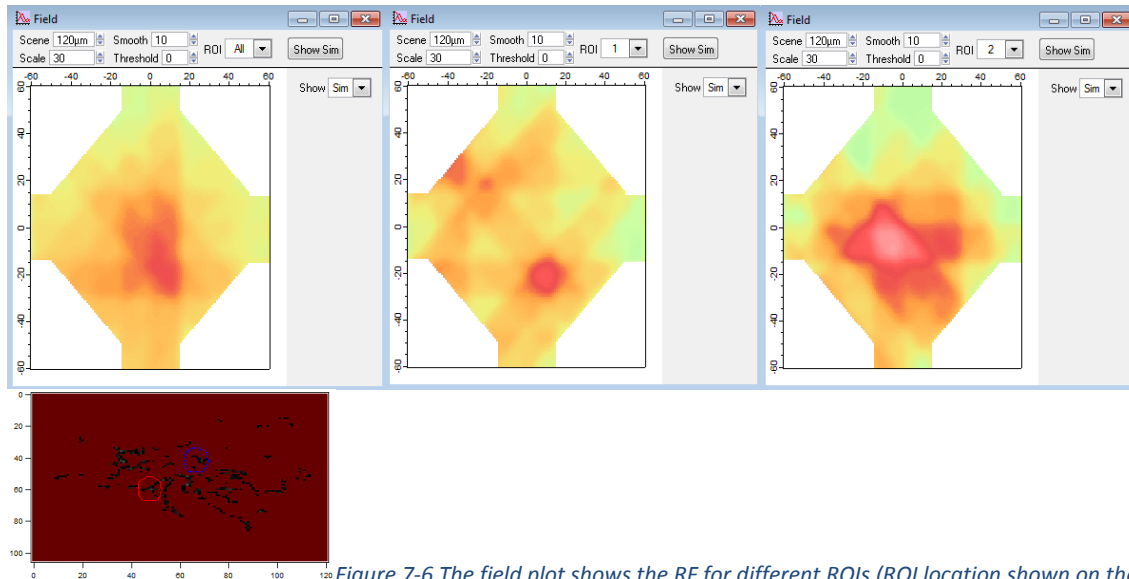


Figure 7-6 The field plot shows the RF for different ROIs (ROI location shown on the bottom)

The field plot shows the reconstructed RF. The size of the reconstructed scene is set by the **scene** field. The **scale** sets the color range. **Threshold** removes all points below the indicated value (this has an effect only on the display, the underlying data remains unchanged). Smooth performs a regular Igor smoothing of both axes. ROI selector indicates the ROI (if done on imaging data) on which the RF was calculated.

7.4. Simulated RF

Because FBP tends overestimate the size of the RF, the user can simulate RFs and see how they would be detected by the stimulus used in the experiment. To simulate a RF, select Show->Sim, this would change the Field display to show the contour of the experimental data.

