PArameter-free Generic Iterative Thresholding Algorithm (PAGITA)

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Introduction

Welcome to the PAGITA [4] Getting Started Tutorial. PAGITA is a generic and parameter-free tool to simultaneously segment and classify 3D/4D nuclei in early embryo image which is packaged as plug-in for ImageJ [2]. The segmentation approaches is based on learning from samples supplied by the user for the existing objects in image. This tutorial explains all necessary steps to achieve good segmentation quality.

Documentation

Training

The classes are created based on geometrical shape and intensity of existing structures inside image. Each class of objects must has a distinct features that permit them to be recognized. Figure 1 shows a possibility to define the classes by cell cycle (*C. elegans* embro) because general shape and intensity distribution from a class to another are different.

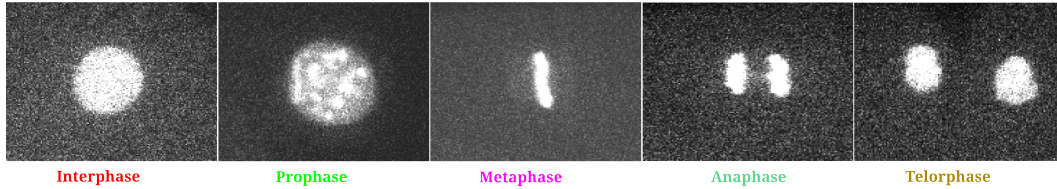Interphase Prophase Metaphase Anaphase Telorphase

Figure 1: Class definition by cell-cycle

When you decide to create necessary number of classes, the next step is to find approximate interval of calibrated volume of each class of objects. In other words, volume must be supplied which may represent the interval of variation of volumes for a class, in the case of embryogenesis, largest objects are found at initial frames and the smallest at the end frames. The best way to accomplish this task is to crop sample by drawing a ROI using imageJ and adjust the threshold manually to detect appropriate boundary of sample contour. Hence, apply threshold to the cropped image and uses statistic *3D Object Counter*[3] tool to determine the volume. Figure 2 shows necessary steps to carry-out this step.
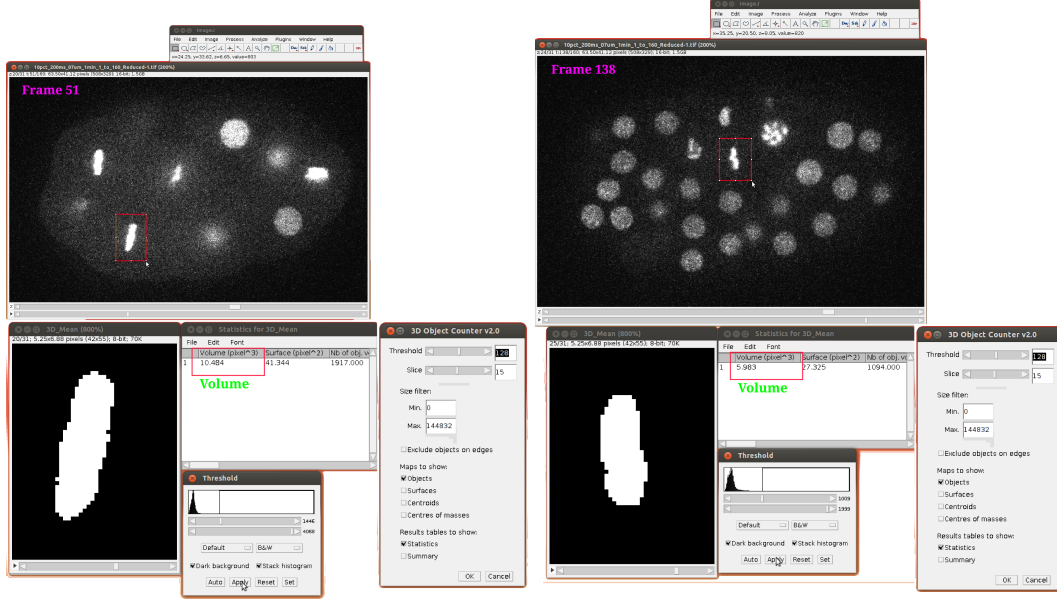
Figure 2: Determination of object's volume using *3D object counter*. Initially, a ROI is drawn around the nucleus, followed by cropping the image. Finally, *3D object counter* is used to obtain the volume by selecting a suitable threshold that gives satisfactory shape of nucleus.

