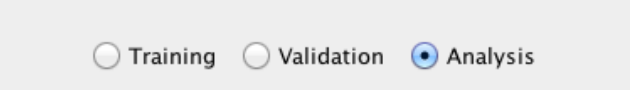
IsletJ - user's guide

IsletJ is a free plugin for Fiji platform. It was developed to allow trainable and fully automated analysis of microscopic images of dithizone-stained isolated pancreatic islets. First validation of the algorithm is described in *Habart et al. Cell Transplantation 2016*.

IsletJ is built around an automated classifier, which needs to be trained on a qualitative range of islet images in order to fully automatically analyze future islet micrographs acquired under similar conditions. Trained classifier creates segmentations of original islet micrographs. Two currently accepted 3D islet models (sphere and prolate spheroid) are used to calculate volumes of individual islets from automatic segmentations. Islet size histogram is constructed using incremental bins of 50µm (Ricordi, Acta Diabetol. Lat 1990).

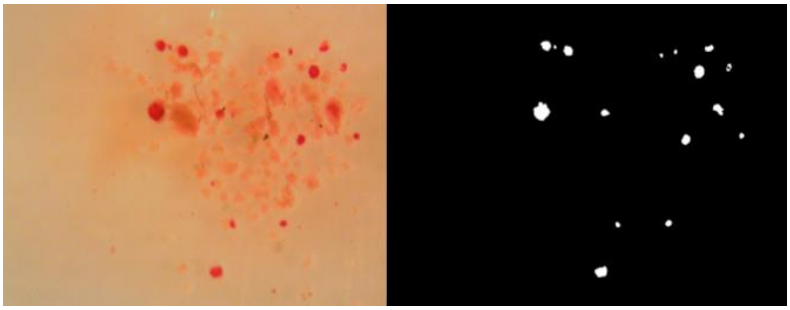
IsletJ user's interface has three modes: Training, Validation, and Analysis. Assuming that classifier is trained and validated only once, but it is repeatedly used for assessment of future images, the Analysis mode is set as the default option.



We suggest for the beginning to create folders 'training', 'validation' and 'analysis' to store the respective inputs and outputs summarized below.

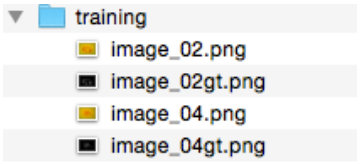
	Inputs		***Outputs***	
Training	islet images GT segmentations	training pairs	trained classifier	training table [csv]
Validation	trained classifier islet images GT segmentations	testing pairs	automated segmentations [png] pixel-to-pixel comparison [png]	validation table [csv]
Analysis	trained classifier (validated) islet images		automaged segmentations [png] islet size histogram [pdf] summary [pdf]	analysis table [csv]

IsletJ uses original islet micrographs obtained by digital camera in RGB image format (png, jpg, tiff or bmp). To train and validate classifier, *IsletJ* uses 8-bit gray-scale binary reference segmentation images (ground truth, GT segmentations) with black background (pixel value = 0) and white islets (pixel value = 255). The GT segmentation images can be created fully manually by delineating islet borders in GIMP or by any other means according to user's preference. Pixel dimensions of the original images and theirs segmentations must equal.



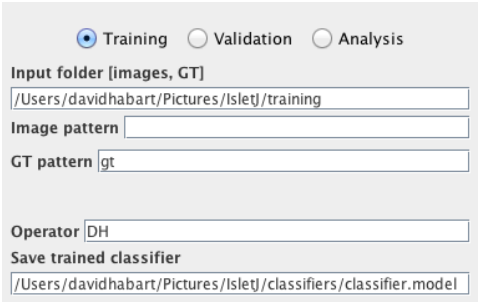
IsletJ can distinguish original islet images and GT segmentations based on RGB vs. 8-bit format and using two strings: 'Image pattern' can be left empty for flexibility of original image names; 'GT pattern' must always be specified.

IsletJ uses numbers to link original image-segmentation pairs for training and validation purposes. The numbers need not be sequential.

