Threshold an image based on the gamma distribution fitting its histogram

GammaFitting fits an image stack with gamma function and masks the image to include only those pixels whose value exceeds a given a mount of times the standard deviation of the gamma fitting function.

This program is especially useful to segment punctate labeling in immunofluorescence confocal images (i.e. synaptic proteins).

Follows the procedure published in Note5/Fig.10 of the book chapter:

> Using Fluorescent Markers to Estimate Synaptic Connectivity In Situ. Hoon M, Sinha R, Okawa H. Methods Mol Biol. 2017;1538:293-320.
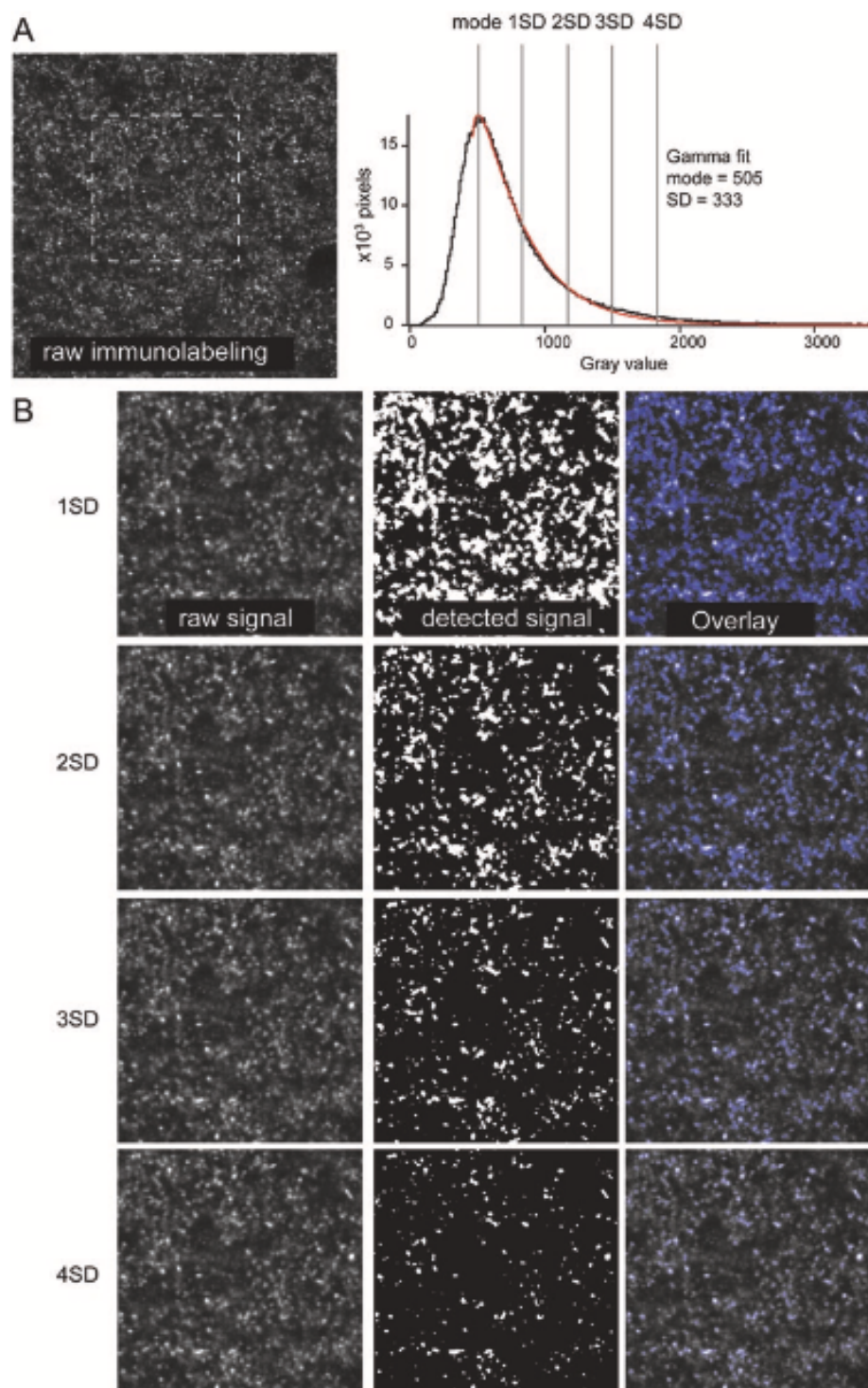
Fig. 10 Determining the threshold during synaptic protein volume estimation analysis. (**a**) Raw image of a synaptic protein immunolabeling (*left*). All the pixels of the image can be plotted against the spread of the gray value intensities (*right*). This plot can be fitted with a gamma function (*red curve*) to determine the value of the noise peak (mode of the distribution) and the values of the standard deviation (SD) above the noise peak. (**b**) Example of the raw signal (*dotted box* in **a**) before and after the application of increasing thresholds. The middle panel illustrates the binarized accepted pixels. Note that at lower thresholds set at 1 SD above the noise peak a lot of the background signal gets erroneously included

