



iDigBio

Integrated Digitized Biocollections



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Focus Stacking for Improved Depth of Field

Leveraging Digitization Practices across Multiple Domains

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Focus or Z-Stacking

Recording and merging several images of a single subject taken from the same angle and from several focus distances through a process that preserves the sharpest pixels in each image to ensure the greatest depth of field (DOF) in the merged image.

Challenges

- Overcome the limitations of aperture and magnification in obtaining maximum DOF.
- Preserve consistent aperture, ISO (light sensitivity of sensor), shutter speed, and white balance in recording layers.

Focus Stacking Options

Software

CombineZP

Helicon Focus

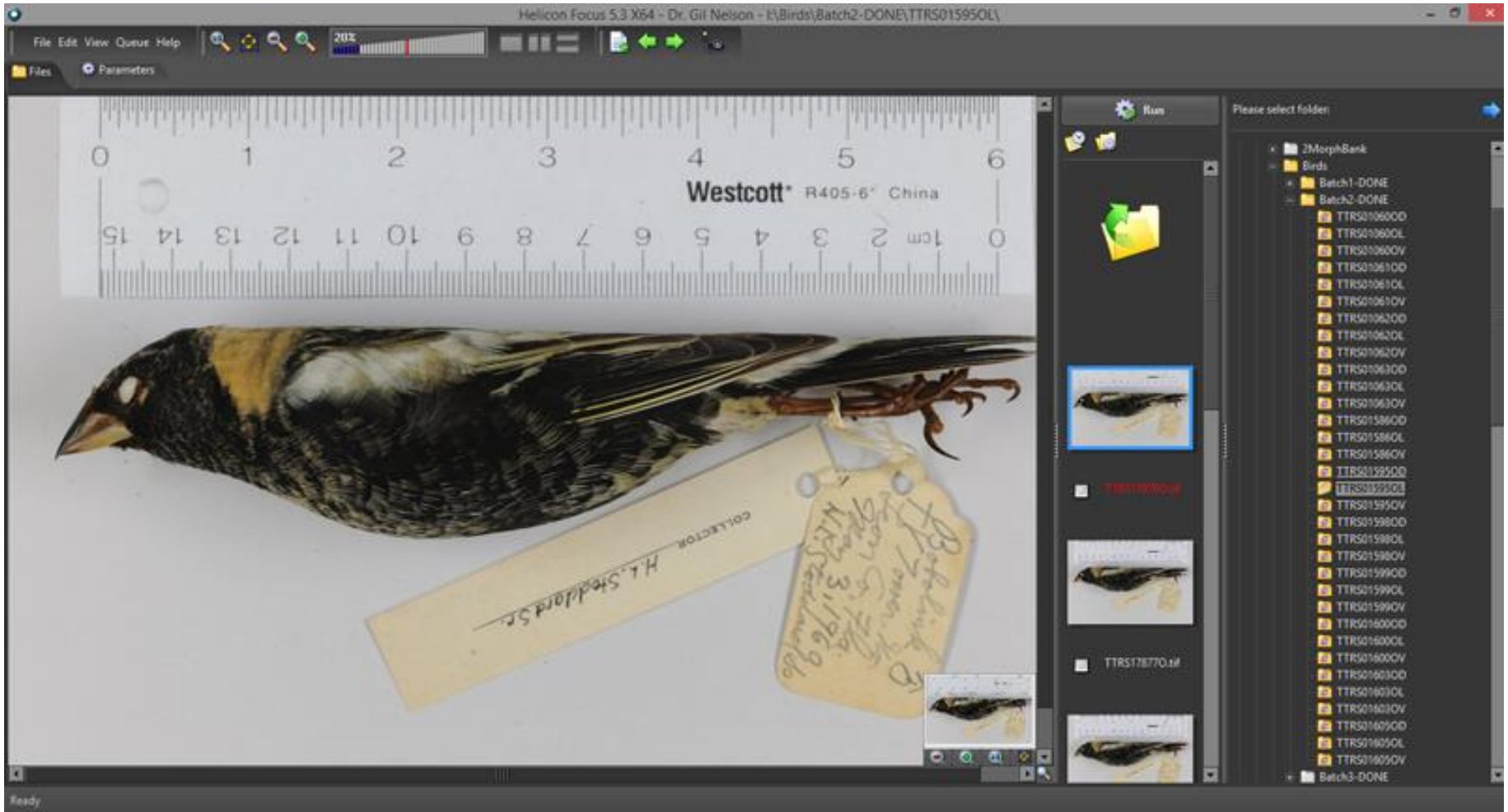
Zerene Stacker

Photoshop

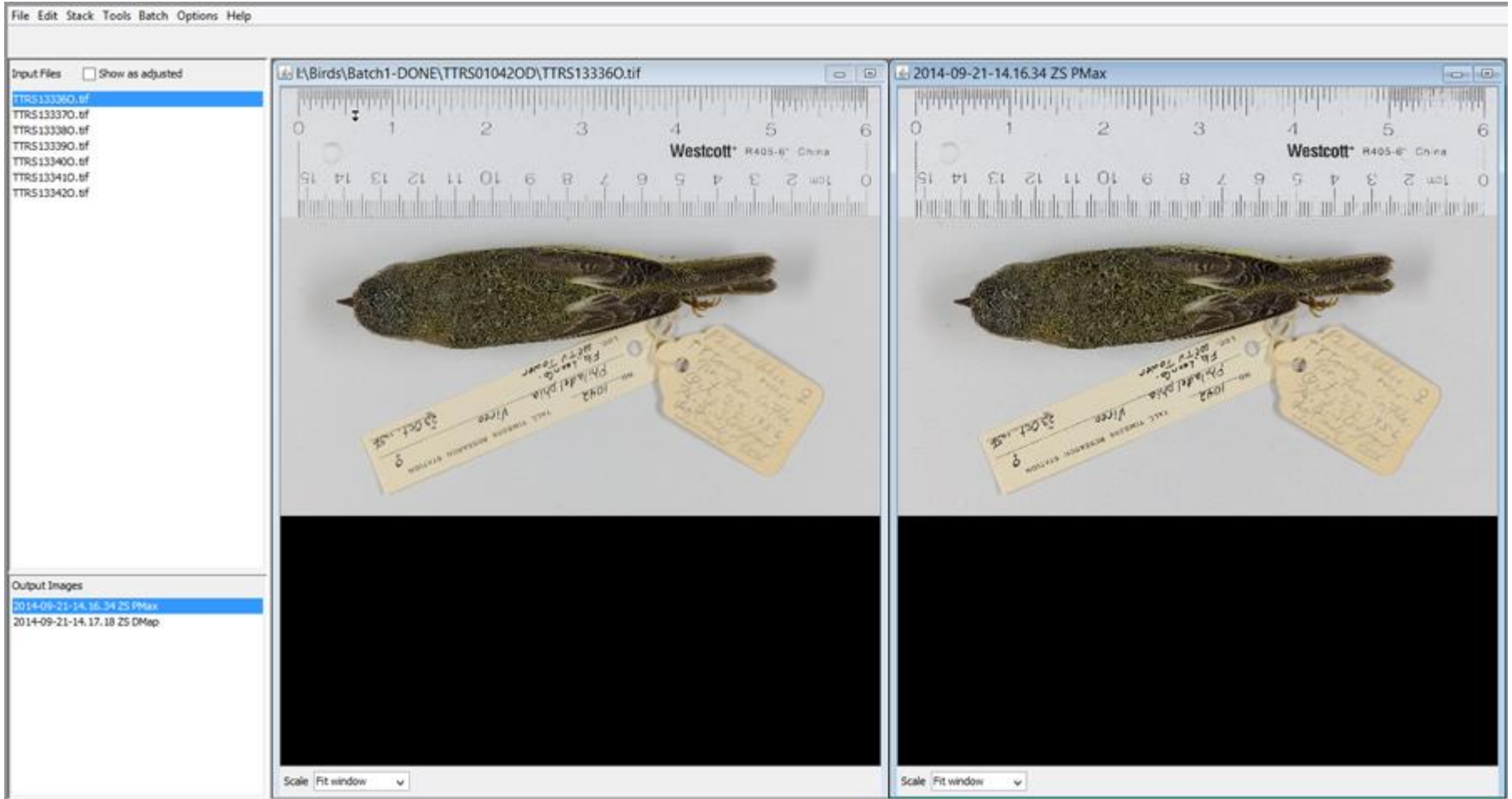
Hardware

Stackshot

Automontage (Syncroscopy)



