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Integrated Digitized Biocollections



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Focus Stacking for Improved Depth of Field

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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

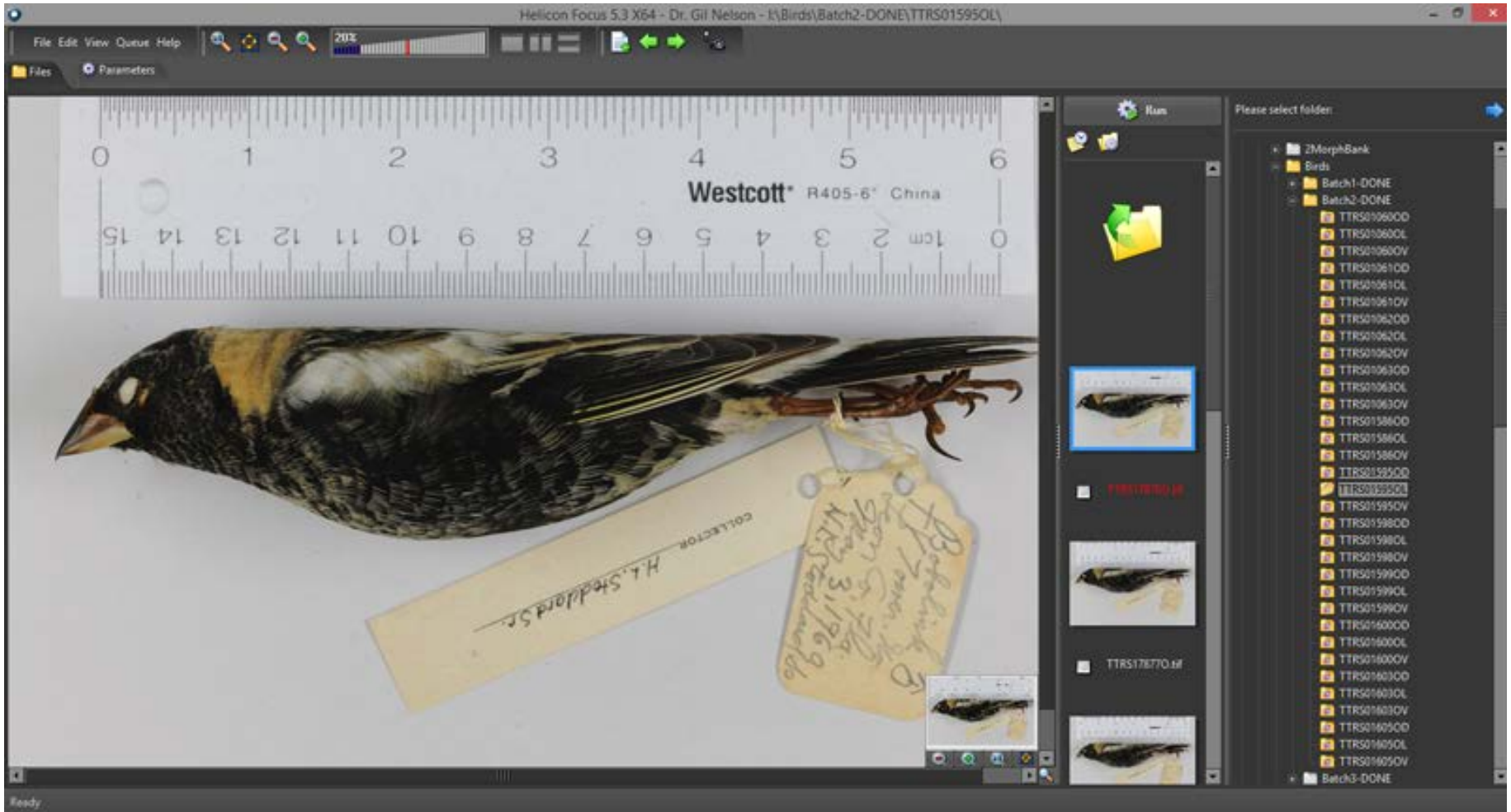
Zerene Stacker

Photoshop

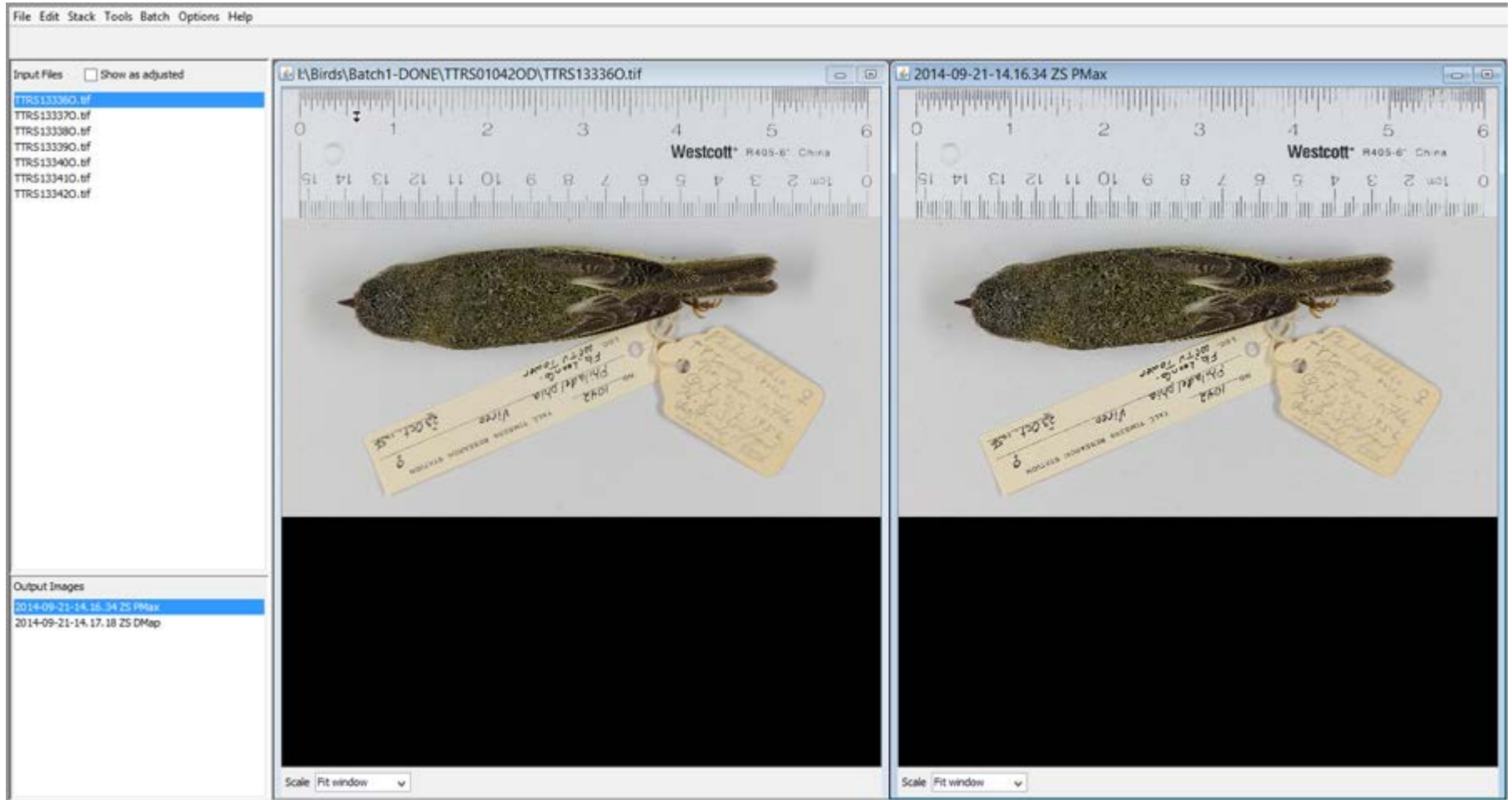
Hardware

Stackshot

Automontage (Syncroscopy)



