

Image Stitching and Extended Depth of Focus with Fein Optic 3DCxM2.3, 3DCxM6.3, 3DCxM12 and 3DCxM20









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Helpful Tip: Focus your image looking at your monitor rather than focusing through the eyepieces. This will give you a clear, crisp and focused image ready for working with the Image Stitching feature.

- Place your specimen on the stage and bring it into focus.
- Using the options in the tool bar to the left, adjust your image by clicking the drop down arrows for the corresponding adjustment option.





- Select Process > Stitch.
- The <u>Stitch icon</u> can also be found on the top tool bar.

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- A tab will appear next to your video image tab showing "Stitch 1".
- A <u>checkered background</u> will be behind your image.
- Your stitch image is "live" meaning if you bump or move your stage or specimen the stitching process will record the movement.

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- Your image will be framed in a <u>bright green border</u>. This rectangle is the real time image. Any image outside of the real time image is from the stitching results.
- At the top left of your image directly above the bright green border there is a "Sharpness" indicator.
- The Sharpness indicator will change from bright green to <u>yellow or red</u>. When the border is green the stitching function is working properly. When the border is yellow or red, the slide should be moved back a bit to the area where the border turns green again. After the border is green again, proceed with moving the slide.





- Very slowly begin to move your stage or specimen in the direction you want included on the image stitching.
- The image section to the left, outside of the green rectangle, is the <u>stitched image</u>. The image section inside the green rectangle is the <u>real time image</u>.





• If you are satisfied with your image, select the stitch icon to stop the live stitching function.





- The stitched image will cut from the checkered background.
- The resulting stitched image is ready for editing and saving.
   Select Image and Process to see functions for editing your
- Select <u>Image</u> and <u>Process</u> to see functions for editing your stitched image.





## Extended Depth of Focus: Manual Capture and Live Capture

- There are two processes for the Extended Depth of Focus feature. One is manually capturing images (Manual Capture) and then layering them together. The other process is done via live imaging (Live Capture).
- See the following instructions for each process.

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#### Items you will need for Extended Depth of Focus

- Microscope
- DCxM2.3, DCxM6.3, DCxM12.3 or DCxM20 camera
- PC or laptop.

#### **Getting Started**

- Once your microscope, camera and computer are connected and properly set up, launch the software.
- Check that you have selected the camera which is found on the tool bar to the left. The camera is recognized by the following model number: <u>E3CMOS02300KPA(DCxM2.3</u>), <u>E3ISPM06300KPS(DCxM6.3</u>), <u>E3ISPM120000KPA(DCxM12</u>) and <u>E3ISPM2000KPA(DCxM20</u>). Once the camera is selected you will see a live video image of your microscope stage. If the camera is not selected you will not see an image.

Helpful Tip: If your microscope has a beam splitter, be sure to check that it is set in the proper position for working with your microscope camera. If it is not set in the proper position you will not see an image.

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Helpful Tip: Focus your image looking at your monitor rather than focusing through the eyepieces. This will give you a clear, crisp and focused image ready for working with the Extended Depth of Focus feature.

- Place your specimen on the stage and bring the closest or furthest depth of your specimen into focus. You will be refocusing your specimen to perform the Extended Depth of Focus feature.
- Using the options in the tool bar to the left, adjust your image by clicking the drop down arrows for the corresponding adjustment option.





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- With the selected section of your specimen in focus, snap an image by clicking on the <u>Snap</u> button on the tool-bar to the left.
- After the image has been captured and before moving your focusing knob, click back on the <u>Video</u> tab. Next, slightly adjust your focus knob and snap an image. Adjust your focus knob again and snap another image. Remember to click back on the video tab each time ensuring you are looking at the live image of your specimen. Continue to bring different depths of your image into focus each time pausing to snap an image.
- The images you are capturing will be numbered and appear across the top of the image screen.





- After you have captured your images, select Process->EDF from the top toolbar. • File Edit View Browse Setup Capture Image Process Layer Measurements Options Wind 1 🔤 👫 Stitch... Shift+T 1 💕 🔛 🎽 Micrometer (µm) V 5x M40 111 High Dynamic Range (HDR)... Camera x 4 00 EDF... Shift+F Ŧ ٨ \$ Camera List Deinterlace Color Composite... E3ISPM06300KPA 0 Segmentation & Count . \$ Capture & Resolution Denoise Sharpen Record Snap Color Toning 200 Shift+1 Filter... 3072 × 2048 V Live: Image Stacking...  $\sim$ 3072 × 2048 Snap: 12-Profile... Format: RGB24 ~ Surface Plot... 400 \* Exposure & Gain Pseudo Color... × White Balance Range... Shift+R × Black Balance Binary... Shift+B
- The EDF icon can also be found directly on the top tool-bar.

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- A box will appear for you to select which images you want to perform the EDF process on.
- Select the desired images individually and then click <u>Add</u> or click <u>Add all</u> to select all images listed in the left hand column.

