Operating Instructions

Macro 1 - a-3D_Close&Fill.txt

1. This macro will try to close holes in the vessel wall dilating and eroding the image by 3D convolution with cross or square kernels according to a number of cycles defined in the Dialog window.
2. Then the macro will execute the ImageJ command "Fill Holes" for every slice in the 3 cartesian orientation of the stack.
3. To avoid filling intervascular spaces, the added areas will be classified according to an user defined threshold and those larger than the threshold will be discarded.

Version hystory:
v. 0.1 released 09.05.2011 first relase
v. 0.2 released 09.05.2011 rewritten to introduce functions
v. 0.3 released 10-05-2011 after the first fill holes it performs a second passage in order to fill additional cavities created by the first fill-up.

Dialog interface:
Select the number of dilation/erosion cycles - default =2
Select the limit of the filled areas - default =1200

Then the macro would present a message, after which it will present a File Input dialog:
- Select the folder grouping all the binary stacks with empty vessels to fill-in

Input files:
binary isotropic stacks in .tiff format

Then the macro would present a message, after which it will present a File Input dialog:
- Select the folder where you want to collect the filled vessels

Output files:
The macro will produce 3 folders: Closed, Filled and Final grouping files at the 3 different steps. The output files are .tif image stacks saved in the Final folder which could be further processed.

Macro 2 - b-ClassifyVessels.txt

Analytical routine for volume filtering using ImageJ Analyze Particles fuction. The macro isolates and recover voxels belonging to particles with area lower than a defined threshold in each of the 3 Cartesian planes passing through the voxel. It starts from a cubic image stack. If the original volume is not a cube, but shows a square base, it is converted into a cube by addition of an appropriate number of slices at the bottom of the stack. These slices are eliminated at the end of the elaboration.
If the original volume is taller than a cube, the routine dilate the canvas of each slice, keeping the image centered, until the volume represents a cube. The modified volume is then reconverted to the original dimensions by cropping at the end of the elaboration.
The macro is dependent from the TransfomJ sets of plugins developed by Meijering J. [https://imagescience.org/meijering/software/transformj/].

The routine filters one or more stacks using user-defined thresholds. For each input stack it generates a set of 7 stacks grouping the desired bandpass voxels. Voxels belonging to particles larger than the largest threshold are not considered and therefore discarded.
Thresholds are defined as it follows:
the lowest threshold is called "offset" and can be choosen among 5 dimensions (in square pixels). It identify the lowest class of calibers, those showing a cross-section comprised between "offset" and 0 square pixels.
the second threshold is a multiple of "offset". This multiple can be choosen among 6 choices ranging from 1.5 to 2.0 values.
similarly, each of the following thresholds will be the choosen multiple of the previous one.

v. 0.1.0 released 24.12.2010 first version, cube-only analyses
v. 0.2.0 released 30.12.2010 analysis of volumes smaller than cubes but presenting a square base.
v. 0.3.0 released 08.02.2011 after the filtering steps, the routine creates the bandpass stacks grouping 3D caliber-filtered particles on the basis of pre-defined thresholds.
v. 0.4.0 released 28.07.2017 bandpass stacks are now created according to user-defined thresholds
v. 0.5.0 released 04.05.2018 this version elaborates also original stacks taller than a cube.

Dialog interface:
You will be asked to define the appropriate set of thresholds. First of all you have to choose an offset from a popup menu with 5 choices. This value will set the lowest threshold and will define the lowest class: that with cross-section values comprised between "offset" and zero.
Sometimes, vessels are very large and small offset define void classes or grouping only background noise.

Then, you will have to choose a base (a multiplier) from a pop-up menu with 6 choices.
This multiplier will define all of the further thresholds. It will be applied to "offset" to define the second threshold, then the second will be multiplied to get the third threshold, and so on until defining a total of 7 classes of calibers.

Then the macro would present a message, after which it will present a File Input dialog:
Select the folder grouping all the filled binary stacks to be classified according to the choosen set of thresholds

Input files:
binary isotropic stacks in .tiff format representing the filled vessels

Then the macro would present a message, after which it will present a File Input dialog:
Select the folder where you want to collect the folders containing the classified vessels

Output files:
Starting from an initial angioarchitecture (a .tiff file) the macro will output a folder, beginning with the "bp" string, containing 7 different stacks characterized by the limits of the used thresholds. Each stack harbours only the voxels classified as belonging to that class.
The "bp" folder must be used in the next step in order to recover the original signal by intersection.