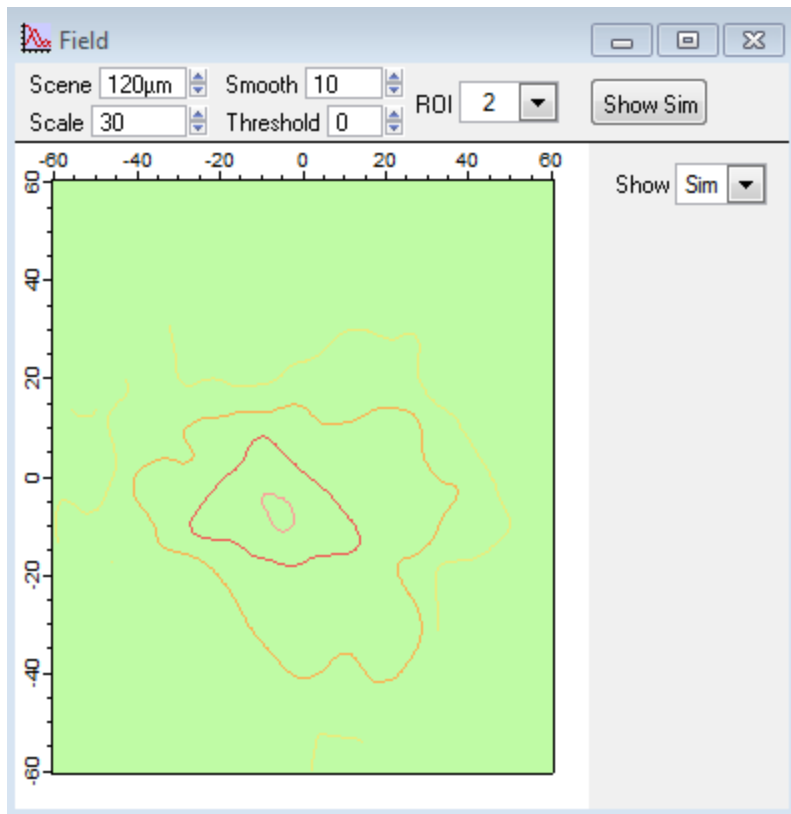


Figure 7-7 Simulated RF step 1 - contour of the experimental data

Next the user can select a marquee at the predicted site of the RF and click on '**Add RF**'

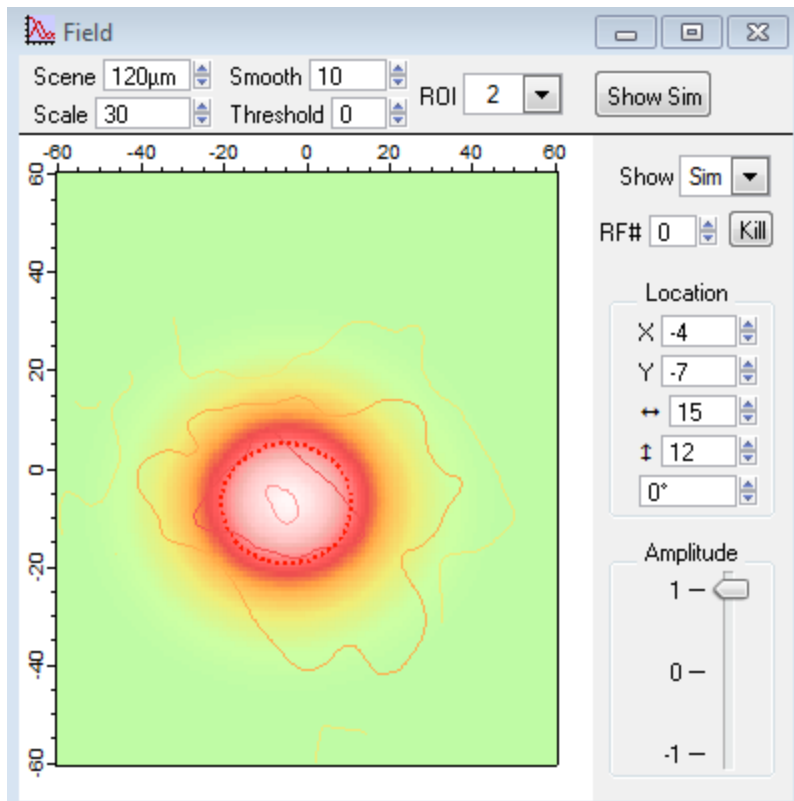


Figure 7-8 Simulated RF step 2 – user generated RF

The newly created RF is the 'actual' RF, to see how it would be estimated by FBP, select '**Show-> Sim RF**'.

