Universal Probe Finder

User Manual

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Overview

The Universal Probe Finder is a multi-species histology processing and probe alignment pipeline. It can read multiple ephys formats so you can use neurophysiological markers to better fine-tune your probe's location in the brain. It can use multiple atlases and calculates the stimulus responsiveness of your clusters with the zetatest using only an array of event-onset times.

The pipeline consists of three modules:

1) SlicePrepper: annotate where the probes left tracks on your histology slices,
2) SliceFinder: align your histology slices to the brain atlas,
3) ProbeFinder: fine-tune your probe's location based on neural response markers.

At this time, the Universal Probe Finder supports the following atlases out-of-the-box:

a. Sprague Dawley rat brain atlas, downloadable at: https://www.nitrc.org/projects/whs-sd-atlas
b. Allen CCF mouse brain atlas, downloadable at: http://data.cortexlab.net/allenCCF/
c. CHARM/SARM NMT_v2.0_sym macaque brain atlas: https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/nonhuman/macaque_tempatl/atlas_charm.html

Moreover, it currently supports the following ephys formats:

a. Clustered spiking data from Kilosort, downloadable at: https://github.com/MouseLand/Kilosort
b. Raw SpikeGLX data, downloadable at: https://billkarsh.github.io/SpikeGLX/
c. Acquipix pre-processed data, downloadable at: https://github.com/JorritMontijn/Acquipix

It is also possible to add your own ephys format or atlas: see the section "Adding an atlas"/"Adding an ephys format".

Installation instructions

Before you start:

1. For best performance, make sure your GPU supports OpenGL-accelerated graphics, so update your drivers if required. If you want to run spike detection on SpikeGLX data, you will need CUDA-supporting graphics card (Nvidia)
2. Download the brain atlas you wish to use, for example:
    a. Sprague Dawley rat brain atlas: https://www.nitrc.org/projects/whs-sd-atlas
    b. Allen CCF mouse brain atlas: http://data.cortexlab.net/allenCCF/
    c. CHARM/SARM macaque brain atlas: https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/nonhuman/macaque_tempatl/atlas_charm.html#download
3. Install the atlas if required (i.e., extract the files to nifti/.nii)

Installing the Universal Probe Finder:

1. All required external files are included in the repository's subfolders so you only need to clone this repository: https://github.com/JorritMontijn/UniversalProbeFinder

1b. Make sure the zetatest submodule is included! If you use git, use this command:

```
git clone --recurse-submodules https://github.com/JorritMontijn/UniversalProbeFinder
```

If you click "Code" and then download the code as a .zip, the submodule is also not included. You can add the zetatest submodule manually from here: https://github.com/JorritMontijn/zetatest.

2. When everything is downloaded and installed, add the main path to matlab and you're done – or, as the English would say: Bob's your uncle!

User guide

Starting the program

Once you have downloaded the atlas you wish to use (see above) and the Universal Probe Finder repository, it's very easy to start. Open matlab and navigate to where you installed the repository (replace the path with wherever it is on your PC):

```
cd F:\Code\Acquisition\UniversalProbeFinder
```

Next, simply type `UniversalProbeFinder`, and it will start the program:

```
UniversalProbeFinder
```

It will then ask you which program you would like to run: The SlicePrepper, the SliceFinder, or the ProbeFinder. If you want to export microscope files, merge channels, or annotate your histological slices with electrode tracks, start with the `SlicePrepper`. If you already have a probe coordinate file, or simply wish to plan your implantation trajectory, start with the `ProbeFinder`.

SlicePrepper

When starting the SlicePrepper, you can select the folder from where you want to import your slice images or load your previous data.

