

MetaMorph Downstream Analysis

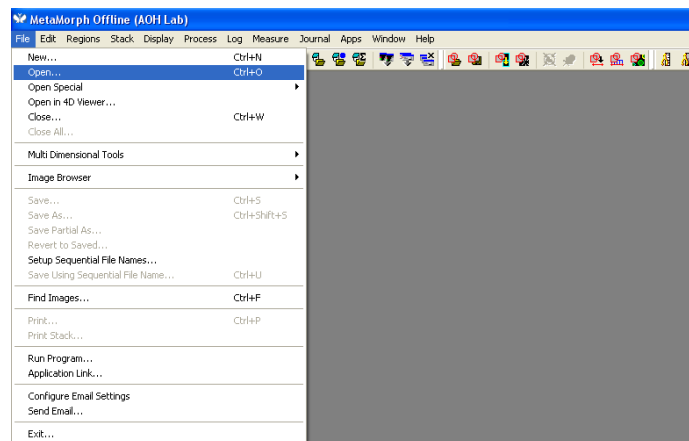
This tutorial was designed for the MetaMorph versions available in the Bacterial Imaging Cluster workstations. Please, use this tutorial and the MetaMorph available at each dedicated workstation to process your fluorescence images

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

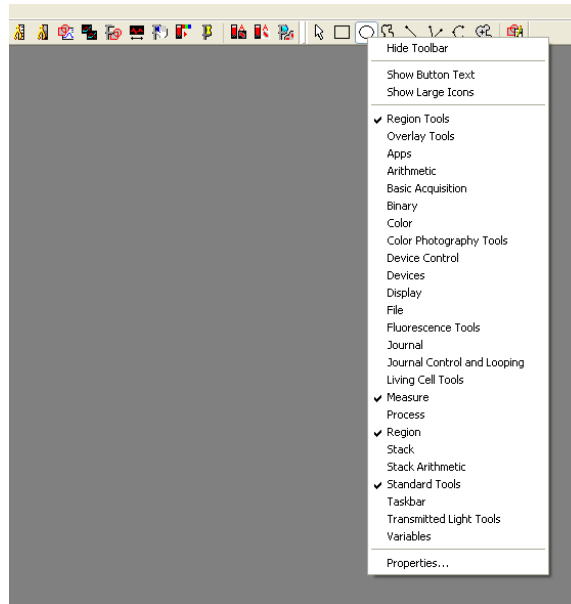
4. Open MetaMorph Offline (icon in the desktop)
5. Go to **File** -> **Open** and search the directory that contains the images



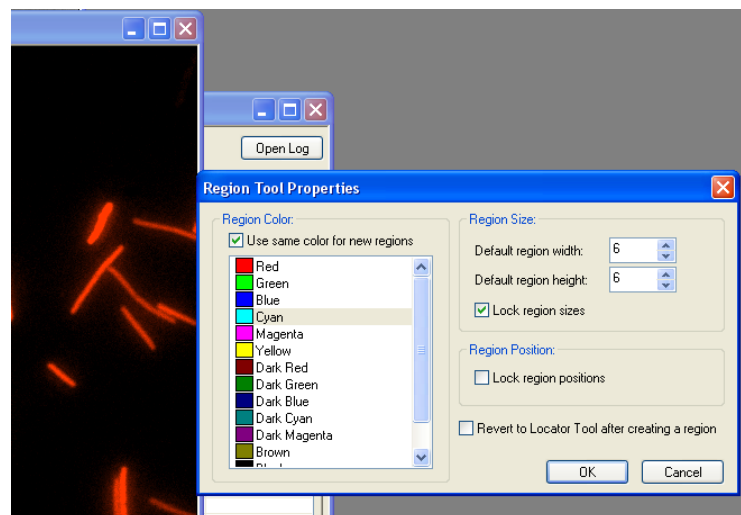
6. Select only the images files to be used. Do not open MetaMorph standard format files (these files are used by MetaMorph to recognize the contents and features of your data set)
7. An image window will open for each image file previously selected