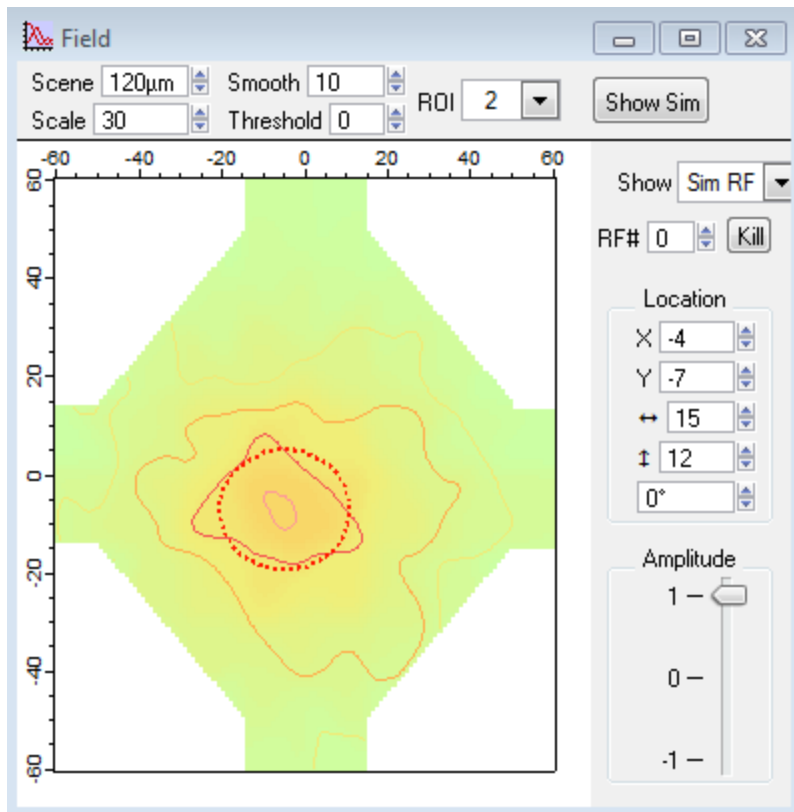


Figure 7-9 Simulated RF step 3 – estimation of the user generated RF

It is possible to move, change the shape and rotate the estimated RF using the **'Location'** menu. The RF can be positive or negative in size and multiple RF can be simulated on the same field by selecting new RFs with the marquee as described above. The goal is to replicate the experimental RF as closely as possible. The fit can be measured from the Field and the RF data plots. Sometimes it is useful to change the scale of the field to get a better fit.

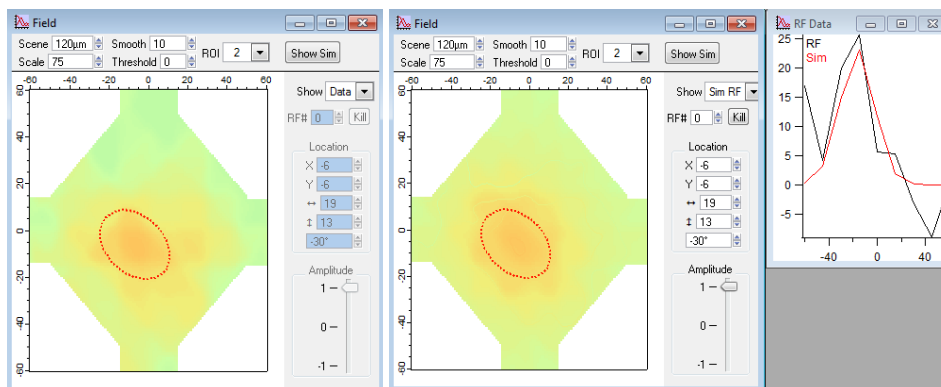


Figure 7-10 Example reconstructed RF. Left - the experimentally determined RF. Right - The simulated RF, note the good overlap in colors. The dotted oval shows the size of the simulated RF. Right - RF data of the experimental (black) and the simulated (red) RF at one of the simulated directions.

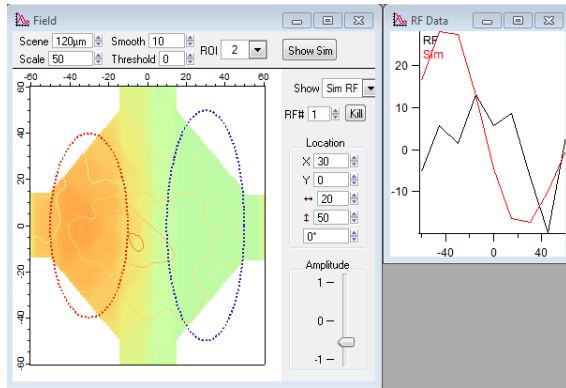


Figure 7-11 Example of 2 component RF, where the blue RF (#1) is inhibitory.

7.5. Statistical tests

Different ROIs may have different RFs, but given the uncertainties of calcium imaging, these RF estimations may differ due to noise. RF mapping module allows to estimate the accuracy of the RF mapping and test if different ROI have different RFs.

7.5.1. Single ROI

The single ROI test examines the accuracy of the center of the RF. The estimation is performed by adding noise to the data, reconstructing the resulting RF and extracting the center of the RF. The process is repeated over a number of iterations as set by the '**Depth**' field. The size of the noise can be set by the user using the '**SNR**' field. SNR =0 is a special option that will take the SNR of the experimental data (when there are multiple repeats of the same stimulus parameters in each direction).

