Quantification walk-through

Atherosclerotic plaque

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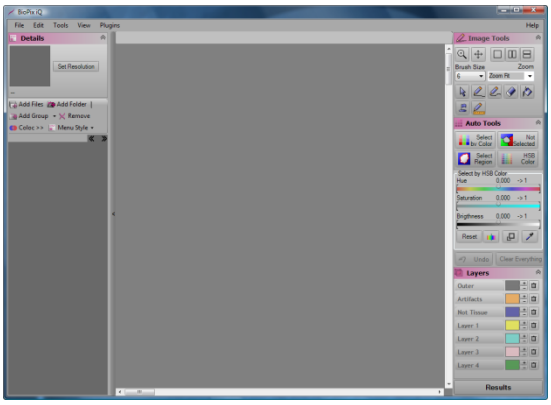
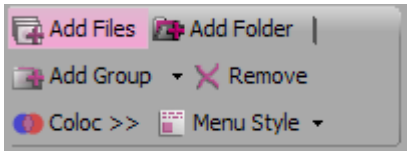
Introduction

This walkthrough demonstrates one way of quantifying a microscopy image of a blood vessel where an atherosclerotic plaque has been stained with a red coloring. The walk-through shows the process for one image.



Adding files

When the application is first started, an empty project is created. If there is currently a project loaded, you can create a new, empty project from the File menu.



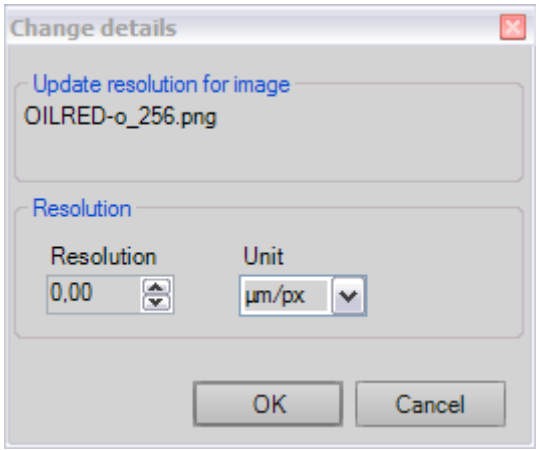
First add images to the project. Click the **Add Files** button, on top of the File List, and select one or more files to add. You can also use the **Add Folder** button. This allows you to add all image files in a folder and its sub folders. The added files will be structured in groups, much like the folder structure on the computer hard drive. You can use drag-and-drop to move images in the structure. Group images together by selecting several (while holding down the Shift or Control key) and right click on the images. Select **Group** from the context menu and enter the name for the group.

Double click an image in the list to open it for quantification.

Setting resolution

Pixel resolution can be set for files and groups, either as **µm/pixel** or **pixel/µm**. This must be done to get correct absolute values in the output data for area and length measurements. Otherwise only relative, pixel values can be given. Select an image or a group of images and press the Set Resolution button 

.



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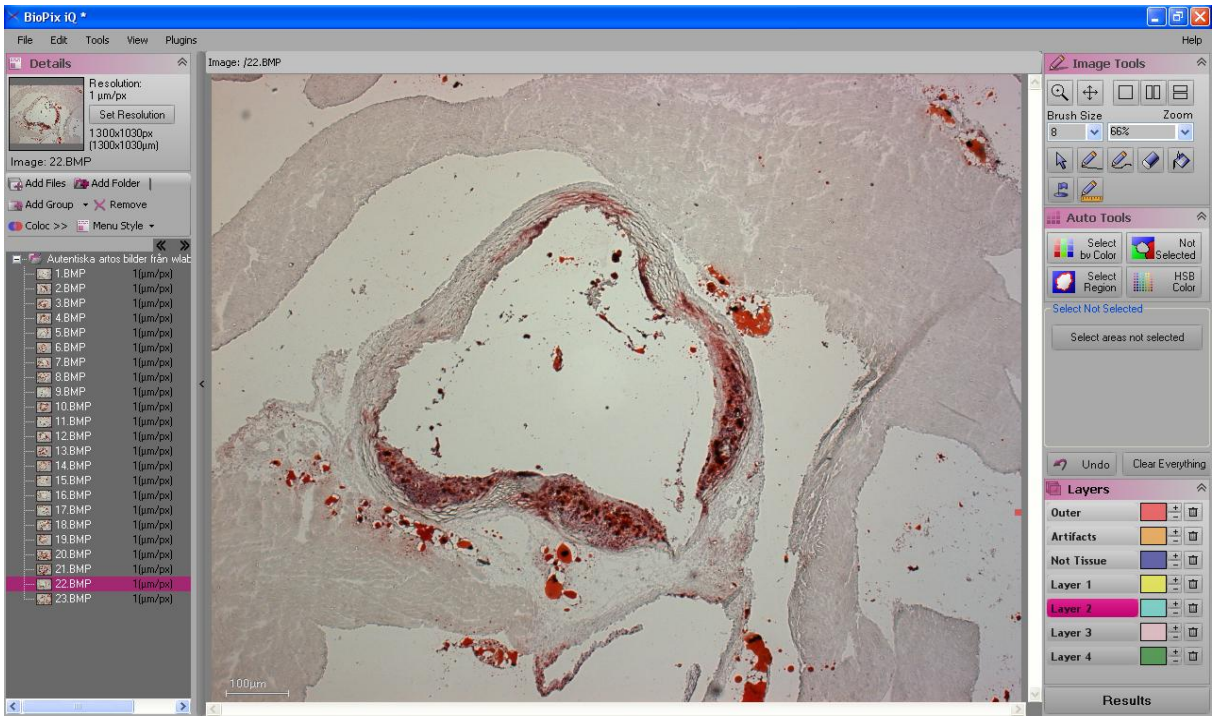
Marking the outer region