Counting and Fluorescence Intensity Quantification

1. In MetaMorph toolbar, right-click on the **Ellipse Region** button  and open **Properties**

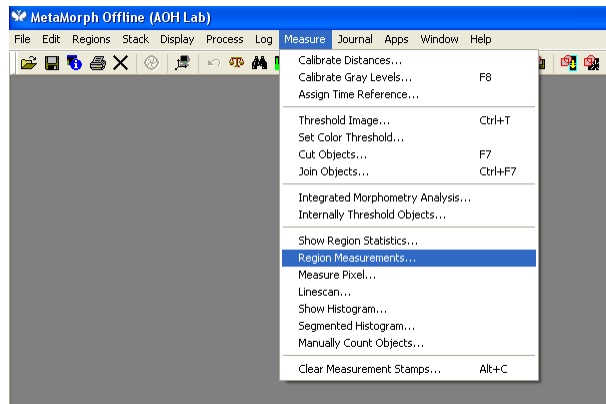


2. In the **Properties** window set a small region size, i.e. 5 X 5-pixels region-like, and make sure to box the **“Lock region sizes”** option

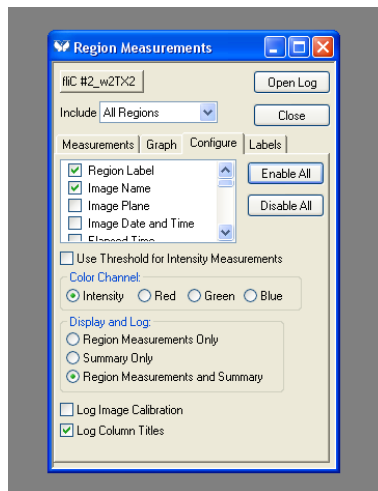


3. Then, go to **Measure -> Region Measurements...**

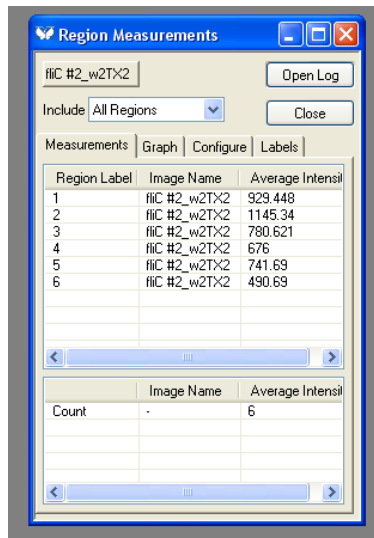
4. A **Region Measurements** window will open



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



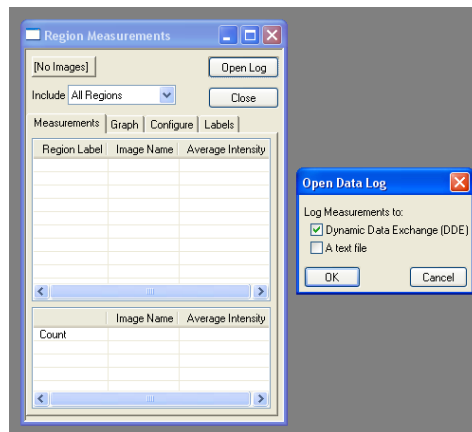
6. G to the **Measurements** tab where you can follow the measurements



