CRISP : Continuous Autofocus System

The Continuous Reflection Interface Sampling and Positioning (CRISP) 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 CRISP solves the focus drift problem that plagues time-lapse experiments at high magnification, and also allows the microscope to follow the sample in a large well that isn't mounted perfectly flat. The CRISP system uses a pupil obscuration method to determine focus from reflective surfaces. The control system allows adjustment of the focal lock position, relative to a nearby surface, once the system is locked.

Software

There are 3 main programs to interact with the CRISP device through a GUI. CRISP can also be operated by sending serial commands and even completely stand-alone using buttons on the controller.

ASI CRISP Control - Micro-Manager Plugin
CRISP Ninja - Standalone Windows Application
ASI Console - Standalone Windows Application (MS-2000 only)

System Overview

The CRISP system consists of optical, electronic, and mechanical components. The optical system injects IR LED light into the microscope, captures the beam reflected from the specimen slide or cover slip, and routes the reflected beam onto a position-sensitive detector (PSD). The signal from the PSD is conditioned by an amplifier circuit in the ASI controller and used as the feedback signal for Z-axis control by the controller. All ASI-made focus devices are supported, and a 0-10V output is available to control an external focus device.

Optical placement

The CRISP unit is a C-mount device that is placed optically conjugate to the camera (and sample). A dichroic beam splitter used to couple the CRISP unit to the microscope system. The dichroic can either be placed in focus space or collimated space.

Dichroic in focus space: A shortpass dichroic is placed between the tube lens and camera, most often using ASI's DCMS (dual C-mount splitter) so that CRISP shares the microscope photoport with a camera. CRISP's IR light is reflected into the CRISP unit and visible light transmits to the camera. **Placement in focus space is usually required when CRISP is used with an existing microscope** because there is no access to the space between the objective and tube lens. This placement induces (usually minor) aberrations on the straight-through camera imaging path due to the (tilted) dichroic.[1)]

Dichroic in collimated space: A dichroic is placed between the objective and tube lens. **Placement in collimated space is preferred when it is possible, e.g. with ASI's modular infinity microscopes.** Placing CRISP closer to the objective improves the SNR (fewer spurious reflections) and simplifies selection of other filters. However, because CRISP is a C-mount device, placement of a dichroic in collimated space requires the addition of a tube lens between the dichroic and CRISP unit. ASI's MIM2 configuration uses a longpass dichroic and most often ASI's C60-TUBE-100 tube lens. ASI's MIM3-PSL configuration uses a shortpass dichroic and includes a 75mm focal length lens.[2)] Ideally this tube lens is approximately “4f” with the objective.

Figure 1: CRISP with DCMS photo-port splitter.

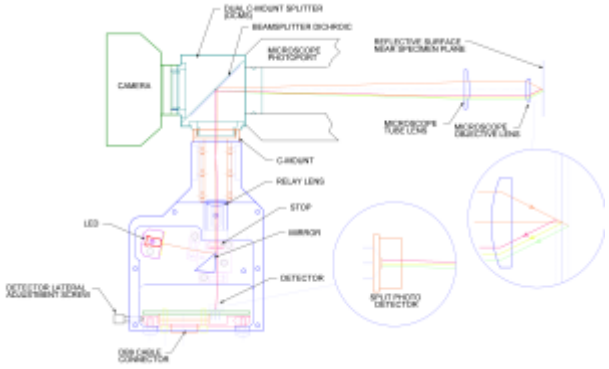


Figure 2: Schematic diagram of CRISP optical system with DCMS.

CRISP LED Options and Filters

CRISP uses an internal LED light source, and many different LED wavelengths are available. The CRISP wavelength should be selected based on filters in the microscope. When the dichroic is placed in focus space (on conventional “Big4” microscope stands), a common CRISP wavelength is 780nm because that can often make it through multi-band filter sets. When the dichroic is placed in collimated space (e.g. with ASI's modular microscopes), there is more freedom to choose an appropriate filter and the default wavelength is 850nm.

The table below shows the LEDs that can be supplied, along with the suggested dichroic beam splitter and blocking filters. With sufficient spectral distance between the LED wavelength and the dichroic and camera block cut-off wavelength, a cleanup filter for the LED may not be required. The detector in the CRISP unit begins to lose sensitivity after about 1000nm limiting the maximum usable

wavelength to about 1050nm.

LED Part Number	LED Color (nm)	Typ. LED Power @50mA (mW)	FW HM (nm)	FW to 2% wings	Shortpass Dichroic Beam Splitter		Shortpass Camera Block Filter		Bandpass LED Cleanup Filter	
					Dichroic Cutoff (nm)	Dichroic Part Number	Shortpass Filter Cutoff (nm)	Shortpass Filter Part Number	Bandpass Filter Cutoff (nm)	Bandpass Filter Part Number
VLCS5830	625	10	18	580-660	600	69216	600	84710	628/32	84087
L660-06	660	3	20	615-700	600	69216	600	84710	650/50	84774
L700-06	700	13	30	650-740	650	69217	650	84712	700/50	84775
L720-06	720	13	30	670-760	700	69218	650	84712	725/50	86943
L735-06	735	18	30	680-780	700	69218	700	84714	725/50	86943
L740-06	740	18	30	685-785	700	69218	700	84714	750/50	84776
L780-06	780	20	30	710-830	750	69219	750	64332	775/50	86944
TSHG8200	830	25	40	750-900	750	69219	750	64332	825/50	86945
TSHG5210	850	27	40	790-930	800	69220	800	64333	850/50	84778
TSFF5210	870	23	40	810-950	800	69220	800	64333	875/50	86946
TSHF5210	890	23	40	830-970	850	69221	850	64334	900/50	84779
L940-06	940	17	50	840-1040	900	69222	900	64335	950/50	84780
L970-06	970	5.5	50	910-1070	900	69222	900	64335	975/50	86948
L1050-06	1050	2.5	50	950-1130	1000	86695	1000	64337	1050/50	85881

Fluorescent Filter Considerations

The CRISP system utilizes a (near) IR LED that is projected onto the sample, most commonly 780nm or 850nm. Proper arrangement of the light filters in the microscope is necessary for the system to function properly. **This is usually trivial if the CRISP dichroic is placed in collimated space, but requires some thought when the CRISP is placed in focus space.** A dichroic beam splitter that reflects the IR light is used in the dual C-mount splitter (DCMS). No other filters can be in the path to the objective that block the IR light. An emission filter that blocks the IR LED should be placed in front of the camera and can be located in the C-mount fitting of the DCMS for the camera. Fluorescence dichorics need to have a “window” in the IR to pass the CRISP LED. See the list of commercial filter sets that work with CRISP below.

The long C-mount adapter on the Olympus IX-71 or BX scopes permits the use of both a filter wheel and the CRISP unit in the provided space. This allows use of specific emission filters in conjunction with either a multi-band dichrioc with an IR pass band, or with a single excitation wavelength and a long pass dichroic in the scope.

Some configurations provide an easier solution to the filter problem. If a spinning disk confocal unit attached to the C-mount port is used for fluorescent microscopy, the filter cube is located in the confocal head and not in the microscope. In this case the CRISP mounted on the DCMS will work fine and not be impeded by any fluorescence filters in the microscope.

