PiAutoStage: An Open-Source 3D Printed Tool for the Automatic Collection of High-Resolution Microscope Imagery

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Abstract

We have created an open-source 3D printable microscope automatic stage and integrated camera system capable of providing a means for imaging microscope slides—the PiAutoStage. The PiAutoStage was developed to interface with the high-quality optics of existing microscopes by creating an adaptable system that can be used in conjunction with a range of microscope configurations. The PiAutoStage automatically captures the entire area of a microscope slide in a series of overlapping high-resolution images, which can then be stitched into a single panoramic image. We have demonstrated the utility of the PiAutoStage when attached to a transmitted light microscope by creating high-fidelity image stacks of rock specimens in plane polarized and cross-polarized light. We have shown that the PiAutoStage is compatible with microscopes that do not currently have a camera attachment by using two different optical trains within the same microscope: one set of imagery collected through the photography tube of a trinocular microscope, and a second set through a camera mounted to an ocular. We furthermore establish the broad adaptability of the PiAutoStage system by attaching it to a reflected light stereo dissection microscope to capture images of microfossils. We discuss strategies for the online delivery of these large-sized images in a data-efficient manner through the application of tiled imagery and open-source Java-based web viewers. The low cost of the PiAutoStage system, combined with the data-efficient mechanisms of online delivery make this system an important tool in promoting the universal accessibility of high-resolution microscope imagery.

Plain Language Summary

The instruction of Earth science courses often relies upon the observation of in-hand specimens which poses a significant barrier to delivering courses in an online format. While there are abundant resources for the digital delivery of 3-dimensional images of rock specimens, there are limited avenues to deliver microscopic materials to students in a manner that approximates the in-person experience. We have developed an accessible solution for creating and delivering microscopic educational materials to students. Our solution is an open-source device that combines a 3D-printed mechanism, to move a sample around the microscope, and an integrated camera that are both controlled by a central, inexpensive computer. The PiAutoStage system can be attached to almost any microscope and is capable of automatically imaging an entire microscopic sample by combining hundreds of collected images into a single panorama. We have found that the images permit an experience comparable to using a microscope and have the additional benefit of allowing students to examine, not only the field of view permitted in a microscope but an entire sample at once. The system is low-cost and utilizes widely available components making it universally accessible to any institution with an existing microscope.

1. Introduction

The ongoing COVID-19 pandemic has forced a re-evaluation of how to deliver traditionally in-person formal and informal laboratory instruction. Shared facilities, such as microscope laboratories, were acutely affected as the sudden pivot to online-only instruction prevented access to these tools. Strategies employed by instructors to overcome these difficulties included: (1) the rewriting of laboratory courses in order to utilize the limited collection of free online microscopy resources, (2) expensive commercial microscopy solutions, (3) the ad-hoc collection of new microscopic imagery by either film scanners with limited optical resolution or laborious manual image collection. While these solutions provided avenues to permit learning continuity, they have not provided an adequate bridge linking students’ pre-pandemic experiences with the
new online paradigm. An optimal solution is one that provides students with imagery of microscope-comparable quality over the entire area of a slide.

Advances in 3D printing, and in particular the high-tolerances required for the fine spatial scales required for microscopy, have allowed for the creation of customizable designs that permit the fabrication of microscope parts. The parallel advances in the development of open-source microcontrollers and small low-cost computers have permitted the motorization and automation of such parts. Finally, progress in the resolution, control options, and interconnectability of computer cameras permits the creation of custom microscope image capture devices. While fully 3D printed microscopes and components are the subject of active development (Collins et al., 2020; Diederich et al., 2020; Maia Chagas et al., 2017; Nuñez et al., 2017; Sharkey et al., 2016), there exists no open-source system that can integrate with the high-quality optics of existing microscopes. We have developed PiAutoStage—a low-cost 3D printed universal automatic microscope stage that is attached to a Raspberry Pi computer and a 12 megapixel camera. The PiAutoStage camera can be connected to a wide variety of existing microscope optics through the inbuilt C-mount bracket. PiAutoStage can capture the entire area of a microscope slide in a series of overlapping images, which can then be automatically stitched into a single high-resolution mosaic image. We describe strategies for the efficient online delivery of this content using a tiled image file format. This system provides a new avenue for instructors to create and deliver online microscopic content that more closely reflects their existing lab courses.

2. Methods

2.1. Component Fabrication Using 3D Printing

The body of PiAutoStage is constructed from easily fabricated 3D printed components, congruous with the increasing use of this technology within geosciences (Arcand et al., 2020; Chenrai, 2021; Ziegler et al., 2020). The design of PiAutoStage is based upon a rack-and-pinon style movement mechanism, originally designed as a drawing machine (MakerBlock, n.d.). This model was modified using computer aided design (CAD). 3D models are available through a GitHub repository (https://zenodo.org/badge/latestdoi/333261932). The most significant adjustments included:

A) The creation of a mounting bracket to facilitate attachment to a microscope stage. This mounting bracket was designed with adaptability to allow its use across a wide range of microscope models. In particular, the use of elongated screw mortices permits adjustability in the width and depth of the mounting screw patterns. Further adjustments to the existing designs are possible by user-driven adjustment to the openly available shape files.

