Continuous Reflective-Interface Feedback Focus (CRIFF)

Instruction Manual

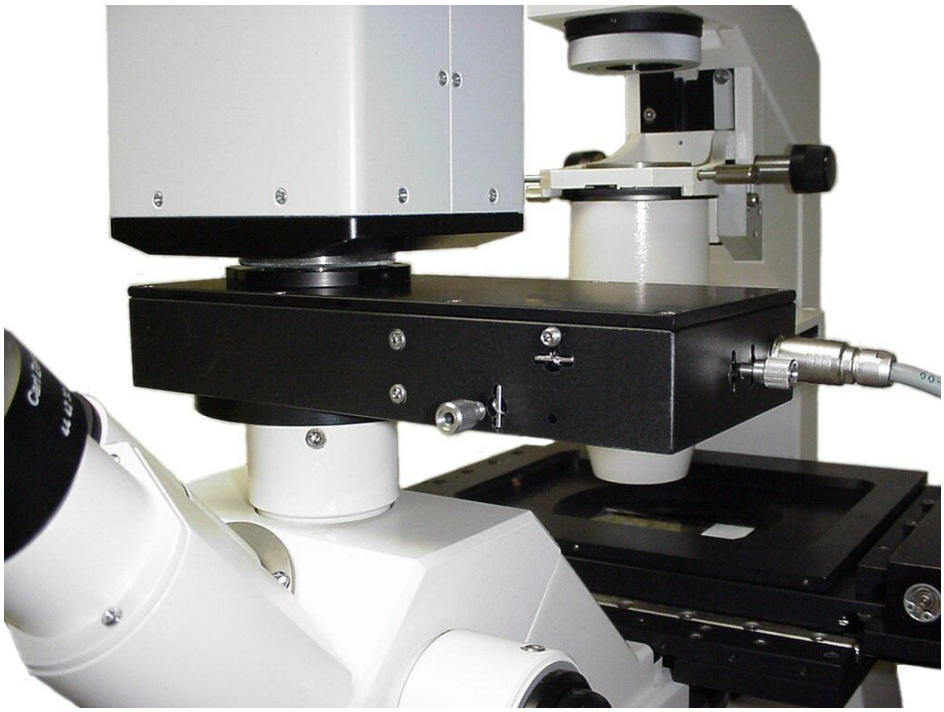

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Table of Contents

Continuous Reflective-Interface Feedback Focus (CRIFF) System

The CRIFF system provides for a very high level of focus stability, allowing a specimen to remain accurately focused for hours at a time with drift <0.1 µm. The system compensates for focus changes caused by temperature variations as well as mechanical drifts of the microscope mechanisms. The CRIFF system promises to be a solution to focus drifts that plague time-lapse experiments at high magnification. The CRIFF system uses an off-axis laser beam reflected from the sample cover slip. The reflected beam is obtained by using total internal reflection from the cover slip – sample interface on systems equipped with an objective lens with NA of 1.4 or more.

System Overview

The CRIFF system consists of optical, electronic, and mechanical components. The optical system injects a laser beam into the microscope, captures the beam reflected from the specimen cover slip, and routes the reflected beam onto a position-sensitive detector (PSD). The signal from the PSD is conditioned by a "position amplifier" and used as the feedback signal for the MS-2000 or MFC-2000 Z-axis control. The MS-2000 Z-axis controller changes the focal position of the microscope either with a servomotor or with a PZ-2000 piezo Z-axis stage.

The CRIFF optical system is illustrated in Figure 1. The optical components are enclosed in a small box that attaches to a photoport of the microscope. The enclosure includes a C-mount coupler so the user can equip the microscope with a camera. Right behind the C-mount there is a dichroic beam-splitter that allows visible light from the sample to pass through to the camera but reflects the IR laser light that the system uses. There is also provision for a barrier filter directly behind the C-mount to restrict light to the camera to only the desired wavelengths. A laser clean-up filter can be provided in the focusing lens mount. This filter is required to attenuate shorter wavelength light from the laser diode from getting through the blocking filter.