It may be possible to place the CRISP in the excitation path or to find an alternative location in focus space (the preferred approach with ASI's RAMM/MIM systems). Although these solutions are better optically, they may require customization of a non-ASI microscope. Contact ASI for details.

Filter Sets Suitable for CRISP

It is important to make sure the filters in your microscope are compatible with CRISP. This is not a concern when using ASI's modular microscopes because CRISP can be introduced in collimated space before any other filters.

When retrofitting an existing microscope, CRISP is usually added near the camera using a DCMS. The DCMS has an internal dichroic mirror and two C-mounts, one for the camera and the other for CRISP. The light from the CRISP needs to make it from the CRISP unit, (reflected off the dichroic in the DCMS), through the microscope to the sample, then back again to the CRISP unit. Most microscopes have fluorescence filters installed inside the microscope. Filter sets can be placed into three categories depending on their transmission at the wavelength that CRISP uses (which can be selected from multiple alternatives, see LEDs/wavelengths). These three categories are as follows:

1. If both the dichroic and emission filter transmit the CRISP wavelength, then the filter set is completely compatible with CRISP and the complete filter set can be used inside the body of the microscope. This is a rare situation.
2. If the dichroic transmits the CRISP wavelength but the emission filter does not, then the filter set will work with CRISP as long as the emission filter is placed just before the camera instead of inside the microscope. This is a common situation. CRISP is usable but there switching between filter sets usually involves physically exchanging a filter just before the camera.
3. If neither the dichroic nor emission filter transmit the CRISP wavelength, then the filter set is not usable with CRISP. This is also a common situation.

The first category is obviously the best. Most often these filter sets are are marketed as simple longpass filters, not bandpass filters.

The second category is the most common. Many filter sets have a single edge longpass dichroic (which allows the CRISP illumination to be transmitted) but an emission bandpass filter that blocks the CRISP illumination. In this case, specify the CRISP wavelength to be as large/red as possible to keep it away from the normal emission, yet still where the dichroic has good transmission. The emission filter must be placed in front of the camera (inside the DCMS C-mount splitter) where it will block the CRISP IR LED from the camera but not block light between the CRISP unit and the sample. If the microscope has room for both a filter wheel and a DCMS (e.g. Olympus IX71/73), a filter wheel can be used to switch between filter sets, with the dichroic switching inside the microscope and the emission filter switching between the DCMS and the camera.

The third category needs no further explaining. CRISP illumination must make it through the microscope for CRISP to function. If you need to use such a filter set please get in touch with ASI so we can quote you a MIM/RAMM system 

Contact ASI with your filter specifications for further guidance.

Using multi-band filter sets with CRISP

Frequently the dichroic beam splitter on multi-band filter sets has limited transmission outside the data-channel color bands. Nevertheless, there are several multi-band commercial filter sets that can be used with CRISP. One particularly interesting filter set is the Semrock five-band with the dichroic filter characteristics below, which falls into the first category above:

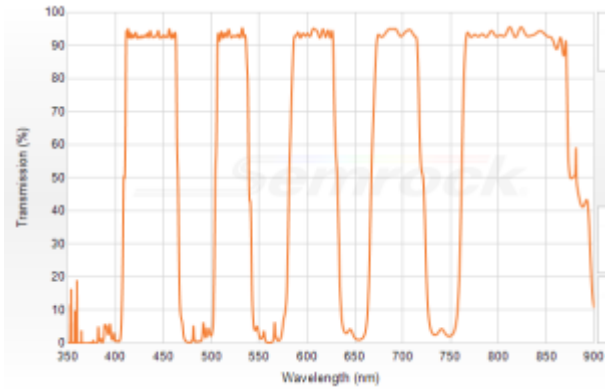


Figure 3: Semrock Dichroic FF408/504/581/667/762-Di01

This dichroic is used with either individual emitters and exciters for each band, or with individual exciters only as a Pinkle set. The upper transmission band of the dichroic is perfect for the standard 780nm CRISP IR LED. Used in this way, this filter set can be installed in the microscope's filter cube in the usual manner. A 750nm IR block is place in the DCMS splitter camera C-mount to block the upper band from the camera.

Another Semrock multi band set, LF405/488/561/635-A-000, has an extended region for the red band that would pass IR light. The pass band above 700nm is open, allowing easy operation with a 780nm IR LED for CRISP. In this case, the emission filter would be installed in the DCMS splitter C-mount in front of the camera and would act as the IR block for the CRISP LED.

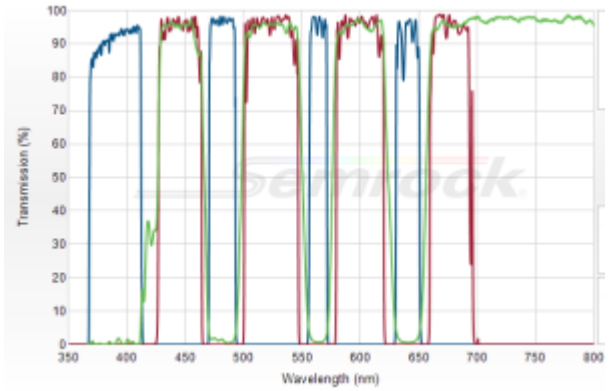


Figure 4: Semrock LF405/488/561/635-A-000

To determine the correct filter set for your application, first check the filters you have to see if there is a pass band in the IR. If not, consider alternatives that have such a pass band. Listed below are several filter sets from major filter manufacturers that will work with CRISP. Some of them require special non-standard LED color.

Semrock multi-band filter sets that will work with CRISP

- DA/FI/TR/Cy5/Cy7-5x-A-000
- Uses FF408/504/581/667/762-Di01 dichroic with passes 780 to 850 IR LED
- This five band set has the top band situated perfectly for CRISP
- Uses a multiband emission filter with pass band in IR so can be used in microscope filter cube.

- LF405/488/594-A-000
- Uses Di01-R405/488/594 dichroic which passes 780 to 800 IR LED
- Uses a multiband emitter that can be placed in the camera's DCMS C-mount

- LF405/488/532/635-4x-A-000
- Uses Di01-R405/488/532/635 dichroic which passes 780 to 820 IR LED

- LF442/514/561-3X-A-000
- Uses Di01-R442/514/561 dichroic which passes 780 to 830 IR LED
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount

- LF488/561-2x-B-000
- LF488/561-A-000
- Uses Di01-R488/561 dichroic which passes 780 to 830 IR LED
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount

- FRET - GFP/RFP –C-000
- Uses FF 495-Di03 dichroic which passes 780 to 850 IR LED
- Requires switched emission filter before camera for two channels

- FRET-CFP/YFP-C-000
- Uses FF458-Di02 dichroic which passes 780 to 850 IR LED
- Requires switched emission filter before camera for two channels

Chroma multiband filter sets that will work with CRISP

- 59004 FITC/TRITC –ET
- 59204 FITC/TRITC
- Uses 59004bs dichroic with available pass band at 740nm – specify 740nm LED for CRISP.
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount

- 59017 ECFP/EYFP – ET
- 59217 ECFP/EYFP
- Uses 59017bs dichroic with available pass band at 650nm – specify 660nm LED for CRISP.
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount

- 69000 DAPI/FITC/TRITC
- 69300 DAPI/FITC/TRITC
- Uses 69000bs dichroic with available pass band at 700nm – specify 700nm LED for CRISP.
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount

- 69008 ECFP/EYFP/mCherry
- 69308 ECFP/EYFP/mCherry
- Uses 69008bs dichroic with available pass band at 735nm – specify 735nm LED for CRISP
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount

- 88000v2 DAPI/FITC/TEXAS RED/Cy5
- Uses 88100bs dichroic with available pass band at 830nm – specify 830nm LED for CRISP
- Uses a multiband emission filter that can be placed in the camera's DCMS C-mount
- This set will also work for CRISP in the microscope's filter cube if the Cy5 channel is used for CRISP – Specify 700nm LED for CRISP for this application, and place 650nm SP block in front of camera.

