Acquipix User Manual

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Contents

Overview

Multi-stream data recorded while performing a visual or optogenetic experiment, can be difficult and laborious to process. If your data includes stimulation, multi-channel spiking, LFPs, running speed, and pupil tracking, you will likely already have to deal with 5 independent data streams that need to be synchronized (fig. 1). Moreover, keeping track of all these different files can be a pain. That's why we created the Acquipix repository, which does all the synchronizing and synthesizing for you. All our code is modular, open source and fully customizable, but we made sure you can also run everything out-of-the-box using only graphical user interfaces without having to write a single line of code.

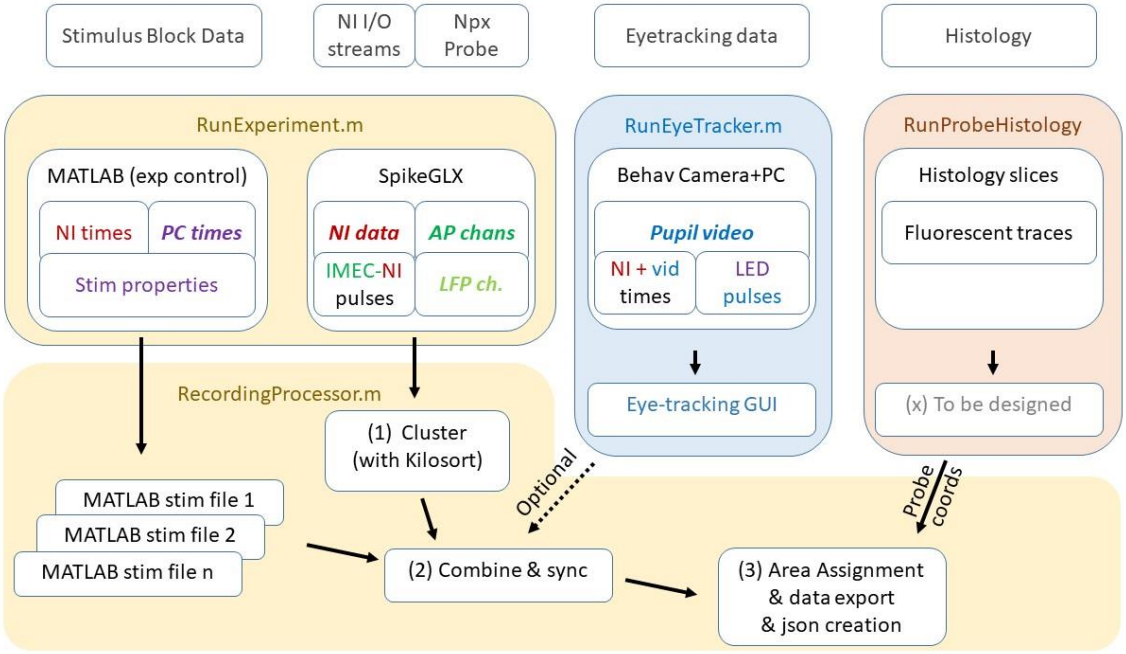


Figure 1. Technical data flowchart showing a single recording. First column from left (*MATLAB (exp control)*): each recording consists of one or more stimulus blocks that contain onset/offset times and stimulus data that are generated using RunExperiment.m and its dependency functions. The stimulation scripts automatically record the time stamps of the national instruments (NI) I/O card and internal stimulus times (PC times) generated by Psychtoolbox. Second column (*SpikeGLX*): although the NI I/O streams and IMEC neuropixels (Npx) data may appear to be monolithic, they are entirely separate data streams with independent clock times. SpikeGLX produces data streams at three different sampling frequencies: the NI I/O data, the AP channels, and the downsampled LFP channels. To synchronize these data streams, you need common pulses; i.e., connect the IMEC pulse generator to an NI I/O stream. Third column (*RunEyeTracker*): optionally, you can use the eye-tracker program to perform online eye-tracking and automatically synchronize your pupil tracking movie to the rest of your data. While recording, the eye tracker program periodically queries SpikeGLX for NI data stream time stamps and saves a log file containing synchronous video time stamps and NI time stamps. Moreover, you can define an ROI for luminance-based high-resolution synchronization (LED pulses). Last column (*RunProbeHistology*): by registering your histology slices to the Allen Brain Atlas, you can extract your probe's location during recording. The RecordingProcessor allows you to easily cluster your spikes with Kilosort (1), combine and synchronize data from multiple sources (2), and automatically extract which brain area each cell was located, using the ABA API (3).