The image is now open with the resolution set. To avoid counting material not belonging to the vessel, the outer region must be marked.



Outer

Press the Outer Limits button to activate the layer. Two tools are ideal for marking the outer region of the vessel; the Line Tool and the Pen Tool.

Line tool

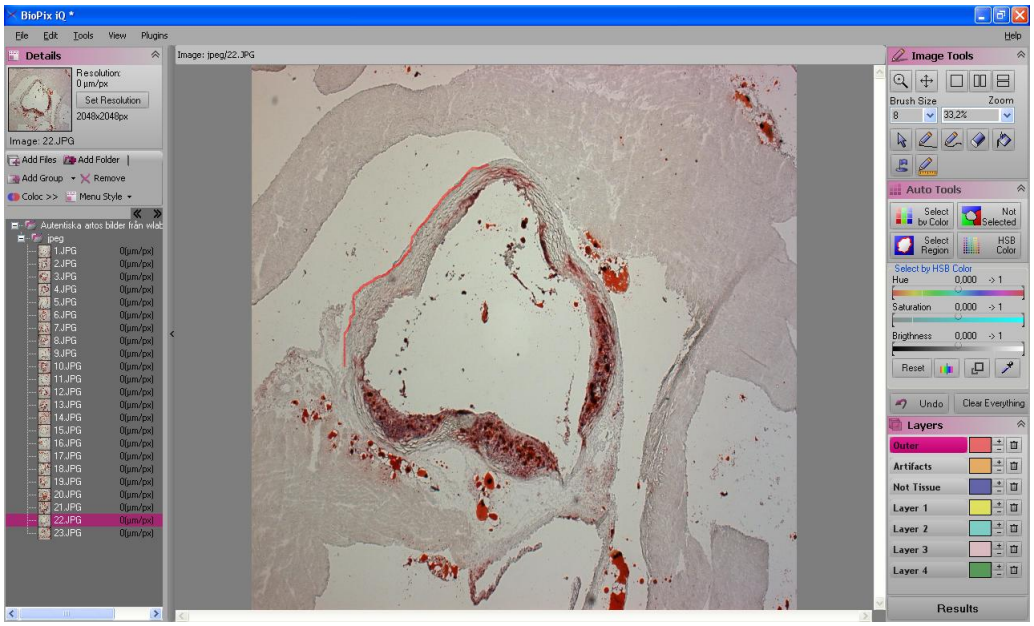
Select the Line Tool with a suitable Brush Size in the image toolbox. Use the left mouse button in the image to produce straight lines stepwise. Right click to stop drawing lines.

