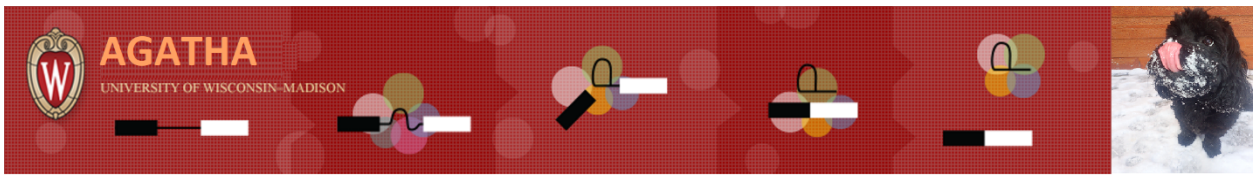


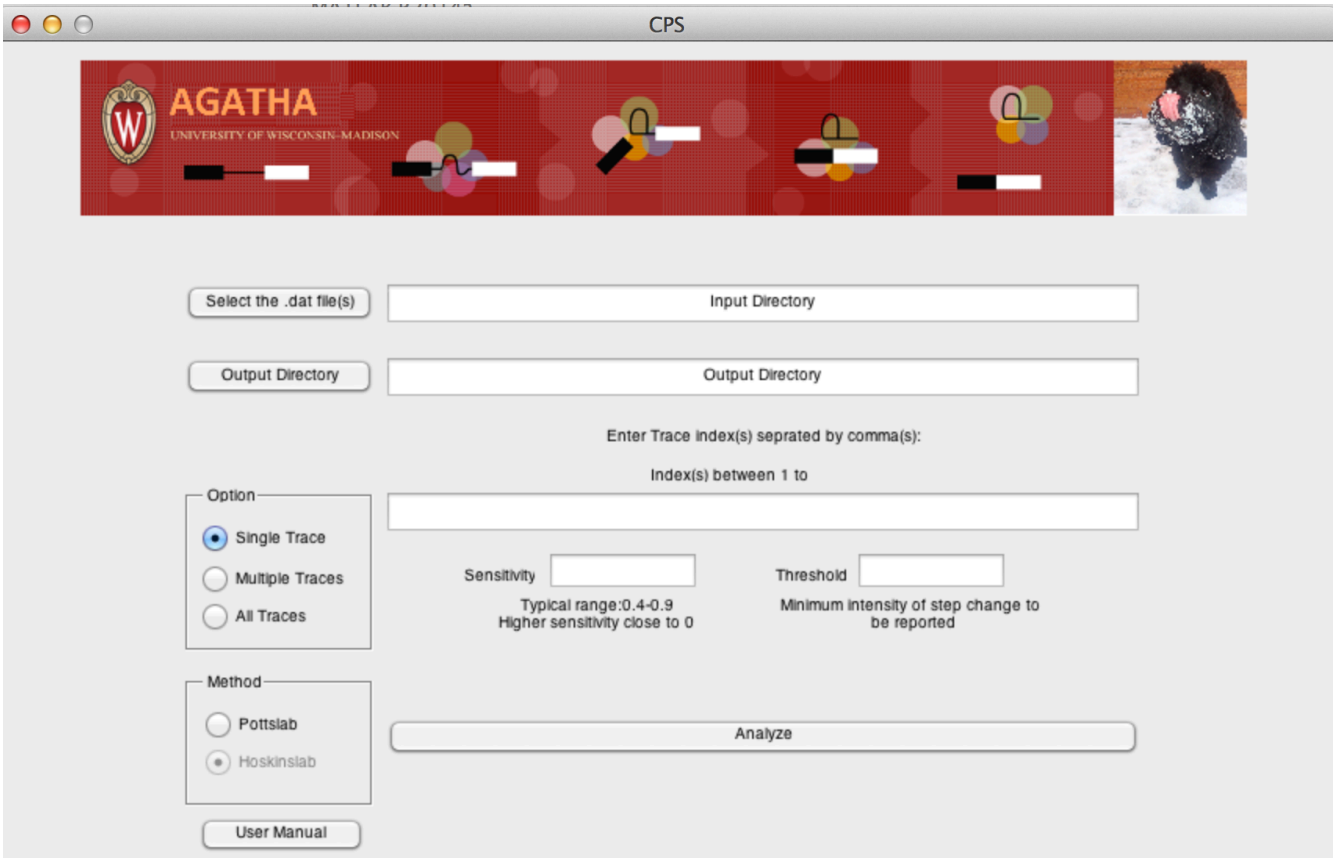
User Manual
Counting Photobleaching Steps