Installation instructions

Before you start:

1. Make sure your computer is up-to-date, and has compatible (not necessarily the newest) CUDA drivers for your Nvidia GPU. If you don't have an Nvidia GPU, you'll have to buy one, because multiple Acquipix functions, as well as the external libraries it uses (Kilosort, Psychtoolbox) make use of CUDA-specific GPU-accelerated computing.
2. You will want a fast SSD for data buffering for various pre-processing operations. You can run everything without an SSD, but it will be noticeably slower.

Installing the external libraries for the recording computer:

1. If you want to run visual experiments on this computer, download Psychtoolbox and follow the installation instructions: http://psychtoolbox.org/download
2. If you want to record Neuropixels on this computer, install SpikeGLX: https://github.com/billkarsh/SpikeGLX
3. If you want to perform pupil-tracking specifically, or generally want to have accurate & automatic synchronization between your recorded videos, stimulation and Neuropixels data, you can you install this Acquipix-independent module on the computer where you're recording the video's: https://github.com/JorritMontijn/EyeTracker

Installing the external libraries for preprocessing your data:

1. Download the Kilosort, Npy-matlab and spikes repositories and follow their installation instructions: https://github.com/MouseLand/Kilosort/, https://github.com/kwikteam/npy-matlab and https://github.com/cortex-lab/spikes. To compile Kilosort's GPU functions, you'll first need to install a compiler. You can for example use visual studio, but note that only specific versions will work in combination with specific versions of matlab: https://visualstudio.microsoft.com/vs/older-downloads/. In all cases, make sure you get the ***Community*** version, and make sure you also install the ***C++ compiler***.
2. Compiling Kilosort's CUDA functions with ***Visual Studio Community 2015*** with Update 3 seems to work with Matlab R2019b, but the version compatibility seems somewhat arcane and random. If your matlab is R2019b or later, try VSC2015 first. If it doesn't work, you can try a different version (i.e., VSC2013).
3. Rename and edit the config and chanmap files to match your preferred settings and copy them to a folder outside the git repositories if you want to make sure they don't get overwritten accidentally

Installing Acquipix and its dependencies:

1. Download the Acquipix repository at https://github.com/JorritMontijn/Acquipix
2. Download the GeneralAnalysis and MNCP repositories from https://github.com/JorritMontijn/
3. If you wish to automatically compute a responsiveness metric for your putative single cells, download https://github.com/JorritMontijn/ZETA

Workflow summary:

To run an experiment:

1. Start SpikeGLX and begin a new acquisition
2. Start “RunExperiment” in MATLAB
3. Select your stimulation and parameters & start the experiment (spikeglx will start recording automatically)

Optional steps for pre-processing:

A) Pre-process your eye-tracking data
B) Pre-process your probe coordinates

To pre-process your spiking data in five mouse clicks:

1. Start “RunRecordingProcessor” in MATLAB and compile your data library
2. Select the recording you wish to preprocess
3. Cluster your data by clicking the button in the GUI
4. Combine data from multiple sources by clicking another button using the GUI
5. Optionally check the results, make some changes if necessary & then export with click #5

User guide

Running an experiment

To run an experiment, run matlab and execute “RunExperiment”