Pen tool

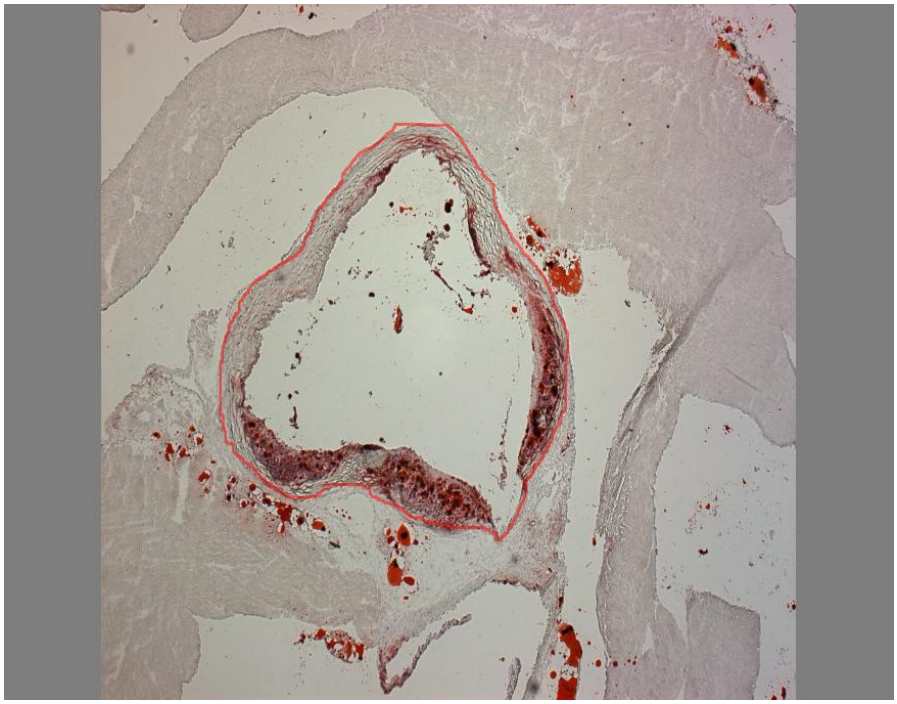
Select the Pen Tool with a suitable Brush Size in the image toolbox. Press and hold the left mouse button to mark the outer region with a continuous line.

Zoom tool

You can use The Zoom Tool to zoom in and out. Holding down the Ctrl key on the keyboard and using the mouse scroll wheel will also zoom the image.



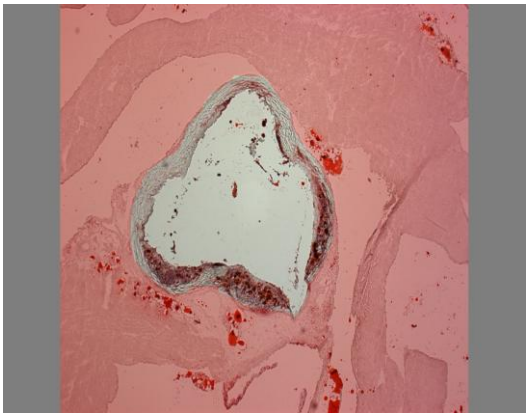
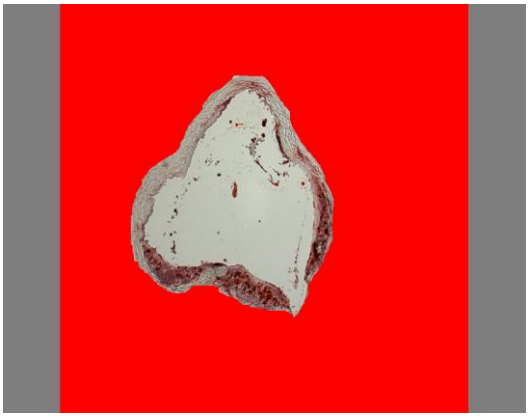
Make sure that the outer region is **continuously** drawn without any gaps or holes before proceeding. Mark the outer regions of all your images in this step. This will speed up the selections in the later steps.



The area around the vessel is now marked with a continuous selection. Select the Paint Bucket tool  and click outside the selection to mark outside the vessel. If the selection is not continuous the paint bucket will fill the whole area. Press the Undo button 

to undo the painting and use the Line or Pen tool to correct the gap in the selection.

Opacity for each area [buttons] can be adjusted to show or hide the background image for areas that have been selected already. Use the plus and minus  to change opacity of a layer.

Marking artifacts

If there are artifacts in the image that should not be part of the data, these can be marked by first pressing the Artifacts layer button and then using any of the tools, for example the Pen Tool with a large brush size, to mark.