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

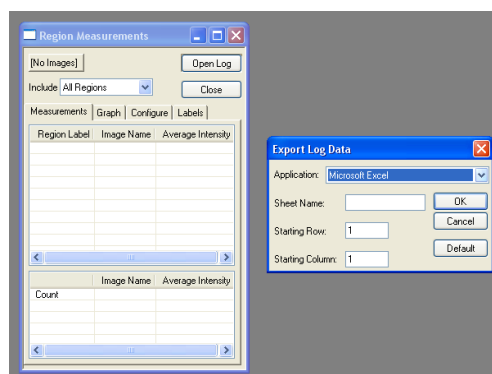

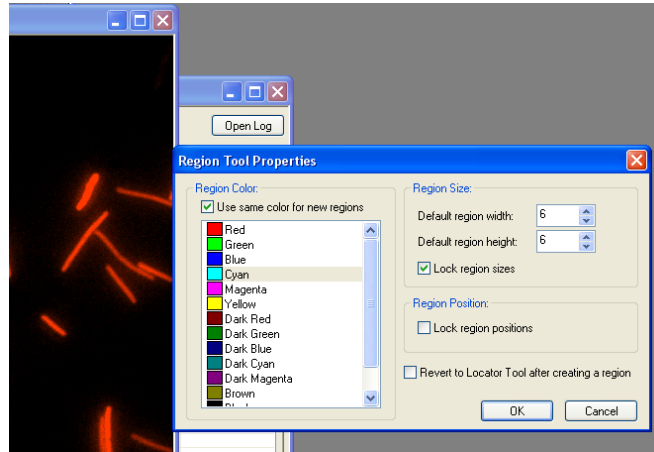
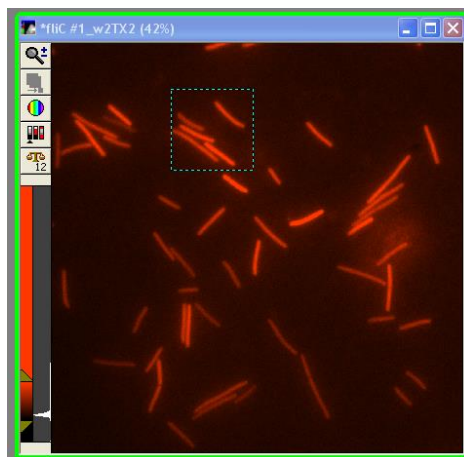


Image Clipping and Region Selection

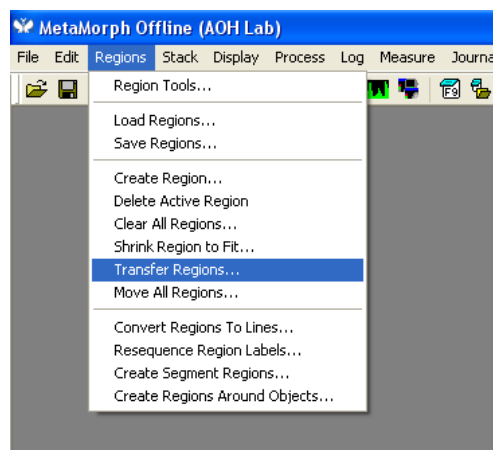
1. In the MetaMorph toolbar, right-click in the **Square Region**  button and open **Properties**
2. In the **Properties** window, set the region size you would like to clip, for instance, a 200 X 200-pixels region-like



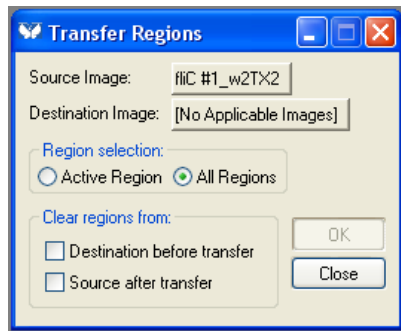
3. Select the zone that you would like to clip or cut out



