
# Automated Multi-focus Imaging

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*The multi-focus ("stacking") technique for photomicrography has allowed micromineral photographers to dramatically increase the depth of field in their photomicrographs. Although the software for this technology has advanced rapidly in competence and in ease of use over the past few years, the manual photographic process is tedious, time-consuming, and requires painstaking care to achieve optimum results. The computer-controlled automated multi-focus imaging system described here alleviates this difficulty, allowing for unprecedented speed and ease in creating multi-focus photomicrographs.*

## INTRODUCTION

Before delving into the details of computer-controlled automation, let's briefly review some basic optical principles as they apply to microscopy, to better understand what multi-focus imaging is all about. We can then see why multi-focus imaging is rapidly becoming a de facto standard for digital photomicrography and why it is also finding applications in macrophotography.

One of the primary parameters of interest in an optical instrument, whether it is a microscope, camera or telescope, is resolution: a measure of the finest detail that we can resolve in an image. Resolution is finite, and is a function of other optical design parameters that the optical designer has to work with in the scope of a particular design. Today's microscopes (aside from some of the cheaper imports) have very good resolution yielding sharp, crisp, high-contrast images; these instruments are capable of producing excellent photomicrography when combined with a suitable camera or electronic imaging device. Increased *depth of field*, however, is something that we soon long for. It is annoying having to focus up and down on a subject in order to explore it all in sharp focus, and more so as the magnification is increased. Why is depth of field such an issue? One would think that space-age, state-of-the-art, computer-aided technology and materials should surely allow optical designers to design microscopes with greater depth of field, but that has not happened. To understand why, let's take a brief look at some basic optical physics in order to develop a better understanding of the problem.

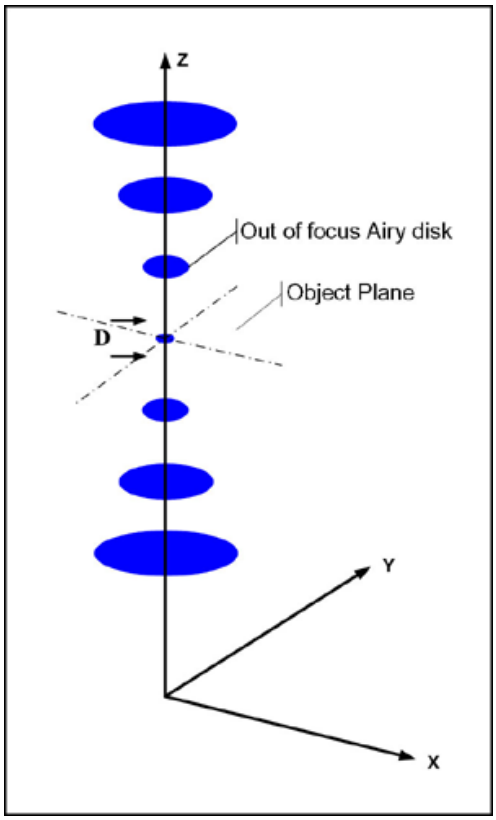


***Figure 1.*** **The Z-axis, or optical axis, of an imaginary microscope, showing the object plane where the object is in best focus. The depth of field (D) is defined as the distance along the Z-axis where the object is in acceptable focus.**

First, let's define and quantify resolution. Looking at Figure 1, we see the optical axis of an imaginary microscope intersecting the object plane, where the object is in best focus. A viewed object, on the object plane, can be considered to consist of an infinite number of infinitely small points. But, because resolution is finite, a point on the object plane will appear to be a small disk, called an "Airy disk," as shown in Figure 2a (Davidson, 2009). This is the result of diffraction and the wave nature of light. Resolution is defined as the shortest distance between two Airy disks at which they both can still be resolved, as illustrated in Figure 2b. Two Airy disks that are not fully resolved are depicted in Figure 2c. Resolution is a function of the wavelength of the light, in which the subject is viewed, and the numerical aperture of the microscope's objective lens. It can be derived from the analysis of the diffraction caused by light passing through a circular aperture (Halliday and Resnick, 1966) and is described mathematically by the following relationship:

**Equation 1:**

$$R = \frac{0.61\lambda}{NA}$$

where :
*R= resolution in nm*
*λ = wavelength of light in nm*
*NA = numerical aperture of the objective lens ( ~ 0.05 - 0.3 for most stereo microscopes)*

