process_plate_reader_data.py documentation

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May 27, 2021

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Location: https://github.tamu.edu/cody-martin/CPT_code/plate_reader_code
Must use your TAMU account You will need to contact me to get access to this repository.

S1: INSTALL PYTHON and SHELL

1. You will need to be somewhat familiar with your computer system's shell or command line tool.
   - For MacOS users, press: *CMD* + *SPACEBAR* and search "Terminal"
   - For Windows users, press: START, type "PowerShell", and choose "Windows PowerShell"
2. To run this script at the command line, you will need to have installed python on your computer. If you're a python beginner, you can install miniconda3: https://docs.conda.io/en/latest/miniconda.html, which has bare minimum python packages.
3. You will then need to install 2 extra python packages:

package	version	build	command
pandas	1.1.3	py38hb1e8313_0	`conda install pandas=1.1.3`
openpyxl	3.0.5	py_0	`conda install openpyxl=3.0.5`
matplotlib	3.3.2	0	`conda install matplotlib=3.3.2`

4. Then you can clone the repository to your local computer by using this command: `git clone https://github.tamu.edu/cody-martin/CPT_code.git`
   - You can delete / ignore all the other folders unrelated to `plate_reader_code`
   - Or you can just manually download the `.py` script needed in this directory.
5. To change directories (folders) on MacOS/Linux, type: `cd ~/directory1/directory2/directory3/` until you are in your desired directory
   - NOTE: "directory1,2,3" should be replaced with the actual folder name
   - For Windows users: I believe you can use the filepath as `C:\dir1\dir2\dir2`

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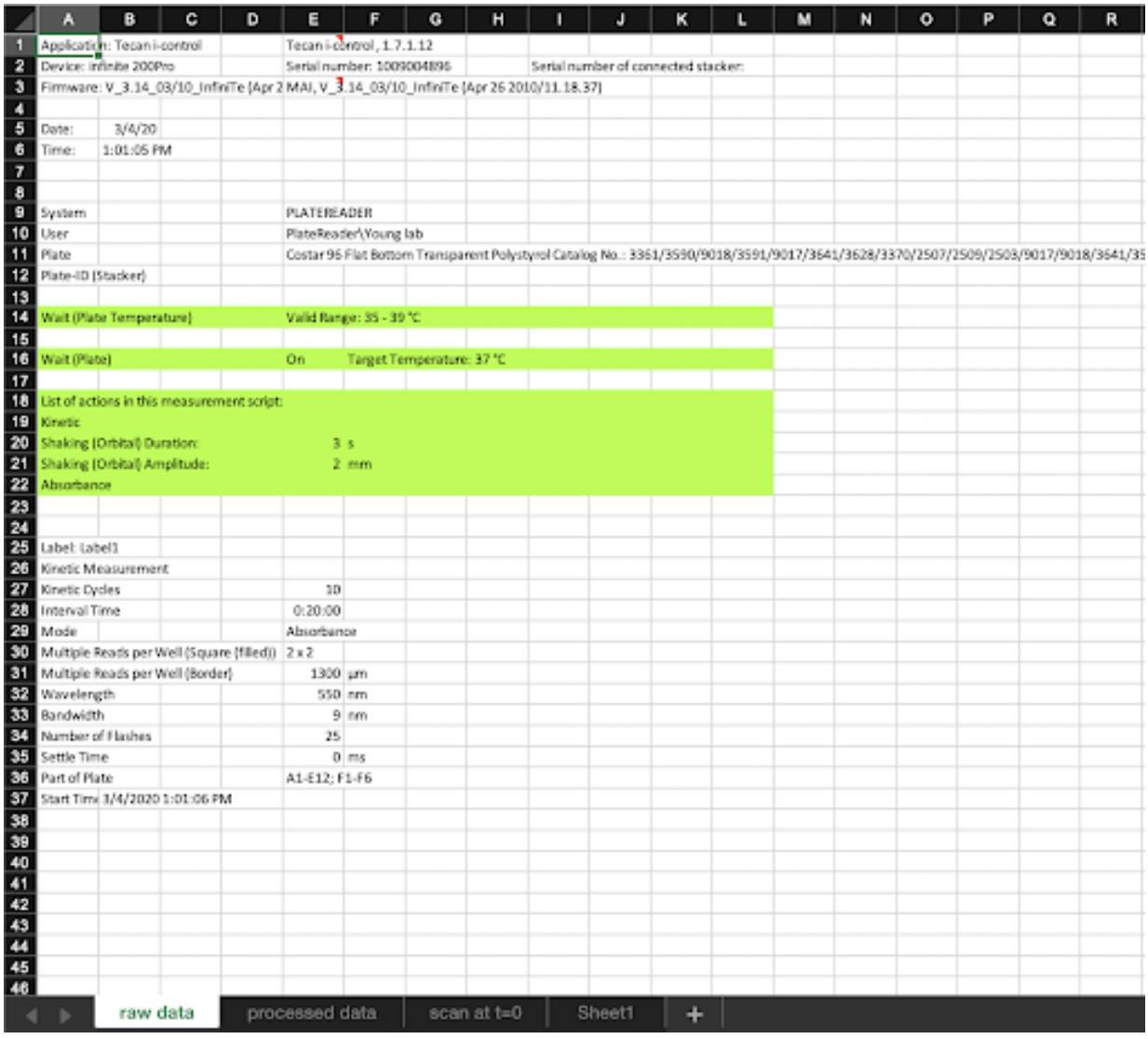
6. You will need to save the code in the same directory (folder) as your raw data from the infinite200Pro plate reader; tecan i-control 1.7.1.12 software. Do NOT edit the data. (Actually, you can just put the full file path, ie `~/Documents/CPT/N4/plate_data.xlsx/`, instead of just the file name.)

