Process Description:

The calibration of a valid noise model is critical for the statistical detection of speckles. From a cropped stack of background, the calibration step computes the mean and the standard deviation of the background intensity. To validate the Gaussian noise assumption, the noise model calibration calculates an additional value called Gauss ratio. This ratio defines by how much the standard deviation of the noise field decreases after filtering with the point-spread function. For regular high-numerical aperture imaging and appropriate pixel size (>5 pixel per PSF radius), the Gauss ratio should be greater than 3. If this is not the case then the image background (and with it most likely the speckle cell area) has high stray-light, the noise pattern exhibits spatial correlation (check the decay of the spatial autocorrelation of the noise), or the sampling (typically pixel size) is inappropriate for speckle imaging. A Gauss ratio between 2 and 3 may allow some analysis, especially of speckle flows, but the results should be interpreted with extra caution. Images with a Gauss ratio below 2 should not be processed.

Parameter Descriptions:

Input Channels:

This allows you to select which channels to use to calibrate noise models. This should be applied to all channels where speckles will be detected. Select the channels by clicking on them in the "Available Input Channels" box and then clicking "Select>" to move them to the "Selected Channels" box. You can unselect a channel by clicking the "Delete" button

Select Window of Interest

This allows the user to select a background region in the movie. A pop-up window should open showing the current channel and the frame. Adjust the rectangular Region of Interest (ROI) to select a region containing only background. Use the frame slider to make sure no parts of a cell move into the ROI during the movie.

First/last stack image

This allows the user to select a frame range for cropping the background stack.

References

Ponti, A., P. Vallotton, et al. (2003). "Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy." Biophys J 84(5): 3336-3352.