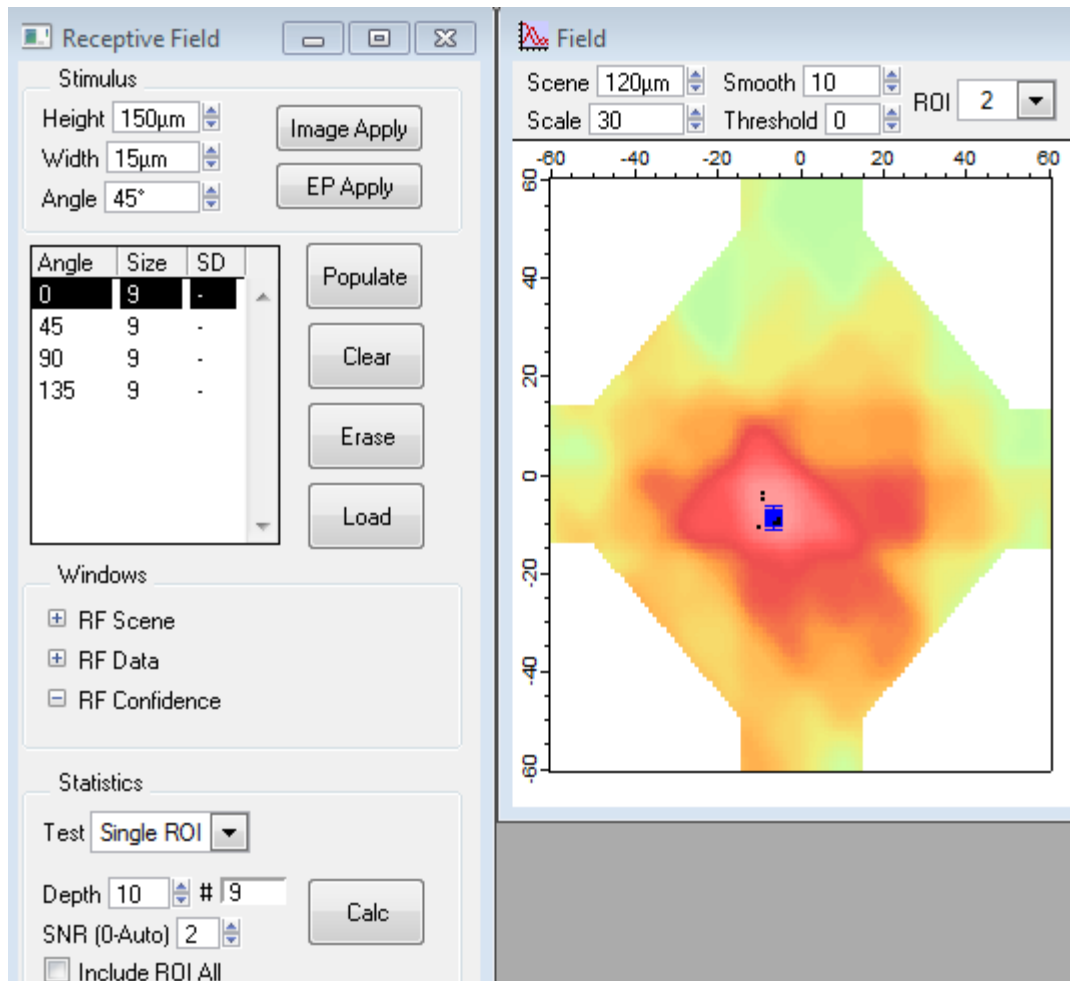


Figure 7-12 Accuracy of RF estimation for a single ROI

7.5.2. Multiple ROI

Different ROIs can be compared using a similar approach.

7.6. Electrophysiology RF mapping

EP data can be mapped in a similar way to imaging. Electrical responses to bars can be imported to RF mapping with 'EP apply' button. Currently, only the first (A) analysis region is considered. With EP, the number of ROIs is set to 1. The data that is imported and used in RF mapping is set by the modality of EP analysis (number of action potentials, subthreshold voltage or raw data) using the regular EP selection, as described [above](#). All other controls will be identical to imaging RF mapping.