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• Additional option boxes will appear. Continue working through the options selecting <u>Next</u> each time you have set your preferences.

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Description Weighted Average method product often gives superior results to FFDSSD met microscopy. It is worth noting that images to furthest to closest with the microscope obje Maximum Contrast method is similar must be in Z order as well. The difference is detail though what detail is preserved will b method of EDF. FFDSSD is a method that does filter transform (FFT) and does synthesis in the s misalignment. Users can manually or autom aligned.	es a soft fusion result but may miss detail hod with deep stacks, such as images pro to be processed must be in Z order (closes ective lens relative to sample on the stage r with weighted average method, and the that the maximum contrast method may e sharper. Maximum contrast method is a ring in the frequency domain using Fast F space domain. FFDSSD can be used for im atically select reference images to make in	. This method oduced by st to furthest or e), e input images loose some a preferred fourier nages having nput images
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- The last option to select is how the EDF will align all the images you have captured.
  - Select <u>Finish</u> once the appropriate option has been selected.

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- The EDF process will begin.
- Note: The processing could be slower depending on how many images were captured and the options selected.

EDF (Extended Depth of Focus)		×
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- The image will appear once the EDF process has completed.
- The image is now ready for any additional editing or saving.





Problem	Solution
No live image displayed	Beam splitter is not in the proper setting. Check the beam splitter setting.
	Camera is not selected. Select the camera on the tool bar to the left.
Live image is dark	Adjust the illumination on the microscope Use the white balance option on the tool bar to the left.
Live image is upside down or backwards	Use the Rotate and Flip features on the tool bar to the left.
Dust or dirt is visible in the live image.	Clean the sample Clean the objective Clean the eyepieces Clean the C-mount Clean the lens on the camera

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#### Items you will need for Extended Depth of Focus

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Helpful Tip: If your microscope has a beam splitter, be sure to check that it is set in the proper position for working with your microscope camera. If it is not set in the proper position you will not see an image.





### **Extended Depth of Focus: Live Capture**



Helpful Tip: Focus your image looking at your monitor rather than focusing through the eyepieces. This will give you a clear, crisp and focused image ready for working with the Extended Depth of Focus feature.

- Place your specimen on the stage and bring the closest or furthest depth of your specimen into focus. You will be refocusing your specimen to perform the Extended Depth of Focus feature.
- Using the options in the tool bar to the left, adjust your image by clicking the drop down arrows for the corresponding adjustment option.





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Select Process > EDF. This EDF Icon can also be found in the tool bar. File Edit View Browse Setup Capture Image Process Layer Measurements Options Window Help Stitch... Shift+T 📴 🛃 🎽 🗁 🚼 🛛 Micrometer (um) 🔽 5x M40 / / / // / ↓ • □ ○ ○ • ◎ & • ヽ Ī ☆ 🖀 🖊 📣 il High Dynamic Range (HDR)... Camera ▼ ₽ X 1 EDF... Shift+F 200 400 600 800 \$ Camera List Deinterlace Color Composite... E3ISPM06300KPA 0 Segmentation & Count \$ Capture & Resolution Denoise Sharpen Record Snap 10 **Color Toning** 200 Filter... Shift+1 Live: 3072 × 2048 Ŧ 3072 × 2048 Image Stacking... Snap: Ŧ ٠ Format: RGB24 12 Profile... Surface Plot... 400 ¥ 🔁 Exposure & Gain Pseudo Color... ¥ White Balance Range... Shift+R O Black Balance ¥ Binary... Shift+B ¥ 🍋 Color Adjustment 600 \$ Bit Depth





- A Live Capture box will appear in the lower left hand portion of screen.
- The larger background box is where your EDF process is being performed.





## Extended Depth of Focus: Live Capture

- Begin to very slowly move your focusing knob to bring the different depths of your specimen into focus while looking at the Live Capture box. Remember which portion of your specimen (closest or furthest depth) you have in focus as this will determine which way you turn your focusing knob.
- It is recommended to stop turning the focusing knob periodically to allow for the EDF process to accurately capture all images being brought into focus.
- The large background box is "capturing" the different focused images and stacking or layering them to achieve the complete EDF picture.
- If the background image is blurry around the edges, this is a result of moving to quickly when bringing the different depths of your specimen into focus. Slow down the focusing and pause periodically as mentioned above.
- If the blurriness is significant, you may want to stop and begin your EDF process again.





# Extended Depth of Focus: Live Capture

• You can stop adjusting your focusing knob once you are satisfied with your image and it is now ready for editing and saving.





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Problem

#### Solution

No live image displayed	Beam splitter is not in the proper setting. Check the beam splitter setting.
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Live image is dark	Adjust the illumination on the microscope. Use the white balance option on the tool bar to the left.
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Dust or dirt is visible in the live image.	Clean the sample Clean the objective Clean the eyepieces Clean the C-mount Clean the lens on the camera

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