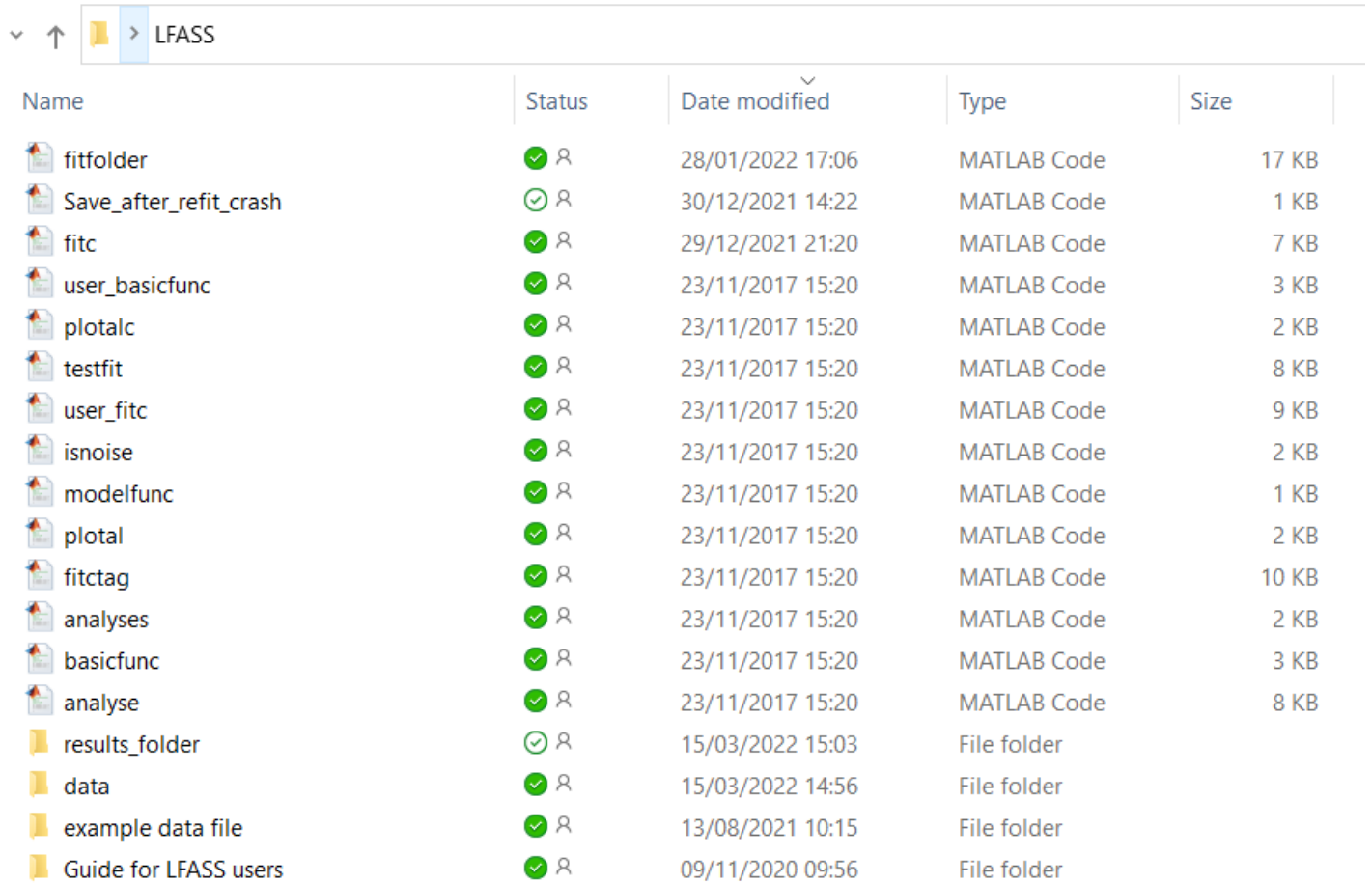
1. Download and install Matlab (Mathworks).
2. Download and copy LFASS folder where needed (https://github.com/ABA80/LFASS).
3. Open LFASS folder:

