

ImageJ Macro Tool Sets for Biological Image Analysis

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ABSTRACT

At the imaging facility Montpellier RIO Imaging we create custom solutions for image analysis and automation tasks based on ImageJ. We provide these solutions in the form of ImageJ macro tool sets. Macro tool sets are easy to install, provide a simple graphical interface, allow to set options and can call plugins for more complex tasks. The first button of each tool set opens the help and installation page of the tool set on the wiki of the facility's project management and task-tracking tool. The wiki contains installation and usage instructions, the tool set macro, links to plugins and other dependencies and example images. A tool set often contains a manual version of the macro that can be used to test the macro on the current image and a batch version that will run the same macro on a set of images. The general approach and a number of available tool sets will be presented here. The tool sets for biological image analysis include: the segmentation of adipocytes, the analysis of multi-well arabidopsis seedling images, the counting of segment like objects, the analysis of virus infections in leaves, the measurement of the interdigitation index and the thickness in skin images and the analysis of wound healing. The tool sets concerning the workflow include: the cropping of regions from big images, the conversion of image formats, the navigation within a set of images, the transformation of ROIs and the order of ROIs in the ROI-manager.

Keywords: ImageJ, microscopy, image analysis, macros, tool sets, automation

1. 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.¹ 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.

While in the past we used the ImageJ based MRI Cell Image Analyzer (CIA) framework,² we switched to the usage of ImageJ macro tool sets³ as the main user interface more recently. The reason for this is that, on the one hand a certain number of features that were missing in ImageJ when the CIA was written are now available. On the other hand macro tool sets are a standard ImageJ feature and need less adaptations as the versions of ImageJ advance. Among the now available features are:

- the **switchable tool bars** allow to provide a set of tools that solve a given problem
- the **options of a tool on the toolbar**, accessible via a right-click, can be set independent from running a macro
- the **waitForUser macro command** allows to have interactive macro tools

Further points that the CIA handled, like for example the independence of the ImageJ operation from the user interface will be solved in ImageJ2.⁴ What is still missing in ImageJ is a more convenient way to specify a list of input images and output folders. We solved this with a plugin Macro.IO.Settings,⁵ that we use in combination with the macro tool sets.

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It has to be noted that 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. Besides of this we wrote a number of tool sets that do not solve biological image analysis problems directly. These are either there to optimize the workflow of a protocol or they make available a functionality used in another tool set which can be interesting in its own right.

2. MATERIALS AND METHODS

We use the web-based project management and task-tracking tool Redmine⁶ to manage the image analysis projects of the facility*. For a new image analysis project an issue is created. Biologists can subscribe to issues and follow the advancement of the work. The tool set macro files and other resources are uploaded to the files-part of the Redmine and ordered by releases. Releases are numbered by the year and the month of the release. The wiki part of the Redmine contains a help page for each macro tool set.

The help page of a tool set starts with a general description of the tool. It contains one or more example images for which the tools work well, using the default options. It explains how to install the tool set and eventually its dependencies and how to get started with it. The meaning of the options of each tool are explained. For each tool 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. This way they can easily be changed. The user can open the tool set macro file by pressing the shift-key while selecting the tool set, change the default values and save the modified file. Macros run in batch mode, by using the `setBatchMode` macro command. Processing in

*<http://dev.mri.cnrs.fr/projects/imagej-macros>

batch mode is faster and safer. Each macro should show the current activity and the progress in a log-window. The ImageJ-progress bar is not appropriate to show the overall progress of the macro, since single commands called from the macro are likely to interfere with it. The macro should clearly show when the processing ended. If the macro worked on a set of files it should list the files that have been processed and those that have been skipped. Macros running on file sets will usually write the measurement results and control images that show the measured objects into a result folder. We use the Macro_IO_Settings plugin to conveniently specify input images and output folders and to access them from macros. 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. This is implemented by running a shell command that closes the operating system session from within the macro. More complex tasks or user interfaces are better implemented as plugins. Macro tool sets can still be used to set the options and to start the tool.

3. RESULTS

In this section a number of macro tool sets developed at Montpellier RIO Imaging will be described. In the first part tool sets that help optimize the workflow or make conversions are presented. In the second part tool sets that solve biological image analysis tasks are described.

3.1 Tool sets for conversions and workflow optimizations

3.1.1 Crop 4D Cells



Figure 1. The Crop 4D Cells tool set.

