

12.2 AutoMeasure (Automatic Measurement)

General

The process of measuring interesting objects in an image automatically is closely linked to the rapid development of the hardware sector. Without this development it would take a huge amount of time to perform these measurements (which can involve several hundred images in some cases).

Thanks to the performance of modern PCs, it is now possible to use increasingly complex functions for image analysis, and to perform an automatic evaluation.

The term automatic measurement is understood to mean, in particular, the **automatic recognition** of interesting objects. In contrast to interactive measurement, it is no longer necessary to use the mouse to outline the objects to be measured in the image. The objects can be recognized automatically on the basis of their brightness and/or color and other descriptive parameters (shape, size etc.).

An automatic measurement usually consists of the six steps briefly listed below. These steps represent the general sequence of an image analysis procedure. They are in no way specific to AxioVision, and should always be carried out in this or a similar form.

1. Image acquisition

Image acquisition is – besides segmentation – the most important step within image analysis. It is only during image acquisition that you have all the original data available. Image information that is poor or, worst of all, incorrect prevents you from achieving accurate measurement results. This can even lead to a situation in which a sample that should be simple to measure from an image analysis perspective is no longer analyzable, as the system can no longer recognize the objects to be measured. In such cases you have to revert to interactive measurement.

Information that is not acquired during image acquisition is very difficult to reconstruct during image enhancement. In many cases this is not possible at

all. You *must* therefore take advantage of all of the technical possibilities offered by your microscope and camera during the preparation of the sample.

Often there is room for improvement with regard to sample preparation. You need to be self-critical, and check whether the quality of your samples could be improved.

2. Image enhancement

In general terms, it is possible to distinguish between two different application situations:

The **optical enhancement** of an image helps the user to correctly interpret the accuracy of the solution suggested by the measurement system. This includes enhancement of the brightness and contrast, and also gamma correction. Enhancing the quality of the image from an optical perspective, however, has no influence on the quality of the recognition results.

Functions for **data extraction** allow you to enhance recognition performance (segmentation) in such a way that, under certain circumstances, images can be measured automatically even when the image quality would lead you to assume otherwise. These functions include, for example, the elimination of illumination errors (shading correction).

3. Segmentation

Segmentation is the key to a correctly functioning automatic measurement. This process involves having the interesting objects determined automatically by the measurement system, and separated from the regions that are not to be measured (the image background). Initially this takes place on the basis of brightness or color information. Reference objects are outlined or highlighted in the image using the mouse. AxioVision then recognizes all other objects independently. It is also possible to directly select the thresholds for the brightness or color ranges that represent the objects using the distribution of the brightness or color information (histograms). However, this requires a certain amount of experience.

The result of segmentation is a "measurement mask", which is represented internally by a so-called binary image. This mask conceals the background regions using black pixels, and shows the objects to be measured as white. You will only come into contact with this measurement mask indirectly. AxioVision shows it as a color image superimposed over the original image.

4. Processing the measurement mask

A measurement cannot be performed until the objects to be measured, and only those objects, have been correctly recognized. Sometimes, however, this is not possible. Artifacts that you do not want to measure may also be acquired, or objects may be touching and may not therefore be recognized as individual objects by the system. These must then be separated before proceeding.

Typical processing steps therefore involve the deletion of artifacts, the filling of gaps in objects and the automatic separation of objects. In difficult cases, subsequent interactive processing (deletion, separation, additional drawing in of objects etc.) may also be required.

5. Measurement

During the measurement, the selected parameters are determined by AxioVision and saved in a results file. Two types of file are distinguished: files for so-called **region specific** and **field specific** image data.

- **Region specific** image data are measurement parameters that are determined for every individual object (an image *region*). These are, for example, the area, circumference and shape of the object in question, or even its densitometric values, such as the average gray value. The measurement results are written within the results file in the form of **one line per region** or object.
- **Field specific** data are measurement parameters that are determined for the entire image *field*. These are, for example, the number of objects, or the percentage of the entire image area represented by all the objects present, etc. Field-specific measurement results lead to **one line per image** in the results file.