B) The cross-bracing of the design facilitates free-standing attachment to a microscope stage. PiAutoStage attaches to a microscope stage such that ~50% of the body of the mechanism overhangs free-space. Thus, a stiff model design is necessary to prevent unwanted flexure during operation.

C) The creation of a microscope slide carriage to permit imaging of standard 45 × 27 mm slides. This carriage was designed to avoid collision with short working distance objectives. Alternative versions of this carriage are provided that can hold slides of different sizes. The slide carriage may be user-modified to suit the specific application—the shape files of the existing carriages are openly available.

D) The integration of alignment and fitting indicators on the design. These features include markers, labels, direction indicators, and user-modifiable calibration tabs. These features assist in both the initial assembly and calibration, in addition to day-to-day operation.

E) Modification of the Y-rack to facilitate the attachment of the slide carriage.

Upon completion of the initial design, the CAD models were sliced into a toolpath using Cura (ver. 4.8.0) and uploaded to a 3D-printer. The primary printer requirement for manufacture of this system is a print bed large enough to manufacture the X-rack (~168 mm long). Other printing factors are determined by the user as there are a wide range of 3D printers available. The two most common printer types are Fused Filament Deposition (FDM) and stereolithography (SLA). FDM-type printers utilize a solid thermoplastic filament that is melted and forced through an extruder nozzle, building up layers of plastic. SLA printers use a photo-sensitive resin that is hardened by a laser beam to build up printed layers. In general, FDM printers are less-expensive than SLA-printers for comparable print volumes, but SLA printers are capable
of printing to much finer resolution than an FDM type-printer. Both methods result in a model built from layers of plastic where the size of each layer determines the quality of the surface finish. Given the size of the gears in this mechanism (4 mm), the fine layer heights (<0.1 mm) achievable with SLA printing are not necessary, making FDM-printers a good choice for manufacturing the components of the PiAutoStage. For the system presented here, all components were printed with a Creality CR-10 Mini printer using 1.75 mm Polylactic Acid (PLA) filament at a layer height of 0.2 mm (additional printing conditions are outlined in Table S3). This layer height gave the best results on our system, producing a smooth surface finish for the gear teeth in the rack-and-pinion mechanism and sufficient detail to read the markings on components. We have not tested layer heights larger than 0.28 mm as this is the maximum height of our system, but it is possible with large diameter nozzles. We acknowledge that it should be possible to use other thermoplastic filaments (e.g., Acrylonitrile Butadiene Styrene or ABS) or print the mechanism on an SLA printer but such tests go beyond the scope of this contribution.

2.2. Hardware and Electronics

The primary controller for the PiAutoStage is a Raspberry Pi 4 model B running the Raspbian operating system with 4 GB of RAM. A Raspberry Pi High Quality (HQ) 12.3 megapixel camera is attached to the Raspberry Pi 4 by ribbon cable. The microcontroller board used is an Arduino Uno, connected to the Raspberry Pi 4 by USB. The microcontroller operates two SG90 micro servo motors that move the x and y axes of the PiAutoStage. Connecting the micro servo motors to the Arduino microcontroller may be achieved by either soldering the components or by using terminal connectors on an electronics breadboard (Figure 1). The supplemental instruction guide describes the breadboard method as it does not require a soldering iron (text S1 and S2).

The Raspberry Pi HQ camera comes with a standard C-Mount bracket, which can be screwed directly (or using widely available adaptors) into the optical train of microscopes with existing attached cameras. Where the microscope does not have a camera attachment point, the Raspberry Pi HQ camera may be screwed into commercially available ocular adaptors and inserted into one of the microscope ocular cavities. Given the short length of the camera ribbon cable, it is suggested the encased Raspberry Pi and Arduino be attached by velcro to the microscope body.
2.3. Software

2.3.1. Arduino “Sketch” Software

The software used to operate the Arduino microcontroller (termed a “sketch”) is constructed from a modified version of the C++ language, edited and compiled using the Arduino Interactive Development Interface. The sketch used in the PiAutoStage (text S3, hosted on https://zenodo.org/badge/latestdoi/333261932) has been adapted and modified from an original script written by Arduino User: Zoomkat. This sketch is divided into a “Setup” and “Loop” portion. The “setup” portion of this code (a) reads and initializes communication with the serial port on the main computer and, (b) begins sending impulses to the servos that encode the neutral position (1,500 µs, default in Arduino Servo Library). While encoding the neutral position is not required, it does prevent the servos from returning to their last position before they were powered down. The “Loop” portion of the sketch retrieves commands sent from the Raspberry Pi serial port and encodes these commands into impulses that are delivered to the servo motors.

Commands sent from the Raspberry Pi computer through the serial port are read byte-wise, and are thus converted into a string that is a series of eight characters. The “Loop” portion of the sketch parses this string into two sets of four characters. For example, if the string “15001200” is passed to the Arduino microcontroller, it is parsed into two strings, “1500” and “1200”. The first set of numbers encodes the pulse rate (in µs) to be delivered to the x-axis micro servo motor, and the second encodes the y-axis micro servo motor. To prevent unintentional movement of the stage, which may damage either the stage or the microscope during initialization, the sketch requires a “GoCode” to attach the micro servo motors to the Arduino microcontroller. A string starting with “5555” will attach the micro servo motors and allow for movement. The initialization sequence of the included Python scripts sends this “GoCode”, allowing subsequent commands to the micro servo motors.

