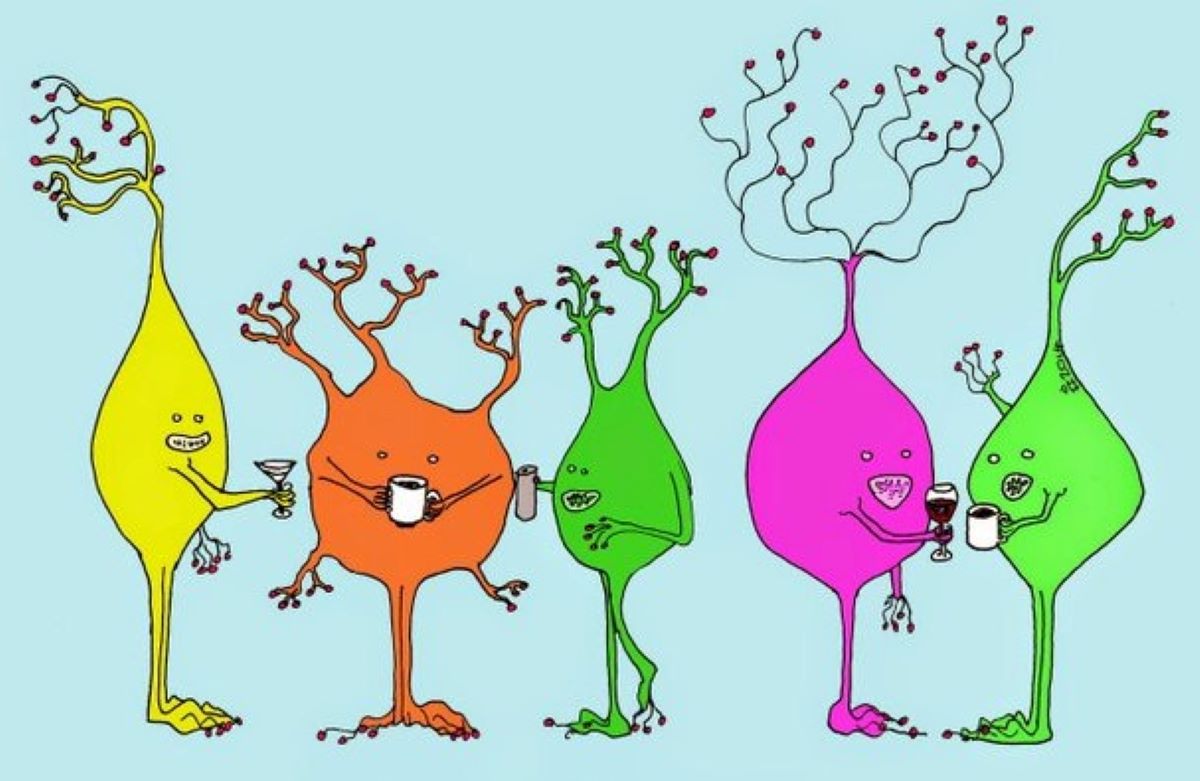
**Processing Axona data**



Collection of scripts and procedures for processing Axona data recorded in the Jezek lab.   
Does not include instructions for sgClust.

Also updated on www.github.com/SusanL82

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# Installation

## Spyder and Anaconda navigator

I used Spyder for most procedures in this document. As someone who is more familiar with Matlab, I find Spyder the most convenient way to interface with Python.   
It should be possible to run all python scripts in Jupyter notebooks as well. It is currently not possible to use my code as functions from the command prompt. I do not know how to convert them for this use yet, but it should be possible and I’ll probably make this update in the future.

## Installing SpikeInterface with klustakwik

Klustakwik and klusta are old legacy software require the following installations of Python and SpikeInterface.

