**READ ME.**

**MATLAB File Name:** Piezo1\_mSMC\_Calcium\_Influx

**MATLAB version:** Matlab2020a (or more recent)

**Programming language**: MATLAB

The code described in this document should be used for research purposes only and is currently available through the following links.

* <https://github.com/ydu1955/Piezo1_mSMC_Calcium-Influx/tree/MATLAB_Piezo1_YD>
* DOI: 10.5281/zenodo.6282081 (MIT License)

**Installation and use instructions:**

This code was written using a student license for MATLAB 2020a and installation instructions can be found through MathWorks website. The Symbolic Math Toolbox will also need to be downloaded after MATLAB is installed. The file can then be added to MATLAB pathway and opened in editor for use.

Green line commentary text is available for each section of code and variable that needs to be adjusted/changed both at the beginning of every section and for relevant lines. The file referenced by the code must be in “.txt” format and data sorted in the first 2 columns without labels, time and intensity, respectively. Crucial lines to note are listed below.

* The text file’s name must be changed in lines 18-20
* Line 53 for baseline calculation and line 54 for manual input (remember to disable line 54 if you decide to calculate baseline).
* Line 55 to adjust datapoint detection for peak bases (typically between 0-0.1)
* Line 65 to adjust cutoff multiplier to determine threshold for peaks (typically a value between 1.0-1.5)

When everything is ready, the calculated variables and arrays for the time and intensity can be exported as an “.xls” file. Column and sheet number can be changed as needed. There are no labels in exported file, reference Lines 219-229 from MATLAB code for column labels.

Please note, this code has not been submitted for community commenting and input.

**Description:**

The key operations of the code are identifying peaks in mSMC calcium influx signaling. First, the baseline is identified through manual input or by calculating the lowest average after dividing the curve into small segments, see Installation/use instructions for more details. Next, a multiplier is used to mark a threshold where data points above this threshold are filtered using loops to find peaks. Datapoints left and right of peak datapoint are filtered using loop conditions to estimate where peak bases are. Peaks, peak bases, and baselines are graphed in a figure for confirmation. All data points are saved in separate arrays where they can be used to calculate the intensity change, time between calcium spikes, time duration of peaks, frequency, and roughly estimate the area under the curve. This code is best used for calcium curves with low background noise and relatively cleaner curves. Messier curves or curves with sloping baselines might require manual deletion/manipulation of data points from the original curve without biasing the final calculated variables.

**Example Data**

The following is a sample of our mSMC PiezoWT calcium influx signal data with a figure to demonstrate the primary function of the code. Numerical values calculated based on the arrays generated (visualized in the figure) are exported in a separate “.xls” file.

File name: Example\_mSMCs\_WT\_04.txt (included below)

Adjusted Lines:

* **Lines 18-20:** Example\_mSMCs\_WT\_04.txt
* **Line 54:** 556
* **Line 55:** 0.03
* **Line 65:** 1.255