CPS is a program that detects stepwise changes in single-molecule TIRF experiments based on the Pottslab toolbox (https://github.com/mstorath/Pottslab[1,2,3]

Input data: a series of ".dat" files from the GLIMPSE-Imscroll program.
Output data: a set of annotated fluorescense intensity traces and a text file detailing the number of steps in each trace

Instructions:

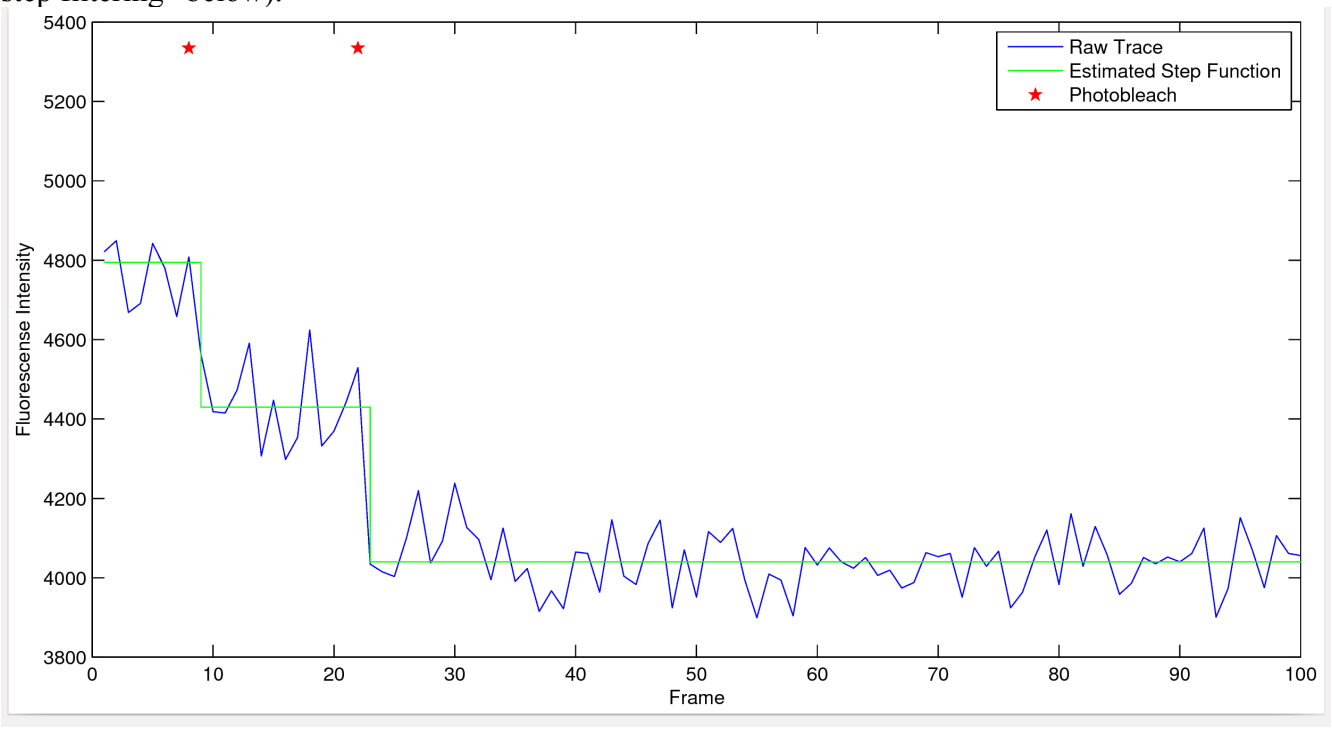
1. Download CPS.zip and extract the folder to a convenient location.
2. Open CPS.m in MATLAB and run the script. The CPS window should open.



3. Press the "Select the .dat file(s)" button. A file explorer window should pop up.
4. Select the .dat file or files you wish to analyze. Multiple .dat files in the same folder can be selected.

4a. If only one .dat file is selected, you have the option to specify individual traces to analyze rather than the full set. Type in the indices of the traces you wish to analyze in the text box.