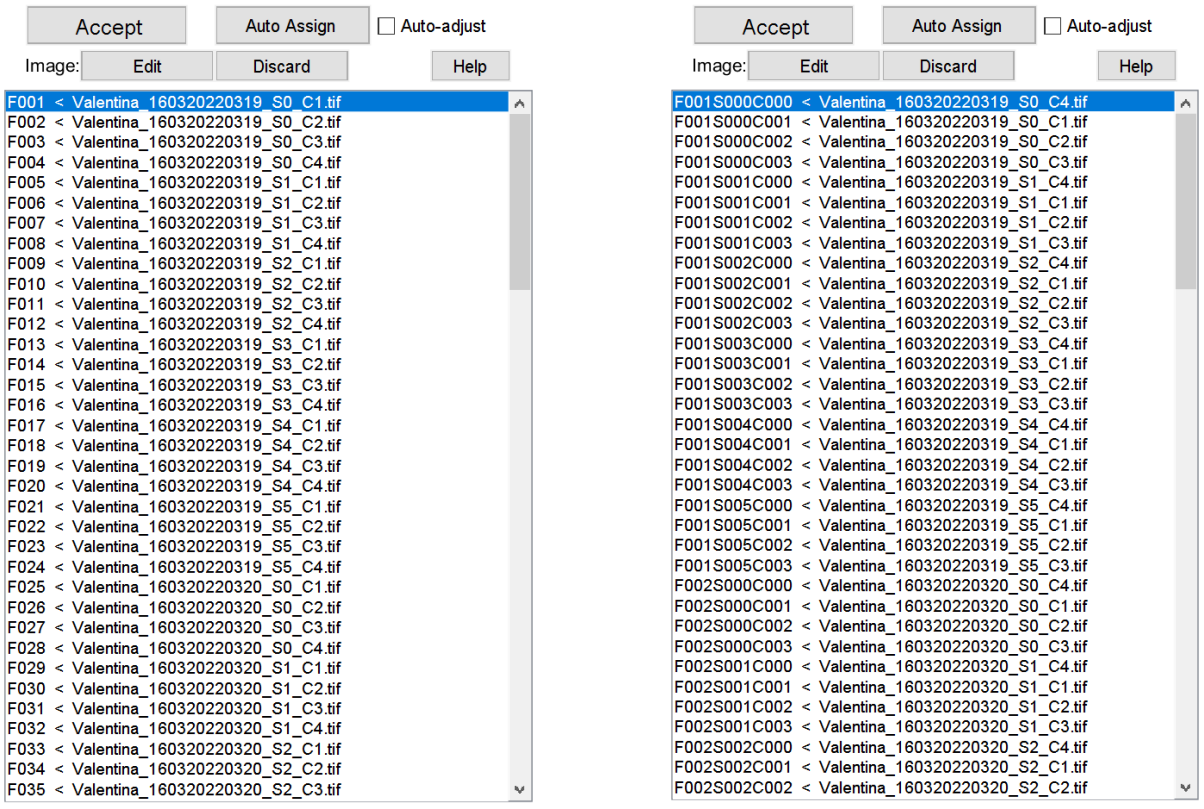
Unpacking files

The SlicePrepper can unpack some formats like .czi files if you have the bioformats toolbox installed: https://www.openmicroscopy.org/bio-formats/downloads/. Some formats save images at multiple resolutions, so you might not want to export the highest possible resolution, as the SlicePrepper will shrink the image size to your screen resolution anyway. It will therefore ask you what resolution you'd ideally like to have (default 1000 pixels width/height), and then it will export the closest image size. It will unpack all images, save them as .tifs, and give them unique names:

[*source file*]_S[*scene number*]_C[*channel number*].tif

Loading images

After exporting, or when you select a folder with images, it will show the following screen where you can select which images you want to load, and how to merge the images into different channels:



Selecting and merging images.

Left: unordered file list.

Right: automatically assigned image destinations after using the magic of regular expressions.

On the left-hand side of the list it will show the destination (e.g., “F001”), an assignment marker “<”, and on the right the source file name. If you click “**Edit**” You can manually assign an image a file number (F), image number (S, for “scene”), destination channel (C), and X/Y tiling location.

It is usually more convenient to automatically assign all images by clicking “**Auto Assign**”. This will show a small pop-up where you can edit the regular expressions the program will use to assign the source file to a destination image. All images with the same file (F) and image scene (S) will be combined into one image, and the source image may be assigned a specific channel (C) and tiling location (X/Y). You can remove images from the list with “**Discard**”.

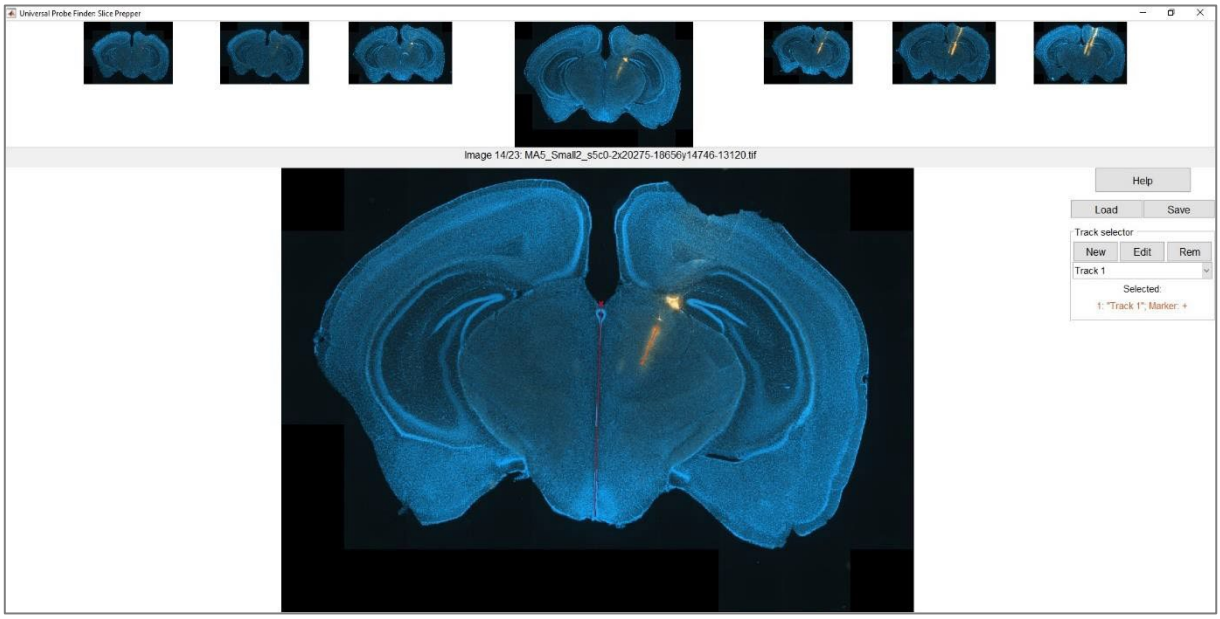
If you want to automatically adjust the brightness of all images, you can activate “**Auto-adjust**”.