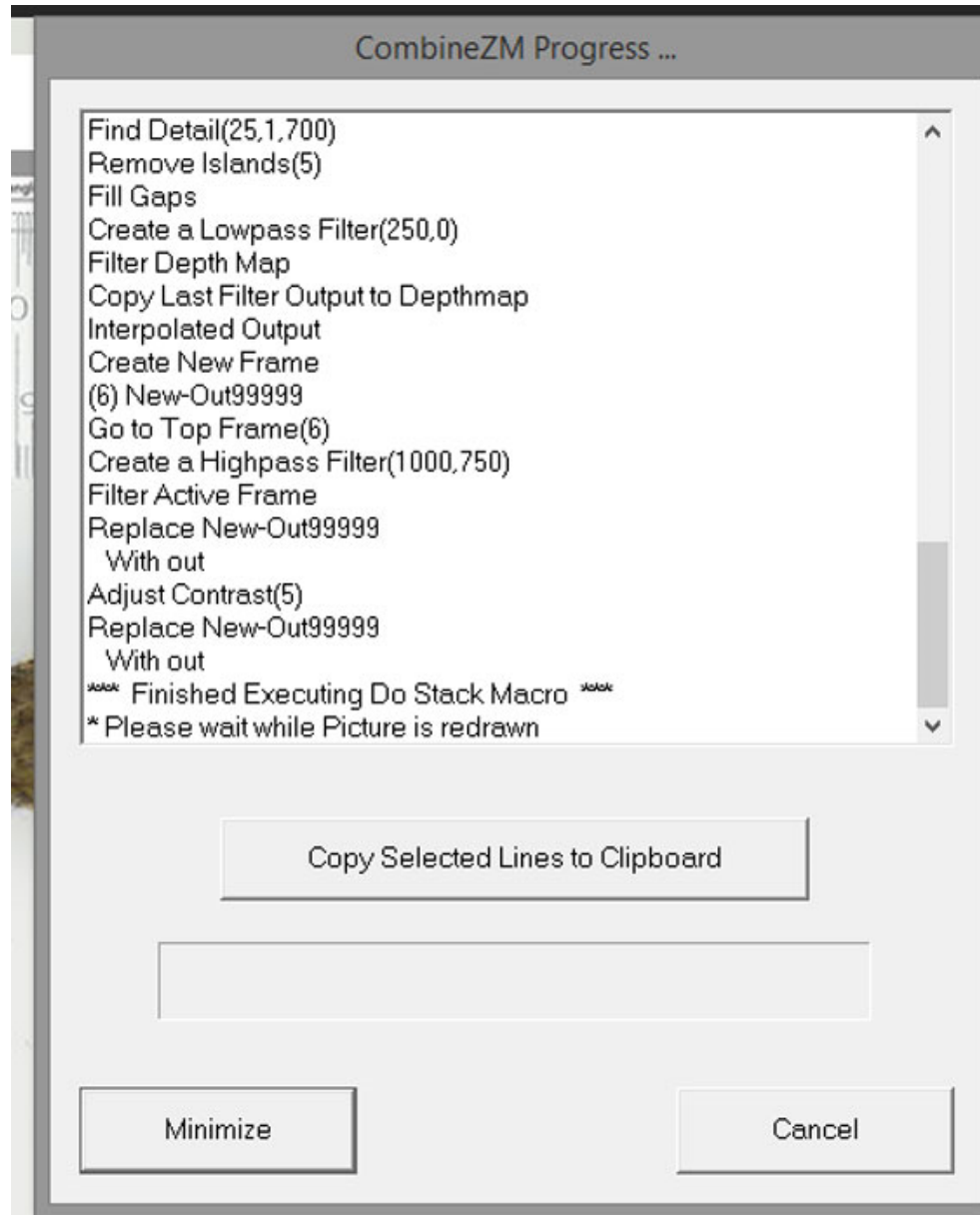
Helicon Focus

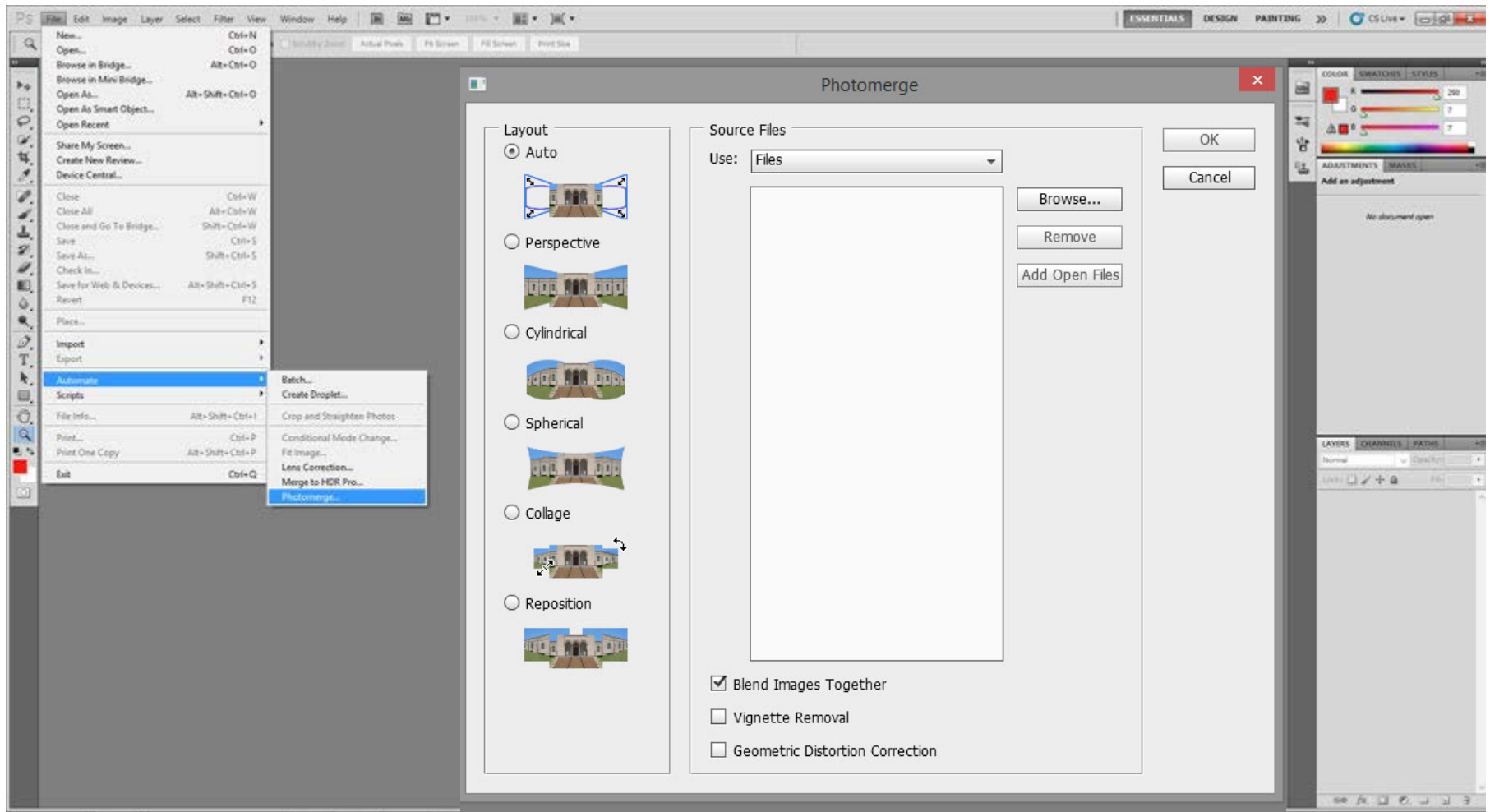


Zerene Stacker



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

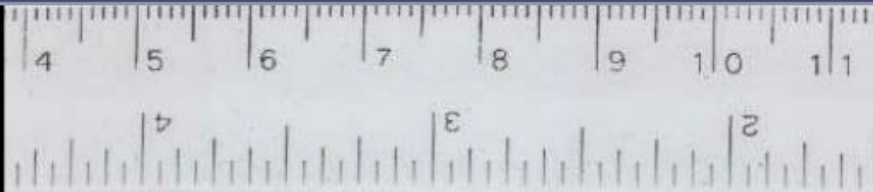
User: Guest [\[click to login\]](#)

