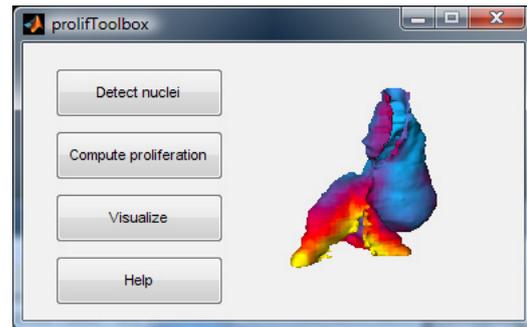


Toolbox interface

The proliferation Toolbox can be used to (I) detect nuclei within images, (II) determine the proliferation/cell cycle length within a tissue or cell type, and (III) to visualize the results. The procedure is described in more detail in [de Boer and van den Berg et al. 2012](#)

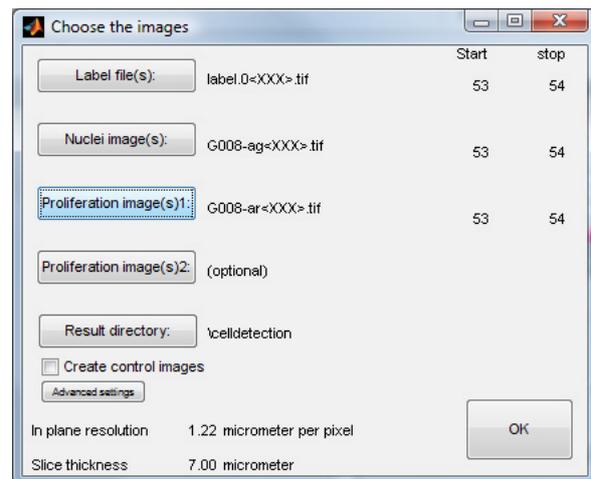


Detect nuclei

To detect the nuclei one should choose a label-file, and corresponding nuclei and proliferation images. These datasets should be aligned. Greyscale tiffs (8,12 or 16 bit), either as multi-page or as a series of tiffs are supported. Note, when using tiff series the last three characters of the filename (excluding ".tif"), are used as file number. Pixel sizes are automatically determined when using a label file which is generated using Amira .

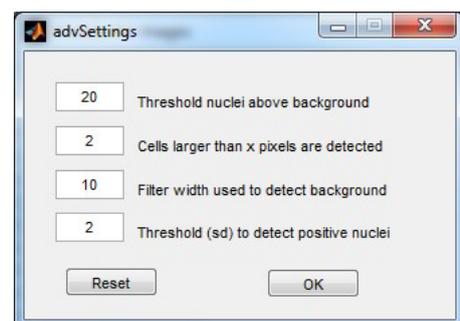
The **label file** is a segmented set of images where each tissue or region of interest has its own unique pixel value. Background is denoted with a zero value. Only nuclei within the labels will be detected.

The **nuclei images** are the images where the nuclei of interest (all nuclei or a subpopulation of nuclei) are stained. The **proliferation images** are images wherein nuclei are stained during a phase within the cell cycle. For the case in which different exposure times for thymidine analogues are used, it is possible to use 2 sets of **proliferation images**. When using one **proliferation** dataset it is possible to compute the fraction of nuclei labelled. On the other hand when using two sets, the cell cycle time can be computed. Only nuclei stained in the nuclei images will be processed and checked whether they are positively stained. After image selection a result directory can be chosen. Default the resulting files will be placed in the sub-directory "celldetection" of the directory where the label file is placed. Optionally control images can be created; In the control images detected nuclei are outlined in red and tissue blue. Binary files (_bin.tif) are created in which the position of the nuclei is stored.



Advanced settings

In the advanced settings one can change the different settings. The first option is the threshold which should be set for the intensity above local background that a nucleus should have to be considered as a nucleus. The second setting is how many pixels a nucleus should consist of before considered a nucleus. The third option is the filter size which is used to determine a local maxima image on which the nucleus threshold is based on. The fourth option



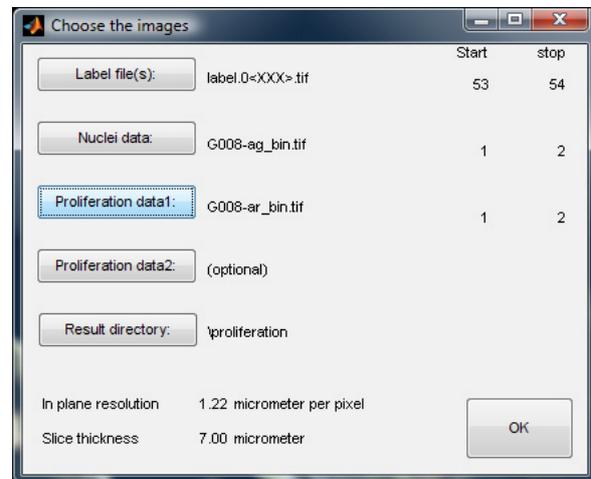
is the number of standard deviations a nucleus should be above background to be considered as positively stained for a proliferation marker.

