Analysis of visual responses to sinusoidal gratings

Contents of zip file

- These notes on setting up the visual stimulation and recording hardware.
- A Matlab program for generating visual stimuli: *SineGratings.m*
- A table of marker codes and corresponding stimulus angles and grating pitches
- A Spike2 script (*AR 05.s2s*) for analysing responses to moving visual stimuli
- An example Spike2 data file so that you can familiarise yourself with the analysis.

Visual Stimulus generation

The stimulation program, *SineGratings.m*, runs from PsychToolBox, which is a Matlab based visual stimulation toolbox.

For Matlab, use 32-bit version of 2014b with Data Acquisition toolbox and a Windows PC with a good quality graphics card (Gforce or similar). The brightnes of the screen presenting the stimuli should be measured with a luminometer (phone apps are freely available) and be close to 32 cd/m2. The Ribic lab. uses a system with an LED screen with a refresh rate of 60 Hz .Screen brightness parameters are set to gamma: 2.4; backlight: 0; brightness: 10 and contrast: 50

Psychtoolbox communicates with the CED 1401 data acquisition interface (Power or Micro) via a National Instruments PCI 6503 (Nidaq). Install this beforehand. Connect the PCI to the 25-way D-type digital input port (male) on the rear panel of the 1401 interface.

Connect the following pins to the appropriate slots on the PCI connector block with insulated cables: 13 (Power1401) to 50 (PCI), 23 to 17, 5-6-7-8 to 33-35-37-39, 18-19-20-21 to 41-43-45-47. These pins on the 1401 digital input connector correspond to digital input bits 0 to 7 plus the *Data available* pin. A full description and diagram of the pin assignments of the 1401 digital input port are available from the hardware handbook of your 1401. You can download this from the CED website.

Connect the other end to the PCI slot in the computer. NI sells the card with all accessories (ribbon cable and connector block).

The Ribic group use PTB version 3.0.11 so make sure to get the right one. Follow the installation instructions to install it (on PTB website). Now, check whether Matlab recognizes the Ni-daq by typing *daqsupport* in the command window. If not, register the device using data acquisition toolbox functions

(https://www.mathworks.com/help/daq/referencelist.html?type=function).

You should now be able to run the Matlab script *SineGratings*. to generate the visual stimulus gratings you require. Spike2 will record markers (time stamps with associated marker codes) at the onset and offset of stimuli (same code for start and stop). The stimuli are arranged in array of angles and frequencies so they are simple to decode using Spike2 markers.

Spike2 sampling configuration notes

Set a sampling rate of at least 25 kHz for each waveform channel of extracellular spikes. This gives sufficient resolution for achieving a good separation of individual units into WaveMarks offline via template matching or principal component analysis.

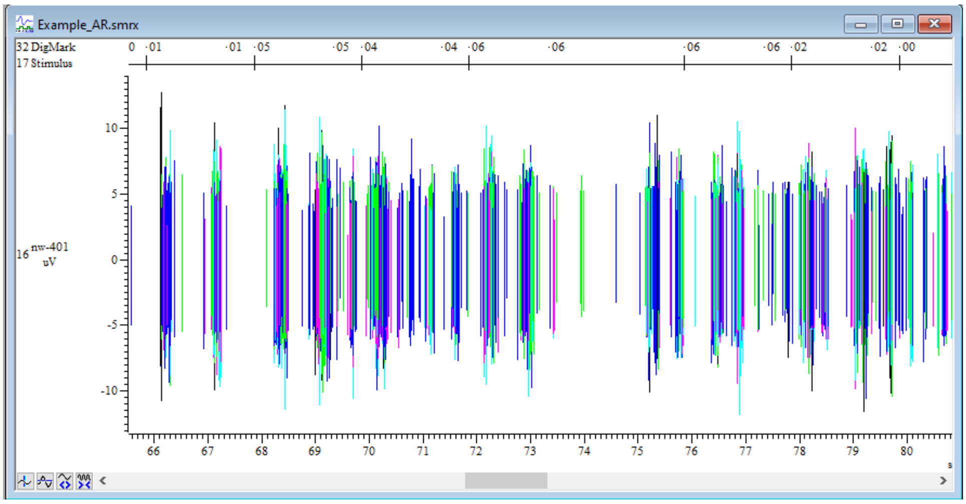
Check the *Resolution* tab of the sampling configuration to ensure that it is optimised for your particular 1401 model and that the internal clocks are adjusted to achieve the sample rates that you requested.

You must enable the digital marker channel (32) to record the time stamps and markers generated by the visual stimulator.

