

## ASI Application note

### **Some Points to consider when adding a motorized stage and Z - axis drive to a microscope**

Adding an automated x,y stage and z-axis drive to your existing microscope can make it a more valuable research tool. By automating these functions the user can screen a large number of samples in a more accurate manner. However, to ensure the integrity of your data and the quality of the microscope's imaging capability a number of points should be considered when selecting an automated xy stage and z -axis drive. This application note will address feedback and encoder selection; x, y stage selection and condenser considerations; as well as, z-axis drive selection.

#### ***Feed back: Open loop versus closed loop and DC steppers versus DC servo motors***

Closed loop feed back differs from open loop systems in that in a closed loop system an encoder is used to feed back the actual position of the stage or fine focus shaft of the microscope. For example, say we have installed an automated z-axis drive on our microscope that employs a closed loop design. With this design we command the stage to move 1.5  $\mu\text{m}$  towards the objective. The command is converted into a DC voltage that powers a motor that is attached to the fine focus, or coarse focus, shaft of the microscope. In this closed loop system the position of the stage or focus shaft is monitored with a high resolution encoder. The encoder allows the control electronics to know when the stage or the shaft actually moves the 1.5  $\mu\text{m}$  that it was commanded to move and then the control electronics can tell the motor to hold its position.

In an open loop system no encoder is used. Instead of using encoders, which can add a great deal of cost to a system, most manufacturers use DC stepper motors. The controller for the DC stepper motor keeps track of the motor's position by counting the pulses it sends to the motor. Since the pulses to the motor usually result in the proper linear movement of the z-axis drive or xy stage, stepper motors are commonly used in these applications. However, if something binds the stage's lead screw or the stepper motor's shaft and motion is lost, then the open loop stepper can lose its position.

Systems that utilize closed loop feedback are usually more precise than those that operate in an open loop mode. This is because the feedback device/encoder ensures that the commanded position is reached. DC stepper motors can be operated in either the open or closed loop mode. To obtain fine resolutions they are usually micro stepped in very small increments. DC servo motors, on the other hand, have an infinite resolution and they are almost always used with a feed back encoder to provide a very precise closed loop design.

## **Encoders & Backlash**

In a closed loop system the overall accuracy of the system is determined by the resolution of the feedback device (the encoder). There are basically two types of encoders that can be used in microscopy applications and these are the rotary and the linear encoder. As their names imply, the rotary encoder measures rotary movement, and the linear encoder measures linear movement. Rotary encoders are usually used to measure the position of the microscope's focusing shaft and, if properly placed on a particular microscope, they can provide a resolution from 0.1  $\mu\text{m}$  to 50nm and a repeatability of better than  $\pm 0.1$  to  $\pm 0.2 \mu\text{m}$ . The advantages of rotary encoders are that they can be easily installed, and they provide a very good price/performance ratio. The only disadvantage of using a rotary encoder is that backlash can exist, which is not accounted for, when you change directions on the focusing shaft. This backlash is the slop between the stage and the focus shaft on the microscope and can vary between a tenth of a micron up to five microns or so, depending upon the design and adjustment of the microscope, as well as the wear that has occurred. Once the amount of backlash is known, it can be accounted for by selecting a band slightly below your area of interest and factoring the backlash out in any calculations or by always approaching the target z-position from the same direction, this can be easily done in the software.

The other way to deal with the backlash is to install a linear encoder on the stage of the microscope and read the actual position from the stage of the microscope. This method does provide a more accurate means of measuring the stage position as the backlash is accounted for. However, high quality sub micron linear encoders are fairly expensive (3 to 6k) and to work properly the linear encoder must be mounted and aligned properly on the microscope. The installation and alignment to obtain sub micron resolution is somewhat involved and does add cost and a degree of difficulty to the design of the system. Greater care must also be taken in the installation and maintenance of the system with linear encoders when working in the nm range.

ASI offers both rotary and linear encoder options with their Z-axis drives and MS-2000 XY stages. Since the MC-2000 XY stage utilizes anti-backlash lead screws, the rotary encoders we use on the motors very closely match the accuracy that would be obtained with linear encoders.

