ImageJ Macro Toolsets for Biological Image Analysis

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Introduction

At the facility Montpellier RIO Imaging we provide the creation of custom image analysis and automation solutions on demand as a service. The biologist contacts us and we find a protocol that solves the image analysis problem. If the protocol can not be executed using existing software packages, we create the necessary tools based on ImageJ. The protocol and the tools are evaluated in cooperation with the biologist, using data provided by him. Once the evaluation done, the biologist uses the provided tools by himself to solve the image analysis task.

The requirements for tools that are mainly intended for the usage of one research group are different from the requirements for tools that are made available to the users of a facility or to a wider community. In the first case a number of macro files with an explanation of how to use them can be enough. Needed in the second case are:

- a possibility to discover the tool
- documentation that is accessible from the tool
  - installation instructions
  - usage instructions
  - meaning and influence of the options
  - a description of the method used
  - an explanation of the results
- the tool must be easy to install and easy to use
- a way to try it on data on which it is known to work
- an easy way to change options
- a way to try it on the data of the user
- a possibility to change default values
- a possibility to adapt the tool

ImageJ tool sets are a simple way to provide tools that can fulfill the above requirements. At the facility we developed a number of tool sets for biological image analysis applications from different areas.

Materials and Methods

A part of the help page of a macro tool set.

We use the web-based project management and task-tracking tool Redmine to manage the image analysis projects of the facility. The wiki part of the Redmine contains a help page for each macro tool set. The help page starts with a general description of the tool. It contains example images for which the tools work well, using the default options. It explains how to install the tool set, eventually its dependencies and how to get started with it. The meaning of the options are explained. The algorithms and methods applied are described. The page can contain hints for the usage of the tools or a description of the workflow. Finally examples of the results of the tools are shown. The first button of each tool set contains either an image that helps to identify the tool

set or a questionmark. Pressing the first button opens the help page of the tool set in the web-browser. There can be one version of the macro that runs on the active image and a batch version that runs on a set of images. The tools can have options that can be set by right-clicking on the button of the tool. The default values of the options are defined as variables at the beginning of the tool set macro file. A macro shows the current activity and the progress in a log-window. Macros running on file sets will usually write the measurement results and control images that show the measured objects into a result folder. Since at the facility the user pays for the time that he uses the image analysis workstations, a batch macro tool can have an option to close the workstation session after the processing finished.

Results

In this section some examples of macro tool sets developed at Montpellier RIO Imaging will be described.

