

Bio-EdIP: An Automatic Approach for in vitro Cell Confluence Images Quantification

User Manual

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This manual provides the documentation to run Bio-EdIP. This is a software tool to automatically quantify cell-covered and cell-free regions using in vitro cell culture images. Bio-EdIP incorporates a user-friendly graphical interface.

Image analysis conducted by Bio-EdIP can be exported and saved in a selected folder.

Bio-EdIP has been developed in MATLAB R2012a. This software has been tested in Windows 7, 8 and 10.

To download this tool uses the following address:
<http://be.itm.edu.co/>

1. Installation

Download the executable file (available at <http://be.itm.edu.co/>)

1. Download “Bio-EdIP.rar” file (373 MB).
2. After downloading, place the file in a new empty folder and then unpack it.

Installing Bio-EdIP

This software tool was compiled in MATLAB R2012a. Although Bio-EdIP does not require MATLAB to run, installing MATLAB Compiler Runtime (MCR v7.8) is needed. This free software is included into “BioEdIP_pkg” file. For computers with MCR v7.8 please download “Bio-EdIP(without MCR).rar”, unzip the file and continue with steps 3 and 6.

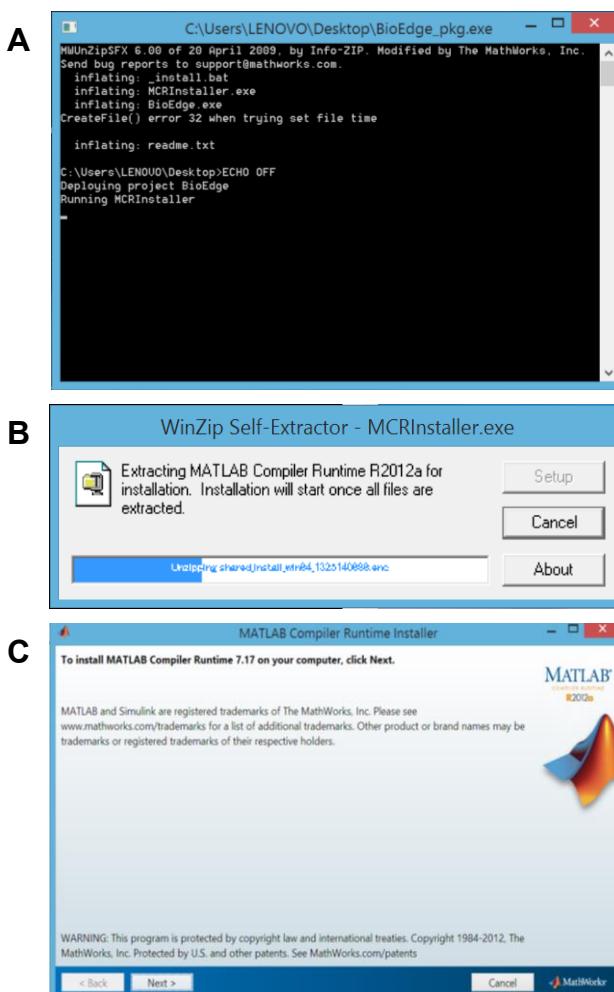
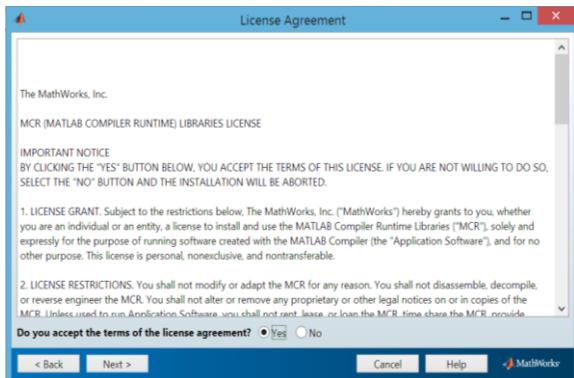


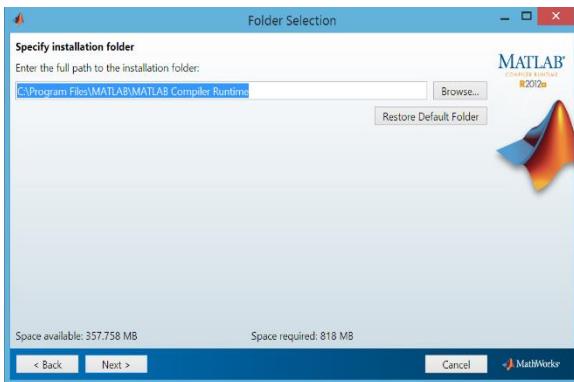
Figure 1. Extract MCR program

3. Open the “BioEdIP_pkg” file. This action will generate an emerging window (Fig. 1A), which must not be closed. Then a new window will appear (Fig. 1B). Wait while MCR program is extracted. When this process is done the installation of MCR will start (Fig. 1C). Please do not close the previous windows.
4. Continue with the installation: accept the terms of use (Fig. 2A), Specify an installation folder or use generated automatically (Fig. 2B), then press “Install” button (Fig. 2C).
5. Finally, press the “Finish” button (Fig. 2D). Automatically all emerging windows will be closed.
6. In this moment Bio-EdIP can be open.

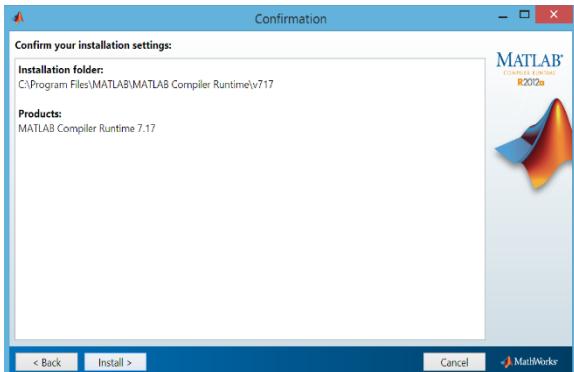
A



B



C



D

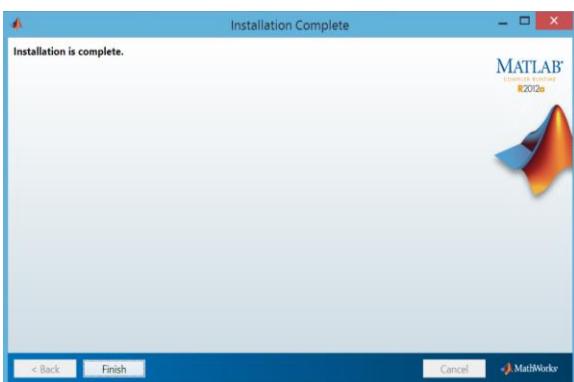


Figure 2. Install MCR program

Initialization of Bio-EdIP

Installation generates three additional files in the folder containing “BioEdIP_pkg” file (Fig. 3). In order to open the software tool, press double click in “BioEdIP” file.