### ***Automated X,Y Stages***

There are a number of manufacturers who offer automated xy stages. Most manufacturers of automated stages utilize D.C. stepper motors in a micro stepping mode to control the movement of the stage. Since stepper motors are usually used in an open-loop configuration without any sort of feedback device to ensure that the stage has arrived at the commanded position, it is important that the stage be well designed and thoroughly tested to ensure accuracy and repeatability of the device. Quality stages will utilize lead screws and precision bearings to ensure accuracy. In contrast, lower cost stages will use

rack and pinions or other low cost gearing. For precision stages, quality control testing with high resolution, highly accurate test equipment such as laser interferometers is also required to ensure the accuracy and repeatability of the device in an open-loop configuration. ASI tests and calibrates each and every MS-2000 stage with a laser interferometer based test jig . ASI also offers lower cost stages including American made lead screw systems and a low cost scanning stage which uses a gear driven rack to move the stage. The scanning stage is not nearly as accurate as the lead screw systems but if accuracy and repeatability is not a major issue, these stages offer a very economical (\$5,500) way to automate the x,y and z-axis of most upright microscopes, as well as a few inverted microscopes.

The three things to look for when deciding what stage is accurate enough for your application is:

- 1) Resolution or the smallest step size that the stage can make
- 2) Repeatability or how close the stage can come back to a given point
- 3) Absolute accuracy or how true the distance moved actually is

Depending upon the application one or more of the above specifications may be more important than the others. ASI offers a range of products for automated xy and z control to fit nearly any application. In fact, we manufacture our own line of closed-loop z-axis drives, xy stages and controllers, as well as open loop devices. We even offer x y stages from Marzhauser to ensure that we have a device to meet nearly every application, and fit nearly any microscope

### ***Condensers***

Whenever you add any scanning stage to your microscope, you will add distance between the condenser and the bottom of the slide on any upright microscope. This is simply a fact and there is no getting around it. How much distance you add is dependent upon the stage, but any of the quality units are going to add about 0.70" to the top of the stage. If the stage plates are thinner than this, then they may flex, or warp, with changes in temperature. Because the stages add distance between the slide and condenser, the condenser carrier must be able to move the condenser up this added distance so that proper Kohler illumination can be obtained. However, nearly all the microscope manufacturers do not allow for this. To overcome this problem ASI offers special condenser extenders that go in between the condenser and the condenser carrier. *Please Note: Because there are so many different condensers out there, many which are no longer manufactured, we may need to get the condenser from the customer to design the adapter for it if we do not have the item in stock.* Typical distances that xy stages can add are in the range of 12 to 20mm to the top of the stage. Since this added distance is to the top of the stage, various slide inserts that fit into the stage to hold the slide or sample can add to or reduce the condenser distance problem.

For example, ASI offers a range of slide inserts for various applications. These include the Silver finger insert (SIF), and the Rotating Slide Insert (SIR). These two inserts affect the condenser distance problem differently. The SIF insert places the slide at the top of the stage so the condenser carrier must be able to move the condenser this total additional distance, say 20mm, to place the condenser next to the slide for proper Kohler illumination. On the other hand, the SIR (rotating) slide insert would lower the slide down towards the condenser by ~7mm so the condenser only has to be moved up ~ 13mm.

In addition to the condenser distance problem, there are other problems which may occur when adding a stage to the microscope. These may or may not be solvable depending upon how they are viewed.

- 1.) On some microscopes, such as the Zeiss Axioplan, the screw that secures the condenser to the carrier "bumps into the microscope and will not allow the condenser to be moved up. We have designed a replacement screw for this condenser that prevents this from happening.
- 2.) If the rotating insert is used to lower the slide down, then on some scopes such as the Zeiss Axioplan the longer high powered objectives may scrape against the side of the insert as they are rotated into place. Since these objectives are spring loaded, they will not be damaged, however when you hear a \$10,000 objective scraping, it is an unnerving sound. The solution here is to simply lower the stage slightly before changing objectives, or use a combination of condenser extenders and customized SIR to correct the matter.
- 3.) On some condensers there is a lens that can be flipped in and out of place. If the condenser is racked up all of the way you may or may not be able to move the lens depending upon its size etc.. The solution is to rack down the condenser a bit, move the lens, and then move it back, again is an inconvenience that ASI may be able to provide a custom solution for, but the solutions do add costs to the system. The bottom line is that, depending upon the microscope and condenser, the user may give up some conveniences (not utility) when an automated xy stage is added. However, in most cases the added utility and performance of an automated stage adds outweigh any inconvenience.