Adipocytes Tool
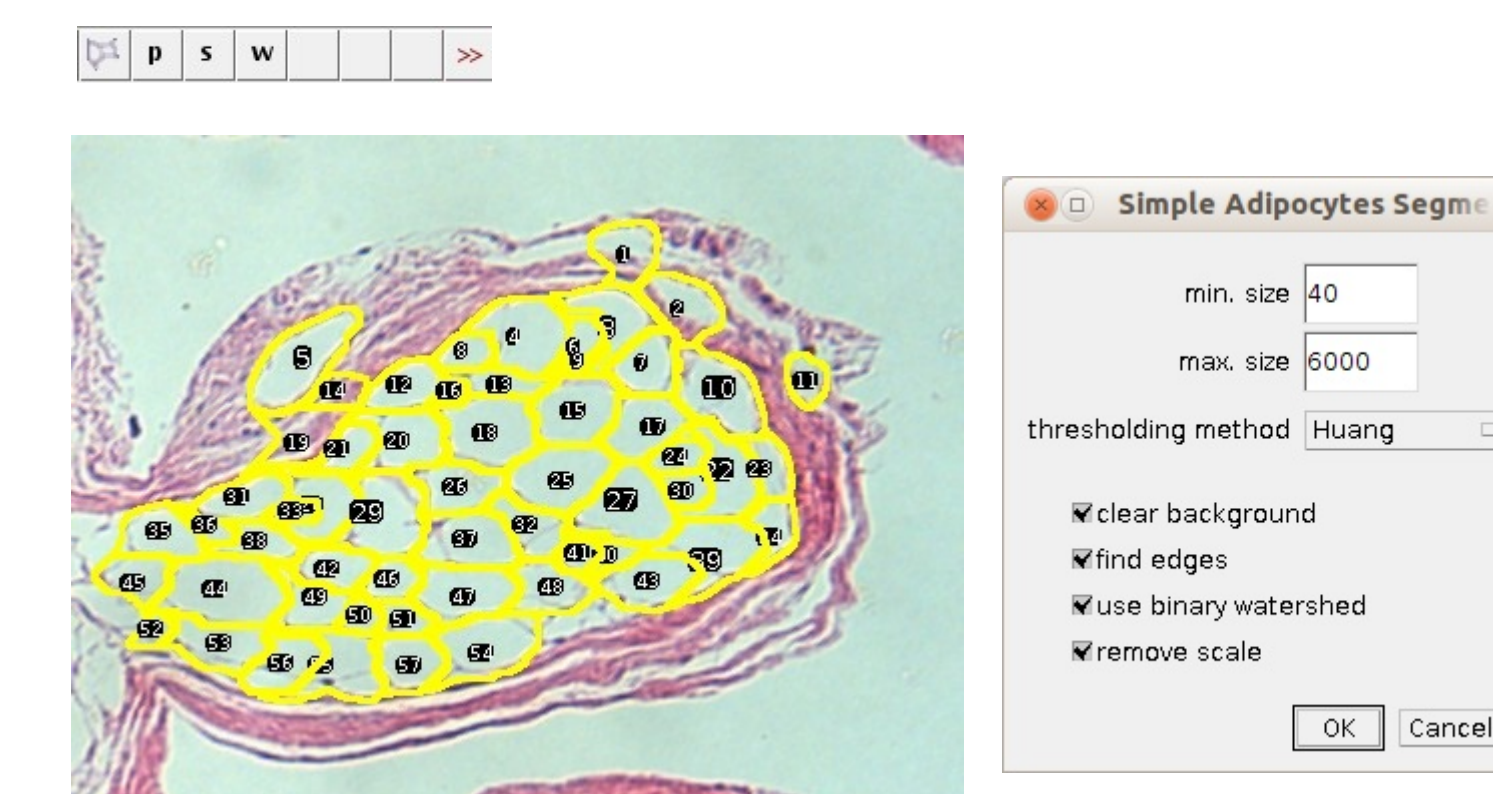
The Adipocytes Tools help to analyze fat cells in images from histological sections in which the membranes are stained.

- preprocessing excludes regions that clearly do not contain fat cells
- simple method uses automatic thresholding and the particle analyzer
- complex method uses a grayscale watershed algorithm
  - after the exclusion of the background, a find edges command is called and the result is smoothed with a Gaussian blur filter before the watershed is applied

As a result a ROI for each detected cell is added to the ROI-manager.