Data analysis

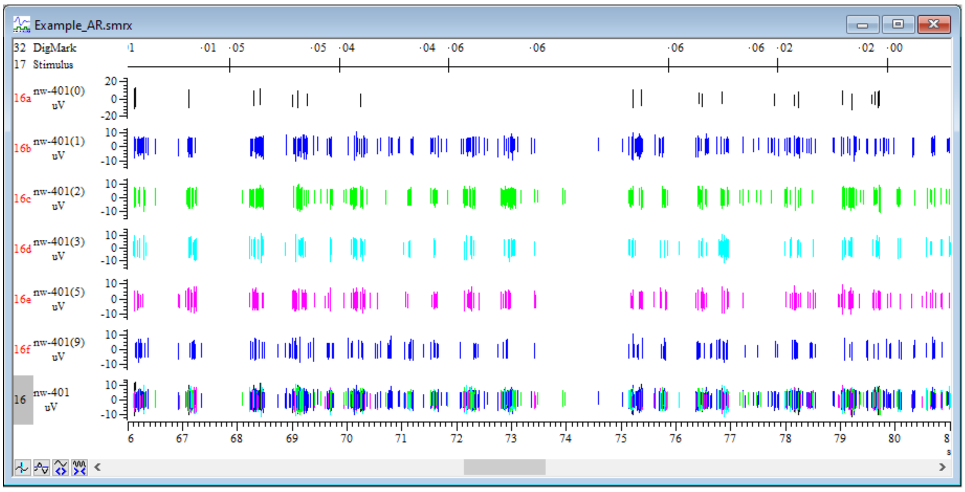
The initial stage of analysis is the identification of individual units within a multi-cellular response and the generation of multi-unit WaveMark channels with spikes from each unit identified by a marker code. This is the starting point for analysis with the spike2 script AR(*n*).s2s where *n* is a serial number. The script has its own user guide which you should read in conjunction with these notes.

Raw data



In this example, multiple WaveMarks have been generated offline from the original waveform recording. On this scale they appear as coloured vertical lines rather than templated spike shapes. Channel 17 (*Stim On*) contains timestamps marking the onset of each visual stimulus. Channel 32 (DigiMark) contains markers at the onset and end of each visual stimulus. This channel is generated via the Onset Chan button on the script toolbar. The marker codes 01, 02 etc., indicate the angle and pitch of the grating. Multiple stimuli of each type are presented in a user-defined sequence.

Split codes

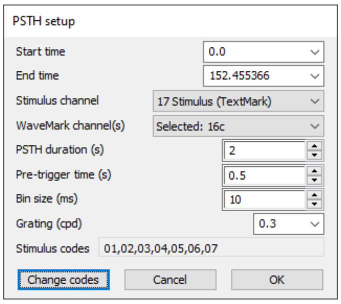


Clicking on the Split codes button creates duplicates of the selected WaveMark channel (highlighted channel nr.) with each duplicate channel displaying responses of a single unit. The next step is to set up the script to create post-stimulus time histograms for each unit and each stimulus angle.

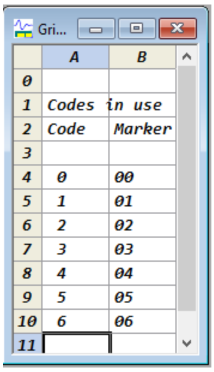
Onset Chan

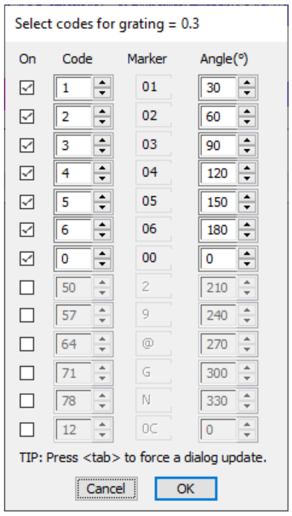
Click here to create a trigger channel containing markers for all the stimuli that you wish to include in your analysis. You can include all the stimuli in the file (default) or include only stimuli within time ranges that you include by bracketing them with cursors. The onset markers are saved as TextMarks that are colour coded to indicate the stimulus type, *Set up* In the *Setup* dialog, you can specify:

- the time range to analyse
- The channel holding stimulus onset markers
- One or more WaveMark channels to analyse
- PSTH parameters: duration pre-trigger and bin size
- Grating pitch (cycles per degree)
- Specify the Stimulus codes to analyse via Change codes



Set Codes When you click on Change codes, a grid view and a dialog open. The grid view lists all of the stimulus codes that were found in the current data file.





In the dialog you can opt to analyse up to 13 codes by checking boxes to enable them. Select each code using the spinner arrows on the Code items in the dialog to select the decimal version of the desired marker code. The hexadecimal equivalent (shown in the data file) is shown in the next column. Finally, select or type in the stimulus angle corresponding to that marker code so that PSTH and Response vs Angle plots are correctly labelled. Click on OK to close the dialog.

Process

Click on the Process button to generate PSTHs and plots of Response vs. Angle for each unit selected in the *Setup* dialog. Results from two units are shown below. Histogram bars and traces are colour coded to match the colour of the WaveMark from which they were derived. Traces in the PSTH plots are presented in the order specified in the *Change Codes* dialog Additional information is stored in the channel comments. Hover with the mouse over the channel titles to view them.

Visual responses

Response vs Angle plots are (mean freq during stimulus ï pre-stimulus frequency) shown with 1 standard error of the mean.

Spike2 has many options for modifying these plots to suit your requirements. Some of these such as showing and hiding channels are available via the context menu when you right-click on a view. Many others are available via the Spike2 *View* menu or the colour palette icon on the toolbar.