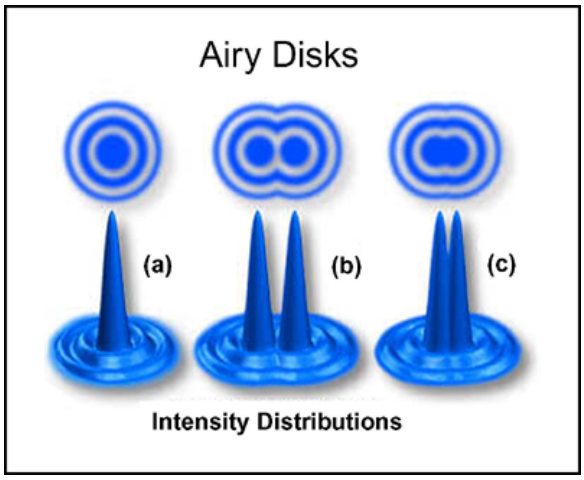


***Figure 2.*** **The simulated Airy disks show the appearance looking down the Z-Axis onto the object plane (upper images) and the intensity profiles as a function of spatial distance from the Z-axis (lower images); (a) is a perfect theoretical Airy disk; (b) illustrates two fully resolved Airy disks; and (c) shows two Airy disks that are not fully resolved. (Image © Florida State University)**

As an example, consider a research-quality stereo microscope that has a numerical aperture (NA) of 0.21. Assuming a wavelength of 550 nm and doing the simple math, we find that $R$ =1.6 µm, which is approximately 625 lines per mm. As an aside, it is interesting to note that a compound microscope, with a maximum NA of 1.6, using immersion oil and a wavelength of 400 nm, results in the maximum theoretical resolution of a light microscope of approximately 0.15 µm.

Perhaps the reader is wondering what relevance this has to photomicroscopy or multi-focus photomicrography. The relationship between resolution and depth of field will soon become apparent, as well as why multi-focus photomicrography is such an exciting technique that can vastly improve our photomicrographs.

Having reviewed the factors involved in resolution; now let us now consider depth of field. Depth of field (D) is defined as the distance above and below the object plane that the object appears to be in focus. D is finite and is a function of the wavelength of light and the inverse of the square of the numerical aperture, and is expressed mathematically as:

**Equation 2:**

$$D = \frac{1.22\lambda}{(NA)^2}$$

Where:
*D = depth of field in nm*
*λ= wavelength of light in nm*
*NA = numerical aperture of the objective lens (~ 0.05 - 0.3 for most stereo microscopes)*

Equation 1 and Equation 2 are easily combined and reduced to give the following important relationship.

**Equation 3:**

$$D = \frac{3.27R^2}{\lambda}$$


***Figure 3.*** **A graphical representation of Eqation 3 showing the relationship between depth of field (D) and resolution (R).**

From Equation 3, it can be seen that depth of field (D) is a function of the resolution squared and the inverse of the wavelength of the illuminating light. Remember that resolution (R) is in nm, so a larger value of R equates to less resolution. Equation 3, for depth of field as a function of resolution, is a very good approximation for high-quality stereo microscopes. This relationship between resolution (R) and depth of field (D) is shown graphically in Figure 3. From Equation 3 and the graph, it is apparent that if we wish to achieve greater depth of field, it is only possible by lowering resolution—something we don't want to do. Using aperture diaphragms, which effectively change the numerical aperture (NA), can increase depth of field at lower magnifications, where resolution can be sacrificed, but not at the resolution limits of the microscope. In short, we are bound by the immutable laws of optical physics, which dictate the maximum depth of field available for a given resolution. Since the days of Anton van Leeuwenhoek in the 17th century, microscopists have despaired over this impenetrable roadblock. Not until the "digital revolution" did a solution become possible—a solution with stunning results.

## MULTI-FOCUS IMAGING:
## Cheating the Immutable Laws of Optical Physics

It has become commonplace to see amateurs using digital cameras, both consumer models and specialized, dedicated microscope cameras, to photograph mineralogical and entomological specimens. With the rapid evolution of the personal computer, computational capabilities have been growing by leaps and bounds over the past decade. Digital imaging and image processing applications, such as Photoshop, have become inexpensive and widely available to the amateur. In the past few years, very sophisticated professional image processing applications have become available at very reasonable prices, ranging from a few hundred dollars to "freeware" available at no cost at all.

