

Segmentation macro for ImageJ to isolate nuclear and cytoplasmic regions in fluorescent microscopy images

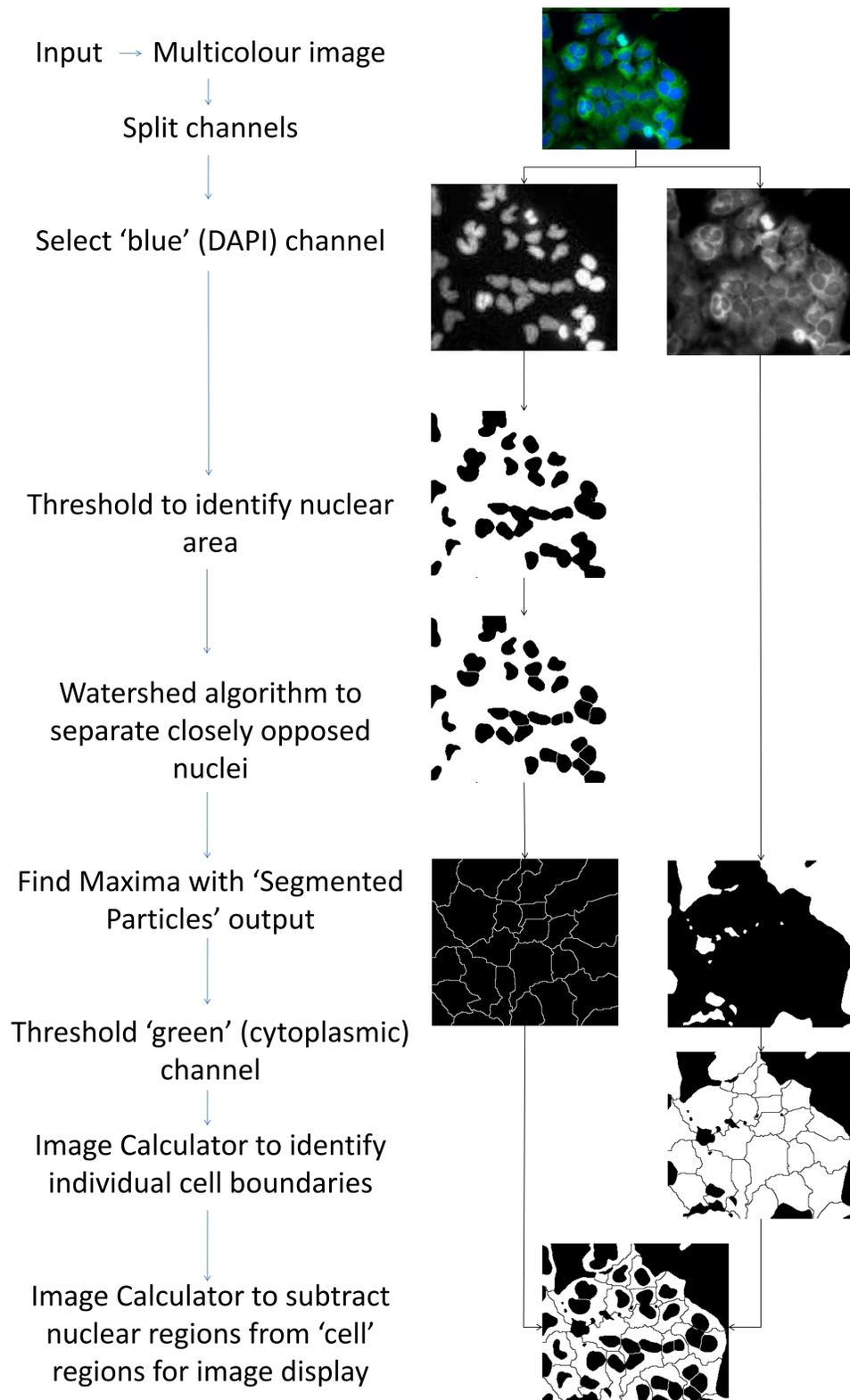
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Introduction