Contact ASI or your filter supplier if you have further questions.

LED Power and Eye Safety

The CRISP system uses an IR LED to illuminate the sample and provide a reflected beam that is used to determine focus. Although relatively bright IR LEDs are used in the CRISP unit, the distributed nature of the LED source, masking of the LED, reduction in aperture and reduce duty cycle combine to make the CRISP light source eye-safe. Never-the-less, please do not stare into the CRISP C-mount when the unit is powered up. IR LED sources do not generate visible radiation, so prolonged exposure is possible and should be avoided.

The maximum measured average power for a typical CRISP unit at the C-mount is less than 100µW (typically about 70 µW) with the LED set to 100% intensity and the internal aperture stop open fully. The CRISP LED mask appears to be about 1.0 mm × 6.8 mm in the image plane. The brightest part of the LED emitter depends slightly on the LED used and the exact focus, but is about 1.0 mm square at the image plane. Based upon the objective used, you can use these numbers to calculate typical maximum intensity of IR illumination at the sample. However, be aware that many objectives will not pass the full aperture at the CRISP aperture stop, so the number you get this way will be a maximum.

For example, a 60× objective will expose some parts of the sample to a maximum of about 0.36W/mm2 of IR radiation.

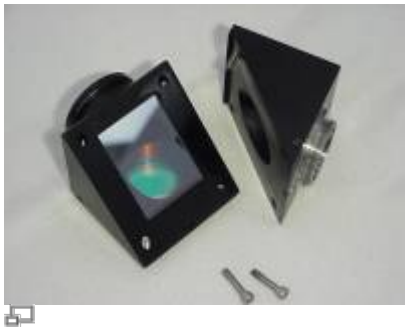
$$\frac{\text{Total Power}}{\text{Area}} = \frac{100\mu W}{1/60\ \text{mm} \times 1/60\ \text{mm}} = 0.36\ \text{W/mm2}$$

You can reduce the radiative power at the sample by using a lower LED intensity and/or reducing the internal aperture stop.

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 CRISP optics.

The CRISP device is designed to be used at a camera C-Mount location. In order to accommodate both data recording camera and the CRISP unit on the microscope photo-port, a dual C-mount splitter, such as the ASI DCMS is used. The DCMS is normally equipped with the appropriate dichroic beam splitter to reflect the CRISP IR LED light into the microscope while allowing the visible light to the camera.



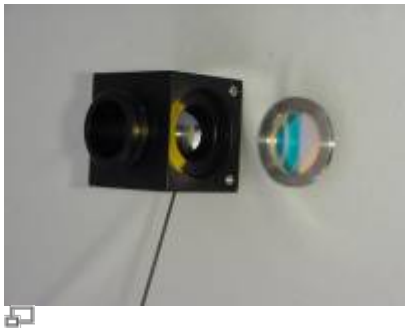


Figure 5: C-mount Splitter (DCMS) contains dichroic mirror and blocking filter

There should also be a blocking filter on the camera C-mount to keep LED light out of the camera.

Mount the CRISP unit on the reflected port of the DCMS.

Mount the camera on the “straight-through” port of the DCMS.

CRISP Cable Connection

Connect the DB9 cable from the CRISP unit to the labeled connector on the back of the MS2000 control unit.

Pin-outs for this cable are shown below.

CRISP DB-9 Connector		
PIN	**SIGNAL**	**INFORMATION**
1	N.C.	Not Connected
2	LED	LED control voltage, 0 to 5v DC input, <100uA*
3	GND	Ground
4	N.C.	Not Connected
5	PD_CH1	Photodiode Channel 1, 0 to 0.5v typical out, 5v max
6	N.C.	Not Connected
7	N.C.	Not Connected
8	+5V	+5V power
9	PD_CH2	Photodiode Channel 2, 0 to 0.5v typical out, 5v max

*50.3KΩ to +5v internal resistor, hi-Z otherwise, I = 99.4uA(0v) to 0uA(5v)

Theory of Operation

The CRISP autofocus device uses a pupil obscuration method for determining focal position. It projects light from a small aperture into the specimen plane but only using half of the optical system's pupil or optical aperture. As a result, light reflected from the specimen back into the CRISP unit appears to move laterally as the focal position is changed. The reflected light is focused on a split photodiode detector which detects the lateral motion of the reflection which corresponds to an axial motion of the specimen reflection, and that is used as a feedback signal for the focus motor to move back to the desired axial position relative to the specimen.

During operation of the CRISP unit – in the “Lock” and “In focus” states – a `focus_error` value is computed continuously (roughly every millisecond). `focus_error` is the difference in the two signals from the split photodiode minus the `focus_offset`. The `focus_offset` is adjusted by the user so that the `focus_error` is zero at the desired axial position of the specimen. In other words the photodiode signals need not be exactly balanced at the desired focal plane if `focus_offset` is non-zero, but the system performs best if the photodiode signals are approximately balanced which is equivalent to saying that `focus_offset` should not be too large.

At a user-specified update rate, the target position of the focus motor is changed by an amount proportional to the averaged `focus_error` and also proportional to `loop_gain`. This adjustment to the motor's target position will reduce `focus_error` in the future. These processes – computing the focus error and adjusting the target position of the focus motor – both continue indefinitely in a manner that the system tries to hold constant the axial position of the specimen.

When the `focus_error` is sufficiently small the CRISP reports that it is “In focus”. Operation continues to be identical to the “Lock” state.

Larger `loop_gain` will make the feedback more responsive and allow tracking faster changes in specimen position, but if the gain is set too large then oscillations will result.

Increasing averaging will reduce the influence of perturbations in the reflected signal but also make the feedback less responsive.

CRISP updates the target position of the focus motor continuously. The speed of the focus motor' response to changes in its target position depends on its mechanical dynamics and PID settings.

ASI's firmware has reasonable default settings for `loop_gain`, the stage motor update rate, and averaging. Please reach out to ASI for suggestions if you are trying to optimize behavior for a particular application.

