Ease of use –
giving your work an effortless feel.

Z-Stacking tool of JENOPTIK GRYPHAX® software

The "Z-Stacking tool" of JENOPTIK 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.

19.1   Preparations:

All camera image parameter (e. g. colour, white balance) must 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" (or use keyboard key: **space bar**)

Magnifier tool with "focus indicator bar":

"**red**" bar – shows the max peak of focus level of magnifier ROI

"**blue**" bar – shows the current relative focus level of magnifier ROI
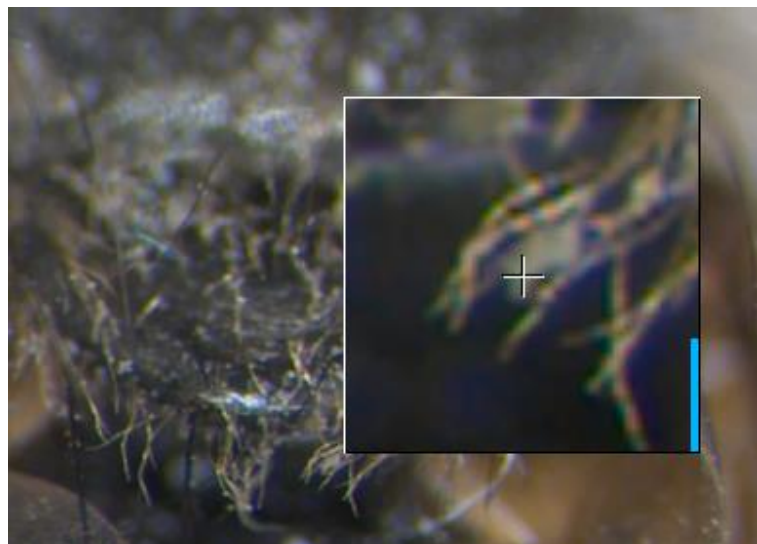
The zoom factor of "Magnifier tool" can be adjusted at software preferences by 3:1 | 2:1 or 1:1.

<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, deactivating of FX tools: "Sharpen", "Dynamic" and "Noise reduction" increases live frame rate as well.

19.2   Start:

After successfully preparation, you can open *Z-Stacking tool* by click on Z-Stacking icon: ⊞
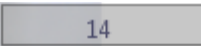
As of JENOPTIK GRYPHAX software version 2.1.0.0 or newer the *Z-Stacking tool* **automatically** corrects the image composition with parallax shift correction of e.g. stereo microscopes during live instantly.

This reduces image calculation time for parallax shift correction compared to previously versions of Z-Stacking tool.

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

The *"Z-Stacking"* tool is displaying the **screen split into two sections**. On the left the composed result preview image and on the right site the live image getting from camera.
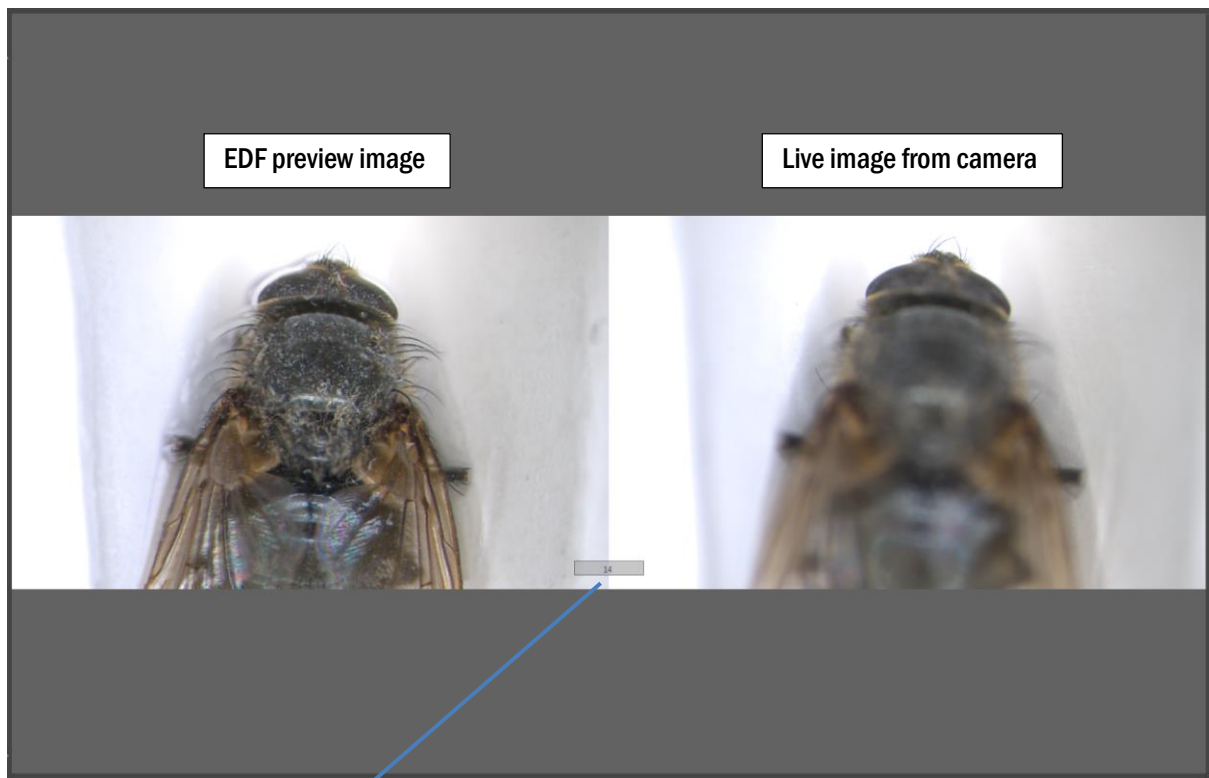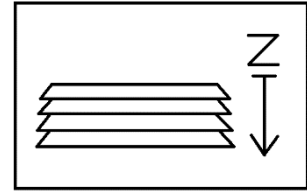

The image counter **14** is displaying the current number of used different focus images.

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.

19.3   Save:

Press the "STOP" button to save the composed EDF image to the 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.

For image composition without parallax shift correction - the EDF image will be saved immediately after press "STOP" to the Gallery.

For image composition with parallax shift correction the EDF image will be generated from the current number of different found focus images.
This will be more time-consuming. The remaining time will be displayed.

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

Limitations:

- Measurement and Annotation are not allowed during *"Z-stacking tool"*.
- Save of separate focus layer images are not available.
- The image number for Z-Stacking images is limited by approx.: 2 GB of memory
  The software will display a warning message as follows:
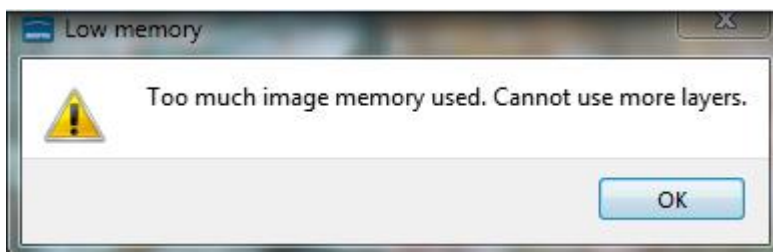
Please stop image combining and save the Z-Stacking image by using "STOP" button.