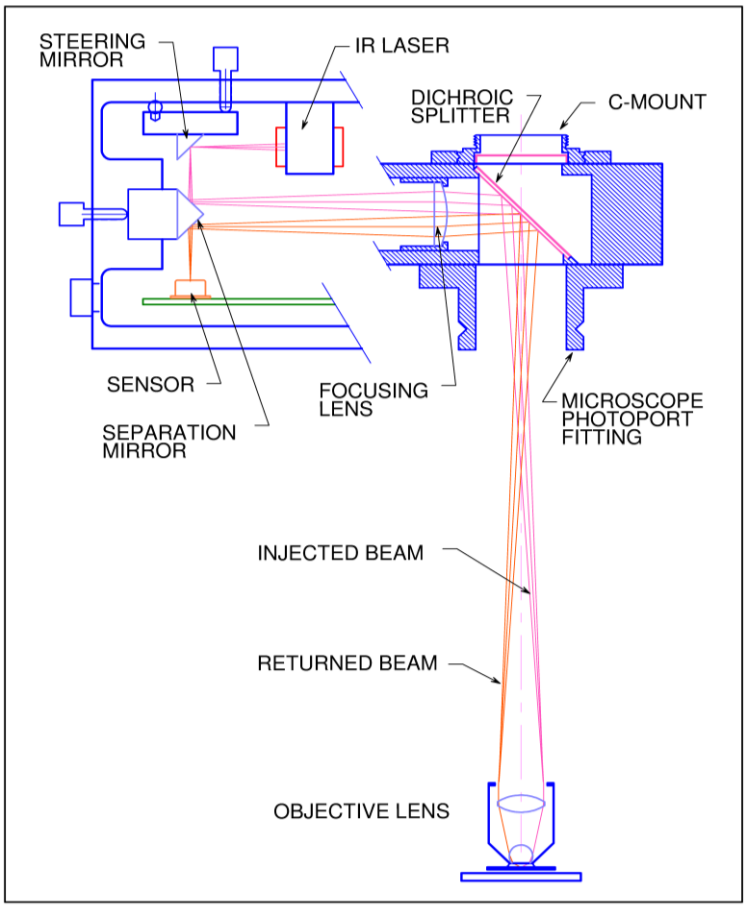


Figure 1: CRIFF Optical Arrangement

The CRIFF laser and steering optics are located off to the side of the camera light path. The laser is directed into the microscope via an adjustable steering mirror, a right-angle separation mirror, and a focusing lens. Ideally, the laser module is focused on the steering mirror. This is accomplished using the adjustable lens on the laser module. The separation mirror sends an off-axis beam toward the objective lens. The laser beam is refocused into the back aperture of the objective lens using the focusing lens. This sets up a symmetric arrangement whereby the laser beam will be focused first at the steering mirror, again at the back aperture of the objective, and finally, the return beam will be focused at the PSD. Because of the off-axis injection, the returned beam will be deflected onto the sensor by the separation mirror. When properly aligned, changes in separation between the objective lens and the cover slip will result in changes in the position of the returned beam on the PSD.

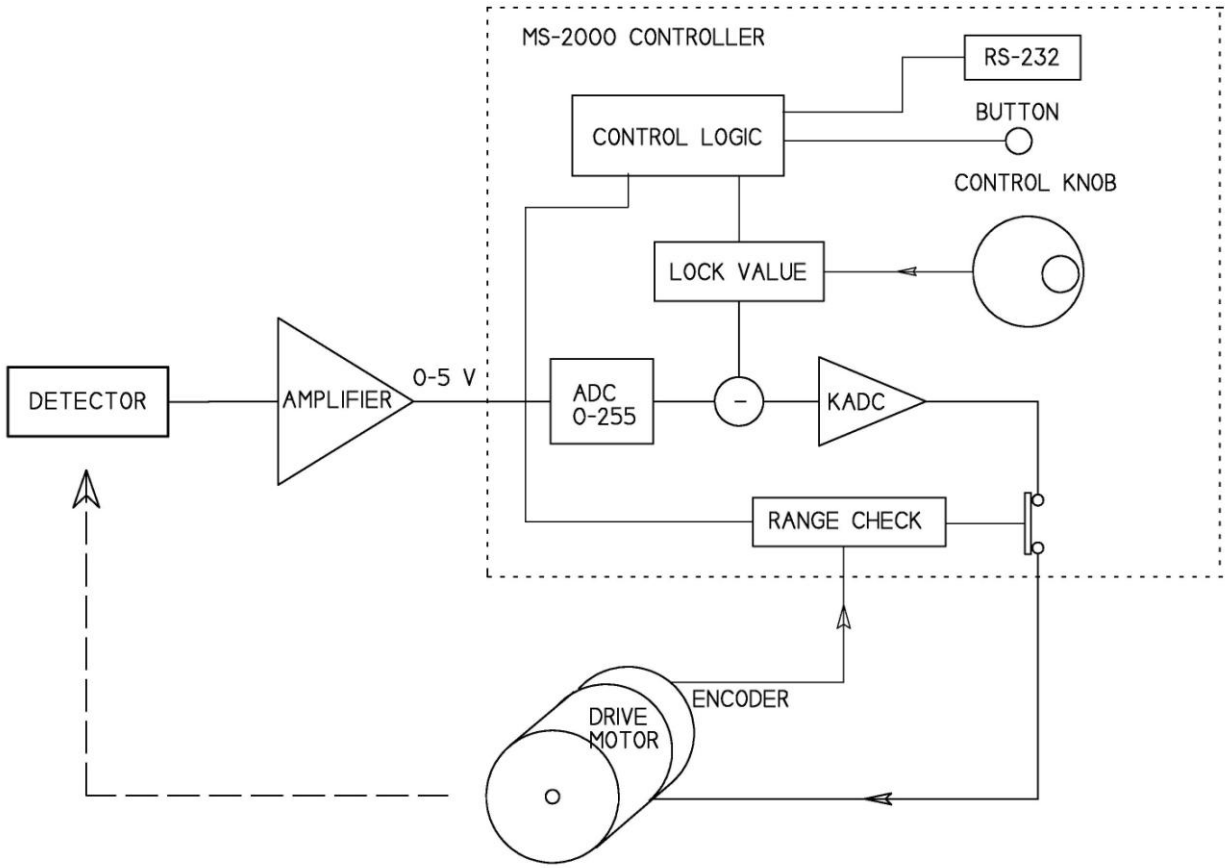
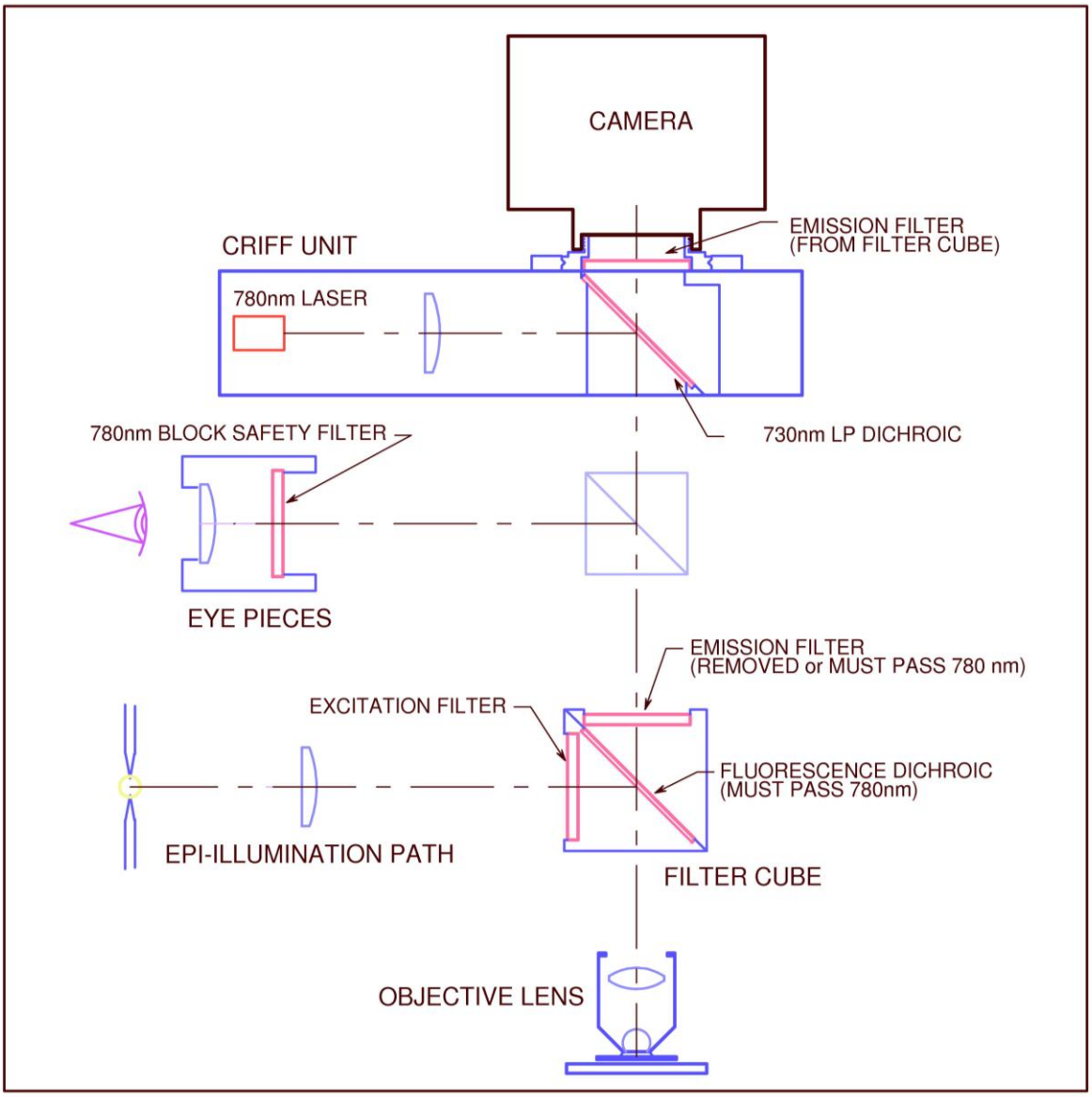