The same procedure of volume determination should be performed for all considering classes. Feature extraction and training process in PAGITA tool is composed of four steps: classes definition, sample selection, sample segmentation and sample validation (figure 3).
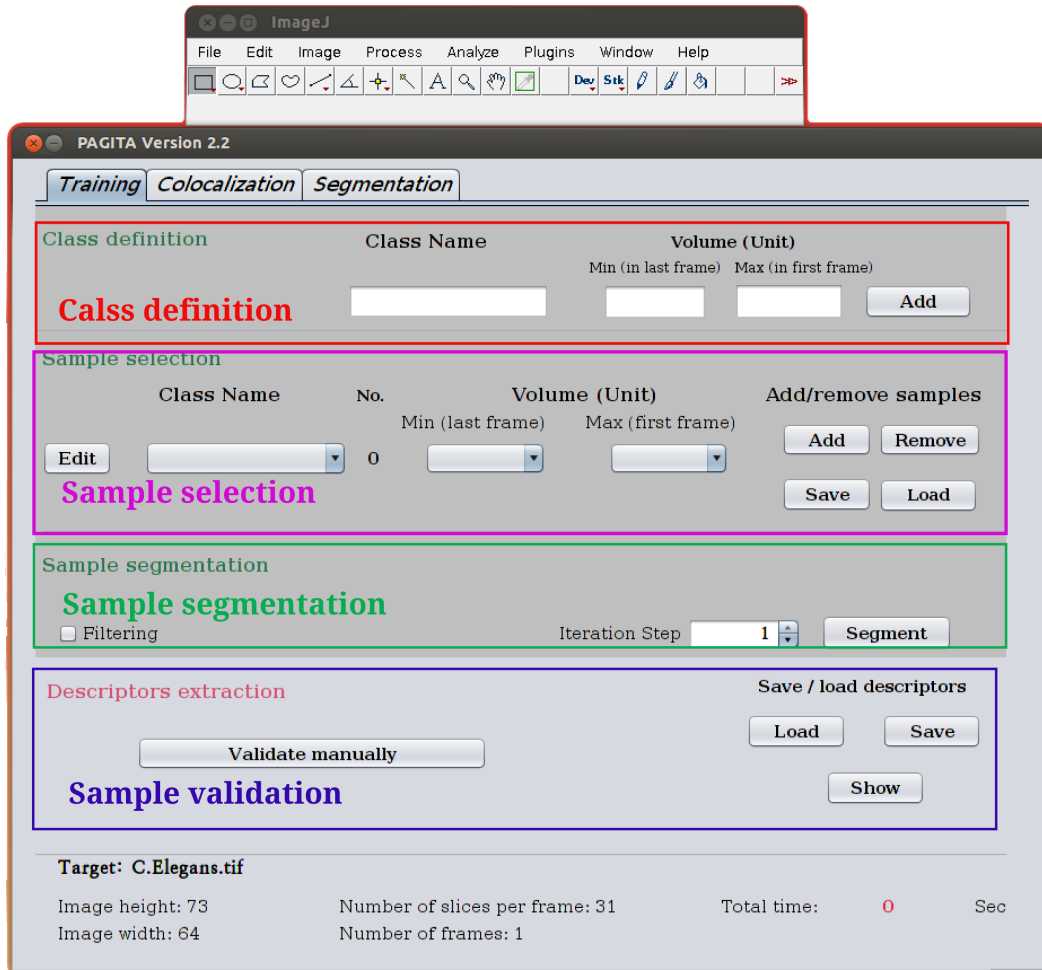
Figure 3: Main steps in training procedure

Class definition
In Analysis tab, enter the class name and maximum and minimum volumes. Figure 4 shows class creation assuming five class of objects (Interphase, Prophase, Metaphase, Anaphase, and Telophase).

