Article supplementary material

Image processing commands and functions

The following document is a step-by-step description of the commands and functions that have been applied for image processing using ImageJ/Fiji software (ImageJ 2.1.0/1.53h/Java 1.8.0_66 (64-bit)).

Downloading *3D Suites* plugin is mandatory to fully perform the following instructions.

After each step, the image should be saved as a new TIFF file.

1. Image conversion and calibration

The stack is converted to 8-bits format using the following functions: *Image > Type > 8-bit*

The stack is calibrated using the following functions: *Image>Properties…>* The voxel width, height and depth are entered.

2. Grey-level segmentation

The extraction of the 2 structures of interest is performed by a segmentation technique based on 2 greyscale-defined thresholds. The threshold values are determined from the histogram of frequency distribution of the voxel greyscale values.

The histogram is generated using the following functions: *Analyze>Histogram>* Click *'Yes'* to consider all the images of the stack. The histogram hence appears in a new window.

A threshold is applied using the following functions: *Image>Adjust>Threshold…>* It should be checked that only '*Dark background*' and '*Stack histogram*' are ticked. The minimum threshold value is set using the command *>Apply>* selecting '*Default*' in *Method* and '*Dark*' in *Background*, then ticking '*Black background (of binary masks)*'" *>OK*

3. Filtering

A 3D median filter is applied using the following functions: *Process>Filters>Median 3D…>* Enter '*3*' as a value for x, y and z radii.

4. 3D Manager Options

The isolation of the glomeruli is performed using the *3D Manager* function of the *3D Suites* plugin.

First, the function had to be programmed with the following functions: *Plugins> 3D> 3D Manager Options>* In '*Measurements*' tick the boxes '*Volume (unit)*', '*Surface (unit)*', '*Compactness*', '*Fit Ellipse*'> In '*Options*' tick the boxes '*Use 32-bits image for segmentation (nb objects > 65 535*' and '*Sync with selected 3D viewer*'> Enter, respectively *'10'* and *'1.8'* as values for *'Distance between centers (pixel)* and *'Distance max contact'*

5. 3D volume segmentation

The isolation of the glomeruli is performed in 3 stages using the following functions: *Plugins> 3D> 3D Manager>* '*RoiManager3D*'. '*RoiManager*' window is open.

The first stage consisting in the elimination of the small non-glomerular labeled objects based on a 3D volume segmentation is performed using the following steps:

a. A 32-bit analyzable version of the filtered imaged is generated by using the following command: Click '*3D Segmentation*' and set '*Low Threshold*' value at '*1*'.

b. Close the previous image.

c. The objects on the image are added individually in the '*RoiManager3D*' using the following command: Click '*Add Image*'.

d. A table with the volume information of each object is generated using the following command: '*Measure 3D*'.

e. The table generated is saved using the following command that: *'Menu'*> *'Save'*.

f. Once the volume threshold using the *'Vol (pix)'* column is obtained, the objects on the table are classified according to their volume by using the following command: Click on '*Vol (pix)'* column.

g. The objects with a volume equal or higher to the volume threshold are selected on the table, and they are shown on the *'RoiManager3D'* window using the following command on the table window: *'Menu'*> *'Show Objects'*.

h. In order to isolate these objects without using a high-capacity computer, and thus avoiding time waste, first, the image is converted to 8-bits format using the following functions: *Image* > *Type* > *8-bit*; and second, the large objects are erased from the stack using the following command *'RoiManager3D'* window: '*Erase*'.

i. The resulting image is saved and the '*3D Manager'* windows are closed.

j. A threshold is applied on the image to binarize it using the following functions: *Image*>*Adjust*>*Threshold…*> It should be checked that only '*Dark background*' and '*Stack histogram*' are ticked. The minimum threshold value is set at *'1'* using the command >*Apply*> selecting '*Default*' in *Method* and '*Dark*' in *Background*, then ticking '*Black background (of binary masks)*'" >*OK*.

k. The initial filtered image is open.

l. The image eliminating the small non-glomerular labeled objects is obtained by fusing the filtered image to the last obtained image using the following commands: *Process*> *Image Calculator…* > Select the initial filtered image as first and the last obtained image as second image > Choose the *'Subtract'* operation > Tick only *'Create new window'* > OK.

m. The resulting image is the 3D volume segmentation result.

6. 3D shape segmentation

The second stage consisting in the elimination of the large non-glomerular labeled objects based on a 3D shape segmentation is performed using the following steps:

a. The '*3D Manager' is* opened by using the following function: *Plugins*> *3D*> *3D Manager*> '*RoiManager3D'* window is open.

b. Steps *4.a to 4.e* are repeated.

c. Once the 'RatioVolEllipsoid' threshold using the *'RatioVolEllipsoid'* column is obtained, the objects on the table are classified on their volume by using the following command: Click on '*RatioVolEllipsoid'* column.

d. The objects with a 'RatioVolEllipsoid' equal or inferior to the 'RatioVolEllipsoid' threshold are selected on the table, and they are shown on the *'RoiManager3D'* window using the following command on the table window: *'Menu'*> *'Show Objects'*.

e. The objects are erased from the stack using the following command *'RoiManager3D'* window: '*Erase*'.

f. The resulting image is saved and the '*3D Manager'* windows are kept open.

7. Artefacts elimination

The last stage consisting in the elimination of the artefactual objects is performed using the following steps:

a. On the table, the objects with a volume inferior to 10,000 pixels are selected and they are shown on the *'RoiManager3D'* window using the following command on the table window: *'Menu'*> *'Show Objects'*.

b. The objects are erased from the stack using the following command *'RoiManager3D'* window: '*Erase'*.

c. A final table with all the desired information is generated using the following command: '*Measure 3D'*.

d. The generated table is saved using the following command that: *'Menu'*> *'Save'*.

e. The final image is saved and the '*3D Manager'* windows are closed.