NOTE: MATLAB standalone applications need few minutes to open. Time required depends on the technical specifications of the computer used.

	Nombre	Tipo	Tamaño
<input checked="" type="checkbox"/>	BioEdIP	Aplicación	12.646 KB
<input type="checkbox"/>	BioEdIP_pkg	Aplicación	372.495 KB
<input type="checkbox"/>	MCRInstaller	Aplicación	361.095 KB
<input type="checkbox"/>	readme	Documento de texto	2 KB

Figure 3. Bio-EdIP application

Appearance

Bio-EdIP (Fig. 4) was designed with the aim to provide a user-friendly experience. In screens with a high pixel density, the interface could appear blurred. To solve this problem, press right click over “BioEdIP” file. In properties select the compatibility tab, then select the option “Disable display scaling on high DPI settings”

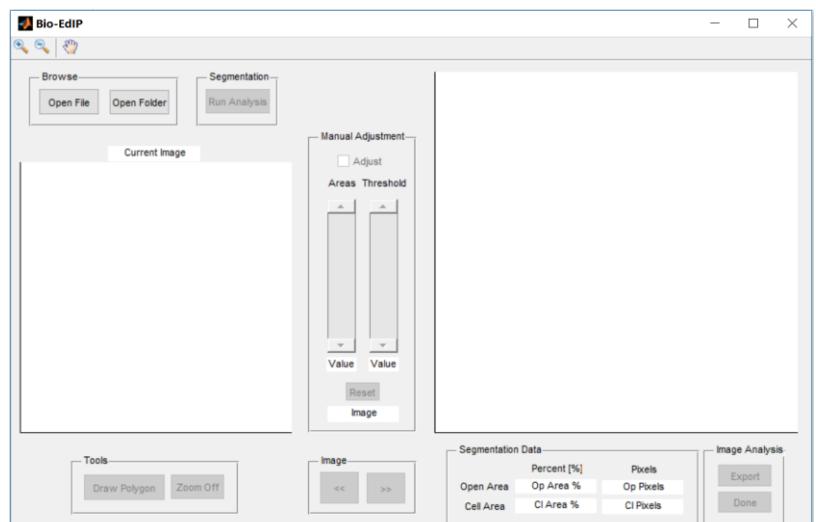


Figure 4. Bio-EdIP user interface

Bio-EdIP uses a segmentation algorithm, which could be summarized as follows:

1. Image pre-processing: here, a given image is converted to gray scale. This image is improved through spatial filtering.

2. Starting pixels' selection: this stage is composed of local processing techniques based on pixel intensities.

Initially, the image binarization is carried out based on histogram mode. Selecting starting pixels is carried out by local processing of binary image.

3. Segmentation: an automatic morphological reconstruction-based segmentation algorithm is used to segment cell-free region, further conserving growth morphology.

Quick Start

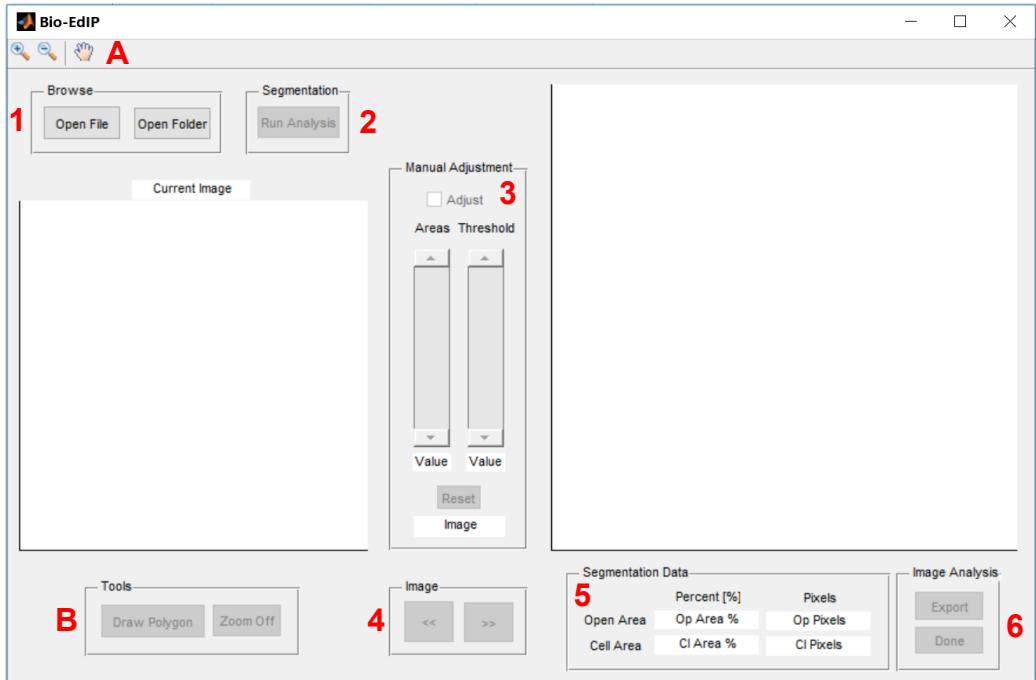


Figure 5. The Bio-EdIP graphical user interface

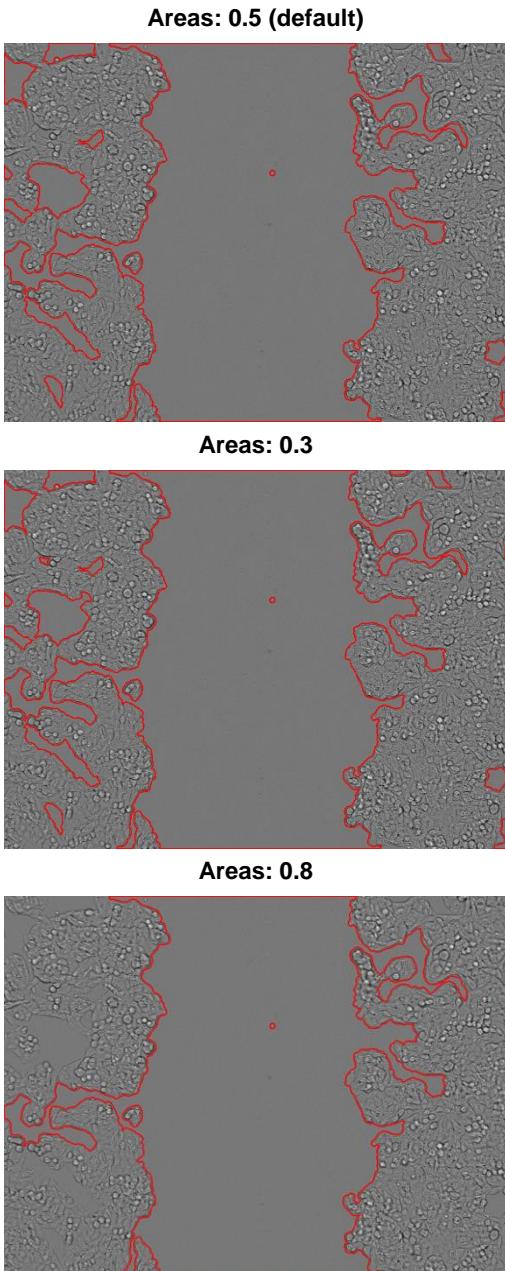
Red numbers (Figure 5) correspond to the image processing panels and the red letters represent additional tools incorporated in Bio-EdIP GUI. The following step-by-step instructions are according to the numbers in image 5:

1. Browse panel: image analysis could be performed using a single image (press the “Open File” button) or selecting a folder that contains several image files (press the “Open Folder” button).

When the image or folder are loaded, the title bar will show the path of the selected object in the computer. Additionally, in the left white box (“Current Image”), a representative image will appear.

2. Segmentation panel: both single and multiple image analysis modes are performed by pressing the “Run Analysis” button. Processing time will be proportional to the number and size of selected images. An emerging window will show the progress whereas in title bar it is specified the status of the segmentation.

3. Manual Adjustment panel: once the automatic segmentations ends, buttons in this panel will be able to use. Segmentation parameters can be manual-set, enabling the check box “Adjust”. Then, use the sliders “Areas” and “Threshold” to modify the segmentation. This process can be done in each image (see number 4). This panel contains a button identified as “Reset”, which restores the segmentation to default.

**Figure 6. Customization of “Areas” slider**

“Areas” and “Threshold” parameters: Bio-EdIP segmentation is performed with two adjustable parameters referred as “Areas” and “Threshold”. “Areas” means the size of segmented areas. When this slider is moved down, the software could detect small cell-free regions in addition to those segmented during automatic analysis. Increasing “Areas” value causes to miss small regions. (Fig. 6). “Threshold” parameter is the variance of the morphological reconstruction-based segmentation algorithm.

NOTE: modifying segmentation parameters is optional, use them only if the segmentation results need to be improved. Otherwise continue with the step 4.

4. Image panel: Buttons here are used to switch between images, they are useful to check and modify (if necessary) the segmentation, when using in multiple image analysis mode.

5. Segmentation Data panel: here, the data obtained from segmentation is shown. Bio-EdIP provides the area occupied by cells (Cell Area) and the cell-free area (Open Area). The area is measured in percentage (respect to image size) and amount of pixels.

6. Image Analysis panel: pressing the button denoted “Export” opens an emerging window, where the user can select or create a folder to save the segmentation results. Segmentation data is exported as a Excel file. Furthermore, for each processed image, Bio-EdIP generates three graphical results: a binary image, an image with outlined edges and the original image with the segmented region. Finally, press “Done” to start a new analysis.

A. Tools bar: it presents three tools: zoom in, zoom out and panning.

B. Tools panel: “Draw Polygon” only will be enable when the user uses a single image. This button allows a single image to be manually segmented. Please see the “Analysis Mode” section for specifications about this kind of analysis. “Zoom Off” is an additional tool to return to image original size.

Title bar: Please make sure to constantly check the title bar to review the segmentation status and the different processes.

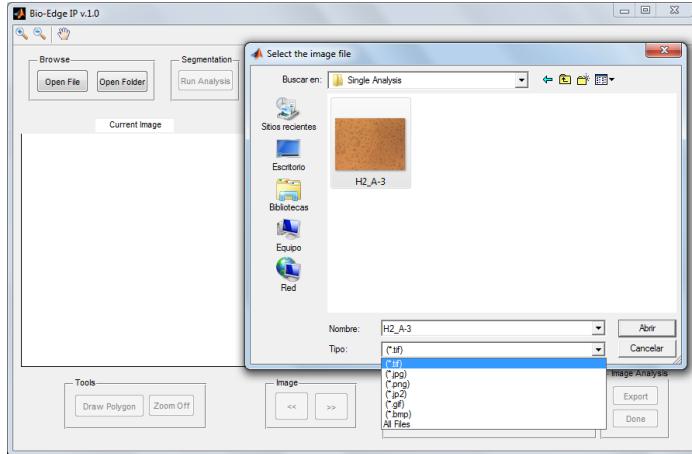
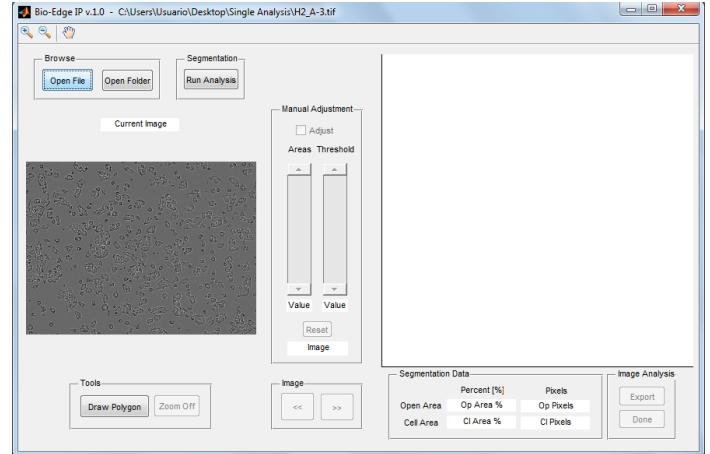
Analysis Mode

This section shows examples of the different image segmentation modes when using Bio-EdIP. Images used for examples can be downloaded from “Constructed dataset.rar” file.