Sample Considerations

There are several classes of samples that are common in microscopy and present very different challenges for focus systems. CRISP relies on reflected light from the sample to detect focus position. Usually the reflected light comes from small refractive index discontinuities at sample surfaces. The amount of light reflected at a dielectric interface is given by

\begin{equation} R = \frac{(n_1 – n_2)^2}{(n_1 + n_2)^2} \end{equation}

where n_1 and n_2 are the refractive indexes of the adjoining dielectric materials. The table below shows the refractive index of several optical materials and the magnitude of the reflection expected at various interfaces between materials. You will note that reflections from an air interface are around 4%, whereas reflections from a water interface is about 1/10 as much. This makes for “easy” and “difficult” focus applications.

Please note that prepared samples made with index matching mounting media will not work with the CRISP system. Many researchers have tried to get CRISP to work on such samples and have failed completely, which makes sense because of the lack of reflection. One work-around is getting coverslips with a special coating that is at least somewhat reflective to the CRISP wavelength but transparent to the imaging wavelength; even a very slightly metalized surface can give enough reflection to operate CRISP with negligible

detriment to the imaging path.

A good test sample for oil objectives is just a fingerprint on a cover-slip-bottom dish with water in it.

Table 1: Reflection Intensity from a Dielectric Interface

Material	Refractive index @ 800nm	Reflectance at interface (%)		
		Air	Water	Glass
Air	1.000	—	2.0	4.3
Water	1.329	2.0	—	0.46
Immersion Oil	1.518	4.2	0.45	0.0003
Glycerol	1.473			0.03
Glass (typical)	1.523	4.3	0.46	—
Plastic (Polystyrene)	1.575	5.0	0.72	0.03
Plastic (PMMA acrylic)	1.483	3.8	0.30	0.02
PDMS	1.410	2.9	0.09	0.43
Fused Silica	1.453	3.4	0.20	0.05

It can sometimes be difficult to discriminate between light coming from two closely spaced interfaces, for example, the two sides of a coverslip, or the variable spacing between a cover slip and a slide. Here CRISP relies on all but one interface being out of focus, and the relative measure of distance is the objective's depth of field. For example, a high-NA objective will have a depth of field of a few microns, so the opposite side of a 170um-thick coverslip will be wildly out of focus.

Photodiode Displacement Signal

The heart of the focus system is the split photodiode displacement sensor. The difference in intensity of the light falling on the two halves of the detector is used to determine the relative position of the reflected light beam. As focus position changes, the lateral position of the reflected beam will shift. The difference of the two signals from the split photodiode is a measure of the relative focal position.

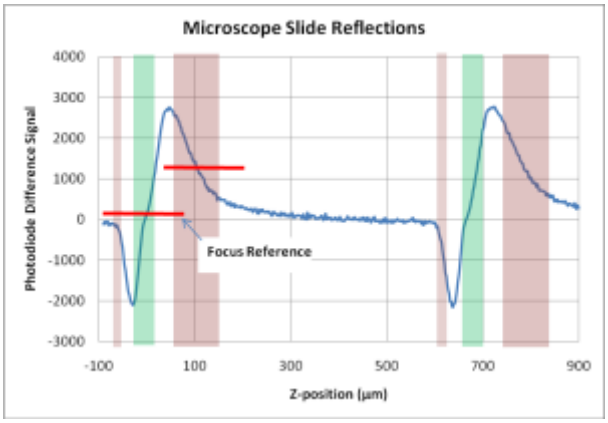


Figure 6: Photo detector difference signal for a scan through a microscope slide.

The figure above shows the difference signal from the photodiode pair as the focus is scanned through a standard microscope slide. You will notice two green shaded zones corresponding to the front and back surface of the slide. Any region with a large slope can be used to lock onto focus. Most often we are interested in viewing right at the reflective surfaces or very near them. A reference

signal level, one of those marked by the two red lines, is used to specify the desired focal position. Any deviation from the reference is an error signal that will direct the stage back toward focus. Changing the focus reference allows you to adjust focus within the shaded regions. Once the system is locked, the MS2000 control knob adjusts the focus reference level, thereby effecting focus changes on the locked system.

The brown shaded regions have opposite slope compared to the regions near the surface. If, for some reason, you wished to use one of those places to lock focus, the servo calibration would need to be done again, and you would expect to get a negative calibration value.

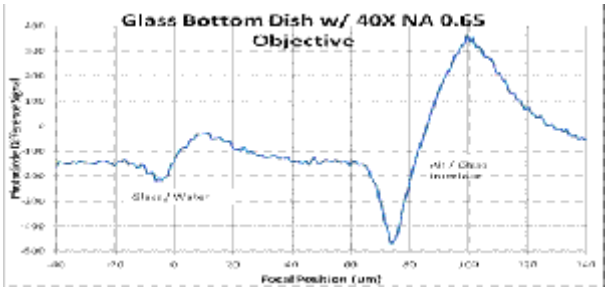


Figure 7: Reflections from a glass bottomed Petri dish.

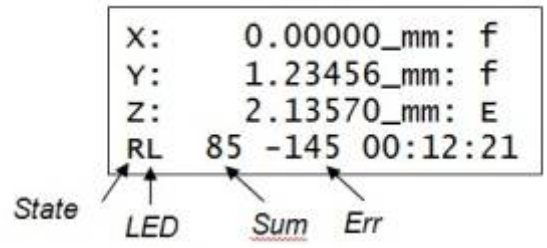
A common typical sample is a glass-bottomed Petri dish with a water sample. Here we can easily see the two reflections again, but note that the glass/water reflection is much less intense that the air/glass reflection.

Control of the CRISP system

To use the LCD display, ensure that the display-mode DIP switches 1 and 2 located on the back of the controller are in the UP position. The MS-2000 controller provides an easy means to turn on and off the CRISP LED as well as to initiate the focus lock. The LCD display shows the status of the system. The figure below shows the typical display.

LCD Display

On the MS2000 controller, the bottom line of the LCD display shows information about the photo-detector signals and CRISP system state.



The meaning of the quantitative information on the display changes depending up on the system state. The first character is the CRISP system state, described in the table below. The next character is L if the LED is turned on, otherwise blank. In most states, the photodiode Sum signal is next, followed by the Err signal. In the Dither state, the Err signal is the change in focus error as the focus is

moved over the `cal_range`. In any other state, the Err number is the `focus_error` value.

For a Tiger controller, the text that would be shown on bottom line of the LCD display is accessible by sending the serial command `[Addr#]EXTRA X?` and parsing the response.

As of Tiger `3.39` and MS2000 `9.2n` there are serial commands to get each individual value in EXTRA X?:

- State: `LK X?`
- Sum: `LK T?`
- Error: `LK Y?`

There are 3 states meant to read sensor values and display them on the LCD:

State Character	Sum Changed	Error Changed
A - Signal	Yes	Yes
B - Balance	Yes	Yes
M - Background Difference	No	Yes

This table shows which states change the Sum and Error result on the LCD display. This also applies when using the `EXTRA X?`, `LK T?`, and `LK Y?` commands.

Button Actions

The @ button is used to manually control the CRISP system. The duration of the button press determines the action.

Function	Button
Advance to next focus state	Press @ briefly and release
Back to Previous state or Advance to Calibration state	Press @ >3 sec. and release
Set Focus Offset to zero from READY state	Press @ >10 sec. and release

CRISP System States

Activating and calibrating the CRISP system is done by moving to the next CRISP state using the @ button on the controller and pressing it for various amounts of time as shown in the “Next State” and “Previous State” columns in the table below. It is recommended that you use software to calibrate CRISP, rather than buttons.