### ***Z-Axis Drives & Focus Controllers***

There are a number of factors to consider when automating the focus of a microscope. Most manufacturers neglect to address most of these concerns. In fact, a number of manufacturers sell drives that simply clamp on, or are stuck to, the fine focus knob in an open-loop configuration. The reason that most manufacturers go this route is because it takes a great deal of time and consideration to custom design a z-axis drive for each and every microscope manufactured. We know that it takes a great deal of time to custom design these drives because this is the way that ASI does it.

ASI manufactures very precise motorized focus drives and controllers which can be utilized to obtain extremely accurate optical sections for stereology, confocal microscopy, deconvolution, lineage analysis, 3-D reconstruction and a number of other video microscopy applications. All of ASI's drives employ a closed-loop positioning system where the command position is constantly monitored with a high resolution encoder. The encoder feedback, along with the drive's mechanical design, ensures precise positioning. The controller offers both remote focusing via a front panel control knob and computer control via standard ASCII commands and constantly monitors the stage position to sub-micron accuracy.

ASI's closed-loop DC servo motor systems provide a number of key advantages over common stepper motor focusing systems. Some of these advantages include:

1. Rather than a one-size-fits-all design, our drives are custom designed for each microscope. When installed, they become an integral part of the microscope. This is the next best thing to being designed-in by the microscope manufacturer.
2. ASI drives use geared D.C. servo motors with position feedback from the focus shaft with rotary encoders, and linear encoders with resolutions down to 10nm can also be added as an option. This means, unlike stepper motors, full torque is available even for very small movements. A closed-loop servo system does the positioning. The stage position displayed on the control console is the actual stage position. It is available for interrogation by a computer.
3. A switch located on the control console operates a clutch that disengages the motor drive from the fine focus shaft when the drive is not needed. When disengaged, the position still displays and is still available for interrogation by computer. This feature lets the researcher note specific focus positions, or for a computer to memorize them, for later use in driving the z-axis. The controller also offers a safety feature where it continually monitors the commanded and actual positions. If they do not agree within a pre-selected amount of time, the clutch disengages and the motor drive shuts off.
4. On all of our drives, the original fine focus knob on the drive side of the microscope (usually the left), is brought out on an extension shaft. On some of our drives, depending upon the microscope, the original coarse focus knob is accessible as well. When the clutch is disengaged, or the motor drive shut off, the microscope can be manually focused from either side with no added torque felt on the knob and no cable to twist up.
5. A remote focus knob on the control console has several useful purposes. It is a convenient means of fine focusing without having to reach in around a lot of apparatus often surrounding the microscope during complex experiments. Where patch clamping is taking place, it allows vibration free fine focusing. It allows either temporary or permanent altering of the focus during multi-plane, time-lapse imaging. If the focus commands issued by the computer are *absolute*, then the effect of adjusting the remote focus knob is canceled at the next command, which lets the

researcher look around between commands. If the computer commands are *relative*, then the remote focus adjustment acts as a correction for changes taking place in the sample being observed.

6. ASI motor drives have lower electrical noise and lower mechanical vibration than do stepper motor drives. This reduces the likelihood of interference with other research equipment. Unlike stepper motors that are driven by fast rise and fall time (electrically noisy) current pulses, our drive signals are D.C. and essentially zero when the drive is not moving. We shield our drive cables and power cords to prevent electrical noise. The vibration is lower because D.C. servo motors, unlike stepper motors, do not have magnetic detents.
7. ASI drives accept standard ASCII commands over an RS232 serial link and easily interface to existing computers and software. They can be daisy-chained on the serial line with our shutter controllers and other devices. This is particularly useful when the controlling computer is a Macintosh or PowerPC since these computers typically have only one available serial port. ASI drives can also be programmed to mimic common command sets, so chances are that they will work with your existing software.

Installation requires no modification to the microscope other than removal of the fine focus knob and replacement of a back plate or base plate, depending on the particular microscope. All of the necessary hardware components, tools and detailed instructions, including videotapes on installing the drive, are provided with every unit.

ASI's z-axis drives and the other components and systems that we offer can turn your existing microscope into a more powerful tool. For more information please feel free to give us a call at (800) 706-2284, e-mail us at: [info@ASImaging.com](mailto:info@ASImaging.com) or visit our web site at: [www.ASImaging.com](http://www.ASImaging.com)