6. Evaluation

Before you can actually make a statement about the image using the data for the images or objects in which you are interested, this data needs to be evaluated. As a wide range of special evaluation programs, such as Microsoft Excel, are now available, AxioVision itself does not feature any evaluation functions. The data are made available in Excel format, and can therefore be evaluated in the environment you are familiar with.

In addition to the pure measurement data, image documentation should also always be used to make clear which objects were used to determine the measurement results. AxioVision gives you the option of displaying the objects, e.g. by outlining their contours, and the measurement results directly in the image.

The concept of the AutoMeasure module

The concept of the AutoMeasure module is based on Carl Zeiss' many years of experience in the field of automated image analysis. It has become clear that in most cases only a small proportion of the range of available functions are actually required and employed. The aim was to arrange these functions in a way that was simple to operate and would also allow less experienced users to perform fast and accurate measurements.

As with automated analyses you are usually not just measuring an individual image, but in some cases are analyzing a very large number of images, the interactive execution of individual steps via menu functions or a workflow is often not a realistic option. Nevertheless, with the AutoMeasure module you have the option of measuring "just" one image quickly and efficiently (further information on this will be provided later).

The two points touched upon – "Complex technology, simply packaged" and "Analysis of large numbers of images" – are reflected by the structure of the AutoMeasure module.

The module therefore consists of two parts:

- The **Automatic Measurement Program Wizard** allows you to generate automatic measurement programs quickly and simply.
- The function for the **Run Automatic Measurement Program** allows you to execute a program you have generated for any number of images. It makes no difference whether these images are acquired via a camera or are already present as files.

NOTE:

- At this point, we should emphasize that the AutoMeasure module is not suitable for resolving every conceivable measurement task. It is intended more as a tool to simplify your everyday tasks.
- More complex applications are possible using the AutoMeasure Plus module. In this module the full range of image analysis functions are available via the AxioVision menus, and can be freely combined. You can therefore even realize exotic applications, although this requires the person generating the program (*not the actual user of the program*) to have some knowledge of the background to digital image analysis.

The wizard for generating measurement programs

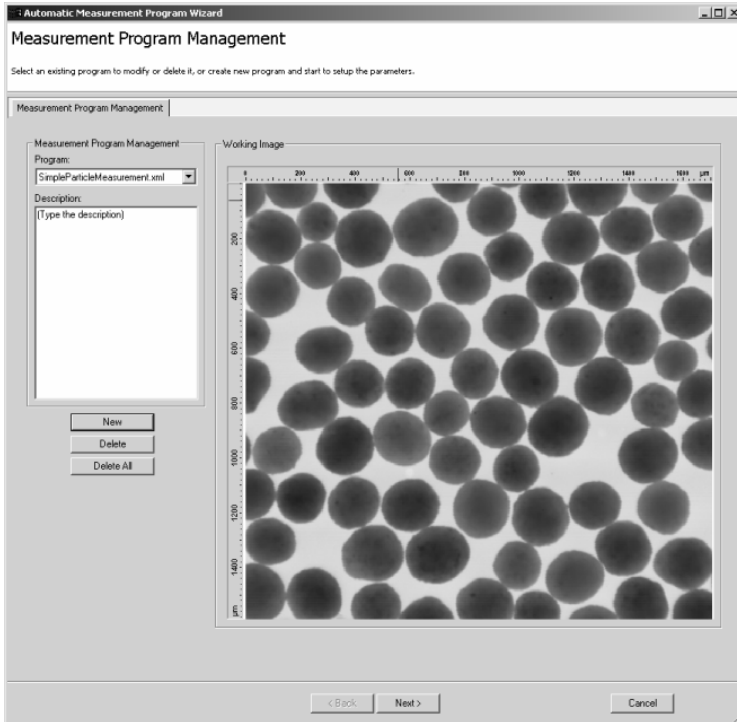
The Automatic Measurement Program Wizard leads you step by step through the individual stages of digital image analysis (please also read the "General" section earlier in this chapter).

Start it by loading an image from the hard disk or acquiring an image over the camera and afterwards execute the **Automatic Measurement Program Wizard** function on the **Measure** menu. Further information on the individual steps, and the functions and functional parameters used, can be found in the online help by pressing the **F1** key.