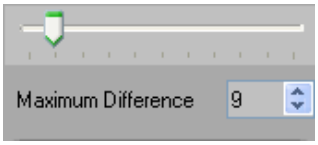
Marking None Tissue area

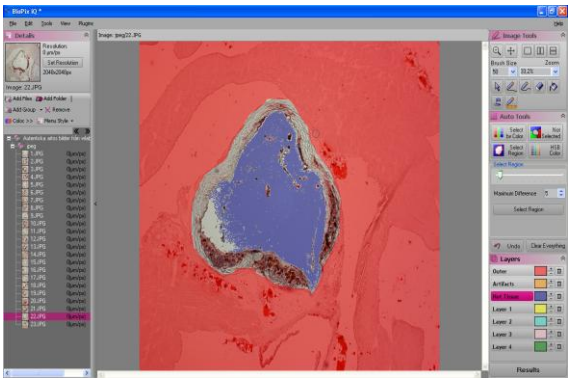
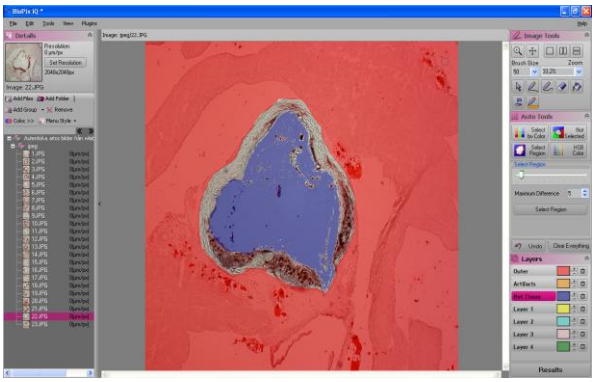
To mark the None Tissue area, in this case the vessel lumen, use the Select Region 

tool. Click on the select region button 

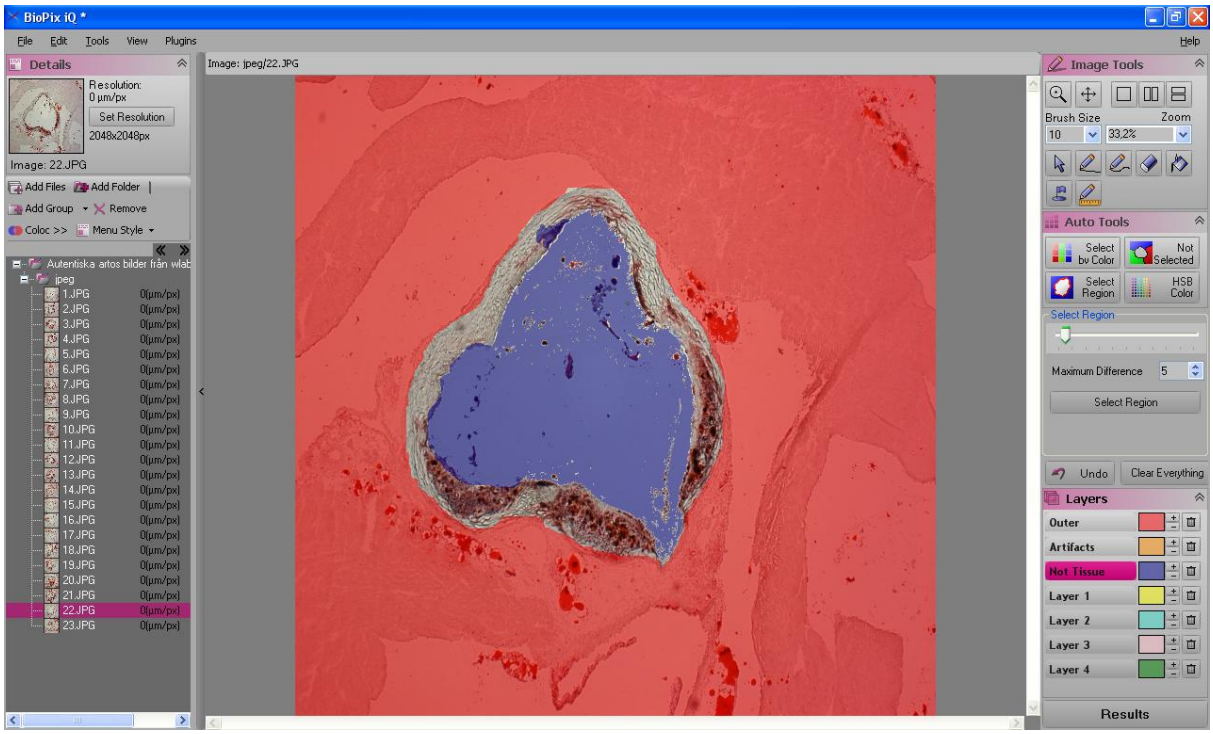
and then click in the area that is non tissue. To extend the selection, click on another location. The tool selects adjacent pixels that have a small difference in color. Adjust the **Maximum** Difference for the tool to select areas of very homogenous color, or areas of more difference. Undo any selections by pressing the Undo Button 