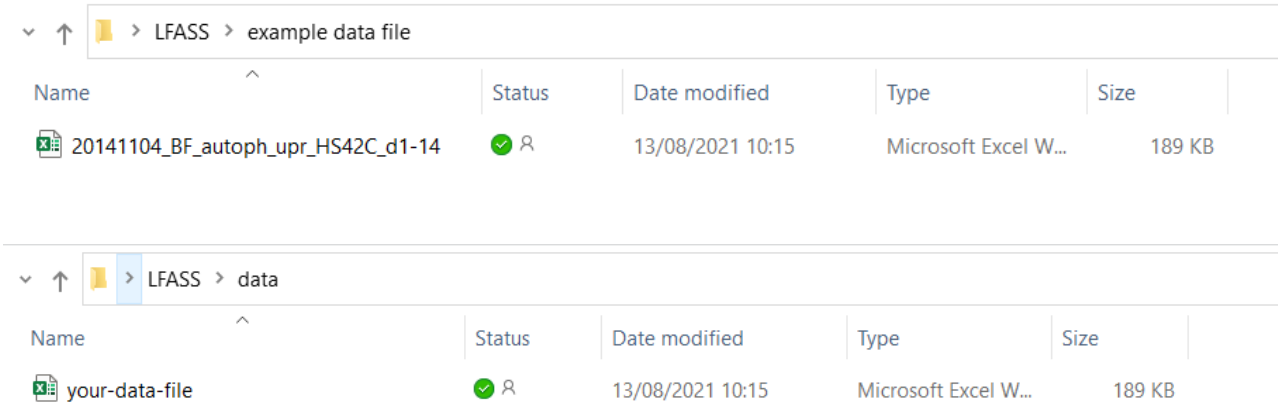


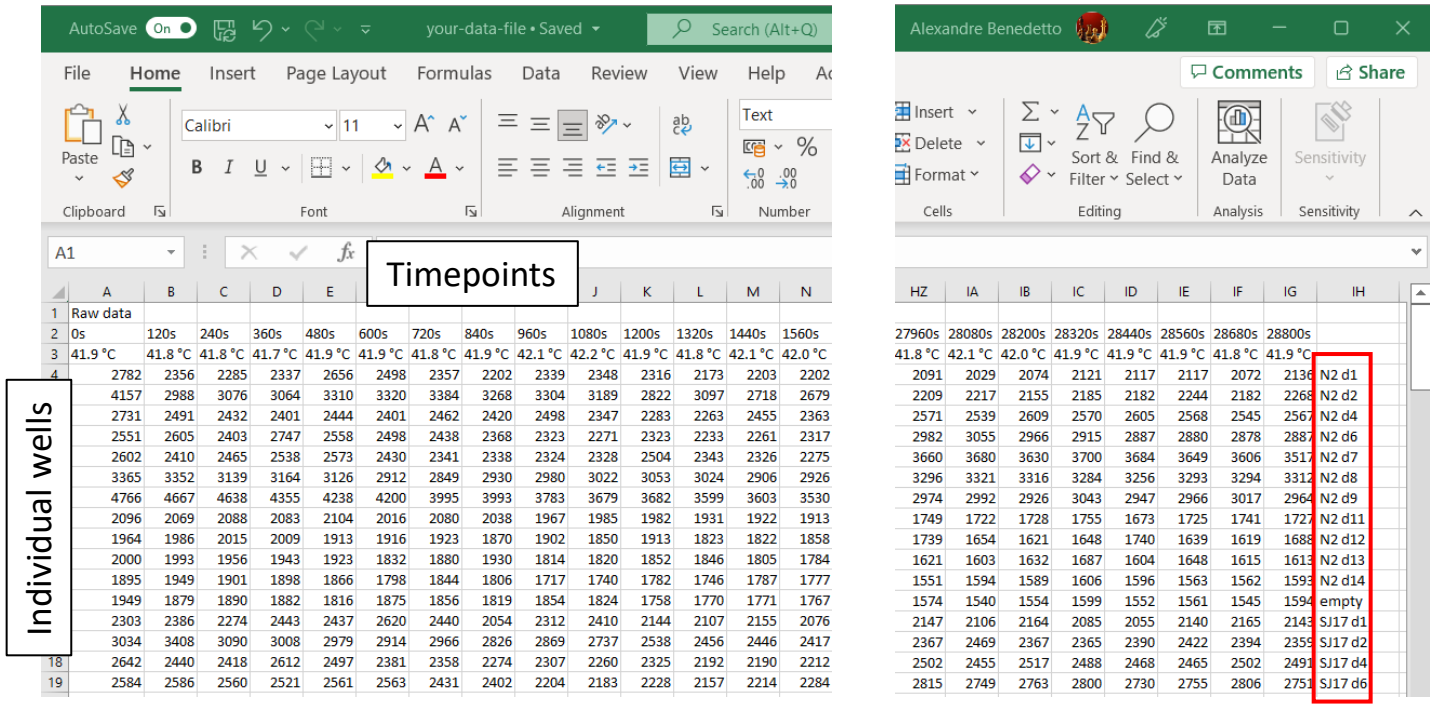
4. ‘results_folder’ may not be there. It will be automatically created when running the routine for the first time.

5. You can use the file in the ‘example data’ folder to practice:

6. Copy it in the ‘data’ folder, or copy your own data file:

7. Your data file should be organised as follows:
   - Wells in rows
   - Timepoints in columns
   - Identifiers for wells in first or last column
   - Non-number rows (headings, temperatures) will be ignored





8. Double-click on the ‘**fitfolder**’ file in the LFASS folder (it will open Matlab automatically)

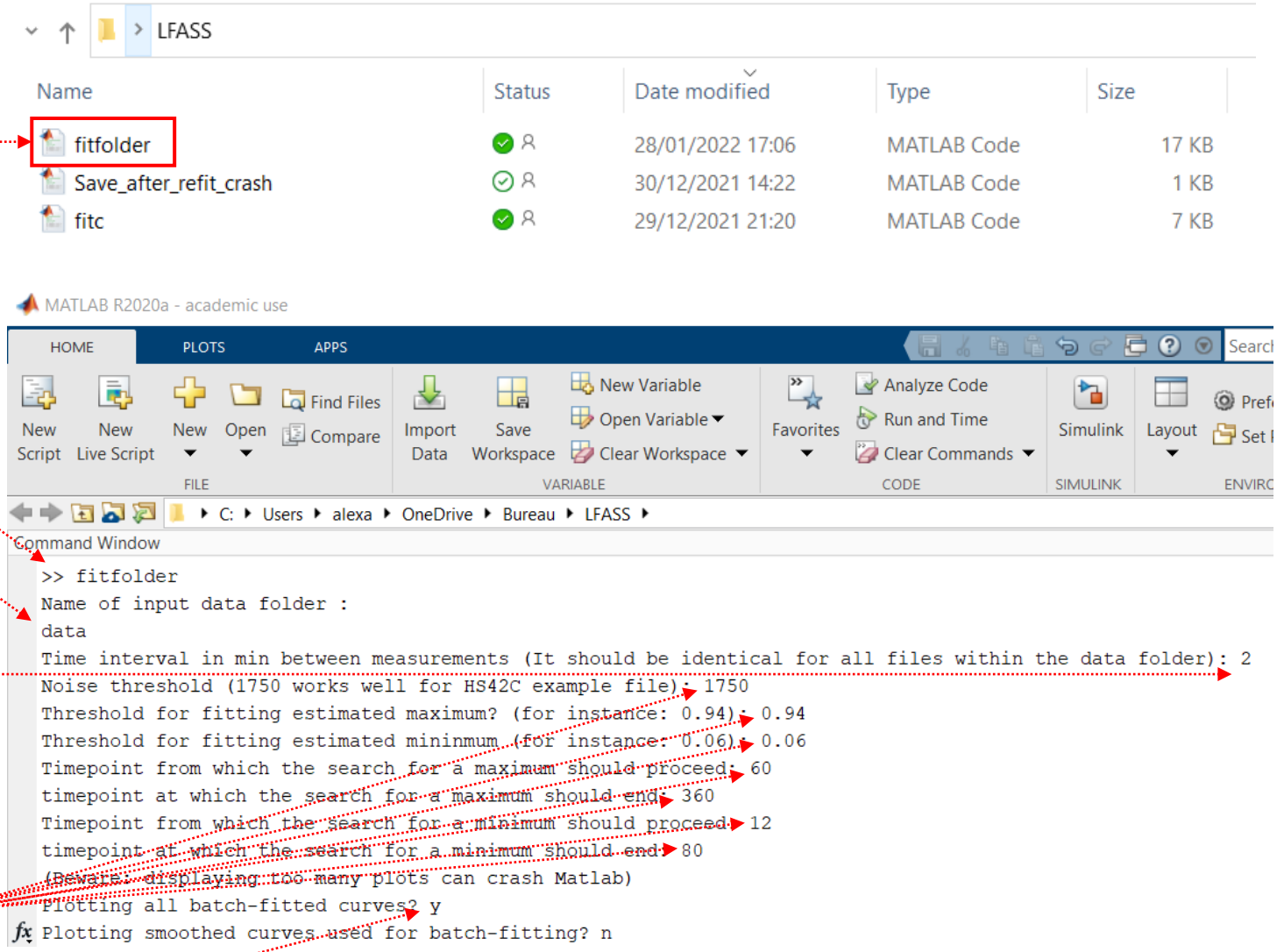
9. In the command window, type ‘**fitfolder**’, press ↵