Once you’re happy with the assignments, you can click “**Accept**” and the images will be loaded.

Annotating probe tracks

The first thing you want to do for each slice is to **align the midline** to vertical. Push the *Ctrl* button on your keyboard and left click somewhere along the top of the midline, then select a second point below it to adjust your slice orientation.

Next, you can create a **new track** by clicking on “New” in the track selector panel. You can now give your track a name, a marker and a colour. Once you have created a track, you can **draw a trajectory** on the slice by left clicking once to a beginning point, and again left clicking to set the end point:



To **navigate through your slices**, you can use the left/right arrows, page up/down, and home/end. You can also swap the position of the current slice with the previous or next slice by using shift + left/right arrow.

Once you have set all the tracks in your slices, you can click “Save” to save the data and continue in the SliceFinder.

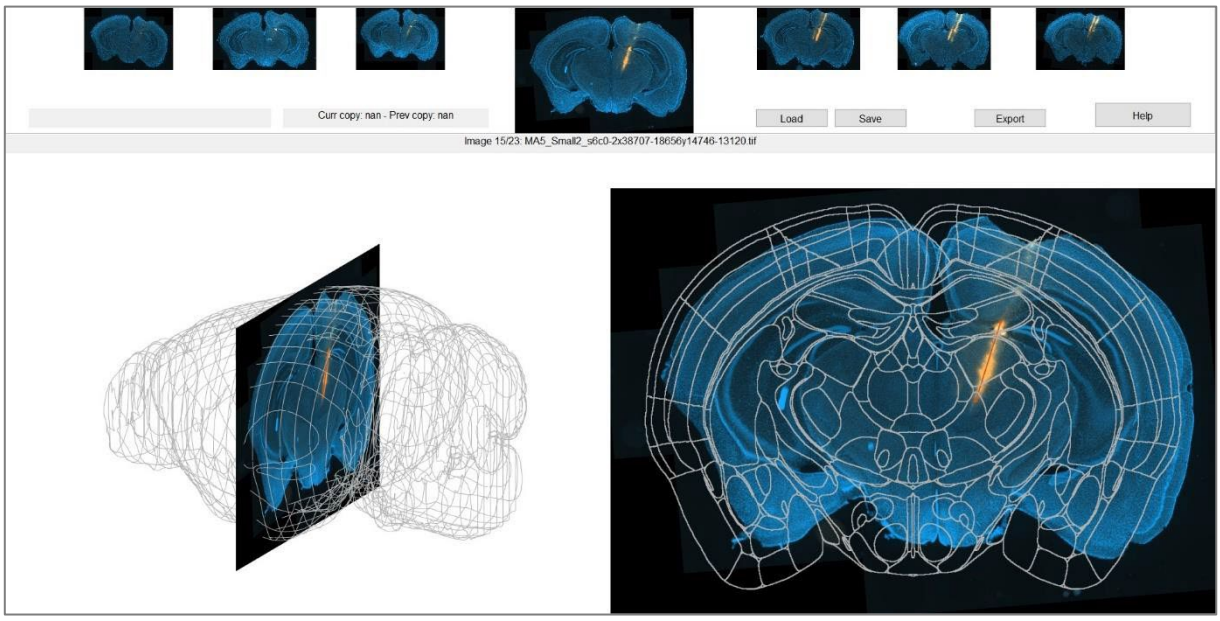
SliceFinder

The basics

Load your data folder as with the SlicePrepper and select the Prepped(…) file to load the tracks you annotated on the images. You will now also be asked to select which atlas you wish to use, which by default is either mouse, rat, or macaque.

If this is the first time you're starting the SlicePrepper or ProbeFinder, it will now ask you where you installed the atlas you selected. Navigate to its directory and click OK.

In the SliceFinder you can move, rotate, and rescale the slices with respect to the atlas. When you start the SliceFinder, all slices will be set to the middle of the atlas on the DV and ML axes, and to the AP position that matches the origin of the atlas (e.g., bregma):



Interpolating slice positions

While you can align each slice one by one, a more efficient approach is to align one slice at the beginning of the range you're interested in, and align one slice at the end, then interpolate the positions + rotations of the intermediate slices. You can **copy** a slice with control+c (e.g., slice #10), navigate to a different slice, copy this second slice by pressing control+c again (e.g., slice #20), and then press **control+b to interpolate** slices 11-19 based on the positions of slices #10 and #20.

Assuming your slices are of similar thickness and they're correctly ordered, this should set all slices close to their true position in a couple of button presses. If you ever make a mistake, you can **undo** your last paste action (control+v or control+b) by pressing control+z.

