

Process Description:

Speckles are detected as local intensity maxima. First, the image is low-pass filtered using a Gaussian kernel of standard deviation equal to the standard deviation of the theoretical point-spread function. Local maxima are then identified within a window of 5x5 pixels and tested for significance against the intensity of three neighboring local minima. The confidence level of this test is a critical parameter in the speckle detection step. According to this procedure, a speckle therefore is a local intensity maximum in the image whose intensity is significantly higher than the surrounding local intensity minima. When performing more than one iteration in the detection maxima associated with existing speckles are subtracted from the image and new speckles are detected using the same procedure as described above. The validity of each newly detected speckle is determined using its relative position and intensity to neighboring speckles of lower order.

Parameter Descriptions:

Input Channels:

This allows you to select which channels you want to perform speckle detection on. 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

Standard deviation for image filtering (pixels):

This value determines the standard deviation of the Gaussian kernel used for low-pass filtering the image. Its default value matches the width of the theoretical point-spread function of the channel.

Detection mask(s):

The masks box lists the channels where masks are available from the previous mask processes. One or several masks can be selected and used for speckle detection. If several mask channels are selected, the intersection of the masks is computed for each frame and then used for speckle detection. Only speckles inside the mask area will be detected.

Noise model parameters:

If a noise model calibration was set up for this movie, the software will use the output of the noise

model calibration for detecting speckles. If the process has been run successfully, the values of background mean, standard deviation and Gauss ratio will be shown in the corresponding edit boxes. If a noise model calibration was not setup for this movie, load a noise calibration model performed on an appropriate background movie. Click on **Load noise model parameters**. Select the MAT file created by running the noise model calibration step on the background movie.

Alpha value for statistical selection of speckles:

This specifies the alpha value used for the statistical selection of the speckles.

Iterative speckle detection:

Maximum number of iterations

This determines the maximal number of iterations to be performed.

Minimum fraction of additional speckles

If the maximum number of iterations is greater than one, this value specifies the minimal fraction of speckles to be detected at each iteration so that the iterative detection is continued.

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.
- Ponti, A., A. Matov, et al. (2005). "Periodic patterns of actin turnover in lamellipodia and lamellae of migrating epithelial cells analyzed by quantitative Fluorescent Speckle Microscopy." Biophys J 89(5): 3456-3469.