About

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TTRS02681L

Meg. larisi
Melrose Plantation
Thomas Co.
Georgia

ex pupa
adult Aug 10, 1964
Leon Neal
first so. GA record



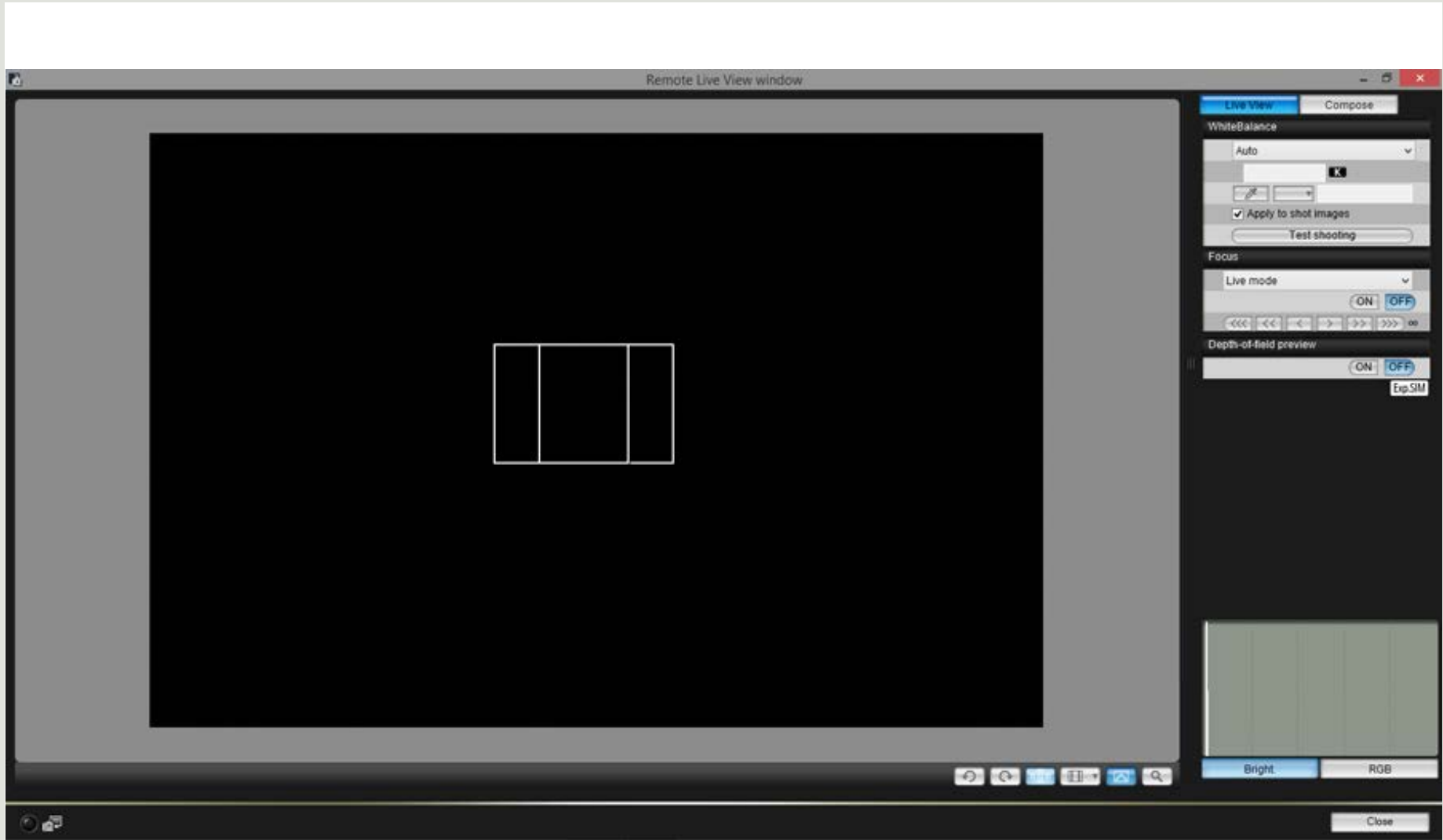
Showing image 782641



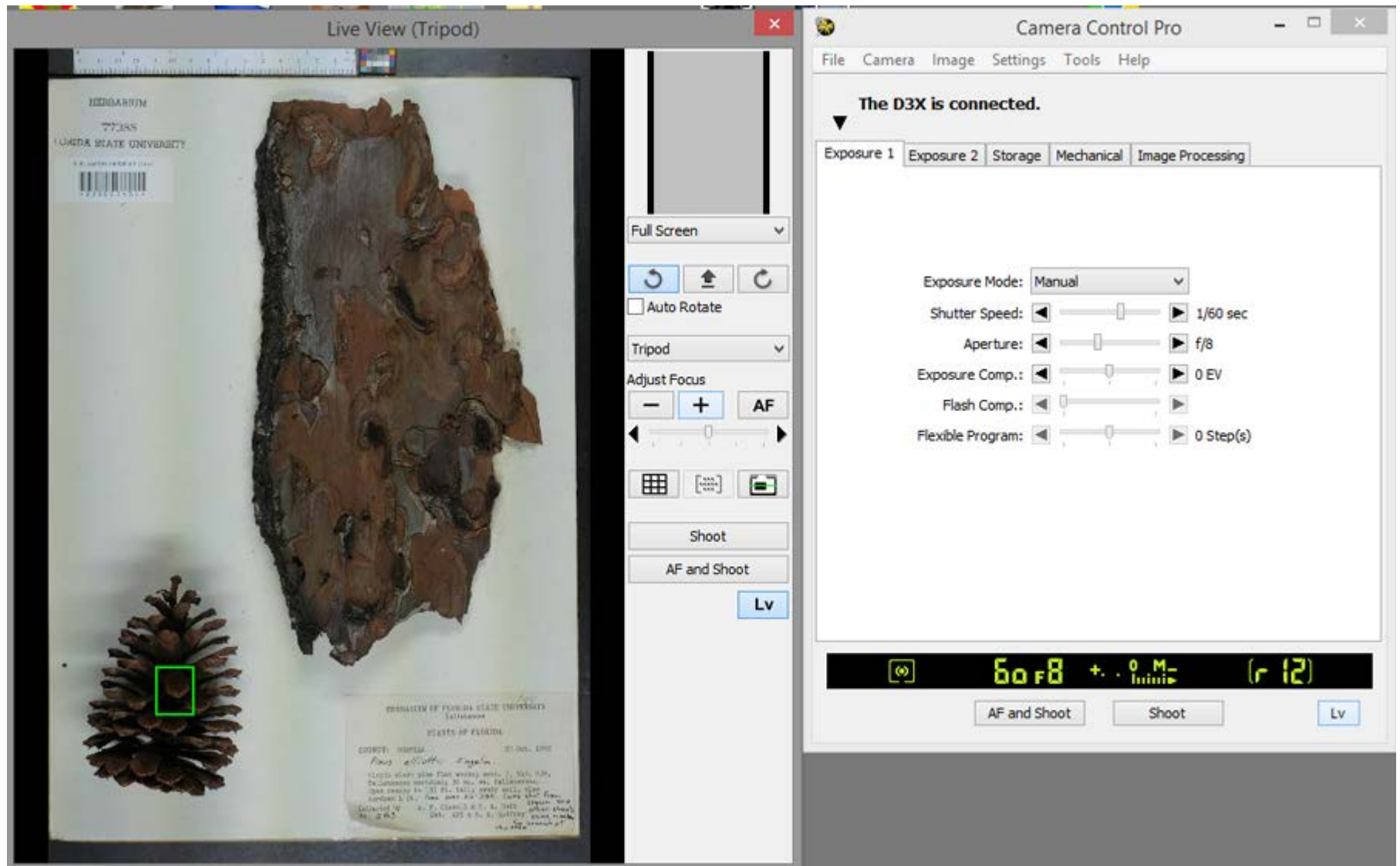


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Showing image 782641



Canon EOS Utility



The screenshot displays the Nikon Camera Control Pro 2 software interface. On the left, a 'Live View (Tripod)' window shows a specimen (a brown, textured fragment) and a pine cone on a white background. A green box highlights a small feature on the pine cone. The specimen is placed on a white card with text: 'HERBARIUM 77385 FLORIDA STATE UNIVERSITY' and 'UNIVERSITY OF FLORIDA STATE UNIVERSITY Gainesville FLORIDA'. Below the specimen is a handwritten label with botanical details.

The right side of the interface shows the 'Camera Control Pro' window. It includes a menu bar (File, Camera, Image, Settings, Tools, Help) and a status message: 'The D3X is connected.' Below this, there are tabs for 'Exposure 1', 'Exposure 2', 'Storage', 'Mechanical', and 'Image Processing'. The camera settings are displayed as follows:

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

At the bottom of the software window, a digital display shows '60 F8 + 0 M (r 12)'. Below the display are buttons for 'AF and Shoot', 'Shoot', and 'Lv'.

Nikon Camera Control Pro 2

Live View (Tripod)

HERBARIUM
77338
FLORIDA STATE UNIVERSITY
6.4 0196 100017 734

HERBARIUM OF FLORIDA STATE UNIVERSITY
Tallahassee
PLANTS OF FLORIDA
CITRUS: WALTER
Nov 21/1911
Trop. a. about 1000 feet, sandy soil, 1 mi. N. of
Macduff's plantation, 10 mi. W. Tallahassee.
Tree 100 to 120 ft., 100 ft. trunk, 100 ft. diam.
at base. Bark 100 ft. diam. at base.
Crown 100 ft. diam. at base.
Bark 100 ft. diam. at base.
Nov 21/1911

Camera Control Pro

The D3X is connected.