5. Press the “Output Directory” button. A file explorer window should pop up.
6. Select the directory you wish to save the data to. One subfolder for each .dat file will be created here for the output data.
7. Press the “Analyze” button.
8. Look at the results in the output folder. Each folder contains a series of images corresponding to the analyzed traces. The raw fluorescence data is shown in blue and the fitted step function identified by Pottslab is shown in green. Photobleaching steps are identified above the trace as red stars (for more information on what constitutes a photobleaching step, see “Technical aspects of step detection and step filtering” below).



The data is summarized in “datfile_steps.tsv”, which contains the following columns:

Column Header	**Explanation**
traceIDs	The trace ID (the same name as the associated image file)
x	The x-coordinate of the spot from the field of view
y	The y-coordinate of the spot from the field of view
numAllSteps	The total number of steps identified in the trace
numBigSteps	The number of steps in the trace that were larger than the root mean square deviation between the data and the step function
numPhotobleaches	The number of steps identified as photobleaching events

9. Plot the number of photobleaching steps as a histogram to infer stoichiometry.
 is a text file that contains the trace ID, the x-y coordinates for the area of interest, the total number of steps identified by Pottslab, the

Technical aspects of step detection and step filtering:

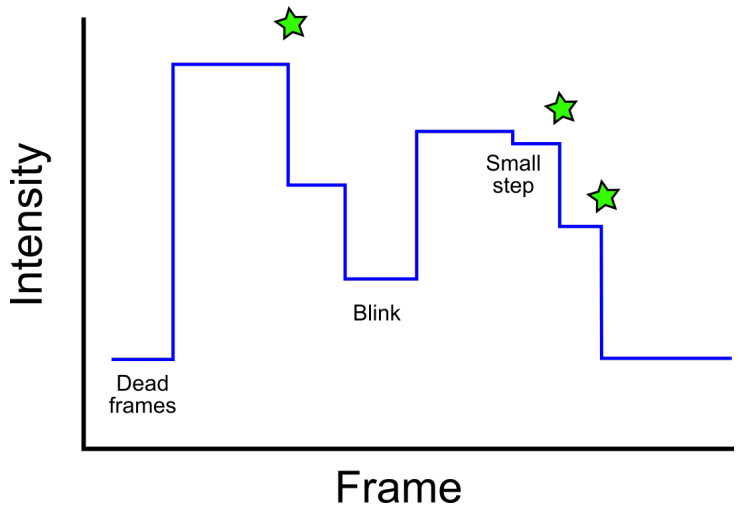
Fluorescense intensity traces are extracted from the aoifits table from the .dat file. These traces are then normalized so that the maximum value is 1 and the minimum value is 0. This normalized vector is then analyzed by the minL1Potts function using the default sensitivity value. The output (a piecewise constant vector corresponding to the step function fit) is then filtered for artifacts to detect "true" photobleaching events.

First, very small steps that are unlikely to correspond to photobleaching events are ignored. The threshold is currently defined as the root mean square deviation between the data and the computed step function; steps smaller than this threshold are ignored.

Next, stepwise changes corresponding to bleaching events are extracted. The Potsslab algorithm identifies both upward and downward steps in the fitted data. For photobleaching analysis, only downward steps that are not accompanied by upward steps correspond to bleaching events. The photobleaching events are identified using a fairly simple heuristic:

*Photobleaching steps must be downward.
*Blinking events (a downward step accompanied by an upward step) are ignored.

The filtering process is schematically illustrated below. Changes accompanied by a star correspond to the photobleaching steps identified by this filtering process. Note that currently, the magnitude of the intensity change is ignored for photobleach detection purposes except in the case of very small steps, as noted above.



Total steps: 7
Big steps: 6
Photobleaching steps: 3

REFERENCE:

1. Storath, Martin, Andreas Weinmann, and Laurent Demaret. "Jump-sparse and sparse recovery using Potts functionals." *IEEE Transactions on Signal Processing* 62.14 (2014): 3654-3666.
2. Weinmann, Andreas, Martin Storath, and Laurent Demaret. "The L^1-Potts Functional for Robust Jump-Sparse Reconstruction." *SIAM Journal on Numerical Analysis* 53.1 (2015): 644-673.

3. Weinmann, Andreas, and Martin Storath. "Iterative Potts and Blake–Zisserman minimization for the recovery of functions with discontinuities from indirect measurements." *Proc. R. Soc. A*. Vol. 471. No. 2176. The Royal Society, 2015.