Single Analysis

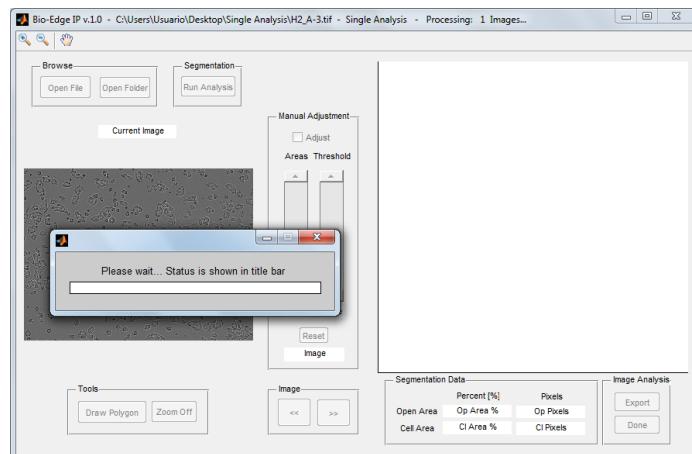
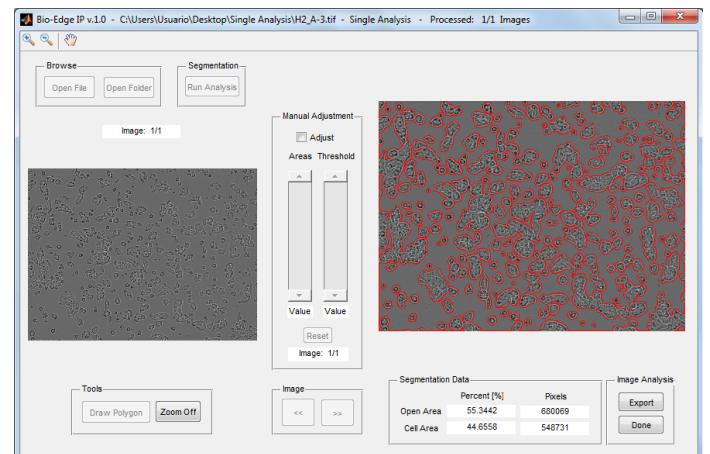
In order to analyze an image, please follow these steps:

1. Press “Open File” and select the image (A).
2. Wait while the image is loaded. In the title bar the path of the loaded image will be shown (B).

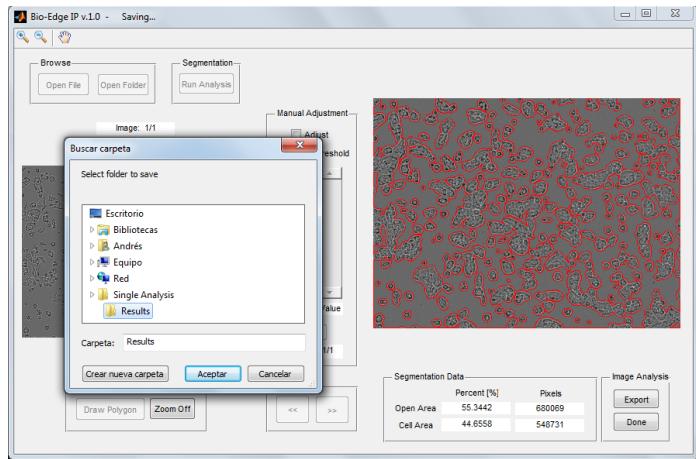
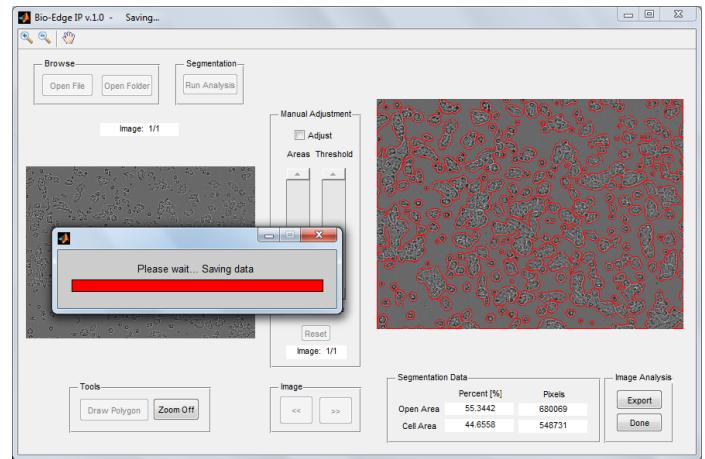
A**B**

3. Press “Run Analysis” (C).

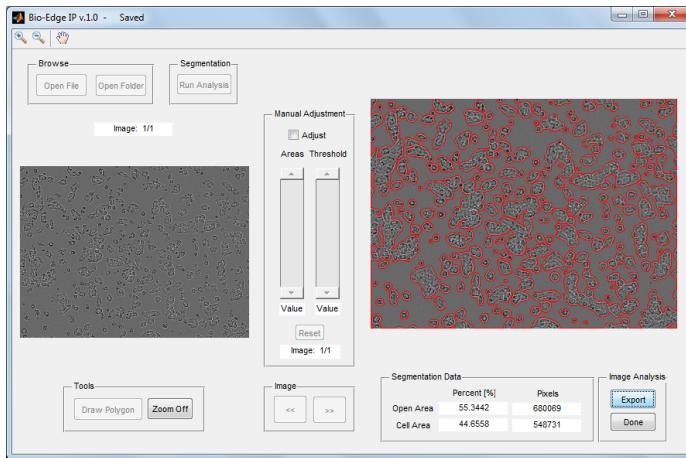
4. Wait a moment, segmentation ends when the segmented image appears, the emerging window will automatically close (D).

C**D**

5. The results can be saved by pressing “Export” in Image Analysis panel (E).
6. Process of exporting data is shown in a new emerging window (F).

E**F**

7. Wait until in title bar the word “Saved” appears. To start a new analysis please press “Done” (G).

G

8. Results:

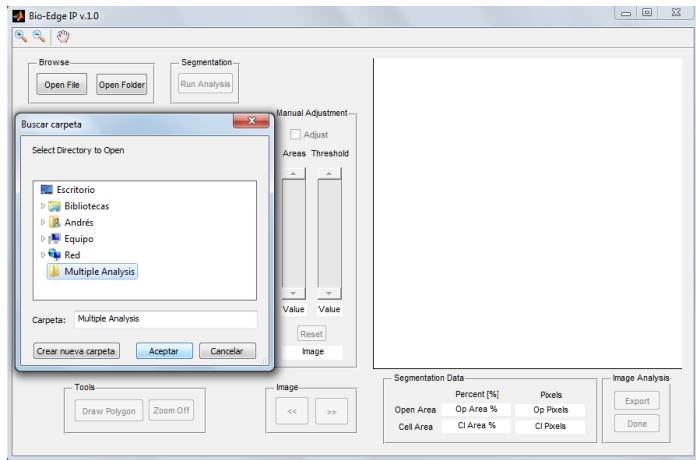
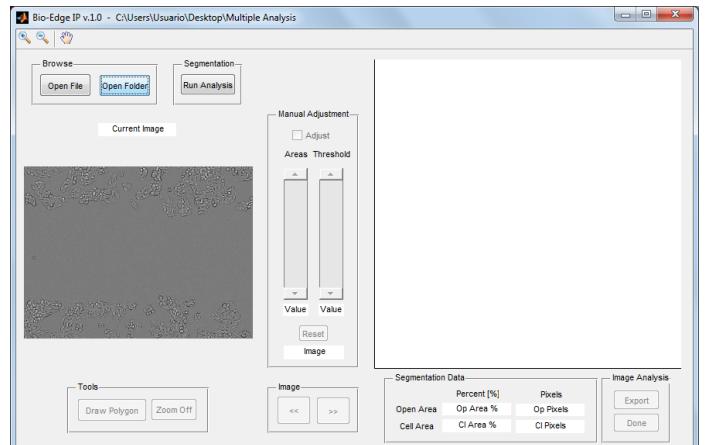
For each processed image is generated three TIFF files denoted like:

ImageName_Binary.TIFF
ImageName_Outline.TIFF
ImageName_Overlay.TIFF

In addition an Excel file is created, which contains the segmentation data.

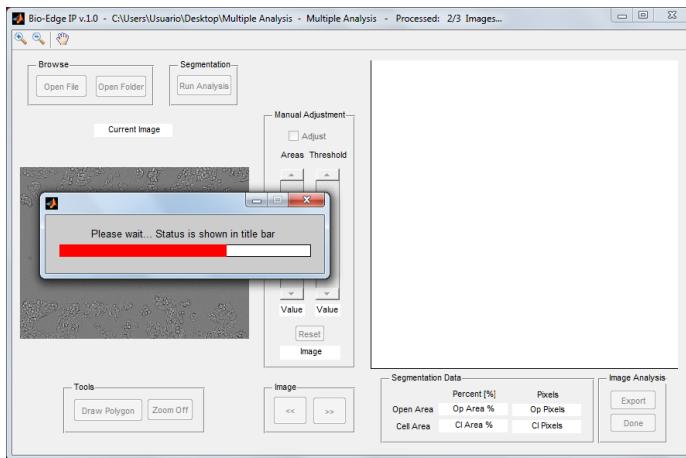
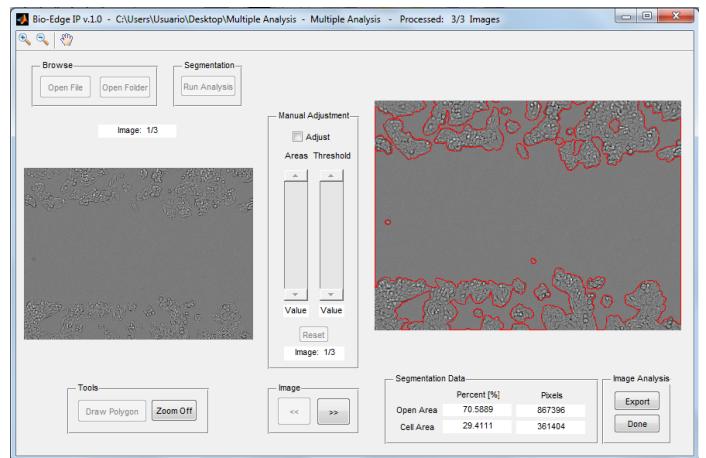
Multiple Analysis

1. Press “Open Folder” in order to select a folder containing several images (A).
2. Similar to Single Analysis mode, when the folder is loaded, an image from the folder will appear in the left side of interface (B).

A**B**

3. Press “Run Analysis” in Segmentation panel (C).

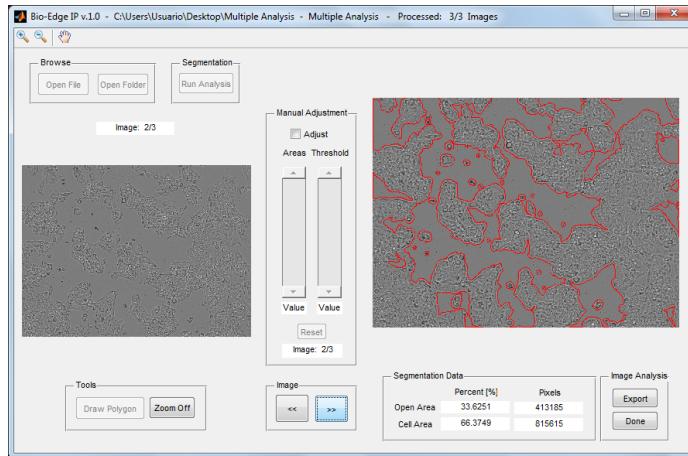
4. Wait until the segmentation process ends. The first segmented image will show in the right side (D).

C**D**

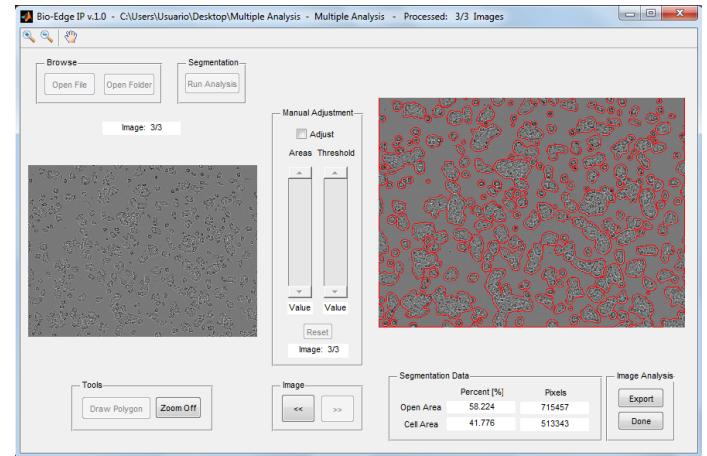
NOTE: Manual Adjustment panel is useful if segmentation needs to be improved. Each image can be modified by moving the sliders after to enable the check box “Adjust”. In Manual Adjustment section, this process is exemplified.

5. In Multiple Analysis mode, user can check the processed images by using the («) and (») buttons in “Image” panel (E and F).