2.3.2. Python Code

PiAutoStage uses a Python code (Python v3.7 and above) to control both the motion commands sent to the microcontroller, and the image collection commands sent to the Raspberry Pi HQ Camera (text S4, https://zenodo.org/badge/latestdoi/333261932). We use the Spyder development environment and execute the codes using that environment and associated Python console. Succinctly, the Python code instructs the PiAutoStage to move the slide carriage into a set position by encoding x and y location commands to the microcontroller, then to capture and store an image at a set resolution by the Raspberry Pi HQ camera. This process is repeated until the full area of the microscope slide is imaged.

The geometry of gridded image collection can be described by assuming that the x position represents a column of images and the y axis represents a row of images. For gridded image collection, the order in which these columns and rows of images are collected can be described by a combing motion or a continuous motion. Based upon the mechanical tolerances of the PiAutoStage, combing motion (where the PiAutoStage steps through each column in the same direction) provides the optimal results due to the better lateral alignment of images (Figure 2). Continuous (or serpentine) motion, which captures images with a minimum of overall stage movement, steps through the rows in adjacent columns in opposite directions (Figure 2). This movement generates images that are prone to lateral offsets that cause alignment issues when creating the composite images. The Python code provided to operate the PiAutoStage thus uses combing motion (text S2). To implement this motion, the Python code generates an eight digit coordinate wherein the first

![Figure 2](https://zenodo.org/badge/latestdoi/333261932)
four digits represent the $x$-motion and the last four represent $y$-motion. This coordinate is encoded as a byte and sent via the serial port to the Arduino microcontroller. The last four digits of the coordinate are progressively changed such that the PiAutoStage travels along the $y$ axis. Upon reaching the end of the $y$ axis, the first four digits of the coordinate are modified to move the $x$-axis and then the process is repeated until the grid commanded by the user is completed. The stage then returns to its home position.

Image collection is achieved using the Picamerax Python package—a modified version of the Picamera library. This package must be installed by the user on the Raspberry Pi 4 computer prior to operating the PiAutoStage. The Picamerax package was selected over the Picamera package as it permits the collection of a series of photos where the brightness, color, and contrast may be set manually and thus provide consistent photos for compositing. The PicAutoStage Python code sets these values during the initial focusing steps. During the initial focusing step, the stage moves to a pre-set representative position within the slide to permit manual microscope focusing. The gains are set during this step and are held constant for all remaining image collection. The python code then moves the stage to four additional positions to allow the user to observe the slide to ensure proper focusing and to obtain additional exposure values. For petrographic thin sections, we have found that selecting the fastest shutter speed (as opposed to an average of the five positions) produces the best results, however, these settings are user-modifiable.

PiAutoStage collects images using a compressed Joint Photographic Experts Group format (jpeg), which results in the loss of some image fidelity when compared with the uncompressed Tagged Image File Format (tiff). The format of image collection is user modifiable. We have selected the compressed image format given the computational limitations associated with the processing of hundreds of images for the creation of a composite mosaic. It should be noted that the web-based delivery of content, discussed subsequently, uses a compressed image format thus there is limited utility in collecting large sized uncompressed images. The issue of computational limitations must also be considered when selecting the resolution of the captured images. The Raspberry Pi HQ 12.3 megapixel Camera has a maximum resolution of $4056 \times 3040$ pixels, however, it may be necessary to use a lower resolution where the computer running the image stitching software (not the Raspberry Pi used for image collection) has insufficient RAM to complete the compositing operation. Capturing images at high resolution may require adjustment to the Raspberry Pi Bios settings to permit more RAM to be allocated to the GPU.

### 2.4. Stage Assembly

Prior to assembling the 3D printed components, it is necessary to first prepare the electronics such that the Y-motor is initialized to its neutral position (1,500 µs; text S2). The micro servo motors should then be inserted into their housing and the gears attached using the hardware provided with the micro servo motors. There are eight printed components, which must be assembled beginning with the Y-stage and Y-rack (Figures 3 and S4–S5). These Y components are then attached to the X stage (Figures S6–S9). This assembled piece comprises the moving component of the PiAutoStage and is then seated into the immobile X-rack (Figures S10–S11). The entire PiAutoStage can now be attached to the microscope stage by either; (1) using screws that fit the user’s microscope stage. The user must ensure the screw pattern of the microscope stage is compatible with the pattern on the PiAutoStage bracket (the design of the bracket is user modifiable). (2) Adhesive strips may be used to affix the PiAutoStage bracket to the microscope stage.

It should be noted that 3D printing can result in slight imperfections in the PiAutoStage components. For example, where parts are too tight, gentle sanding may be necessary. More significant removal of material, such as that required to remove extra material from tabs used to fit the Y-rack between the two motor housings (see Figures S7 and S15), can be done with a utility knife. When removing material to improve the fit of components, the user should check the fit often to prevent it being too loose, which can result in poor stage stability.