Figure 2: Block diagram of the servo control system

The position of the returned beam is detected on the dual-lateral photodiode of the PSD. An amplifier conditions the position output. Figure 2 shows a block diagram of the servo system. The output of the position amplifier is sent to the MS-2000 Z-axis drive controller. The controller generates a motor drive signal based upon the difference between a reference "lock value" and the signal obtained from the PSD. When locked-on to the reference value, the controller still monitors the stage position to detect run-away conditions. The CRIFF system can either be used with a motorized Z-axis focus, or with ASI's PZ-2000 Piezo Z-Top XY stage.

Fluorescent Filter Considerations

The CRIFF system utilizes a 780 nm laser injected into a photoport. Proper arrangement of the light filters in the microscope is necessary for the system to function properly. The figure below illustrates the basic light paths in a fluorescent microscope and the placement of filters and beam splitters when used with the CRIFF system.



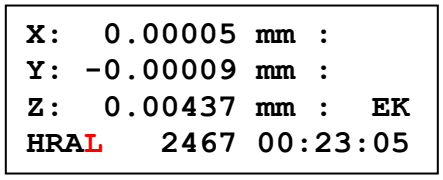
The CRIFF's 780 nm laser must be able to get to the objective lens. This requires that the emission filter and the dichroic beam splitter in the microscope's filter cube allow the 780 nm laser light to pass. Usually the filter cube's dichroic beam splitter has a long-pass characteristic that will pass the 780 nm light. A good emission filter, on the other hand, is designed to block all light except the desired wavelength, so, in general, will not pass the 780 nm laser light. To solve this problem, the emission filter should be removed from the filter cube and placed in the C-mount of the CRIFF unit. With the emission filter removed from the filter cube, the eyepieces

will not have the benefit of the blocking filter. The best solution to this problem would be to have filter cube emission filters designed with a 780 nm window for the CRIFF laser light. ASI can supply several filter sets that have been specially designed with a 780 nm window. Part numbers and specifications are listed in Appendix I. A high quality IR blocking filter is placed in front of the C-mount to clean up leakage through the CRIFF's dichroic splitter.

Laser safety at the microscope eyepieces must not be neglected. If the CRIFF is used on any port that does not direct 100% of the light to the camera, then ASI supplies Schott BG22 colored glass safety filters for the eyepieces that will block the 780 nm laser light. In a TIRF system, removing the emission filter from the filter cube will expose the eyepieces to the TIRF laser's wavelength as well. Best practice is to interlock the TIRF laser to the eyepiece shutter and use the camera for observations instead.

Manual Control of the CRIFF System

The MS-2000 controller provides an easy means to turn on and off the CRIFF laser as well as to initiate the focus lock. The LCD display shows the status of the system. The figure below shows the typical display. Be sure the display-mode DIP switches 1 and 2 located on the back of the controller are in the UP position if the display looks different than in the figure.



On the Z-axis line, the "**E**" indicates that the Z-drive's clutch is engaged and the MS-2000 has control of the microscope Z-axis. The "**K**" indicates that the system is in a locked state using the ADC feedback.

The fourth character on the bottom line shows the current CRIFF state. The integer number in the center of the bottom line is a monitor of the position sensor amplifier. Either a Sum or Difference monitor signal is shown, depending upon the CRIFF state. The chart below shows the meaning of the various CRIFF states.

