10.4 Extended Focus

General

The **Extended Focus** function is available as an optional module for AxioVision. This function enables you to display the sharp areas of different focus positions of your sample in a single image.

Essentially, the **Extended Focus** module is operated interactively. You do not need a motorized microscope. Several images are acquired at different focus positions. The sharp areas are extracted from each of the images and automatically combined. You immediately obtain a sharp 2D image. The individual images of the different focus positions are not saved, but are combined straight away. The focus is driven manually across the entire z-range until all the details have been displayed sharply. There are no specifications, recommendations or formulae that say how many images have to be acquired or how large the distance between two focus positions should be. This always depends on the sample in question. Generally speaking, it can be said that it is better to acquire too many images than too few.

Alternatively, an image with extended depth of field can also be calculated using a z-stack. To do this, you need a z-stack image, the acquisition of which requires a motorized microscope and the **Z-Stack** module (see also section 10.8 "z-stack images").

Acquiring images with Extended Focus

The **Extended Focus** function can be run via the work area on the left of the AxioVision window. If the work area is not displayed, activate it using the

Standard toolbar Workarea .

Alternatively, select the **Windows** function in the **View** menu and then select the **Workarea** entry.
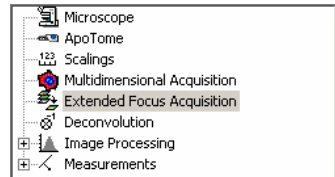
NOTES:

▪ The work area may in fact be open but covered by the workflows. If that is the case, simply click on the **Workarea** tab at the bottom of the work

area .

➢ Switch the light path to the camera. Open the live image window by clicking on the live image icon in the **Standard** toolbar. Alternatively, select the **Live** function in the **Acquisition** menu.
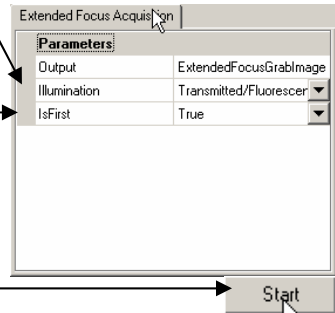
➢ In the work area select **Extended Focus Acquisition**.

➢ Select **Illumination**.

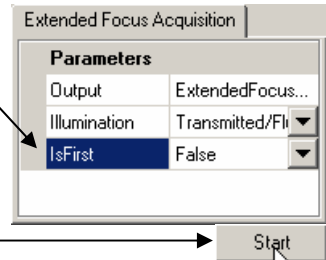➢ Make sure that the **True** setting is active for **IstFirst**.

NOTES:

▪ **Yes** creates a new resulting image. If one already exists it is overwritten.

➢ Click on **Start** to acquire the first image.

➢ Now go to the next focus position.

➢ Make sure that **False** is now set for the **IstFirst** parameter (this normally happens automatically once the first image has been acquired).

➢ Click on **Start** to acquire the next image.

NOTES:

▪ The image of the second focus position is now automatically combined with the first image.

▪ You can now acquire further images until the result is satisfactory.

▪ If results are not satisfactory you can also test the other type of illumination. In many cases the result is then improved.
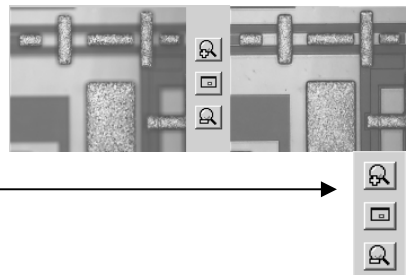
Using z-stack images for Extended Focus

➢ Activate the image to which you would like to apply the **Extended Focus** function. To do this, click on the image or select it via the tabs at the top of the document area.
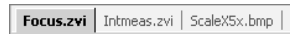
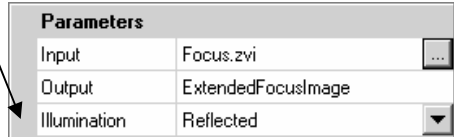➢ Now select the **Extended Focus** function in the **Processing** menu.
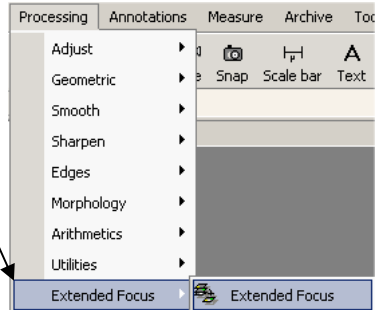
➢ The two thumbnails show the original image (left) and the resulting image (right).

To select a specific frame, you can magnify, choose any frame from, or reduce the thumbnails.

➢ Select **Illumination**.

Parameters		
Input	Focus.zvi	...
Output	ExtendedFocusImage	
Illumination	Reflected	▼

➢ Click on **Start** to check the result.

☑ Automatic Preview

Start   Cancel   Close

➢ Now click on **Close** to exit the function.