**CELL ANALYSIS PROTOCOL – UPDATED ON 08/14/2017**

1. First run extract\_cells\_withsegmentation  
   Note that this only runs on mat files generated from cell cropping and alignment for Crop\_Align\_Cell. This step list all mat files in current folder and runs cellfind\_multijumps\_greenthresh\_minimization. The code thresholds green channel and picks put pixels that stay above the threshold throughout the video. No user input required other than going to said folder at start of code or making a list of all folders to run this code on.
2. Then run findjumps\_withsegmentedcells  
   This will further analyze \*cells.mat generated by step 1. This code runs findjumps\_multijumps that picks jump positions and assigns the full trace to specific jumps for further analysis. User will need to pick peaks/enter value for auto peak recognition. User then approves jump picking. The code generates split trace variables jump\_traces\_ (green, red, da and time) and appends them to the said \*cells.mat workspace.
3. Start = 1; append\_cellsize\_cellmat
4. Initialize cell “folders” with folder names of folders containing desired folders with cells to average. For example, to average all cells with mHSP and mEGFP-PGK3 distributed over three days; folders may contain all such folders with these cells to be averaged.
5. Finally run Compile\_all\_cells(folders) which will then allow the user to look at traces and select them to either be averaged or excluded from averaging. A line is drawn through the raw traces using “pchip” interpolation between 20 to 46 C with a 2 C temperature step. The traces are normalized and corrected for quantum yield slope by fitting the four starting points to a line and subtracting the slope. Finally these traces are averaged.

**CODE DESCRIPTIONS**

1. **cellfind\_multijumps\_segmentation\_greenthresh\_minimization\_wtemp**Thresholds green channel, does pixel minimization that keeps only those pixels that stay bright up to 46 C. Needs jump.xls
2. **cellfind\_multijumps\_segmentation\_greenthresh\_minimization**Thresholds green channel, does pixel minimization that keeps only those pixels that stay bright up to highest temperature point, ~47 for small jumps and 52 for big jumps
3. **Compile\_all\_cells**Final step for analysis. Calculates da and averages for different PGK types and implements interpolation
4. **findjumps\_withsegmentedcells**Script runs findjumps multijumps on workspaces created by cellfind. **cellfind\_multijumps\_withsegmentation\_greenchannel**Thresholds green channel
5. **findjumps\_multijumps**Pick jumps and split full trace into individual temperature points
6. **extract\_cells\_withsegmentation**Script runs cellfind on a folder. Needs jump.xls
7. **cellfind\_multijumps\_withsegmentation**Thresholds red channel.
8. **cellfind\_multijumps\_withsegmentation\_greenchannel\_mini2\_SS**Minimize the threshold to always include set number of pixels in all frames
9. **cellfind\_multijumps\_withsegmentation\_greenchannel\_mini2\_SS2**
10. **cellfind\_multijumps\_withsegmentation\_redchannel\_hist\_thresh**Thresholds red channel frame-by-frame with histogram thresholding method. **<**thresh(j,i) = floor(GaussB.b1 - (0.5\*(GaussB.c1/sqrt(2))));>
11. **cellfind\_multijumps\_withsegmentation\_redchannel\_hist\_thresh2**Thresholds red channel frame-by-frame with histogram thresholding method. Try minimization of gaussian threshold cutoff to keep pixel included constant.
12. **cellfind\_multijumps\_withsegmentation\_redchannel\_hist\_thresh\_sfr**Thresholds red channel with histogram thresholding method. However, threshold only one frame and use the mask from thresholding on all other frames. Iteratively removes very dark pixels from masked frames.
13. **cellfind\_multijumps\_withsegmentation\_redchannel\_hist\_thresh\_sf2**Thresholds red channel with histogram thresholding method. However, threshold only one frame and use the mask from thresholding on all other frames.
14. **cellfind\_multijumps\_withsegmentation\_rchannel\_hist\_thresh\_jump2**Threshold a frame every jump. frames\_to\_thresh = [1;120;600;1080;1560;2040;2520;3000;3480;3960;4440]; Use threshold for all frames in the jump
15. **cellfind\_multijumps\_withsegmentation\_rchannel\_hist\_thresh\_sjump**Threshold a frame every jump. Use the mask from thresholding on other frames in the jump