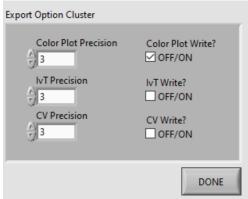
## Instructions for "The SSIM Method" for FSCV Adenosine Data Analysis.

Updated: March 11, 2020 by Pumidech Puthongkham

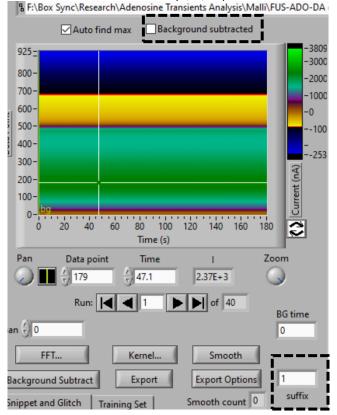
The SSIM Method was developed in MATLAB 2019b. Make sure that your MATLAB also has the "Signal Processing Toolbox," which can be added when you installed MATLAB. The software can be downloaded from <a href="https://github.com/maxchem6/imgADanalysis">https://github.com/maxchem6/imgADanalysis</a>. Please download the whole folder, then extract it to have every file in the same folder.

To analyze the adenosine FSCV data...

1. Open HDCV Analysis, then open .hdcv data file. Click "Export Options" and check only "Color Plot Write." Click Done.



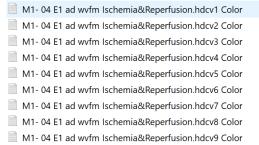
2. Uncheck "Background subtracted." Put file number (1, 2, ...) in "suffix" box. Then click "Export."



3. Open MATLAB. Click "Browse for folder" icon, and choose the program folder.



- 4. For adenosine data, double-click "imageFSCVAnalysis.m" and run.
- 5. In the Command Window, the program will ask if you want to build you own reference ("internal references") or use the "standard library."
  - For "internal reference," type 1 and Enter. Then, put the file number and rise time (not peak time!) for six transient adenosine events from your data. (i.e. you have to quickly go through your data before using the program) Wide range of concentration and duration is recommended: two large (> 0.25 μM), two medium (0.1-0.25 μM), and two small (< 0.1 μM) transient events.
  - For "standard library," type 2 and Enter.
- 6. Type the calibration factor (peak current for 1 uM adenosine) and Enter.
- 7. Choose the FSCV .hdcv Color files that you exported. They do not have to be in the same folder as the program, but the file number (suffix) must be in the correct order. Click "Open."



- 8. The program should analyze the data now. It takes 5-7 min per 1 h of experiment (20 3-min files).
- 9. Once finished, the program will save the analysis result in the Excel file named "adenosineResult\_YYYY-MM-DD\_HHRR.xlsx" in the program folder.
- 10. To read the Excel file, open it from Windows folder directly, or Right-click in the MATLAB and choose "Open Outside MATLAB."

File	Seed_s	SSIM	PeakTime_s	Curr_nA	Conc_uM	HalfWidth_s	Noise3_nA	PassSN	InterTime_s
1	82.1	0.632591966	82.7	1.547586531	0.051586218	1.280772929	0.65447738	1	82.7
2	6.7	0.668964949	7.2	3.204680983	0.106822699	1.264166879	0.659310528	1	104.5
3	92.9	0.646278721	94	2.163939384	0.072131313	1.768712233	0.600552293	1	266.8
3	137.9	0.704180864	138.5	1.686710291	0.056223676	0.679213616	0.600552293	1	44.5
4	133.5	0.729573787	134.2	3.474531339	0.115817711	1.192998001	0.656324034	1	175.7
6	160.1	0.522474481	160.9	4.192193938	0.139739798	1.122563041	0.652724363	1	386.7
7	37.3	0.76072036	38.4	10.83839901	0.361279967	2.022920421	0.767097556	1	57.5
7	161.7	0.529184729	162.8	11.02037171	0.367345724	1.34958351	0.767097556	1	124.4
7	174.3	0.574332626	175	2.051838702	0.068394623	1.9	0.767097556	1	12.2
8	173.9	0.717474447	175.2	30.69272541	1.023090847	3.028352762	0.841140541	1	180.2
10	23.3	0.641109567	23.4	1.145837824	0.038194594	0.8	0.623876813	1	208.2
10	91.9	0.603762419	93	1.894818991	0.063160633	1.081875219	0.623876813	1	69.6
10	120.9	0.524553297	121.7	1.797913529	0.059930451	1.688346988	0.623876813	1	28.7
11	12.1	0.510571292	12.9	1.977133396	0.065904447	0.976669761	0.664353486	1	71.2

Columns: A. file number, B. seeding time (s), C. SSIM index, D. peak time (s), E. current (nA), F. concentration (uM), G. peak duration (s), H. S/N=3 level (nA), I. Pass S/N?, and J. inter-event time (s). May need only column F, G, and J for your experiment.