The following functions are available in the individual steps:

Program management

Program management gives you an overview of the measurement programs available, and a brief description on them individually:



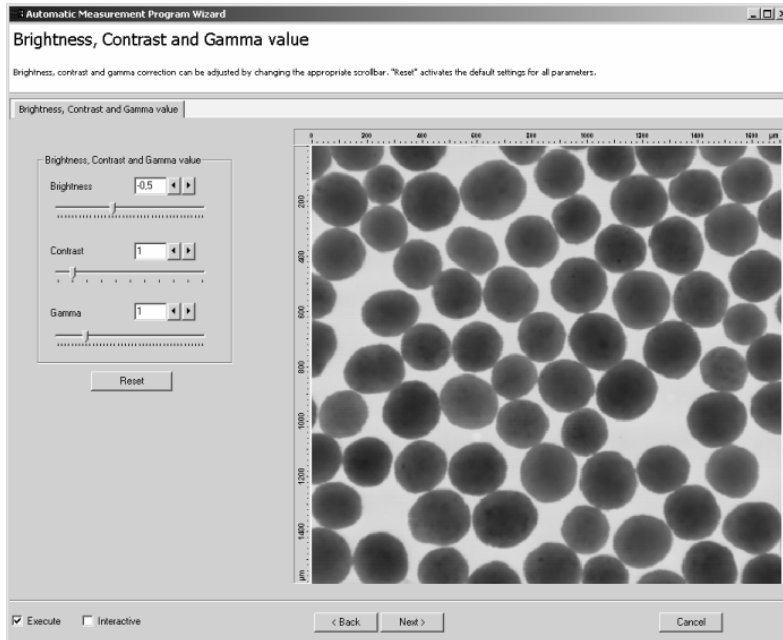
Program overview with brief description on the selected program

NOTE:

- The measurement programs are saved by default in the folder "My Documents\Carl Zeiss\Data\Automatic Measurement Projects".

Image enhancement

For optical image enhancement, functions are available for brightness adjustment, contrast enhancement and gamma correction.

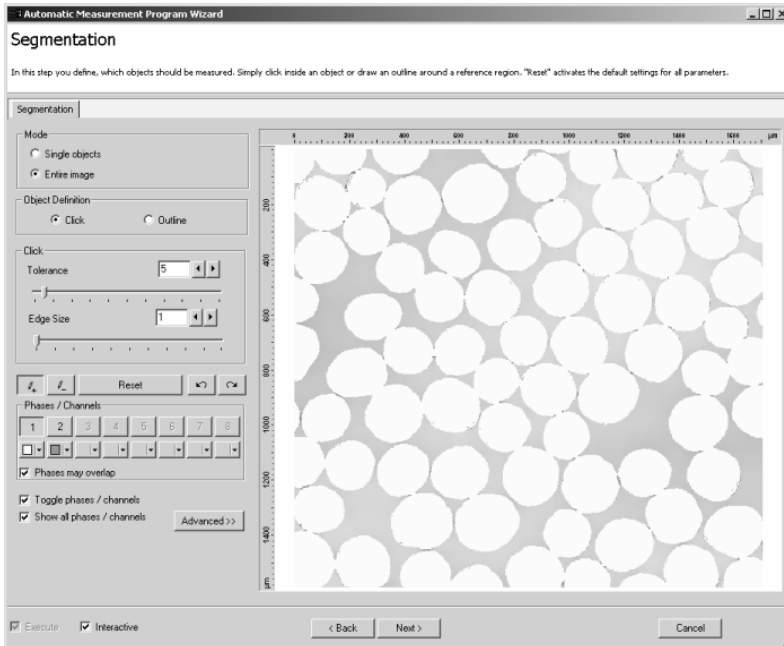


Enhancement of brightness, contrast and gamma value

To improve recognition performance, images can be smoothed (Sigma filter), illumination errors corrected (Shading correction), and object edges sharpened (Delineation).

Segmentation

Segmentation is probably the most powerful function of the AutoMeasure module.