ProbeFinder

Loading and pre-processing the data

When starting the ProbeFinder, select your atlas. If you wish, you can now also import:

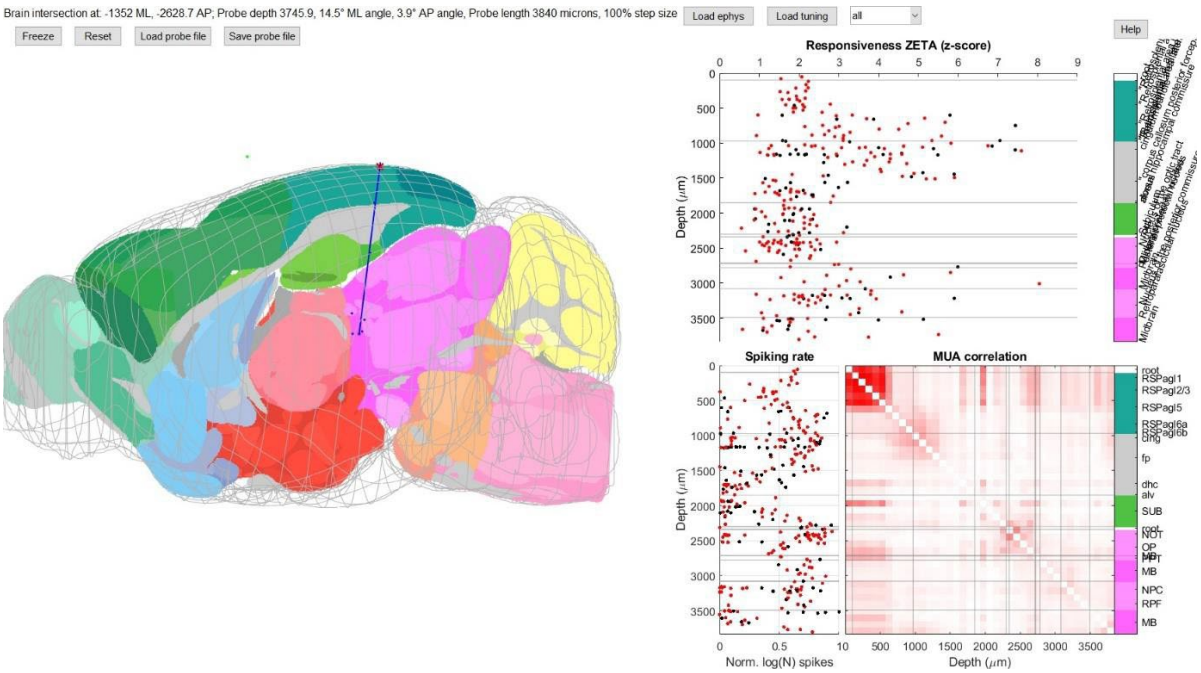
1) A probe file of various formats, such as:
    a. AP_histology output
    b. SHARP-track output
    c. ProbeFinder output
2) Electrophysiological data, such as:
    a. A kilosort 2.5 or kilosort 3 output folder
    b. SpikeGLX raw data
    c. Spike-detected ProbeFinder data
3) An event-time or cluster-responsiveness file, such as:
    a. A matlab file containing event onset times
    b. An Acquipix file
    c. A file containing ZETA responsiveness values

If you load raw SpikeGLX data, the ProbeFinder will first run spike detections on all channels to convert the data to multi-unit spike times. You can save this pre-processed data for later use so you don't have to run the spike detections again.

Similarly, if you select unprocessed event times for your responsiveness file, it will compute the neural responsiveness per cluster or channel based on the spike times and the event onsets you provided with the zeta-test (https://elifesciences.org/articles/71969). When it's done, you can save the file for future use.

Using the program

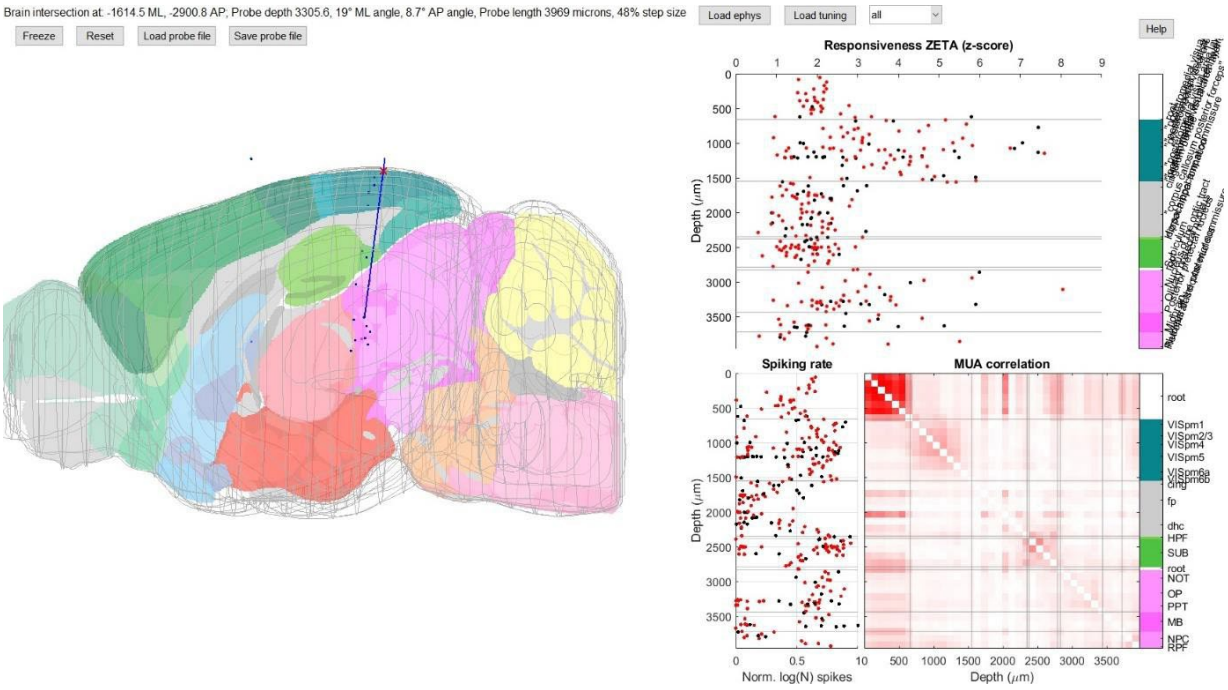
Once you've loaded a probe, ephys, and responsiveness file, you will see something like:



On the left (previous page), you can see an outline of the brain and a slice somewhat orthogonal to your viewing direction that goes through the probe. If you imported a probe location file, you will see blue dots that indicate where you thought the probe tracks were located on your histology slices. The green dot floating outside the brain is Bregma.

Normally, this histology-based location is what you would export and use to assign areas to the clusters you recorded on your probe. But often this is not entirely correct! The depth is clearly wrong, but only moving the probe up is not giving a good match either. The spiking rate and the MUA correlation (bottom right) give a pretty good indication of where the top of the cortex is located (MUA correlation is strong for noisy contact points above the brain) and where the white matter bundles lie (clusters show low firing rate). The exact boundaries between different cortical and subcortical areas are more difficult to determine, however.

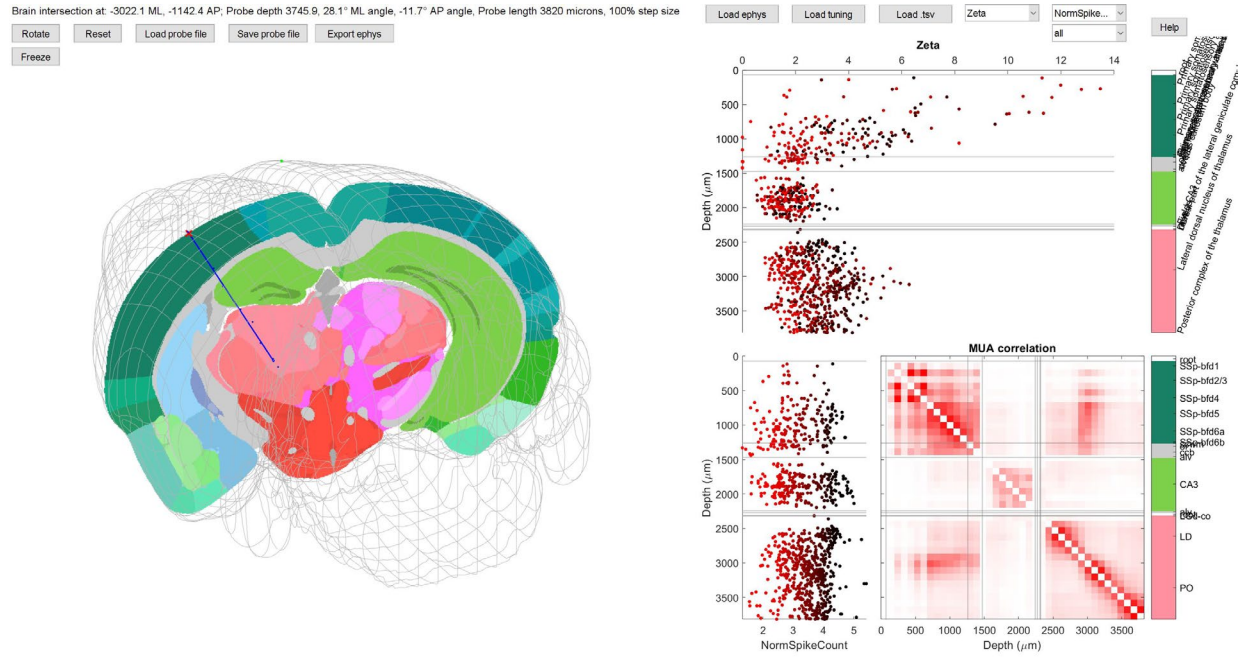
In this experiment, I showed drifting gratings and natural movies, so calculating the responsiveness ZETA scores using their onset times shows a clear delineation of visually-responsive and non-visually responsive regions (top right). Tweaking the probe location, length and angle a bit, I ended up with this alignment (not perfect yet, but clearly a lot better than before):



Notice how the subiculum has a high MUA correlation, the NOT has a couple of highly visually-responsive cells, and the boundaries of the visual cortex are very sharp when looking at the responsiveness ZETA.