E



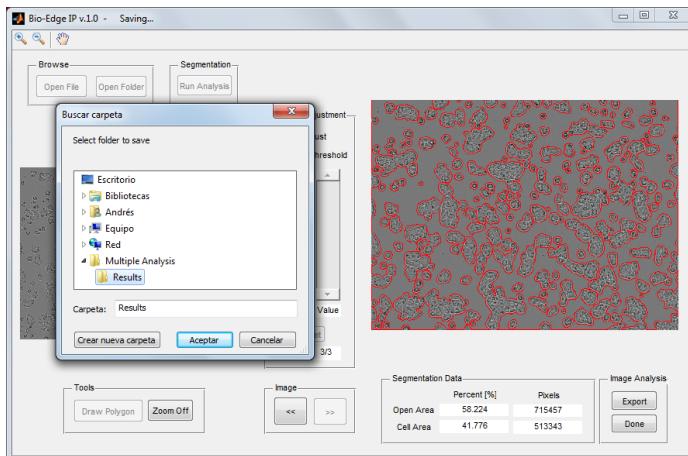
F



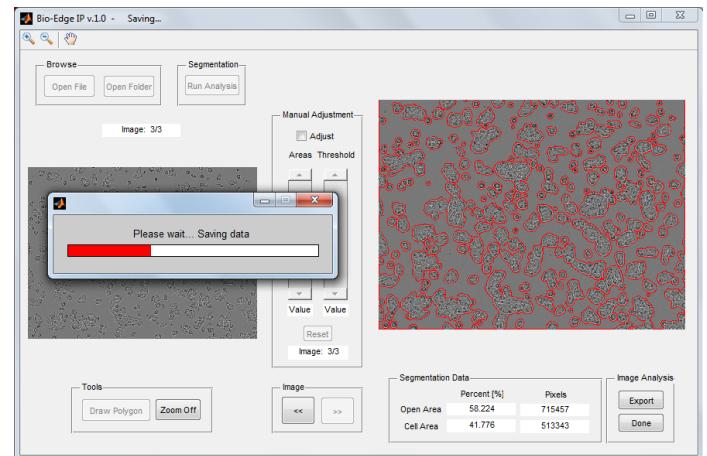
6. Data from all processed images in Multiple Analysis mode is saved by pressing “Export”. Similar to Single Analysis mode, the user must select the folder to save (G).

7. Please wait while Bio-EdIP saves the precessed images and generates the Excel file (H).

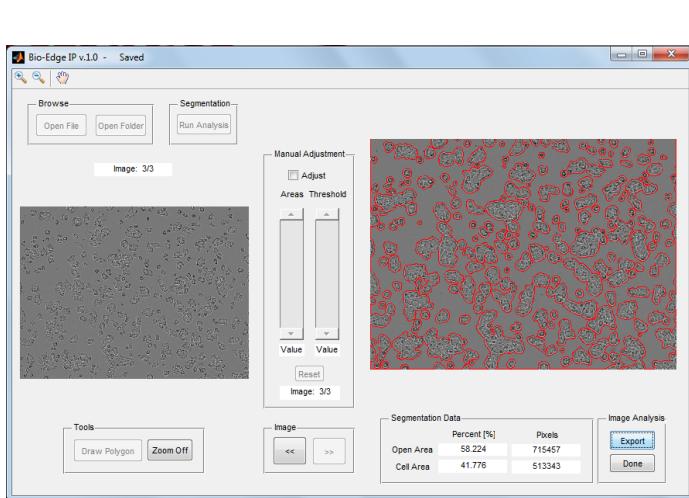
G



H



8. Wait until in title bar the word “Saved” appears. To start a new analysis please press “Done” (!).



9. Results: when saving process is done, Bio-EdIP generates the following results:

For each processed image is generated three TIFF files denoted like:

ImageName_Binary.TIFF

ImageName_Outline.TIFF

ImageName_Overlay.TIFF

In addition an Excel file is created, which contains the segmentation data for all processed images:

J

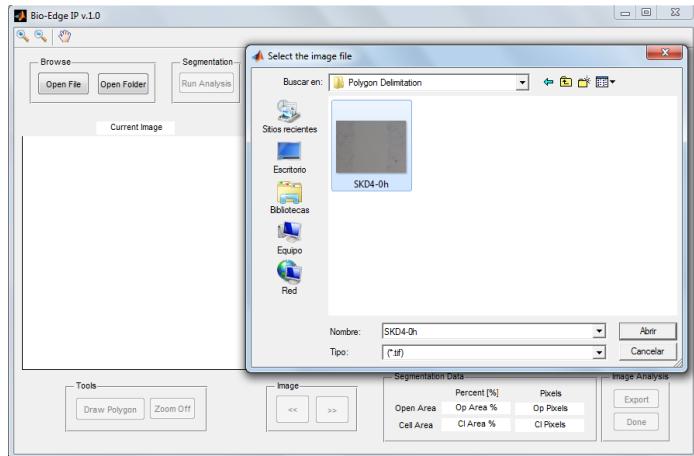
	A	B	C	D
1	Image	H2C2-0h.tif	H2_3c-3.tif	H2_A-10.tif
2	Open Area [%]	70,5888672	33,6250814	58,2240397
3	Open Area	867396	413185	715457
4	Cell Area [%]	29,4111328	66,3749186	41,7759603
5	Cell Area	361404	815615	513343
6	Blocks	0,5	0,5	0,5
7	Threshold	3	3	3

Single Analysis – Polygon Delimitation

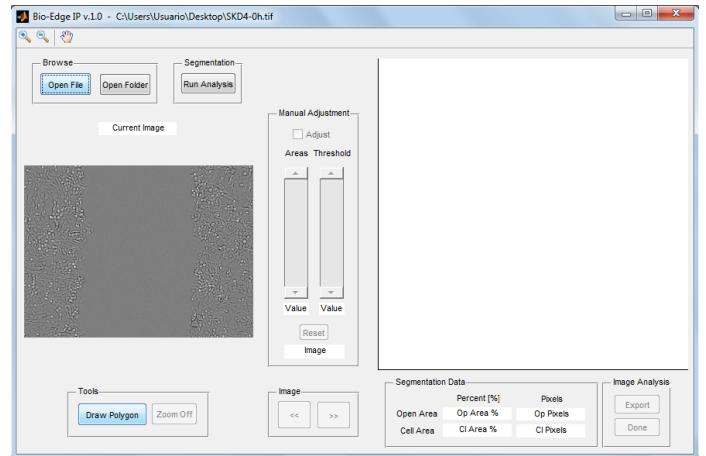
In order to manually segment an image, please follow these steps

1. Press “Open File” and select the image (A).
2. Wait while the image is loaded. In the title bar the path of the selected file will be specified (B).