Exposure 1 | Exposure 2 | Storage | Mechanical | Image Processing

Exposure Mode: Manual

Shutter Speed: 1/60 sec

Aperture: f/8

Exposure Comp.: 0 EV

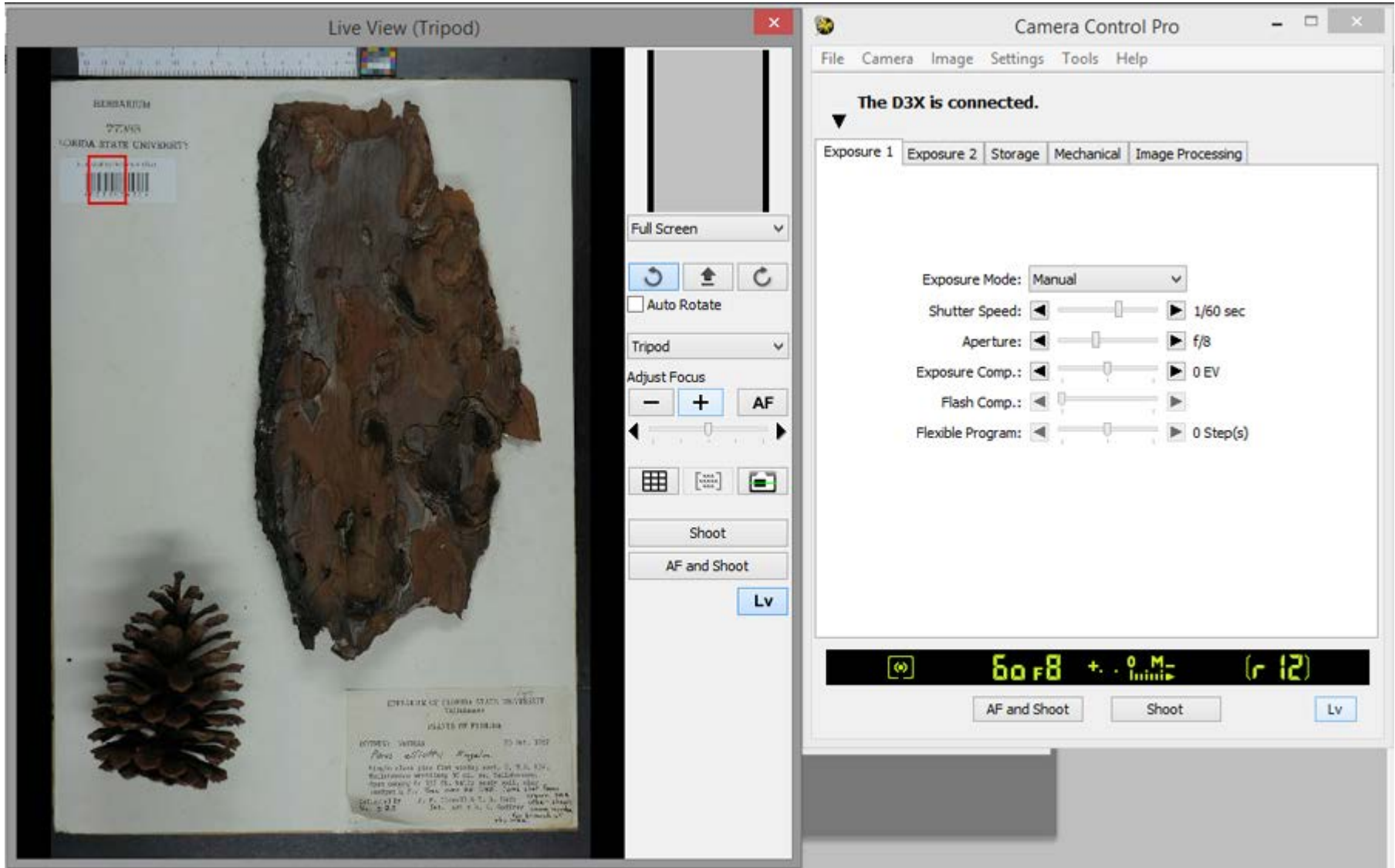
Flash Comp.: 0 EV

Flexible Program: 0 Step(s)

60 f8 + 0 M (r 12)