1. **Create a conda environment with Python 3.7**I used the anaconda navigator for this and created the environment *klusta.* You can name your environment whatever you like.
2. **Install klustakwik**In the conda command prompt, activate your environment:   
   conda activate klusta  
     
   Then install klustakwik and its prerequisites with pip. Type:  
   pip install Cython h5py tqdm  
   pip install click klusta klustakwik2
3. **Install SpikeInterface 0.95.1**Klustakwik requires SpikeInterface <0.96. Use:  
   pip install spikeinterface[full]==0.95.1
4. **Install Spyder**  
   I installed spyder via the anaconda navigator menu, this automatically set to Spyder 5.3.3.
5. **(optional?) in spyder**  
   when running import spikeinterface.full as si in Spyder, I got the following warning C:\Users\susan\anaconda3\envs\klusta\lib\site-packages\tqdm\auto.py:21: TqdmWarning: IProgress not found. Please update jupyter and ipywidgets. See https://ipywidgets.readthedocs.io/en/stable/user\_install.html from .autonotebook import tqdm as notebook\_tqdm   
   It looks like this prevents spikeinterface from detecting klusta correctly. The warning seems to depend on the version of Spyder.  
   Solve this by running pip install ipywidgets from the command prompt for the klusta environment.

## Using other sorters

Most other sorters will work with Python >3.7. The latest version of SpikeInterface requires Python ≥3.8.

If you’re not using klustakwik, you can just install the latest versions instead.

The code listed in this document should work with newer versions of SpikeInterface and Python, but I have not tested this extensively.

# Preprocessing .set files

Each recording from Axona consists of a large .bin file with the recorded signals, and a small .set file with information about the recording. SpikeInterface uses the .set file to determine recording parameters.   
The collectMask lines are used to determine the presence of tetrode signals. If collectMask is set to 1, the tetrode will be read, and if it is set to 0, the signal will not be read.

For example:

collectMask\_1 1  
collectMask\_2 0  
collectMask\_3 1

In this case signals for tetrode 1 and 3 will be read, but not signals from tetrode 2.

In Jezek lab recordings, collectMask\_1 is set to 1, and all others to 0. To read.bin files with all 16 tetrodes in spikeinterface, open the .set file in a text editor and change collectMask\_1 to collectMask\_16 from 0 to 1. If there are 8 tetrodes in a recording, only change collectMask\_1 to collectMask\_8.

You will need to do this for each .set file.

# Extracting LFP traces

This is a procedure for extracting LFP traces from selected tetrodes for a number of .bin files. Signals from separate .bin files can be merged if desired. Signals are downsampled by a factor 4 (from 24kHz to 6kHz in our recordings), and then stored as variable Sig in a separate .mat file for each tetrode.

It is not possible to merge and output an entire multi-hour recording in one step with this script (the file will be too large). The best way to get traces for an entire recording is to split the recording into multiple .bin file groups and merge those.

This script does not take bad channels into account and does not skip them. All 4 channels will be extracted for the selected tetrode.

Then, in matlab, merge the downsampled recordings (optional: average the signals per tetrode first).

All 4 channels for the tetrode are extracted, there are no channels excluded in this script. The script assumes file names BaseName\_01.bin and BaseName\_01.set for the files.

To downsample by a different amount, edit signal.decimate() in line 61 of ReadAxonaBins.py

## Use ReadAxonaBins.py (script)

**Inputs**:   
*allnames*: a list with session names for each recorded to-be-merged session. Should be the same size as allsess. E.g: allnames = ["pre","post1"]

*allsess*: list that defines which .bin files are read and merged.   
E.g: allsess = [[1,2],[4,5]] . In this case, .bin files 1 and 2 will be read and merged, and bin files 4 and 5 will be read and merged.

*Tetnums*: numbers of the tetrodes you want to process. These numbers are 1-based, tetrode 1 is the first tetrode (just like on the drive).  
E.g: tetnums = [1, 5, 7] will export signals for tetrodes 1, 5, and 7.

*InFolder*: path to the directory with the .bin files you want to process. E.g: InFolder = "E:/SEPSIS/RAW/BIN/HD214-2004/"

*OutFolder*: path to the directory where the generated .mat files are stored. E.g: OutFolder = "E:/SEPSIS/RAW/SIconvert/"

*BaseName*: the common part of .bin file names for the processed recording. This name is also used as an output file name. E.g: BaseName ="HD214-2004\_" for HD214-2004\_01.bin, HD214-2004\_02.bin, HD214-2004\_03.bin etc.

**Outputs**:   
A .mat file containing variable Sig (4 x N). E.g: HD214-2004\_tt1\_pre.mat

# Clustering with SpikeSort3D

Spikes recorded with Axona can be exported to .ntt files than can be manually clustered in SpikeSort3D.   
First, using ExtractPeaksAxonaSI.py, the spikes are detected from .bin files, a 32-sample waveform for each spike is collected, and timestamps (in samples, starting at 0) and waveforms are saved in a .mat file.  
Second, the generated .mat file is converted to a .ntt file with writeNTTfromSI\_ax.m.