A



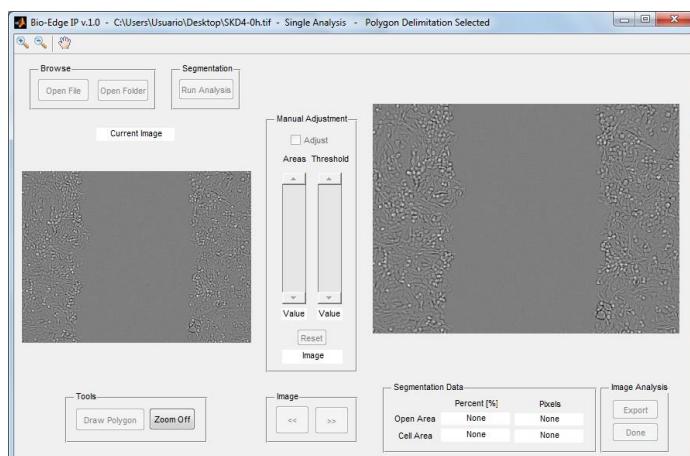
B



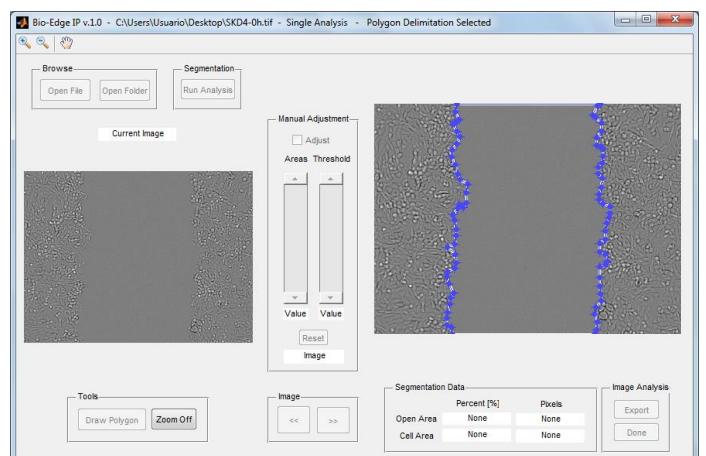
3. Press “Draw Polygon”. In the right side of the interface will appear the image. Place the cursor on the image, the cursor must change to a cross (C).

4. This analysis consist in drawing a polygon using the left click to make vertex. Each click will generate a polygon vertex. In order to complete the polygon, user must link the initial and final vertices (D).

C

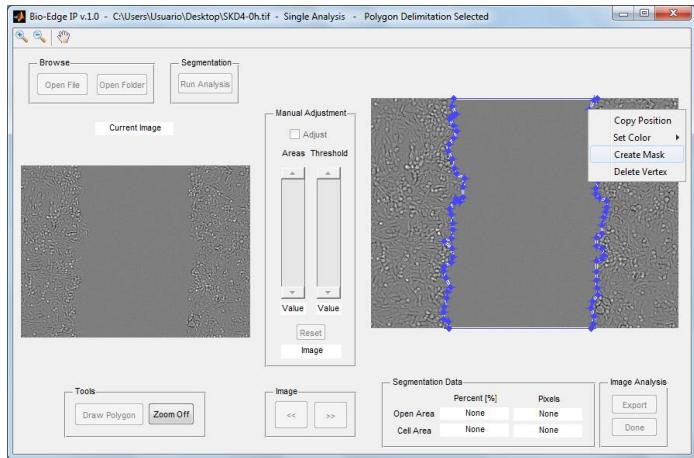


D

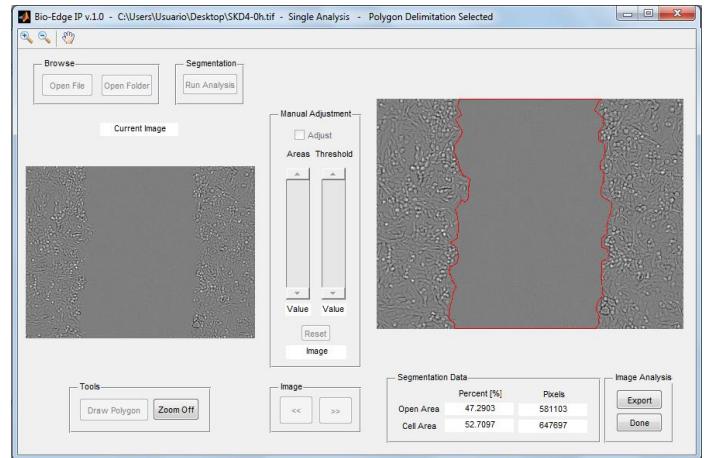


5. Place the cursor on any point, the cursor will change to a circle. Press right click and select “create mask” (E).
6. Immediately, the manual-segmented image must appear (F).

E



F



7. After this step, the manual analysis can be saved as previously described in Single Analysis mode.

8. TIPS:

- * Press Esc in order to cancel the polygon delimitation.
- * Use the zoom and panning tools to facilitate the manual-segmentation.
- * Avoid to use a lot vertices. This analysis mode uses the roipoly MATLAB function.

Manual Adjustment

Manual-setting of default segmentation parameters

As described in quick start section (pag. 4), “Areas” and “Threshold” parameters could determine the quality of the image segmentation. Once the automatic image processing ends, user can modify the value of these parameters if necessary. These parameters are controlled by two sliders. How to use these parameters is shown in the following step-by-step example:

Example

1. Open File or Folder: modification of default segmentation parameters is available in automatic segmentation modes (Single and Multiple). In this example, three images were selected in order to show how “Areas” and “Threshold” parameters work. First, open and load a file or folder as described in Single and Multiple Analysis mode sections.