Multi-focus (or "stacking" as it is commonly called) is an image-processing technique that can overcome the deficiencies of optical systems and their inherently limited depth of field. Stacking relies on the computer to process a set of individual images taken at focal intervals, combining the in-focus portions of each image to produce a single composite image in which all portions of the subject are sharp. More accurately stated, a series of images must be made with the following requirements:

(1) Take multiple images of the subject at intervals along the optical, or Z, axis.

(2) The depth of field for each image must overlap that of the next image.
(3) Take enough images such that the total required depth of field is covered.

A synthesized, or composite, image is then created by processing the set of images with stacking software such as CombineZ, HeliconFocus and others (Wikipedia, 2009). The resulting image can have as much depth of field as one would like, contingent upon the number of images taken and processed. As an example, Figure 4 (left) clearly shows the limitations of the microscope's depth of field. The foreground crystals are in focus while the background crystals are out of focus. Figure 4 (right) shows the effect of stacking four images taken at equal intervals along the optical axis, providing an image with greatly increased depth of field.

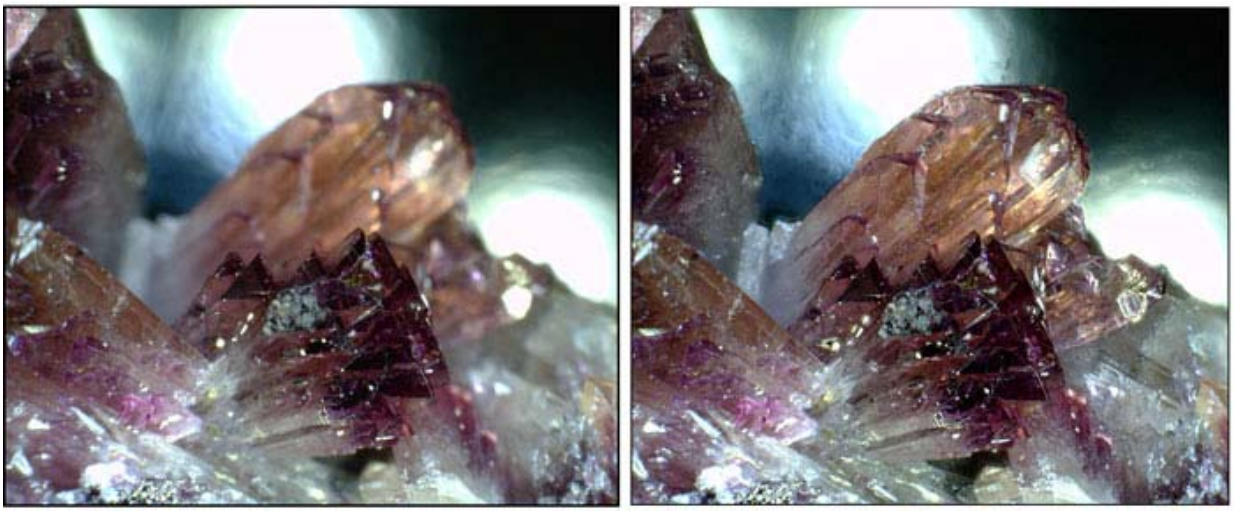

***Figure 4.*** **Adamite (Mn-rich), from Mapimi, Durango, Mexico; collection of E. Cisneros. Photographed with a Nikon SMZ-2T and 1.3 MP EM-130 digital microscope camera. The field of view is 5 mm wide. (a) A single image showing lack of depth of focus. (b) Image composited from a stack of four exposures, showing greatly increased depth of field and sharpness.**

As simple as the concept of multi-focus is, actually producing an excellent image can be difficult and time-consuming. A major issue that must be overcome is how to mechanically increment the microscope focus in precise steps of the correct distance along the Z-axis. There is no easy answer to this problem, and many ingenious methods have been devised to accomplish this task (Betz, 2005). The simplest and most obvious method is to carefully turn the microscope focus knob in very small increments, calibrated by eye. If one is very careful and is able to step the focus in somewhat even amounts, a satisfactory image may result, though repeatability is poor at best. Some have affixed calibrated scales to the focus knob so as to better control the size of the increments. Though these methods may produce usable images, they will seldom be of optimum quality.

