



MS57 Training guide: 2D+ Focus stacking imaging (RBINS & RMCA)

1. Acquiring: StackShot workflow	1
2. Stacking: Zerene Stacker Workflow	5
3. Making scales to add to Focus stacked images	7
4. Adding a scale using ImBatch	8
5. Tips	9
6. Troubleshooting	9
7. Credits	9

1. Acquiring: StackShot workflow

- Before switching on the StackShot Controller by connecting it to the Electrical network, make sure the following steps are ok:
 - a. The camera is connected to the Stackshot controller (Shutter cable)
 - b. The StackShot controller is connected to the StackShot Rail
 - c. The camera is connected to the PCAll ok? Plug in the StackShot Controller

- Switch on the camera
 - a. Open 'EOS Utility' (Fig. 1)
 - b. Select 'Camera settings/Remote Shooting'
 - c. Now the window appears to select your camera settings etc. Follow further instructions



Figure 1: EOS Utility start screen

- Switch on the light inside the closet
- Switch on 'Live View Shoot' (Bottom left in the EOS Utility window)
- Set the Shutter speed on 'BULB' (click on the shutter speed and press ↓)
- Set the power of the speed lights (if you use a Grey Background)
 - d. ½ or ¼ for 3x to 5x magnification
 - e. 1/8 to 1/16 for 1x to 3x magnification
 - f. 1/32 or 1/64 for regular macro lenses
 - g. test if the flash lights work by pressing the remote
- Place the specimen in the closet in the center of the lens
- If you use the MP-E 65 mm Macro Photo, set the approx. desired magnification

Magnification	Sensor filling size
1x	22.5 mm
2x	11.3 mm
3x	7.5 mm
4x	5.6 mm
5x	4.5 mm

- Make sure that 'Mode: Auto-Dist' is selected on the StackShot controller, if not press 'up' or 'down' on the controller till it appears (Fig.2).



Figure 2: StackShot controller

- Move the camera up or down by pressing 'Fwd' or 'Back' till the specimen is in-focus (Confirm through Live View in EOS Utility).
- Set the distance which the camera needs to travel between each picture by pressing 'select' once and 'up' or 'down' to increase or decrease the 'Dist/Step'. Guidelines for the Step Size:

Magnification	Step Size
60mm 1:1 – 1:10	400 μ m to 2 mm
1x	150 μ m to 300 μ m
2x	120 μ m to 200 μ m
3x	75 μ m to 150 μ m
4x	50 μ m to 80 μ m
5x	30 μ m to 60 μ m

Write down the magnification you used, as this is needed to add a scale after stacking the images. Normally you'll end up with a stack of 15 to 40 pictures for the mean values.

- Set the appropriate f-stop and speed light power
 - h. 1:1 to 1:5: f/5.6 to f/7.1, flash 1/16 to 1/64
 - i. 3x to 5x magnification f/4.0 or f/4.5, flash ½ nd or ¼th
 - j. 1x to 3x magnification f/4.5 to f/5.6 flash 1/8th till 1/16th
 - k. Press test shooting in the Live view window, a new window appears
 - l. Close the Live View window, otherwise the speed lights won't flash
 - m. Press the white square button upper right
 - n. Alter the f-stop and/or flash power if needed

- Select the upper most part of the specimen by traveling upwards
 - o. Press 'Back' on the controller till the entire specimen is out of focus
 - p. Press 'Fwd' till a part (the upper most part), is in focus
 - q. Press 'up' or 'down'
 - r. 'Select End Pos' appears

- Select the most distant part
 - s. Press 'Fwd' on the controller till the entire specimen is out of focus
 - t. Press 'Back' till a part (the most distant) is in focus
 - u. Press 'up' or 'down'

- Close Live View window and check again if the speed lights work (press the button of the transmitter on the camera)

- Select the folder to save the pictures and make sure you take both RAW and Large pictures if desired.