ProbeFinder improvements with v1.1

With the 1.1 version, the internal data structure is completely reworked. It can now load the channel map from a SpikeGLX IMEC meta file, and the GUI has a couple of extra buttons, so it can show you more information than before. The program now looks like the image below:



List of changes in v1.1

The main change from v1.0 is that you can now **load any .tsv** file that has a "cluster_id" column and some other property. This means it's now fully compatible with phy, and other programs. If you load .tsv files with different numbers of entries, it will show you a warning, because you're probably combining .tsv files from pre- and post- manual curation.

You can show two cluster properties at the same time by selecting them from the dropdown selection menus. The first one will be displayed in the top graph (here, Zeta), and the second one in the bottom graph (here, NormSpikeCount). Moreover, the clusters will be coloured according to the second property, so you can compare property 1 vs 2 vs depth in the top graph. If the second property has under <10 unique values, you can also use those values to select subcategories. For example, if you record from a multi-shank probe, you can now select only clusters from Shank 1. Or if you have manual cluster categories, such as PV, Pyr, SOM, etc, **you can selectively show or color these clusters**.

An additional bonus from the reworked data structure is that the **cluster_id property is now identical to those in Kilosort, phy and/or other .tsv files**. If you click the button "Export ephys" it will export all data you combined in the ProbeFinder to an easily-accessible data structure you can directly compare with your Kilosort cluster ids.

Finally, the graphs now show you **data tips** with the properties for a cluster when you hover over them. Unfortunately, MATLAB has a "feature" where data tips don't work if *any* subplot allows 3D rotations, so you first have to toggle the button "*Rotate*" to "*Data tips*" before the data tips will show up. If the toggle is set to "Data tips", the brain on the left cannot be rotated. Please submit your complaints to Mathworks.

ProbeFinder file format descriptions

Format of the input file

The initial probe location is calculated from a set of points in atlas-space. In order for the ProbeFinder to read a file successfully, the file should contain the variable "`sProbeCoords`" with at least:

- cellPoints [1 x N]: cell array where each cell contains points for a single probe *i*, i.e.:
  - cellPoints{i} [P x 3]: P points with [ML AP DV] native-atlas format coordinates

You can also add the following optional field to supply a name for each probe:

- cellNames [1 x N]: cell array where each cell contains the probe's name

Note that if you use the SlicePrepper and SliceFinder, these files are automatically generated when you click the "Export" button.

Format of the output file (updated for v1.1!)

The current probe location is saved in the field "`sProbeAdjusted`" of the structure "`sProbeCoords`". The `sProbeAdjusted` structure contains the following fields, of which the more useful fields are marked in bold:

- probe_vector_cart [2 x 3]: cartesian tip and base coordinates in [ML,AP,DV] atlas space
- probe_vector_sph [1 x 6]: spherical coordinates: [ML AP DV deg-ML deg-AP length]
- probe_vector_intersect [1 x 3]: coordinates of brain entry
- probe_vector_bregma [1 x 6]: bregma-origin format: [ML AP ML-deg AP-deg depth length]
- probe_area_ids_per_depth [1 x N]: vector of area IDs per point along the probe
- probe_area_labels_per_depth [N x 1]: cell array of acronyms per point
- probe_area_full_per_depth [N x 1]: cell array of full names per point
- probe_area_boundaries [1 x P+1]: locations along the probe of area boundaries
- probe_area_centers [1 x P]: centers of areas
- probe_area_ids [1 x P]: id per area of the above
- probe_area_labels [P x 1]: acronym per area
- probe_area_full [P x 1]: full name per area
- **stereo_coordinates:** table with the probe's location in stereotactic coordinates:

Name:	**Content:**	**Unit**
ML	Probe's brain entry location along ML (medial-lateral) axis, relative to the atlas origin (e.g., bregma)	Microns
AP	Probe's brain entry location along AP (anterior-posterior) axis, relative to the atlas origin (e.g., bregma)	Microns
AngleML	Angle of the probe in the ML direction (see below)	Degrees
AngleAP	Angle of the probe in the AP direction (see below)	Degrees
Depth	Depth below the brain entry of the lowest recording channel of the probe (i.e., usually the depth of the tip)	Microns
ProbeLength	Length of the probe after stretching/shrinking	Microns

- **probe_area_ids_per_cluster**: area IDs per cluster
- **probe_area_labels_per_cluster**: area acronyms per cluster
- **probe_area_full_per_cluster**: full name of the area per cluster
- **cluster_id**: original id of the cluster (e.g., the template # in kilosort). ***N.B.: these indices are now identical to the source ids, and are no longer offset by +1 (in contrast to v1.0)***

Note that these final cluster-based variables only have values if ephys data was loaded.

Definition of ML/AP angles in the ProbeFinder

Defining a single axis of 360-degree rotation in R^3 is trivial and defines a unique set of points. Unfortunately, combining two 360-degree axes of rotation in 3-dimensional space leads to a non-unique spherical projection space, where multiple combinations of the two angles describe the same point. Describing these problems in detail is beyond the scope of this manual (see https://en.wikipedia.org/wiki/Spherical_coordinate_system#Unique_coordinates for more details), but it is important to note that converting between cartesian and spherical coordinate systems can be tricky.

