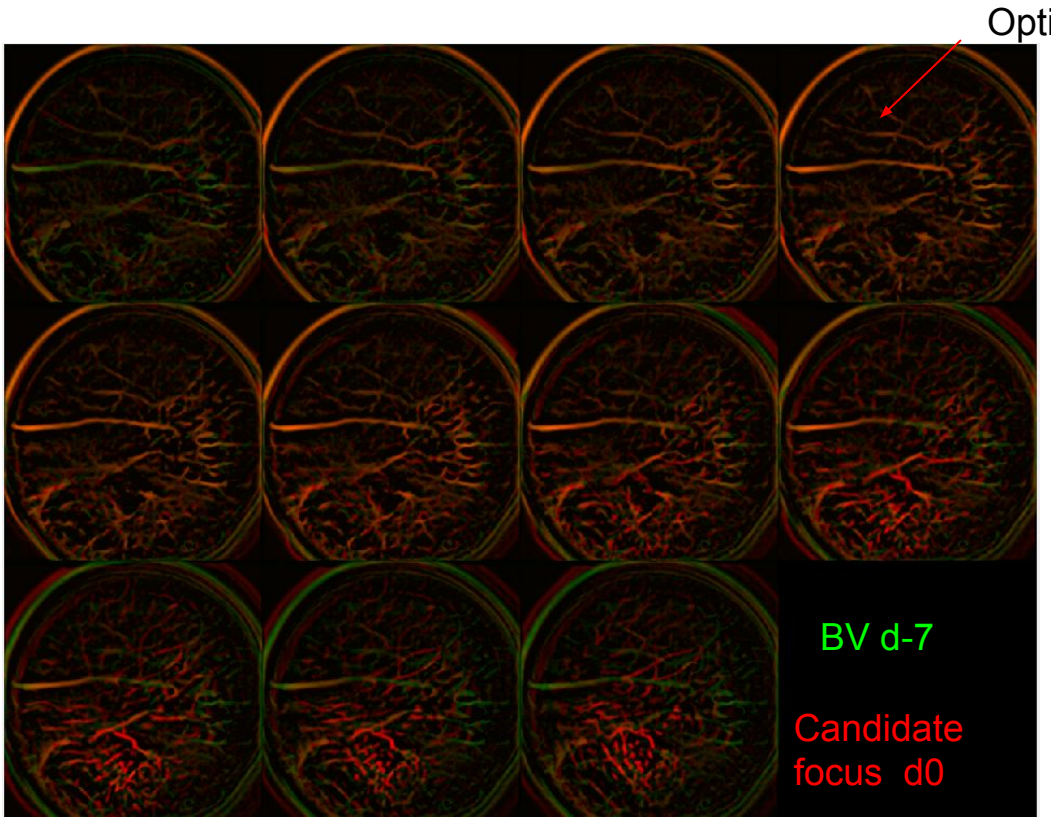
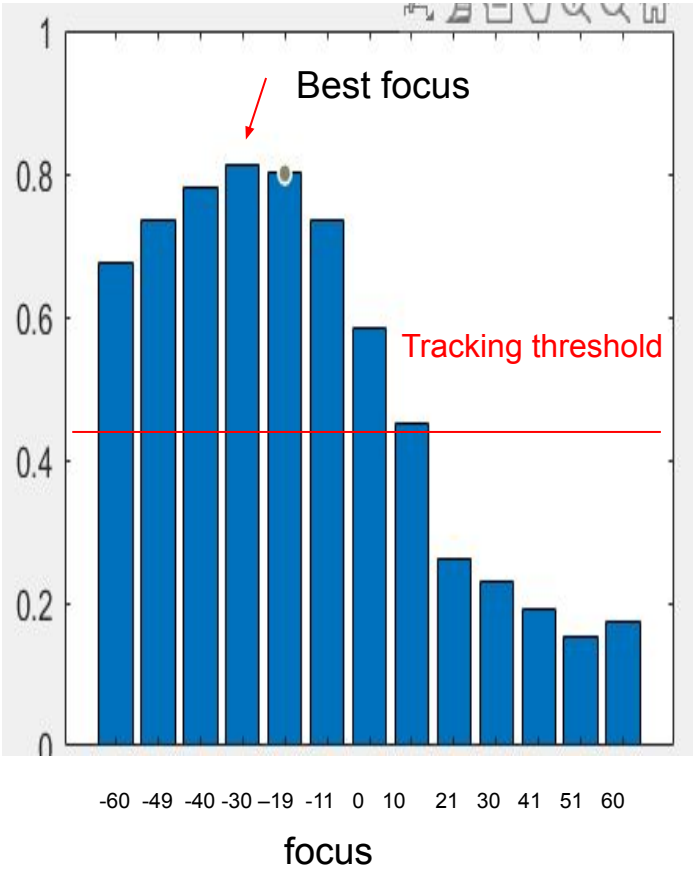
