**fSummary of how the code works:**

**recursiveOSAnalysis** search for all the movies in the directory and runs **OSAnalysis** in each of them using the set parameters (numSteps, startFrequency, endFrequency, baselineTime, peakDistance, minPeakProminence, minMinProminence, minMinWidth). After the analysis is complete it runs **OSPostAnalysis** and executes **compileData.**

You can change the number of workers for parallel processing in this function

**OSAnalysis**

1. Checks if the movie has already been analyzed
2. If not (or if the reAnalize flag is on (flag1 from **recursiveOSAnalysis**), it analyzes it
   1. **loadData** loads the movieStack (this is the part of the analysis that takes longer)
   2. **getOSProtocol** Calculates the light trace using the stimulation parameters
   3. **cutVideoToOS** cuts the movieStack to the length of the stimulation. *The light stimulations starts with 5 seconds of darkness, followed by 5 seconds of red light ON, and then the stim happends. The code finds the moment the red light turns ON, and sets that as the beginning of the movie, then calculates the time the stimulation is ending and sets that as the end of the movie.*
   4. **getOSContractilityTrace** generates the contractility trace for the tissue, comparing to a baseline frame (baselineTime)
   5. **getPeakHeightThresholdsOS** calculates the threshold for what it will be counted as a peak. Right now this is set as at least 25% of the maximum of the trace. I find this to work well, except in some rare occasions when tissues are not contracting. This parameter can be adjusted in the Post Analysis (see below).
   6. **Saves all the info generated to this point into an output struck so the movies can be reanalyzed without needing to load the movieStack again**
   7. **analyzeTraceOS** finds the peaks and the minima of the contractility trace (the minima are used to calculate the start point of the contraction)
   8. **matchPulsesToOSContractions** correlates the contractions with the light stimulation. Briefly, it counts as triggered contractions those that start in the 200 ms interval after a light pulse (**maxMatchLength**). This parameter can only be changed inside this function. If it is too big, more contractions will be counted as triggered, but the probability of contractions happening in that interval will also be much higher so a higher correction will be applied. I find 200 to be a good balance. Calculates the score of the tissue and saves it.
3. **Labels the movie as analyzed so it won’t be analyzed again unless the reanalyze flag is set to 1**

**OSPostAnalysis.** This function displays to you the result of the analysis using the preset parameters and asks if the analysis was correct. If is not, it gives you 3 options

1. **Reset the baseline frame.**
2. **Change the height threshold.**
3. **Change the analysis parameters.**

This function is recursive so it will keep you asking what you want to change until you select nothing (introducing 0).

Once you’re done it labels the move as postanalyzed so it won’t give you the option to run the postanalysis again unless you set the second flag to 1.

**compileData.** Generates a single excel file with the results for all the tissues.

**How to use the code:**

1. Name your files as: day\_(condition)\_plate\_tissue\_experiment
2. Open recursiveOSAnalysis
   1. Adjust:
      1. Stimulation parameters according to the protocol used while imaging the tissues (numSteps, startFrequency, endFrequency)
      2. Initial analysis parameters
         1. baselineTime: this is the time point used as baseline for calculating the contraction, so you need to pick it while the muscle is resting. I normally choose 0.1 seconds and works but sometimes there’s an spontaneous contraction happening and you need to change it. This is one of the parameters you can adjust during the reanalysis (see below)
         2. peakDistance: how close peaks can be. It’s not a very important paramenter but it helps getting rid of noise sometimes.
         3. **minPeakProminence**: how large the contractions need to be for you to count them. The code is very sensitive and can pick up small contractions but sometimes you want to only count bigger contractions, for example, only the ones that result in pillar bending. For these tissues I have been using 100 or 150 but it’s not unusual for me to adjust this parameter later once I have made the movies and see what I am picking up as contractions. This parameter can be adjusted during the reanalysis but I do not like using different thresholds for different tissues so I rarely change it then (instead I will redo the whole analysis with a new value from the beginning). *Note: due to how the code works, there is some self-correction happening even when you change this parameter: if you choose a small prominence threshold the code will pick up tons of contractions but also calculate a high probability of random contractions happening during light pulses, so the score will be reduced when applying the correction. If you only pick up large contractions most likely you won’t be picking up the spontaneous activity and the total number of contractions will be low, so the correction to the score will be minimal.*
         4. minMinProminence and minMinWidth. The same but for the minima instead of the peaks.
3. Run recursiveOSAnalysis(DataDirectory, flag1, flag2). When both flags are 0, it would only analyze and postanalyzed movies that haven’t been analyzed before. You can use the first flag to force the reanalysis of the movies (e.g. if you have changed some parameter in the code), and the second flag to re run the postanalysis only (this is not that common unless you made a mistake while running the postanalysis and want to fix it).

When is done running it will show you the graphs generated for each tissue and ask if the analysis is correct. Hit Yes if it looks correct or No if it doesn’t, and the 1,2 or 3 depending on what you want to change.

1. **Reset the baseline frame.** You do this when the trace looks incorrect (inverted peaks). This will reload the movie and start the analysis from the beginning.
2. **Change the height threshold.** This lets you manually select the threshold.I do this when (1) some peaks are not being detected when it looks like they are the right size (this happens rarely) or (2) the tissue is clearly non responsive but some random things are being picked up as peaks. In that case I just set the new threshold higher than the trace.
3. **Change the analysis parameters.** This is the most frequent case of adjustments that need to be made. It’s quite frequent that the minimum is misidentified and results to happen before the light pulse, which leads to the peak to not be counted as triggered. To help with this when you run this option it will show you the peaks (x) and minima (circles). To fix this you reduce the minMinProminence and minMinWidth. If you reduce those parameters too much the minima will be labeled close to the peaks and also result in the peak not being counted (too far from the light pulse).

At the end of this you’d have an excel file named **CompileData**

1. Generate graphs from the **CompileData** file using **barGraph1var** or **barGraph2var**. barGraph1var makes bar graph and calculates statistics for just one variable (first parameter from the name of your file which is the first column of the excel file). barGraph2var makes bar graph and calculates statistics for 2 variables (first 2 parameters from the name of your file which are the 2 firsts column of the excel file). The function will let you chose the response variable, which in this case will be the **score**. This function saves the graphs both as pictures and as Matlab files so you can modify the colors, etc later.
2. (Optional) Run recursiveOSMovie to generate movies with the trace. This takes very long and I normally only do it for selected movies that I want to show in a presentation, etc. It uses the results from the analysis to generate the movies.