Macro 3 - c-OriSignalRecovery.txt

This macro uses a set of stacks containing voxels from solid vessel masks and classified as belonging to a defined vascular cross-section, to intersect signals from an original binary angioarchitecture and decomposing it into a set of stacks with caliber signal voxels.
The macro needs two input folders and can work only with strictly equal numbers of masks and angioarchitectures. It verifies the correspondence of the name of the original stack with the name of the caliber masks and abort the macro if something is wrong.

Version hystory:
v. 0.1 released 26.05.2011 first relase

The macro would present a message, after which it will present a File Input dialog:
Select the folder grouping all the original unfilled binary stacks whose voxels are to be classified according to the calibered masks obtained in the previous step.

Input files:
binary isotropic stacks in .tiff format

The macro would present a message, after which it will present a File Input dialog:
Select the folder grouping all the folders containing the calibered stacks to be used as intersecting masks. Each subfolder is referring to a single original angioarchitecture.

Service files:
binary isotropic calibered masks in .tiff format

Then the macro would present a message, after which it will present a File Input dialog:
Select the folder where you want to collect the classified voxels.

Output files:
The macro will output a folder for each angioarchitecture grouping the stacks showing the location of the original voxels classified by cross-section.

The macro can abort for two different reasons:
1. the number of stacks in the input folder is different from the number of subfolders in the Service folder
2. the title of the original image is different from the title of the service folder - once the last has been removed of the additions inserted in the previous steps.

BEWARE: Images and masks are paired by alphabetical order. Therefore, it is important do avoid renaming images in the course of the analysis. The developed Macros are tailored to cope each other and renaming the processed images at any step can cause a crash in the processing. In this respect images are usually ordered by an alphabetical prefix which states the order by which they are processed.

Macro 4 - d-BuildProgressiveArchitectures.txt

ImageJ script for constructing partial angioarchitectures by cumulating sequential classes of vessels with decreasing calibers.

This script sums passband images which SHOULD be organized in a 2-level nested folder and named in order to be listed from lower to higher calibers.

Version history:
v. 0.1.0 released 26/05/2011 first release
v. 0.2.0 released 29/08/2011
v. 0.3.0 released 08/01/2019 main changes: dialog interface and reconstruction of angioarchitectures from vessels classified according to different sets of thresholds.

The macro would present a dialog interface, in which it asks for the set of thresholds used to classify vessels. Sets are characterized by a specific ratio among thresholds ranging from 1.6 to 2.0.

The macro would present a message, after which it will present a File Input dialog:
Select folder grouping folders with images to be combined.

Input files:
a folder contining a set of subfolders, each referring to an original angioarchitecture. Each subfolder present 7 binary isotropic stacks in .tiff format containing voxels classified as belonging to vessels with specific cross-sections. Stacks are listed in alphanumeric order ranging from lower to higher calibers.

Then the macro would present a message, after which it will present a File Input dialog:
Where should I save combined images?

Output files:
The macro will output a subfolder for each angioarchitecture (subfolder) grouping the 7 stacks in .tiff format representative of progressively reconstituted angioarchitectures.

Macro 5 - e-NormalizingVolume.txt

This macro performs the assessment of the percent volume occupied by signal (black voxels) in a binary volume

Version hystory:
v. 0.1 del 21.11.2010
v. 0.2 del 08.01.2011 version for independent samples
v. 0.3 del 19.12.2018 version corrected for images with measured sizes

The macro would present a message, after which it will present a File Input dialog:
Select the folder containing subfolders with the progressively reconstituted angioarchitectures.

Input files:
a folder contining a set of subfolders, each referring to an original angioarchitecture. Each subfolder present 7 binary isotropic stacks in .tiff format representative of partially reconstituted angioarchitectures.

Then the macro would present a message, after which it will present a File Input dialog:
Select the folder where you want to collect the result files.

Output files:
The macro will output a .txt file for each angioarchitecture (subfolder) grouping the results from the stacks representative of progressively reconstituted angioarchitectures.

Before proceding to the analysis of signal amount and dispersion, the indicated normalizing volume must be duplicated, renamed and copied into each subfolder to be analyzed.