Wound Healing Tool
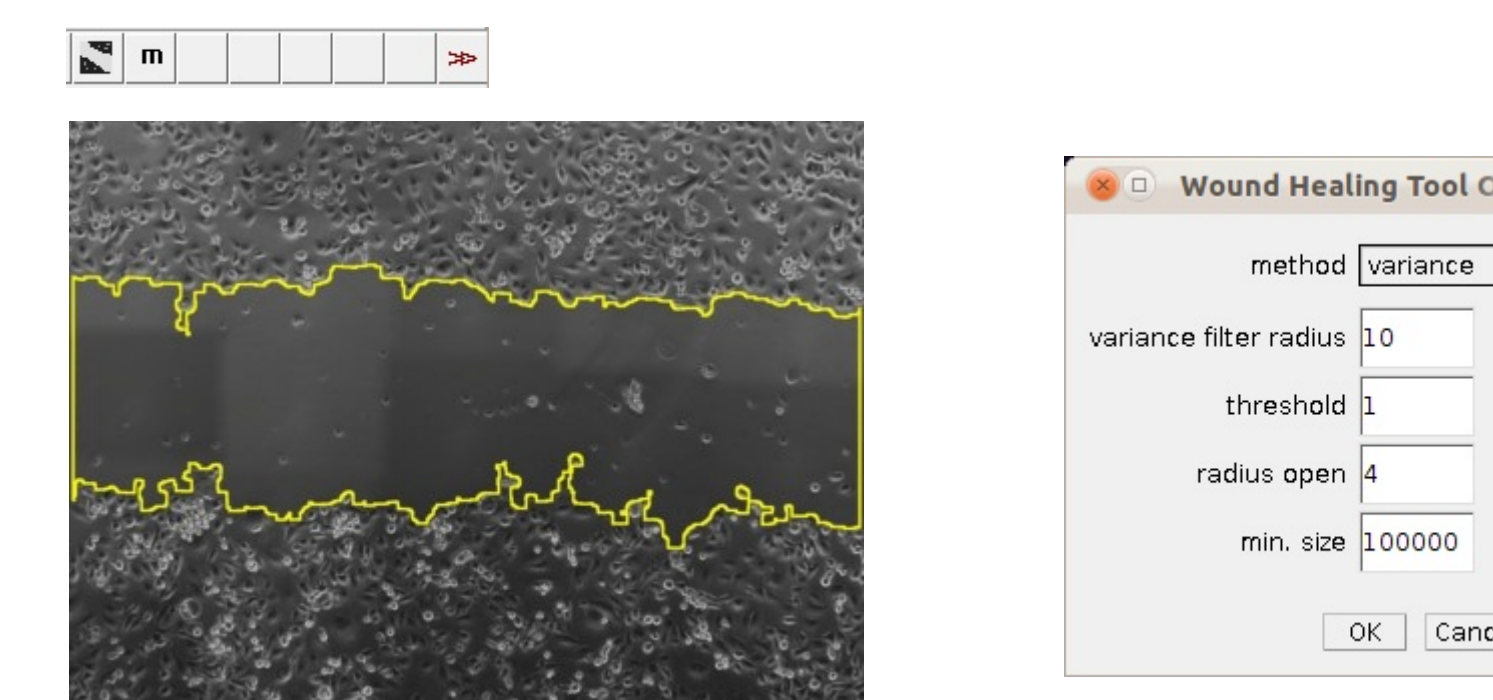
The wound healing tool measures the area of a wound in a time series of images of cellular tissue. The tool will measure the area of the wound, i.e. the area that does not contain tissue, in each image. The segmentation is based on the fact that the image is more homogeneous in the region of the wound as in the region of the tissue. Via the options, one of two methods to detect the empty area, can be selected. The first uses edge detection, the second a variance filter. Holes in the detected tissue are filled using morphological operations.