You can use the serial command `LK F=<decimal number>` as shown in column 2 of Table 2 below to force the system to enter a CRISP state. For example, to set the CRISP state to Balance, issue the serial command `LK F=66`. (Use with care, as out-of-sequence events are not necessarily handled smoothly!)

The state character in column 1 of Table 2 shows up as a character on the LCD display on MS2000.

Next State - @ button short press
Previous State - @ button long press

Table 2: CRISP System States

State	Code	State Name	Next State	Previous State	Description
I	79 (O)	Idle	R	G	LED is turned off going from Ready to Idle
R	85 (U)	Ready	K (D)	I	LED on - @ button locks
D		Dim	(R)	I	Low returned light signal (prevents Ready state)
K	83 (S)	Lock	R(k)	R	Active but not within focus tolerance; @ button unlocks
F		In Focus	R	R	Active and within focus tolerance; @ button unlocks
N		Inhibit	R	I	Low returned signal (unlocks system)
E		Error		R	Usually Out-of-Range Error
G	72 (H)	loG_cal	R	1	Initiate basic Log-Amp Calibration
	67 (C)	gain_Cal	(2,3,B,f)		Initiate Servo-Gain Calibration
f (g,h,i,j)	102 (f)	Dither	R	R	Dither Z for optical adjustments
†c	97 (a)	Curve	(R)		Generate focus curve data
†B	66 (B)	Balance	R		Display shows signal from each half of detector. Use to balance optics.
†o	111 (o)	Set Offset	(R)		Resets focus offset to zero for present focal position.
†Y	89 (Y)	LED Hold On			LED on without anomaly checking e.g. for low light. MS-2000 v9.53 required.

†States can only be initiated with the LK F=<Code> command.

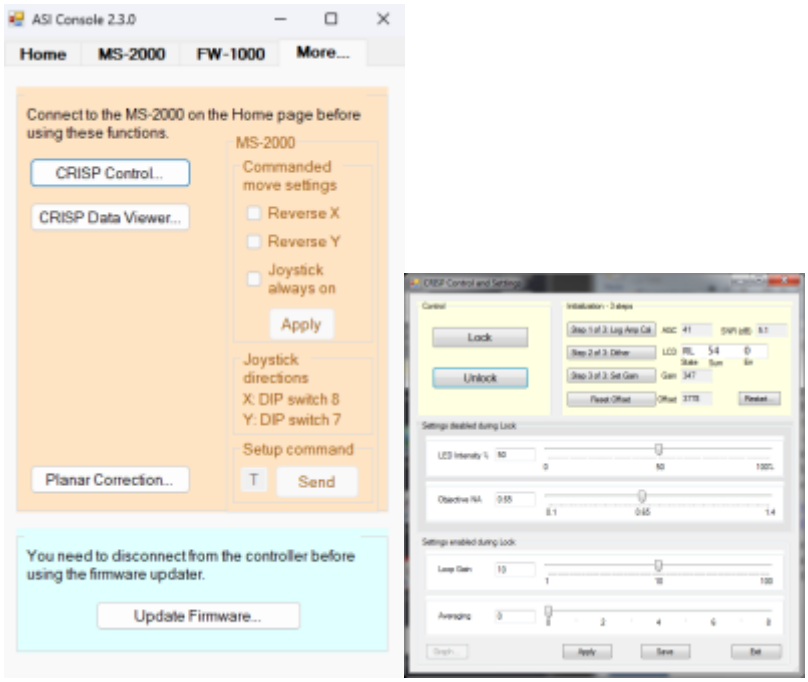
CRISP States - Detailed Version

Note: many of these states are substates and should not be entered directly, use the states from the table above. This table is useful to find out what the result of LK X? means.

CRISP States			
State	**Code**	**Name**	**Description**
A	65	Signal	
B	66	Balance	
C	67	Calibrate	
D	68	Low Light	
E	69	Error	
F	70	In Focus	
G	72	Log Cal Complete	
H	72	Log Cal	
I	73	Idle	
K	75	Lock	
L	76	OOR Limit	
M	77	Background Diff	
N	78	Inhibit	
O	79	Stop	
S	83	Start	
R	84	Ready	

CRISP States			
State	**Code**	**Name**	**Description**
U	85	Unlock	
Y	89	LED Hold On	
Z	90	LED On	
o	111	Set Offset	
p	112	Lock When Ready	
Calibration Routine			
1	49	Cal 1	
2	50	Cal 2	
3	51	Cal 3	
4	52	Cal 4	
5	53	Cal 5	
Focus Curve Routine **(MS-2000 only)**			
a	97	Curve 1	
b	98	Curve 2	
c	99	Curve 3	
d	100	Curve 4	
e	101	Curve 5	
Dither States			
f	102	Dither Start	
g	103	Dither 1	
h	104	Dither 2	
i	105	Dither 3	
j	106	Dither 4	
t	116	Dither Stop	
l	108	Dither End	

ASI Console support for CRISP



The ASI Console program has built-in support for CRISP that makes it easy to setup and calibrate the CRISP unit. Using the ASI Console program eliminates the need to learn all of the special button presses to accomplish the calibration steps.

Download ASI Console from the ASI website.

In operation, the CRISP control is found on the MORE tab. Clicking on the CRISP button will bring up the main CRISP control panel.

The main initialization steps are presented with three buttons. Lock and Unlock functions are provided as buttons as well. Set-up parameters are presented at the bottom of the CRISP window as sliders for setting LED intensity, Objective Numerical Aperture (used to determine the range of calibration moves), relative Loop gain and signal averaging.

After you have calibrated the system with the three steps indicated, you may wish to obtain a plot of the focus curve. The Graph… button will generate the focus curve. The z-depth of the focus range for the graph is determined by the Objective NA setting – smaller NA, longer travel.

Once the system is basically working, the Loop Gain slider is the easiest way to optimize the performance. If you have plenty of signal (dither Err number > 200) you can probably increase the Loop Gain to obtain faster focus and tighter focus position. If the system is marginally unstable, reduce the Loop Gain and it will become more stable.

CRISP Operations

The following guide assumes that the default CRISP parameter settings are adequate and will provide an adequate focus lock with many objectives and sample types. Focus on your sample.

Quick Start Instructions Using ASI Console

1. Download and install ASI Console
2. Using ASI Console, connect to the MS2000 controller and navigate to the CRISP control panel via the More… page.
3. Follow the three step initialization and calibration procedure in the CRISP control window. Use the Lateral adjustment thumb screw to maximize the ERR signal for Step 2 dither.