- Press the 'Up' or 'Down' button to start the process of taking pictures

2. Stacking: Zerene Stacker Workflow

- Open Zerene Stacker (not the safe mode)
- Press 'ok' on the welcome screen
- Click 'add files' in the 'File' menu or skip to 23 on if you have multiple folders (views) to stack.
 - v. Select all the images in the selected folder
 - w. press 'add'
- In the 'Stack' menu select one of the following parameters:
 - x. Align and Stack PMax
 - y. Align and Stack DMap
 - z. Align and stack both
 - aa. it is possible to stack without aligning by selecting the images in the 'input' window and select 'Stack selected (PMax)' or 'Stack selected (DMap)' in the Stack menu
- After the stacking process is done, select all the output files and press 'Save output images' in the 'File' menu.
- Click on 'Show batch dialog' in the Batch menu tab (a new window appears) (Fig. 3).

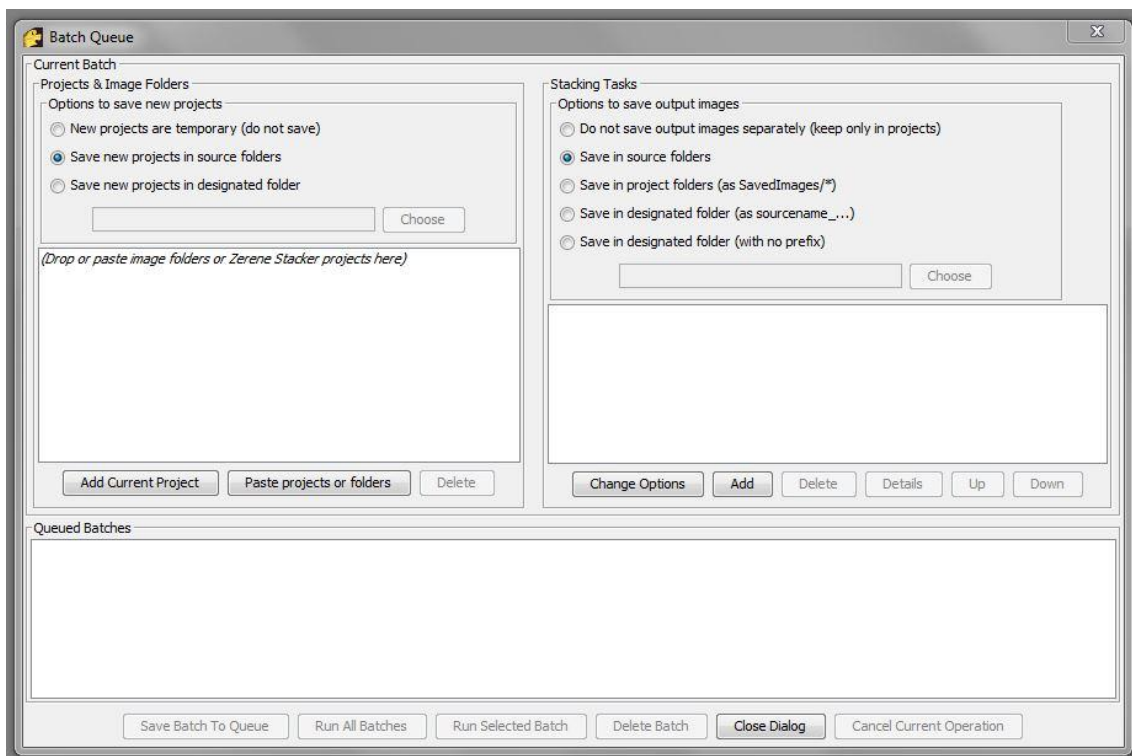


Figure 3: Batch Queue Window of Zerene Stacker.

- In the Batch Queue window the left part is to select folders and to choose what to do with project files. The right part is to decide how to stack and save the stacked images.
- LEFT: Drag the folders containing the images in the window (important each folder is one view). Decide to Save the project folders in case of type material.