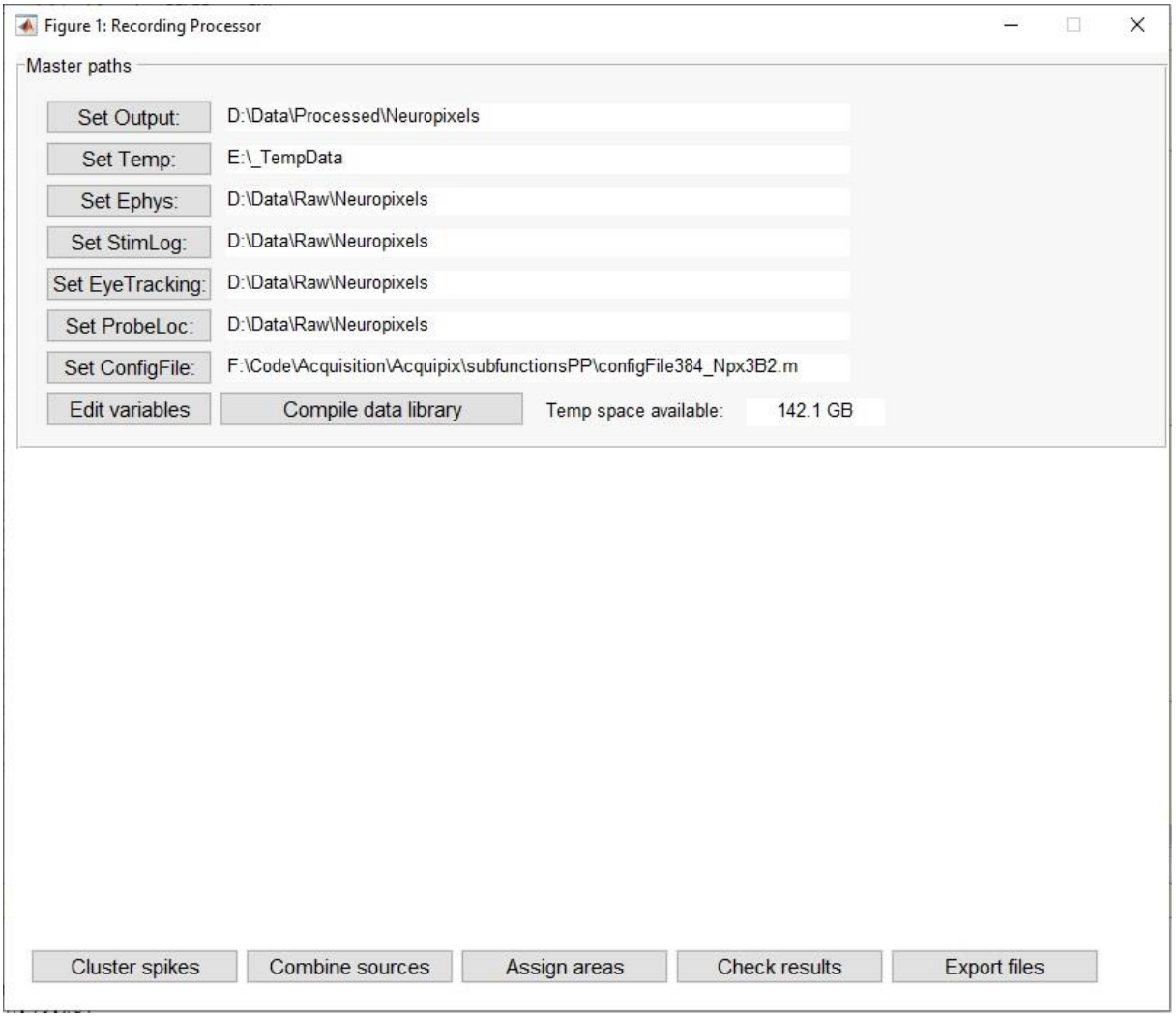
Using the online analysis tools

Acquipix comes with a set of online analysis tools that allow you to stream SpikeGLX data in real-time and calculate MUA tuning curves per channel: runOnlineOT for Orientation Tuning, runOnlineNM for Natural Movies, and runOnlineRF for Receptive Field mapping.

Preprocessing your data

Setting the variables and parameters

To start preprocessing, run matlab and execute “runRecordingProcessor”. The startup screen will look like this:



To start pre-processing your data, you will first need to set the correct paths:

“Set Output:” specifies where the aggregated and pre-processed data files will be stored.

“Set Temp:” path to fast SSD for data buffering (e.g., used by KiloSort).

“Set Ephys:” root path of your SpikeGLX data files. The actual data files can be in subdirectories. The recommended path structure is something like /root/experiment-set/recordingdate/. To avoid having to compile huge libraries on startup, it is also recommended to use a different root directory for each user and project, but that is of course all up to you.

