(**M**edical **I**mage **P**rocessing In **Py**thon)

User's Guide

Table of Contents

About

Purpose

The goal of the software is to perform post-processing and statistical analysis on a set of images through the Fiji platform. The plugin allows any number of images to be opened and sent through an arbitrary pipeline of processes. After processing, the results will be passed on to statistical software that will provide a final analysis from the image input.

Recommended Users

Recommended users would be those that have programming experience in R. However, users without such experience are given a default R script included in the software to run a generic analysis on inputted images.

Description

The software takes a collection of images as input, either in a folder, or from URL addresses. The user then provides a Fiji macro, designed to operate on a specific file, and generalizes it to operate on all of the provided files. After the processing of the input images, the R script is run and the data from the images is pulled and placed in the output folder selected by the user for use with an external statistical program. The software includes a template R script to aid the user in writing their own R script to accommodate their specific needs, as well as a sample Fiji macro.

Preparation

Prerequisite Software

1. **Fiji** (Fiji is just ImageJ)
    a. Download here: http://Fiji.sc/Downloads#Fiji
    b. Requires **Java**: http://www.oracle.com/technetwork/java/javase/downloads/jre8-downloads-2133155.html
    c. After installing Fiji, update to the latest version by opening the '*Help*' menu and selecting '*Update ImageJ…*'

   d. **NOTE:** Reading the documentation and instructions on Fiji's website is recommended
2. **R**
   a. Required for statistical analysis
   b. To download, go to https://cran.r-project.org/mirrors.html and select a mirror site
   c. **IMPORTANT:** Windows users should take note of where R is installed

Installation

To install the plugin, download the '*.zip*' file below and extract to the '**plugins**' folder in FIji.
https://github.com/pwlandoll/cs470-image-processing/raw/master/Medical_Image.zip.

1. Windows/Linux
   a. Extract the zip file to the '**plugins**' folder of the Fiji.app folder downloaded earlier. If there is an option to create a new folder for the extracted files, do not select it.
   b. Restart Fiji
2. OS X
   a. Double-click/extract the zip file
   b. Open a Finder window, and from the '**Go**' menu, select '**Go to Folde**r'
   c. In the text field, type '*/Applications/Fiji.app/plugins*'
   d. Drag the extracted folder into the open plugins folder
   e. Restart Fiji

Uninstallation

To uninstall the plugin, make sure Fiji is not running and delete the '**Medical_Image**' folder from Fiji's '**plugins**' folder.

Using the Software

Starting the Plugin

1. Start Fiji
   a. On Windows and Linux, the executable file will be in the '*Fiji.app*' folder
   b. On OS X, run the Fiji application
2. In the Fiji '**Plugins**' menu, scroll down and towards the bottom there will be the '**Medical Image**' folder. Inside of this folder select the '**Medical Image Processing**' plugin. Selecting the plugin will run a command to open a window containing the user interface.

Finding the R Executable

If the plugin cannot automatically detect the directory of the R executable in the local computer's file system then the user has to manually find and select the executable. This can result from the R program being moved, missing, or being installed in another location. The R path can be changed by selecting the '**File**' menu option in the plugin window and choosing the '**Change R Path**' option. This option will prompt a file navigation window to popup to find and select the location of the R executable. The '*Rscript.exe*' file needs to be selected in the '**bin**' folder of the R program file. If the location of the R executable is unknown then it is suggested to re-download R and to take note of where it is installed for future reference.

Preparing the Images

All images that you wish to process should be contained in a folder within a directory that **you have access to**. If you do not have permission to access the root folder containing your pre-processed images, Fiji will *not* be able to open the images to perform any processing to them. The folder may contain images of differing file extensions (e.g., *.jpg*, *.png*) and may be of any size, shape, file name, etc. If there are images that do not have one of the default file extensions, you can add a custom extension through the file menu (see *step 5* of the '**Running**' section below).

Create/Generalizing a Macro

MIPPy is designed to use custom user-made Fiji macros to process inputted images. An example Fiji macro is included with the plugin that can be used as a model for developing custom macros. All macros must be written in ImageJ Macro Language and be in the '*.IJM*' file format. The macros must be generalized in order to run on all images during image processing.