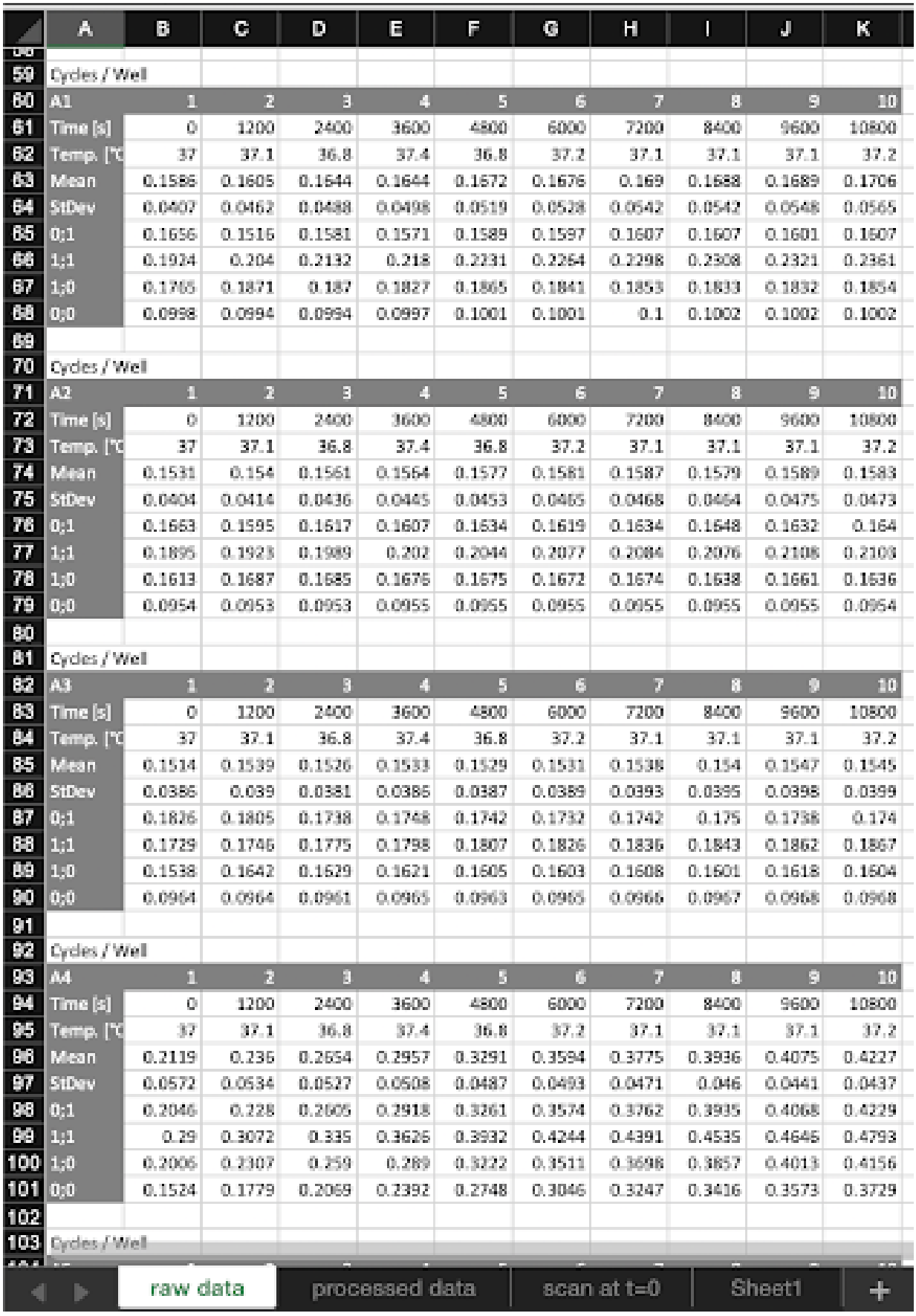
S2: INPUT FILES

1. You will need to input **two** files to run this code: your raw data and your plate setup file.
2. Your raw data must be the unedited .xlsx excel file that is output from the tecan i-control software. It should look very similar to this:



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Application: Tecan i-control				Tecan i-control, 1.7.1.12													
2	Device: Infinite 200Pro				Serial number: 1009004895				Serial number of connected stacker:									
3	Firmware: V_3.14_03/10_InfiniTe (Apr 2				MAI, V_3.14_03/10_InfiniTe (Apr 26 2010/11.18.37)													
4																		
5	Date:	3/4/20																
6	Time:	1:01:05 PM																
7																		
8																		
9	System				PLATEREADER													
10	User				PlateReader\Young lab													
11	Plate				Costar 96 Flat Bottom Transparent Polystyrol Catalog No.: 3361/3590/9018/3591/9017/3641/3628/3370/2507/2509/2503/9017/9018/3641/35													
12	Plate-ID (Stacker)																	
13																		
14	Wait (Plate Temperature)				Valid Range: 35 - 39 °C													
15																		
16	Wait (Plate)				On	Target Temperature: 37 °C												
17																		
18	List of actions in this measurement script:																	
19	Kinetic																	
20	Shaking (Orbital) Duration:				3	s												
21	Shaking (Orbital) Amplitude:				2	mm												
22	Absorbance																	
23																		
24																		
25	Label: Label1																	
26	Kinetic Measurement																	
27	Kinetic Cycles				10													
28	Interval Time				0:20:00													
29	Mode				Absorbance													
30	Multiple Reads per Well (Square (filled))				2 x 2													
31	Multiple Reads per Well (Border)				1300	µm												
32	Wavelength				550	nm												
33	Bandwidth				9	nm												
34	Number of Flashes				25													
35	Settle Time				0	ms												
36	Part of Plate				A1-E12; F1-F6													
37	Start Time	3/4/2020 1:01:06 PM																
38																		
39																		
40																		
41																		
42																		
43																		
44																		
45																		
46																		