10. Then enter the name of your data folder: ‘**data**’

11. Enter the time interval between consecutive measurements of the same well (it should be an integer, here ‘**2**’ for 2 min)

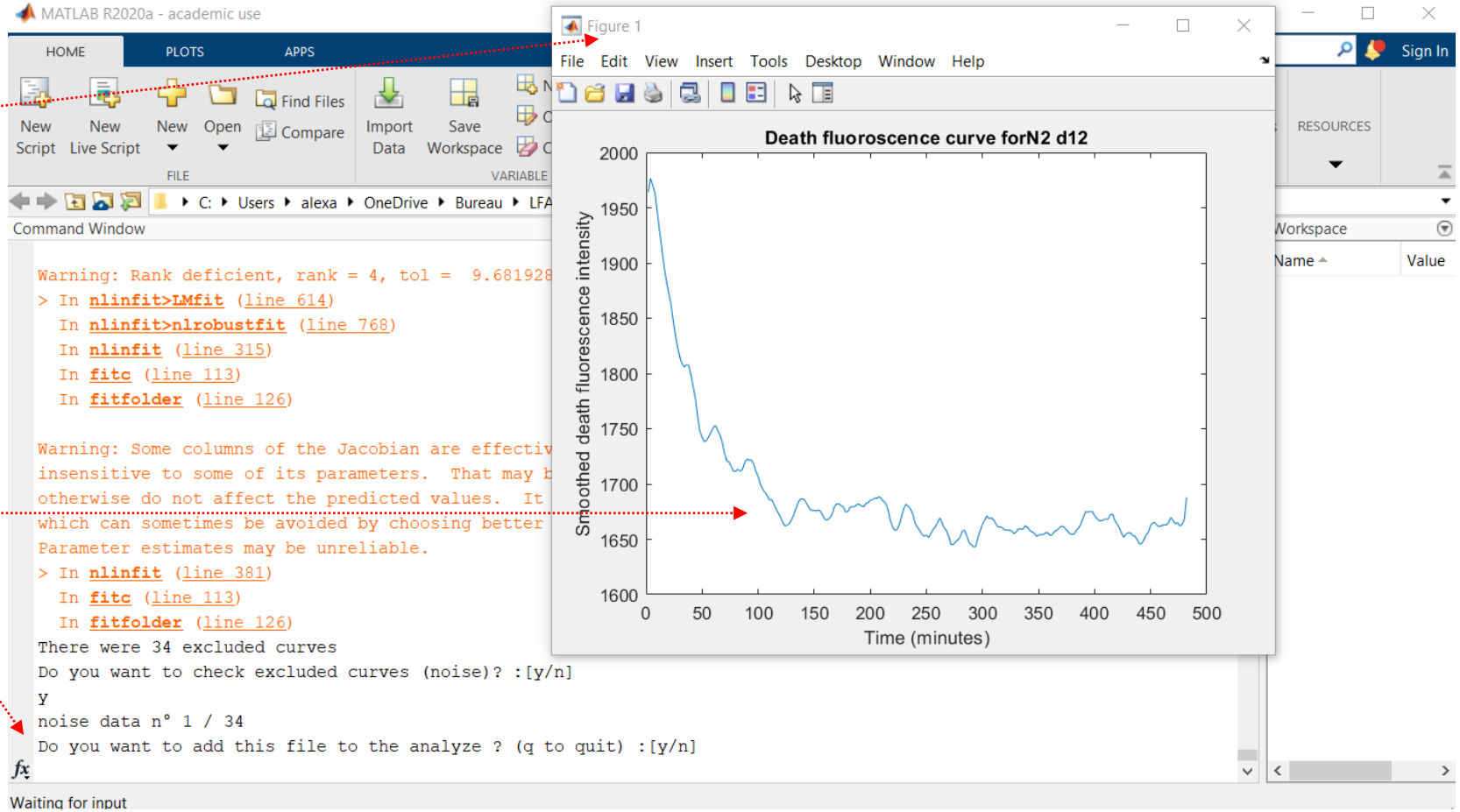
12. Enter other parameters pressing ↵ each time.