There is currently no option to skip bin files in the python script. Tetrodes with fewer than 3 good wires are not processed by the python script.

If there are only 3 good wires, the good channels will be located on the first 3 channels of the .ntt file and the bad channel will be the fourth. E.g: if tetrode wires 1, 3, and 4 were recorded, the .ntt will have spikes on channel 0 (tetrode wire 1), channel 1 (tetrode wire 3) and channel 2 (tetrode wire 4). Channel 3 will only have zeros.

There is no explicit time vector in these converted recordings and in the processing of the spikes. So, if two bin files are processed, the first sample of the second file follows directly after the last sample of the first file. Please keep this in mind if the clustered recording needs to be separated in time periods later (lengths of each .bin file are stored in the .set file, but starting times may not be). As far as I can tell, it is not possible to keep recording segments separated in an .ntt file, although it may be possible to generate some kind of event file.

For automated clustering, recorded segments (e.g. bin files) can be processed together and kept intact via SpikeInterface.

## Use ExtractPeaksAxonaSI.py (script)

**Inputs:**

*Tetlist:* Tetlist.txt is a comma separated text file that indicates good and bad wires . It contains one line per tetrode with 5 values per line (tetrode number, value for wire 0, value for wire 1, value for wire 2, value for wire 3). There is no header. Values for good wires are set to 1, bad wires are set to 0. See example in the code chapter. Tetlist.txt should be located in InFolder.

*InFolder*: path where the to-be-processed .bin files are stored. E.g. InFolder = "D:/SEPSIS/"

*OutFolder*: path where the the generated .mat files are stored. E.g. OutFolder = "D:/SEPSIS/ntt\_test/"

*BaseName*: the common part of .bin file names for the processed recording. This name is also used as an output file name. E.g: BaseName ="HD214-2004\_" for HD214-2004\_01.bin, HD214-2004\_02.bin, HD214-2004\_03.bin etc.

*numsess*: the number of sessions/.bin files in the recording. E.g. numsess = 4, will read bin files 01, 02, 03 and 04.

*Tetnums*: numbers of the tetrodes you want to process (1-based, same as on the rat/rec). Tetnums = [2,4,5,6,7,8,9,11]

*spike\_thresh*: setting for spike detection, the spike detection threshold is spike\_thresh \* median signal SD. E.g: spike\_thresh = 5

*spike\_sign*: setting for spike detection, defining the direction of the detected peaks. Can be: 'neg', 'pos', 'both'. E.g: spike\_sign = 'neg' detects negative peaks only.

**Outputs**:   
A .mat file containing variables Timestamps (1 x N) with spike times for each spike (in samples) and Spikes (32 x 4 x N) with 32-sample waveforms on each tetrode channel for each spike. 8 samples are stored before to the peak, and 24 after the peak.

## Use writeNTTfromSI\_ax.m (function)

**Function**: [Filename] = writeNTTfromSI\_ax(Inpath, Outpath, Filename)

**Requirements**: This function uses the Mat2NlxSpike provided by Neuralynx

**Inputs:**

*Inpath*: path where .mat file generated by ExtractPeaksAxonaSI.py is located. E.g: Inpath = 'D:\SEPSIS\ntt\_test'

*Outpath*: path where .ntt file will be generated.E.g. Outpath = 'D:\SEPSIS\ntt\_test’

*Filename*: name of the input file (generated by ExtractPeaksAxonaSI.py) E.g: Filename = 'HD190-1511\_tt2.mat'. This is also used for the output file name.

**Output**:

An .ntt file with the signals in the selected .mat file. Peak and valley values are added for each spike.   
I think the other features used for clustering are calculated on the fly by SpikeSort3D (they are not included in the .ntt files generated by the Jezek lab Cheetah system).