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

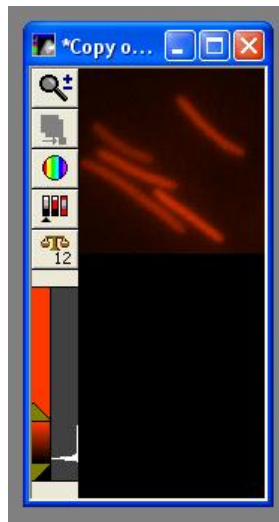


5. In the **Transfer Regions** window, select only **All Regions** and nothing else, and then press **OK**



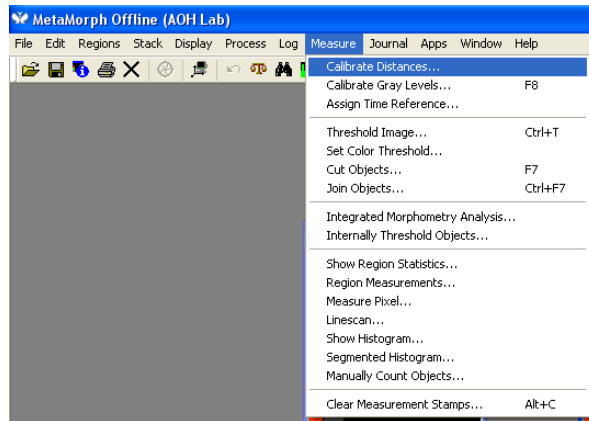
6. The square region will be present at the same location in all open images

7. Press **CRTL + D** to create new images only from this square region

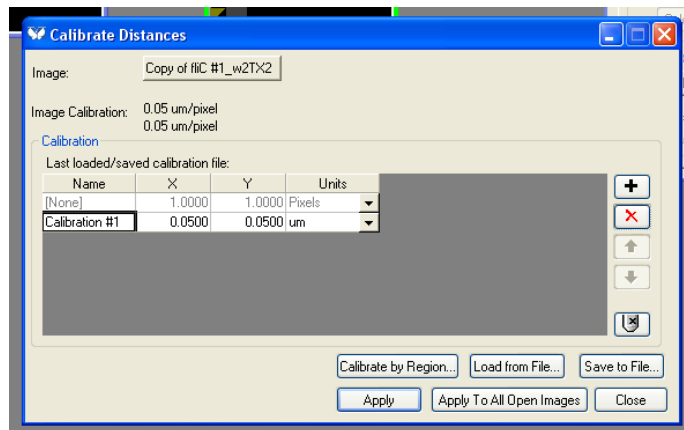


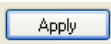
Set Scale Bar

1. Go to **Measures** -> **Calibrate Distances**

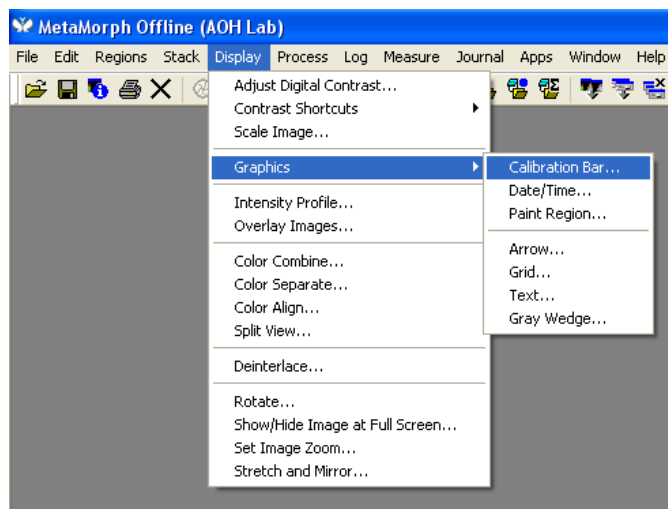


2. In the **Calibrate Distances** Window, select the “**Calibration #1**” for a standard scale bar (X: 0.05 μm ; Y: 0.05 μm)