AF and Shoot Shoot Lv

Nikon Camera Control Pro 2



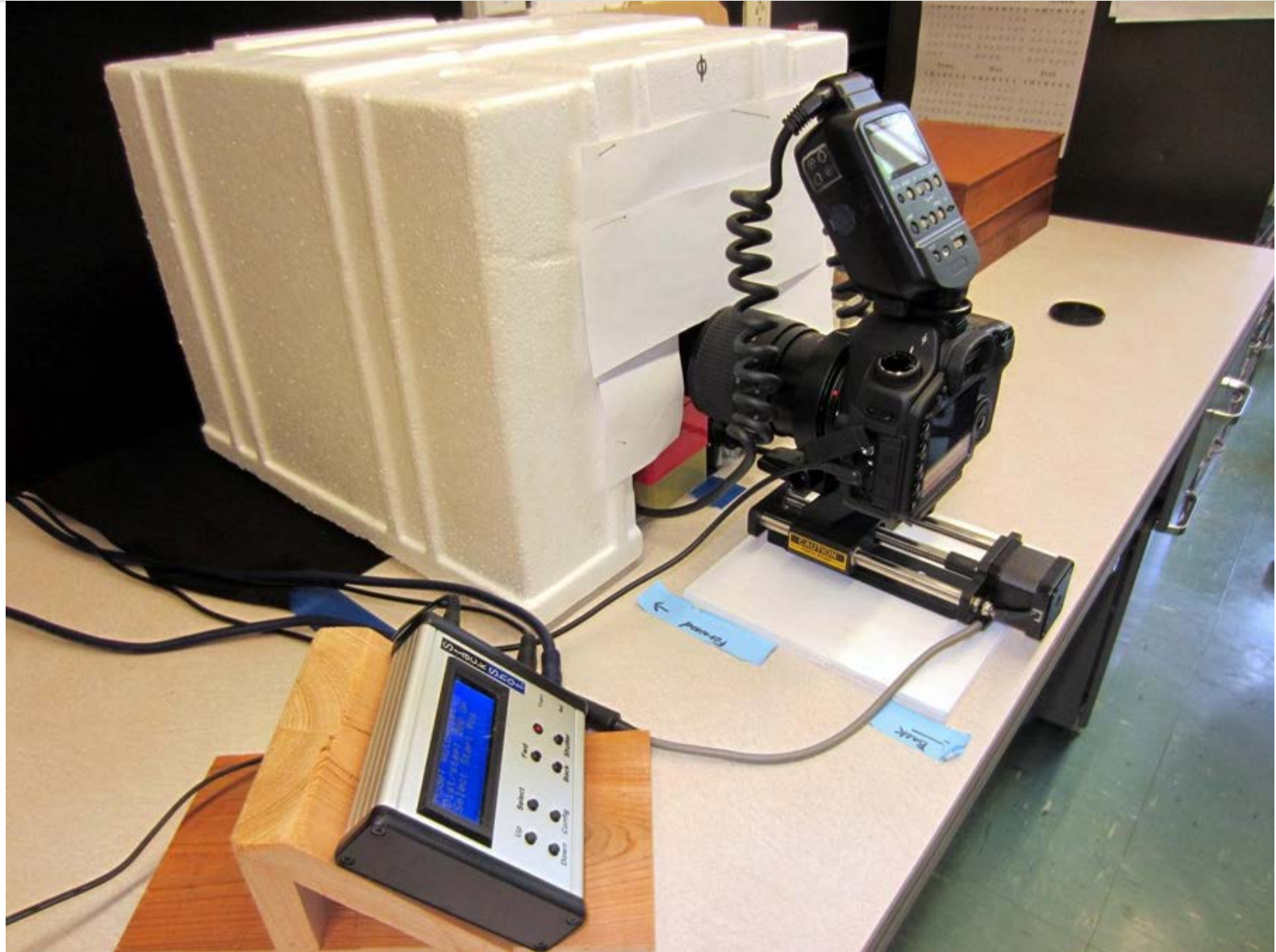
Nikon Camera Control Pro 2



Stackshot Rail



StackShot Controller



Sam Droege (USGS) Stackshot setup



AutoMontage

GIGAmacro.com

- http://gigamacro.com/viewer/gigaview/index_beta.php?image1source=gigamacro&image1id=79&bg=FFFFFF



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 a single specimen into a discreet folder, preferably a folder within a master folder of all images to be processed.
3. Convert all images to uncompressed TIFF or JPEG using 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. If cropping is required, crop the resulting TIFF or JPEG images.
5. Archive raw images.
6. 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.



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