Quick Start Instructions Using Controller Only

- Press @ button for 3 seconds to achieve reflectivity calibration. Verify that LCD shows at least 2.0 dB SNR on the LCD display and that the status indicators on the Left side of the LCD show GL, indicating the Log Amp calibration is complete and the LED is on.
- Press @ button for 3 seconds to initiate the Z-axis focus dither. After a few seconds it should be apparent that the focus system is moving rapidly back and forth a small distance. The number in the middle on the LCD status line indicates the magnitude of the *focus error change* over the dither range.

```
hL  75  145  00:12:21
```

- Adjust the detector lateral adjustment screw on the CRISP unit for maximum absolute value of the focus error change. Motion of the detector will give large temporary values, so pause after changing the adjustment to observe the reading. For best performance you would like to have a value >50 with only modest fluctuations. When you have discovered the best spot for the detector…
- Press @ button briefly to advance to the READY state. You can verify that you have a good calibration by changing the focus of the sample and observing the change of the Err value. You should see Err respond proportionally to the change in focus, going positive in one direction and negative in the other.
- Press @ button briefly to advance to the Lock state. If the focus is not perfect, you can use the knob on the controller to change the lock reference and hence the focus. If the lock state is “nervous” or “sluggish”, see details below for how to adjust the loop gain and averaging for more desirable behavior.
- Press @ button briefly to unlock and return to the READY state. Subsequently you can just use a quick-press of @ to toggle the focus lock on and off.

For optimum performance, please refer to the more detailed instructions below.

Engaging the LOCK for Normal Operation

In addition to the quick start instructions above…

If you have calibrated the system, but then perhaps changed samples or significantly disturbed the system, you may find that the focus-error shown on the LCD is nowhere near zero when in the Ready state prior to locking. If you try to lock, the system could easily run away. Instead reset the offset by holding down the @ button for >10s first. When you release the button, the Err numbers should fluctuate about zero, and the transition to the lock state should be smooth.

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.

To unlock the system, again, a short-press of the @ button to will do it, returning to the Ready state.

When the Lock is engaged, any commanded move to the focus axis will fail and will generate a COD 47 error.

Saving Calibration and Offsets

Once you are satisfied with the focus performance and adjustments, you can save the calibration parameters to the controller so that in the future you don't have to go through the entire calibration procedure again. Merely back out of the READY state, to the IDLE state, with a long-press (3 seconds) of the @ button. In the IDLE state, hold down the @ button for >10 seconds to save settings to flash memory.

Now, as long as you stay with the same sample preps and objective lens, you should not need to go through the first three steps above. When you power on the controller, advance from the IDLE state to the READY state with a brief press of the @ button. A brief press again, and the system is locked.

LK M? will query the LogAmp_AGC value set by the calibration routine and you can use LK M=# to set it manually. This is the value that you would be saving to the controller's flash memory with the steps above.

You can use AL X? to query the LogAmp_AGC value directly from the potentiometer, LK M? queries the value saved in flash when the controller was calibrated. This can be used to verify that LK M=# is changing the internal settings.

If you are trying to save the calibration state manually by querying serial commands, you will also need the **cal_gain** (LR X) and the **lock_offset** (LK Z) variables. These parameters are set when the controller is calibrated.

You will need all 3 values:

1. LogAmp_AGC
2. lock_offset
3. cal_gain

If you plan to switch between objectives, you will also need to set the Objective NA with LR Y.

Calibration Details

Different samples and objective lenses can result in dramatically different levels of signal of returned light and different sensitivity of the detector to focus error. For this reason, there are two "single button" calibration steps that need to be done before the system is ready to use.

Log-Amp Calibration

Before calibration, choose your objective and focus on your sample.

This calibration step is initiated from the Idle state by a long press (3 sec.) of the @ button. It also can be initiated using the LK F=72 command.

During this step the controller automatically adjusts the range of the internal log amplifier so that the light level on the photodiode corresponds to ~75% of full scale. The resulting LogAmp_AGC value is set automatically and can be queried using the LK M? command. During this step the LCD display shows a signal-to-noise number that is the signal level on the photodiode compared to when the LED is turned off. For best results aim for SNR > 4.0 dB. If you have low levels, be sure your sample is in focus, and increase the LED intensity using the UL X=n% command (default LED level is 50%).

Focus Sensitivity Calibration and Detector Lateral Adjustment

Before this step, first focus on the sample and perform the Log-Amp Calibration described above. This calibration step can be initiated from the loG_CAL complete state (G) by long-press (3 sec.) of the @ button, or with the serial command LR Y=NA, where NA is the numerical aperture of the objective you are using. Using the serial command with the correct numerical aperture will allow the system to use an optimal distance for the focus moves it needs to make. The default is NA=0.65 which generates move distances suitable for a wide range of objectives, if not ideal. This calibration step moves the focus up and down a few microns to determine the focus sensitivity of the system and then proceeds to the Dither state where the focus is continuously moved back and forth a small amount.

Focus Dither for Optical Adjustments

In the Dither state the focus is changed by up and down by the cal_range amount. The difference in focus error signal is displayed on the LCD. The system will remain in the dither state, moving the focus up and down, until commanded to turn off. During the dither, the LCD Err number shows the change in focus signal from the top to bottom of the dithered focus move. Now you can make changes to the optical alignment while maximizing the displayed Err number.

Slowly adjust the detector lateral adjustment screw for a maximum absolute value of the Err number displayed[3)]. Large negative numbers are just as good as large positive numbers for obtaining a lock. When you make any optical adjustments using the Dither function you should keep an eye on the Sum indicator on the LCD display as well. If the signal level on either detector half gets out of range for amplifiers, then Sum will read either 0 or 100 for saturated low or high levels respectively. You may find that best Err reading results in a lower or higher Sum signal that you started with. If the Sum signal is outside the range 50-80 it is best to redo the log-amp calibration step.

When satisfied that the focus slope is the best possible, a short press of the @ button will cause the controller to return the stage to the initial position, check and set the error offset to zero, and leave the system in the Ready state.

Parameters used with the CRISP system

The serial commands give the user access to several parameters used with the CRISP system. Advanced users may find that they have a need to change particular settings from the default values for specific purposes.

cal_range	Sets the distance the stage moves gain calibrations, dither moves, and focus curve generation. This can be set directly using the LR F=cal_range command, or indirectly using the LR Y=NA command \begin{equation} Cal Range = \frac{1.5µm}{NA^2} \end{equation} where NA is the numerical aperture of the objective lens used.
lock_range	Specifies a maximum range of travel from the point of lock at which point the controller will disable the lock function and halt motion. This prevents runaway conditions from damaging objective lenses and sample. Set with the LR Z=lock_range command.
loop_gain	Sets the strength of the feedback applied to the focus motor. Higher numbers represent more overall loop gain. Set and queried using the the LR T command. Higher setting for loop gain make the system more responsive to sudden changes in focus, but can also lead to feedback instability and oscillation.
cal_gain	Sets the relative gain of the detector system to the focus motor. Higher numbers represent less overall loop gain. This value is set during the calibration routine. The value can be queried or set with the LR X command. Users are encouraged to use the LR T=# command to change the loop_gain rather LR X=#, although either parameter can be used to similar effect. The LR X parameter is primarily used to restore a saved calibration.
lock_offset	This parameter is a signed integer number representing the focus error on the detector that corresponds with the desired focus point. The lock_offset is set upon calibration, changes when the control wheel is turned when locked, and can be reset to the value that will cause no change in position when the @ button is pressed for >10s in the Ready state. The user can directly read and write this value with the LK Z command.
LED_Intensity	The LED light level can be controlled with this parameter. The default value of 50% is adequate for many applications. Improved signal to-noise can be obtained using more light. Set with the UL X=LED_Intensity command.
LogAmp_AGC	This parameter is set automatically during the log amp calibration step. A digital potentiometer is set such that the signal on the photodiode fills, but does not saturate, the ADC converter input range. This number will increase with higher LED_Intensity and more reflective samples.
in_focus_mm	This parameter sets the point when the status changes from Locking (K or k) to In_Focus (F). Specified in the AFLIM Z=in_focus_mm command, using a smaller value will increase the time to achieve the level of precision desired for the state switch. Doesn't change actual behavior, only reporting of K and F state.