Ensuring that the slide carriage is coplanar with the microscope's stage platform is essential for maintaining focus during image capture. We have found that the most reliable way to attach the carriage to the Y-rack is to perform the task when the stage mechanism is fixed to the microscope stage platform. By applying a bead of hot glue to the carriage mast and placing the carriage flat on the microscope stage platform, the user can slide the carriage up to the Y-rack and press the carriage into the end of Y-rack (Figures S12 and S13). It is...
useful to check periodically that the bottom of the carriage remains flat to the platform. We have observed that one side of the carriage may gradually lift from the platform either due to extended exposure to the warm microscope light that heats the plastic causing some deformation or because of the buildup of debris under the forks of the slide carriage. If this happens, simply peel the slide carriage off, clean off the old glue and debris, and reattach.

2.5. Calibration of the PiAutoStage

The coordinate system utilized by the PiAutoStage is based upon the function of the micro servo motors. The micro servo motors function by interpreting the rate of pulsed electrical signals as a degree of rotation. By default, the neutral position of the micro servo motors is 1,500 microseconds (µs), which should be the center position in both the x and y directions of the stage as denoted by markings on the stages and racks of the PiAutoStage. The SG90 micro servo motors are capable of rotating 90° from neutral in either direction. Therefore, the space that the PiAutoStage operates within are defined by the limits of the micro servo motors—i.e., to a range of pulse rates between 544 and 2,400 µs. The length of the X and Y racks are calibrated to this range of rotation. Movement of the PiAutoStage within this space is accomplished by adding or subtracting microseconds from the neutral pulse rate. Increasing the pulse rate moves the x-direction to the right and the y-direction inward. Decreasing the pulse rate delivered to the micro servos moves the X-Stage to the left and pushes the carriage in the y-direction outward (Figure 2). However, it is unlikely that the full range of the stage will be utilized by a user, especially in the y-direction where movement of the carriage is somewhat limited by the X-rack and some short working-distance microscope objectives. These two limits will need to be determined by the user based on their own microscope arrangement. Determining the limits of a user’s particular system can be accomplished by iterating stage positions through the serial monitor in Arduino IDE or by sending serial commands through the Python console (text S2).
3. Results

3.1. Image Collection

3.1.1. Precision of Mechanism (Reoccupation)

The horizontal precision of the PiAutoStage mechanism can be condensed into a test of the ability of the stage to reoccupy locations on a microscope slide. We examined the capacity for the system to reoccupy positions within the \( x \)-\( y \) plane, shown in Figures 4 and 5. For the \( y \)-direction, the maximum error in reoccupation of positions is 49 pixels in a 2,028–1,550 pixel image or 3% deviation over three cycles of image capture. The deviation in the \( x \)-direction is substantially larger, with a maximum deviation of 405 pixels in the same size image amounting to \( \sim \)20% deviation. This deviation is the sum of imperfections in the stage mechanism. Given that the PiAutoStage is constructed from 3D printed components, and that every 3D printer is

Figure 4. Sequence of four photomicrographs corresponding to four unique stage positions \( (X = \#\#\#\# \; Y = \#\#\#\#) \) while observing a mantle xenolith (see Figure S1, for full image). Collection of these images was repeated for three cycles to determine reoccupation accuracy of the PiAutoStage in the \( y \)-direction. Images of cycle 1 were used to determine the anchor point (white arrows) which calibrated the anchor line (blue dashes). The deviation in pixels from anchor point in cycles 2 and 3 to the anchor line is the reoccupation error. The maximum \( Y \)-reoccupation error is 49 pixels.
geochemistry, geophysics, geosystems

 capable of differing levels accuracy during printing, it would be expected that each individual PiAutoStage would have somewhat different precisions that should be examined by the user prior to first use.

Maintaining planer movement such that there is minimal deviation in the vertical direction is required to ensure that the focus of the microscope is consistent across the specimen. For images collected using a 2X and 4X objective, we have found that images are in focus across entire specimens. We can therefore conclude that the vertical deviation of the mechanism is within the objective manufacture’s tolerances for working distance. Vertical deviation of the system from the focal plane is thus adequate for the presented applications. End user experiences will depend primarily upon the accuracy of the 3D prints and the optical tolerances of their specific microscope.

### 3.1.2. High Magnification and Spherical Lens Aberration

High degrees of magnification or the use of ocular mounted focal reducers can impart a spherical aberration into the optics of the system, thus limiting the effective field of view. The presence of spherical aberration caused by focal reducers or poor optics, require the clipping of images such that images only capture the sharp, in-focus region. This clipping can be passed as an argument to Picamerax during image capture, and an example is presented in the supplement (text S4).

Clipping reduces the effective field of view, requiring more images to be collected at smaller steps in order to maintain image overlap. A critical limitation is the effective step size of the micro servo motors, which in the system described here, are accurate to about 1° of rotation. Step sizes at or below that threshold result in inconsistent micro servo motor operation, resulting in poorly aligned images that cannot be reliably stitched together. This limitation is of particular concern at higher magnifications, where very small step sizes may be needed. PiAutoStage has been tested successfully using 2x and 4x microscope objectives.

