

## Downloading Octave and installing packages:

**NOTE:** this section only needs to be performed once the first time you use the script.

- Download and install the latest version of the Octave graphical user interface (gui). Make sure you download the correct version for either Mac or Windows.

**Octave for Mac:**

<https://octave-app.org/Developer-Downloads.html>

**Octave for Windows:**

<https://www.gnu.org/software/octave/download.html#ms-windows>

- Download the io and nan packages using the links below.

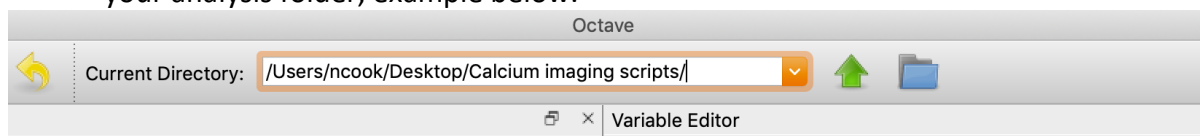
**Io:**

<https://octave.sourceforge.io/io/index.html>

**Nan:**

<https://octave.sourceforge.io/nan/>

- Create a folder to perform the analysis in and copy the io and nan files into the folder.
- Open Octave and change the path in the “Current Directory” bar to be the path of your analysis folder, example below.



- In the Command Window, install the packages io and nan using the commands below:

```
pkg install io-2.6.1.tar.gz
```

```
pkg install nan-3.4.5.tar.gz
```

**NOTE:** ensure the name of the package highlighted in green is **identical** to the name of the package you downloaded

## Detrending using Octave:

- Copy the entire length of the YFP/CFP ratio into the excel\_input\_template.xlsx file with one column per trace.

**NOTE:** blank values in the middle of the trace where you have been focusing etc. will still allow the script to run but uncalculated values such as #DIV/0! are not permitted and must be removed!

- Enter the “start II” (highlighted in red) as 0 for each trace.
- Put the time of the last value of the trace in “end II” (highlighted in red).
- Save and rename the template in the folder you wish to do the analysis. An example of the top of the input file can be seen below.

**NOTE:** there must be the same number of columns filled in for exp.# as there are traces you want to analyse, if there are more or less exp.# then this causes the script to fail.

I15								
	A	B	C	D	E	F	G	H
1			exp. #	1	2	3	4	
2			start I					
3			end I					
4			start II	0	0	0	0	
5	only phase II is analysed		end II	7674	6576	6573	7179	
6			time					
7	"time" column should as long as the longest experiment		0	1.35812966	1.31756586	1.29498058	1.33971227	
8			3	1.36063121	1.32185061	1.30494502	1.34695135	
9			6	1.34353113	1.31147857	1.28953982	1.34318526	
10			9	1.32649123	1.32028813	1.29283116	1.34646534	
11			12	1.33530272	1.31694724	1.2984768	1.34925854	
12			15	1.32352534	1.3082548	1.29012524	1.34806852	
13			18	1.314573	1.30887623	1.29064354	1.35041741	
14			21	1.31410168	1.32905563	1.29698089	1.3484308	
15			24	1.30361441	1.3032438	1.29316595	1.35040463	
16			27	1.30308641	1.31202879	1.3017533	1.35552826	
17			30	1.30386893	1.32108869	1.29993555	1.35026133	

- Copy the Loading\_Analysis\_and\_Saving\_Det.m and INPUT\_file.m files into the same folder as the renamed excel\_input\_template.xlsx containing your data.
- In Octave open the INPUT\_file.m and change the "Genotype\_name", "Excel\_file\_name", "Excel\_sheet\_name" and Final\_Output\_Excel\_file" fields. The Genotype name and output excel files can be called whatever you like. The Excel file name and sheet name **must match exactly** the name you have given the renamed excel\_input\_template.xlsx file and sheet name contained within it.
- The "Excel\_references" field refers to the region of the excel sheet that will be analysed but does not need to be changed.
- Save the INPUT\_file.m file.
- Open the Loading\_Analysis\_and\_Saving\_Det.m file in Octave.
- Click on the "Save file and Run" icon to run the script.
- The output of the script will give two excel files into the same folder as the renamed excel\_input\_template.xlsx. The file name which contains DETREND has the detrended YFP/CFP ratios for each of your traces. The other output file contains information about the amplitude, frequency, rising phase and falling phase of the calcium spiking and **should be ignored unless experimental set up has been installed. To be discuss with Myriam.**