# Clustering with **Klustakwik** and Phy

* in progress -

# Code

## Make a single tetrode .prb file

The following code can be used to generate a .prb file with a single tetrode. For a 3-wire tetrode, you can edit the resulting .prb file.

from probeinterface import ProbeGroup, Probe, generate\_tetrode, write\_prb, read\_prb

Outfile = 'C:/Users/susan/Desktop/klustatest/tet4\_probe.prb'

# 4-wire tetrode

probegroup = ProbeGroup()

new\_chans = [0, 1, 2, 3]

tet4\_probe = generate\_tetrode()

tet4\_probe.set\_device\_channel\_indices(new\_chans)

probegroup.add\_probe(tet4\_probe)

write\_prb(Outfile, probegroup)

## Tetlist.txt

This is an example of Tetlist.txt. In this file, tetrode 2 has 2 bad wires (wire 1 and 2), tetrode 4 has one bad wire (wire 3), and tetrode 12 has 3 bad wires (wire 0, wire 2 and wire 3).

**Note**: all 16 tetrodes must be listed in Tetlist.txt.

1,1,1,1,1

2,1,0,0,1

3,1,1,1,1

4,1,1,1,0

5,1,1,1,1

6,1,1,1,1

7,1,1,1,1

8,1,1,1,1

9,1,1,1,1

10,1,1,1,1

11,1,1,1,1

12,1,0,0,0

13,1,1,1,1

14,1,1,1,1

15,1,1,1,1

16,1,1,1,1

## ReadAxonaBins.py

import spikeinterface as si

import spikeinterface.extractors as se

from scipy.io import savemat

import numpy as np

from scipy import signal

##

allnames = ["pre","post1","post2a", "post2b","post2c"]

allsess = [[1,2],[4,5],[7,8,9,10],[11,12,13,14],[15,16,17,18]]

Tetnums = [2,4,5,6,7,8,9,11]

InFolder = "E:/SEPSIS/RAW/BIN/HD214-2004/"

OutFolder = "E:/SEPSIS/RAW/SIconvert/"

BaseName ="HD214-2004\_"

##

for sess in range(len(allsess)):

Sessnums = allsess[sess]

RecName = allnames[sess]

files = list()

#make file list

for f in range(np.size(Sessnums)):

InName = InFolder+BaseName+"%02d" % Sessnums[f]+".bin"

list.append(files, InName)

recording\_list = list()

for f in range(np.size(Sessnums)):

#read axona file

list.append(recording\_list, se.AxonaRecordingExtractor(files[f]))

##

MergeRec = si.concatenate\_recordings(recording\_list)

##

TheseSigs = []

for tet in range(np.size(Tetnums)):

thistet = Tetnums[tet]

gettet = thistet-1

a = 4;

chanIDs1 = gettet\*a

chanIDs1 = chanIDs1

chanIDs2 = chanIDs1 + 4

chanlist = range(chanIDs1,chanIDs2)

chanlist = ["{:01d}".format(x) for x in chanlist]

TheseSigs = MergeRec.get\_traces(channel\_ids = chanlist)

TheseSigs = signal.decimate(TheseSigs, 4, axis = 0)

thistet = str(thistet)

outname = OutFolder+BaseName+"tt"+thistet +"\_"+ RecName + ".mat"

savemat(outname,{'Sig': TheseSigs})

del TheseSigs

TheseSigs = []

print('\a')

## ExtractPeaksAxonaSI.py

import spikeinterface as si

import spikeinterface.extractors as se

from scipy.io import savemat

import numpy as np

from spikeinterface.sortingcomponents.peak\_detection import detect\_peaks

from spikeinterface.preprocessing import bandpass\_filter

from tqdm import tqdm

InFolder = "D:/SEPSIS/"

OutFolder = "D:/SEPSIS/ntt\_test/"

BaseName ="HD190-1511\_"

numsess = 2 #number of sessions/ .bin files

Tetnums = [2,4,5,6,7,8,9,11] #tetrodes to process (1-based, same as on the rat/rec)

spike\_thresh = 5 # detection threshold for spike detection is spike\_thresh\* signal SD

spike\_sign = 'neg' #detect negative peaks only. can be: 'neg', 'pos', 'both'

# !!! need to manually edit the .set files so that all channels get read.

##############################################################################

allsess = range(numsess) #count sessions zero-based

# make file list

files = list()

for f in allsess:

thissess = allsess[f]+1 #1-based session number

InName = InFolder+BaseName+"%02d" % thissess+".bin"

list.append(files, InName)

# make list of SI extractor recordings

recording\_list = list()

for f in allsess:

list.append(recording\_list, se.AxonaRecordingExtractor(files[f]))