For our purposes of defining a probe's location, it is most useful to have two axes that align more or less with our two most important axes: ML and AP. Moreover, a natural "origin" for the probe would be to define the direction going straight down as 0 degree ML and 0 degree AP. This requires rotating the spherical coordinate system (fig. 1).

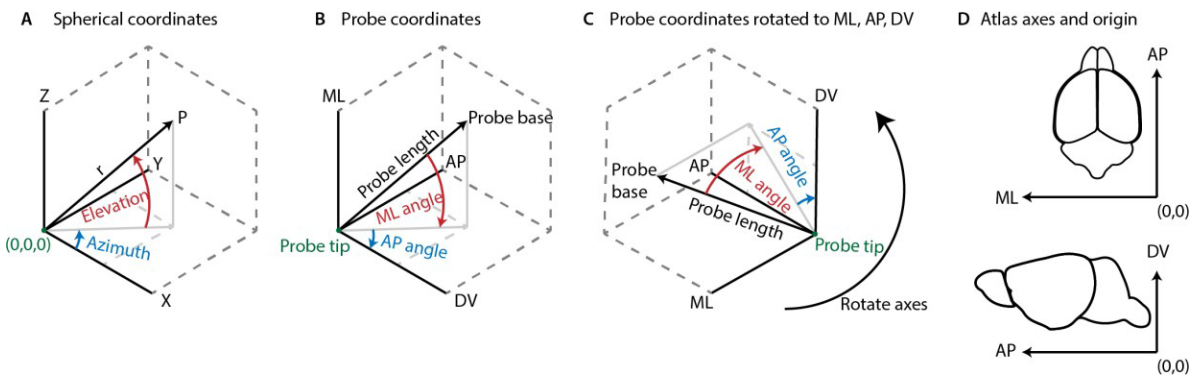


Figure 1. Definition of ML and AP angles. A) Standard spherical coordinate system using elevation and azimuth. Azimuth is defined on the interval [-π, π] and elevation on [-π/2, π/2]. B) X,Y,Z, replaced by stereotactic axes. Notice the change in angle direction. C) Rotated axes aligned with atlas directions. D) Overview of atlas axis directions and origins.

Furthermore, to ensure that the necessary discontinuities in the rotations occur when the probe is upside-down (which presumably is not a common recording orientation), rather than close to its "origin" position, we invert the AP-axis and apply the following transformations:

```
if dblAngleAP < -90 && dblAngleML > 0
    dblAngleAP = dblAngleAP + 180;
    dblAngleML = -dblAngleML + 180;

elseif dblAngleAP < -90 && dblAngleML < 0
    dblAngleAP = dblAngleAP + 180;
    dblAngleML = -dblAngleML - 180;

elseif dblAngleAP > 90 && dblAngleML > 0
    dblAngleAP = dblAngleAP - 180;
    dblAngleML = -dblAngleML + 180;

elseif dblAngleAP > 90 && dblAngleML < 0
    dblAngleAP = dblAngleAP - 180;
```

The specific implementations can be found in the functions PH_CartVec2SphVec, PH_SphVec2CartVec, PH_BregmaVec2SphVec, and PH_SphVec2BregmaVec.

Adding an atlas

The ProbeFinder reads a configuration .ini file to find which atlases are installed. If no configAtlas.ini file is present, it will create a default file containing metadata on the Allen Brain mouse atlas, Sprague-Dawley rat atlas, and CHARM/SARM macaque atlas. If you wish to add an atlas, you can edit the .ini file by adding another atlas entry set. For example, the first entry looks like this:

```
[sAtlasParams(1)]
name='Mouse (AllenCCF)'
pathvar='strAllenCCFPath'
loader='AL_PrepABA'
downsample=2
```

It specifies:

- name: the name of the atlas,
- pathvar: the name of path variable that is saved in configPF.ini (the atlas's path location)
- loader: the name of the function that pre-processes the atlas files (see below)
- downsample: the amount of downsampling when plotting an atlas slice

The name, pathvar, and downsample are self-explanatory, but the loader requires some more explanation. The syntax of a loader function is:

```
sAtlas = name_of_loader_function(str_atlasname_Path)
```

The loader function reads the atlas files at the specified path and outputs a structure sAtlas with the following fields:

Field name	Size	Description
.av	[ML x AP x DV]	Annotated volume, where a value in av denotes an area ID that can directly index into .st; i.e., sAtlas.st(sAtlas.av(x,y,z)).name gives the full name of the area at location x,y,z.
.tv	[ML x AP x DV]	Grey-scale template volume in range [0 255]
.st	[N x T] table	N-entry table, where N is highest area index in .av
.Bregma	[1 x 3]	Origin of atlas in native atlas voxel coordinates [ML AP DV]
.VoxelSize	[1 x 3]	Size of a single voxel in microns [ML AP DV] Note: currently only isometric voxels are supported
.BrainMesh	[P x 3]	Mesh of brain outline, where each vertex is a [1 x 3] point, and curve-ends are denotes by a [nan nan nan] entry. Brain meshes can be created from a .av or .tv volume using `getTrace3D`
.Colormap	[N x 3]	N-entry RGB color map, specifying a color for each area in .av
.Type	string	Name, e.g.: Allen-CCF-Mouse

The area-table .st must have least the following fields:

Field name	Description
st.id	ID of area (numeric)
st.name	Full name of area (string)
st.acronym	Short name of area (string)
st.parent_structure_id	ID of parent area

If you wish to add an atlas to the program, we are happy to help out. You can contact us by e-mail or via the github repository.