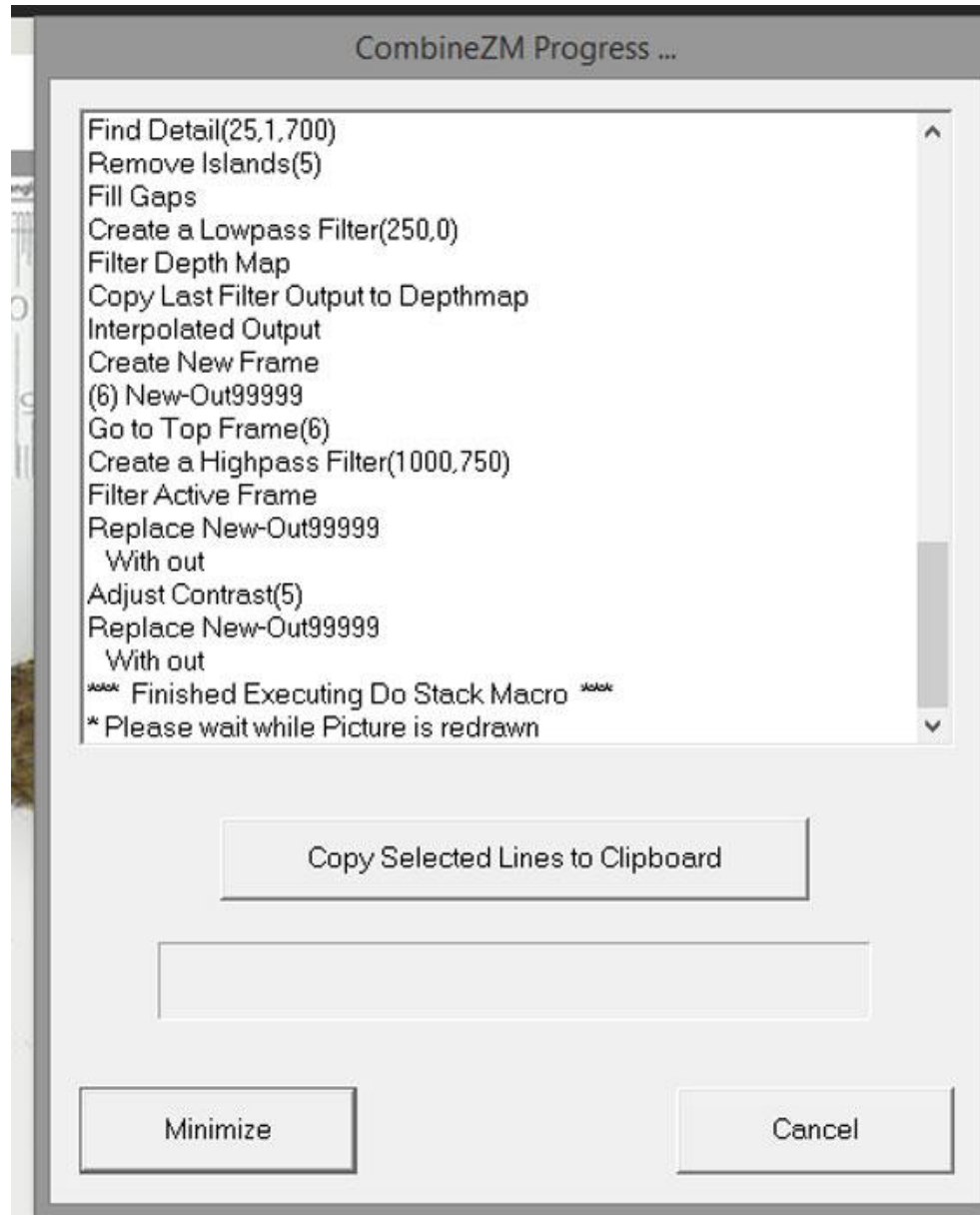
Helicon Focus

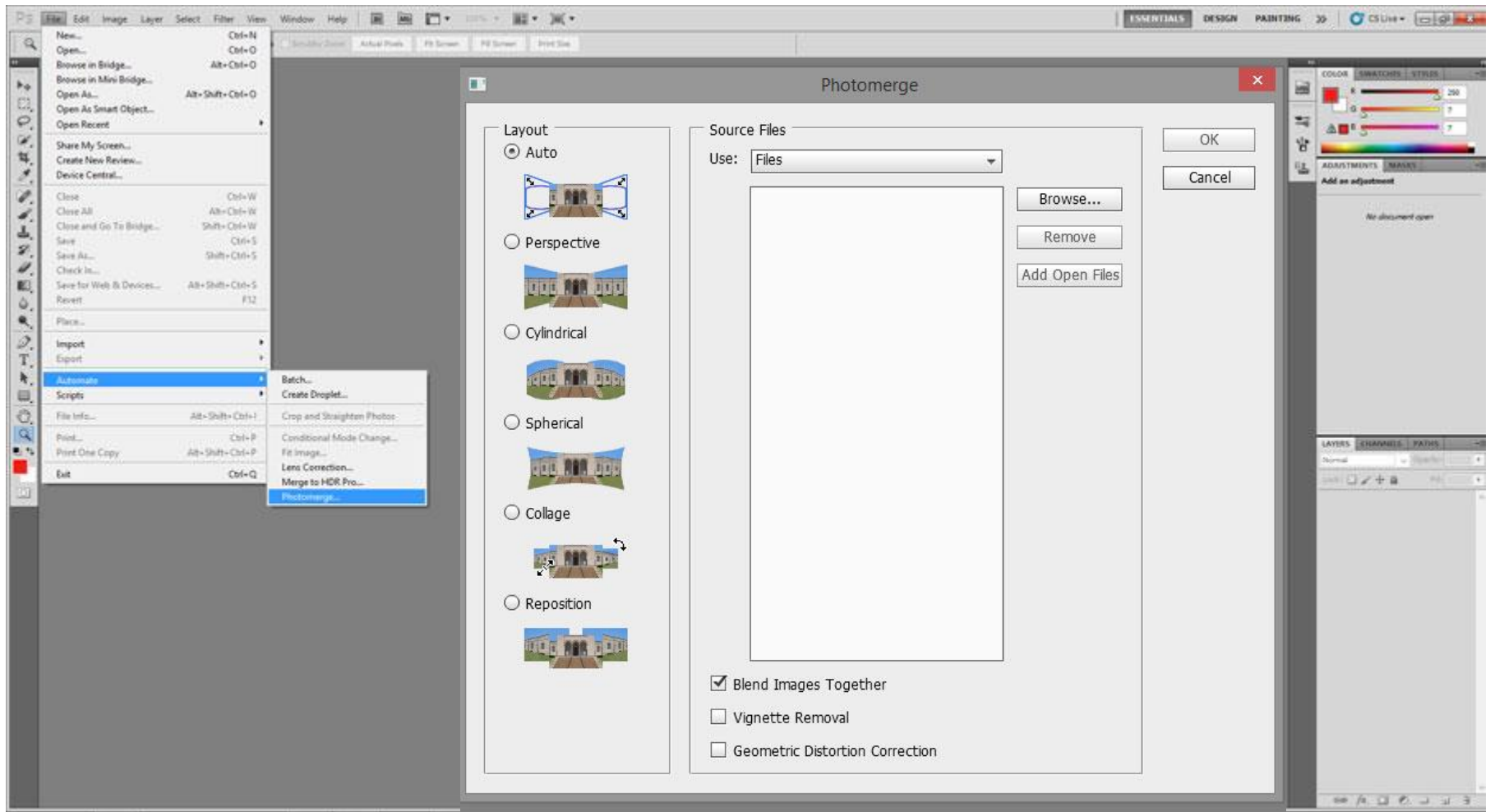


Zerene Stacker



CombineZ





Photoshop CS5

Methods for building stacks

Adjusting focus

- manually with focus ring

- manually with tethering software and remote release

- automatically with batch remote shooting

Moving the subject

- Moving the camera (rail system, StackShot)

Advantages of moving the camera versus internal focusing?