Compute proliferation

The necessary input fields are already filled in when in the same session nuclei are detected. Else the label file and binarized nuclei and proliferation images have to be manually selected. The second proliferation file is optional and needed for the computation of cell cycle lengths.

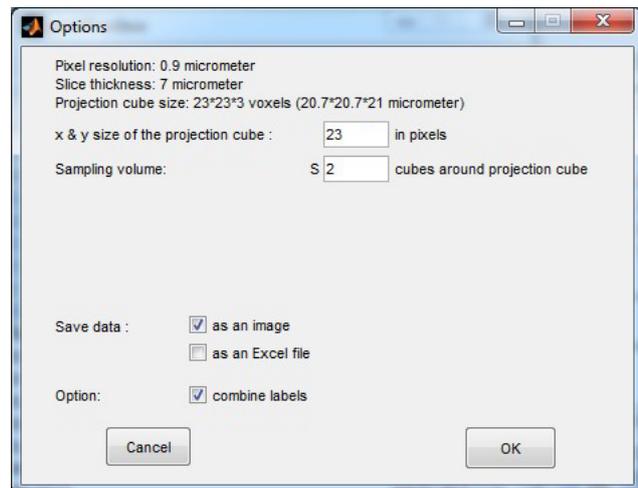
This step can produce the following files:
label file name + label +

- `_i` (labelling index)
- `_i(1)` (labelling index first proliferation file)
- `_i(2)` (labelling index second proliferation file)
- `_e` (average cell size)
- `_tg` (cell cycle length)
- `_ts` (length of s-phase)
- excel file of summary



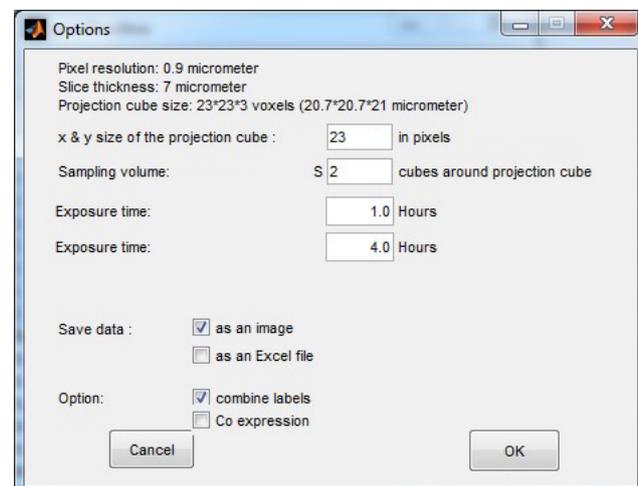
Parameters labelling index

The size of the projection cube and sampling volume need to be filled in. The sampling volume is important for the reliability of the measurements. The optimal size of the sampling volume is dependent on the tissue density. A size should be chosen which ensures that at least 171 nuclei are present in a majority of sampling volumes.



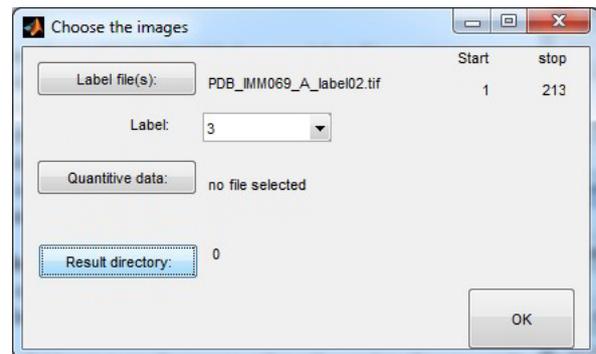
Parameters cell cycle length

In addition to the parameters that are used for the labelling index it is also necessary to provide the exposure time to the Thymidine analogues



