

Supplementary File 5

DATA PROCESSING USING MATLAB COMMAND-LINE INTERFACE:

1. Download the MATLAB programs for NPS data analysis:
<https://github.com/sohnlab/NPS-analysis-JOVE>.
2. Tune preprocessing parameters using mNPS_fastQC
 1. This program enables visualization of datasets for the purposes of tuning preprocessing parameters: ASLS_param, N
 2. ASLS_param are used to create a baseline fit using asymmetric least-squares smoothing²⁶.
 1. Parameters should be chosen to enable close fitting to the current signal when no pulses are present, but no visible “dip” in baseline across an entire cell event.
 3. N specifies the downsampling rate and should only be >1 if the sample rate is >10,000 samples/s
3. Note the information passed as arguments to mNPS_procJOVE
 1. Device/data info: ch_height: channel height, De_np: D_e , wC: contraction channel width, sampleRate
 2. Processing parameters: [thresholds], [ASLS_param], [eventlength_filt]
 1. thresholds: a two column vector including a (column 1) “top” and (column 2) “bottom” threshold. Thresholds are used to identify local minima and maxima in the difference signal (**Figure 3B**), and can be adjusted for each cell event during analysis if needed.
 1. The “bottom” threshold should be less than the local minima and maxima due to sub-pulses in the sizing and recovery pores, but greater than background noise.
 2. The “top” threshold should be greater than the local minima and maxima due to sub-pulses in the sizing and recovery pores, but less than the local minima and maxima due to the contraction pore.
 2. ASLS_param: should be set to values chosen in Step 2
4. Note the parameters that have to be hard-coded in mNPS_readJOVE
 1. In “SECTION 0: device parameters” of the code parameters will need to be adjusted given different device geometry
 1. “segment layout”: total_segs: total number of pores, num_ref_segs: number of sizing pores, num_rec_segs: number of recovery pores
 2. “mask geometry”: L: overall channel length (excluding reservoirs), npL_ref: lengths of each sizing pore (as a vector), npL_rec: lengths of each recovery pore (as a vector), sql: length of the contraction pore, wNP: width of the sizing and recovery pores
 3. “SECTION 1: load data and perform basic signal conditioning” set N: downsample factor