- RIGHT: Click on 'Add' and Select 'PMax' and 'DMap' (Align and Stack) and press OK
- Select 'Save in Source Folders' and Press 'Run all Batches'. You can close the Batch Queue Window.
- If you want that automatically the name of the folder is saved as name for the stacked image, do as follows:
Open the 'Preference' submenu (Fig. 4) in the 'Options' menu and choose 'Image saving'.
Below you find 'Output Image Names' and write the following in the space below:
{sourcefolder} ZS {method}
Press on 'Apply' and OK

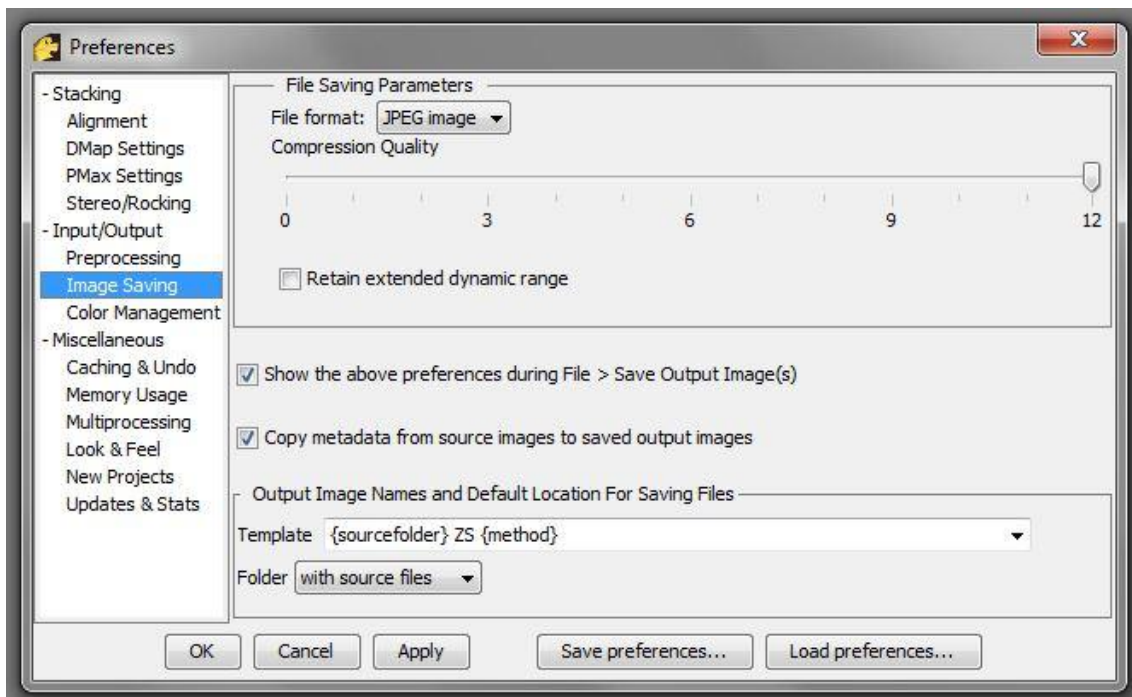


Figure 4: Preference menu in Zerene Stacker to change the output template

- After the Stacking process is done it is possible to add a scale using ImBatch.

3. Making scales to add to Focus stacked images

- Picture a microscope measuring slide with the camera at high magnification or a tape measure for images of larger sized specimens (Fig. 5).