Figure 4: Class definition

You can edit class's name and volumes using *edit* button in sample selection (figure 5)

Figure 5: Class Editing

Sample selection

In this step, the samples of defined classes are selected. You can save defined classes, their volume intervals, and and sample's coordinates. Moreover you can load previously defined classes and samples (figure 6).
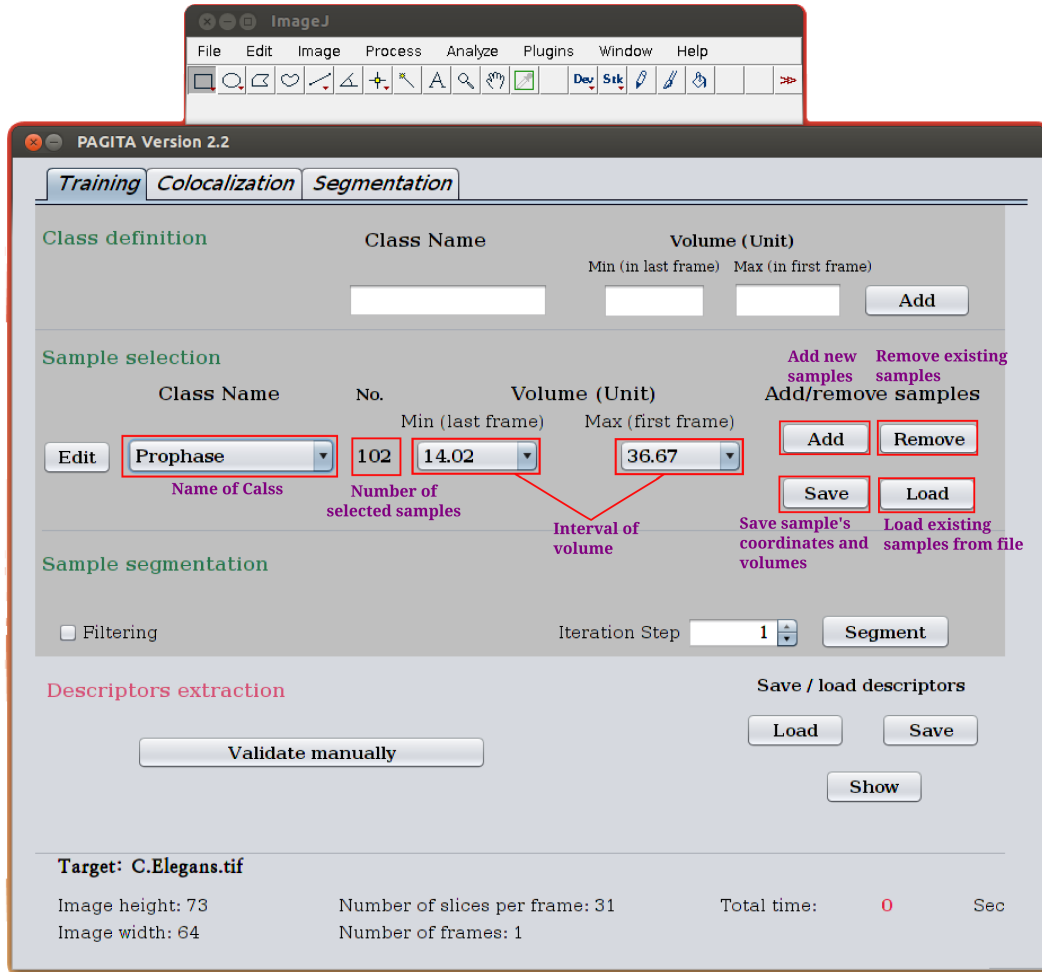

Figure 6: Sample selection details

To select a sample:

- Click on *Add* button.