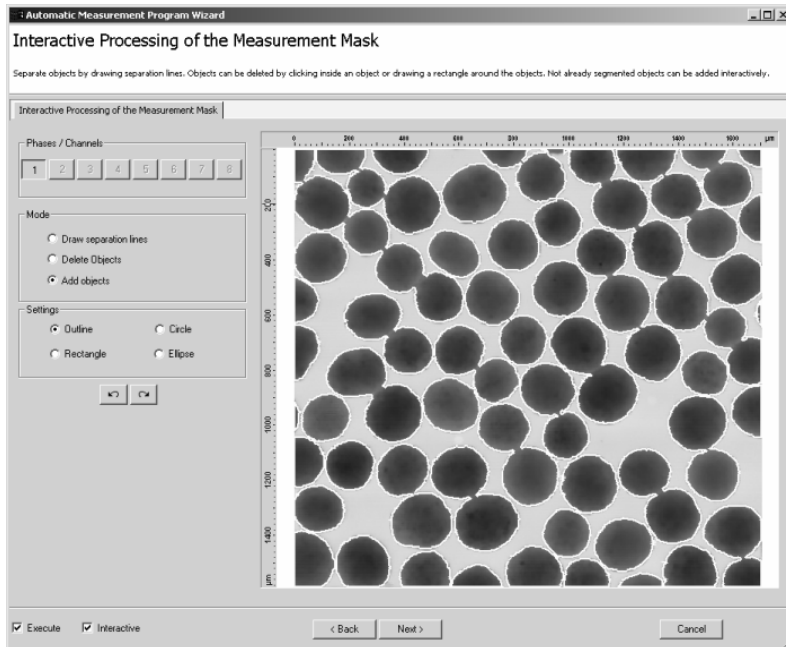
Dialog for image segmentation

Here you can separate the objects to be measured from the background simply by clicking on or outlining reference objects. Select whether you want individual objects only, or the entire image, to be segmented.

With multidimensional images, you also have the option of performing segmentation separately for each channel of a multichannel fluorescence image. If "normal" gray level or color images are used, up to eight different phases can be defined (in place of channels).

Processing the measurement mask (binary image processing)

In most cases, the measurement mask generated during segmentation has to be processed subsequently to ensure that only the objects you actually want to measure are highlighted.



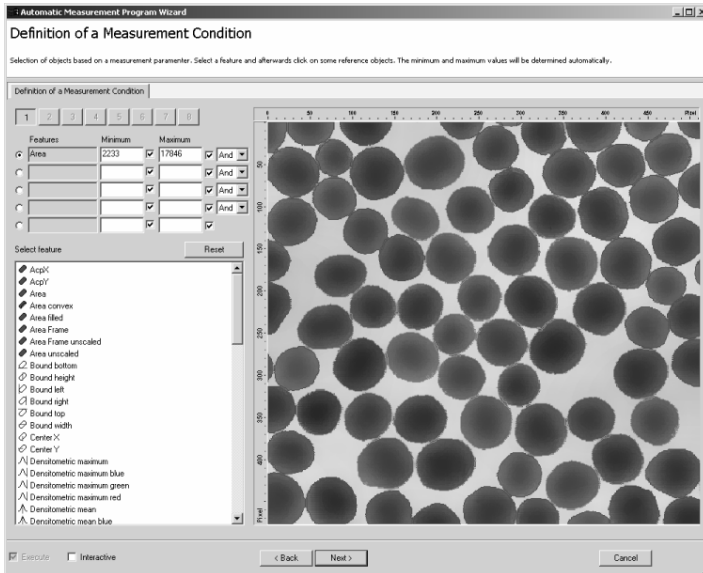
Step for interactive correction of the measurement mask

The measurement wizard therefore contains a function for the automatic separation of touching objects (Erosion/Dilation, Watersheds) and for the subsequent interactive processing of the mask (drawing in separating lines, deleting and adding objects).

Both steps start with a function for eliminating artifacts and for filling gaps ("cleaning" of the measurement mask).

Measurement

The actual measurement procedure consists of several steps. These are used to define how the measurement should be carried out in detail.