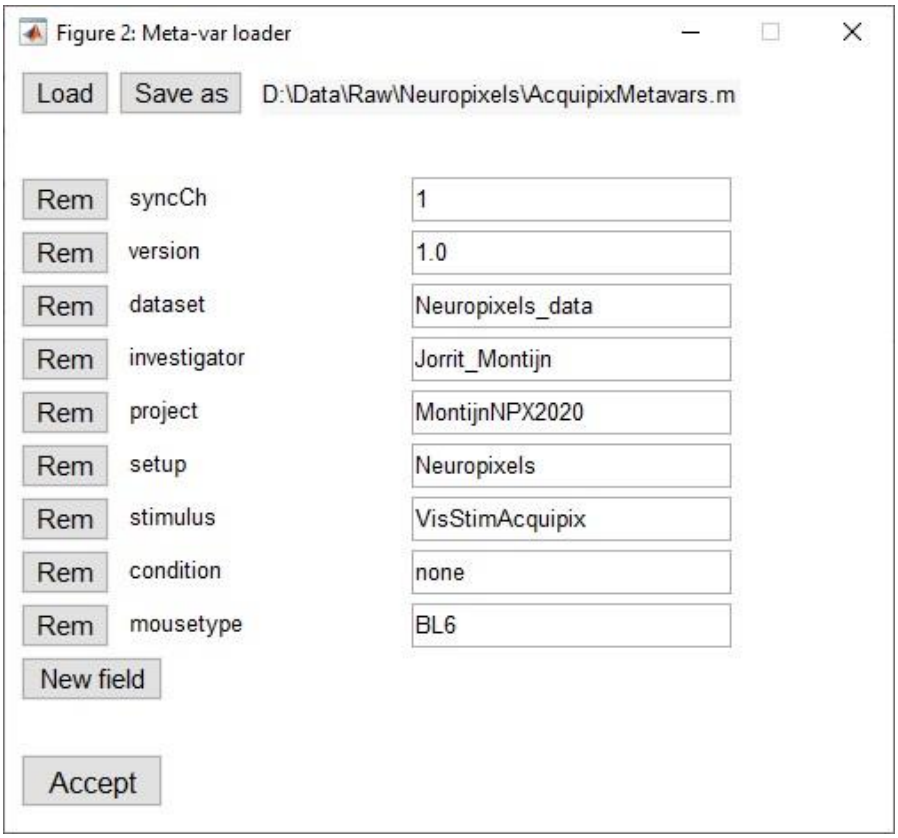
“Set StimLog:” Same as above, but for the stimulus logs produced by RunExperiment’s subsidiary stimulation functions (e.g., RunDriftingGrating). If you wish, you can put these files together with the SpikeGLX files in the same folder and set the same root. The library compiler combines files from the same recording based on the SpikeGLX run name if they are stored as a variable in the file. If not, it defaults to using the experiment ID in the file name. It is therefore recommended to not change the file names yourself, as this might potentially cause incorrect file attributions.

“Set EyeTracking:” Same as above, but for the output of the EyeTracker repository. Use of the EyeTracker is optional.

“Set ProbeLoc:” Same as above, but for the probe location files exported by RunProbeHistology (NB: still to do). During data synthesis generation, each cluster will be assigned a brain area from the Allen Brain Atlas, based on the supplied probe location and depth of the cluster’s dominant probe channel.

“Set ConfigFile:” Path to the preprocessing configuration file used by KiloSort. A default file for the Neuropixels 1.0 probe is supplied in the /Acquipix/subfunctionsPP/ folder (configFile384_Npx3B2.m). You can edit the file yourself to match your probe properties, but it is recommended to save this file to a separate directory so you don’t make changes to files the /Acquipix/ directory. This way you can easily update the code with github if a new version is released. If you start locally overwriting repo files, you might have to create a fork and merge commits manually.