To create a macro, first open the '**Plugins**' menu in Fiji, and select '**Macros**' then '**Record…**' to begin recording inputs. Open an image, and perform any of Fiji's image processing features on that image. Take note of the filename and extension (*e.g. 'testimage.jpg'*) as you will be asked for it later.

After completing the macro, the plugin needs to generalize it so that it can be used on any image. To do so, in the plugin's '**File**' menu, select '**Create Generalized Macro File**' option and select the macro that you just created. When prompted, input the name of the image file you used earlier (*e.g. testimage.jpg*). Then, save it to any location (*you will be asked for the generalized macro file later*).

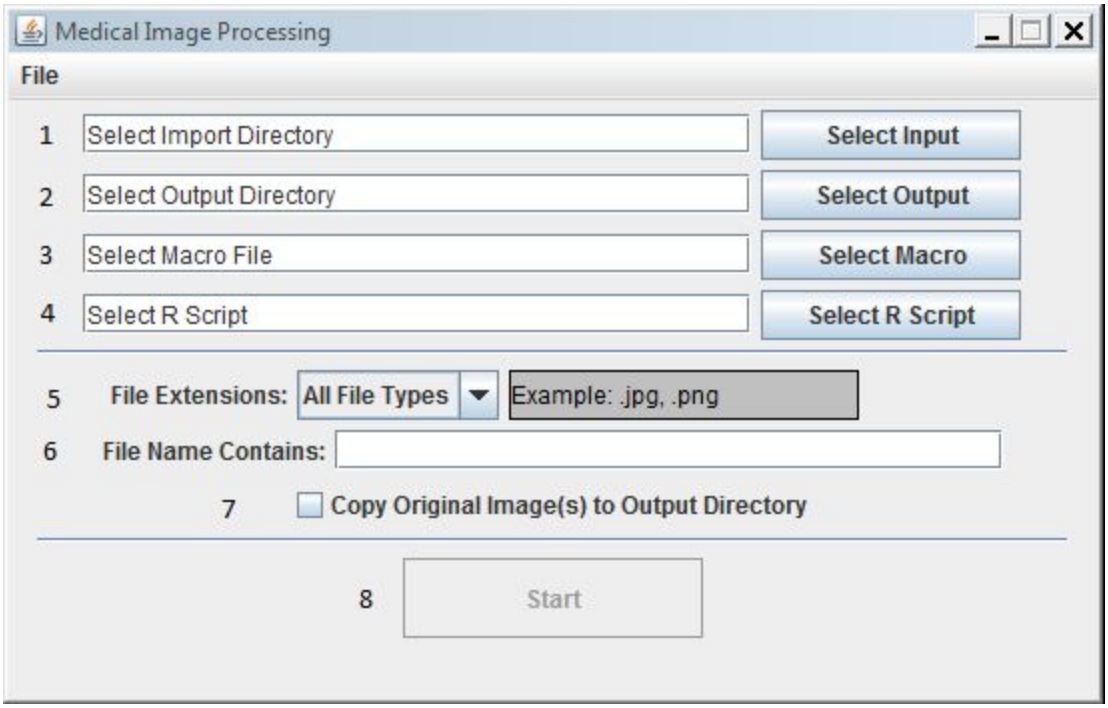
(Optional) Create a Custom R Script

MIPPy is designed to run custom user-made R scripts to evaluate the image(s). Along with the default R script included with the plugin, a R script template called '**template.R**' is also included to model new scripts after. The R script template contains directions and tips on using the template to develop custom scripts that will run with the plugin.

Running

Selecting the '***Medical Image Processing***' option from Fiji's plugin menu will bring up the following window:

Fig. 1



Menu Components (see Fig. 1):

1. Input Directory - Can be navigated to and selected by pressing '**Select Input**' button.
2. Output **Directory - Can be navigated to and selected by pressing the 'Select Output**' button.
3. Directory for the generalized ImageJ macro - Can be navigated to and selected by pressing the '**Select Macro**' button.
4. R script directory - Can be navigated to and selected by pressing the '**Select R Script**' button.