### 3.1.3. Collected Images

We present three sets of images that demonstrate both the effectiveness of the PiAutoStage at generating high-quality imagery and the adaptability of the system to different applications. Two sets of transmitted light images were collected through two optical arrangements in the same Nikon Eclipse E600 POL microscope:

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**Figure 5.** Sequence of four photomicrographs corresponding to four unique stage positions (X = #### Y = ####) while observing a mantle xenolith (see Figure S1, for full image). Collection of these images was repeated for three cycles to determine reoccupation accuracy of the PiAutoStage in the x-direction. Images of cycle 1 were used to determine the anchor point (white arrows) which calibrated the anchor line (blue dashes). The deviation in pixels from anchor point in cycles 2 and 3 to the anchor line is the reoccupation error. The maximum X-reoccupation error is 405 pixels.
Camera attached to a trinocular head unit and using a 2X objective lens. This setup requires the collection of 320 images to fully recover the area of a standard petrographic thin section (45 × 27 mm). The resolution of each image was set to 2028 × 1550 pixels, with the aim of limiting the total data collection to ~1 gigapixels per view to facilitate efficiency of subsequent image stitching.

Camera attached to a commercially available C-mount ocular adapter and inserted into the left microscope ocular. We used the 4x objective to simulate a common setup on petrographic microscopes (Figure 6). This setup requires the collection of 1,216 images to fully recover the area of a standard petrographic thin section. The resolution of each image was set to 800 × 600 pixels, resulting in 0.58 gigapixels per view.

For transmitted light images, we collected three different views of the same thin section using different configurations of the microscope. The first image was captured using plane polarized light, the second with cross-polarized light with the stage at 0°, and a third with cross-polarized light with the stage set at 45°. When combined, the images collected under these three configurations best simulate the common usage of a petrographic microscope.

To examine the applicability of the PiAutoStage system beyond transmitted light applications, we attached the stage to a dissection microscope using a commercially available C-mount ocular adapter and inserted into the left microscope ocular cavity. We then collected images of foraminifera mounted to a slide with glue using reflected light. This setup requires the collection of 170 images to fully recover the area of a mounted section (effective viewing area of 47 × 20 mm). The resolution of each image was set to the maximum possible for the camera (4,056 × 3,040 pixels), resulting in 2.1 gigapixels per view. Given the samples being imaged by this method are 3-dimensional, three different focus levels were captured to ensure sufficient vertical resolution.

3.2. Mosaic/Panorama Creation

The generation of a high-quality series of optically consistent overlapping images permits the use of image compositing software in order to render a single panoramic image. There are a number of software options available to undertake this work, and below we explore some of the more popular options. The stitching process is computationally demanding and will need to be done on a computer more powerful than the Raspberry Pi.

3.2.1. Microsoft™ Image Composite Editor

This software was developed by the Microsoft Research Computational Photography Group and is freely available to users of the Microsoft Windows operating system. This software provides the most direct method by which both panoramic images and tiled images may be created. Images may be loaded into the software with or without a specific structure assigned to them and stitched using the planar motion option with no further user input. Once cropped to the desired shape, a high-quality panorama of the stage can be exported in multiple image formats.

3.2.2. ImageJ/Fiji

The powerful Fiji/ImageJ suite was developed by the United States National Institutes for Health (Abramoff et al., 2004; Schneider et al., 2012) and is a multiplatform open-source image processing package that, among many other uses, can produce composite images. This software suite has a number of stitching plugins that are specifically designed for microscopy. The first, titled "Stitching" (Preibisch et al., 2009) was...
designed to generate composite images of biological specimens using the Fourier Shift Theorem. The second is titled the Microscopy Image Stitching Tool (MIST) and was also developed for biological applications but contains a number of error correction tools (actuator backlash and stage repeatability), and provides quantitative accuracy measurements (Chalfoun et al., 2017). Both Stitching and MIST require information about the structure of the image array and overlap between images. MIST has additional options specifying overlap in the $x$ and $y$ directions, the overlap uncertainty, and the repeatability of the stage. For use of these stitching tools, the user will need to specify the number of rows and columns and the direction of image collection. This information is specific to the optics of the microscope used (see notes on calibration above).

### 3.2.3. Panorama Output and Fidelity of Images to Sample

While the images collected by the PiAutoStage accurately reflect the field of view observed at that time, it was necessary to confirm that artifacts are not introduced during the stitching process to create mosaics. To test this, we created mosaic images in multiple stitching platforms (Image Composite Editor and ImageJ/MIST) using blending and overlay modes (Figure 7). We found that the overlay image was functionally indistinguishable from the blended mosaics. All crystals are in the same position and have the same morphology. One observed difference are changes in color saturation within crystals where the crystal is sufficiently large to require many frames to fully capture.

Using three conditions outlined above, the final composite image sizes were: 0.3 gigapixels for the 2x objective, 0.22 gigapixels for the 4x objective, and 0.36 gigapixels for the reflected light slide. It should be noted that the decrease in the total number of pixels from the sum of the collected image frames to the final composite image reflects the overlap between individual image frames.

