ImageJ Macro code supporting: Cheng, Y-W, Anzell, AR, Morosky, SA, Schwartze, TA, Hinck, CS, Hinck, AP, Roman, BL and Davidson, LA (2024) 'Shear stress and sub-femtomolar levels of ligand synergize to activate ALK1 signaling in endothelial cells', (In revision) : 2024.

The objective of this analysis is to provide an unbiased way to analyze signaling through ALK1, a TGF-beta type receptor expressed on human umbilical endothelial cells (HUVECs) and other cultured endothelial cells. Once a receptor is activated it will phosphorylate SMAD, a transcription factor present in the cytoplasm. Phospho-SMAD, i.e. pSMAD, then moves into the nucleus where it may drive gene expression changes. This ImageJ Macro calculates the intensity of pSMAD in the cytoplasm and in the nucleus, and exports those values in a spreadsheet for later numerical and statistical analysis.

This repository includes the ImageJ macro ("ASI Quantitation Macro.ijm") and a sample XYZC image ("Sample Image Stack.tif") to quantify levels of intracellular signaling from two-channel confocal stacks. There are two channels in this image stack, one from fluorescence from DAPI to identify the nucleus, and one from immunofluorescence staining showing the presence of pSMAD.

To run this Macro, open ImageJ (version 1.54f or later). Install the macro. Press [1], [2], [3], [4] to complete image analysis and generate two csv files.

(Note A) After [1], you may find nuclei are not correctly identified. In this case, before continuing you may adjust the threshold levels. Use the same threshold levels when identifying nuclei in samples from the same experimental run or staining procedure. These adjustments should be made before proceeding to [2].

(Note B) After [2], you may find regions-of-interest (ROI) of overlapping nuclei or defective staining regions. These should be removed manually from ROI manager before proceeding with [3]. The sample stack contains regions where there are overlapping nuclei in the DAPI channel and a defective straining region in the pSMAD channel.

(Note C) The mean and median of the pSMAD intensity in ROIs from nuclei and adjacent cytoplasm can be found in csv files after [4].