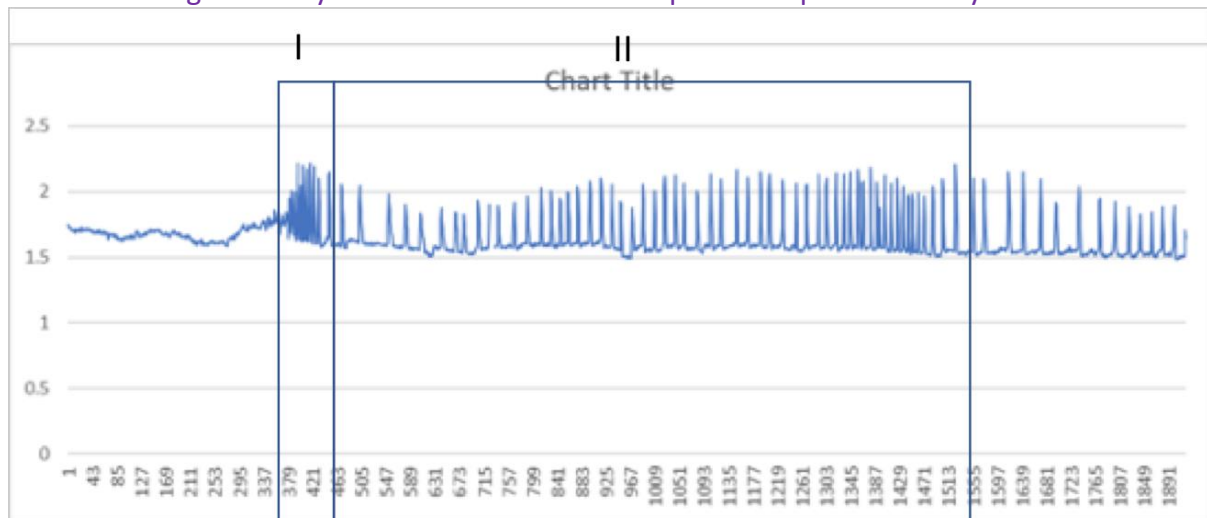
## Analysis of Ca spiking using Octave:

- Copy the entire length of the YFP/CFP ratio into the excel\_input\_template.xlsx file with one column per trace.

**NOTE:** blank values in the middle of the trace where you have been focusing etc. will still allow the script to run but uncalculated values such as #DIV/0! are not permitted and must be removed!

- Enter the time of the start of phase II spiking in the “start II” (highlighted in red) box for each trace. An example of phase I and phase II spiking can be seen below.

**NOTE:** make sure you understand the difference between phase I and phase II spiking before starting the analysis. For a more detailed explanation please ask Myriam.



- Enter the time of the end of phase II spiking in the “end II” (highlighted in red) box for each trace.
- Save and rename the template in the folder you wish to do the analysis. An example of the top of the input file can be seen below.

**NOTE:** there must be the same number of columns filled in for exp.# as there are traces you want to analyse, if there are more or less exp.# then this causes the script to fail.

- Copy the Loading\_Analysis\_and\_Saving\_Det.m and INPUT\_file.m files into the same folder as the renamed excel\_input\_template.xlsx containing your data.
- In Octave open the INPUT\_file.m and change the “Genotype\_name”, “Excel\_file\_name”, “Excel\_sheet\_name” and Final\_Output\_Excel\_file” fields. The Genotype name and output excel files can be called whatever you like. The Excel file name and sheet name **must match exactly** the name you have given the renamed excel\_input\_template.xlsx file and sheet name contained within it.
- The “Excel\_references” field refers to the region of the excel sheet that will be analysed but does not need to be changed.
- Save the INPUT\_file.m file.
- Open the Loading\_Analysis\_and\_Saving\_Det.m file in Octave.
- Click on the “Save file and Run” icon to run the script.
- The output of the script will give two excel files into the same folder as the renamed excel\_input\_template.xlsx. The file name which contains DETREND has the detrended YFP/CFP ratios for each of your traces. The other output file contains



information about the amplitude, frequency, rising phase and falling phase of the calcium spiking for each of the traces. An average across all of the traces for each of these parameters is printed in the command window once the script is complete which can be copied and saved into the output excel file.