### 3.3. Image Alignment

The creation of multiple panoramic images of the same microscope slide using different microscope configurations constitutes what is commonly termed a “stack” of images. Viewing this stack of images can be achieved through commercial software such as Adobe Photoshop™ or the freely available ImageJ. In both cases, images are loaded into the software as layers that can be viewed with different rendering options. A critical aspect of stack creation for local viewing or remote delivery (see Section 5) is the alignment of images. The panorama images created during stitching will contain slight differences that may result in misalignment when viewing the stacked images. Both pieces of software contain options for stack alignment (e.g., Linear Stack Alignment with SIFT: Lowe, 2004) that should be executed to ensure the best results. It is suggested that these aligned images be exported and used as the basis for any subsequent visualization (including the creation of tiled imagery for online delivery).

### 4. Discussion and Applications

#### 4.1. Applications

We demonstrate several applications of the PiAutoStage within the Earth sciences, but note that the adaptability of this system extends to almost any optical microscope capable of supporting the stage hardware.
We examine the utility of the PiAutoStage system for making petrographic observations and assess the capacity of the system to replicate the experience of conducting microscopic observations. In particular, we focus on the application of PiAutoStage attached to a petrographic microscope and a binocular reflected light microscope. The discussion below does not seek to make novel textural insights into the particular samples chosen to demonstrate the PiAutoStage, but instead show that the system presented is able to adequately replicate aspects of a student’s microscope experience. We note that the carriage of the PiAutoStage is user modifiable such that other applications not discussed below are possible (e.g., reflected light microscopy on polished materials). The primary limitation of such applications is the microscope to which the PiAutoStage is paired.

4.1.1. Transmitted Light Petrography

One of the most valuable characteristics of the petrographic microscope is the ability to make observations of mineral optical properties by examining a petrographic thin section in both plane-polarized and cross-polarized light. Replicating this experience therefore requires the capture of two sets of images under two different microscope configurations. However, the appearance of crystals under cross-polarized light can change significantly depending on the orientation of a crystal relative to the polarizer. Information on zoning and twinning of crystals, for example, can be gained by rotating the microscope stage to take advantage of this property. We have therefore collected three sets of images under the following microscope configurations: (1) plane polarized light, (2) cross-polarized light at a 0° starting stage position, (3) a second cross-polarized image with the stage rotated 45° from the previous position (Figure S1).

In order to demonstrate that petrographic observations can be made from images collected with the PiAutoStage system, we present image mosaics of a mantle xenolith from Kilbourne Hole (United States) (Byerly & Lassiter, 2012; Perkins & Anthony, 2011; Reid, 1976) and a Cenozoic porphyritic lava from the East African Rift System, southeast of the Kenyan town of Lodwar, within the Lothagam tectonic block (East African Rift) (McDougall & Feibel, 1999; Schofield et al., 2021).

Key petrographic features are evident at a range of scales in all of the images (Figures S1 and S2), comparable with observations that could be made using a standard petrographic microscope. In plane polarized light (Figures S1 and S2), there are subtle differences in color between mineral phases where olivine is colorless, orthopyroxene is brown, and clinopyroxene is green (Figure S1). Such an observation would be difficult if not impossible if the exposure and color setting of the set of images was not held constant, a feature integrated into the PiAutoStage system. In cross-polarized light, the interference colors readily allow for the identification of olivine, and in combination with the plane polarized light image, distinguish between olivine, orthopyroxene, and clinopyroxene.

The presentation of two cross-polarized images allows for important mineral observations, which if only given a single image may result in ambiguity. For example, having two different cross-polarized light orientations allows for a distinction to be made between anisotropic and isotropic minerals. Isotropic minerals such as spinel appear as black under the two presented orientations of cross-polarized light (Figure 8).
This facilitates distinction from anisotropic minerals such as olivine, which may be extinct in one or other the cross-polarized views but not both. Examples where two cross-polarized images are necessary are the identification of crystal deformation features and compositional zoning. Deformation features have been cited as important observations in published literature on Kilbourne Hole (Kil & Wendlandt, 2004), and are among the crystal features a typical undergraduate student would be expected to note when working on such a sample using a petrographic microscope. While kink-banded olivine is readily observable in cross-polarized light, the orientation of the crystal relative to the polarizer can result in identification difficulties (Figure 9). In the example shown, kink-banding is much more evident in image B of Figure 9. The observation of compositional zoning in crystals is similarly dependent on the crystal’s orientation relative to the polarizers. For example, large clinopyroxene crystals in the lava sample (Figure S2) exhibit concentric compositional zoning that is most apparent in the large crystals when the stage is in the 45° orientation. At 0°, many of the large crystals are near extinction making the zoning within the crystals difficult to observe. It is therefore apparent that presenting multiple orientations of the slide allows students to make a larger range of observations using digital imagery. Given the time and computational limitations in capturing images at different microscope configurations, consideration must be made by the user of the PiAutoStage system as to the most appropriate microscope configurations for their specific application, but at a minimum the three configurations presented herein are necessary for petrographic investigation of a sample.