I have devised two methods that are very useful in consistently producing optimized images. The first is to attach a machinist's dial gauge indicator to the microscope (Fig. 5), such that the plunger is in contact with, and follows, the microscope focus carriage. Although the dial gauge allows for measurement of the focus carriage position to 0.001-inch accuracy, or about 25 µm, the microscope focus mechanism movement may not be fine enough to fully take advantage of this.

The second method is to de-couple the microscope focusing mechanism from the process altogether. This is accomplished by employing a Z-axis translation stage (Fig. 6), a device designed for highly accurate positioning of laser components and other optical devices. A resolution of 5 µm is not uncommon for these devices. Aside from rather high cost, around $400-$500, these devices provide all of

the Z-axis resolution and repeatability required to consistently produce excellent multi-focus images. The only drawback to this method of stacking is that it, too, can become rather tedious when producing a stack of ten or more images. In a continuing quest for greater depth of field, I have routinely been processing stacks of ten to 30 images. This tedium inspired the next logical step.

***Figure 5.*** **A machinist's dial gauge indicator that has been mounted on Nikon SMZ800 microscope to facilitate accurate measurement of the Z-axis position of the microscope head.**

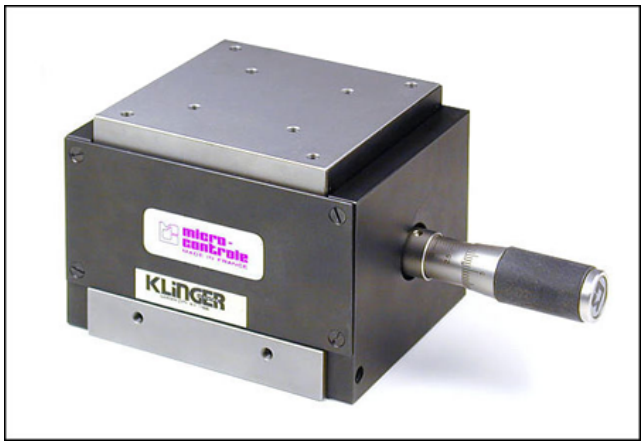


***Figure 6.*** **An example of a small industrial Z-axis translation stage.**

## THE PROJECT: a Better Mouse Trap

Abhorring the tedium of hand-stepping large stacks of images provided the impetus to automate the stacking process. Although automated multi-focus systems exist at the professional level, they are very expensive and are generally not available to the amateur. Most commercial devices are also proprietary hardware and software and are therefore only applicable to a specific model of microscope, usually attaching to the fine-focus knob.

Conceptually, an automated multi-focus system consists of three parts; mechanical, electronical and software. The most important criteria are as follows.

*Criteria:*
(1) Emphasis on low cost, using off-the-shelf components and surplus parts.
(2) The device must be transportable between microscopes and support various imaging devices.
(3) Easily constructed, not requiring special tools or skills.
(4) Use as much existing software as possible to minimize the programming effort.
(5) The device should be simple, reliable and intuitive to operate.

Since this is a low-liability device and wouldn't be flying into space, nor would anyone's safety depend on it, there is no need to design it to environmental, safety or commercial specifications or requirements. Therefore, the following very minimal specification will satisfy the needs dictated by the various microscopes and imaging devices that are commonly used by amateurs for photomicroscopy.

*Specifications:*
(1) Positional Range ± 3 mm
(2) Positional accuracy 10 µm
(3) Repeatability 10 µm
(4) Digitally controlled by a personal computer running Windows XP or Vista
(5) Support TWAIN-compliant cameras that require software image capture
(6) Support a hardware-triggered shutter release for DSLR cameras and other digital cameras.
(7) To address electrical safety issues, the device will only require 12-volt DC for operation.