Adding an electrophysiology format

Similar to when loading an atlas, the ProbeFinder reads a configuration .ini file to find which ephys formats are installed. If no configEphys.ini file is present, it will create a default file containing metadata on Kilosort, SpikeGLX, Acquipix, and the native ProbeFinder ephys format. If you wish to add a format, you can edit the .ini file by adding another entry set. For example, SpikeGLX's entry looks like this:

```
[sEphysParams(2)]
name='SpikeGLX'
loader='EL_PrepEphys_SG'

reqfiles='.*[.]imec.*[.]ap[.]bi+n,.*[.]imec.*[.]ap[.]met+a,.*[.]nidq[.]bi

+n,.*[.]nidq[.]met+a'
reqisregexp=1
```

It specifies:

- name: the name of the format,
- loader: the name of the function that pre-processes the format (see below)
- reqfiles: comma-separated list of files that *must* be present for the format to be valid
- reqisregexp: specified whether the file names are regular expressions or plain-text

Most formats will need to use regular expressions, as the filenames can change between recordings. However, in the case of kilosort for example, it always produces the same set of file names, so these must be an exact match; and therefore the switch `reqisregexp` will be 0.

The loader function reads the ephys files at the specified path and outputs a structure sClusters. **Note that this data structure has changed from v1.0 to 1.1.**

Field name	Size/type	Description
.ProbeLength	float	Length of the probe in microns
.UseClusters	[1 x N]	List of cluster entries
.CoordsS	[1 x C]	Shank per channel
.CoordsX	[1 x C]	X location per channel
.CoordsD	[1 x C]	Depth per channel
.Clust	[1 x N]	Template structure with N entries, containing the fields:
.Clust(i) cluster_id	int	Cluster id
.Clust(i).NormSpikeCount	float	Spike count (default: log10(spike count))
.Clust(i).Shank	int	Shank number
.Clust(i).Depth	float	Depth along the probe in microns
.Clust(i).SpikeTimes	[1 x S]	Spike times
.Clust(i).x	[1 x 1]	Any additional field belonging to cluster/channel i. This can be a numeric value or a string (e.g., " "mua", "good")

If you wish to add a format to the program, we are happy to help out. You can contact us by e-mail or via the github repository.

Acknowledgements
This work is based on earlier work by people from the cortex lab, most notably Philip Shamash and Andy Peters. See for example: https://www.biorxiv.org/content/10.1101/447995v1

This repository includes various functions that come from other repositories, credit for these functions go to their creators:

https://github.com/petersaj/AP_histology https://github.com/JorritMontijn/Acquipix
https://github.com/JorritMontijn/GeneralAnalysis https://github.com/kwikteam/npy-matlab
https://github.com/cortex-lab/spikes https://github.com/JorritMontijn/zetatest

I would also like to thank Alexander Heimel, Nora Jamann, Valentina Riguccini, Frederic Michon, Chris Eva, Chris Klink, and Robin Haak for their feedback on the program.

If you use the Universal Probe Finder, please cite our paper:
https://www.biorxiv.org/content/10.1101/2022.06.20.496782v1.

Happy probe finding!

Troubleshooting

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

Answer: Restart your PC, make sure the UniversalProbeFinder is on your matlab path, and try again.

Q: *The program crashes because it says it's missing a file?*

A: You've probably downloaded the code as a zip file (see "*Installation Instructions*"). If you download it as a zip, the zetatest module and its dependencies are missing. If you clone it, or download and extract the zetatest repository manually, it should probably fix your problem. If not, please make a bug report on github.

Q: *Why is it so slow?*

A: You're probably not using OpenGL rendering. If you're from the future, your matlab version might have broken the OpenGL rendering switch. If so, please file a bug report. If you're using anything that's R2022a or earlier, you might need to update your GPU or your graphics drivers.

Q: *I'm using R2022a or earlier, updated my drivers, have a compatible GPU, and it's still slow.*

A: You might want to consider editing the configAtlas.ini file to increase the downsampling for your atlas (Note: values are rounded to the nearest integer). For example, if you're using the Allen Brain atlas, you will see:

[sAtlasParams(1)] name='Mouse (AllenCCF)' pathvar='strAllenCCFPath' loader='AL_PrepABA' downsample=2

You can change the line "`downsample=2`" to (e.g.) "`downsample=5`", restart the program, and see if it makes a difference.

Q: *I found a bug*

A: If you've fixed it, you can make a pull request, otherwise you can create a bug report here: https://github.com/JorritMontijn/UniversalProbeFinder/issues. Please copy/paste the error message and provide 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.