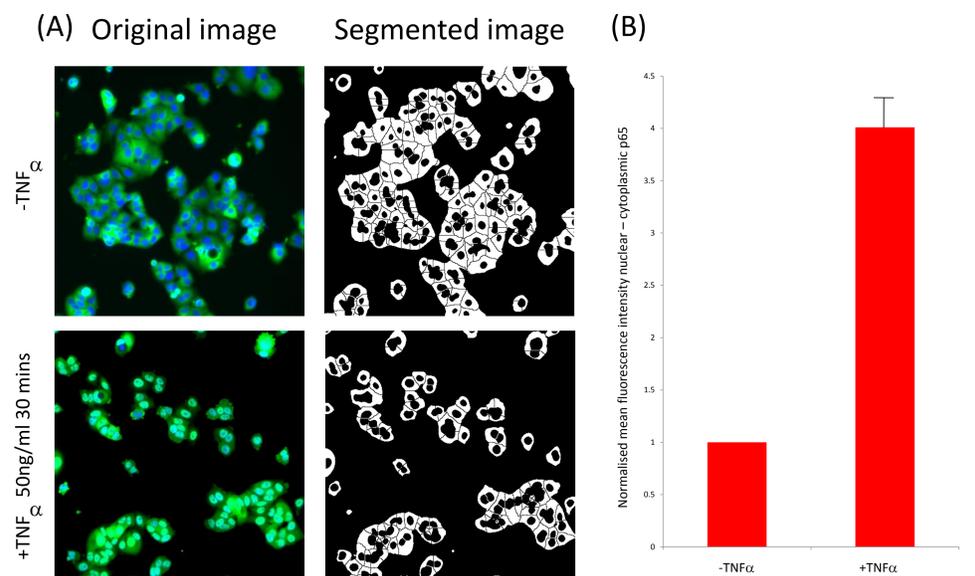
The open source programme Image J is a major image analysis tool employed by a huge number of researchers in the field of image analysis. The programme contains many of the key tools for undertaking very complex analysis routines. However, in order to get the most from the programme it is necessary to utilise these tools in the most effective manner, often through the development of multi-step macros. The macro presented here demonstrates how one can identify individual cells in a field and ascertain their cellular boundaries by a simple sequence of segmentation steps. Utilising this macro the distribution of NFκB/p65 is analysed in HeLa cells treated with the apoptosis agent TNFα. The macro will be integrated into an ImageJ plugin that will allow the user to optimise various settings to get the most effective segmentation.

Image processing steps

The following steps are utilised to segment the nuclear and cytoplasmic regions of a field of cells. These tools are all available within the standard ImageJ distribution and do not require additional plug ins to be installed:



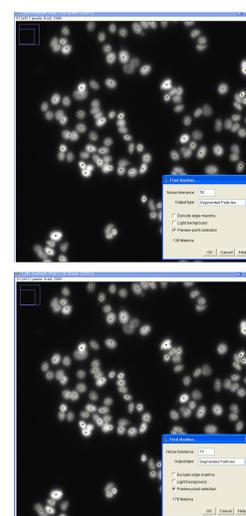
Application of segmentation macro in analysing NFκB nuclear translocation



NFκB Translocation assay (A) HeLa cells were treated with 50ng/ml TNFα for 30 mins prior to fixation with methanol and staining with mAb against NFκB/p65 and Alexa 488 secondary. Cell nuclei were counterstained with DAPI. Images were captured on a Zeiss Axiovert 200 using an AxioCam HR in monochrome mode using AxioVision software. (B) Image analysis shows a 4-fold increase in the mean fluorescence of NFκB/p65 in the nucleus of HeLa cells following treatment with TNFα. Output data from the image analysis was assembled in Microsoft Excel. The mean fluorescence intensity of >1500 individual cells was analysed from 4 separate fields of view.

Optimizing the macro

A number of elements can be optimised within the macro to improve the accuracy of the segmentation process and structure identification.



- **Find Maxima Noise Tolerance.** This is utilised in order to identify the central regions of the cell nucleus (left hand panels). Using the *Segmented Particles* as an output provides us with a method to subsequently identify the boundary of each cell.

- **Thresholding algorithm** – we use the default *Make Binary* command to segment the image in this macro, but more sophisticated thresholding approaches are available as additional plugins e.g. Auto-Threshold.

- **Analyse Particles** – this is the tool for generating regions of interest and enabling quantitation. We can control the structures we consider suitable for analysing by filtering them by size, shape or location.

Future Developments

We plan to develop this macro into an integrated plugin within ImageJ so that it can be utilised in multiwell screens and live cell tracking experiments. Utilisation of this macro in a plugin would enable tools such as defining channels, find maxima, thresholding algorithms and filters to be easily optimised by the user in order to achieve the most effective segmentation.



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