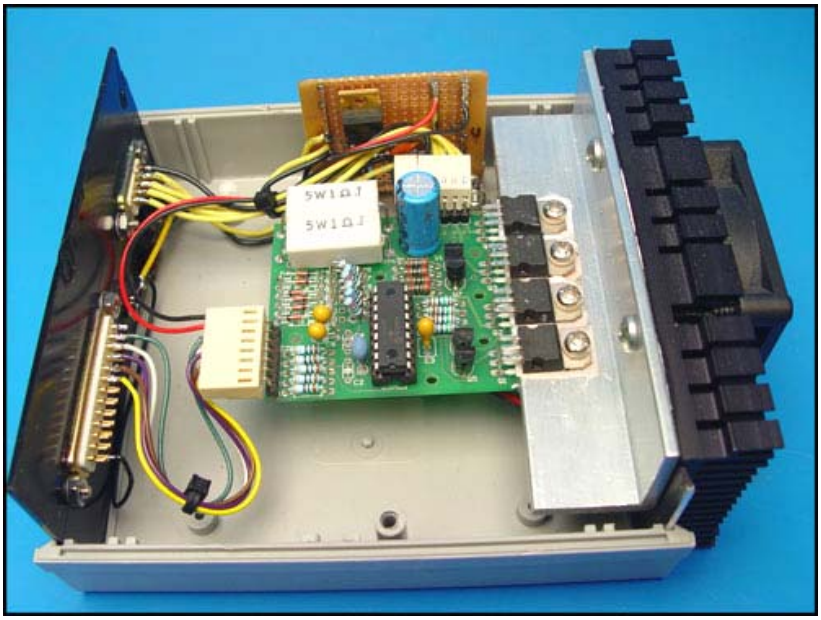

***Figure 7.*** **Stepper motor driver electronics assembly.**

A review of widely available stepper motors and drivers reveals that the wheel need not be reinvented. A motor driver can be selected from many that are available and widely used for computer-controlled lathes, milling machines and other motion control applications. The motor controller chosen here was purchased for $35 in kit form and was easily assembled in a couple of hours. A very minimal amount of extra support electronics is required. The motor controller electronics are housed in a plastic electronics project box and appropriate connectors and other hardware are installed (Fig. 7). A further

simplification of the project is to utilize commonly available and inexpensive computer cables for interconnection between the computer, the motor controller and the motor. This completes the electronics part of the system and only the mechanical and software aspects of the motorized stage then need to be worked out. A $2 surplus stepper motor will do the work of turning the micrometer on the Z-axis translation stage. A small aluminum plate and two pieces of angle aluminum are all that is needed to complete the motor-driven stage, and a rubber O-ring is used to greatly simplify the drive mechanism. The completed electronics unit, with power supply and motor-driven stage, is shown in Figure 8. The open connector is the interface to the PC. Figures 9 and 10 illustrate the ability of the automated stage to be easily used with various types of microscopes.

***Figure 8. (left)*** **Z-axis translation stage with stepper motor; (*right*) stepper motor driver electronics and 12-volt power supply (above).**