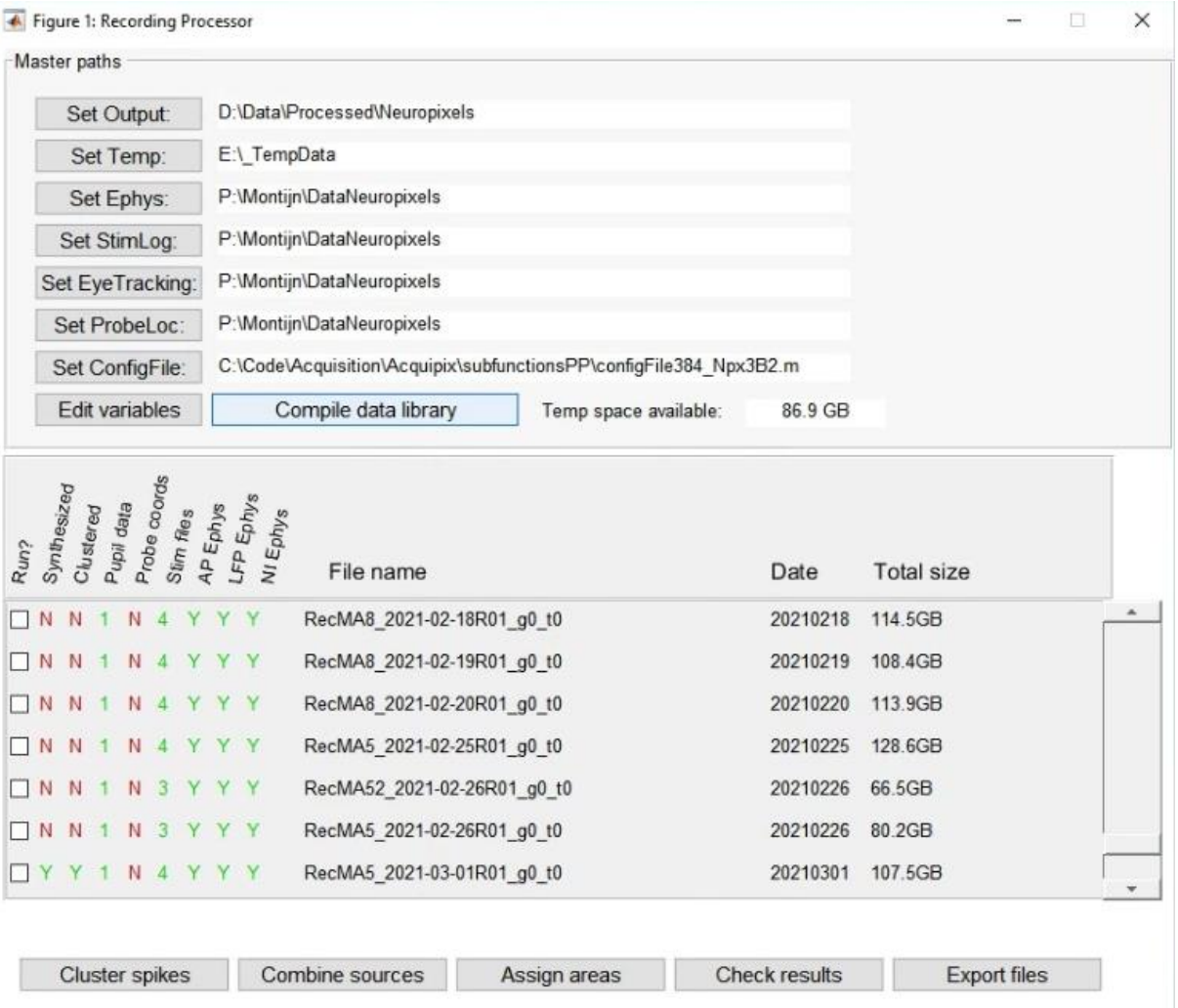
Next, you will want to edit the variables using the “Edit variables:” button. This will make another screen appear, where you can set some metadata that you want to be exported as a json file along with your aggregate data file:



You can add and remove variables as you wish. There is only one “special” variable here, and that is “syncCh”. syncCh specifies the channel in your National Instruments data file (nidq) that can be used to synchronize stimulus onsets. E.g.: on my setup, we use a photodiode stuck to the upper right corner of the screen where a white rectangle is presented at the beginning of each stimulus presentation. This photodiode then sends a voltage signal to NI channel #1 so we know exactly when the visual stimulus appeared on screen. It is not required to use this additional synchronization, but if you don’t, there will likely be multiple milliseconds of uncorrected lag+jitter between when your stimulus PC logs the stimulus onset and when it actually appears on the screen.