This tool set allows to copy the interesting parts of big images and to save them to separate image files. Only one region of interest needs to fit into the memory of the machine at a time. The tool set displays a number of buttons to navigate within a defined list of images. These images can be in any format handled by loci-bioformats.⁷ The list of images can be set as an option of the tools of the tool set. Images are opened as virtual stacks. The user makes a selection and the regions of interest are saved when he adds a new one and automatically loaded into the ROI-manager when he opens an image. Once all interesting parts selected on all images the user can run the export, that saves the result images in ics-format⁸ into the results folder.

3.1.2 Image Conversion Tools

The tool converts all images in a folder from the formats lif, lsm and zvi[†] into tif-images.⁹ Each channel of a multichannel image is saved separately and an RGB-snapshot is saved as well. The options allow to apply a z-projection of either all slices or a subset of the slices and to select the channels that will be used in the RGB-snapshot image. The colors of the converted images will correspond to the colors of the original images including for the conversion of the zvi-images.

3.1.3 NDPI Tools

This tool set is useful for the work with images from histological slices. The first tool converts images in the ndpi format[‡] used by the Hamamatsu Nanozoomer into tif-images. The user can select a resolution present in the ndpi-image for the export. The highest resolution is not supported. The second tool tries to select automatically the different cuts on a slide. The arrangement of the cuts can be set in the options of the tool. The selections are added to the ROI-manager and can be corrected by the user. The last tool exports the cuts according to the ROIs in the ROI-manager, each one as a separate image.

[†]Leica Image File Format (lif) is a file format owned by Leica Microsystems GmbH, the Laser Scanning Microscope (lsm) file format and the Zeiss Axio Vision file format are owned by the Carl Zeiss MicroImaging GmbH

[‡]The NanoZoomer Digital Pathology Image format is owned by Hamamatsu

3.1.4 Next Image as Hyperstack Tool

This tool set has two tools. The first converts the current RGB-image into a hyperstack. The second opens the next image in the current folder as a hyperstack. In the options the display mode of the hyperstack and the channel selected by default can be changed. The tool set allows to work conveniently on a given channel in a series of RGB-images.

3.1.5 ROI Converter Tools

These tools have been developed in the context of the Skin Tools[§]. The first tool creates a one-dimensional ROI from an area ROI. The resulting ROI consists of the upper border between the leftmost and rightmost points of the area ROI. With the help of this conversion the tool allows to measure the length of the upper border of an area ROI. The second tool creates a point ROI from a one-dimensional ROI. The resulting points are the local extrema of the original ROI. The options allow to exclude small local extrema. The third tool takes a multiple-point ROI, traces for each point a vertical line from the first pixel above with intensity bigger than zero to the next pixel with intensity zero and adds it to the ROI-manager.

3.1.6 Roi Shuffle Tools

This tool set has been developed for the Leaf Infection Tools[¶]. It allows to easily change the order of the ROIs in the ROI-manager. The up-button moves the selected ROIs one position up in the ROI-manager if possible and the down-button moves them one position down. It is possible to open a separate window containing the two buttons.

3.2 Tool sets for biological image analysis

3.2.1 Adipocytes Tool

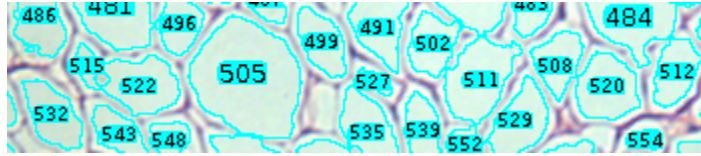


Figure 2. Adipocytes segmented with the Adipocytes Tool.

The Adipocytes Tools help to analyze fat cells in images from histological sections in which the membranes are stained. The preprocessing excludes regions that clearly do not contain fat cells. It is integrated into the two other tools. It is nevertheless available as a separate tool in order to allow to find the right options more easily. The simple method uses automatic thresholding and the particle analyzer to detect the cells. The options allow to apply a binary watershed¹⁰ to separate touching cells. The last method uses a grayscale watershed algorithm. After the exclusion of background areas, a find edges command is called and the result is smoothed with a Gaussian blur filter¹¹ before the watershed is applied. As a result a ROI for each detected cell is added to the ROI-manager.

3.2.2 Arabidopsis Seedlings Tool

The tool allows to measure the surface of green pixels per well in images containing seedlings in multiple wells. It uses color-thresholding in the CIELAB color space¹² for the segmentation. The options allow to change the threshold values and to adapt the macro to the distribution of the wells. It can be run in batch mode on a series of images. The result is a spreadsheet file with the measured area per well and a control image showing the detected surface for each input image.