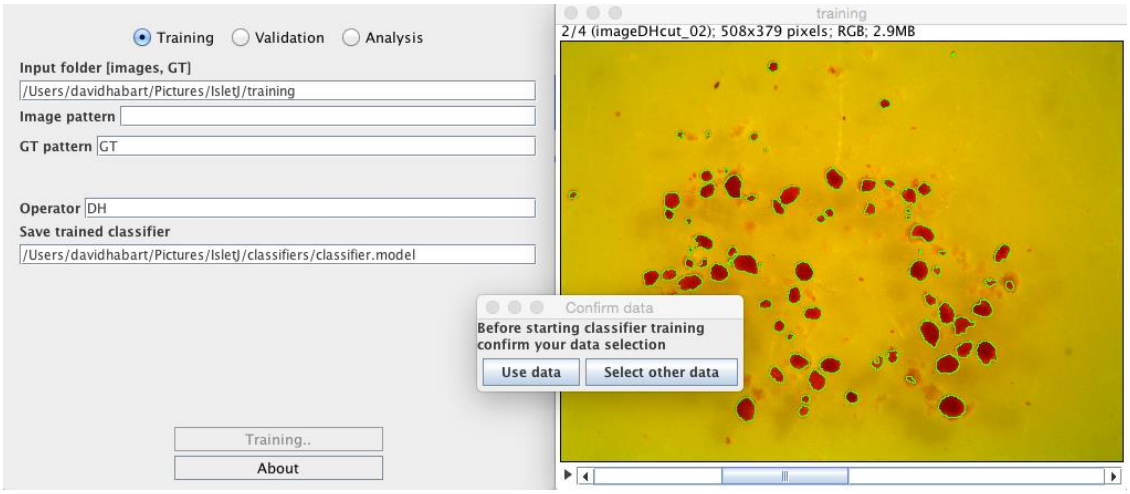


Training Mode

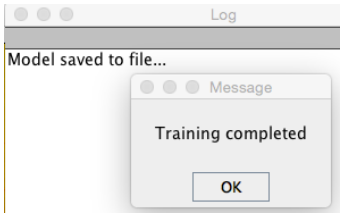
Classifier is trained on a set of original islet images and corresponding ground truth (GT) segmentation images - *training pairs*, which form a training set. All images comprising a training set are stored in 'training folder' and must have same dimensions. Operator's ID can be recorded. Output of the training phase is 'trained classifier'. We suggest creating an output folder 'classifiers' in order to store trained classifiers together 'training tables', record training conditions of each classifier. Trained classifier can have any name; recommended extension is '.model'. Training table will have the same name as the classifier.



After pressing button 'Train' all the image-segmentation pairs will be loaded and visualized in a new window. Islet borders determined by GT segmentation are green and can be toggled by L-click. To parse the training pairs move slider with a mouse or use R/L-arrows on your keyboard. After verifying correct alignment of all training pairs press 'Use data' to proceed with the training.

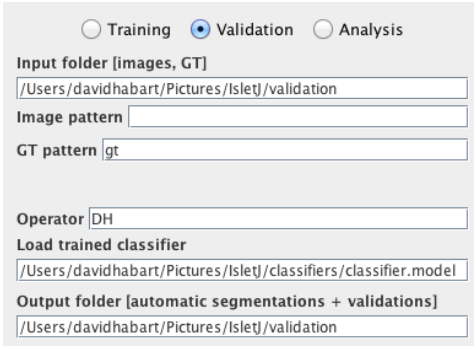


Training can be lengthy depending on the number and size of the islet micrographs used, so please be patient. You will be prompted to note completion of the training.