Character	State Name	Next State	Monitor	Comment
I	**Idle**	**L**	Sum	Laser Off
L	**Laser_ON**	**1**	Sum	Laser ON – Align system for strong Sum signal.
1	**Cal_check_1**	**2**	--	Move + 5 µm
2	**Cal_check_2**	**3**	--	Move – 5 µm
3	**Cal_check_3**	**G** or **B**	--	Check PSD gain
G	**Cal_OK**	**k** or **O**	Difference	Ready to Lock
B	**Cal_Bad**	**I**	Sum	Check Alignment
k	**Locking**	**K**	--	
K	**Lock**	**G** or **O**	Difference	
E	**Error**	**G** or **O**	Sum	Usually Out-of-Range Error
O	**Laser_OFF**	**I or k**	Sum	Serial **Unlock** and **Relock** commands turn the laser off and on.

Button Actions

On MFC-2000 Z-axis-only systems, the @ button is used to control the CRIFF system. On MS-2000 XYZ systems, the **HOME** button is used to control the CRIFF system, (the @ button is used with the saved-position buffer). The duration of the button press determines the action.

Function	**MFC-2000 Z-axis-only**	**MS-2000 XYZ system**
Advance to next CRIFF State	Press @ for two seconds.	Press **HOME** for six seconds. (or @ for two seconds – Version 7.4+)
Initiate LOCK (toggles lock on/off)	@ short press and release	Press **HOME** for two seconds.
"Home" System to coordinate zero.	**HOME** short press and release	**HOME** short press and release

Engage/Disengage Switch

If the system is locked, disengaging the drive will unlock the system and put it in the **Laser_OFF** state. Re-engaging the drive will cause the system to relock at the previously locked location. (See the **RELOCK** command).

Engaging the LOCK for Normal Operation

You must first align the system. See the section below.
Once the system is aligned, use the following procedures to lock onto your sample.

a. Focus on your sample.
b. Advance CRIFF state from **Idle**, to **Laser-ON**, to **CAL_OK**.
c. Toggle to the **LOCK_ON** state.

Once the LOCK is engaged, the Z-axis control knob on the controller can be used to manually adjust the reference lock value. This allows manual focus adjustment of the locked system.

Laser Safety

The CRIFF systems are provided with a < 5 mW 780 nm near-infrared laser. The laser is adjusted to supply about 1 mW of power. It is important to follow eye-safety precautions when aligning and using the system.

WARNING: The laser should never be viewed directly! Permanent eye damage could result. The 780 nm laser light looks dimly red to the naked eye, but it is actually intensely bright! DO NOT BE FOOLED INTO COMPLACENCY BY THE BARELY-VISIBLE BEAM!

Unless your microscope is equipped with 100% photo ports, ***DO NOT USE THE MICROSCOPE WITHOUT THE IR BLOCKING FILTERS IN THE EYEPIECES.***

With TIRF systems, be sure you understand when the TIRF laser can be seen in the eyepieces, and the interaction with the filter arrangement required for the CRIFF system.

Installation

Install the Z-axis drive or PZ-2000 stage as described in its manual. Become familiar with the functions of the Z-axis focus control system before installing the CRIFF optics.

The CRIFF module comes with the appropriate photoport adapter for your microscope. Install the CRIFF module securely in the photoport. Orient the module so the adjustment screws are accessible. Attach the interface cable between the MS-2000 controller and the CRIFF module. If you can view through the eyepieces while sending light to the camera port with the CRIFF module, then you do not have a 100% photo port and ***you must install the IR blocking filters provided into the eyepieces of the microscope.*** Most microscope oculars have a place where the filter can be mounted. Remove the microscope's eyepieces and install the filters into both lenses.

Alignment

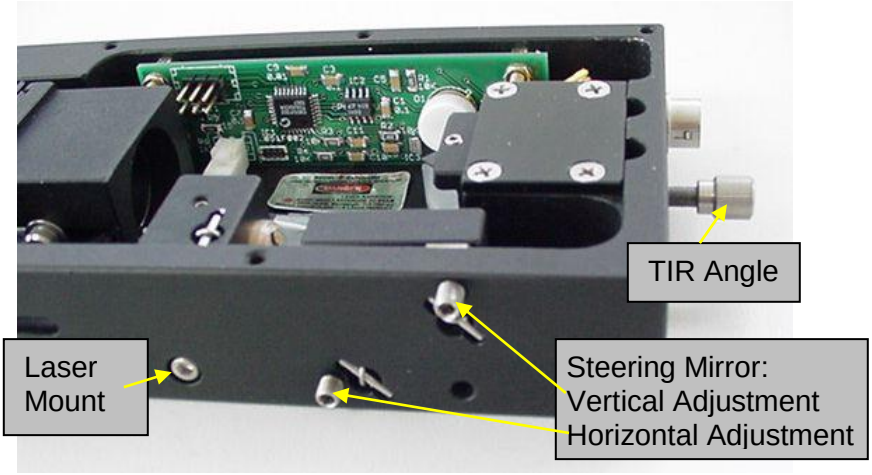
During the alignment procedure you will need to be able to visualize the laser beam position and focus. For the 780 nm laser most often used, the laser is barely visible to the naked eye. The simplest way to visualize the beam is to interpose a piece of white paper and observe the reflected laser beam off of the paper. ***Never look directly into the laser beam path!*** For laser safety, it is better to work in a well-lit room rather than in a dark room. In the event of an accidental exposure, the iris of your eye will reduce the amount of light that can enter your eye. For some alignment steps, especially if working with light reflected from the air/glass interface, you will need to dim the room lights in order to see the returned beam clearly enough to complete the alignment steps. Be especially aware of the position of the laser beam exiting the objective lens during these alignment steps, and be very careful.