13. Enter ‘**y**’ for YES to plot fitted curves



14. The routine will display excluded curves one by one querying whether it should be fitted

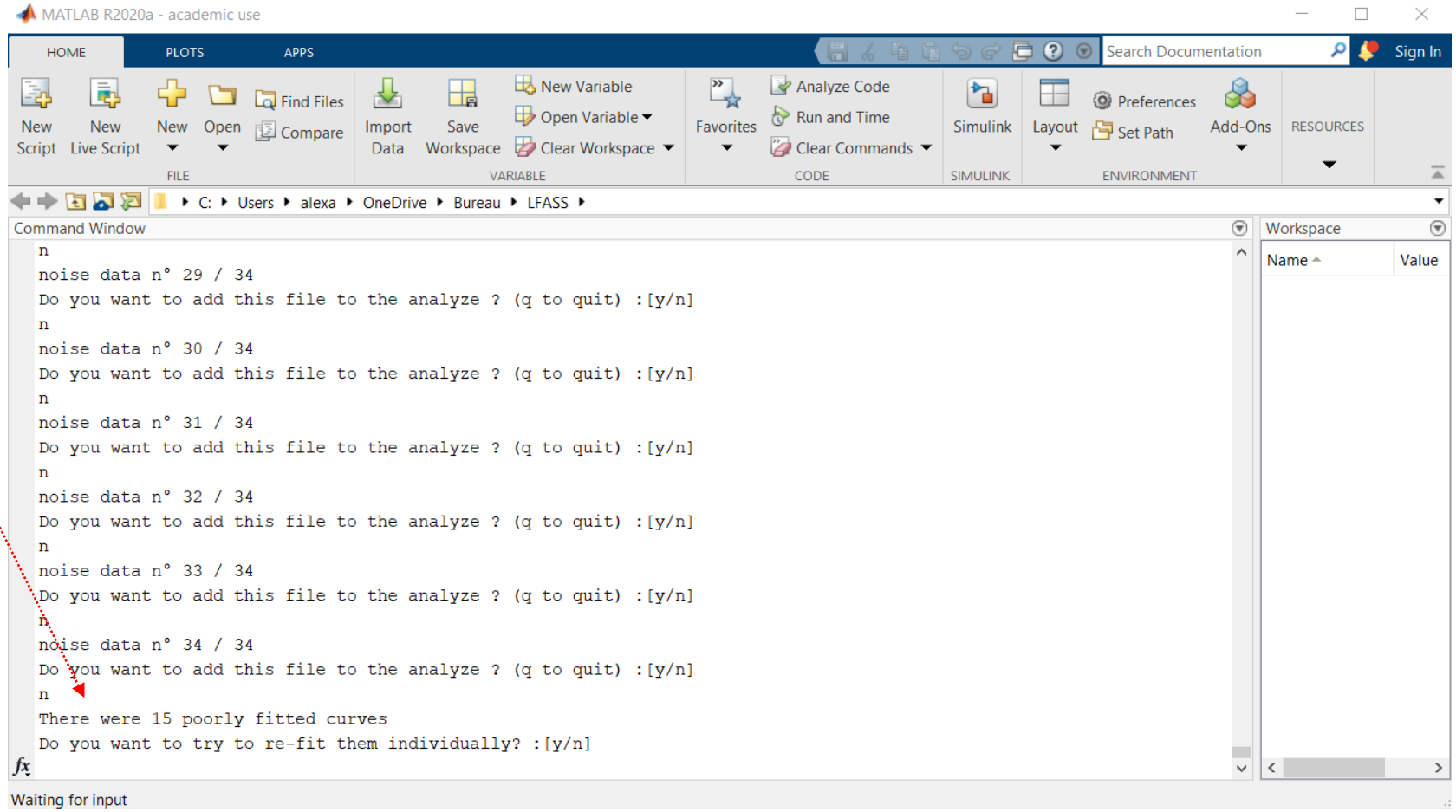
15. Enter '**y**' to try to automatically refit the curve and '**n**' if not (here the curve does not display any DF peak and is excluded from the analysis)



16. After all excluded curves have been checked or ignored, poorly-fitted curves are reviewed next.

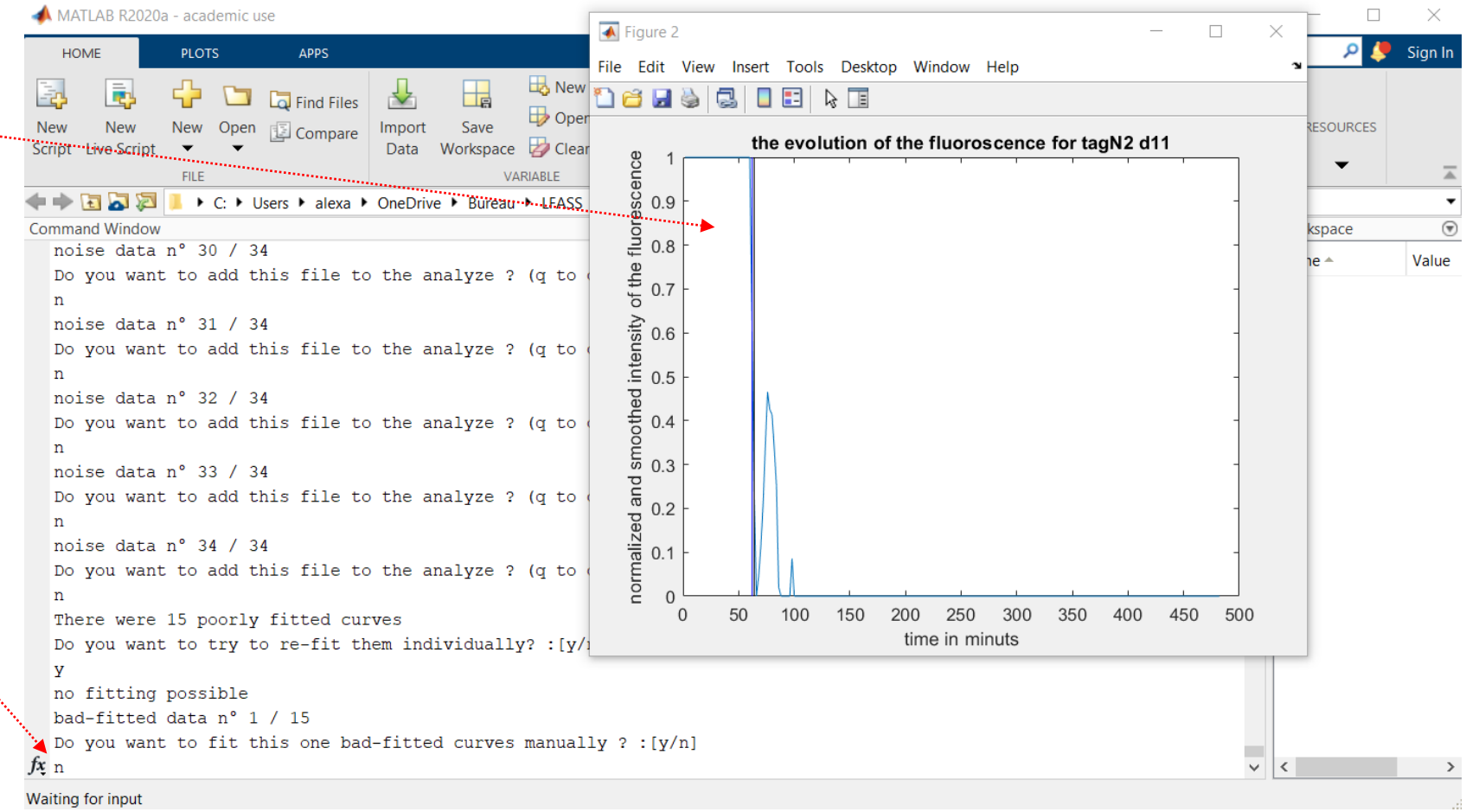
17. Usually, these will be rechecked one by one and refitted using new time boundary parameters.