Compiling the library and pre-processing the data

Now that all parameters have been set, you can compile the data library by clicking ”Compile data library”. The program will now compile a list of all your data files and group them by experiment. The leading file is the SpikeGLX nidq meta file:



This will show you a list of all experiments, their dates and sizes, and whether certain (preprocessed) files are present or not. Hovering over the different indicators will give you further detailed tooltip information.

You can now select multiple recordings (mark the check box under the “Run?” column) and click “Cluster spikes”, after which the program will run Kilosort on all your selected files in serial. When the clustering is finished, you can combine all files of one recording and synchronize the timestamps of the various data into a single synthesized aggregate by clicking “Combine sources”. Finally, you can assign an area to each cluster using “Assign areas” (to do), check and tweak the results with “Check results” (to do), and finally export everything with “Export files” (to do).

Technical descriptions

If you want to make your own stimulation and/or online analysis scripts, you can use the modules present in Acquipix. I've tried to make everything as modular as possible, so it should be easy to plug in experiments of your own design.

Experiment scripts

Overview

We will use "`RunDriftingGratings.m`" as the example here, but other files work the same. All experiment scripts are built so they can be run as a script in the base workspace. The idea here is that your variables will be available in the base workspace even in the case you get an error, forget to save your data, there's a network drive malfunction, etc. I've also built the experiment script so it can be run both independently with default parameters, and as a subfunction of the `RunExperiment` GUI with supplied inputs.

Description of inputs and structure of an experiment script

When you press "Start Experiment!" in `RunExperiment`, it runs the following code block:

```
%run!
assignin('base','sExpMeta',sExpMeta);
assignin('base','sStimParamsSettings',sStimParamsSettings);
assignin('base','sStimPresets',sStimPresets);

evalin('base',strStimType);
```

As mentioned above, you can see that this assigns three variables to the base workspace: `sExpMeta`, `sStimParamsSettings`, and `sStimPresets`. The experiment you wish to run is contained in `strStimType`; for example `runDriftingGratings`.

`sExpMeta` has fixed fields filled by `RE_genGUI`:

```
sExpMeta.boolUseSGL = boolUseSGL;
sExpMeta.boolUseNI = boolUseNI;
sExpMeta.dblPupilLightMultiplier = dblPupilLightMultiplier;
sExpMeta.dblSyncLightMultiplier = dblSyncLightMultiplier;
if boolUseSGL
    sExpMeta.strHostAddress = sRE.strHostAddress;
    sExpMeta.hSGL = sRE.hSGL;
    sExpMeta.sParamsSGL = sRE.sParamsSGL;
    sExpMeta.strRunName = sRE.strRunName;
end
if boolUseNI
    sExpMeta.objDaqOut = sRE.objDaqOut;
end
```

It contains some required static information about this recording; whether you wish to use SpikeGLX (boolUseSGL), the National Instruments I/O box (boolUseNI), gain values for the pupil and synchronization LEDs. If SpikeGLX is connected, it supplies the server's IP address (strHostAddress), a

handle to the SpikeGLX object (hSGL), a parameter structure supplied by SpikeGLX (sParamsSGL), the name of the current recording (strRunName); and if the NI I/O box is used, it supplies a Matlab Daq Interface object objDaqOut.

`sStimParamsSettings` is a structure with parameters that are dynamically extracted from the experiment script code itself. If you look in `runDriftingGratings.m` you can see the following block:

```
%% input params
fprintf('Loading settings...\n');
if ~exist('sStimParamsSettings','var') || isempty(sStimParamsSettings)
    %general
    sStimParamsSettings = struct;
    sStimParamsSettings.strStimType = 'SquareGrating';
    sStimParamsSettings.strOutputPath = 'C:\_Data\Exp'; %appends date

    %visual space parameters
    sStimParamsSettings.dblSubjectPosX_cm = 0; %cm; relative to c of scr
    sStimParamsSettings.dblSubjectPosY_cm = -2.5;%cm; rel to c of scr

    (...)
else
    % evaluate and assign pre-defined values to structure
    cellFields = fieldnames(sStimParamsSettings);
    for intField=1:numel(cellFields)
        try
            sStimParamsSettings.(cellFields{intField}) = ...
                eval(sStimParamsSettings.(cellFields{intField}));
        catch
            sStimParamsSettings.(cellFields{intField}) = ...
                sStimParamsSettings.(cellFields{intField});
        end
    end
end
```