Much of the discussion above focused on how the PiAutoStage system can replicate a student experience with a microscope. However, the panoramic images of thin sections that are generated by PiAutoStage make it possible to examine features that would otherwise be too large to observe within a single field of view in a microscope. Large cm-scale textural or mineralogical heterogeneities can now be viewed in the context of the entire specimen instead of students attempting to describe heterogeneous regions independently of one another, or by relying solely on reflected light hand-lens observations. To demonstrate the utility of the panoramas generated by the PiAutoStage system, we present an image mosaic (Figure 10) of a sample from the Caledonian Leinster Granite, SE Ireland (Fritschle et al., 2017; O’Connor & Brück, 1978). This image clearly shows cm-scale mineralogical domains dominated by either feldspar or micas that would be difficult to fully appreciate within the field of view of a standard microscope.

### 4.1.2. Reflected Light Microscopy

By modifying the carriage that carries the specimen and affixing the mechanism to a dissection microscope, we reconfigured the PiAutoStage system to image a microfossil slide. The specimens in this example are 3-dimensional, requiring image capture to be performed at a range of focal heights. The example we present is of foraminifera, testate single celled eukaryotes, that are commonly used in biostratigraphic analyses of seafloor sediments (Dubicka, 2015; Hadi et al., 2019) (particularly in the petroleum industry) and paleoenvironmental studies (Burkett et al., 2020; Spero et al., 2003). At a most basic level, it is possible to classify the foraminifera shells (tests) based upon the chamber arrangement (Loeblich & Tappan, 2015) into uniserial, biserial, spiral, and globular morphologies (Figures 11a–11d). On tests that are oriented properly,
Figure 10. Composite image mosaic (2x objective, 320 images, cross-polarized light) of part of the Leinster Granite (Ireland). The sample is texturally and mineralogically heterogenous, where feldspars dominate the left side of the sample and micas dominate the right. This image demonstrates additional utility of image mosaics to observe large scale features that could not be fully appreciated in the field of view of a typical microscope.

Figure 11. Screen capture from image mosaic (Figure S3) showing various morphological characteristics of foraminifera (Eocene Shubuta Formation). The broad test morphologies that can be observed are uniserial (a), biserial (b), spiral (c), globular (d). Specific features are observed, such as the aperture (d),(e), pores (a),(e), and spikes (f).
it is possible to determine the geometry of the aperture (Figures 11d and 11e) as well as identify pores (Figures 11a and 11e). There are also several examples of tests with spines or spikes (Figures 11f). The ability to make these observations on test morphologies demonstrates the quality of images collected with this system and the utility of these images for teaching laboratory courses virtually.

The ability of this system to quickly (and inexpensively) capture images of microfossils could be a powerful tool when coupled with machine learning in the form of image classification algorithms (Wong & Joseph, 2015). Classification algorithms such as those described by Zhong et al. (2017) require multiple images of the same specimen to be collected under different light conditions. Zhong et al. (2017) collected their imagery of one test at 16 different light orientations. Utilizing the automated technique presented here, it may be possible to rapidly collect large data sets required for machine learning. Such a capacity could be a powerful tool in biostatigraphic analyses that require large numbers of specimens to be sorted, a process typically done by hand.

4.2. Remote Delivery of Stitched Images

The creation of a composite panorama of high resolution imagery of a microscope thin section can result in large-sized image files. Using the optical and camera resolution parameters outlined above, a stitched file size that is on the order of 23,000 by 13,000 pixels is created. A lossless 8 bit RGB TIFF format with these dimensions generates a file with a size of ~850 MB. Compression in a jpg format that preserves the thin section size and features generates file sizes that vary depending on the complexity of the image but range from ~40 to 90 MB based on the images we have captured (further image compression is possible depending on the application the image is needed for). Assuming that an online laboratory exercise for undergraduate students might require the use of five petrographic sections, each with three different optical arrangements then 1.3 GB of data would be needed in order to download the compressed imagery. Aside from being a significant data load, the simultaneous viewing of multiple large image files can place a sizable computational strain on students’ computers.

The delivery of high resolution imagery, optimized for the zoom level of the specific view requested by the user, is implemented via tiling of large images. This technique reduces the data needed to view high resolution imagery by delivering only the tiles necessary to render the specific view commanded by the viewer. One implementation of this concept is the Deep Zoom file format, developed by Microsoft™, which permits a user to observe portions of an image at different zoom levels, loading only those tiles necessary to deliver the current view of the larger image. A Deep Zoom Image format consists of two parts: (1) an xml or dzi file that contains information on tile size, image format, and image dimensions, and (2) the creation of a folder containing a collection of tiled images at different zoom levels. The creation of Deep Zoom images can be achieved through multiple existing software platforms (e.g., Microsoft™ Image Composite Editor) and is not discussed further here.

