MetaMorph Downstream Analysis

This tutorial was designed for the MetaMorph versions available in the Bacterial Imaging Cluster workstations. Please, use this tutorial and the MetaMorph available at each dedicated workstation to process your fluorescence images

Loading Images

1. Switch on the computer and log in into your group account. Alternatively, other and external users to the ITQB should log in into the "other users" account

2. Connect your storage device which contains previously acquired images alongside to MetaMorph standard format files (.nd)

3. Hint: You can copy and paste the folder having your files on the computer's internal memory. This may improve the speed with which the images are queried and processed inside MetaMorph's environment. If you do not have a directory yet, make your own directory by following the path: D:\ALL Users\Your Name

DO NOT forget to save all files in your own personal storage device. Clean, as well, all files and images created during your occupancy

- 4. Open MetaMorph Offline (icon in the desktop)
- 5. Go to File -> Open and search the directory that contains the images

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6. Select only the images files to be used. Do not open MetaMorph standard format files (these files are used by MetaMorph to recognize the contents and features of your data set)

7. An image window will open for each image file previously selected

Counting and Fluorescence Intensity Quantification

1. In MetaMorph toolbar, right-click on the Elipse Region button O and open

Properties



2. In the Properties window set a small region size, i.e. 5 X 5-pixels region-like, and

make sure to box the "Lock region sizes" option



3. Then, go to Measure -> Region Measurements...

4. A Region Measurements window will open



5. In the **Region Measurements** window, go to the **Configure** tab and box the **Region Label**, **Image Name** and **Average Intensity**. In the **Colour Channel** and **Display and Log** options select **Intensity** and **Region Measurements and Summary**, respectively



6. G to the Measurements tab where you can follow the measurements

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7. Go to the image you would like to measure and start clicking on regions of interest (See Erro! A origem da referência não foi encontrada. Erro! A origem da referência não foi encontrada. and Erro! A origem da referência não foi encontrada.)

- 8. To export data, click on the Open Log button
- 9. In the dialog box select the Dynamic Data Exchange (DDE) box and press OK



10. Then, choose "**Microsoft Excel**" in the dropdown box and set the row and column you would like to export the data. If you have an open excel format file, the data will be exported to such otherwise one will be created

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Image Clipping and Region Selection

1. In the MetaMorph toolbar, right-click in the **Square Region** button and open

Properties

2. In the **Properties** window, set the region size you would like to clip, for instance, a 200 X 200-pixels region-like



3. Select the zone that you would like to clip or cut out

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4. If you intend to select the same region in other images of different contrast methods or filter sets, go to **Regions** -> **Transfer Regions...**



5. In the **Transfer Regions** window, select only **All Regions** and nothing else, and then press **OK**



- 6. The square region will be present at the same location in all open images
- 7. Press CRTL + D to create new images only from this square region



Set Scale Bar

1. Go to Measures -> Calibrate Distances



2. In the Calibrate Distances Window, select the "Calibration #1" for a standard scale bar (X: 0.05 μ m; Y: 0.05 μ m)

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3. Press the **Apply** button to set 0.05 μ m per pixel to the currently selected

image

4. Then, go to **Display** -> **Graphics** -> **Calibration Bar...**



5. In the Calibration Bar window, set the bar size to 1 μm

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6. Press the **Stamp** button to apply the scale bar to the currently selected

image	

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Image Overlay

1. Go to Display -> Overlay Images...



2. In the **Overlay Images** window, set the number of images you would like to combine

3. Select the channels by clicking on the coloured square and picking the most suitable one according to the filter set (emission colour) of the images

4. If you notice some misalignment between images, try to correct it using the X Align and Y Align columns

5. Press the **Apply** button to overlay the open images

👽 Overlay Images	
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6. A new image window will pop up with the overlaid images

Save Images in a Universal Format File

Note that images previously acquired or created inside MetaMorph's environment are written on a 12-bits range. Therefore, a clear majority of software is uncappable to open these images

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- 1. Go to Display -> Scale Image...

2. In the Copy to 8-bit image section, press the Copy button



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3. A new image window will pop up as an 8-bit copy of your original image



- 4. Finally, go to File -> Save as...
- 5. Search for your own directory and save both, original and 8-bit copy, images