or by pressing **Ctrl+Z**. The Trashcan icon  next to each layer clears all selections in that layer and lets you start over.



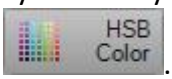
To correct or extend the selection use the Pen Tool  and paint on areas that has not been selected. Use the Eraser Tool  to remove selections.



As with the outer region, it is best to perform this for all images to be analyzed before continuing to the next step.

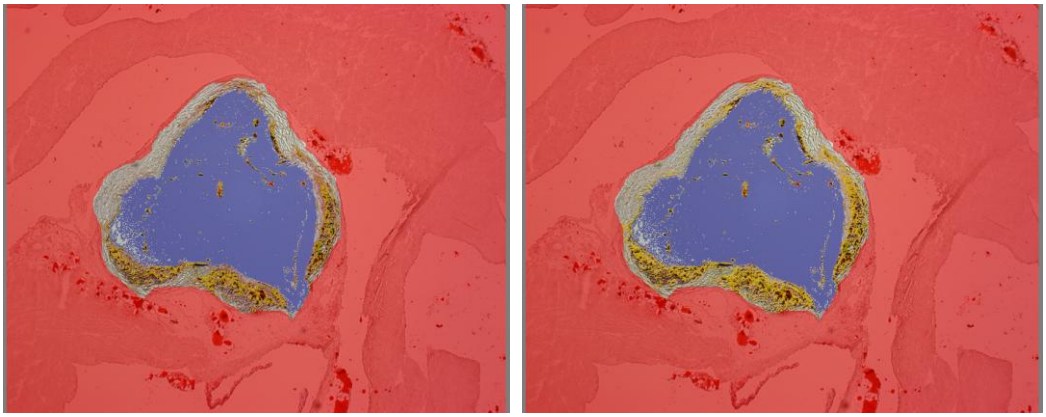
Marking Plaque

To begin editing a tissue area, press any of the Layer buttons 

. To select the plaque in the image use the HSB tool 

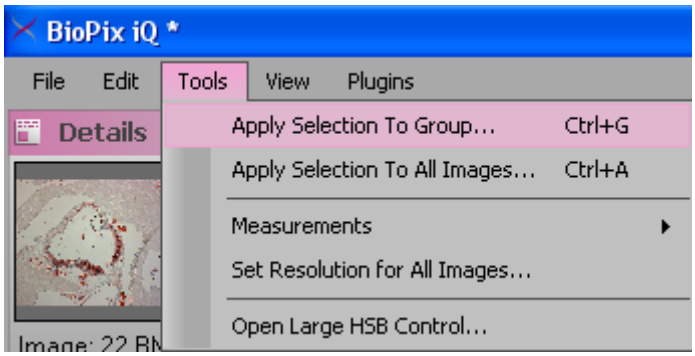
. The pipette button lets you select a color from the image. Adjust the settings on hue, saturation and brightness to fine tune the settings.

With the **Color Marker** tool  markers can be set in the HSB-tool that corresponds to the colors in the image. Left click in the image on areas that are to be included and right-click on areas to exclude. **TIP**: Use plenty or markers! This makes it easier to find a good HSB settings.



You can use the same setting on all images using the **Apply Selection to Group** or **Apply Selection to All Images** functionality found in the **Tools** menu. This can also be done by right-

clicking any number of selected images and groups in the File List and selecting for instance **Apply HSB Tool**.