In addition, users choose whether a single SD threshold is selected and applied to the entire image stack or individual thresholds will be calculated for each Z-plane of the image stack. The latter allows to compensate for changes in the noise distribution due to uneven antibody penetration in the tissue or laser power correction applied during image acquisition. The distribution of applied thresholds is plotted as a function of Z-position in the image stack. The raw fitted values are smoothed (red line) to allow for errors of the nonlinear fitting estimators.

- If only one threshold is applied to the entire image, the output will be a distribution of the pixel intensities, with overlayed the gamma fittinng curved used to calculate the threshold value
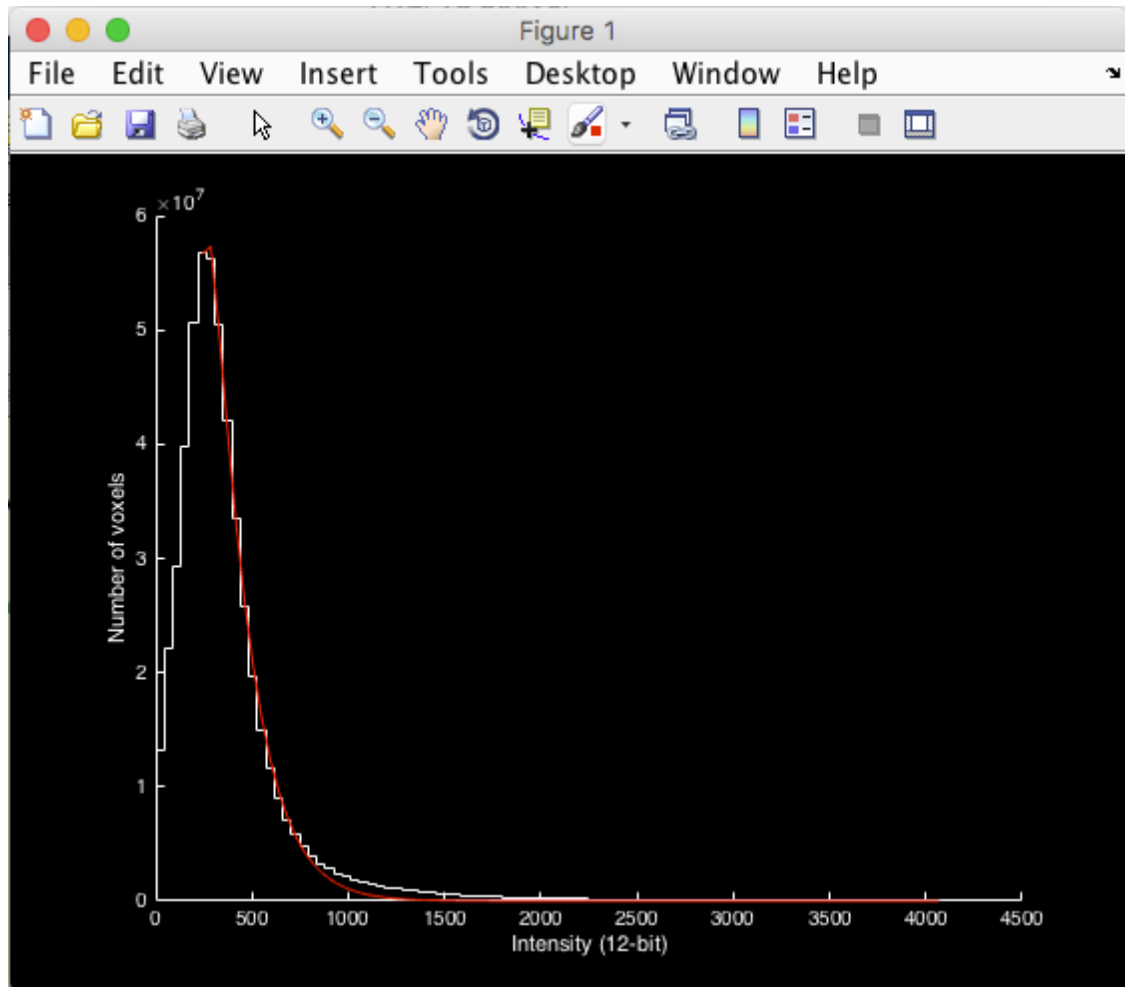
- If multiple thresholds are calculated, the output will be the distribution of threshold values as a function of depth in the volume