Colony Blob Count Tool
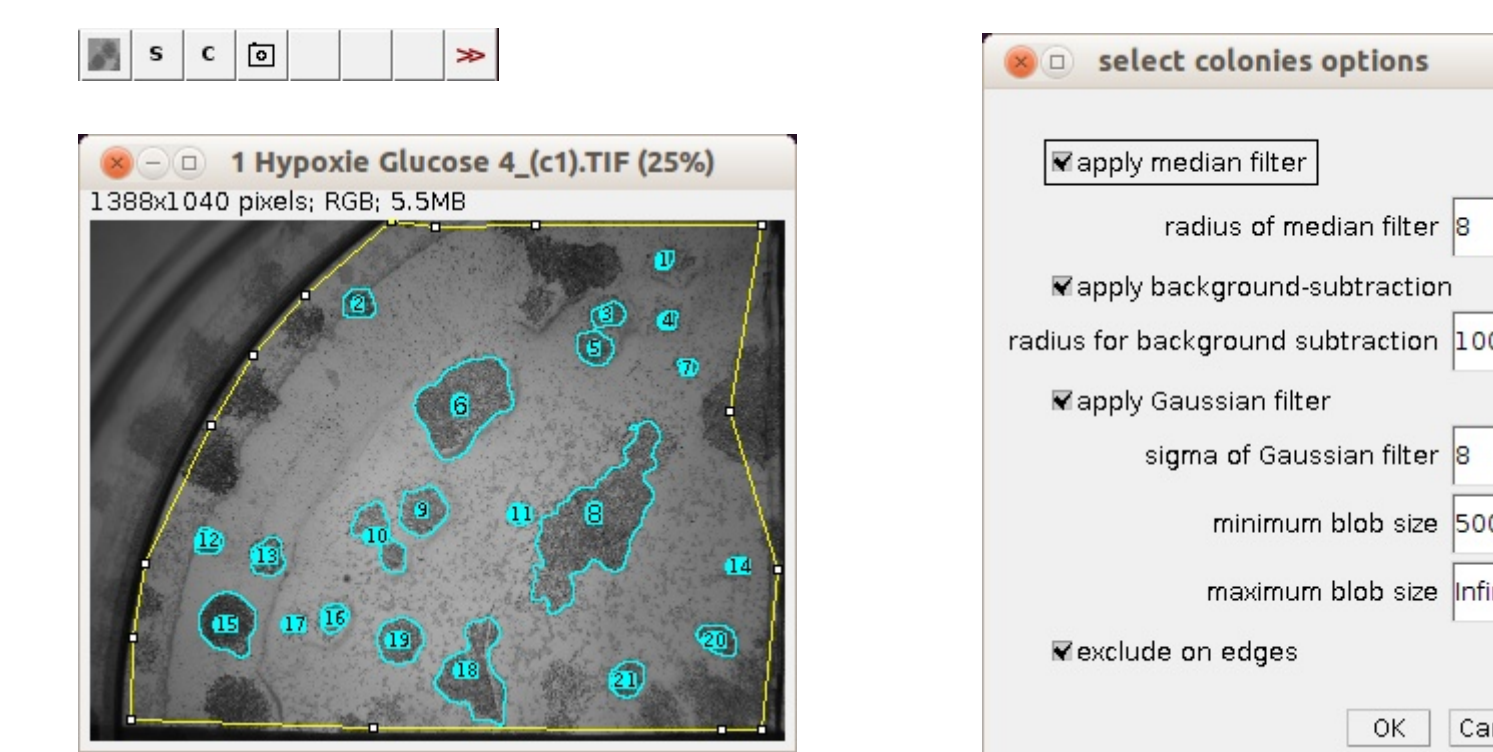
The Colony Blob Count Tool helps to count colonies and measure the areas of the colonies. The bacteria colonies are supposed to appear as blobs on the image, in contrast to cases where they appear as small spots. If the image has a selection, only the selected region is taken into account.