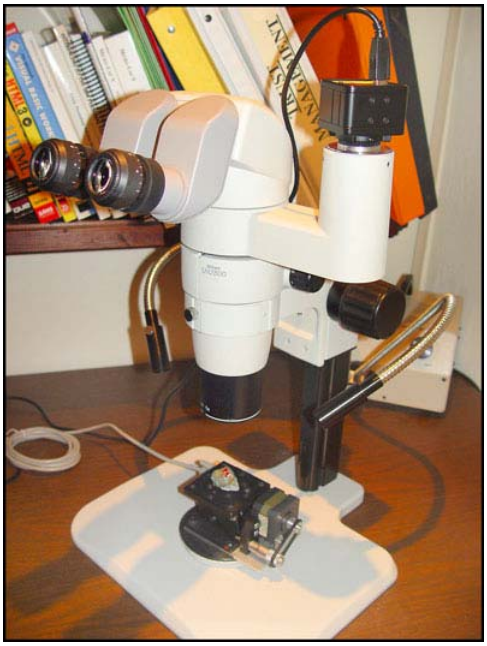

***Figure 9.*** **A research-grade Nikon SMZ800 trinocular microscope with digital camera and automated Z-axis stage.**

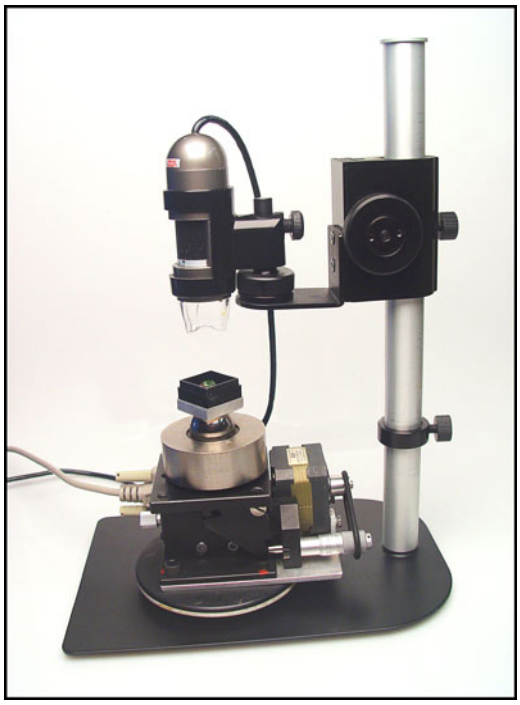

***Figure 10.*** **A USB DinoLite digital microscope set up for imaging with the automated Z-axis stage. Several of the mineral photos in this article were taken with this simple, compact and very easy to use setup.**

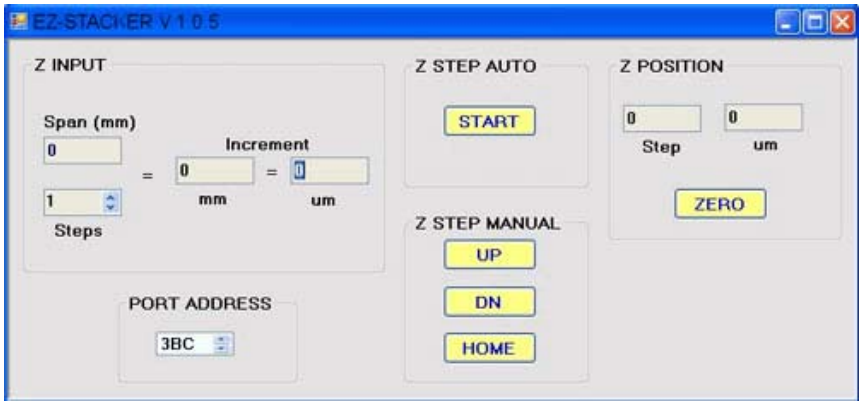


***Figure 11.*** **A screen capture of the EZ-STACKER application control panel.**

The control application program, which I call EZ-STACKER, was written in Visual Basic 2009 for ease of programming for the Windows environment, and because digital control of external devices is easily implemented. EZ-STACKER interfaces with MiniSee, a TWAIN-compliant commercial image acquisition application, commanding it to capture the stack images and save them to a preselected directory. EZ-STACKER also controls the motor-driven Z-axis stage, commanding it when to increment, the size of the increment, and the number of increments. Figure 11 shows the EZ-STACKER user interface window. The parallel port address for the host computer is selected from the PORT ADDRESS selector. The total Z-axis excursion, required for the multi-focus image, is entered into the Span box and then the total number of steps required is selected from the Steps selector. Perhaps, for example, you would like to have a total excursion along the Z-axis of 2 mm and you would like 10 steps of 0.2 mm each. Enter 2 into

the Span box and select 10 from the Steps selector, and the Increment boxes immediately update and display the step size in mm and in µm.

Before starting the automated imaging it is possible, using the Z STEP MANUAL UP/DOWN buttons, to manually step the stage up and down to check the limits of your presets previously entered. Last-minute fine focusing may also be done with the manual UP/DOWN buttons, usually focusing on the highest part of the specimen, while viewing the live image on the computer monitor. Clicking on Home, at any time, will return the stage to its starting position. When the final fine focus is done, the ZERO button is clicked and the Z POSITION boxes are reset to zero without moving the stage. To start the automatic process of collecting the images to be stacked, the START button is clicked. The process starts by capturing the first image immediately and saves it to a pre-specified directory; preferably the one that the stacking program looks at. There is a 2-second delay to allow for the image download and for any vibrations to damp out. The next image is captured, and so on, until the stack is completed.