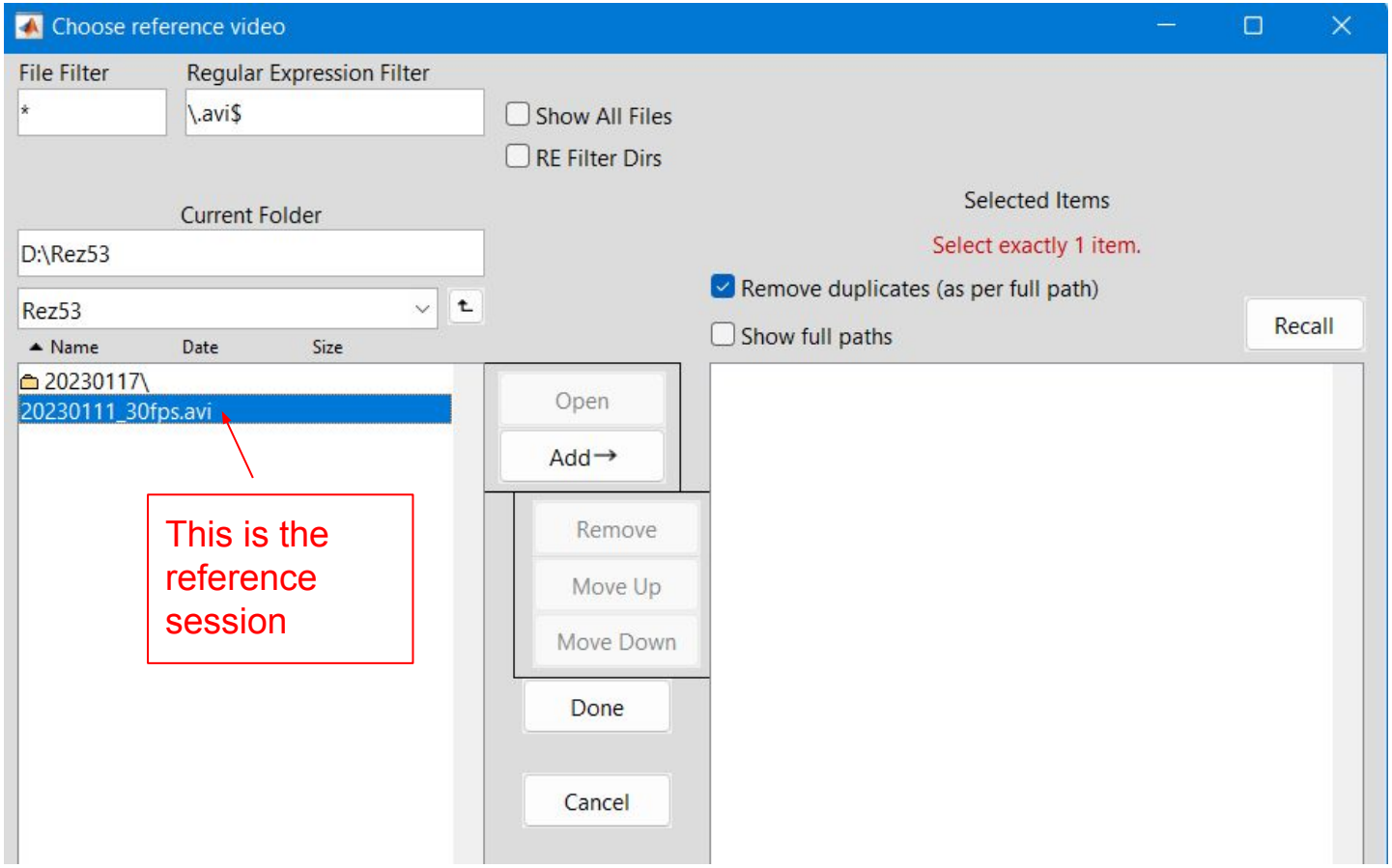
Example results:





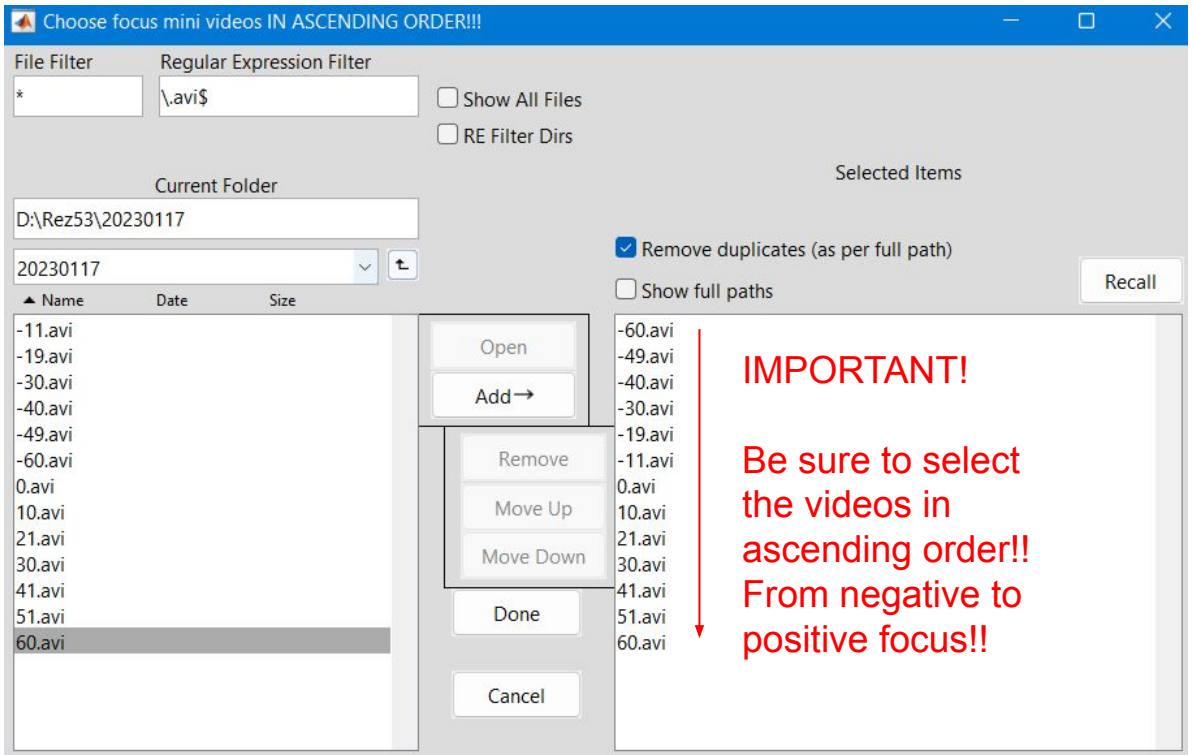
How to estimate optimal focus part 1:

1) Record 11 small video (i.e ~1s) at different focus.
   a) The focus used in each video should be: F-25, F-20, F-15, F-10, F-5, F, F+5, F+10, F+15, F+25, where F is the Focus used in last session.
2) Once the video files are saved, open Matlab.
3) Run “optimal_focus_miniscope()” in the command line (without “ ”).
   a) Pablo will install Matlab and CaliAli in the recording computer. If errors occur contact Pablo ASAP.
4) A window will ask you to select the recording session to which you want to align the candidate focus (i.e. the reference session):
   a) Press Add→ and done.



How to estimate optimal focus part 2:

5) After choosing the reference sessions you need to select the videos files recorded at different focus:



6) Wait for the program to run (check the command window to sii its progress)

```
>> optimal_focus_miniscope()
Now reading D:\Rez53\20230111_30fps.avi
Processing:  100%  |########| 100/100it [00:00:00<00:00:00, 676.64 it/
Appling filter:  100%  |########| 100/100it [00:00:00<00:00:00, 237.48
Elapsed time is 0.651879 seconds.
Now reading D:\Rez53\20230117\-60.avi
Processing:  100%  |##########| 38/38it [00:00:00<00:00:00, 729.35 it/
Appling filter:  100%  |##########| 38/38it [00:00:00<00:00:00, 167.47
```

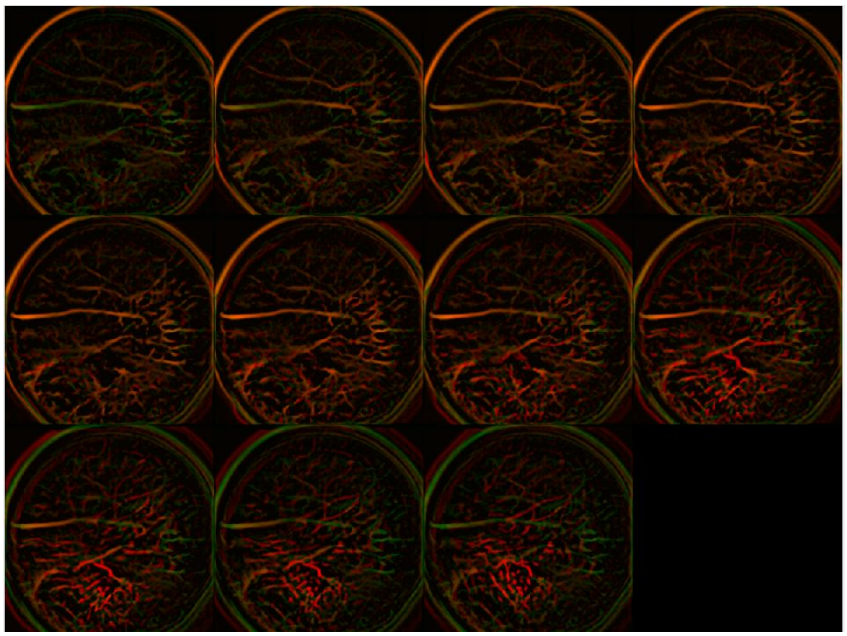
How to estimate optimal focus part 3:

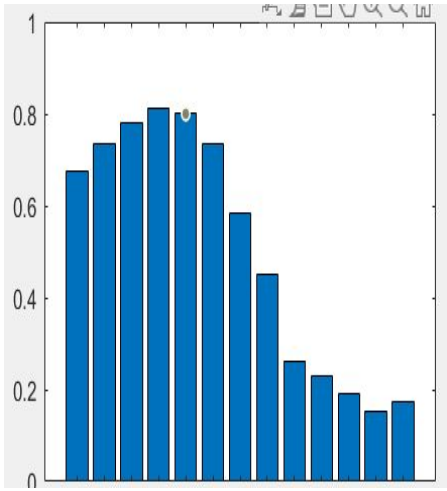
7) Once the programm finish check the last two lines written in the command window: The program will want you if none of the focus are good. If everything is okay, you should see something like this.

```
Maximum BV correlation (0.814) is suitable to track neurons!
Optimal focus is #4!!
>>
```

Note: This will tell you which file has the best focus (#1 is the first file you added to the list, #2 is the second, and so on…). If none of the focuses are good (i.e. the maximum BV correlation in bellow 0.45, then suspend the experiment and contact Pablo ASAP).