Initial Alignment

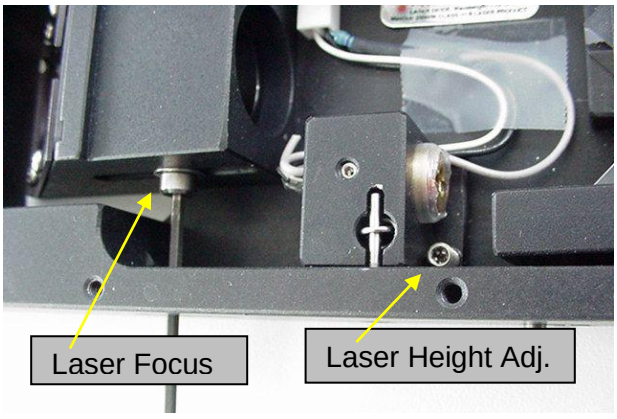
1. Remove the sensor cover from the CRIFF module by removing the four flathead screws on the cover.
2. Turn on the MS-2000 controller. Disengage the Z-drive with the switch. Power-on the laser using the buttons as described above, or using the "**LK**" command via the RS-232

interface. When the laser is on, you should see an "**L**" as the fourth character of the bottom line of the LCD display.

The figure below shows several of the mechanical adjusters on a CRIFF module with the sensor cover removed.

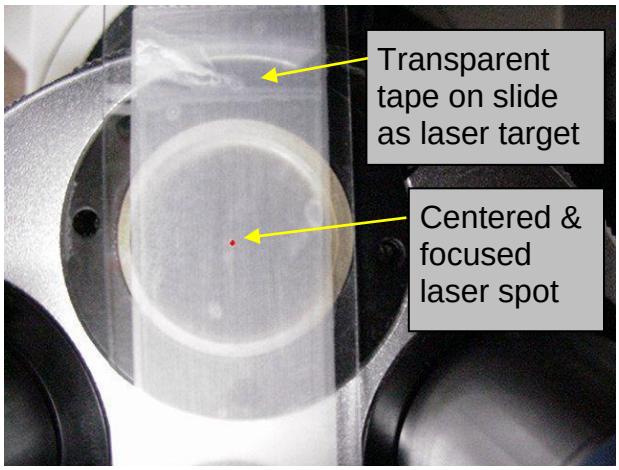


3. Begin with all adjusters in their 'neutral' position: the separation mirror's TIR Angle adjuster should be backed off until the screw no longer pushes on the mirror assembly, and the steering mirror's horizontal and vertical adjusters should be adjusted to hold the mirror holder parallel to the main body.
4. The following initial adjustments in this step should already have been done at the factory, but are presented here for completeness. With the separation mirror pulled out of the way, the laser should be shining directly on the PSD photo detector. The pinhole over the PSD photo detector provides a target upon which to observe the laser. Adjust the horizontal and vertical steering mirror adjustors so the beam is centered on the pinhole. The laser beam is not circular. The long axis should be oriented vertically, parallel to the tip of the separation mirror. This is achieved by rotating the laser in its holder, if necessary, after loosening the laser mount screw. Using a piece of paper placed in the laser beam, verify that the beam waist (best focus) of the laser is located near the steering mirror. The position of the beam waist is adjusted by rotating the lens on the front of the



laser module in or out.

5. The first goal will be to center and focus the injected laser beam in the back aperture of the objective lens.



   a. Turn the objective turret to an empty position and remove the dust cap if necessary.

   b. Place a slide with a piece of transparent tape over the threaded opening to act as a diffused target for the laser.

   c. Leave the TIR angle adjuster fully retracted and adjust the Horizontal steering mirror so the laser is reflected into the microscope. You should see the beam on the target as shown in the picture above. ***Be aware of the laser beam leaving the microscope. It is an eye hazard, especially when well focused.*** Note how moving the TIR angle adjuster inward moves the laser spot away from the center. Now adjust the laser height adjuster (first loosen the Laser Mount screw) and observe how the beam moves orthogonal to the movement when adjusting the TIR angle adjuster. Use the laser height adjuster and the TIR angle adjuster to move the beam into the center of the objective mount opening. Use the Vertical and Horizontal Steering mirror adjusters to optimize the quality of the beam on the target. Note that the steering mirror adjusters will interact somewhat with the laser height adjuster and the TIR Angle adjuster. Since the tip of the separation mirror is on the optical axis, the centroid of the laser beam reflected into the microscope will always be off-axis a small amount. Hence, it may not be possible to for the TIR Angle adjuster to get the beam perfectly on center. Observe the movement of the laser spot across the aperture as you advance the TIR angle adjuster. Return the TIR adjuster so the beam is centered (it should be nearly fully retracted).

   d. Finally, use the Focus lens adjuster to bring the laser to a sharp focus on the target. Do this by loosening the focus clamp adjuster screw and sliding the screw and lens assembly forward or backward to obtain best focus on the target.

6. Switch the turret to the desired objective lens. Now we want to obtain a returned beam from a sample slide. The procedure will be similar whether you are using a reflective cover slip, using a TIR objective, or using the natural reflection from the air/glass interface with an air objective.

   A simple alignment sample that will work with oil objectives of NA < 1.4 can be made for dry TIR. Take a standard cover slip and smear a fine layer of sample, e.g., a fingerprint will do. Tape the cover slip to a microscope slide, sample-side against the slide. View the sample with any oil immersion objective using transmitted light. Any objective with NA > 1.0 will be able to achieve the TIR condition with the dry sample.