raw data | processed data | scan at t=0 | Sheet1 | +



	A	B	C	D	E	F	G	H	I	J	K
59	Cycles / Well										
60	A1	1	2	3	4	5	6	7	8	9	10
61	Time [s]	0	1200	2400	3600	4800	6000	7200	8400	9600	10800
62	Temp. [°C	37	37.1	36.8	37.4	36.8	37.2	37.1	37.1	37.1	37.2
63	Mean	0.1586	0.1605	0.1644	0.1644	0.1672	0.1676	0.169	0.1688	0.1689	0.1706
64	StDev	0.0407	0.0462	0.0488	0.0498	0.0519	0.0528	0.0542	0.0542	0.0548	0.0565
65	0;1	0.1656	0.1516	0.1581	0.1571	0.1589	0.1597	0.1607	0.1607	0.1601	0.1607
66	1;1	0.1924	0.204	0.2132	0.218	0.2231	0.2264	0.2298	0.2308	0.2321	0.2361
67	1;0	0.1765	0.1871	0.187	0.1827	0.1865	0.1841	0.1853	0.1833	0.1832	0.1854
68	0;0	0.0998	0.0994	0.0994	0.0997	0.1001	0.1001	0.1	0.1002	0.1002	0.1002
69											
70	Cycles / Well										
71	A2	1	2	3	4	5	6	7	8	9	10
72	Time [s]	0	1200	2400	3600	4800	6000	7200	8400	9600	10800
73	Temp. [°C	37	37.1	36.8	37.4	36.8	37.2	37.1	37.1	37.1	37.2
74	Mean	0.1531	0.154	0.1561	0.1564	0.1577	0.1581	0.1587	0.1579	0.1589	0.1583
75	StDev	0.0404	0.0414	0.0436	0.0445	0.0453	0.0465	0.0468	0.0464	0.0475	0.0473
76	0;1	0.1663	0.1595	0.1617	0.1607	0.1634	0.1619	0.1634	0.1648	0.1632	0.164
77	1;1	0.1895	0.1923	0.1989	0.202	0.2044	0.2077	0.2084	0.2076	0.2108	0.2103
78	1;0	0.1613	0.1687	0.1685	0.1676	0.1675	0.1672	0.1674	0.1638	0.1661	0.1636
79	0;0	0.0954	0.0953	0.0953	0.0955	0.0955	0.0955	0.0955	0.0955	0.0955	0.0954
80											
81	Cycles / Well										
82	A3	1	2	3	4	5	6	7	8	9	10
83	Time [s]	0	1200	2400	3600	4800	6000	7200	8400	9600	10800
84	Temp. [°C	37	37.1	36.8	37.4	36.8	37.2	37.1	37.1	37.1	37.2
85	Mean	0.1514	0.1539	0.1526	0.1533	0.1529	0.1531	0.1538	0.154	0.1547	0.1545
86	StDev	0.0386	0.039	0.0381	0.0386	0.0387	0.0389	0.0393	0.0395	0.0398	0.0399
87	0;1	0.1826	0.1805	0.1738	0.1748	0.1742	0.1732	0.1742	0.175	0.1738	0.174
88	1;1	0.1729	0.1746	0.1775	0.1798	0.1807	0.1826	0.1836	0.1843	0.1862	0.1867
89	1;0	0.1538	0.1642	0.1629	0.1621	0.1605	0.1603	0.1608	0.1601	0.1618	0.1604
90	0;0	0.0964	0.0964	0.0961	0.0965	0.0963	0.0965	0.0966	0.0967	0.0968	0.0968
91											
92	Cycles / Well										
93	A4	1	2	3	4	5	6	7	8	9	10
94	Time [s]	0	1200	2400	3600	4800	6000	7200	8400	9600	10800
95	Temp. [°C	37	37.1	36.8	37.4	36.8	37.2	37.1	37.1	37.1	37.2
96	Mean	0.2119	0.236	0.2654	0.2957	0.3291	0.3594	0.3775	0.3936	0.4075	0.4227
97	StDev	0.0572	0.0634	0.0527	0.0508	0.0487	0.0493	0.0471	0.046	0.0441	0.0437
98	0;1	0.2046	0.228	0.2605	0.2918	0.3261	0.3574	0.3762	0.3935	0.4068	0.4229
99	1;1	0.29	0.3072	0.335	0.3626	0.3932	0.4244	0.4391	0.4535	0.4646	0.4793
100	1;0	0.2006	0.2307	0.259	0.289	0.3222	0.3511	0.3698	0.3857	0.4013	0.4156
101	0;0	0.1524	0.1779	0.2069	0.2392	0.2748	0.3046	0.3247	0.3416	0.3573	0.3729
102											
103	Cycles / Well										

raw data | processed data | scan at t=0 | Sheet1 | +

- NOTE: your file name must not have spaces! Rename it to have no spaces (just use underscores _). This is good coding practice.

3. Your plate reader setup must be a .csv that looks like the example below.

(a) You MUST have the headers ”row..1..2….12” AND the row letters in CAPS!

(b) Name all replicates EXACTLY the same (just copy and paste)

(c) Label your media-only wells as ”Blank”

(d) Leave empty wells blank

(e) If for some reason, you scanned wells that you do not want/need to analyze, label them ”air.” The code will then know to ignore those wells.