The file `RE_getParams.m` reads the experiment script file and loads the field name, value and comment as tooltip information. Any default value you put/change in your experiment file will therefore automatically pop up in the `RunExperiment` GUI. Moreover, if you change values in the GUI, it will supply them in the `sStimParamsSettings` structure and load them in the `else` block. These are usually semi-static variables, like the experiment script's stimulus type (i.e., "`SquareGrating`"), the location of the mouse relative to the screen, etc.
Finally, `sStimParamsSettings.strRecording` is always added by `RunExperiment` to identify the current recording.

`sStimPresets` has dynamic parameter values that you might want to change often and save as a separate pre-set; e.g., the number of repetitions, orientations, stimulus duration, etc. You can change these values easily in the GUI, and on first run, it generates pre-set files in `assertPresets.m`. For example, you can see the following block here:

```
elseif strcmp(strExp,'RunDriftingGratings')
    for intSet=1:3
        if intSet == 1
            sStimPresets = struct;
            sStimPresets.strExpType = strExp;
            sStimPresets.intNumRepeats = 100;
```

```
            sStimPresets.vecOrientations = 0:15:345;
            sStimPresets.vecOrientationNoise = 0;
            sStimPresets.dblSecsBlankAtStart = 3;
            sStimPresets.dblSecsBlankPre = 0.4000;
            sStimPresets.dblSecsStimDur = 1;
            sStimPresets.dblSecsBlankPost = 0.1000;
            sStimPresets.dblSecsBlankAtEnd = 3;
        (...)
```

If you wish to make your own experiment file, you will have to edit `assertPresets` and add your own default values here. Note that you can make it into anything you wish here, with the exception of the `strExpType` field: this is compulsory to differentiate the different experiment scripts. It must match the name of your experiment. For example, because the experiment script is called `RunDriftingGratings.m`, `strExpType` takes the value `'RunDriftingGratings'`.

Structure of the experiment script & output file

You can basically do anything here, as long as you observe the restrictions imposed by the three input variables described above, and the structure of the output file. Looking in `RunDriftingGratings`, we can see that the output file is saved as:

```
save(fullfile(strLogDir,strFilename), 'structEP','sParamsSGL');
```

The `sParamsSGL` structure is the same as supplied by `sExpMeta`, so you can use the following:

```
sParamsSGL = sExpMeta.sParamsSGL;
```

The `structEP` structure has four obligatory fields:

`structEP.ActOnSecs` is the actual stimulus onset time in seconds (e.g., the PsychToolBox Screen flip time of the first frame of your stimulus)
`structEP.ActOffSecs` is the actual stimulus offset time in seconds (e.g., the PsychToolBox Screen flip time of the first blanking frame after your stimulus ended)
`structEP.ActOnNI` is the actual stimulus onset in National Instruments (nidq) time stamp
`structEP.ActOffNI` is the actual stimulus offset in National Instruments (nidq) time stamp

You can obtain the National Instruments time stamp using:

```
hSGL = sExpMeta.hSGL;
intStreamNI = -1;
dblSampFreqNI = GetSampleRate(hSGL, intStreamNI);
dblTimeStampNI = GetScanCount(hSGL, intStreamNI)/dblSampFreqNI;
```

That said, it is of course wise to save more information than that; in the case of drifting gratings this would be the orientation during each trial, the spatial frequency, etc. To ensure I always have all meta information, I also include the `sStimParams` structure before saving:

```
structEP.sStimParams = sStimParams;
structEP.sStimObject = sStimObject;
structEP.sStimTypeList = sStimTypeList;
save(fullfile(strLogDir,strFilename), 'structEP','sParamsSGL');
```