5. File Extensions drop-down menu to either '*Include*' or '*Exclude*' image file types in the input directory during processing.
6. File Name Contains textbox to contain which image file types the system will process based on the selection of the above drop-down menu (**See #5**).
7. Option to copy the original, pre-processed, images into the output folder.
8. Start Button to begin the image processing.

1. Select an Input Directory

The **input directory** (*see #1 above*) can be chosen by pressing the '**Select Input**' button. This button will bring up a menu to either '**Select Directory**' or to '**Select URL File**.' The '**Select Directory**' button will open a file navigation window to find and select the intended folder or archive containing the input image(s). The '**Select URL File**' option will open up the same navigation window but instead of allowing a folder or archive with the pictures to be selected, it allows a text document that contains the URL address(es) to be selected. In order for the plugin to process images from selected URL address(es), the computer must be connected to the internet.

2. Select an Output Directory

The **output directory** (*see #2 above*)can be chosen by pressing the '**Select Output**' button. This button will open up a file navigation window to find and select a folder in which to store the image processing output. An internet connection is required to store the output in a non-local file repository.

3. Select a Generalized Macro

The **generalized macro** (*see #3 above*) can be selected by pressing the '**Select Macro**' button. Pressing this button will open a file navigation window to find and select the desired macro to run on the image(s) during processing. The selected macro must be written in ImageJ Macro Language and be in the "*.IJM*" file format (see the "**Create/Generalizing a Macro**" section above).

4. Select Desired R Script

The **R script directory** (*see #4 above*) can be selected by pressing the '**Select R Script**' button. This button will open up a file navigation window to find and select the desired R script to run during image processing. The R script must be written in the R programming language and be in the "*.R*" file type (see the "**Create a Custom R Script**" section above).

5. (Optional) Include or Exclude Specific File Types

The '**File Extensions**' drop-down menu (*see #5 above*) contains options to either '*Include*' or '*Exclude*' image file types from the chosen input directory. Selecting to include or exclude file types from the drop-down menu allows certain image file types to be specified in the '**File Name Contains**' (*see #6 above*) textbox next to the drop-down menu in the format '*.jpg, .png*'. Selecting the *'Include*' option allows for *only* the file types specified in the '**File Name Contains**' textbox to be included in image processing. Selecting the '*Exclude*' option allows for the file types specified in the '**File Name Contains**' textbox to be excluded from image processing.

6. (Optional) Copy Original Images to Output Directory

The original input pictures can be included in the output directory by checking the '**Copy Original Image(s) to Output Directory**' (*see #7 above*) check box.

7. Start Image Processing

The '**Start**' button (*see #8 above*) will be enabled after selecting the input, output, R script, and the generalized macro. Clicking the '**Start**' button will run the generalized macro on the selected image(s) in the input directory. The R script will then pull the data from the images and place it into the output directory.

Note: A window with a progress bar will open during the image processing. Closing the progress window will cancel the operation.

8. Accessing Program Output

The output from the image processing can be accessed by opening the output folder specified by the output directory before the start of the processing.