***Figure 12.*** **A screen capture of the automated multi-focus system operating environment. The background window shows the directory that the stack images are sent to. The live image is viewed in the upper left window. EZ-STACKER, left upper window, is the automated Z-axis stage control window.**

During the process, the Z POSITION boxes dynamically indicate the current step that the stage has been commanded to, and the Z position relative to the zero starting position. Figure 12 shows the entire operating environment, when using a TWAIN compliant camera, consisting of three active windows. The background window displays the contents of the directory that the images are stored in. As the automatic capture process proceeds, the images sequentially appear there. The larger of the 2 foreground windows

displays the live video image and can be resized to full screen for critical focusing on the uppermost part of the specimen prior to initiating the automatic process. The last window is the EZ-STACKER control panel, previously described.

**HOW DOES IT ALL PLAY?**

All of the design criteria and specifications have been met and the system functions superbly, considering its surplus and junk-box heritage. The automated stage makes the task of taking any number of images, within its design range along the Z-axis, a trivial task. A stack of 20 images can be taken, and delivered to the directory that the stacking software looks at, in about 40 seconds. Without the drudgery of having to hand-step each Z-axis increment, many specimens can be routinely photographed in a short time. Some images taken with this system in conjunction with different microscopes are shown in Figures 13-16.

As previously stated, cost was at the head of the criteria list, and this is perhaps the most important consideration for most hobbyists. The prototype unit was constructed at a cost of ~$200 utilizing a surplus Z-axis translation stage, the most expensive and necessary component. Diligent shopping may reduce the cost even more. Using all new components, the completed automated multi-focus system would come to ~$600 (2009).

***Figure 13.*** **Boltwoodite from the Rossing Mine, Arandis, Swakopmund District, Erongo Region, Namibia; collection of E. Cisneros. The field of view is 2 mm wide. (a) A single image taken with a DinoLite AM413T Digital Microscope showing little depth of field. (b) Image composited from a stack of 25 exposures, showing greatly increased depth of field and sharpness.**

***Figure 14.*** **Neptunite from Mina Numero Uno, San Benito County, California; collection of E. Cisneros. The field of view is 2 mm. (a) A single image taken with a DinoLite AM413T Digital Microscope, showing lack of depth of field. (b) Image composited from a stack of 25 exposures, showing dramatically increased depth of field and sharpness.**

***Figure 15.*** **Conichalcite from Gold Hill, Tooele County, Utah; collection of E. Cisneros. Image composited from a stack of five exposures taken with a Bausch & Lomb SZ7 and 1.3 MP EM-130 digital microscope camera. The field of view is 4 mm wide.**

***Figure 16.*** **Spessartine from the Wushan spessartine mine, Tongbei, Yunxiao County, Zhangzhou Prefecture, Fujian Province, China; collection of E. Cisneros. Image composited from a stack of eight exposures taken with a Bausch & Lomb SZ7 and 1.3 MP EM-130 digital microscope camera. The field of view is 3 mm wide.**

## WHERE DO WE GO FROM HERE?

EZ-STACKER is a first attempt at addressing software control of the stage. Through experience gained in operating the system, I have identified some areas where the software can be improved. One of these improvements will be a software upgrade to allow the system to automatically find the computer port address so that the user doesn't have to know the specific address for his particular computer. In the long term, a USB interface may be implemented, but the parallel interface control appears to be the most cost effective and the easiest to implement at this time. The hardware seems robust enough and will probably not undergo any changes. Should there be enough interest in this project, I could publish a website outlining the complete details of how to construct the system, including parts lists, parts sources, mechanical details, etc. For those who are not up to programming the control software, EZ-STACKER will be provided without cost (or warranty).

This is an exciting time in the field of photomicroscopy, where use of the complementary technologies of electronics, computing and precision hardware are extending the usefulness and abilities of our optical microscopes. Other applications of these, and new technologies, are currently being investigated and may possibly lead to improvements in photomicroscopy illumination.

## ACKNOWLEDGMENTS

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## ABOUT THE AUTHOR

Gene Cisneros and his wife Sharon have been interested in mineral collecting since they first met in high school, and during the following years field collecting became a passion. In 1966 they formed Mineralogical Research Company, a purveyor of mineral specimens, supplies and related optical equipment. Concurrently, Gene was employed as an electrical engineer at the SLAC National Accelerator Laboratory, designing diagnostic and control systems for the two-mile linear accelerator. In 2003, after 38 years of service, Gene retired from his position as a Project Manager and Head of the Instrumentation and Controls Section. He has now joined Sharon in operating the mineral business full time, and also pursues his hobbies of mineralogy, astronomy, ham radio, sailing and physical fitness.

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