The values of some of the parameters that are set during calibration will be sent to the serial port if the verbose mode VB X=16 is set.

Optical Adjustment

The CRISP unit is pre-adjusted at the factory, and should not need major adjustments. However, this guide will allow anyone to test and check the proper adjustment. The recording camera is helpful for adjusting the primary mirror position. In order to see the illumination light, any blocking filter in front of the camera needs to be removed.

Adjusting the Relay Lens position

The relay lens should be set to the center of its range. There is usually no reason to change this.

Adjusting Position of the LED Light Source

Focus on a glass slide with a 10X or 20X objective so that a typical glass/air reflected beam is obtained. Remove the transmitted light and obtain an image of the reflected light on the camera. (be sure the L LED indicator is showing on the LCD display) Be sure the mirror is intercepting the beam. (You may wish to slide the mirror as far away from the LED holder as possible to ensure adequate light entering the microscope.) Loosen the Adjusting screw on the LED holder and move and twist the holder so that the image of the LED slit is in the center of the camera sensor. Focus slightly deeper until the LED element comes in view and then twist the LED holder so that the active element is showing near the center of the slit. Tighten the screw to hold the LED in place.

a)  b)  c) 

Figure 8: Reflection from glass slide of a) LED exit slit and b) focused deeper, the LED emitter, when the LED holder is properly aligned by moving c) LED holder.

Sometimes you will notice an unfocused glare from the LED in the camera. This can be due to light reflecting from within the microscope, often directly from the front surface of the tube lens or other windows in the light path. Adjusting the position of the LED slightly off center or with a small tilt can reduce this glare and significantly improve the performance of the CRISP unit.

Adjusting the Primary Mirror

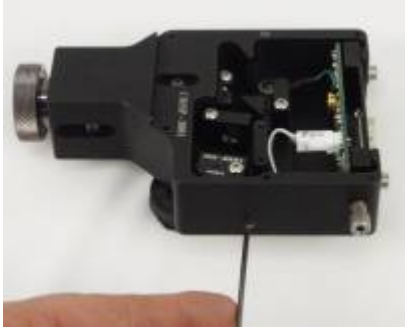


Figure 9: Mirror Adjusting Screw

The most critical alignment in the CRISP device is the primary mirror that injects the LED light into half of the optical aperture and allows light from the other half of the optical aperture to reach the photo-detector. It is important that this mirror reflect the light exactly onto the optical axis and that

the edge of the mirror exactly stop at the centerline of the system as well.

One approach for adjusting the mirror position is to maximize the error signal received when in Dither mode. First center the photo-detector board so it is in the middle of its travel range. Use the mirror adjusting screw to move the mirror back and forth.

You should also find that when the detector and LED are aligned so that you are getting a reasonable dither ERR, you can adjust the mirror position to maximize the Sum signal. However, a high dither Err number is most important for proper operation, so be sure you can return to a good Err number is you use the Sum as a guide to the mirror adjustment. In general, you will need to adjust the Lateral offset adjustment thumb screw as you change the mirror position to maintain maximum dither ERR.

With the mirror properly adjusted, you should observe a strong lateral movement of the LED mask image in the camera as the focus is changed back and forth.

Advanced Techniques

Offset the locking plane from the imaging plane

A common issue is that the CRISP system will be able to hold focus best at a location that is not in the center of the best focus range for the sample. This can typically happen because the reference cover-slip is slightly in front of the region where the sample is best in focus. Often, adjustment of the lateral position of the photo-detector will be enough to get acceptable operation. For more extreme cases, the solution is to move the entire CRISP unit further back from the C-mount using the sliding C-mount built into the CRISP body; putting the CRISP detector in a different (axial) focal plane from the sample (accepting that the objective's performance is worse at this different axial position). The distance, D, to move the back depends upon the objective magnification, M[4)], and the required depth of field change, δ, in media of refractive index n. The dependence is:

$$D = \frac{\delta}{n} M^2$$

For high magnification objectives, small focal plane changes can require substantial extension at the C-mount. Looking 5µm deeper into a water sample with a 100X objective would require about a 38mm extension. There may be the desire to focus slightly above the interface on biological samples, so the CRISP C-mount can be pulled back up to 29mm (see photo). This might be handy, especially with 100X objectives. If longer extension is required, additional extension tubes could be employed.

Figure 10: C-mount extension.

The C-mount extension lengths recommended for various objectives to optimize the focus range of

the system within the sample are included in the table below.

Table 3: Focus Properties for Typical Objectives

Microscope Objective	Relative EPI Light Gathering Power	Objective Pupil Diameter (mm)	Depth of Field (µm)	C-Mount Extension Length (mm)	Typical Focus Range (µm) (w.r.t. glass/water surface in 150 µm glass-bottom dish)	Typical Capture Range (µm)
100X NA 1.25	2.4	4.5	0.43	14-29	+/-3	> +/- 10
60X NA 1.4	10.7	8.4	0.34	0-15	+/-2.5	> +/- 8
40X NA 1.3	17.8	10.7	0.40	0-15	+/-3	> +/- 20
40X NA 0.7	1.5	6.3	1.4	0-15	+/-10	> +/- 20
20X NA 0.75	7.9	15.0	1.2	0	+/-10	> +/- 20
20X NA 0.4	0.64	7.2	4.2	0	+/-25 †	> +/- 50
10XNA 0.25	0.39	9.0	10.8	0	+/-50 †	> +/- 100

†Glass/air interface provides focus signal while focused on glass/water interface for low NA objectives.

The focus range of the system is largely determined by the numerical aperture of the objective used. Once the reflective interface is several times the depth of field (DOF) away from the focus plane, little useful light is returned to the detector and it is difficult to capture focus.

The amount of light available for CRISP depends not only on the type of reflective interface, but also on the light gathering ability of the objective lens. CRISP both illuminates and collects through the objective, so the relative brightness goes as NA^4/M^2. Examples are shown in Table 3.

With low power, low numerical aperture objectives, the light reflected from the air/glass interface will begin to significantly contribute to the focus signal. If this is desirable (the glass is flat and uniform), then an extension tube will enhance the focus signal from the air/glass interface when focused on the glass/water interface. If you wish only to look at the glass/water interface, there are various tricks you can play to enhance the separation between the light obtained from the two closely space interfaces. You can preferentially detect the deeper reflection by the position of the detector. Turn the detector lateral adjustment screw as far counter-clockwise as you can and still obtain a good dither response magnitude with non-zero signal on both detector halves. 20X objectives seem to be about the most difficult to separate the glass/water signal from a nearby much larger glass/air interface. Tricks might include using thicker glass bottom dishes or coverslips so the interface is further away, or just maintaining a very clean and uniform glass/air surface so what contamination of signal from that surface that there is will not affect the overall focus position.

