Deep Focus 3.4

User Guide

Deep Focus 3.4

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Preface

The Deep Focus 3.4 is an additional software module that creates digital images with depth of field much higher than the standard use of optical microscopes can provide (so called focus stacking or z-stacking). The Deep Focus 3.4 module can be used in conjunction with stereomicroscopes as well as with other types of optical microscopes for observation in transmitted or reflected light. The module is also suitable for macro-imaging.

Principle of Function

The image with extreme depth of field is composed from several "slices" - images taken at different focus distances. From each of the slices, only a well focused area is used. The completely focused image is composed from these well focused areas. Possible shifts and scale changes between slices are compensated automatically.

Installation

Note

For the successful Deep Focus 3.4 module installation it is necessary to have the *QuickPHOTO CAMERA*, *QuickPHOTO MICRO* or *QuickPHOTO INDUSTRIAL* program in version 3.1 installed on your computer.

Note

In case you do not have the permission to use the Deep Focus 3.4 module contained in your dongle or you are installing the module into a trial version of the one of the above mentioned programs (no dongle is present), the Deep Focus 3.4 module will run in a mode of a demo version. The demo version provides all the functions of a full version, but a watermark will be inserted into the created images. Each time you will run the demo version you will be informed about that fact. If you want to purchase a full version, contact your software supplier.

Insert the installation CD of Deep Focus 3.4 module into your CD/DVD-ROM drive. An autorun screen will appear. If the autorun screen does not appear, launch the *autorun.exe* file from the installation CD manually. Select *Install Deep Focus 3.4* option.

An installation wizard will appear. Select the desired language and continue by the *Next* button. The wizard will search your computer for installed *QuickPHOTO CAMERA*, *QuickPHOTO MICRO* or *QuickPHOTO INDUSTRIAL* programs. In case there is more then one *QuickPHOTO* version installed on your computer, choose the desired version into which the Deep Focus 3.4 module should be installed from the displayed list and click the *Next* button.

Continue following the on-screen instructions.

Settings for Motorized Image Capturing

The whole process of creating the digital images with extreme depth of field can be automatized by the use of an optional module for motorized focus control or a motorized microscope.

To automate the process of image capturing, the Deep Focus 3.4 module cooperates with a software module for motorized focus control. This module called *Microscope* is installed together with the Deep Focus 3.4 module and can also be run separately by clicking the ⑤ icon in the *Toolbar* of the main program.

The *Microscope* module supports several types of control units, motorized focus drives, stands and motorized microscopes. This module needs to be configured prior to its first use.

In order to set the motorized focus control proceed as follows:

1. Make sure the focusing unit is properly installed and connected to its control unit. Further, make sure the control unit is switched on, connected to the computer and its device drivers are properly installed.

2. In the main program activate the *Modules → Microscope* function or click the ⑤ icon in the *Toolbar*.

3. In the opened *Microscope* dialog box in the *Device settings* part select a desired control unit first, from the *1. Control box* drop-down menu, then select a motorized device from the *2. Motorized device* drop-down menu, and finally select the *2. Motorized device* drop-down menu, and finally select a microscope type from the *3. Microscope* drop-down menu. It is important to select a proper microscope type in order to get the proper z-axis calibration.

4. In the *Serial port* part select the COM port the control unit is connected to. Use the *Detect port* button to find out the correct port.
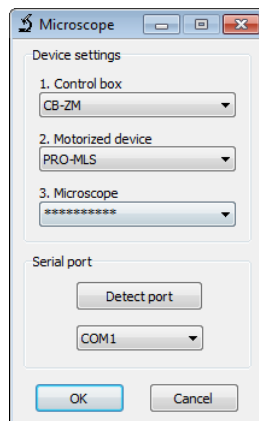
5. Click the *OK* button to confirm the settings.

6. The *Microscope* module will switch to the operational mode. Use the *Up* and *Down* buttons to test its functionality.

7. If the *Microscope* module properly controls the motorized device, close it.

After this procedure you can start using the functions for motorized image capturing of the Deep Focus 3.4 module.

If necessary, you can switch the *Microscope* module to the configuration mode at any time by clicking the *Device settings* button.

Note

The CB-ZM control unit is connectible to the computer via a USB cable, however it communicates with a PC in the manner of a serial port (COM). This device includes a special converter and there will be installed a USB serial port in the operating system. The CB-ZM device is usually installed on the COM port with the highest number.

Manual Capturing of Images for Composition

The first step to create an image with extreme depth of field is capturing of a set of images taken at different focus distances - the so called "slices". In each slice the different area should be focused. The change of focus distance is performed by the focus knob of a microscope and it should not be greater than is the depth of field of the microscope. It means the well focused area should move smoothly over the specimen in the series of the captured images.