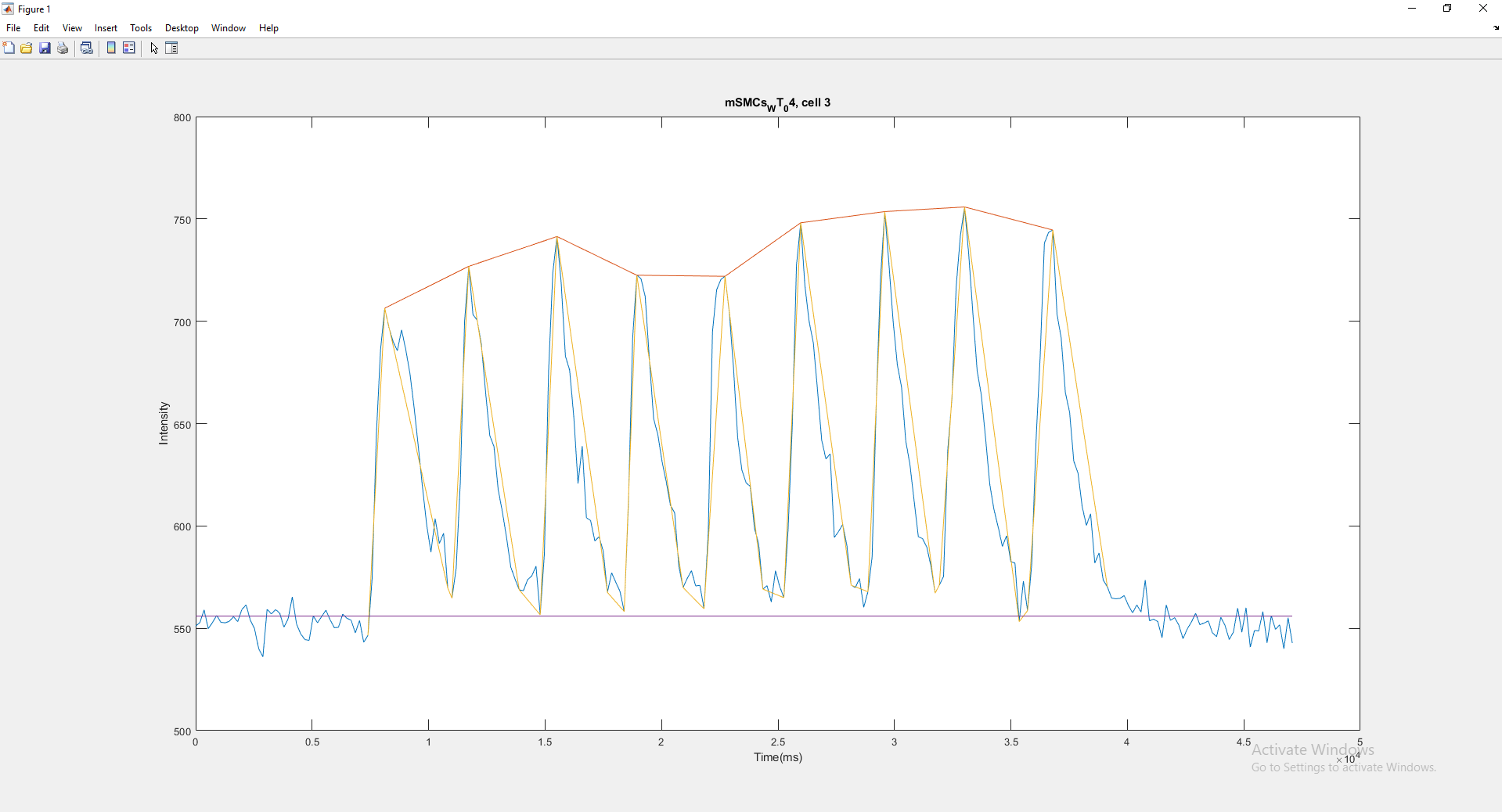
**Figure:**

**Blue Curve:** Original curve exported from zeiss software

**Purple Curve:** Baseline

**Orange Curve:** Identified peak datapoints

**Yellow Curve:** Identified peak and peak base datapoints



Example\_mSMCs\_WT\_04.txt

0 551.0967742

180.0103 552.7741935

361.0206 558.9677419

541.0309 549.8709677

721.0412 552.8064516

902.0516 556.2903226

1082.0619 552.9354839

1262.0722 552.6774194

1443.0825 553.3870968

1623.0928 555.6451613

1804.1032 553.2903226

1984.1135 559.1935484

2164.1238 561.4516129

2345.1341 553.9677419

2525.1444 549.8709677

2705.1547 540.0322581

2886.1651 536.0967742

3066.1754 559.2580645

3247.1857 557.0322581

3427.196 559.1290323

3607.2063 557.4193548

3788.2167 550.516129

3968.227 554.5806452

4148.2373 565.3548387

4329.2476 552.1290323

4509.2579 547.2258065

4689.2682 544.483871

4870.2786 544.1290323

5050.2889 556.0322581

5231.2992 552.6774194

5411.3095 555.8064516

5591.3198 558.8387097

5772.3302 554.1290323

5952.3404 550.2903226

6132.3507 550.483871

6313.3611 556.9354839

6493.3714 554.9032258

6673.3817 553.9677419

6854.392 547.7419355

7034.4023 553.7741935

7215.4127 543.1612903

7395.423 546.5483871

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7756.4436 644.3870968

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9920.5674 600.1935484

10100.5777 587.1935484

10281.5881 603.5806452

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11363.65 621.9032258

11543.6603 699.6129032

11724.6706 726.8387097

11904.6809 703.2580645

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12445.7118 665.4516129

12626.7222 644.1612903

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13167.7531 606.8387097

13347.7634 594.2258065

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13888.7944 568.8387097

14068.8047 568.3225806

14249.815 573.7419355

14429.8253 575.6129032

14610.8357 580.3225806

14790.846 556.5806452

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27958.5991 590.4193548

28138.6094 571.0645161

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28499.6301 574.2580645

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