(f) Make sure there are not any extra spaces anywhere

(g) You can make a .csv file in excel. Just save it as .csv

- Your sample names can now be whatever you want, as long as replicates are named EXACTLY the same.
- However, I would suggest you use actual sample names instead of a code.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	row	1	2	3	4	5	6	7	8	9	10	11	12
2	A	Blank	Blank	Blank	MG1655	MG1655	MG1655	N4	N4	N4	2	2	2
3	B	4	4	4	5	5	5	6	6	6	7	7	7
4	C	9	9	9	10	10	10	11	11	11	14	14	14
5	D	15	15	15	16	16	16	17	17	17	18	18	18
6	E	19	19	19	20	20	20	21	21	21	R98Q P	R98Q P	R98Q P
7	F	R98Q L	R98Q L	R98Q L	N4r- 2-1	N4r- 2-1	N4r- 2-1						
8	G												
9	H												

S3: USAGE

1. At the command line, once the script and your input files are in the same directory, you can use this code like this:

   ```
   python process_plate_reader_data.py -i <rawdata.xlsx> -p <plate_setup.
      csv> -a <active_sheet_num>
   ```

2. A complete command could look like this:

   ```
   python process_plate_reader_data.py -i 200221
      _CCM_n4_lysis_curve_testing.xlsx -p 200221_plate_setup.csv -a 1
   ```

3. There are several arguments that can be input when you call the script, but the 3 that are most important are -i, -p, and -a.

4. -i specifies the input data file

5. -p specifies the plate reader setup

6. -a specifies the sheet from your input data that has the raw data

7. **Note: computers count from 0**, so down below, I would enter 0 (actually you wouldn’t have to do anything because the default setting is the first sheet). If the second sheet had the data, I would enter

   (a)

8. Additionally, for help you can pass -h as your argument to get the list of possible args

Sheet = 0 Sheet = 1 Sheet = 2 Sheet = 3

9. Here are all the possible arguments you can pass and their descriptions:

Arg	Description
-i	input must be a .xlsx excele file from infinite200 pro plate readerl tecan i-control 1.7.1.12 software
-p	plate setup file must be structured a certain way as a .csv – see the example NOTE: replicates must be named exactly the same AND your background media samples called ”Blank” Should you have samples you don’t want analyzed or wells that were scanned but you don’t want analyzed, label them as ”air”
-a	type an integere for the sheet in your .xlsx file with the raw data NOTE: computers start counting at 0 so if your 2nd sheet has the data, type ”1” DEFAULT=0 (first sheet)
-gs	type your intended graphing program options: ”excel”, ”R” DEFAULT=”excel” ”excel” will output wide formatted data ”R” will output long formatted data for use in R with ggplot2
-pp	bool to autogenerate a python plot DEFAULT=True
-y	y-axis label DEFAULT=”OD600”
-gm	type of graphing method for python created graph options: ”time series”, ”time diff” DEFAULT=”time series” NOTE passing ”time diff” does nothing yet TODO
-pw	type an integer for number of points (readings) per well DEFAULT=4

S4: HOW DOES IT WORK

1. The code takes your plate setup file, and groups all replicates together. Then it will find the well coordinates for all data in your plate, grouping the coordinates for replicates together.

2. Then it parses all the data tables in your raw data file and matches the data table with a sample labeled in your plate setup file.

3. Then it will concatenate all data tables together for all replicates of a single experimental group. In other words, if you have 3 replicates of ”rapid_lyser_2” in wells B2, B3, and B4, it will take the data for wells B2,3, and 4, and create one data table of all 3 replicates for ”rapid_lyser_2.”

4. Then it takes the average and standard deviation of all data points for a given experimental group at each timepoint. For the tecan i-control software, the data includes a certain number of readings per well, and then that number is multiplied by the number of replicates. The default readings per

well number is 4, and if you have 3 replicates, that equals 12 data points for each group at each timepoint.

5. The average background signal at each timepoint is then subtracted from the average signal at the corresponding timepoint for each experimental group. This value is what is present in the final output. Additionally, the unchanged standard deviation is also reported in the final output because it will not change by subtracting background signal. (See statistic distribution theory)

6. For the time series plotting, it will plot the relative time (hours) on the x-axis, and whatever units your measurements are in on the y-axis. For each point, it will also graph the standard error of the mean OD ± SEM. at that timepoint. SEM = standard dev / sqrt(n), where n is the number of replicates * number of readings per well.

7. The outputs of this code are a `datafile_PROCESSED.csv` file and a `datafile_PROCESSED.pdf` file. The .csv file contains for all experimental groups, the average signal (with background subtracted) and standard deviation at each timepoint, as well as the time in hours. The .pdf is a python plot.