

## Nuclei Detection Description

The nuclei detection option allows users to detect labeled cell nuclei (e.g. by expression of a histone protein marker) from fluorescent images. Details of the detection methods are described in: *Ng et al. Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility. J Cell Biol, 199, 545-563 (2012)*

In brief, the gradient of the fluorescent nuclei image is calculated using a Sobel filter, then normalized to the intensity range of the raw nuclei image and subtracted from the latter. All pixel values that fall below background value due to the subtraction are set to background level. A double logarithm is applied to the processed image, and the image is then filtered with a broad Gaussian with a standard deviation of  $r$  pixels (where  $r$  is defined by the user – see below – based on the average radius of the nuclei). A local maximum filter of circular support with  $r$  pixels is then applied to detect all local maxima in the image. To cut off the background, either an uni-modal thresholding algorithm is applied to the intensity histogram of the local maxima, or, in the case of confluent monolayers where images contain very few background pixels, a smooth spline is fitted to the intensity histogram of the local maxima, and the first local minimum of the spline function is used to determine the threshold level.

### ***Input channels:***

Select the folder of the fluorescent channel that contains images of nuclei you wish to detect.

### ***Parameters:***

**Radius of the nuclei to detect:** Enter the approximate pixel values of the nuclei radius.

**Detect nuclei in confluent monolayer:** Check this box if the images contain very few background pixels (e.g. if the images are of a confluent cell monolayer without a wound region). Usually this box does NOT need to be checked if the images are of densely-seeded cells migrating in a wound-healing assay, because the “wound” region presents sufficient background pixels for nuclei detection. For details, see Ng et al., 2012.

**Frames To Use:** Enter the frames you wish to detect nuclei.