18. Type '**y**' for YES, to enable review of ill-fitted curves one by one.



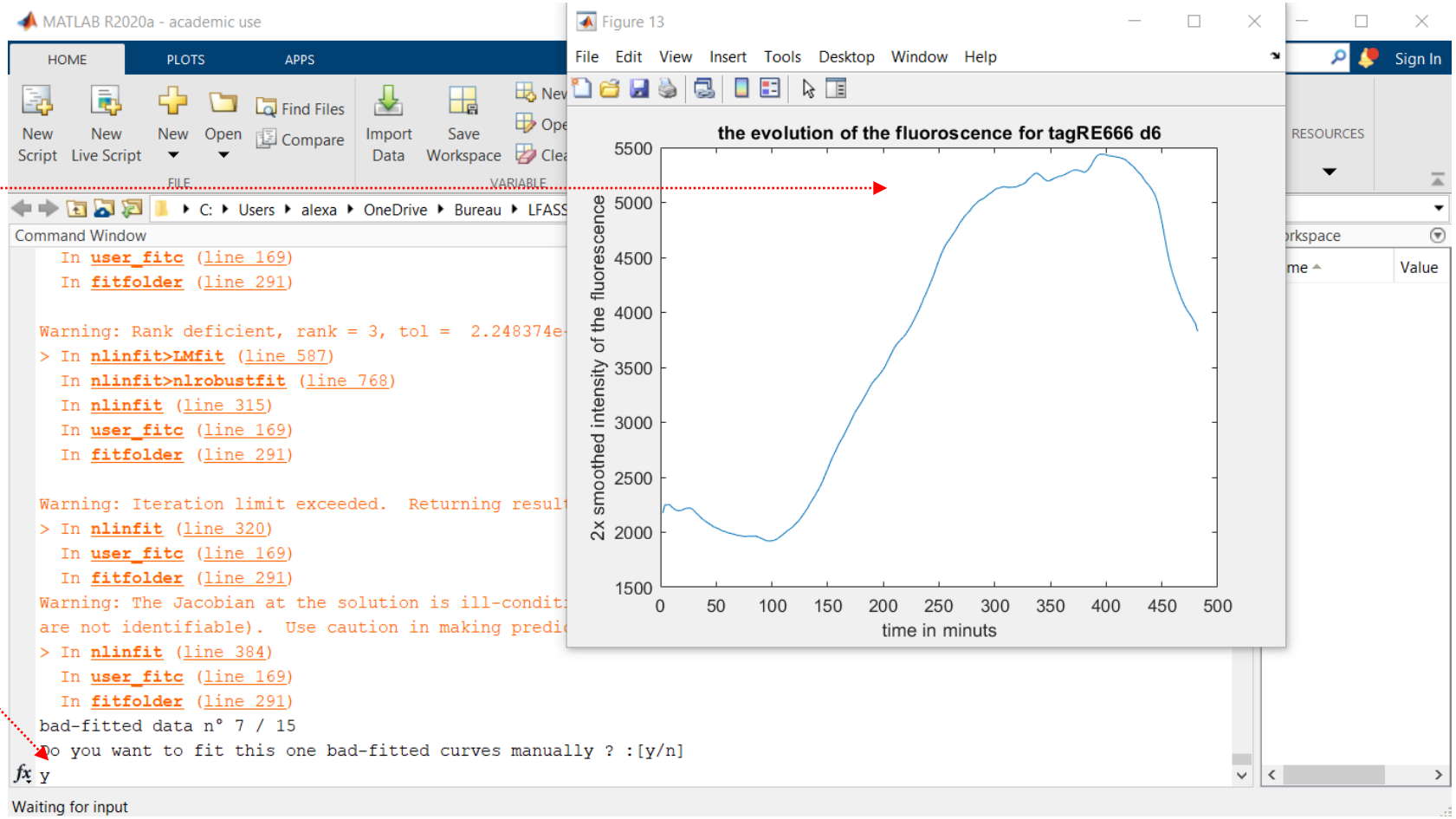
19. Here is an example of a failed fit where the curve does not show any clear DF peak.

20. Visual inspection here confirms that the curve does not contain useable data. Enter '**n**' for NO to reject re-fitting.