Recommended Procedure for Capturing Images

1. In the main program open the control panel of your imaging device.

2. Set the illumination and imaging device's parameters that way, the illumination conditions remain unchanged when changing the focus distance. It is recommended to set the manual exposure mode of the imaging device. In case the automatic exposure mode is set, it is recommended to use the *Auto Exposure Lock (AEL)* function.

3. Using the live view focus the microscope by rotating the focus knob to the bottom part of the specimen.

4. Acquire an image and change slightly the focus distance, using the focus knob of the microscope, towards the highest part of the specimen.

5. Repeat the step 4) until the highest part of the specimen is focused and its image is acquired.

How to Use Deep Focus 3.4 Module

The Deep Focus 3.4 module can be used in four ways:

1. Compose images selected in the *Thumbnail Browser*

2. Compose saved images

3. Acquire and compose images automatically

4. Acquire images automatically (without composition)
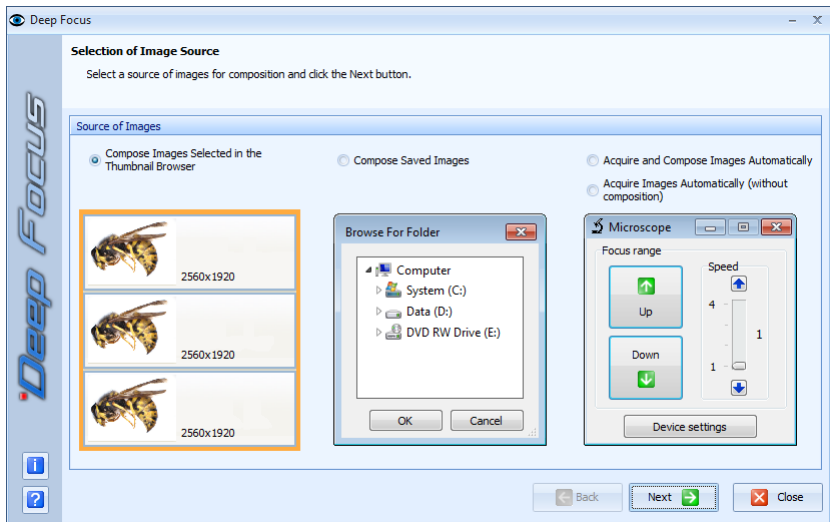
1. Compose Images Selected in the *Thumbnail Browser*

This option is intended for composition of images opened in the main program.

The procedure of use is following:

1. Select the images (intended for composition) opened in the main program by selecting their thumbnails in the *Thumbnail Browser*. The selected images will be indicated by

frames. The selection can be performed similarly to the selection of files in Windows®
Explorer (all images can be selected by [Ctrl+A] key shortcut, the continuous selection
can be done while holding the [Shift] key or respectively [Ctrl] key for discontinuous
selection).

2. Launch the Deep Focus 3.4 module from *Modules → Deep Focus 3.4* menu or by
    clicking the ◉ icon.

3. In the opened dialog box select the *Compose Images Selected in the Thumbnail Browser*
    option and click the *Next* button.

4. The next page of the wizard *Composition Parameters* will display. Continue according
    to the chapter Setting the Parameters of Composition - for more information see
    "Setting the Parameters of Composition" on page 17.

Important

All images to be processed must have the same dimensions in pixels
(e. g. 1024 x 768).

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2. Compose Saved Images

This option is intended for composition of images stored on your hard drive, flash memory
drive etc.

The procedure of use is following:

1. Launch the Deep Focus 3.4 module from *Modules → Deep Focus 3.4* menu or by
    clicking the ◉ icon.

2. In the opened dialog box select the *Compose Saved Images* option and click the *Next*
    button.

3. Use the *Open* dialog to select the images to be composed. Make sure the selection
    does not contain any unwanted images.

4. The next page of the wizard *Composition Parameters* will display. Continue according to the chapter Setting the Parameters of Composition - for more information see "Setting the Parameters of Composition" on page 17.

Important

All images to be processed must have the same dimensions in pixels (e. g. 1024 x 768).

# 3. Acquire and Compose Images Automatically

This option is intended for automated acquisition and composition of images with extreme depth of field using the module for motorized focusing of a microscope.

The procedure of use is following:

1. Make sure the device for motorized focusing or motorized microscope is properly connected, the control unit is switched on and connected to your PC.

2. Launch the Deep Focus 3.4 module from *Modules → Deep Focus 3.4* menu or by clicking the ◉ icon.

3. In the opened dialog box select the *Acquire and Compose Images Automatically* option and click the *Next* button.

4. The control panel of active imaging device will open along with the *Microscope* module for focus control.

5. Set the illumination and imaging device's parameters. The illumination conditions should remain unchanged when changing the focus distance. It is recommended to set the manual exposure mode of the imaging device. In case the automatic exposure mode is set, it is recommended to use the *Auto Exposure Lock (AEL)* function.