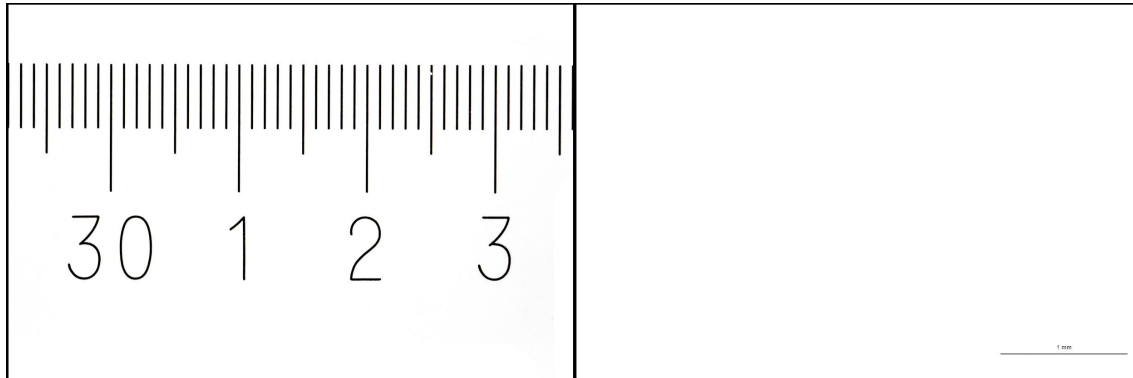


Figure 5: Left: picture of a measuring slide pictured at 5x magnification. 1mm equals 1162pix. This is used to draw a 1162pix line on a transparent .png file with the same dimensions as the picture on the left. The resulting file can be seen on the right.

- Do this for all the magnifications. To ease the work one can calculate the amount of pixels 1mm represent using the following equation:
 $(A/B)*C$
A= Magnification
B= Sensor size (mm)
C= Image width (pixels)

For a magnification of 5x using a Canon EOS 700D (sensor size 22,3 mm) and an image width of 5184 pixels, 1 mm is represented in the picture by 1162,3 pixels, which is the same value as measured using the microscope slide.

- Once all the scales are made, one can start adding these to the focus stacked images using a batch program.

4. Adding a scale using [ImBatch](#)

- Open ImBatch and set up the following parameters:
 - Add Task: Watermark (Fig. 6) Save as (Fig. 7).

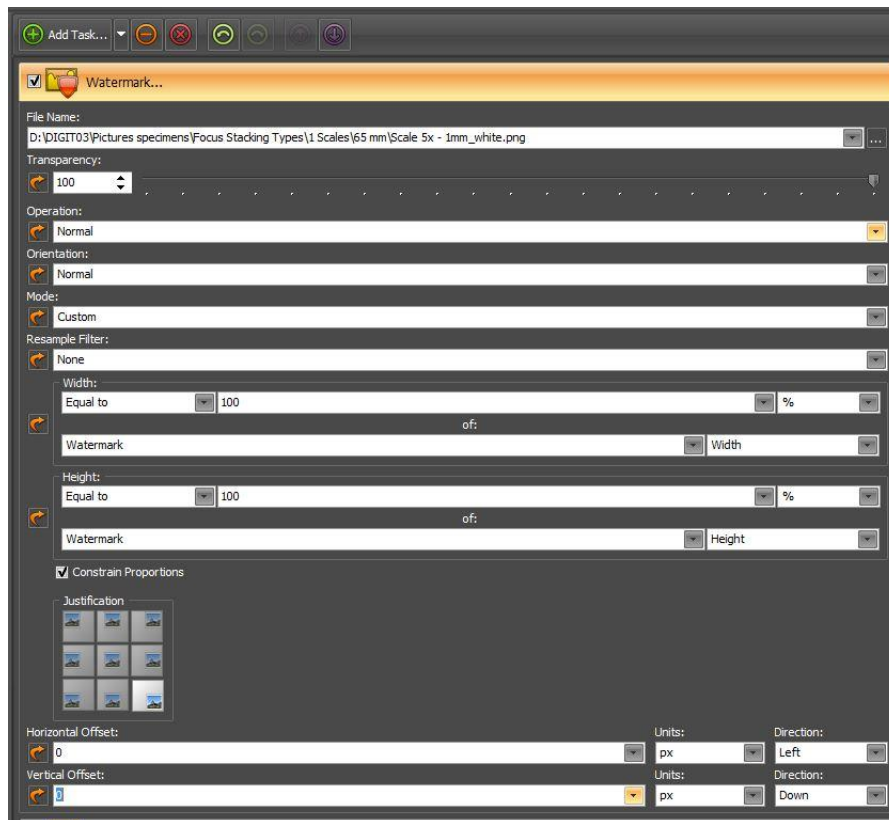


Figure 6: Parameters for the Watermark task.