- Select the samples at different time-points by clicking near the center of nuclei (as shown in figure 7).

- When some samples are erroneously selected for a class, they can be removed using *Remove* button.
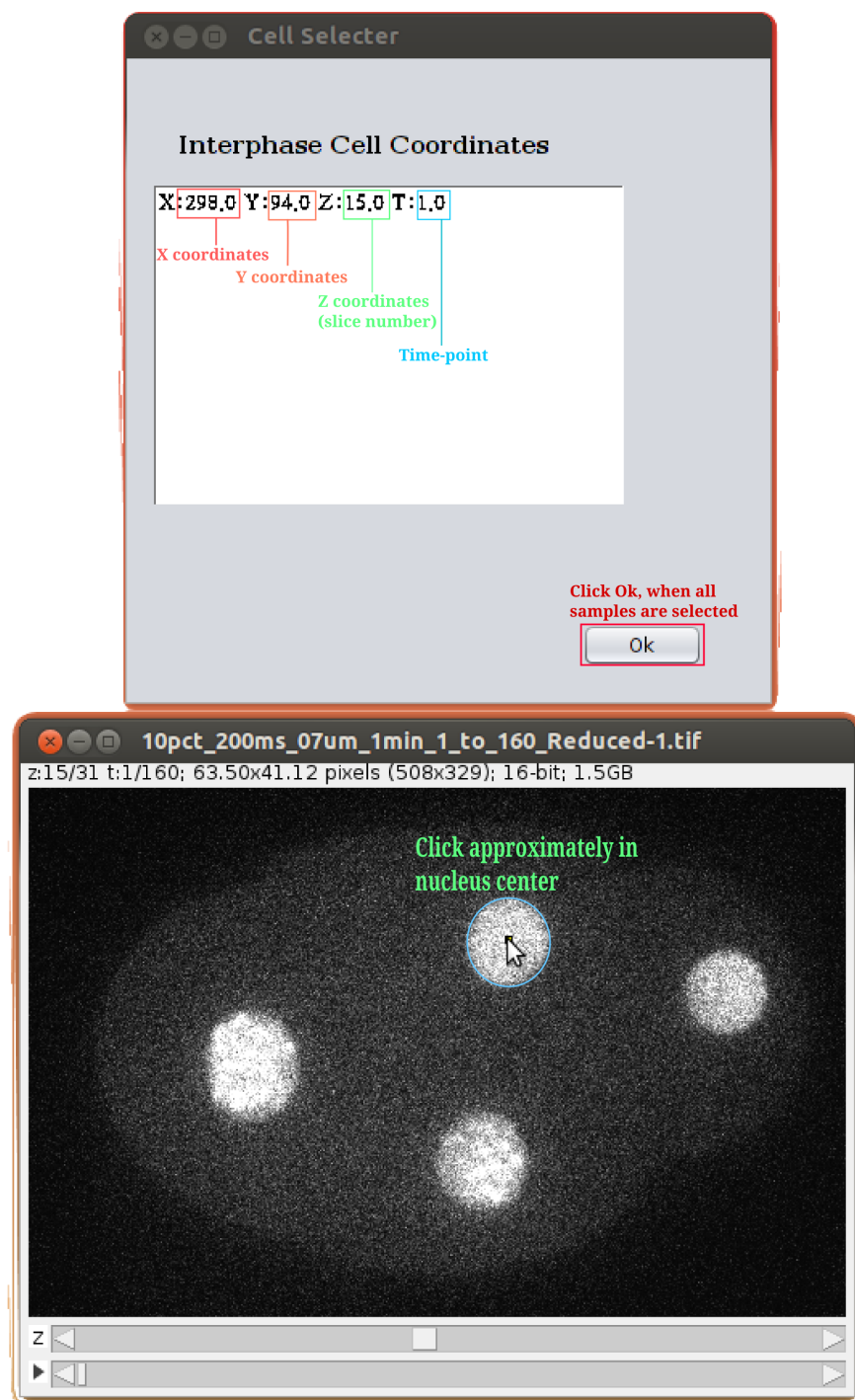
Figure 7: Samples selection