6. In *Focus range* part of the *Microscope* dialog box proceed as follows:

    a. Use the *Up* or *Down* buttons to focus (using the live view) the microscope to the bottom part of the specimen. Click the *Set bottom* button to set the lower limit of the focus range. The *Not set* text on right side of the *Set bottom* button will change to *Set*.

    b. Use the *Up* or *Down* buttons to focus (using the live view) the microscope to the highest part of the specimen. Click the *Set top* button to set the upper limit of the focus range. The *Not set* text on right side of the *Set top* button will change to *Set*.

    The focusing can be also controlled by the keyboard (using the up and down arrow keys). Focusing speed can be set by the *Speed* slider or also by the [+] or [-] keys.
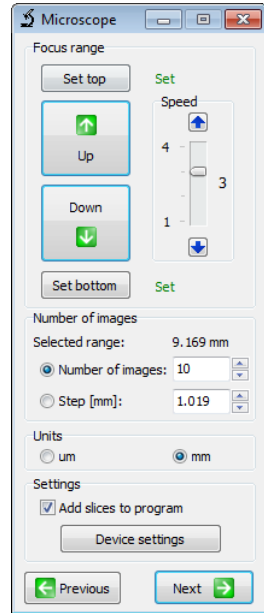
7. In *Number of images* part enter the desired *Number of images* (slices) to be acquired or enter the *Step* (distance) between slices. The corresponding second parameter will always be calculated automatically. The minimum number of slices is two. The *Selected range* indicator shows distance between lower and upper limit of the set focus range in units selected in *Units* part.

8. If you want to keep the slices from which the image is going to be composed, check the *Add slices to program* checkbox. The slices will appear in the main program after acquisition.

9. After clicking the *Next* button in the *Microscope* dialog box there will appear the *Composition Parameters* page of the wizard. Continue according to the chapter Setting the Parameters of Composition - for more information see "Setting the Parameters of Composition" on page 17.

Important

In order to begin the process of automated capturing of slices and image composition, it is necessary to set the lower and upper limit of the focus range and the number of slices to be acquired or a step between them.

Focus distance changing during the image acquisition process will be performed with the speed set by the *Speed* slider in the *Microscope* dialog box.

In case the desired *Step* between slices is selected, the set focus range will be divided into integral multiple of the selected step size and the remaining rest of the range, e.g. set focus range is 170 microns, the selected step is 50 microns - the focus range will be divided into 3 steps of 50 microns and one step of the remaining 20 microns.

4. Acquire Images Automatically (without composition)

This option is intended for automatic acquisition of slices without composition. The slices will be displayed in the main program immediately after their acquisition and the process of composition will be omitted. The procedure of use is similar to article *3. Acquire and Compose Images Automatically*.

Setting the Parameters of Composition

1. On the *Composition Parameters* page, select the type of images to be composed.

    There are two options available:

    • **Aligned Images (Images from Upright and Inverted Microscopes)**

    Select this option for composition of images acquired from a microscope which does not cause shifting of the specimen in the field of the imaging device's view during changing the focus distance (upright and inverted microscopes). Composition of aligned images is much faster than the composition of shifted images (images from stereomicroscopes).

    • **Other Images (Images from Stereomicroscopes and Macro Images)**

Select this option for composition of images acquired from stereomicroscopes, macroscopes and for macro images. Stereomicroscopes generally cause shifting of the specimen in the field of the imaging device's view while changing the focus distance. Within macro images the minor changes of a scale (magnification) usually take place. The Deep Focus 3.4 module compensates the shifts and scale changes between images automatically in the *Other Images* mode.
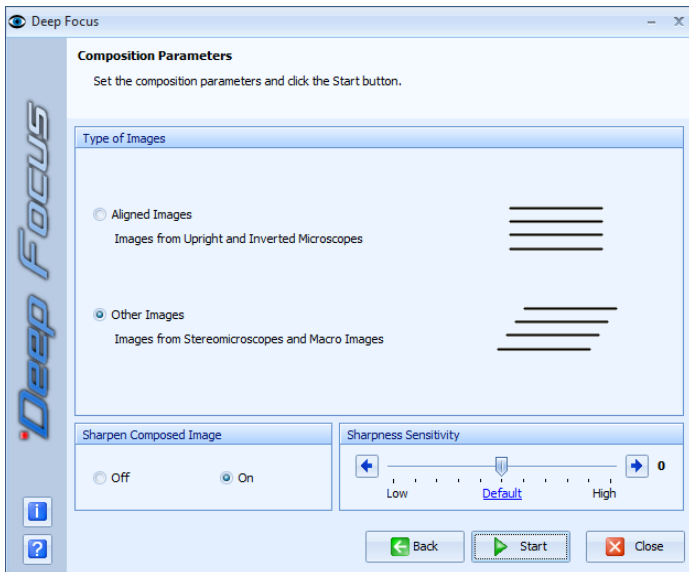
2. In the *Sharpen Composed Image* part select whether you want to post-process the composed image with a sharpen operation. This operation enhances edges in the image.