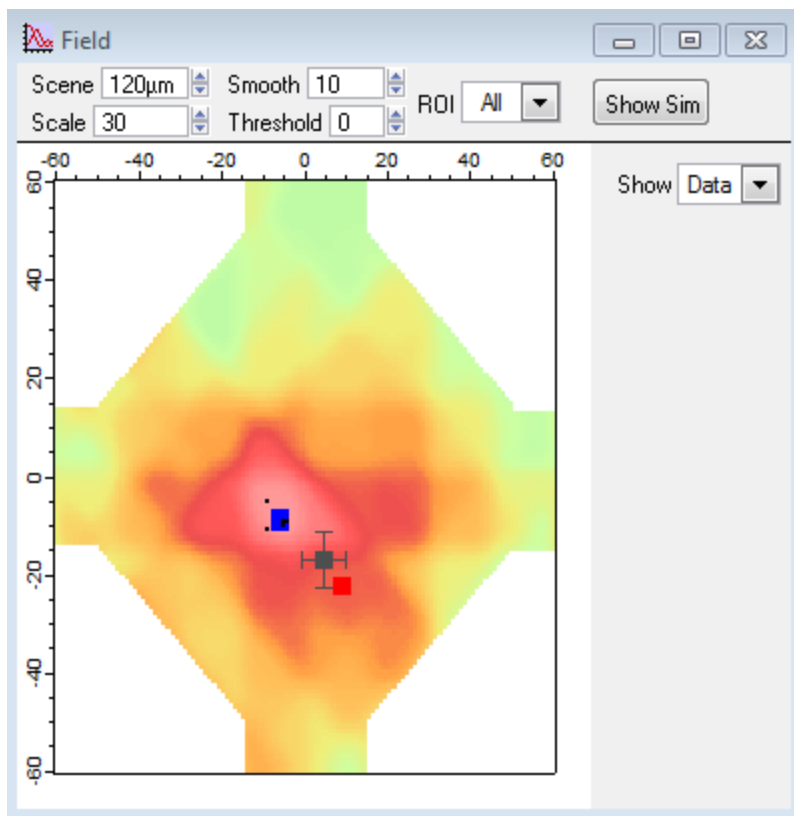


Figure 7-13 Estimated centers of RF for ROI all (grey), ROI 1 (red) and ROI 2 (blue)


RF_ANOVA_RES:RF_ANOVA_Result,...			
R0 C0		5.33662e-10	
Row	RF_ANOVA_Resu	RF_ANOVA_Resu	T_TukeyDescriptors
	0	1	
0	5.33662e-10	2.00728e-08	RF_ANOVAY2 vs RF_ANOVAY1
1	0.0301843	8.52177e-05	RF_ANOVAY2 vs RF_ANOVAY0
2	2.84574e-07	0.00780752	RF_ANOVAY0 vs RF_ANOVAY1
3			

Figure 7-14 RF ANOVA table

The locations of the estimated RFs are compared using ANOVA, and the p values of the X and Y differences are shown in RF ANOVA table.

8. Appendix A - Example usages

8.1. Starting a new electrophysiological acquisition

1. Start Igor
2. Open iPhys
3. If iPhysiology window is not present, select macro->main.
4. Select new output directory with iPhysiology->File, new Path
5. iPhysiology -> Seal test to check electrode resistance
6. iPhysiology -> Run to record data
7. AutoSave before recording to save data or click on the iPhysiology -> save button () post recording.
8. Data trace plot will display the acquired information and trace number

8.2. Setting recording parameters

1. Go to iPhysiology -> I/O -> Channels to set the number of active channels and acquisition rate
2. Go to iPhysiology -> I/O -> Output to determine if seal test is needed and what output parameters are required
3. iPhysiology -> Duration, Repeat # and Repeat (s) set the length of the acquired trace, the number of sweeps and the interval between sweeps.

8.3. Setting output options

For the purposes of this example the experiment involves setting the voltage on the electrode connected to channel one (A1) to -80mV for 100 ms and then increase it by 10mV steps between sweeps for 1000ms.