Sample segmentation

The previously selected samples are segmented using an iterative thresholding procedure. Firstly, the sample's image are cropped from a given time-point based on the largest volume of nuclei. Secondly the cropped images are filtered, if it is necessary, using proposed filtering methods (Median, Adaptive, Mean). Finally, for each iteration, the volume of the segmented object (closest to center of box) is computed and compared to the user supplied volume for that class at a given time-point. Once the object volume is below or equal to this volume, the iteration is ended and another sample is taken. Iteration of thresholding (in figure 8) must not be too big (1-3) for 8 bit format and (1-8) for 16 or 32 bit format.
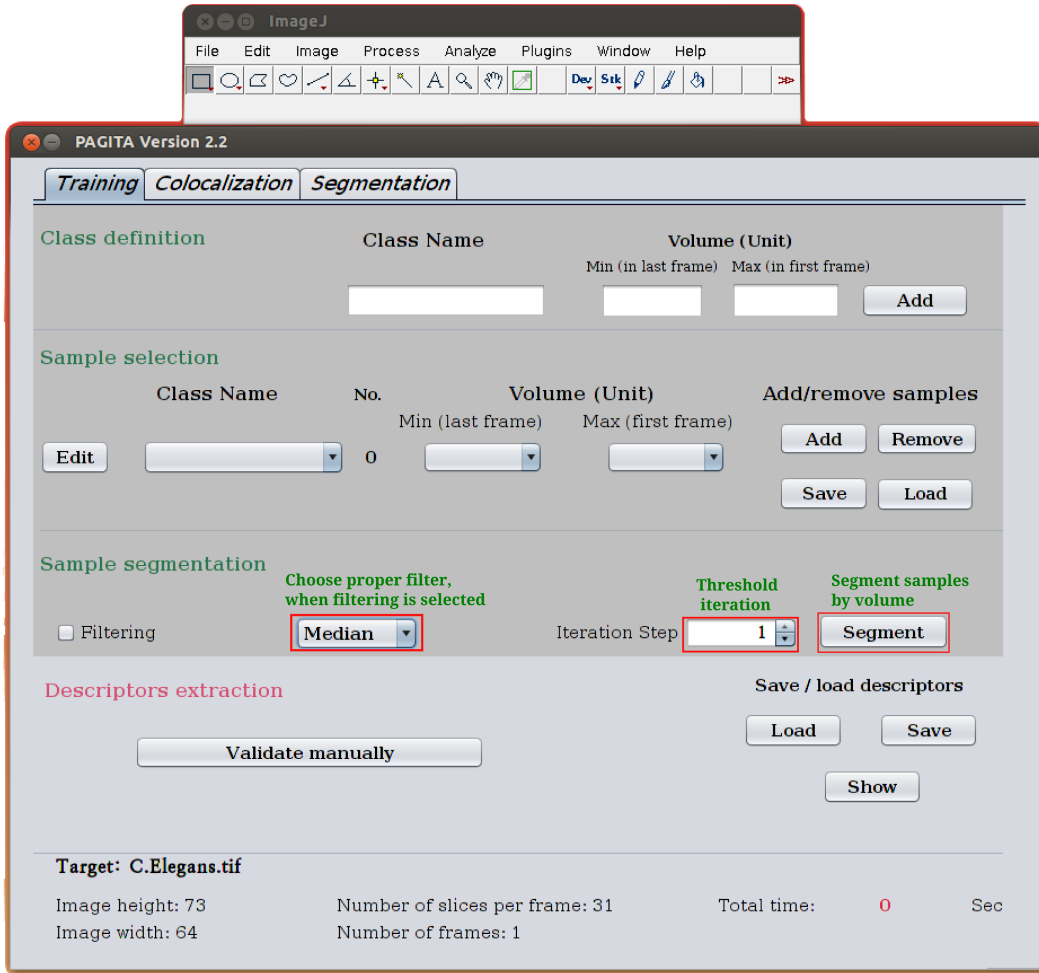