But of course you can decide differently for yourself. A minimal experiment script could therefore look something like this:

```
% evaluate and assign pre-defined values to structure
if ~strcmpi(sStimParamsSettings.strStimType,'StupidExperiment')
    sStimParamsSettings = struct;
    sStimParamsSettings.strStimType = 'StupidExperiment';
    sStimParamsSettings.strOutputPath = 'C:\Data\';
    sStimParamsSettings.strTempObjectPath = 'C:\Temp\';
else
    cellFields = fieldnames(sStimParamsSettings);
    for intField=1:numel(cellFields)
        try
            sStimParamsSettings.(cellFields{intField}) = ...
                eval(sStimParamsSettings.(cellFields{intField}));
        catch
            sStimParamsSettings.(cellFields{intField}) = ...
                sStimParamsSettings.(cellFields{intField});
        end
    end
end

% set output locations for logs
strRecording = sStimParamsSettings.strRecording;
strOutputPath = sStimParamsSettings.strOutputPath;
strTempPath = sStimParamsSettings.strTempObjectPath;
strThisFilePath = mfilename('fullpath');
[strFilename,strLogDir,strTempDir,strTexDir] =
RE_assertPaths(strOutputPath,strRecording,strTempPath,strThisFilePath);

%get SGL data
hSGL = sExpMeta.hSGL;
strRunName = sExpMeta.strRunName;
sParamsSGL = sExpMeta.sParamsSGL;
intStreamNI = -1;
dblSampFreqNI = GetSampleRate(hSGL, intStreamNI);

%run trials
structEP = struct;
hStartTic = tic;
for intThisTrial = 1:sStimPresets.intNumRepeats
    %"inter-trial" interval
    pause(0.5);
    structEP.ActOnSecs(intThisTrial) = toc(hStartTic);
    structEP.ActOnNI(intThisTrial) = GetScanCount(hSGL, ...
        intStreamNI)/dblSampFreqNI;

    %"stimulus" period
    pause(1);
    structEP.ActOffSecs(intThisTrial) = toc(hStartTic);
    structEP.ActOffNI(intThisTrial) = GetScanCount(hSGL, ...
        intStreamNI)/dblSampFreqNI;
end

%save data
save(fullfile(strLogDir,strFilename), 'structEP','sParamsSGL');
```

Integrating your experiment file into Acquipix

If you've followed the descriptions above, the only thing you need to do now is edit the following files:

- `RE_defaultValues.m` creates the default values, including which files are experiment scripts. You want to add your new experiment to the `sRE.cellStimSets` field.
- Add the default parameters and values for an experiment preset to `assertPresets.m`.
- `RE_evaluateStimPresets.m` calculates how long your experiment will take based on your preset parameters and ensures there are no incompatible values

Online analysis scripts

Requirements for your experiment script

As above.

Structure and modules of online analysis scripts

As above.

Troubleshooting

Question ("actually, it's more of a comment"): *It doesn't work*

Answer: Restart your PC

Q: *I downloaded everything, but it says files are missing*

A: Double check you have added all folders to the path in Matlab, you have the required Matlab toolboxes installed (Curve Fitting, Parallel Computing), and you're using a supported matlab version: R2019b is tested and works; anything earlier than R2016b will fail for sure; other versions might work. You may also need additional toolboxes, such as Image Processing and Acquisition to perform eye-tracking. If it's still not working after you've tried the above, google the filename and reinstall its source repository. If it still fails, create a report here: https://github.com/JorritMontijn/Acquipix/issues.

Q: *I cannot compile Kilosort's GPU code*

A: First try installing VSC2015 (make sure you have the Visual Studio ***Community*** version) and make sure you install the C++ compiler. Then ***restart your PC*** and try again. If it doesn't work: uninstall and try VSC2013. If neither of them work, look for help here: https://github.com/MouseLand/Kilosort/

Q: *I cannot run any GPU code in matlab (i.e., gpuArray() fails)*

A: Make sure that you have the correct CUDA drivers installed for your GPU. Note that if you're using anything other than a (modern) Nvidia GPU, you cannot run CUDA.

Q: *I found a bug*

A: Great! Or at least, it's great that you found it, not that it's there. If you've fixed it, you can make a pull request, otherwise you can create a bug report here: https://github.com/JorritMontijn/Acquipix/issues. Please copy/paste the matlab error message and as much detail as you can about what you were doing when it happened. If I cannot recreate the issue, I probably won't be able to fix it.