3. Leave the *Sharpness Sensitivity* slider in the *Default* position for most specimens. In case the composed image contains some unfocused parts although they are well focused in some of the slices, increase the *Sharpness Sensitivity* value and repeat the composition process. The higher sensitivity you set, the more significant presence of

noise, which is present in all digital images, can be. The noise level depends mainly on quality, type and settings of the used imaging device as well as on illumination conditions. For example, when using a cooled digital camera, the noise level will be rather low. Conversely, when using a consumer digital camera with high ISO sensitivity set in poor illumination conditions, the noise level will be quite significant. The noise impact, when the *Sharpness Sensitivity* value is set too high, is usually manifested by a presence of many unsharp areas (islands) in the composed image. In some cases decrement of *Sharpness Sensitivity* value may also be required.

4. Click the *Start* button to begin the composition process. The *Start* button will change to *Stop* button, which can be used to interrupt the process of composition. The composed image will be displayed in the main program window after finishing the composition process. It will be named *f_#####.suffix*, where ##### is the serial number assigned to the image by the main program. If necessary you can interrupt the composition process and close the Deep Focus 3.4 dialog box by clicking the *Close* button. The termination process can take a while.

Note

The dimensions of a composed image will be slightly smaller than are the dimensions of the slices, due to an automatic crop function.

Automatic Image Acquisition Using Imaging Devices Controlled Via TWAIN Interface

Deep Focus 3.4 module enables automated image capturing also from imaging devices controlled via a TWAIN interface. These control panels are controlled externally by the Deep Focus 3.4 module, which implies minor limitations.

After you begin the image acquisition (by clicking the *Start* button) using the TWAIN control panel an informative dialog box will be displayed, which will ask you to click the button for image capturing in the control panel of the imaging device. After you click the corresponding button, initialization image will be captured (which will not be included in the composition), which will enable the Deep Focus 3.4 module to identify the position of the button for image capturing in the TWAIN control panel. Information dialog box will automatically disappear after capturing of this image and the image acquisition process

will begin. To ensure successful process of image acquisition, do not move or click the mouse anymore until the whole image acquisition process is finished.

Control of Motorized Focusing

After installation the Deep Focus 3.4 module you can control the motorized focusing of the microscope in the main program also without launching the Deep Focus 3.4 module. For the focus control you can use the *Microscope* software module, which can be launched by clicking the 🖅 icon in the main program *Toolbar*.

Using the *Down* button you can focus to the lower parts of the specimen. Using the *Up* button you can focus to the higher parts of the specimen. Focusing can be also controlled by the keyboard, using the up and down arrow keys. Speed of the focus movement can be adjusted by the *Speed* slider or also by the [+] or [-] keys.
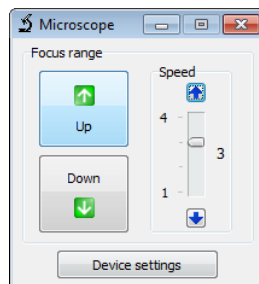
Tips for Work with Deep Focus 3.4

Here there are some recommendations to achieve the best results with Deep Focus 3.4 module:

1. Place the specimen to the centre of imaging device's field of view.

2. Capture the whole series of slices with the same settings of the imaging device and the microscope. Do not change the zoom value during capturing of slices.

3. It is recommended to use manual exposure mode or *Auto Exposure Lock (AEL)* function when using the automatic exposure mode.

4. Do not change the illumination conditions during the capturing of slices.

5. The best results can be achieved when capturing larger amount of slices with small changes of the focus distance.

6. Make sure there is no unwanted image in your selection of images intended for composition.

7. All slices to be composed must have the same dimensions in pixels (e. g. 1024 x 768).

> **Note**
>
> The higher the resolution and number of slices is, the longer time the composition process takes.

Minimal System Requirements

Minimal Computer Requirements:

- Single-core 2.4 GHz or multi-core processor
- 1 GB RAM
- CD/DVD-ROM drive
- Microsoft$^{®}$ Windows$^{®}$ XP(SP3)/Vista$^{®}$/7/8 operating system

Supported QuickPHOTO Program Versions:

- QuickPHOTO CAMERA 3.1
- QuickPHOTO MICRO 3.1
- QuickPHOTO INDUSTRIAL 3.1

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