1. Open iPhysiology -> I/O -> Output window
2. Click on Output Composer
3. On D/A Output window select channels A1
4. Click on 'name' field to change the name to 'baseline'
5. Set duration to the Duration of the trace (for example, 2 seconds equal 2000ms), amplitude -80, start 0
6. Click on 'Duplicate'
7. Select the newly created tab (still named 'baseline')
8. Rename it to 'step' (use the 'name' field)
9. Set duration 1000, amplitude 0, Delta amplitude 10, start 100

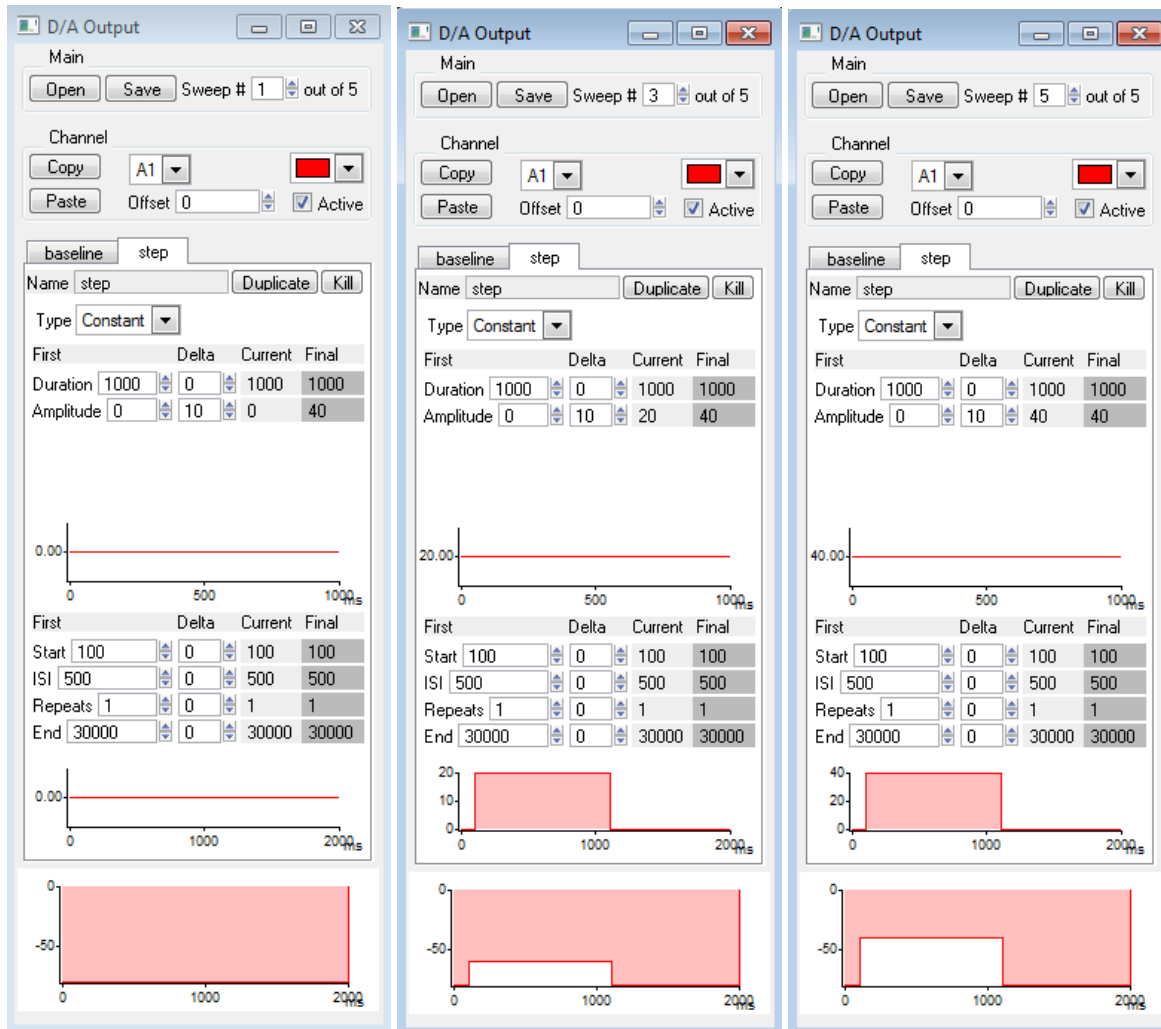


Figure 8-1 Example set voltages at the first, third and fifth sweeps

In next example a digital channel 2 needs to be set to 'high' for 10 ms every 1 second