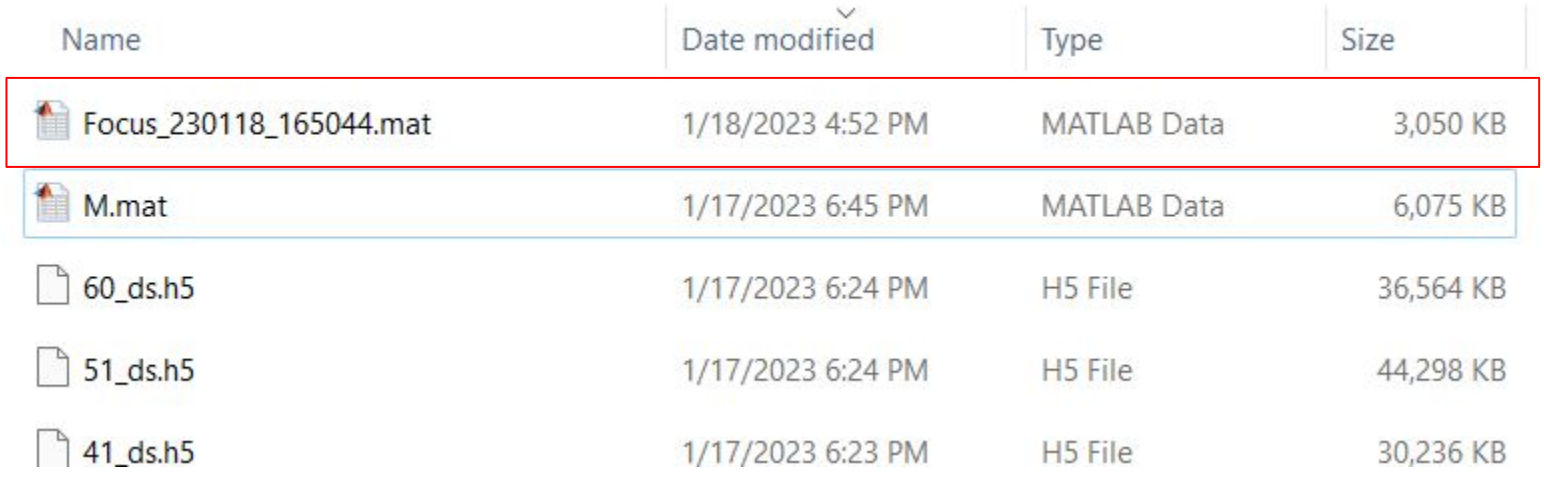
8) The code will automatically show two images, #1: An overlay of the BV obtained with different focus (Red channel) and the BV from the reference session (green channel). #2: the BV correlation obtained with the different video files. Check these images in case there is something obviously wrong and notify Pablo if in doubt.





How to estimate optimal focus part 3:

8) A file with all the relevant information will be created in the folder in which the focus files are stored (or in the folder containing focus #1, if files are in different folder):



Name	Date modified	Type	Size
Focus_230118_165044.mat	1/18/2023 4:52 PM	MATLAB Data	3,050 KB
M.mat	1/17/2023 6:45 PM	MATLAB Data	6,075 KB
60_ds.h5	1/17/2023 6:24 PM	H5 File	36,564 KB
51_ds.h5	1/17/2023 6:24 PM	H5 File	44,298 KB
41_ds.h5	1/17/2023 6:23 PM	H5 File	30,236 KB

Send this file to Pablo for reference.

9) Proceed with recordings using the optimal focus.