- Used mostly in field-recorded images.
- Generally not recommended for studio-recorded images.
- Risks camera shake and misalignment in final image.



Manual focus

When to use focus stacking?

Virtually any image of a specimen will be improved by focus stacking.



Zooming Viewer for 782641

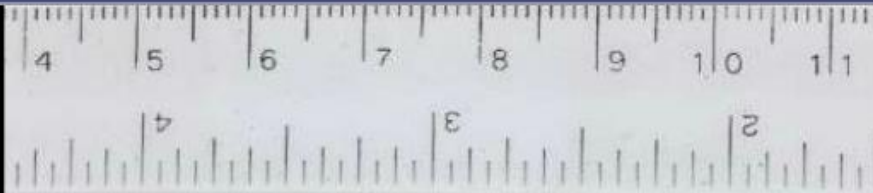
User: Guest [\[click to login\]](#)

About

Browse

Tools

Help



TTRS02681L

<i>Meg. larisi</i> ♀ <i>Melipotis Monticola</i> Thomas Co., Georgia
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Ex pupa
Adult Aug 10, 1964
Leon Neal
First So. GA RECORD

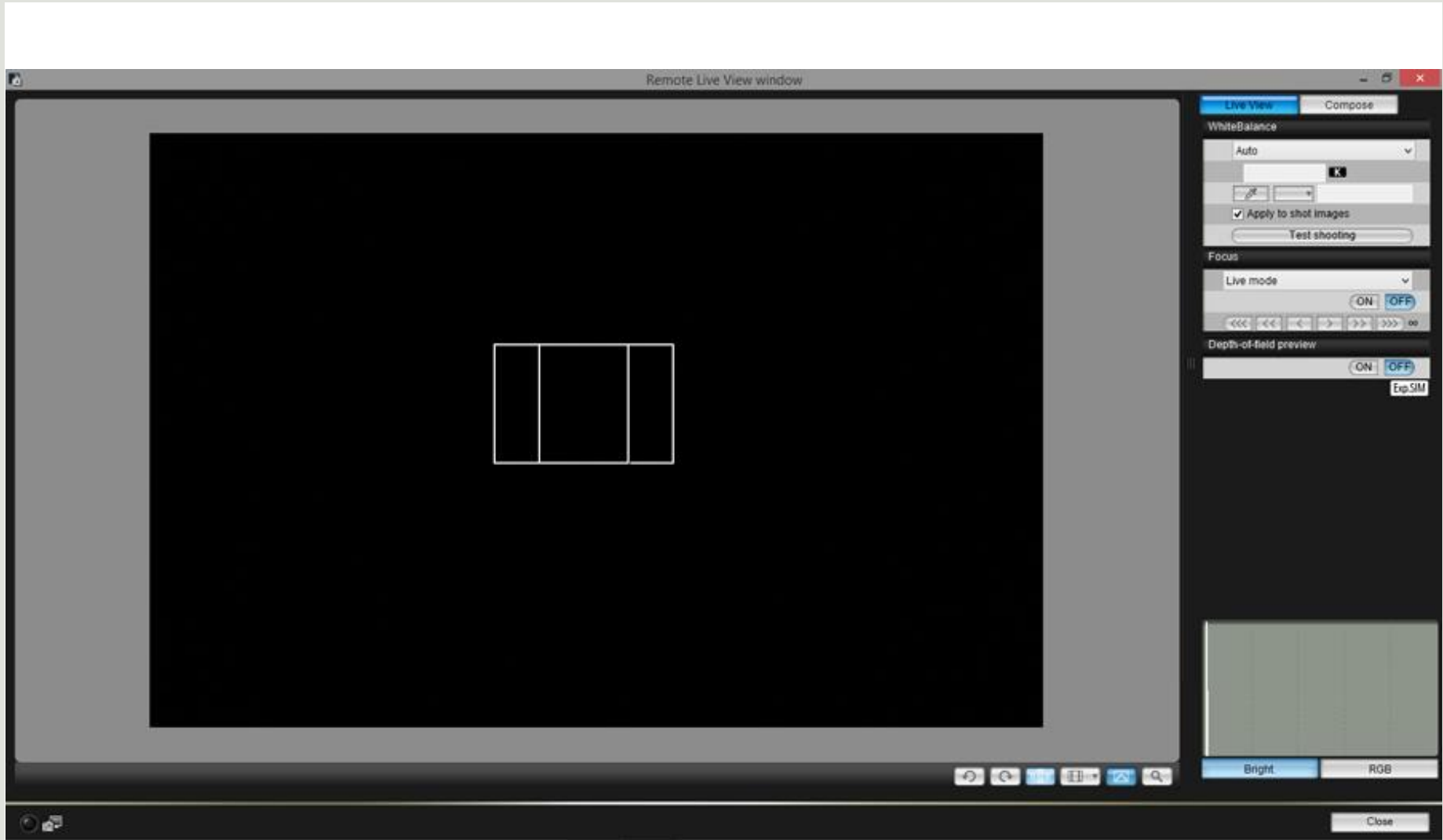


Showing image 782641

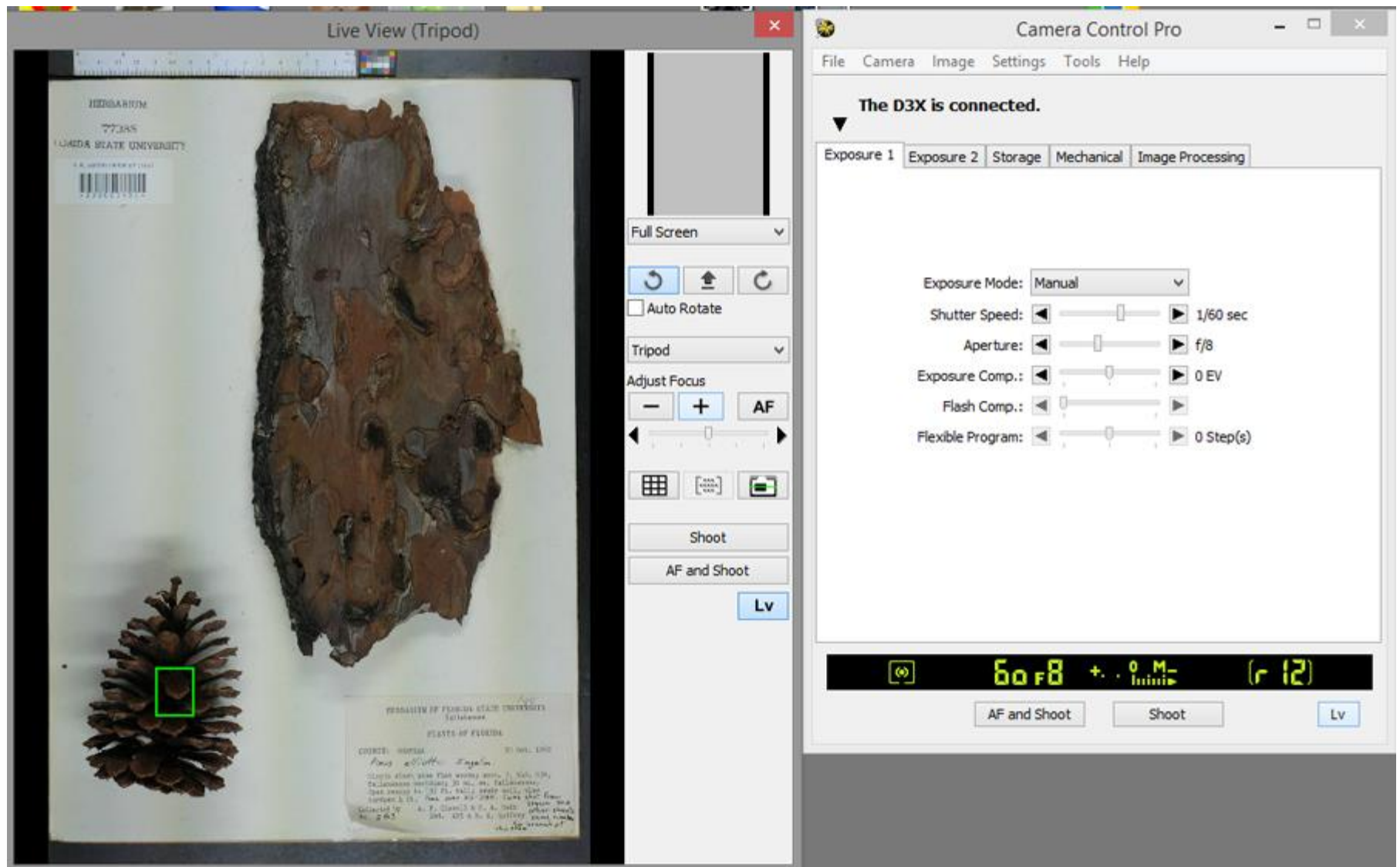




Showing image 782641



Canon EOS Utility



Nikon Camera Control Pro 2

The screenshot displays the Nikon Camera Control Pro 2 software interface. On the left, a 'Live View (Tripod)' window shows a photograph of a specimen, likely a piece of bark or wood, with a green square highlighting