Figure 8: Class definition

Sample validation

The final step in training process is sample validation where samples that are well segmented and have a satisfactory shapes are validated. Click *validate manually* button, then choose the well segmented sample by clicking inside object which are arranged by column. The classes are displayed as 4D image, scroll in time to display different classes, at different frame (as long as number of class). When a new sample is validated, all its features are extracted and will be shown inside a table (figure 9). At the end of validation, extracted features must be saved and used for other datasets. For a new dataset, you just have to load sample's name and volume and features without need to repeat all mentioned steps.
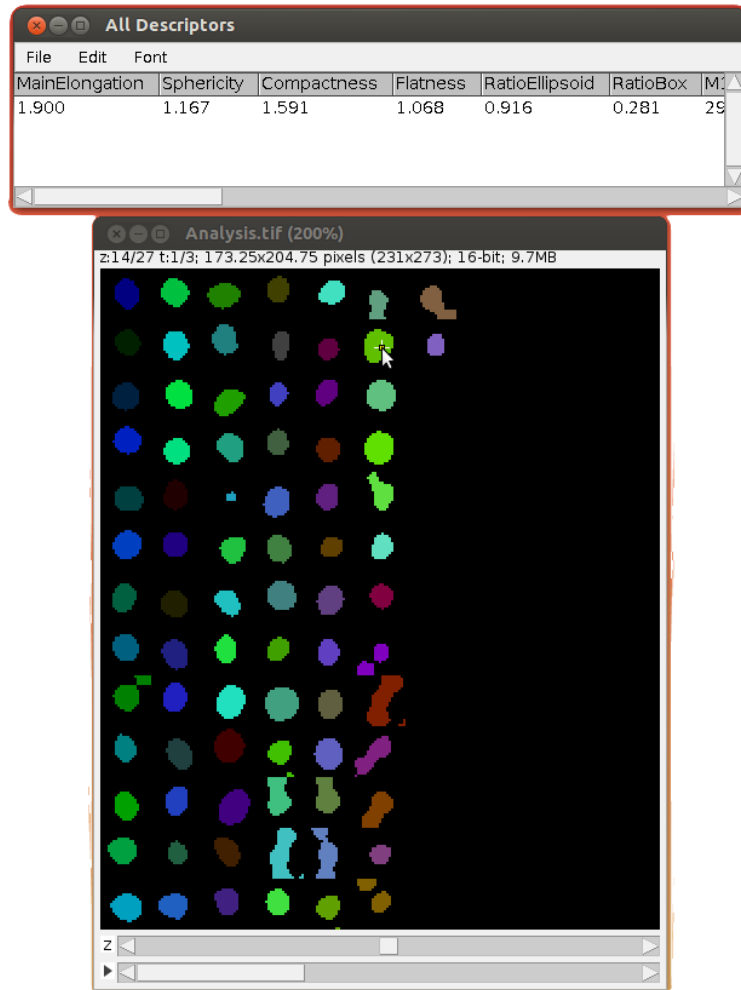