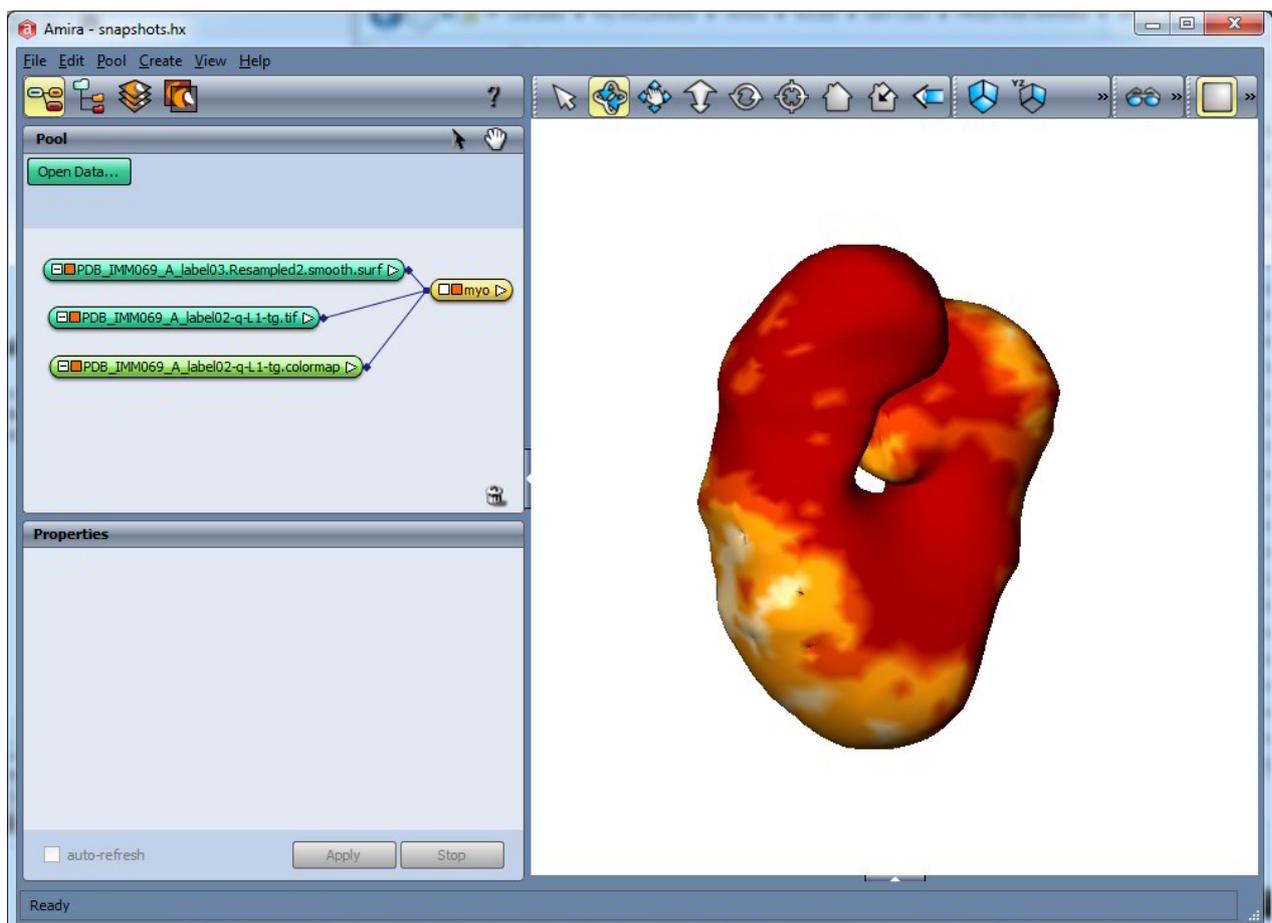
Visualize

Labelling indices and cell cycle length data can be mapped on the label images. One can choose to map on a single label or on a combination of all labels



Visualization in Amira

In Amira a surface file needs to be created (how to do this can be found in the amira documentation). The computed labelling indices or cell cycle length can be opened in a Amira. When opening those files, a colormap will be available automatically. Now connect a surface view to your surface file. The colorfield option of the surfaceview should now be connected to the Tiff-file with the quantitative data and the colormap option to the colormap (Available when the white square in the surfaceview is clicked).



References

- [de Boer and van den Berg et al.](#) Measurement and 3D-Visualization of Cell-Cycle Length Using Double Labelling with Two Thymidine Analogues Applied in Early Heart Development. PLoS ONE 7(10): e47719, 2012