Microtubules Tool (3D)
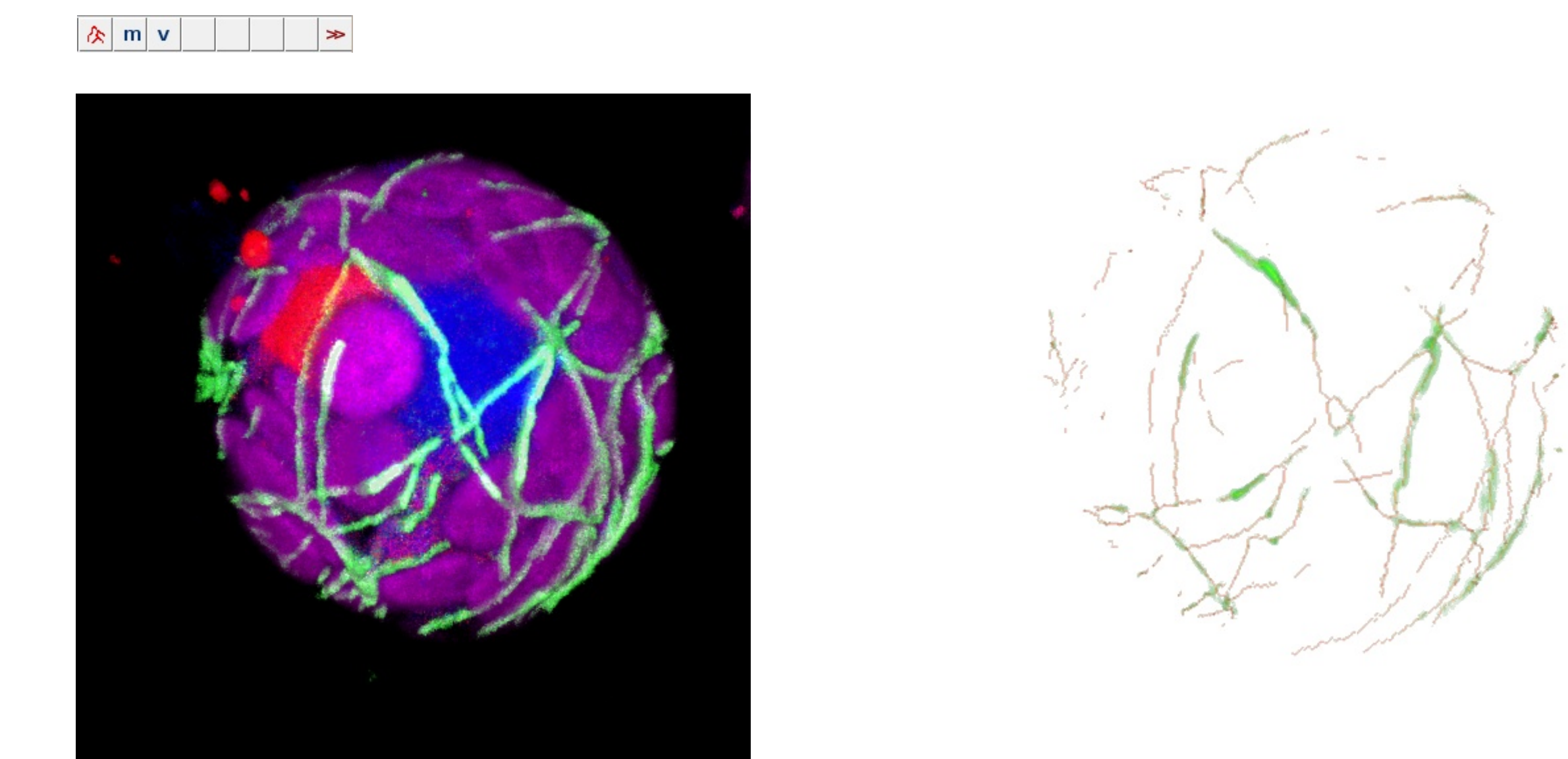
The tool measures the total length of the microtubules in a 3D image. The channel containing the microtubules is smoothed and and segmented by thresholding according to the selected options. The Skeletonize3D plugin written by Ignacio Arganda-Carreras is used to extract the centerlines of the microtubules. The AnalyzeSkeleton plugin from the same author provides the lengths of all the branches of the skeleton. The macro adds all branch lengths to calculate the total length of the microtubules.