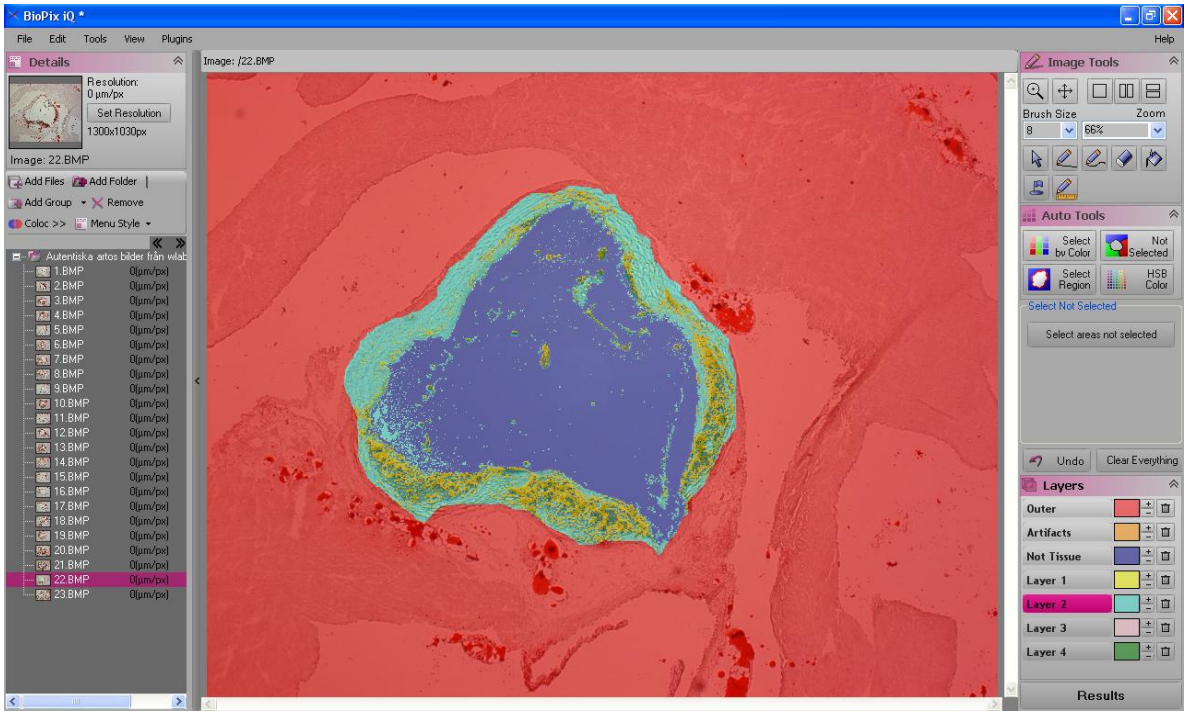
Make sure the outer regions for all images have been selected as described in the steps above before you apply the selection to the images.

Marking the vessel wall

Select a new layer for the vessel wall by clicking on the **Layer 2** button and then selecting the **Not selected tool** 

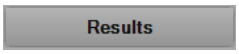
and pressing 

. This automatically selects all area that has not previously been selected in any other layer.



Now all areas of interest in the image have been selected. In order to keep track of your work progress, you can right click on the image name in the image structure and select **Set Done**. This shows you the progress and automatically selects the image for output data in the last step.