a specific area. Below the live view are controls for 'Full Screen', 'Auto Rotate', 'Tripod', 'Adjust Focus', and 'Shoot' buttons. On the right, the 'Camera Control Pro' window shows the camera's status and settings. The status bar at the bottom of the Camera Control Pro window displays '60 f8 + 0 M (r 12)'. The settings panel includes: Exposure Mode: Manual; Shutter Speed: 1/60 sec; Aperture: f/8; Exposure Comp.: 0 EV; Flash Comp.: 0 EV; Flexible Program: 0 Step(s). The interface also shows a menu bar with 'File', 'Camera', 'Image', 'Settings', 'Tools', and 'Help'.

Nikon Camera Control Pro 2

The screenshot displays the Nikon Camera Control Pro 2 software interface. On the left, a 'Live View (Tripod)' window shows a specimen on a white background with a ruler at the top. The specimen is a dark, textured, elongated piece, possibly a piece of bark or wood, with a pine cone placed below it for scale. A label on the left of the specimen reads 'BERBARTH 7748 FLORIDA STATE UNIVERSITY' with a barcode. A label at the bottom right of the specimen reads 'DEPARTMENT OF FLORIDA STATE UNIVERSITY Tallahassee PLANTS OF FLORIDA' and contains handwritten notes. The 'Live View' window includes a 'Full Screen' dropdown, a 'Tripod' dropdown, 'Adjust Focus' controls (minus, plus, AF), a focus slider, and 'Shoot', 'AF and Shoot', and 'Lv' buttons.

On the right, the 'Camera Control Pro' window shows the camera's status and settings. The status bar at the top indicates 'The D3X is connected.' Below this, there are tabs for 'Exposure 1', 'Exposure 2', 'Storage', 'Mechanical', and 'Image Processing'. The 'Exposure 1' tab is active, showing the following settings:

- Exposure Mode: Manual
- Shutter Speed: 1/60 sec
- Aperture: f/8
- Exposure Comp.: 0 EV
- Flash Comp.: 0 EV
- Flexible Program: 0 Step(s)

The status bar at the bottom of the 'Camera Control Pro' window displays '60 f8 + 0 M (r 12)'. Below the status bar are 'AF and Shoot', 'Shoot', and 'Lv' buttons.