# merge recording objects

MergeRec = si.concatenate\_recordings(recording\_list)

# add highpass filter

MergeRec\_f = bandpass\_filter(MergeRec, freq\_min=300.0, freq\_max=6000.0, margin\_ms=5.0, dtype=None)

# detect peaks per tetrode and get trace segments for waveforms

for tet in range(np.size(Tetnums)):

thistet = Tetnums[tet]

print('processing TT'+str(thistet))

gettet = thistet-1

a = 4;

chanIDs1 = gettet\*a

chanIDs2 = chanIDs1 + 4

chanlist = range(chanIDs1,chanIDs2)

chanlist = ["{:01d}".format(x) for x in chanlist]

TheseSigs = MergeRec\_f.channel\_slice(channel\_ids = chanlist)

TetPeaks = detect\_peaks(TheseSigs, method='by\_channel', pipeline\_nodes = None, gather\_mode='memory', folder=None, names=None,

peak\_sign=spike\_sign, detect\_threshold= spike\_thresh, exclude\_sweep\_ms = 0.75)

#exclude sweep is set to the same length as used by our NLX recordings

#TetPeaks has fields: sample\_index, channel\_index, amplitude, segment\_index

# get waveforms. must be 32 samples long, I'm setting peak at sample 8 (same as NLX)

prepeak = 8

postpeak = 24

print('extracting waveforms')

allWFs = np.zeros([32,4,len(TetPeaks['sample\_index'])], dtype = 'int16')

for p in tqdm(range(len(TetPeaks['sample\_index'])), desc="collecting waveforms"):

sf = TetPeaks['sample\_index'][p] - prepeak

ef = TetPeaks['sample\_index'][p] + postpeak

thisWF = TheseSigs.get\_traces(segment\_index = None, start\_frame = sf, end\_frame = ef)

#write only complete spike waveforms (might skip last spike if too short)

if np.size(thisWF,0) == 32:

allWFs[:,:,p] = thisWF

del thisWF

#save peaks to mat file (for later processing with Mat2NlxSpike to generate .ntt file)

print('saving to mat file')

outname = OutFolder+BaseName+"tt"+str(thistet) +'.mat'

savemat(outname,{"Timestamps":TetPeaks['sample\_index'], "Spikes": allWFs})

## writeNTTfromSI\_ax.m

function [Filename] = writeNTTfromSI\_ax(Inpath, Outpath, Filename)

%% load SI data

%get waveforms and timestamps

load([Inpath,'\',Filename],'Timestamps','Spikes')

%% add fourth channel if missing

if min(size(Spikes)) == 3

filler = zeros(1,length(Spikes));

Spikes = [Spikes,filler];

clear filler

end

%% write .ntt

Outname = strsplit(Filename,'.mat');

Outname = Outname{1};

NTTname = [Outpath,'\',Outname,'.ntt'];

% FieldSelectionFlags(1): Timestamps (1xN vector of timestamps, ascending

% order

% FieldSelectionFlags(2): Spike Channel Numbers

% FieldSelectionFlags(3): Cell Numbers (here: 0, no cells sorted yet)

% FieldSelectionFlags(4): Spike Features (8xN integer vector of features

% from cheetah: peaks for 4 channels and valley for 4 channels.

% FieldSelectionFlags(5): Samples 32x4xN integer matrix with the datapoints

% (waveform) for each spike for all 4 channels.

% FieldSelectionFlags(6): Header

AppendToFileFlag = 0; %new file will be created or old file will be overwritten

ExportMode = 1; %export all

FieldSelectionFlags = [1,1,1,1,1,0];

numspikes = numel(Timestamps);

ScNumbers = zeros(1,numspikes); %set to 0 (cheetah also does that)

CellNumbers = ScNumbers; %all cells in cluster 0

Features = nan(8,numel(Timestamps));

for s = 1:numel(Timestamps)

Features(1:4,s) = max(Spikes(:,:,s));

Features(5:8,s) = min(Spikes(:,:,s));

end

disp(['exporting to ', Outpath])

Mat2NlxSpike(NTTname, AppendToFileFlag, ExportMode, [], FieldSelectionFlags, Timestamps, ScNumbers, CellNumbers, Features, Spikes)

disp(['created ',Filename,'.ntt'])

end