Distribution of thresholds across z-axis, blue=original fit, red=smoothed fit)
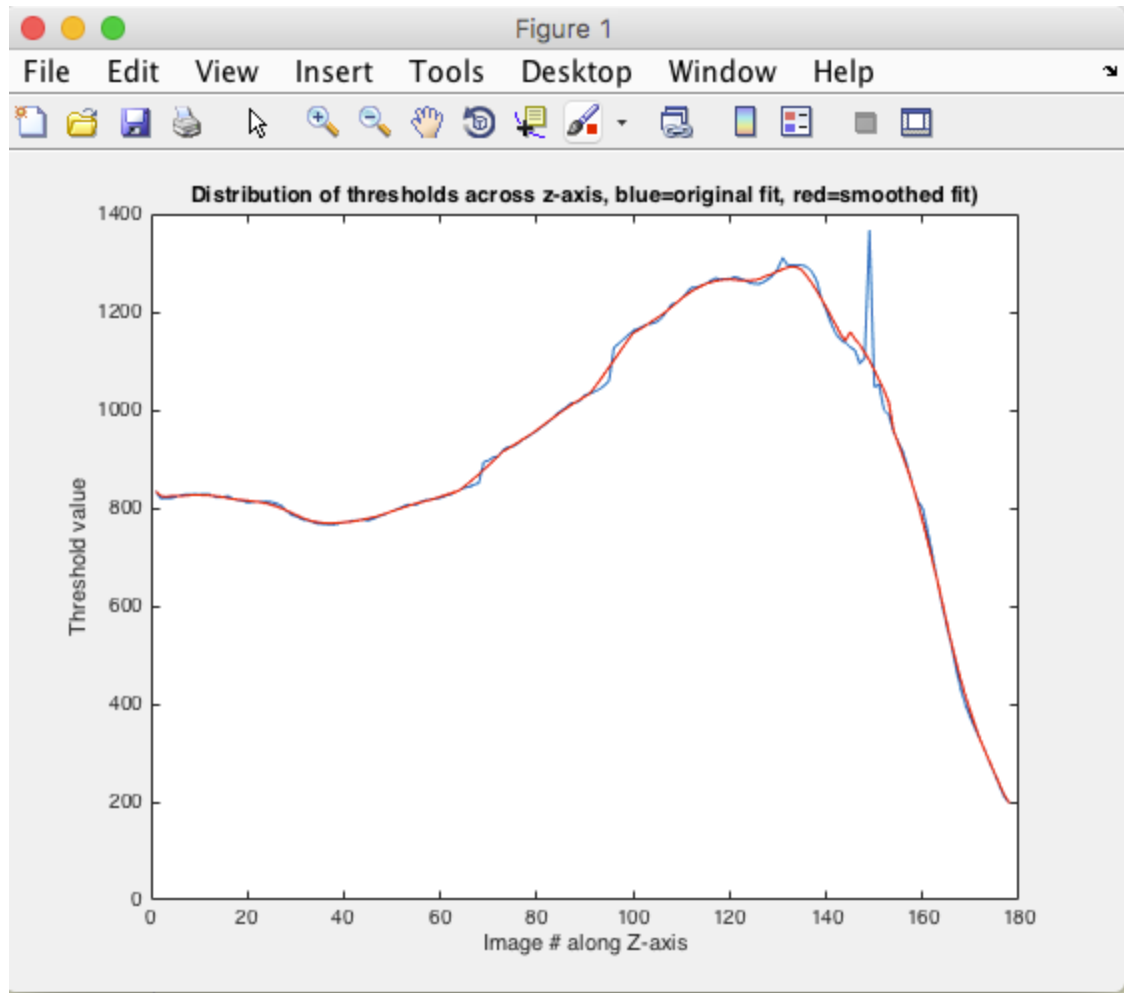
Input:

- Source signam image (single-channel TIF image stack)

Output:

- Binary mask image (single-channel TIF image stack)

Dependencies:

- textprogressbar.m

Change log

Version 2.5.1 *created on 2017-09-08 by Luca Della Santina*

```
+ Reformatted documentation using proper MATLAB markup format
```

Version 2.5 *created on 2017-07-21 by Luca Della Santina*

```
+ Allows inserting multiple SD threshold values to test which is best
% Output plot in single threshold shows properly scaled x-axis
```

Version 2.4 *created on 2017-05-04 by Luca Della Santina*

```
% Image histogram distribution is smoothed (kern=3) before gamma fit
   to prevent problem with images containing a lot of black voxels
% Single-gamma mode: Threshold is plotted (blue line) on the final plot
```

Version 2.3 *created on 2017-04-08 by Luca Della Santina*

```
% Changed smoothing of the threshold curve from default moving averate
  To the robust 2nd order polinomial rloess to remove outliers
  happening when an error occurs during fitting
% Fixed typo in debug output text
```

Version 2.2 *created on 2017-04-06 by Luca Della Santina*

```
+ Errors during fitting loop are reported at the end with z-plane #
% Removed gamma function amplitude and start x from nlinfit beta0
  as these parameters can be precisely calculated from the histogram
  so they should not be further optimized (and changed) by fitting
% Histogram for 8-bit images is now computed and fitted at full
  resolution to minimize fitting errors due to undersampling
```

Version 2.1 *created on 2017-04-05 by Luca Della Santina*

```
+ 8-bit input images are now recognized
+ User is allowed to input custom initial paramenters for the gamma fit
+ Progress bars displayed during each computationally-intensive step
% Output threshold vs z-depth plot now scales from 0 to image max
% Changed default initial gamma fit decay to 0.1 (more consistency)
% Changed gamma fit SD computation to not return error when poor fit
```

Version 2.0 *created on 2017-04-03 by Luca Della Santina*

```
+ User can choose whether to fit the entire stack's histogram with a
  single gamma function or to fit each image along z-axis with different
  gamma functions. The latter allows more effective masking of labelings
  whose noise distribution changes along the z-axis.
  These differences across z-axis usually arise due to:
  . Penetration issues of the primary antibody
  . Labeling more intense in some cell types than others (CtBP2)
  . Laser correction along z-axis applied during image acquisition
+ Thresholds are smoothed along z-axis to compensate errors of nlinfit
  in some planes particularly hard to fit.
+ Auto-detection of histogram peak (before value was user-requested)
+ Threshold is asked to user via graphic dialog, proposing 4 as default
+ Threshold distribution is plocltted against image number along z-axis
% Default value for beta0(1) = 1 instead of 0.5. (more consistent fits)
% FitStartX now matches histogram peak, before it fell 1 bin after peak
```

Version 1.0 *created by Haruhisa Okawa*

```
+ Fitting parameters are calculated once over the entire image stack
+ User is asked to choose the intensity value of histogram's peak
+ Output mask image is saved as uncompressed TIF stack
```