Additional Options

Fig. 2

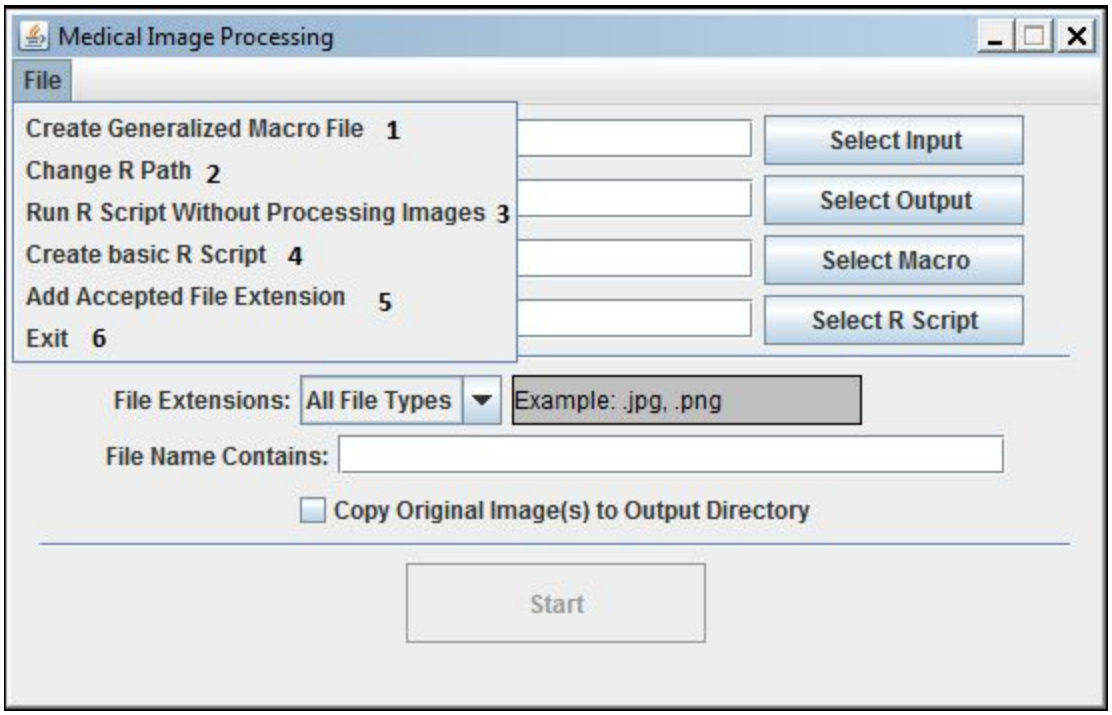
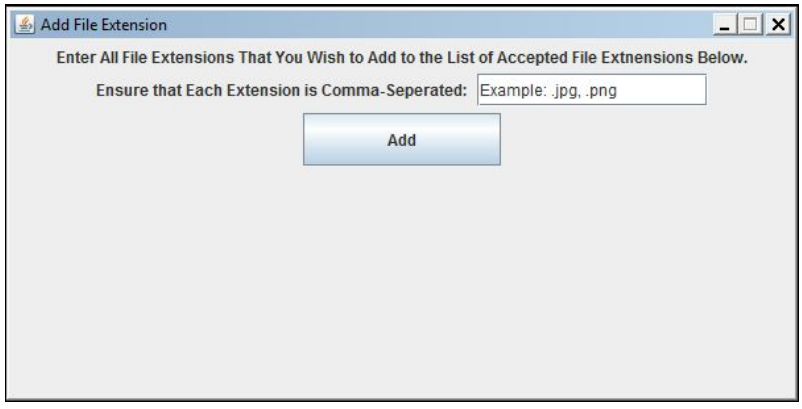


Fig. 2.1



File Menu Options (see Fig. 2):

1. Create Generalized Macro File - After a macro is recorded, it must be generalized so that it can be used in the processing pipeline. Start by selecting the menu option and specifying the original macro file. Then, if during the macro recording process an image

was opened, enter the name of the image (e.g. some_image.png). If no image was opened during the recording process, enter nothing. Then specify a location to save the new generalized macro file.

2. Change R Path - Changes the system's path to your installed R executable (*'RScript.exe*'). Although the system will look for this file by default, you may also manually specify its location via this menu feature (e.g., you wish to use a different version or R). See the "**Finding the R Executable**" section above for more details.
3. Run R Script Without Processing Images - This menu feature grants the ability to perform statistical analyses of images through R without performing any image processing through Fiji. **Note:** You must have an Input and Output directory specified before attempting to run this menu feature.
4. Create Basic R Script - After processing images, the resulting .csv data files can be used to create a basic R script. It will prompt for a *.csv* file, and then two data columns to use to generate basic graphs. The resulting .R file can be entered into the R script box **(see Fig. 1, #4)**, and then can be run with the '*Run R Script Without Processing Images*' menu option (**see Fig. 2, #3**).
5. Add Accepted File Extensions **(see Fig. 2.1)** - Brings up a new window for the user to add file extensions (e.g., *.jpg*) to the list of file extensions accepted by the system. The system comes with a default list of accepted file types (located in Fiji's plugins directory) called '*acceptedFileExtensions.txt*.' If the system is unable to recognize one or more of your files' extensions, you must use this menu feature to inform the system that your file is valid for processing. **Note**: Directions to remove accepted file types are outlined in the "**Troubleshooting**" section below.
6. Exit - Closes the application.