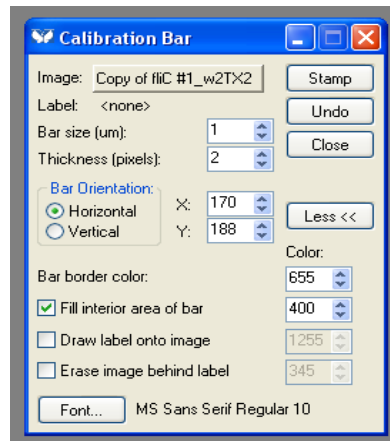


3. Press the **Apply** button  to set 0.05 μm per pixel to the currently selected image

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



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



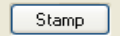
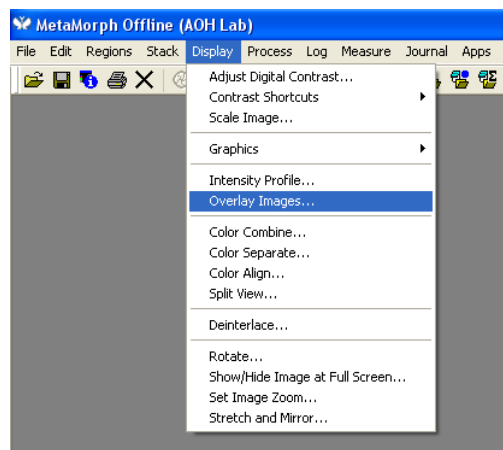
6. Press the **Stamp** button  to apply the scale bar to the currently selected image




Image Overlay

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

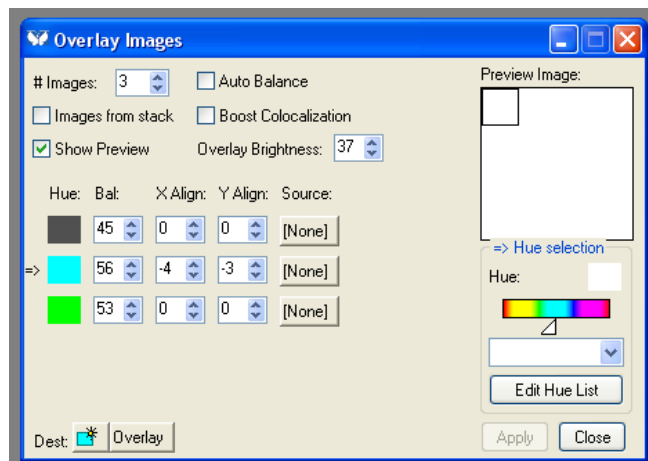


2. In the **Overlay Images** window, set the number of images you would like to combine

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

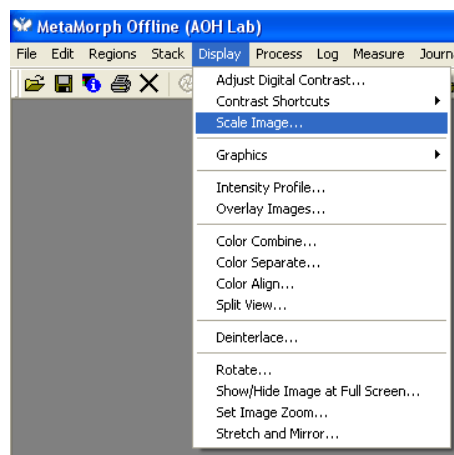


6. A new image window will pop up with the overlaid images

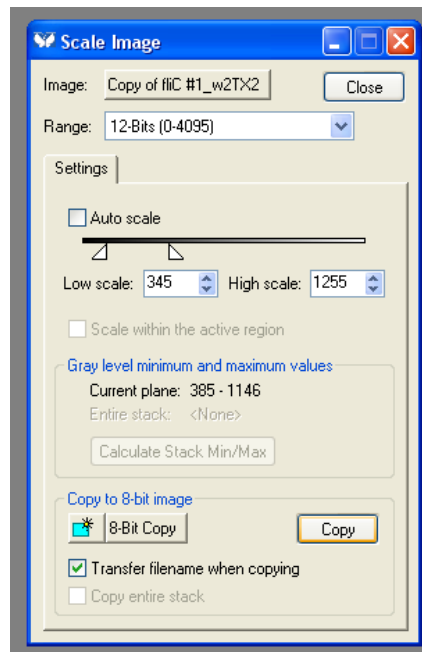
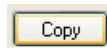
Save Images in a Universal Format File

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

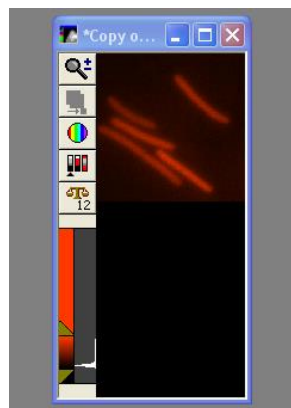
1. Go to **Display -> Scale Image...**



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



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