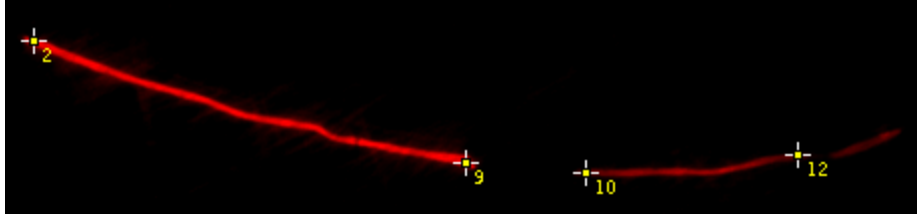


Figure 3. The count segments tool finds the end points of segments.

3.2.3 Count Segments

This tool estimates the number of filament like segments in an image. The segments can cross each other. The idea is to count the end point of segments rather than trying to separate and to count the segments themselves. A blurred version of the image is subtracted from the original image. A Gaussian-blur filter is applied to the result and the image is segmented by using the IsoData-auto-threshold. The result is converted into a mask and skeletonized. The end points of the segments are detected as pixels that have exactly one neighbour.

3.2.4 Leaf Infection Tools

The aim of these tools is to measure the areas of two different epifluorescent stainings and the area of the overlap region of the stainings in images of plant leaves. Each image contains multiple leaves. There is one image for each channel. For each leaf the user adds a rectangular selection in the larger image and the exact selection of the leaf in the cropped region to the ROI-manager. The tool calculates for each leaf the regions of the red staining, the region of the green staining, the region of the overlap of the stainings and Pearson's correlation coefficient¹³ between the two channels. The aim of the experiment is to show that there is mutual exclusion of the two stainings in some cases and not in other cases.

3.2.5 Skin Tools

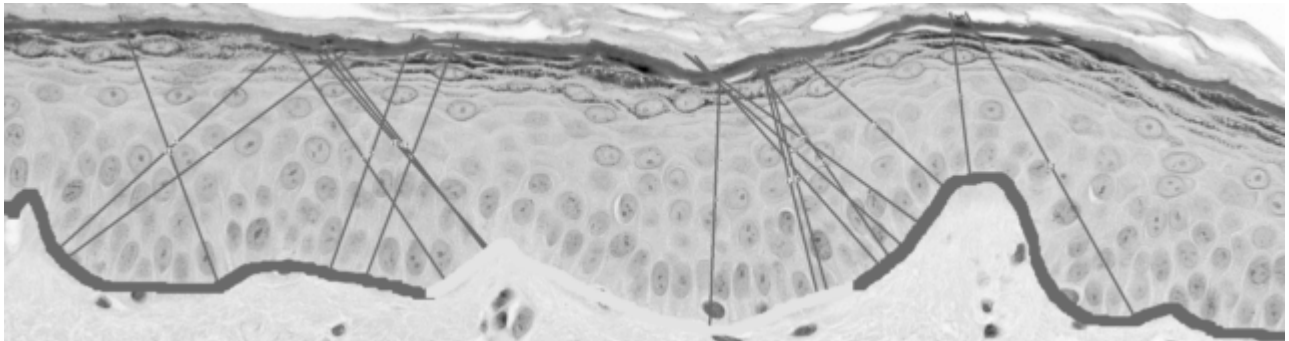


Figure 4. The tool selected the lower border of the epidermis in 3 parts and drew perpendicular line segments to the other side.

The skin tools measure the thickness of the epidermis and the interdigitation index.¹⁴ The input images are masks that represent the epidermis and that have been created from images of stained histological sections. The mask must touch the left and right border of the image. The dermal-epidermal border must be on the lower site of the image. 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. The line segments start at the lower border, are perpendicular to the lower border and end at the opposite border of the mask.

[§]see section 3.2.5

[¶]see section 3.2.4

3.2.6 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.

4. CONCLUSIONS

We provide solutions for image analysis and automation tasks in the form of ImageJ macro tool sets to the users of our facility. 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 for conversions and workflow optimizations and a number of tool sets for biological image analysis have been developed by us and are publicly available.

In the future we plan to combine this work with our central image database project "Web Image and Data Environment" and the project "Remote ImageJ" in order to run batch image processing from client machines and from a web application on distant server machines.

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