Definition of a measurement condition ("object filter")

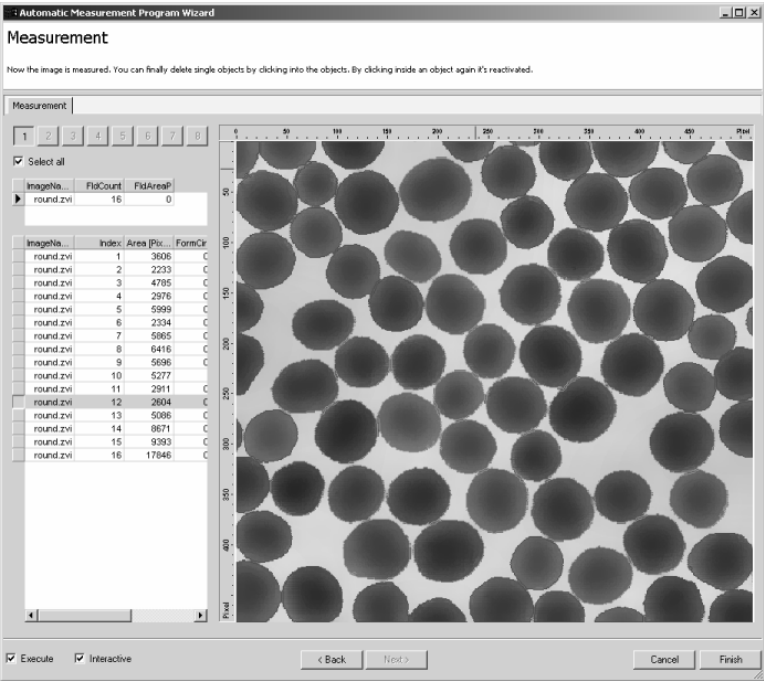
The individual steps

- Determination of region-specific (individual objects) and field-specific (entire image) measurement parameters.
- Definition of the way in which the measured objects should be highlighted in the resulting image at the end of the measurement (contour, measurement parameters etc.).
- Generation of a measurement condition. This defines the conditions that an object must satisfy in order to be measured. This is a kind of "object filter", which can be used, for example, to specify that only objects within a certain size category should be counted.

- Definition of a measurement frame, which is used to specify how objects that are cut off by the image edge, and are therefore likely to distort the measurement result, should be handled.

Evaluation

In the final step, AxioVision shows you the image with the objects selected for measurement again. Here you can make any final changes, e.g. deleting individual objects by clicking on them.



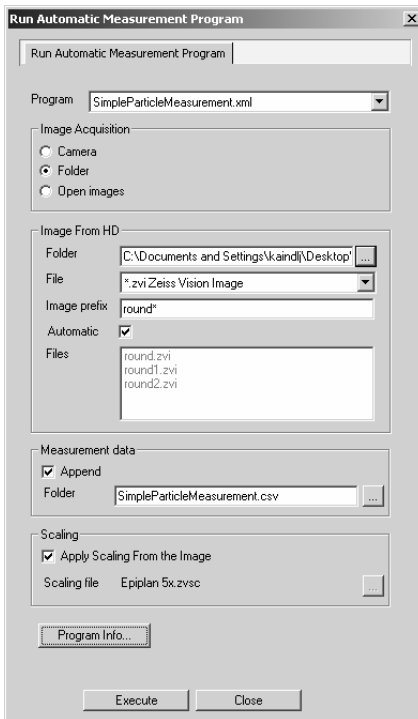
The measurement data are saved at the end of the measurement in so-called CSV format (**C**omma **S**eparated **V**alues).

This is a pure text format that separates the individual values from one another using special text symbols. The specific country settings that have been set in the Microsoft Windows control panel apply.

Files in CSV format offer two advantages: on the one hand, they are a special Microsoft Excel format, and can be opened and processed directly by that program. On the other hand, as the files contain pure text data, you can import the data into practically any other program.

Executing measurement programs

Once measurement programs have been generated, they can be applied to any number of images for series analyses. To do this, start the **Run Automatic Measurement Program** function from the **Measure** menu. Further information on the functional parameters can be found in the online help by pressing the **F1** key.



Function for executing measurement program

NOTE:

- As the tables of measurement results are available at the end of the measurement wizard, the wizard can also be used to measure an individual image. A useful feature is that rather than a new measurement program being generated each time for this purpose, an "auxiliary program", which always has the same name (e.g. "individual image measurement"), is used.