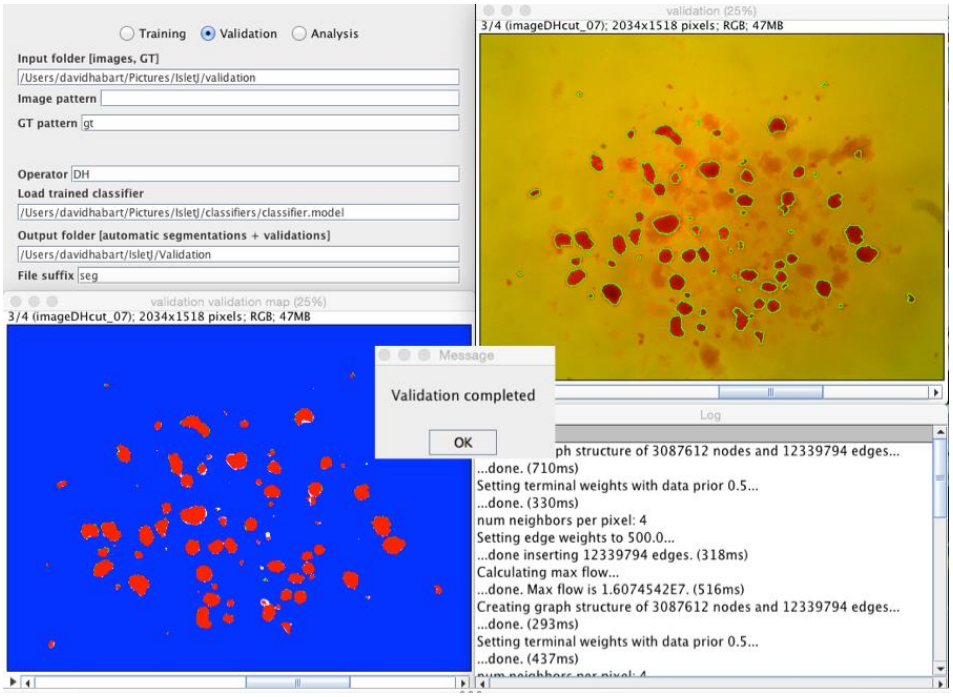


Validation Mode

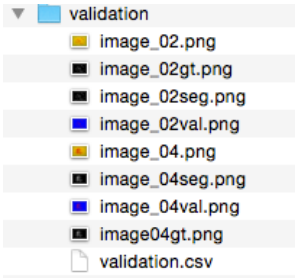
Quality of 'trained classifier' needs to be validated on images from multiple additional donors. Select 'validation folder' containing 'testing pairs' of original micrographs and corresponding GT segmentations. Operator's ID can be recorded. *IsletJ* remembers the path to the latest trained classifier. The output folder needs to be specified.



After pressing button 'Validate', *IsletJ* will load testing images to a new window. Trained classifier will gradually produce automatic segmentations (green line) which can be toggled using L-click. Next, automatic segmentations will be pixel-to-pixel compared to the 'GT segmentations'. 'Validation images' will be created and visualized in an additional window. 'Validation images' use color-coded indices of binary statistics (blue: true negative, red: true positive, green: false negative, white: false positive).

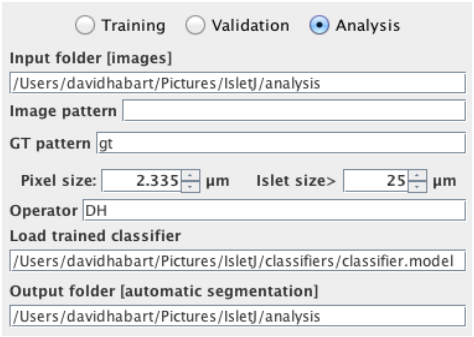


The 'automated segmentation images' (suffix '_seg'), 'validation images' (suffix '_val') and 'validation table' (validation.csv) are saved automatically in the output folder. Quality of validated classifier can be expressed in terms of relative error RE=|(TP+FP)-(TP+FN)|/(TP+FP) and relative islet area RIA=(TP+FP)/(TP+FN), which are recorded in 'validation table' for individual images. Average ± SD should be calculated.