Nikon Camera Control Pro 2



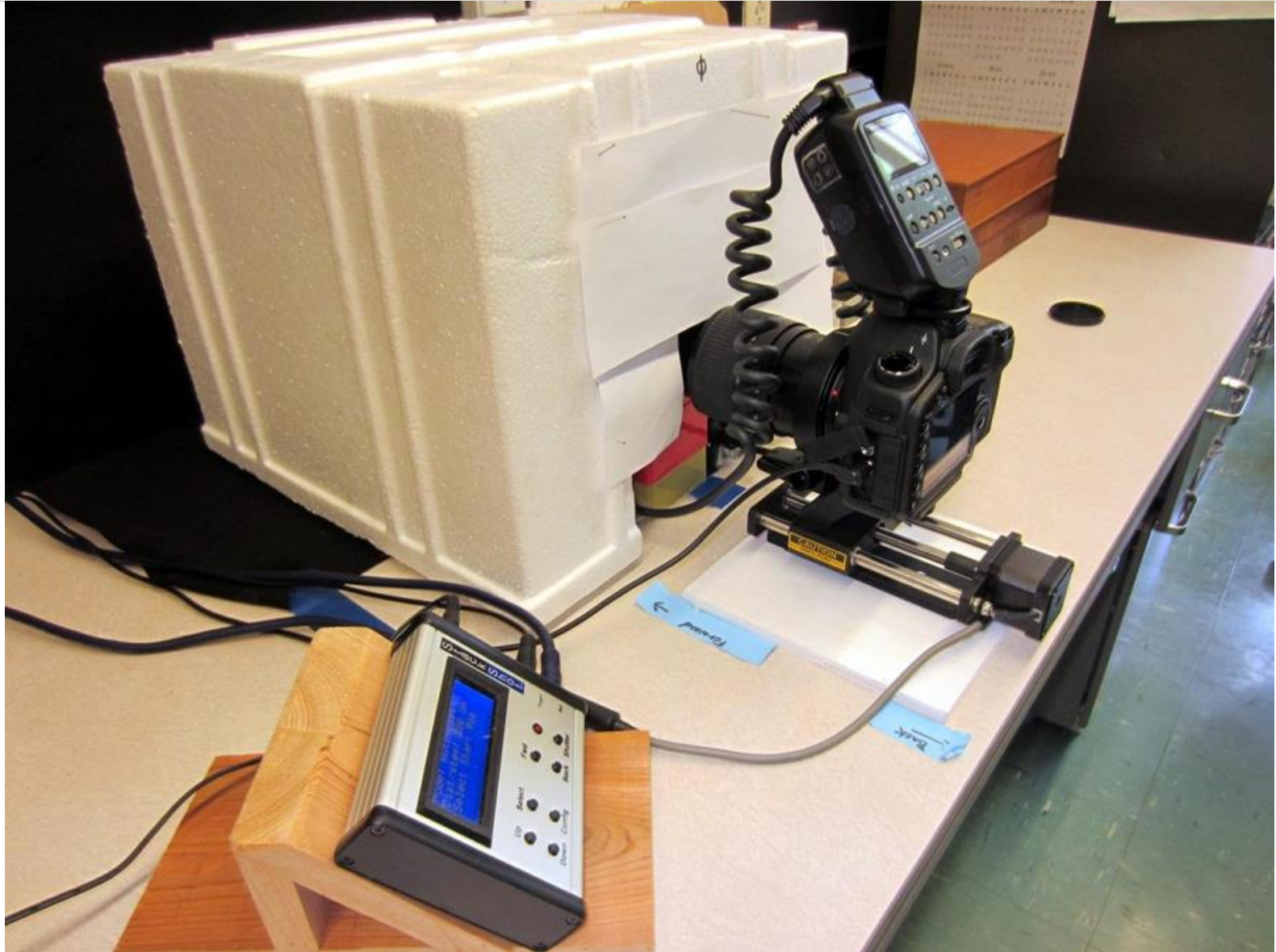
Remote Release



Stackshot Rail



StackShot Controller



Sam Droege (USGS) Stackshot setup



AutoMontage

Recommended Workflow

1. Shoot to raw (NEF, CR2, etc.) using live view and camera manufacturer or 3rd party tethering software that allows targeted focusing and remote shutter release.
2. Save all images of each specimen into a discreet folder, preferably a folder within a master folder that contains the specimen folders.
3. Convert all images to uncompressed TIFF or JPEG using the same software and consistent parameters, preferably as a batch process using manufacturer software or a 3rd party image processing software (e.g. Photoshop). Converted images should be saved within the folder referenced in Step 2 provided that the stacking software used will not accept raw images, otherwise, save converted images into a second set of folders.
4. Process image stacks, directing output to a discreet output folder. Output file type and size should be determined by intended use of the image files.
5. If cropping is required, crop the resulting TIFF or JPEG images, not the intermediary images produced in Step 3.
6. If step 4 results in archival TIFF images, use these images to create other derivatives, such as JPEG files for web display; avoid editing the TIFF archival images.
7. Archive raw images.



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