The remote delivery of Deep Zoom images and their associated files on a browser requires a web-tool to render the images. OpenSeadragon is a customizable java-based Deep Zoom web viewer initially copyrighted by the CodePlex Foundation and later by OpenSeadragon contributors. This open-source software comprises a viewer that is embeddable into a website. The viewer is designed to function on both desktop and mobile devices, expanding greatly the reach of Deep Zoom images, and promoting accessibility where access to desktop units may be limited. OpenSeadragon is broadly customizable, with a diverse array of plugins that may facilitate both research and teaching applications. Of particular note for microscopy, OpenSeadragon features: (1) a Scalebar plugin developed by the United States National Institutes of Standards and Technology (NIST) that permits conversion between pixels and measured units. (2) Configurations that permit the simultaneous viewing of multiple Deep Zoom images (e.g., PPL, XPL images) on the same webpage, for example by calling multiple OpenSeadragon viewers. This combination of the OpenSeadragon web viewer and Deep Zoom file formats is currently used to deliver histology microscope slide images for the Australian Breast Cancer Tissue Bank, and is thus a proven method for accurate and efficient delivery of microscope images (Khushi et al., 2013).

We have successfully tested the online delivery of PiAutoStage composite imagery by: (1) conversion of composite panoramas into the Deep Zoom format using both Microsoft™ Image Composite Editor and the
HDMAKE executable that accompanies Microsoft™ HD View utilities, (2) rendering of these images online via the OpenSeadragon web viewer using the NIST Scalebar plugin, (3) remotely delivering this content to undergraduate students entirely contained within the Desire2Learn (D2L) learning management software, (4) tested delivery of the same content using a standard Apache webserv.

4.3. Education and Teaching Microscopy

An essential component of practical laboratory-based petrology education is student interaction with suites of related rock samples both in hand specimen format and through the exploration of linked microscope thin sections. Most petrology classrooms use sample collections intended to teach specific topics with laboratory exercises built around those samples. The rapid pivot to online delivery of such content during the current pandemic has presented a challenge to educators who have largely approached the problem by either using available online 3D and microscope imagery (e.g., 3D model repositories, online virtual microscopes), or by digitizing in-hand sample collections. Using existing online resources removes the technical challenges inherent to digitization of collections, but requires the assembly of suites of unrelated rock samples and the creation of new student exercises that do not replicate the content of the prior in-person instruction. The alternative digitization approach preserves the content of existing laboratory exercises but can be difficult to implement.

Photogrammetry has provided an opportunity for hand samples and even field sites to be utilized in the classroom or for remote learning (Carrivick et al., 2016; Cho & Clary, 2017, 2019; De Paor, 2016; McCauley, 2017). Examination of 3D models of both hand specimens and field sites have generated positive experiences, which are likely to improve further with the development of virtual reality systems that can immerse students in a pseudo-field experience (Cho & Clary, 2020; Merel, 2015). The widespread interest in the implementation of photogrammetry relates in part to its low-cost—virtually every person possess the necessary hardware (i.e., a camera). The parallel hardware needed to construct high resolution composite microscope imagery, is however, typically bespoke to a particular microscope and can cost tens of thousands of dollars. As of the time of writing of this paper, the cost of components necessary for the construction of a PiAutoStage system is less than US$300. We therefore contend that the expanding toolbox for virtual instruction in Earth science education can benefit greatly from the implementation of the PiAutoStage system to deliver microscope imagery. Implementing the PiAutoStage system to capture imagery of sample suites used by students during in-person instruction will allow educators to utilize laboratory materials that have been carefully curated to teach specific topics.

5. Summary and Conclusions

Within this contribution we have: (1) provided step-by-step instructions on the fabrication and assembly of a universal microscope automated stage and camera; (2) demonstrated a method to digitize microscope slides using this automatic stage, and (3) described how to efficiently deliver this content online. We have established the utility of the PiAutoStage system for imaging microscope slides using a transmitted light microscope by creating high-fidelity and resolution images of petrographic thin sections. The adaptability of this system was shown by our use of two different optical trains: one imaged through the photography tube of a trinocular microscope and another through a camera mounted to the ocular. The versatility of the PiAutoStage system was demonstrated by configuring the system to capture images of microfossils through the ocular of a stereo reflected light microscope.

An important advantage of this system over existing remote-petrographic instruction materials is that it allows the instructor to utilize specimens and laboratory materials already present in the course curriculum. During the current pandemic, such options facilitate the continuity of instruction; however, the PiAutoStage system may have significant utility during non-pandemic times by providing an alternative parallel instruction platform for students that may be unable to use a microscope. Critically, the low cost and our reliance on well-established and widely used components, makes the PiAutoStage system accessible to all institutions that have an existing microscope. This potential universality in ownership equalizes instructional options between institutions with and without the necessary resources to pay for commercial solutions.
The adaptability and affordability of the PiAutoStage permits this system to be utilized for a potentially wide range of research applications. The open-source nature of the system allows researchers to customize their PiAutoStage for their specific application. With requisite modification to the image collection pattern, almost any specimen that would normally be observed in a microscope could be imaged in high-resolution.

Data Availability Statement
All 3D files, Arduino IDE sketch, and Python codes are available from the PiAutoStage GitHub repository at https://zenodo.org/badge/latestdoi/333261932.

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**Erratum**

In the originally published version of this article, Tables S1, S2, and S3 were missing from the Supporting Information section. These tables are now included in the Supporting Information. This may be considered the authoritative version of record.