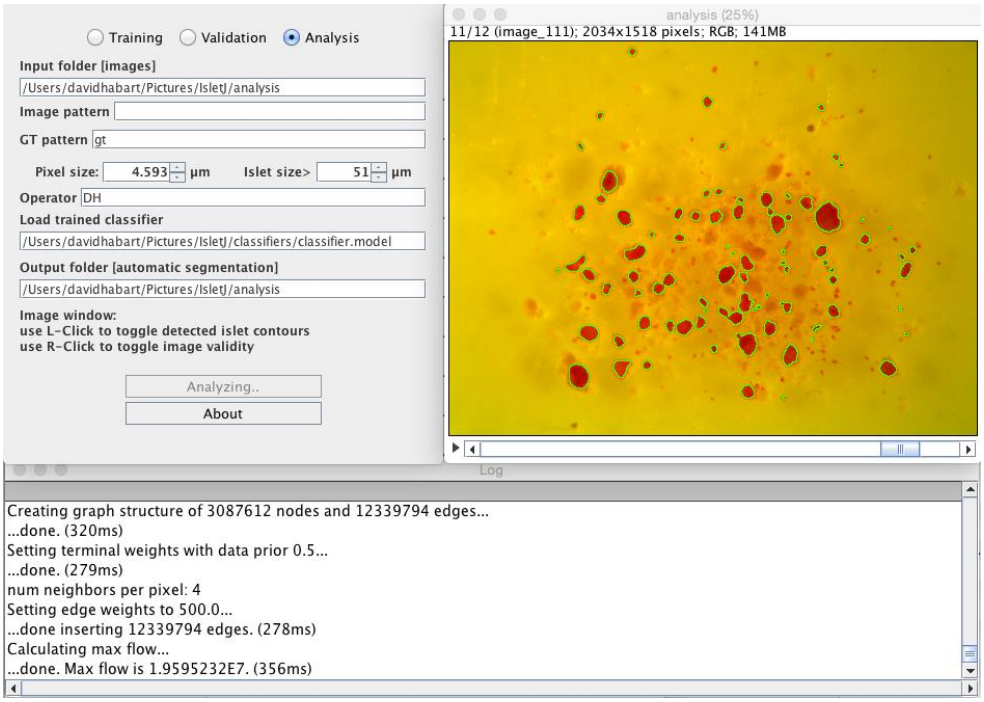


Analysis Mode

Trained and sucessfully validated classifier (e.g. RE<0.10; 0.90<RIA<1.10) can be used for fully automated analysis of new images similar to some of the images used for the training. Specify 'pixel size' for a particular microscope, camera and magnification (the procedure is described in Appendix). Depending on image resolution (pixel size) specify the smallest islet size to be included in analyis. Analysis proceeds in two steps. Individual islets are first segmented by 'trained classifier'. Next, currently accepted 3D islet models are used to calculate volumes of individual islets.

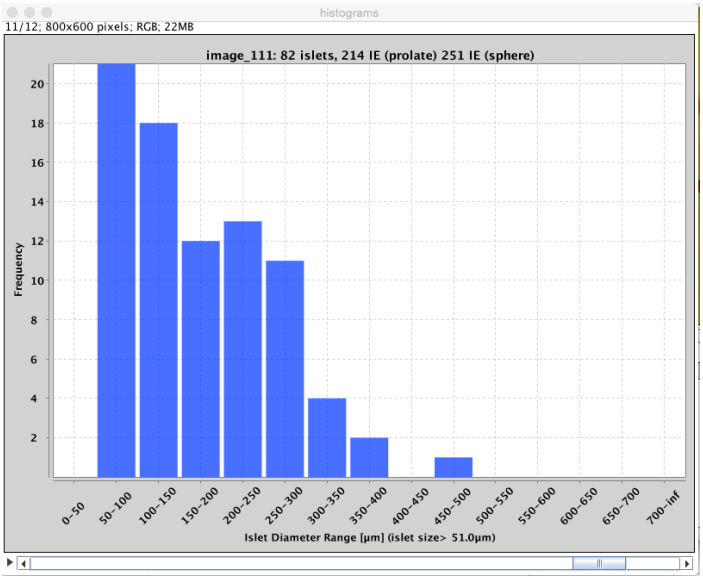


After pressing button 'Analyze', *IsletJ* will load all islet micrographs to a new window. Trained classifier will gradually produce automatic segmentations (green line), which can be toggled using L-click. The images can be browsed through using bottom slider or keyboard R/L-arrow.