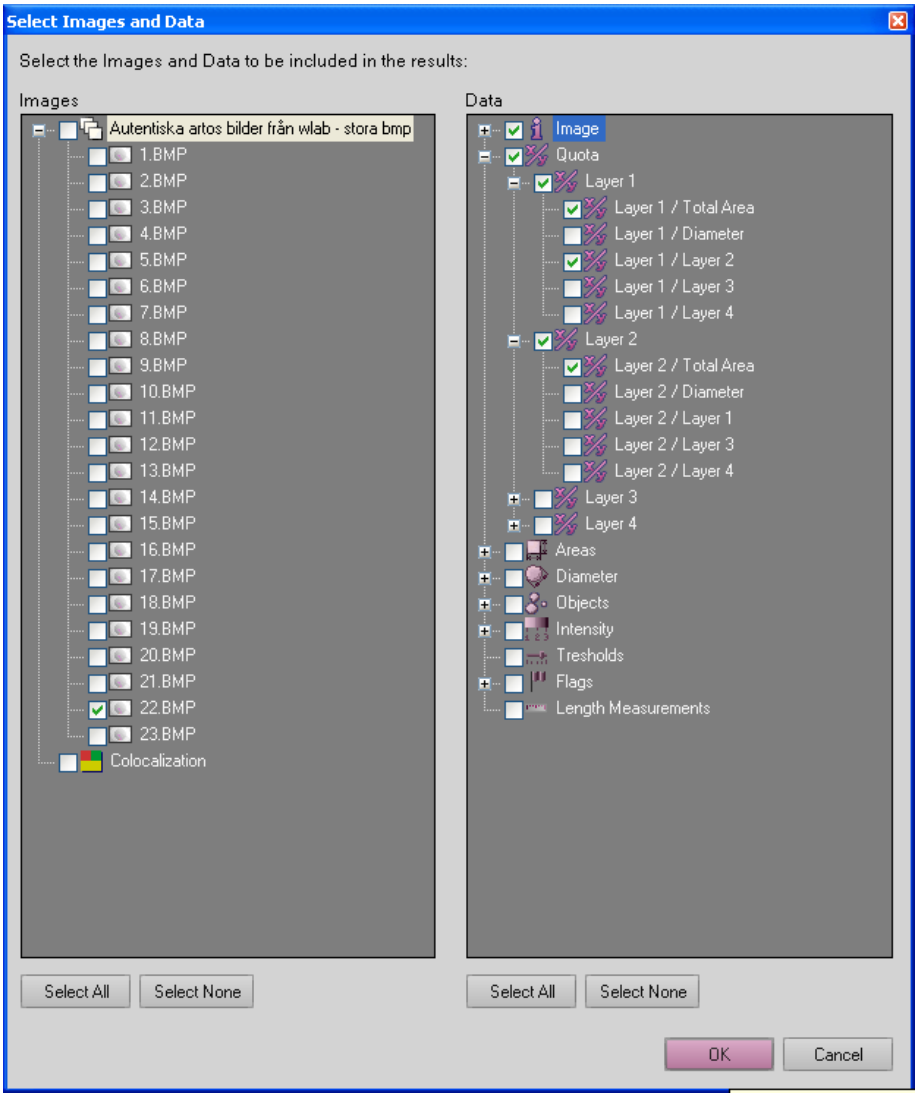
Results

Now it is time to calculate the results. The **Result** button 

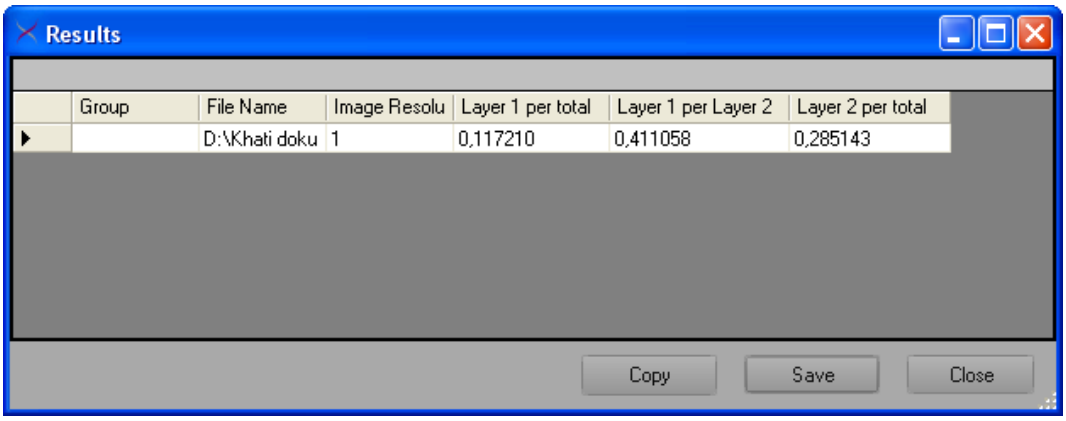
displays the dialog for selecting output data. On the left-hand side you chose which images should be included in the calculations and on the right-hand side you select the analyses to perform on each image.

Images marked as **Done** are automatically selected on the left-hand side. Select the output data that matches the analysis you want to perform. In this case the following calculations are selected:

- **Image**, information on image **name**, **group** name, and **resolution**.
- **Quota** -> **Layer 1** -> **Layer 1 / Total Area**.
- **Quota** -> **Layer 1** -> **Layer 2 / Total Area**.
- **Areas** -> **Area inside outer limit**
- **Areas** -> **Area of Layer 1**
- **Areas** -> **Area of Layer 2**



The results can be viewed in a table or saved as a tab separated text file that later can be imported into for example Microsoft Excel or any other statistical application.



Press the **Save** button to save the data to a file, or **Copy** to copy it to the clipboard so that it later can be pasted in for example Excel. Press **Close** to go back to the program.