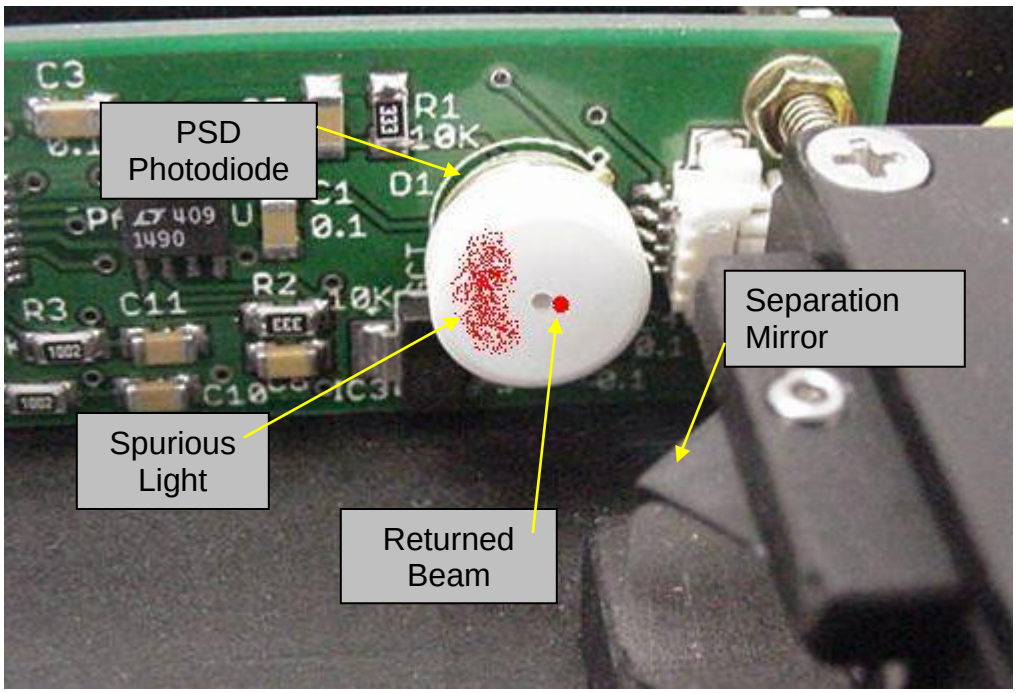
Another simple test sample consists of an empty glass bottom dish with a fingerprint on the surface. You can set up the system easily with a dry sample where the "sweet region" is significantly larger. Then once successful with the dry sample, add water to the dish and fine tune with the more representative conditions.

Alternatively, you could use a sample consisting of fluorescent beads in a Petri dish, and your finest TIRF objective, to closely approximate experimental conditions. Whatever the sample, the laser should be leaving the objective and going through the sample nearly vertically if the adjustments in the previous step were completed successfully.

Note: Prepared slides will usually not work with the CRIFF system. The mounting medium usually has a high index of refraction that prevents TIR.

7. Now advance the TIR Angle adjuster until the beam exiting the objective is nearly horizontal. Continue to advance the TIR Angle adjuster until the transmitted light just disappears if you are using the TIR sample discussed above, or are using a TIRF objective lens.

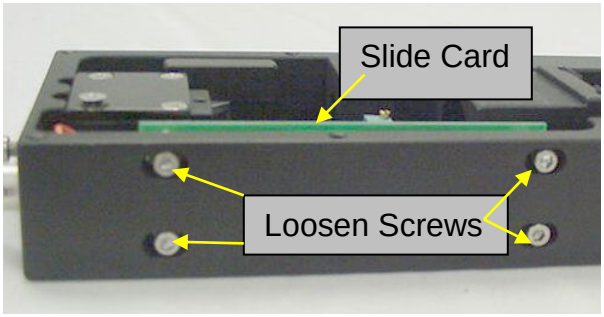
8. Now the goal is to find the returned beam. This job is much easier with a true TIRF objective NA $\geq$ 1.45 than with lower NA objectives. The pinhole over the PSD provides a good target for the returned beam (as long as it is not already going in the pin hole, our eventual goal). You may wish to use a small slip of paper to observe the beam spot since it will be invisible if it is going through the pinhole in the PSD. A darkened room, low power magnifying glasses, and/or an IR viewing card are helpful during this step. There will always be some spurious reflections from various glass interfaces within the microscope. However, only the desired return beam will be focused at the PSD photodiode because of the symmetry of the injection and detection optics. Look carefully for the focused returned beam. One way to know for sure that you are seeing the returned beam is to slightly rotate the objective lens out of its click stop. As you do so, you should see the returned beam spot move across the pinhole, unlike the majority of the spurious



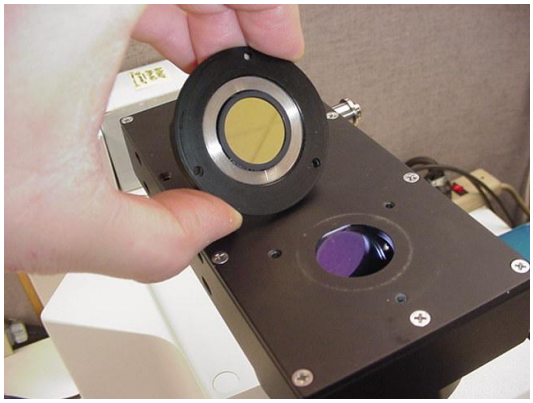
light, which will remain stationary. Adjust the TIR Angle adjuster, watching for the bright returned beam when at the TIR angle. Tweak the TIR Angle adjuster for maximum brightness of the returned beam.

9. Once you know you have found the returned beam, readjust the focusing lens to obtain the smallest spot possible at the photo detector.

10. Next, make sure the returned beam is level with the pinhole in the photo diode mask. This can be achieved by tweaking the *laser height* adjuster. You can also get a millimeter of adjustment by sliding the stand-offs on the amplifier card up or down as well. Tighten the laser mount clamp screw when you are satisfied with the vertical alignment.