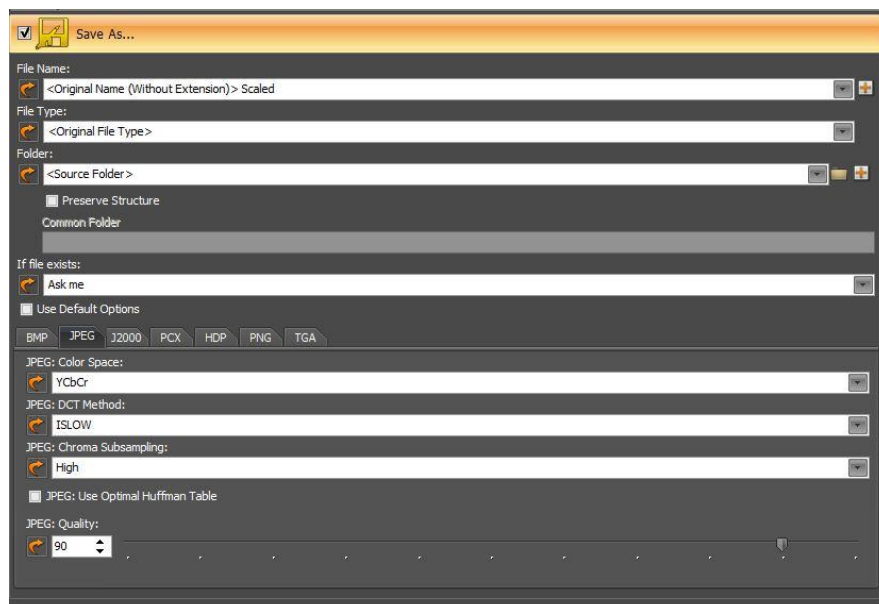


Figure 7: Parameters used for the Save As task in ImBatch

- Once done drag the image to the left part of the screen and set up the corresponding scale in the Watermark task. Check if the scale doesn't cover the specimen, otherwise change its position by changing the Horizontal or Vertical Offset. Once approved, process the pictures.

5. Tips

- The setup can be used by persons with very low knowledge of photography, after a small training course of half a day. However it is important to judge the skills of handling small, fragile and unique specimens. Best is to let them start with groups of solid, difficult to damage specimens, before switching to the other.
- Positioning specimens in alcohol can be challenging from time to time, but the key is to add just enough alcohol in the holder (petri dish, glass beaker, ...) to submerge the specimen. In case too much is added the specimens tend to float or move around while picturing.

6. Troubleshooting

- The stacking process (picturing) is started, but there aren't any pictures taken by the camera.
 - a. Check if the shutter cable is well connected to the camera and the stackshot controller
 - b. Check if the light of the Stackshot is blinking when a picture should be taken
 - c. Check if the 'Live Shoot' window from the EOS Utility window is closed
- The pictures taken fluctuate in light intensity, some are well exposed, others are too dark.
 - a. The batteries of the flash, working in 'Slave-mode', are low in power. Change batteries, preferably of both flashes.
 - b. In case you do not have spare batteries, make the time in between 2 pictures longer (config) to give more time to recharge the batteries. To do so press 'config' on the stackshot controller and press select once to change the settling time (Tsettle) or twice to change the time in between picturing and movement of the rail (Toff).
- Some pictures are black, others are well lit.
 - a. Check the batteries of the flash with the receiver on it. It is possible the batteries are without power, so recharge them.
 - b. It is possible the batteries of the receiver are low on power.
 - c. It is possible that only if the transmitter is low on energy, change the batteries of the transmitter or receiver if changing the batteries of the speedlites didn't work.
- During stacking with Zerene stacker, there is a warning that the selected file doesn't contain any pictures.
 - a. Check previous folders for the pictures, it is possible that you saved your pictures in another folder.

7. Credits

Author: Jonathan Brecko

Date: December 2021

Version: 1.0

CC: CC BY