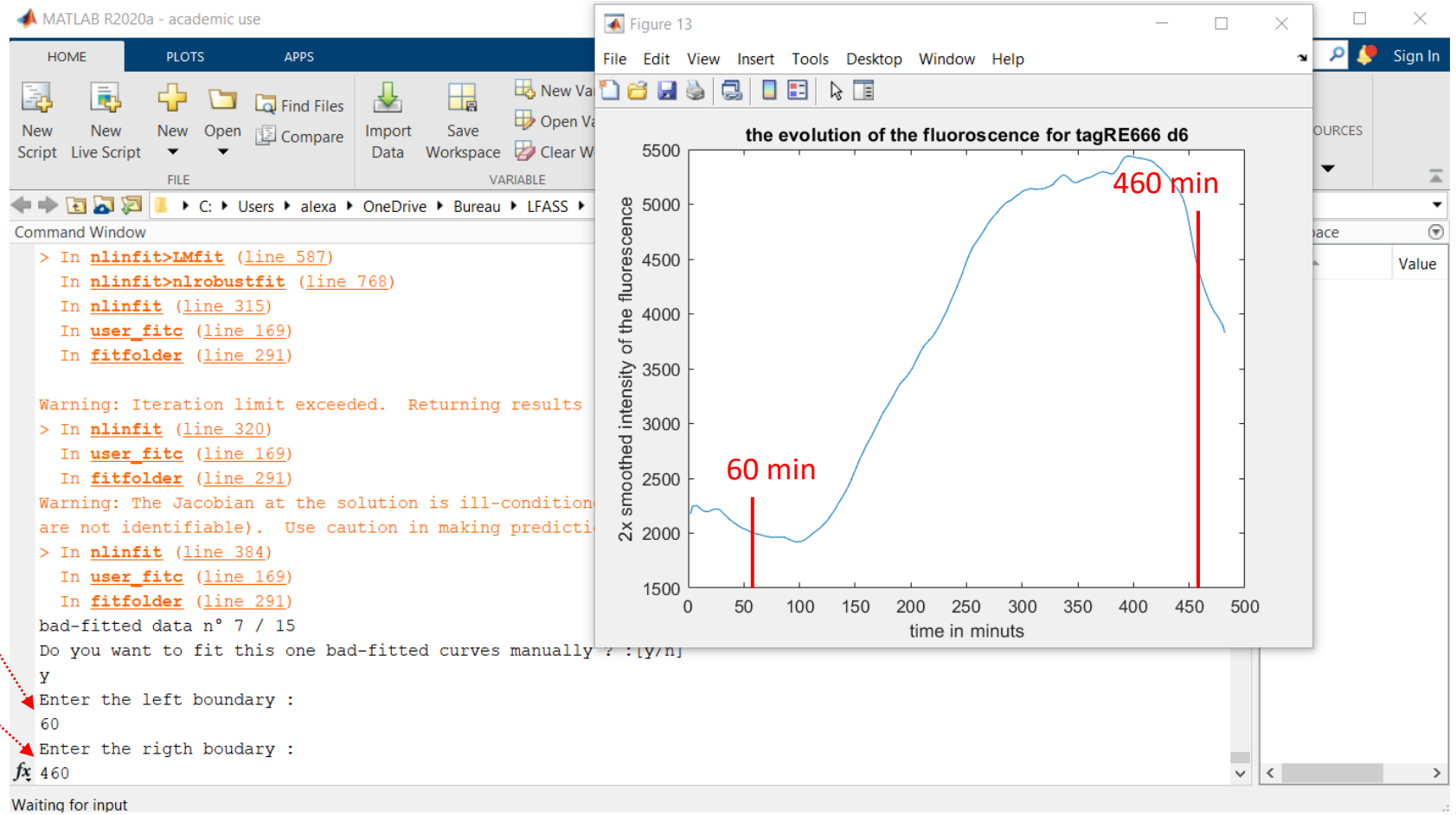


21. Here is an example of a failed fit where the curve shows a clear DF peak. The fit failed because the maximum (at ~400min) is found after the upper limit set earlier for the batch analysis (360 min, see step 12).

22. In this case the curve needs to be refitted. Enter ‘y’ for YES to re-fit.

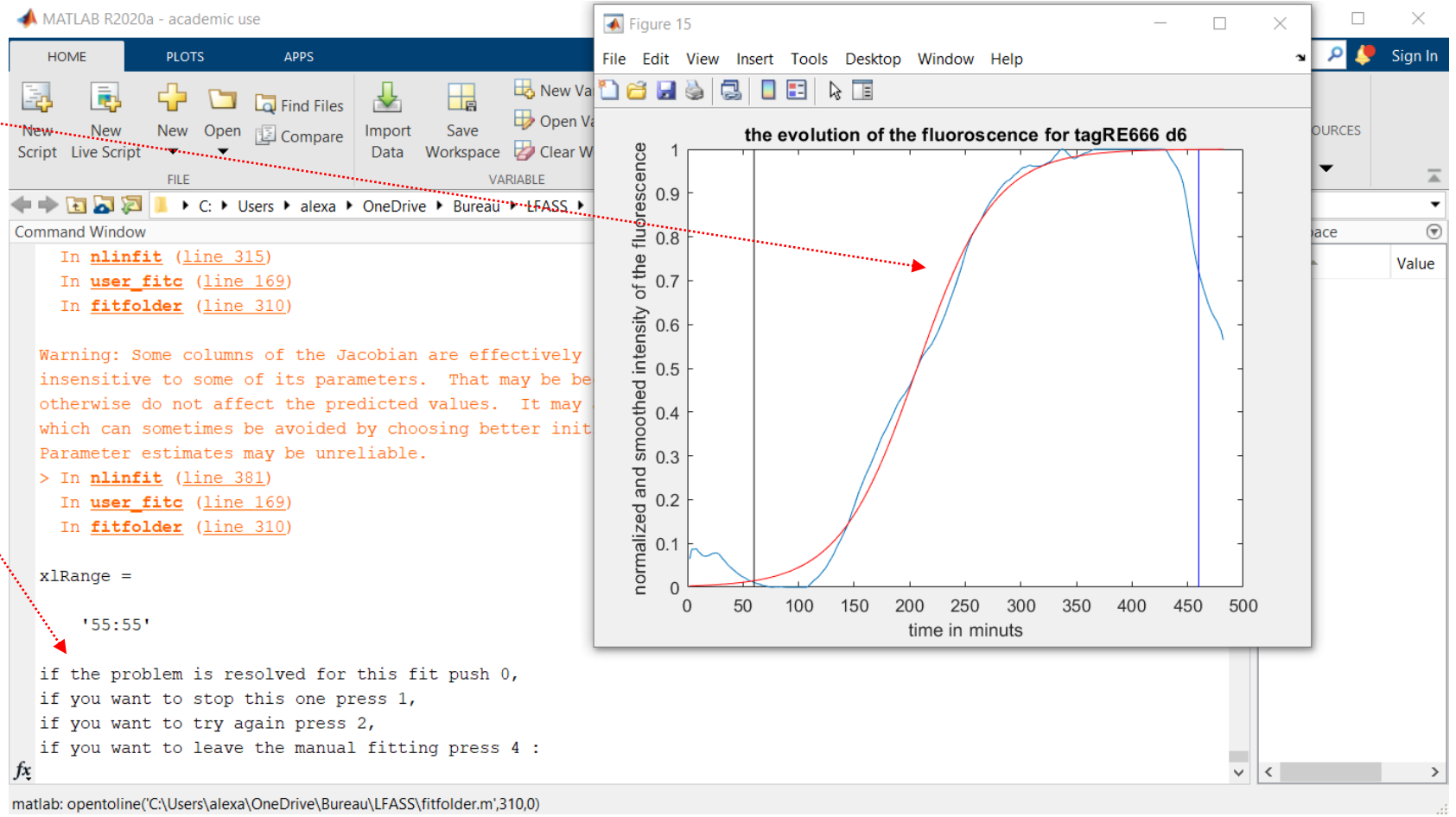


23. Enter new time boundaries that encompass the region of the curve where a sigmoid fit can be applied (for instance here between 60min and 460min)