11. Now loosen the thumbscrews attaching the photodiode amplifier card, and slide the card forward or back so that the returned spot goes through the pinhole. If there is not enough travel, it may be necessary to move the separation mirror on its mount. If you have to do this, loosen its setscrew and carefully slide the mirror in the appropriate direction, being careful to avoid touching the mirror surfaces. Be sure the mirror is pushed back against the backstop, and re-tighten the setscrew. If you have to slide the mirror, it may be worth checking the basic alignment of step 5 once again.



12. This completes the basic alignment of the CRIFF system. Re-install the cover(s). Connect your camera to the C-mount adapter. An emission filter can be installed between the C-mount and the cover as shown in the photo at the right.

Using the CRIFF System – Fine Alignment

1. The next few steps will cover fine alignment of the beam for best performance. Re-focus the microscope on the sample before we do the fine adjustments. This will require using the LCD display on the controller to monitor the output of the CRIFF amplifier.

2. In the **IDLE** state, when the laser is off, the monitor signal readout on the LCD display should be just a few counts. If the laser is not on, switch to the next CRIFF state using the buttons. This will switch the laser on and the "**L**" will indicate the **LASER-ON** CRIFF state. The display will now show the level of the Sum monitor signal on the photodiode. Tweak the TIR angle adjuster, and the horizontal and vertical adjusters to maximize the Sum signal on the LCD display. Then verify that you are looking at a valid returned beam by observing the number on the display as the objective is slightly rotated off its click stop. The Sum signal should substantially decrease. The signal level can vary depending upon the objective used, the method of obtaining the return beam, the presence of light splitters in the microscope optics, etc. The PSD amplifier saturates when the

display show 32000, so if the sum signal gets to this level, adjustments will be required to the circuit to reduce the gain. Sum signal levels anywhere from 3000 to 25000 should be fine.

3. With a good returned beam signal, we are ready to advance the CRIFF state to the calibrate mode. On motorized drives, be sure the Z-drive is *Engaged* so that the controller can change the focus in this step. Press the buttons to advance to the next CRIFF state. The firmware will command the microscope to focus on focal planes at +3 μm and −3 μm from the initial set-up position. The system is placed in Differential measurement mode, and the PSD amplifier gain coefficient is determined from measurements taken at the ±3 μm positions. The gain value is displayed for a few moments on the LCD display, e.g., **`Gn: 25`**. The gain value is approximately the number of PSD counts per 20 nm of Z-axis change. This number will depend upon the objective power, system alignment, and internal ADC gain for the PSD signals. If the calibration is successful, the CRIFF state will advance to the **CAL_OK** state, signified by the letter "**G**" on the display. If the calibration is not successful, (gain value less than one) the CRIFF state will advance to the **CAL_BAD** state, signified by the letter "**B**" on the display.

4. Once a **CAL_OK** state is achieved with the "**G**" on the display, check to see that the system shows monotonic behavior which focus. In the "**G**" state, the LCD display shows the lock error signal, rather than the laser intensity. The numbers on the display are scaled using the gain value obtained previously to read approximately in nanometers. As the focus is changed, the error signal should change predictably and monotonically with the focus position. As you move the focus in one direction, the signal should increase, and decrease as you move the focus in the other direction. At some point the error signal will begin to change in the opposite direction. The point where this occurs defines the range over which the CRIFF will function. Be sure the suitable range includes the focal region of interest. If for some reason, the returned beam spot disappears as you approach the desired focal position (this can happen with NA 1.4 objectives where the "sweet range" is small) you can try to optimize the best spot intensity by simultaneously adjusting the TIR mirror and the Horizontal steering mirror adjuster. These two adjusters perform similar functions, and by moving the pair together it is possible to tilt the beam slightly and optimize the returned beam quality for the focal range you will be interested in. Using the LCD power meter helps in this process.

5. If the calibration is successful, then the system is ready to proceed to the lock state. A short @ button push on an MFC-2000, or a two second **HOME** button press on an MS-2000 system, will toggle to the **LOCK** state, signified by the letter "**K**" on the display. Once locked, you will notice that the focus drive will compensate if you press lightly on the stage to change the focus position. If you have a piezo stage, move the scope's fine focus slightly and watch the piezo compensate. You can vary the focal position of the locked system by using control knob on the MS/MCF-2000 controller to change the internal "lock target." This provides a simple way to tweak the focus without requiring unlocking and relocking.

6. The overall loop gain of the system was set when the calibration step was performed. The PSD signal is translated into error correcting motion after being massaged by user-controlled averaging and gain modification.

   $$\text{Motion Error Adjustment} = \text{Average}|_{\mathbf{N\_AVE}} [\text{PSD} \times \mathbf{KADC}]$$

   The number of averaged samples, **N_AVE**, is set using the command **`RT F=N2`**, where **N_AVE** = $2^{\mathbf{N2}}$. A sample is obtained from the CRIFF unit every TSTEP (1-3 ms depending on total number of axes controlled by MS-2000 controller). More averaged samples will result in slower correction of the stage, but increased stability and noise immunity. Shorter or no averaging will allow quick response but require strong clean signals to avoid spurious motion correction. If more sensitivity is required, the software parameter **`KADC`** can be used to increase the loop gain of the system. Instability will result if the **`KADC`** value is increased too much. Once the optimum values of **`N2`** and **`KADC`** are found for the application, the values may be saved to nonvolatile memory using the "**`SS Z`**" command.

7. If, for some reason, the system runs away, the firmware will detect when the stage has moved more than the **`LOCKRANGE`** limit, and will kill the lock mode and put the system in the error state.

8. For some installations, the motion of the spot on the PSD will be larger or smaller than a "typical" system. It is important that adequate sensitivity from the PSD is available while also not generating numbers so large that they overflow the 16-bit integer values allowed. If the **`GN:`** number from the calibration step is less than 5 (or more than 100), consider increasing (or decreasing) the ADC gain on the CRIFF unit using the **`LR Y=n`** command.

Computer Control of the CRIFF System

The CRIFF system uses several commands dedicated to controlling the feedback system. Please see the MS-2000 Programming Manual for further information about using serial commands.