Figure 9: Sample validation

Inclusion

According to the localization of structures, it may exiting co-localization of two or more structures inside another structure. You can make a decision between suppression and keeping of containing and contained objects. This step is carried-out by selecting all possible classes that could be inside one class (figure 10). In this case, the containing objects are kept and contained objects are deleted.
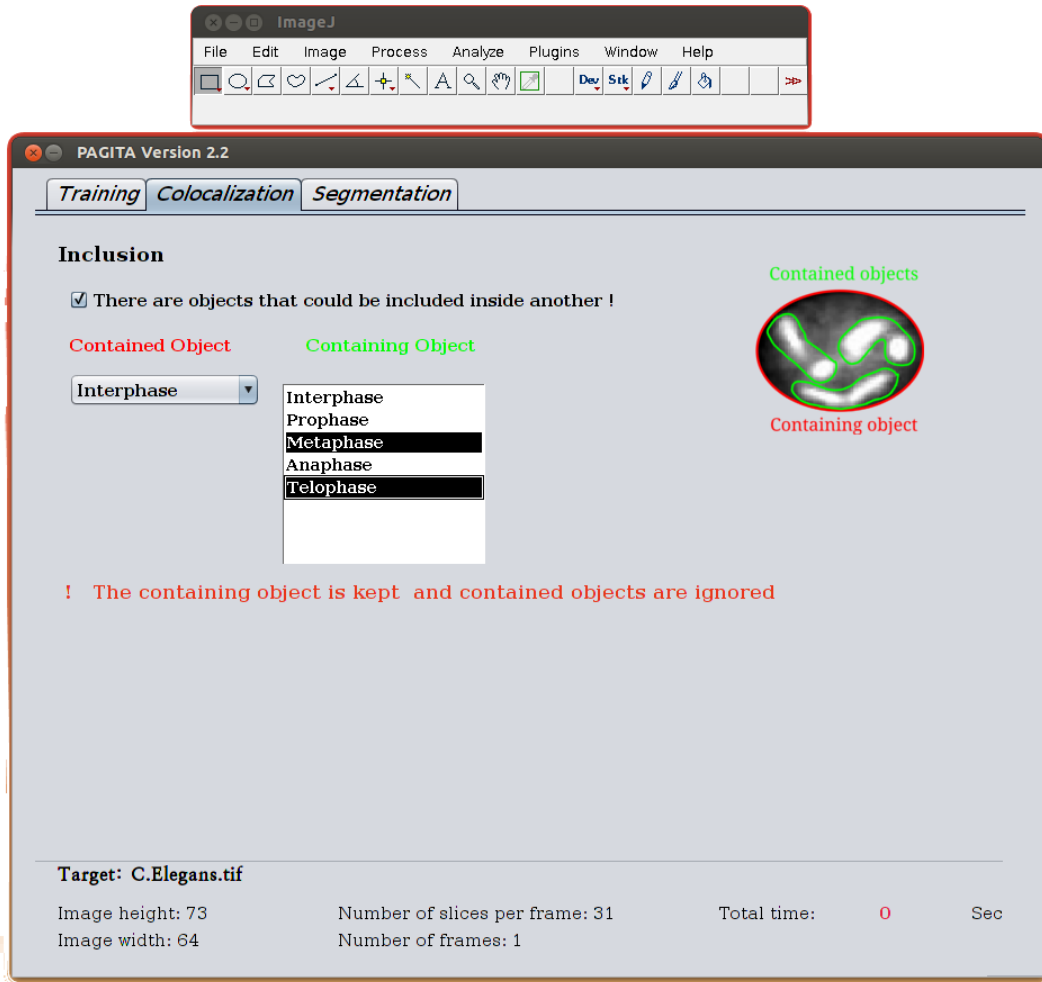

Figure 10: Segmentation

Segmentation

After extracting different features(3D shape, intensity-based, quantitative, 3D moment) from the validated nuclei at previous stage, the classifier (Random forest) can be trained and the segmentation can start. You can choose between Maximum Stability Class (bottom-up thresholding) [1] or Classified Region Growing (top-down thresholding) segmentation technique which is most suitable for your image. Maxi-

mum Stability Class is fast and suitable for nuclei which are not overlapped strongly. However Classified Region Growing is more complex and suitable for inhomogeneous and overlapped nuclei. and The same filtering method which has been applied in training phase must be used (figure 11). The next step is to choose appropriate combination of features (shape, 3D moments, 3D moments invariant, 3D quantitative,..) that will be used in training and testing classifier during segmentation process. All threshold will be tested inside iterative segmentation, however you can specify the range of thresholds to be used especially for Classify Region Growing where the plug-in could be blocked due to using too low threshold.