The plugin can be closed by selecting the '**File**' menu option and clicking '**Exit**' or by simply closing the plugin window.

Note: If any errors occur during the running of the application, please reference the "**Troubleshooting**" section of this guide included below.

Troubleshooting

1. ***"Permission Denied"***

- When the plugin menu option in Fiji is selected, the "Medical Image" folder should be towards the bottom of the plugin list. This folder should only contain the "Medical Image Processing" and "View Step" commands. If the folder doesn't contain these commands or it contains other files please uninstall and reinstall the latest version of the plugin with the above files. There might also be a problem with one of the data storage files. Ensure that the user has permission to read and write from the 'Medical_Image' folder in Fiji's 'plugins' directory, in particular the 'user_paths.txt' file.

2. ***"System Cannot Find R Path"***

- Upon running the application, the system will attempt to find the location of your R executable file (*RScript.exe*) by checking the default R installation directory. If the system is unable to find the file, it will prompt you to locate the file yourself. If you have forgotten or are unsure of the file's location, the best workaround is to re-download R and ensure to take note of where it is being downloaded to. R can be downloaded from https://cran.r-project.org/bin/windows/base/.

3. "***Selected Directory is Empty: Please choose a directory that contains at least one image"***

- This error occurs when the chosen input directory contains no images that match the valid image types. It also occurs when attempting to use a URL file where each attempt to download an image is unsuccessful. Ensure that your computer has a functioning internet connection, and check the file of the URL addresses to ensure that each one is valid

4. ***How to remove a previously added accepted file extension***

- If you wish to remove an extension that you added to the list of accepted types (e.g., perhaps due to a misspelled name), you must manually delete it from the text file - '*acceptedFileExtensions.txt*.' This file is located within Fiji's installation directory. Specifically, the file can be found in "**Fiji.app** -> **plugins** -> **Medical_Image** -> **acceptedFileExtensions**." Find the extension(s) you wish to remove, delete them from the file, and save it.

Glossary

- **Fiji (Is Just ImageJ)** - An image processing package also . It can be described as a "batteries-included" distribution of ImageJ (and ImageJ2), bundling Java, Java3D and a lot of plugins organized into a coherent menu structure. The main focus of Fiji is to assist research in life sciences. (*from: http://Fiji.sc/Fiji*)

- **ImageJ** - An open source image processing program designed for scientific multidimensional images. (*from: http://imagej.net/Welcome*)

- **ImageJ Macro Language (IJM)** - A scripting language built into ImageJ that allows controlling many aspects of ImageJ. It is used to create Macro files that are necessary for the application to perform image processing.
(*from: http://rsbweb.nih.gov/ij/docs/macro_reference_guide.pdf*)

- **Input** - The image(s) that you wish to process. You may choose to provide a Directory, URL File, or Archive File.

- **Input Archive Directory** - The location of a compressed file (*.zip* format) that contains images.

- **Input Directory** - The location of the folder that contains your images (e.g., C:\Users\My Pictures).

- **Macro** - Programs written in the IJM. Can be used to perform sequences of actions in a fashion expressed by the program's design.
(*from: http://rsbweb.nih.gov/ij/docs/macro_reference_guide.pdf*)

- **Non-local** - A computer, network, or server separate from the computer used to run Fiji.

- **Output Directory** - The location of the file directory that you wish to save your processed image(s) to (e.g., C:/Users/My Documents/Output Folder). You may also choose to create a new folder within the file browser itself.

- **R** - A language and environment for statistical computing and graphics. It is a GNU project which is similar to the S language and environment which was developed at Bell Laboratories (formerly AT&T, now Lucent Technologies) by John Chambers and colleagues. R is available as Free Software under the terms of the Free Software Foundation's GNU General Public License in source code form. It compiles and runs on a wide variety of UNIX platforms and similar systems (including FreeBSD and Linux), Windows and MacOS. (*from: https://www.r-project.org/about.html*)

- **R Path** - The location of your R executable file (*RScript.exe*). By default, this file is contained in your specified R installation directory.

- **R Script Directory** - The location of an R Script file ('*.R*' format) that will perform your desired statistical analyses.

- **URL Input File** - The location of a file ('*.txt*' format) that contains file paths/URL locations of images.