Command: LOCK

Shortcut: LK

Format: LK [X] [Y] [Z] [F=*code*]

Function: The LOCK command without any arguments X, Y, or Z advances to the next CRIFF state until the **Cal_OK** state is reached. Once a good calibration is obtained, a subsequent **LK** command initiates the **Lock** state in which the servo loop error signal is supplied from the CRIFF amplifier. The lock is made with respect to reference values of the current location. If the system is in the **Laser_OFF** state, a subsequent **LK** command will lock the system at the current location. (See **RELOCK** command.)

LK X? returns the single character indicating the current CRIFF state as described in the table on page 6 of this manual.

LK Y? returns the present value of the PSD signal that is also shown on the LCD display.

LK Z unconditionally advances to the next CRIFF state (same as long button press).

LK F=*code* will unconditionally set the CRIFF state. ***code*** is the ASCII decimal equivalent for the 'state' character that is displayed on the LCD. For example, to unconditionally enter the 'G' state the command would be **LK F=71**.

Reply: "**:A**" is returned upon receipt of the command.

Example: **LK X?**

:A L shows the system is in the LASER_ON state.

Command: UNLOCK

Shortcut: UL

Format: UL [X]

Function: This command unlocks the servo from the CRIFF system and returns control to encoder feedback from the Z-axis drive. Current lock reference values are saved for eventual use by the **RELOCK** command. With **UL X** the CRIFF laser is turned off and the CRIFF system is placed in the **Laser_OFF** state. **UL** without the X argument places the system in the **Cal_OK** state with the laser still on.

Reply: "**:A**" is returned upon receipt of the command.

Command: RELOCK

Shortcut: RL

Format: RL

Function: Turns on the CRIFF laser and initiates a **LOCK** state using previously saved reference values.

Reply: "**:A**" is returned upon receipt of the command.

Command: LOCKRG

Shortcut: LR

Format: LR [X=*cal_gain*] [Y= *gain*] [Z=*lock_range*] [F=*cal_range*]

Function: The **LOCKRG** command allows the user to control of several system variables. The X parameter, *cal_gain*, is the gain variable normally obtained from running the calibration sequence. Although not recommended, it can be changed with this command, but it will be reset upon running the calibration sequence. The Y parameter controls the internal CRIFF-unit ADC gain. Valid values are 16, 8, 4, 2, 1, and 0. Default value is 8. Use lower values if spot motion is large. The Z parameter controls the maximum excursion of the stage before the system generates an error condition and unlocks. The value *lock_range* is in units of millimeters. The default value is 0.05 mm (50 microns). The F parameter controls the excursion of the stage when going through the calibration sequence. The default value for *cal_range* is 0.002 mm.

Reply: "**:A**" is returned upon receipt of the command.

Query: **LR Z?** returns the lock range.

```
A: Z = 0.050
```
(for example)

Command: KADC

Shortcut: KA

Format: KA Z=*n*

Function: Adjusts a gain parameter in the servo loop where *n* is a signed integer. Use to change the polarity of the feedback. Default *n=4*, (use *n=5* for PZ-2000).

Reply: "**:A**" is returned upon receipt of the command.

Query: **KA Z?** returns the current value.

```
:A Z=1
```
(for example)

Command: RT

Shortcut: RT

Format: RT [X=*report_time*] [Y=*pulse_length*] [Z=*delay_time*] [F=*num_aves*]

Function: The X argument Sets the time interval between report events when using *IN0_mode* = 5, TTL triggered serial interface asynchronous reporting. The *report_time* value has an acceptable range from 20 to 32700 milliseconds. The default value is 200 ms.

The Y argument sets the length of the TTL output pulse when using any *OUT0_mode* that generates a TTL pulse.

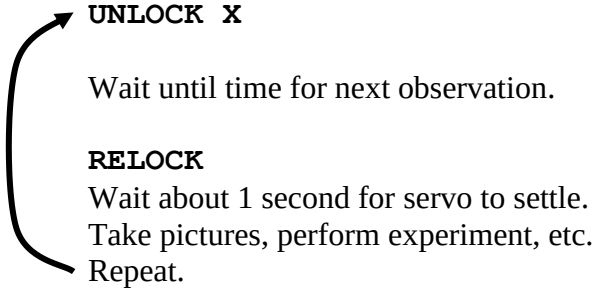
The Z argument sets the post-move delay time for sequenced arrays.

The F argument sets *num_aves*, the power-of-two exponent for the number of samples to be averaged. Used with the CRIFF system.

Reply: "**:A**" is returned upon receipt of the command.

Using CRIFF with the Serial Commands

For long time-lapse experiments, the user may wish to turn off the CRIFF laser for as much of the time as possible to minimize interaction with the sample. Once a **LOCK** condition is established, the sequence of events could go as follows, where the user's software would issue the serial commands to the controller shown in **BOLD** type:



UNLOCK X

Wait until time for next observation.

RELOCK
Wait about 1 second for servo to settle.
Take pictures, perform experiment, etc.
Repeat.

Appendix I: Filter Sets with 780 nm Window

Chroma Part Number	Description
E740SP	Shortpass Blocking Filter for camera, Max block 780/20
71000a /w 780T	FURA2 - Chroma 71000a Set with bs/m both transmit 780nm
41017 /w 780T	EGFP - Chroma 41017 Set with bs/m both transmit 780nm
61008 /w 780T	CFP/YFP/DSRED - Chroma 61008 Set with bs/m both transmit 780nm
61000v2 /w 780T	DAPI/FITC/TRITC - Chroma 61000v2 Set with bs/m both transmit 780nm
61002 /w 780T	DAPI/FITC/TEXAS RED - Chroma 61002 Set with bs/m both transmit 780nm
41008 /w 780T	CY5 - Chroma 41008 Set with bs/m both transmit 780nm