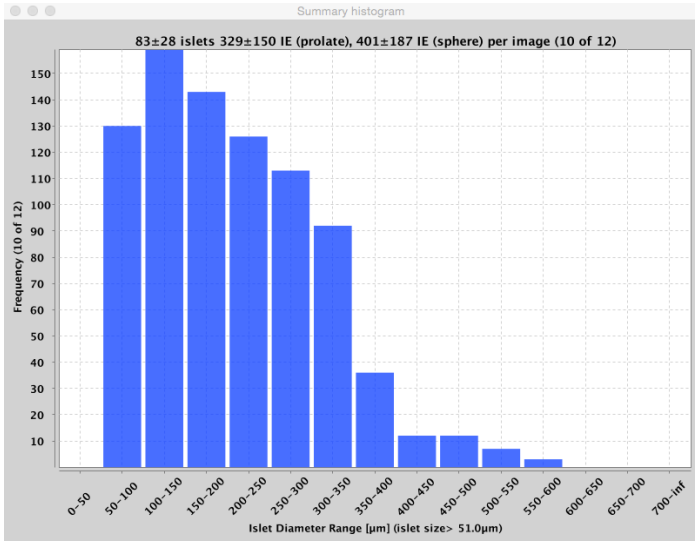
IsletJ uses the automated segmentations to count individual islets, and to calculate their volumes using either prolate spheroid or sphere islet models. For all individual images islet size histograms are created and can be browsed through using bottom slider or keyboard R/L-arrow. The header shows names of original images, islet counts and sum of the islet

volumes calculated by the two respective methods. In addition to individual histograms analysis.csv is created to store detailed results for all analyzed images.

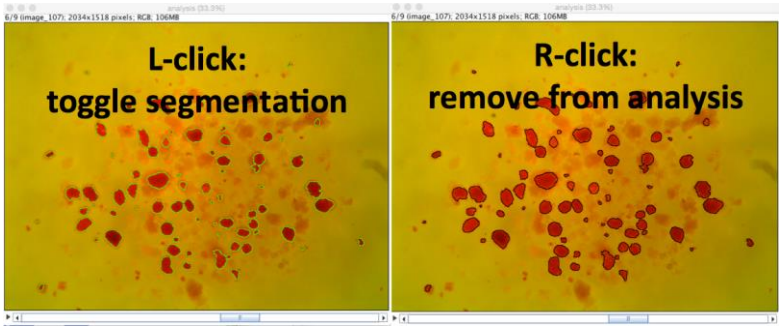


Completion of the analysis is announced by:

IsletJ is intended to improve and facilitate quality control of clinical islets isolation by rapid and reproducible analysis of multiple samples drawn from individual tubes. It is therefore assumed that the input folder 'analysis' contains a number of images from a single tube exclusively and therefore is its possible co calculate average ± SD for islet count and volume in that tube. Graphical summary is created showing average and SD for islet count and volume for all images in the analysis folder.



It is possible to remove individual images from the summary using R-click into the image (segmentation borders will change from green to black).



Images excluded from the summary will be marked in tine ‘no’ in *Analysis table* and moved to the bottom of the list.

Image	Date	Time	Valid	Trained Classifier	Islet number	Volume (Prolate) [IE]	Volume (Sphere) [IE]
image_101	19.07.2016	20:47	y	classifier.model	67	344	426
image_102	19.07.2016	20:47	y	classifier.model	72	298	357
image_103	19.07.2016	20:47	y	classifier.model	134	672	826
image_104	19.07.2016	20:47	y	classifier.model	65	159	201
image_105	19.07.2016	20:47	y	classifier.model	58	216	261
image_106	19.07.2016	20:47	y	classifier.model	95	473	599
image_107	19.07.2016	20:47	y	classifier.model	71	337	406
image_110	19.07.2016	20:47	y	classifier.model	53	176	208
image_111	19.07.2016	20:47	y	classifier.model	82	214	251
image_108	19.07.2016	20:47	n	classifier.model	65	787	1040
image_109	19.07.2016	20:47	n	classifier.model	114	575	719

Appendix

Pixel size determination using Fiji

Note microscope type, camera and magnification. Photograph a stage micrometer (here DIV=0.1 mm). Open the image in Fiji. Measure the distance from 0 to 4 (yellow line). In Analyze/Set scale the distance will be filled automatically. Fill in known distance in µm. Invert the Scale (0.4245 pixel/µm) to obtain pixel size (2.356 µm/pixel).