1. Open iPhysiology -> I/O -> Output window
2. Click on Output Composer
3. On D/A Output window select channels D2 (LSM)
4. Set duration to 10ms, Amplitude to 1 (digital high), Start 0ms, ISI to 1000, repeat to 10 (or higher based on recording length), 'End' to a value above recording length

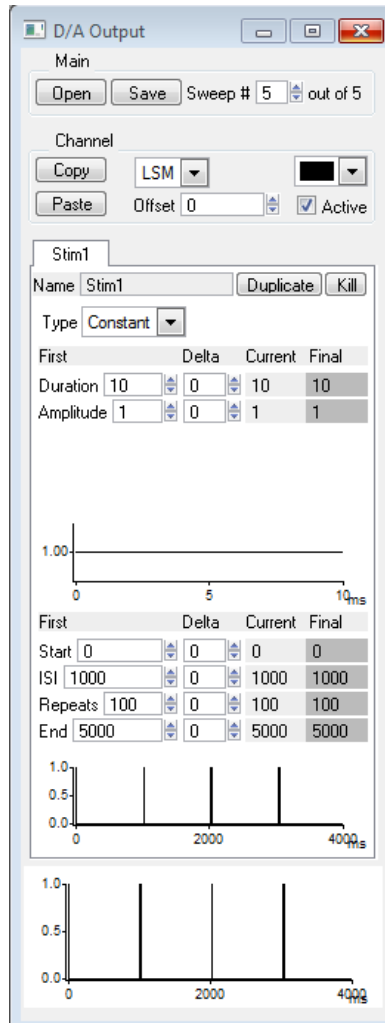
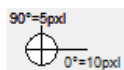


Figure 8-2 Example digital output

8.4. Creating a new visual stimulus

1. If LCD window is not visible select iPhysiology->Windows->Display
2. Check Display to be active
3. Select Communication
4. Select Beacon
5. A target beacon will appear on the display window
6. Move the display window with X and Y fields to position the beacon at the desired location (center of field of view for sub-condenser display)
7. If arranging the stimulator for the first time, measure the distance of the long arm of the target, divide by 10, and enter the number in Pixel field.
8. If arranging the stimulator for the first time, rotate the display till the actual image is similar

to the schematic



9. Select Surface/Shape/Movement/Time to set the attributes of the stimulus
10. [Duplicate](#) the stimulus/Elements if more than one stimulus is needed.

8.4.1. Example 1: create a bright spot with an increasing diameter between sweeps

1. iPhysiology->Windows->Display
2. Select LCD -> Surface, set the background to 50
3. Right click on 'background' tab, select duplicate
4. Right click on the right 'background' tab, rename to 'Spot' by typing in the name field
5. Select LCD -> Surface, set the background to 100
6. Select LCD -> Shape/Overlay
7. Set type to circle, Diameter 50, this is the initial diameter of the spot
8. Click on Shape/Overlay -> Full
9. Set Delta diameter to 20, this is the increase in spot diameter
10. If more than one sweep is used in the experiment, change sweep number to see the resulting shape
11. To limit the time of sweep presentation, Select LCD -> Time, set Start to 1000ms, Duration to 1000ms.
12. Play the movie on the LCD panel to see the shape

8.4.2. Example 2: create a bright ring with an increasing diameter between sweeps

1. Repeat previous example, set 20 to delta Ring diameter

8.4.3. Example 3: create a bar moving in different directions between sweeps

1. Repeat previous example, Change Stim type to Rectangle
2. Select LCD -> Time, Set Start to 0, Duration to inf
3. Select the first sweep
4. Select LCD -> Movement
5. Select 'move shape'
6. Select 'Rotate'
7. Click on the top radio button
8. Click on the 45-degree button to set the 'advance' field to -45

Movement

Full

Movement

Show

Angle

	First	Delta	Current	Final
Start				
Angle	270	-45	270	45
Radius	2000	0	2000	2000
Time	0	0	0	0
End				
Angle	90	-45	90	-135
Radius	2000	0	2000	2000
Time	4000	0	4000	4000
Speed	1000	0	1000	1000

Move Back

☒

Move Shape

Rotate

Force Time

Directions (sweep 1)

135°

☒

45°

180°

☐

90°

☐

0°

225°

☐

315°

Advance

-45°

0°

45°

90°

180°

Movement

Figure 8-3 The Movement window at the end of the example