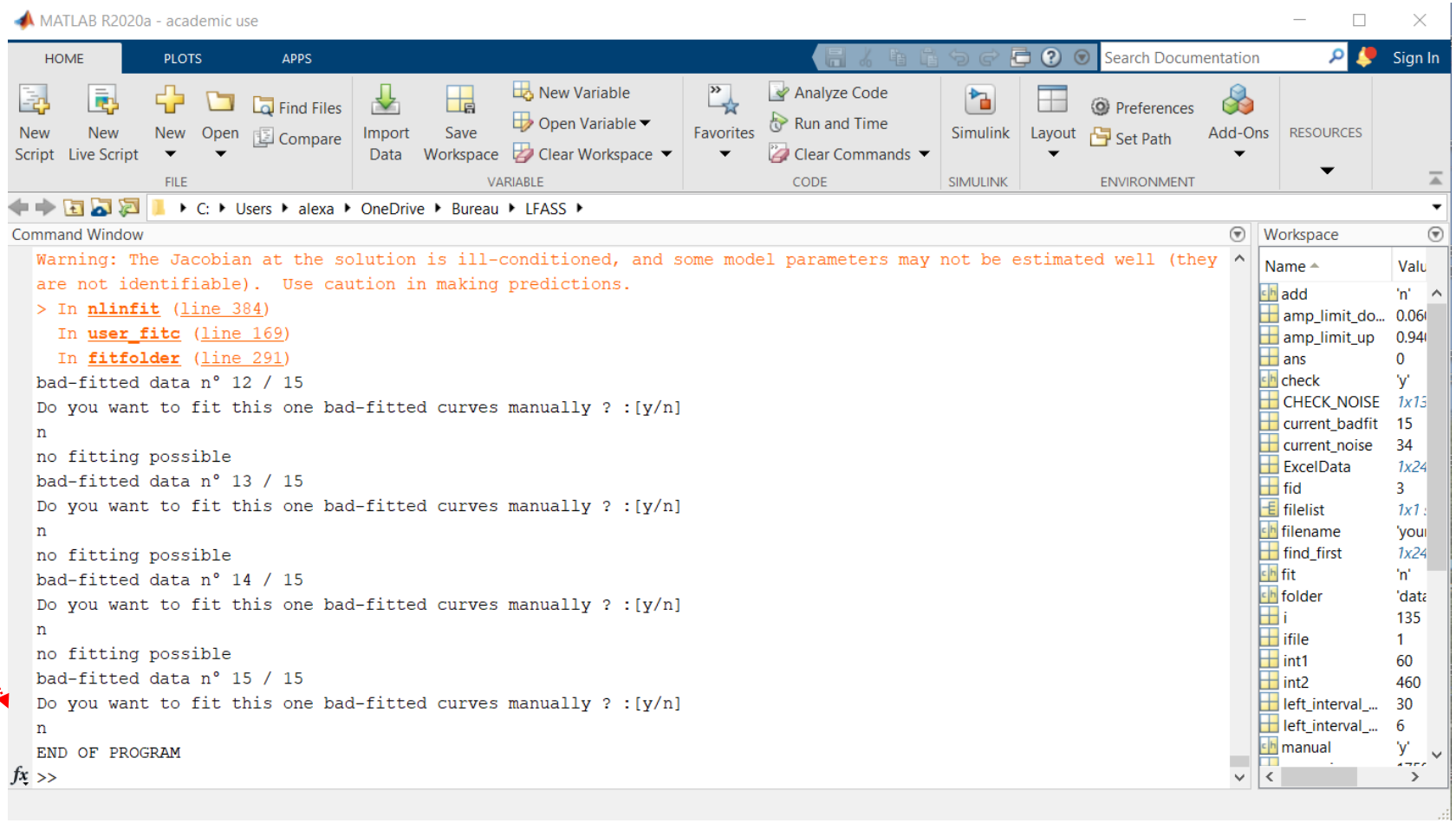


24. After fitting is reattempted, the result is displayed.

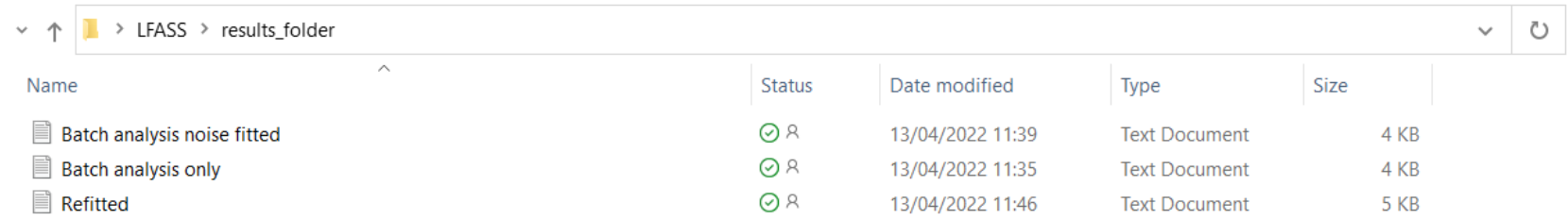
25. A choice is then given to accept the new fit (type '**0**'), or to reattempt it (type '**2**'). Typing '0' automatically moves to the next curve.



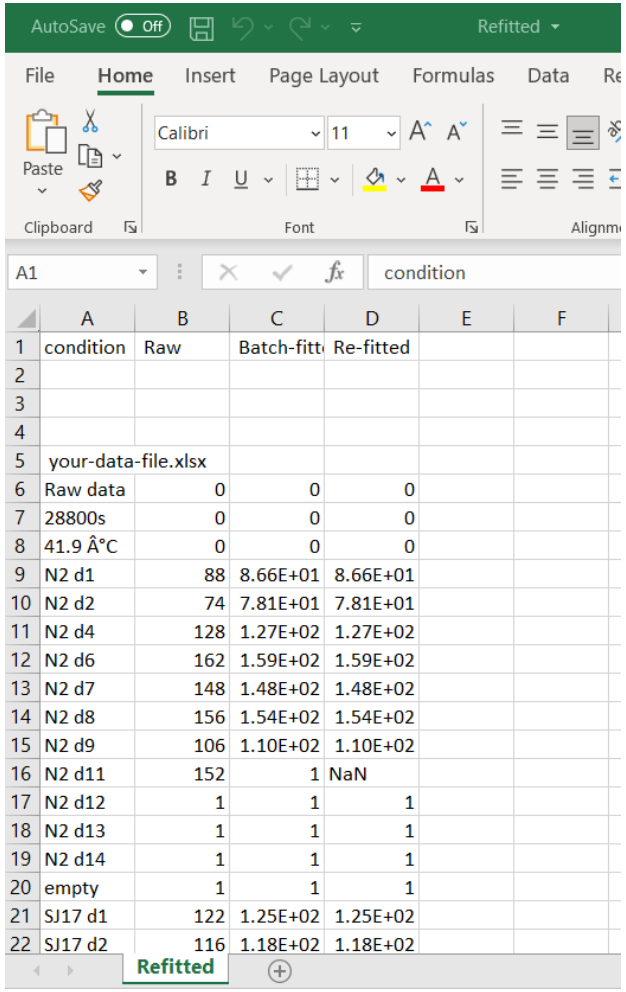
26. After refitting has been attempted or declined for all remaining curves, the program ends.



