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User Guide for Z-Stacking tool of PROGRES GRYPHAX[®] software

The "Z-Stacking tool" of PROGRES GRYPHAX® software enables user to create EDF (extended depth of focus) images of specimen <u>without</u> motorized z-stages at live! The sharp areas of the specimen are <u>automatically</u> recognized and combined to one consistently sharp, so called, EDF image.

Preparations:

All camera image parameter (e. g. colour, white balance) should be set in advance before you start with Z-Stacking image combining tool.

To reach to best results with *Z*-Stacking tool you have to adjust the focus level of specimen to the top focus point and change to "Expert" exposure control <u>before</u> you start using *Z*-Stacking tool.

To find the best focus start point you can use the focus indicator bar of "Magnifier tool" (keyboard short cut: **space bar**)

Magnifier tool with "blue" focus indicator bar:

<u>Note:</u> The zoom factor of "Magnifier tool" can be adjusted at the software preferences.



<u>Note:</u> To improve the Z-Stacking image creating the camera should deliver highest live frame rate. Reduction of live resolution and exposure time enables highest live frame rate. Furthermore, deactivate of FX tools: "Sharpen", "Dynamic" and "Noise reduction" increases live frame rate as well.



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Start:

After successfully preparation you can open Z-Stacking tool by click on Z-Stacking icon:

Z-Stacking tool delivers two different composition modes.

- a) "Fast" very fast image composition without correction for parallax shift
- b) "Precise" high quality image composition with parallax shift correction of e.g. stereo microscopes

Composition mode	
○ Fast	
• Precise	

Please define the Z-Stacking composition mode for image combining <u>before</u> you start image combining!

Press "REC" button **TEC** to start Z-Stacking image combining.

The *"Z-Stacking"* tool is displaying the split screen. On the left the composed <u>result preview image</u> and on the right site the <u>live image</u> getting from camera.



Additionally, the "REC" button change to "STOP" **STOP** to save the composed EDF image.







To start image combining please move the microscope stage at "Z-axis" gently to compose "Z-Stacking" image starting from top focus to bottom like as follows in one not changing direction.

During stage movement the Z-Stacking tool detects and tracks the sharp image areas automatically and accordingly save the different focus images to the internal memory. The result preview image is painted and shows where the Z-Stacking tool has found different sharp area and combined them to one image.

Save:

Press the "STOP" **STOP** button to save the composed EDF image to the GRYPHAX Gallery, at any time if you are satisfied with the result image. After save of EDF image the software returns back to *Z-Stacking tool* to start a new image creation.

With composition mode "Fast" the EDF image will be saved immediately after press "STOP" to the Gallery.

With composition mode "Precise" the EDF image will be generated from the current number of different found focus images. This will be more timeconsuming. The remaining time will be displayed.

The image calculation can be aborted by press "Cancel" button.

Limitations:

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- a) Measurement and Annotation are not allowed during "Z-stacking tool".
- b) Save of separate focus layer images are not available.

SERIES

Low memory

c) The image number for Z-Stacking images is limited by approx.: 2 GB of memory The software will display a warning message as follows:



Please stop image combining and save the Z-Stacking image by using "STOP" **STOP** button.



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We looking forward to hearing from you and remain sincerely,

Your PROGRES GRYPHAX[®] Team