2. Automatic segmentation: select “Run Analysis” and wait while image processing is carried out.

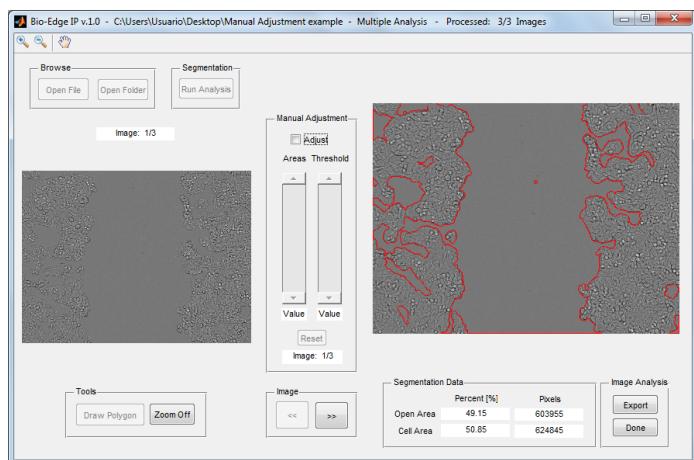
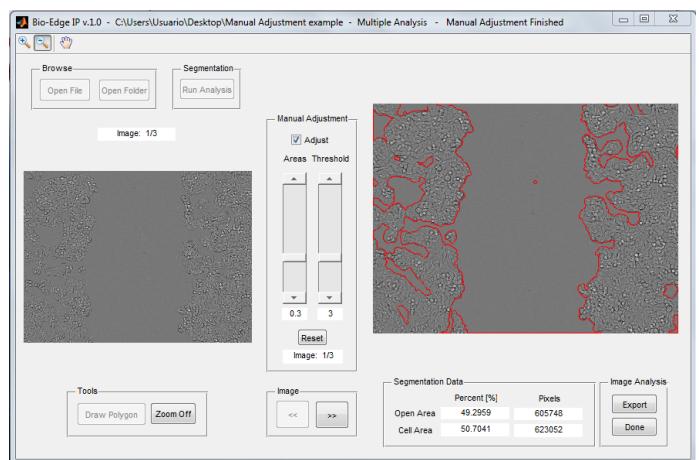
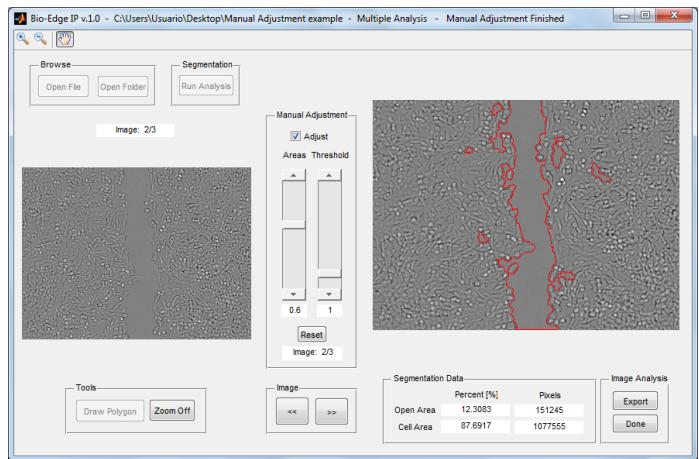
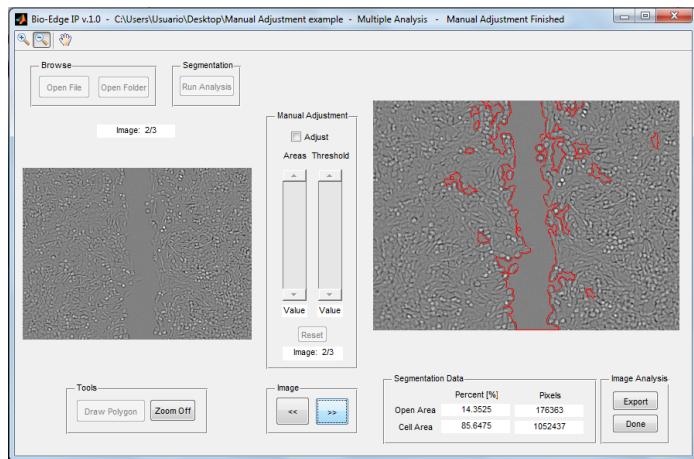
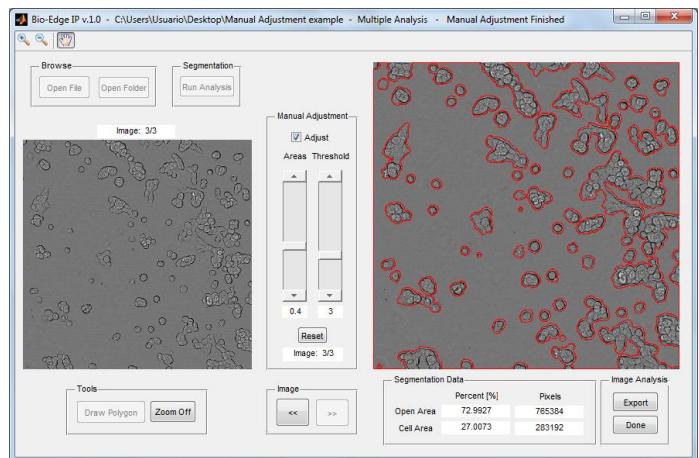
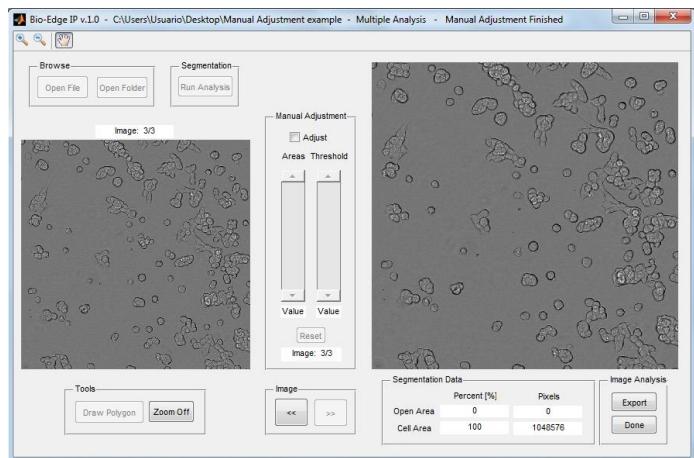
3. Manual Adjustment panel: Each image is managed independently, so changes in default parameters for an image do not affect the remaining images. In order to modify an image, enable the check box “Adjust” in the Manual Adjustment panel (Fig. 5). Then, customize “Areas” and “Threshold” parameters by moving their corresponding sliders, whose default values are 0.5 and 3 respectively. Use the Image panel to change between images. The procedure described can be applied to each image.

Figure 7 shows the three selected images after automatic processing and manual-setting segmentation. In the first image (Fig 7A) the “Areas” slider was moved to 0.3 in order to segment additional small cell-free regions. The second image presents some incorrect segmented areas, therefore the “Areas” parameter was increased to 0.6 and the “Threshold” parameter was decreased to 1 (Fig. 7B). The last image was not segmented using the default parameters, for these cases “Areas” segmentation parameter could be decreased (Fig. 7C).

3. “Reset” button: the changes done in segmented images can be reverted selecting the option “Reset” in Manual Adjustment panel. However, changes done in every single image must be reseted independently.

4. Save the results: press “Export” as described in previous sections. Excel file will contain the modified data.

	A	B	C	D
1	Image	H2C1-0h.tif	SKC5-12h.tif	SN90_C_6_00
2	Open Area [%]	49,2958984	12,30834961	72,9927063
3	Open Area	605748	151245	765384
4	Cell Area [%]	50,7041016	87,69165039	27,0072937
5	Cell Area	623052	1077555	283192
6	Blocks	0,3	0,6	0,4
7	Threshold	3	1	3

A**Default****Custom****B****C****Figure 7. Manual Adjustment example**

Contact

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