27. The result files are found in the '**results-folder**'

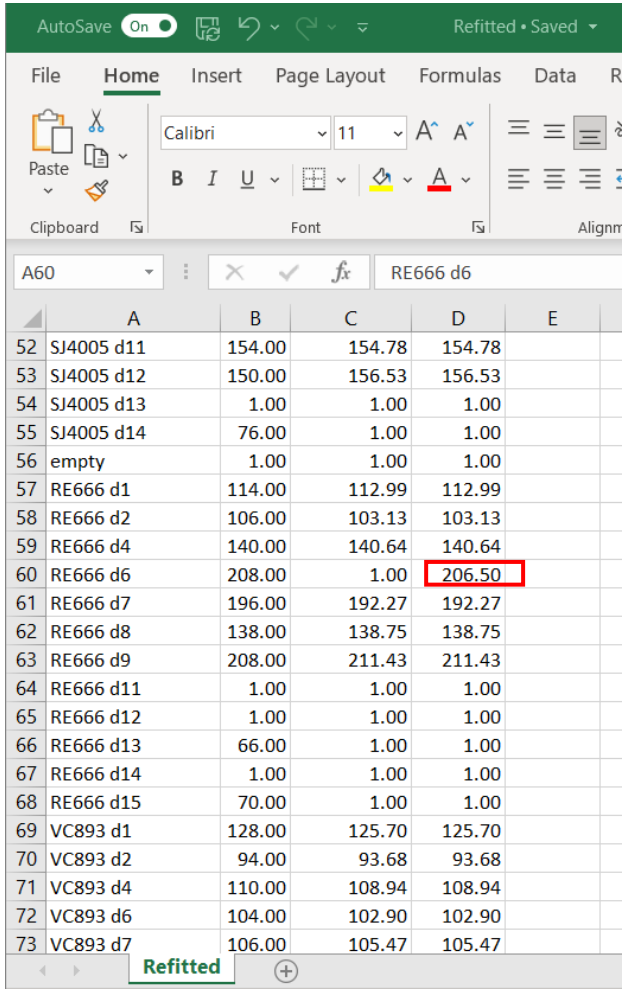


28. Open the '**refitted.txt**' file with Excel or equivalent (contains all the data)



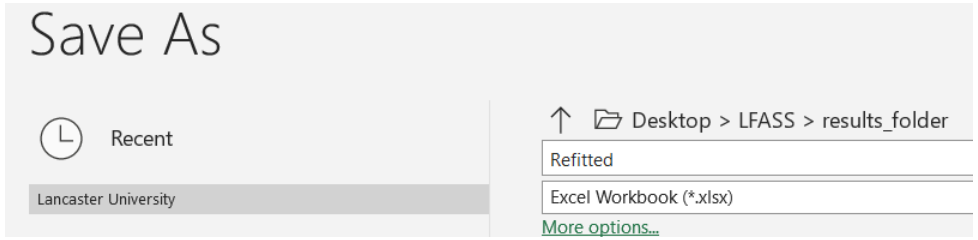
	A	B	C	D	E	F
1	condition	Raw	Batch-fitt	Re-fitted		
2						
3						
4						
5	your-data-file.xlsx					
6	Raw data	0	0	0		
7	28800s	0	0	0		
8	41.9 Â°C	0	0	0		
9	N2 d1	88	8.66E+01	8.66E+01		
10	N2 d2	74	7.81E+01	7.81E+01		
11	N2 d4	128	1.27E+02	1.27E+02		
12	N2 d6	162	1.59E+02	1.59E+02		
13	N2 d7	148	1.48E+02	1.48E+02		
14	N2 d8	156	1.54E+02	1.54E+02		
15	N2 d9	106	1.10E+02	1.10E+02		
16	N2 d11	152	1	NaN		
17	N2 d12	1	1	1		
18	N2 d13	1	1	1		
19	N2 d14	1	1	1		
20	empty	1	1	1		
21	SJ17 d1	122	1.25E+02	1.25E+02		
22	SJ17 d2	116	1.18E+02	1.18E+02		

29. Convert data to preferred number format (here .00, note re-fitted data line 60)



	A	B	C	D	E
52	SJ4005 d11	154.00	154.78	154.78	
53	SJ4005 d12	150.00	156.53	156.53	
54	SJ4005 d13	1.00	1.00	1.00	
55	SJ4005 d14	76.00	1.00	1.00	
56	empty	1.00	1.00	1.00	
57	RE666 d1	114.00	112.99	112.99	
58	RE666 d2	106.00	103.13	103.13	
59	RE666 d4	140.00	140.64	140.64	
60	RE666 d6	208.00	1.00	206.50	
61	RE666 d7	196.00	192.27	192.27	
62	RE666 d8	138.00	138.75	138.75	
63	RE666 d9	208.00	211.43	211.43	
64	RE666 d11	1.00	1.00	1.00	
65	RE666 d12	1.00	1.00	1.00	
66	RE666 d13	66.00	1.00	1.00	
67	RE666 d14	1.00	1.00	1.00	
68	RE666 d15	70.00	1.00	1.00	
69	VC893 d1	128.00	125.70	125.70	
70	VC893 d2	94.00	93.68	93.68	
71	VC893 d4	110.00	108.94	108.94	
72	VC893 d6	104.00	102.90	102.90	
73	VC893 d7	106.00	105.47	105.47	

30. Save the updated file in a convenient format for further analyses such as data manipulation and statistics (here .xlsx)



The End