You can specify all necessary output result from segmentation. By default, there will be segmented image, however you can obtain segmented image visualized by class and also by membership probability.
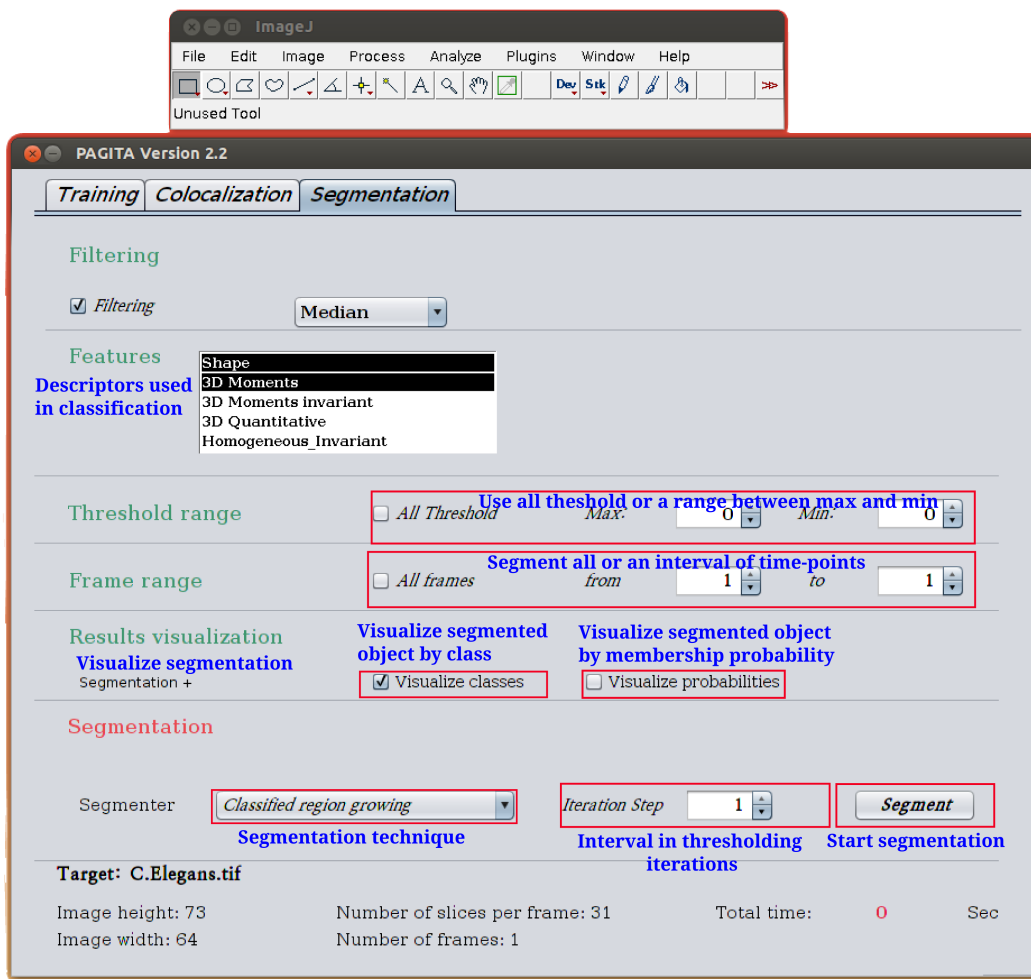

Figure 11: Inclusion

Bibliography

[1] A generic classification-based method for segmentation of nuclei in 3D images of early embryos,BMC Bioinformatics,GulMohammed, J. and Arganda-Carreras, I. and Andrey, P. and Galy, V. and Boudier, T. BMC bioinformatics.

[2] Image Processing and Analysis in Java, http://rsbweb.nih.gov/ij/download.html

[3] 3D Object Counter, http://rsb.info.nih.gov/ij/plugins/track/objects.html

[4] Generic Segmentation 3D in biological development,http://imagejdocu.tudor.lu/