Skin Tools
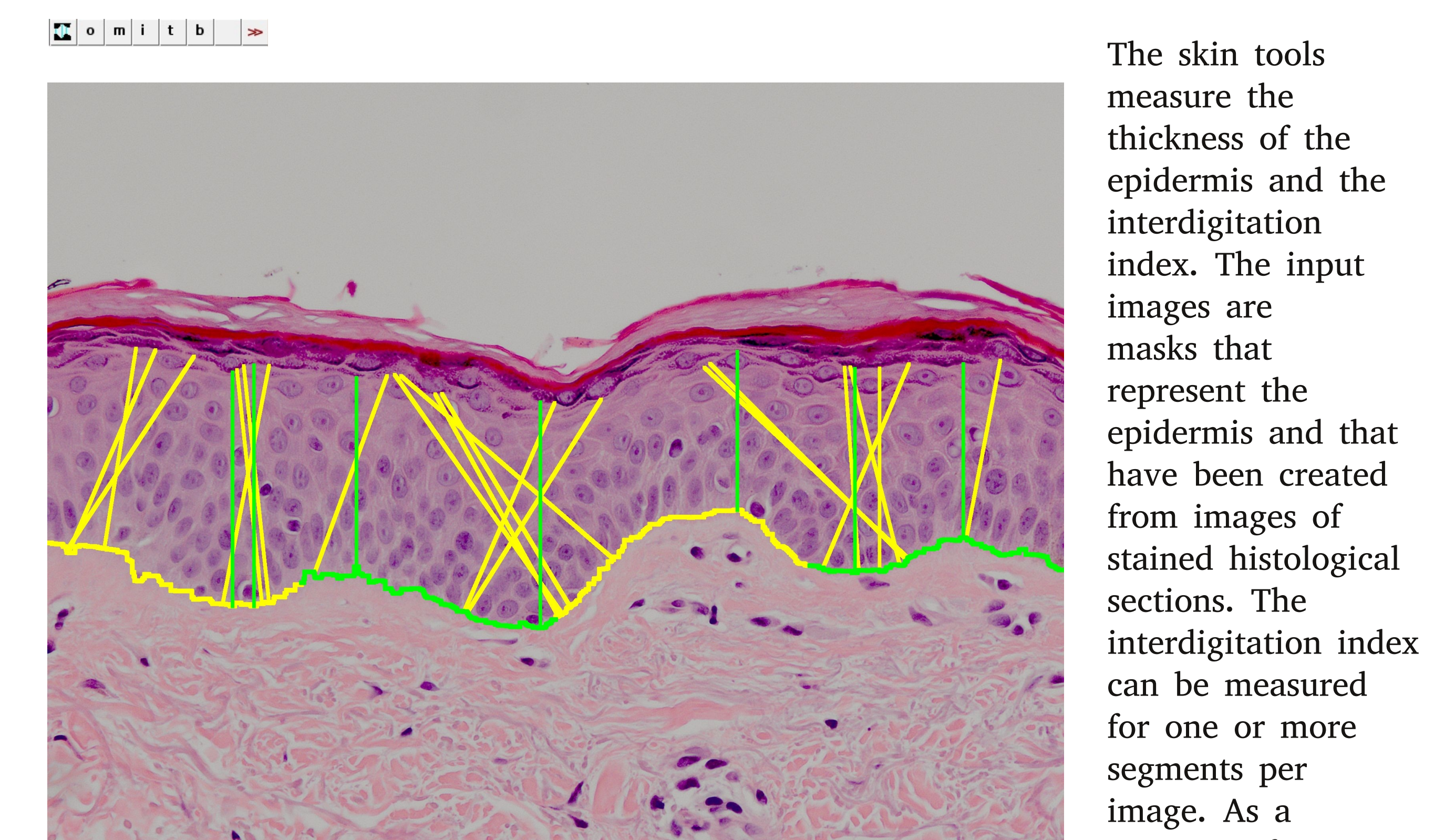
The skin tools measure the thickness of the epidermis and the interdigitation index. The input images are masks that represent the epidermis and that have been created from images of stained histological sections. The interdigitation index can be measured for one or more segments per image. As a measure of the thickness of the epidermis the lengths of a number of random line segments are measured.

Conclusions

We provide solutions for image analysis and automation tasks in the form of ImageJ macro tool sets. We found the macro tool sets to be an appropriate tool for this purpose. For project management and documentation we use the Redmine project management tool. A number of macro tool sets to for conversions and workflow optimizations and a number of tool sets for biological image analysis have been developed by us and are publicly available at:

http://dev.mri.cnrs.fr/projects/imagej-macros/wiki/Help

In the future we plan to combine this work with the central image database OMERO in order to run batch image processing from client machines and from a web application on distant server machines.