Using the Iris LED Beam Stop

The internal iris in the CRISP unit can be used to improve the returned beam quality by reducing the amount of stray LED light that cannot be accepted by the objective aperture. The system is shipped with the iris all the way open so as to be able to accommodate high brightness objectives. Use the dither calibration to optimize the iris size. You may discover that the Err signal remains relatively constant as you decrease the aperture, but the Sum signal decreases, reflecting the decrease in background light level. Decrease the aperture size until the Err signal begins to decrease, and then redo the log-amp calibration.

Using Focus Curve Generation for Optimizing Adjustments

Sometimes it's helpful to plot the focus error response so you can determine the usable lock range and capture range of the system. There are two programs that can generate and display the focus curve data:

- **Tiger:** ASI CRISP Control (Micro-Manager 2.0 plugin)
- **MS2000:** ASI Console (Standalone)

The MS2000 uses a hardware-based method that can be initiated through serial commands. The Tiger controller relies on the CRISP plugin and uses a software-based method to capture the focus curve.

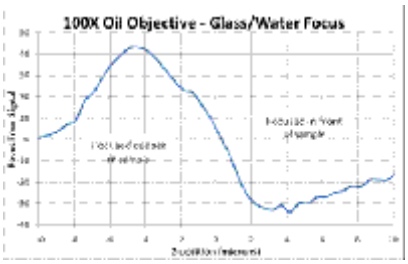
Note: Obtaining the Tiger focus curve requires version 2.5.0 of the CRISP plugin.

To obtain the focus curve, first lock focus and adjust the lock optimally. Unlock to the Ready state and press the Zero button to set the Z-in-focus position to zero. Then issue the command LK F=97 to initiate the focus curve generation (or press the button if using the above software). Below is the serial output from such a run. The second column is time in milliseconds, the third is Z-position in microns, and the fourth is the relative focus error.

Prior to Whizkid firmware v9.51, if you change the units multiplier (UM) from the default, the Z-position column will no longer be output in microns.

```
LK F=97
:A a
T:     0  -10.4     0
T:    50  -10.4    -1
T:   100   -9.9     1
T:   150   -9.4     2
T:   200   -8.9     4
T:   250   -8.4     7
T:   300   -7.9     9
T:   350   -7.4    18
T:   400   -6.9    22
T:   450   -6.4    29
T:   500   -5.9    35
T:   550   -5.3    40
T:   600   -4.8    43
T:   650   -4.3    43 # +Peak
T:   700   -3.8    41
T:   750   -3.3    37
T:   800   -2.8    32
T:   850   -2.3    27
T:   900   -1.8    23
T:   950   -1.3    22
T:  1000   -0.8    16
T:  1050   -0.3    10
T:  1100    0.2     2 # Focus
T:  1150    0.7    -6
T:  1200    1.2   -16
T:  1250    1.7   -25
```

```
T:  1300    2.2   -30
T:  1350    2.7   -32
T:  1400    3.2   -33
T:  1450    3.7   -31
T:  1500    4.2   -34 # -Peak
T:  1550    4.7   -30
T:  1600    5.2   -30
T:  1650    5.7   -27
T:  1700    6.2   -27
T:  1750    6.7   -25
T:  1800    7.2   -24
T:  1850    7.7   -22
T:  1900    8.2   -22
T:  1950    8.7   -19
T:  2000    9.2   -19
T:  2050    9.7   -19
T:  2100   10.1   -16
end
```



Example graph: You can see the strong slope near the surface (Z=0) that provides the focus feedback. For a focus at the interface, the capture range includes any Z-position that has the correct polarity of focus error signal to bring the stage back to the lock point. In this case the capture range extends to about 10µm into the sample and even further before the surface. The useful lock range depth into the sample is any region of the focus error curve with the correct slope – allowing for a little capture range beyond the lock point. In this case beyond about -3.5µm (into the sample) it would be hard to hold lock since it is very close to the focus peak.

Focus Variation Reduction by Averaging

In many instances, the focus lock mechanism is used to hold the subject in focus for long periods of time. Dynamic performance is secondary, and stable focus on a weak interface may be more important. In these cases, turning on averaging of the error correction signal can improve performance. Use the RT F=N command to average 2^N samples. Beware that excessive averaging adds a time delay that can effect stability. If oscillation occurs, either reduce the number of averaged samples or reduce the loop gain. Using N of 5 to 8 with reduced loop gain (`LR T=1 to 5`) can significantly smooth out marginal focus situations and hold the focus very still even on weak interfaces where the purpose of the device is mainly thermal drift compensation.

This feature performs a rolling average on the error correction signal (LK Y?), where the window size is the number of samples (2^N).

Dynamic Performance Optimization

For application of automated image acquisition, the speed of the autofocus can limit the throughput of the system. In these instances, it is important that the focus respond quickly to error conditions that change as the stage moves in XY. Default loop gain established by the calibration procedure results in modest speed and moderate stability. To push the speed, there is a separate variable set by “KA Z=m” that is used as a gain multiplier. The default KA value is 10. Usually the system will be stable increasing the value to 20 or higher, with significant speed improvement, but increasing KA above 10 is best used only when you have a strong reflection with low noise variation. Averaging should be turned off (RT F=0) when higher gain is used since the time delay associated with the average can introduce instability. There is no substitute for a strong reflected signal if you need to focus quickly and accurately.

Troubleshooting Steps

If you cannot get sufficient Err difference in the Dither state, check these troubleshooting steps.

- Verify that the electronics are working. Using the CRISP control in ASI console…
- Set LED Intensity to 0%. Apply.
- Click Step 1 Log Amp Calibration. Verify that the AGC number returned is less than ten. If so, that indicates that the LED can be controlled, and the amplifier has good noise performance. If the AGC number is >10, contact ASI.
- Set LED Intensity to ~70%. Apply. Save. Be sure you can get AGC value >35.
- Verify that you have an appropriate sample that is in focus. Prepared fixed samples usually will not return a good reflected beam because of the index matching mounting media. Choose a simple glass/air interface if you are having trouble before graduating to glass/water interfaces.
- Verify that the IR light can reach the objective. Remove filter cubes if unsure of their properties.
- Flat glass windows and prism surfaces can reflect more IR light than is coming from the desired interface. Be sure you are using a 100% photo port without intervening prisms.
- If problems persist, achieve and image of the CRISP LED on the system camera by removing the IR block from the optical path. Usually there is sufficient light that leaks through the dichroic to be able to see the CRISP LED light on the camera when there is no other illumination. Verify that the CRISP LED light is present at the sample an go through the steps described in the Optical Adjustment section of this manual.

Electromagnetic Interference

Interference from certain cellular phones, microwave ovens, and other devices that emit radiation in the 0.1-10 GHz range has been known to interfere with normal CRISP operation when operated in close proximity and simultaneously. The main issue is the cable between the CRISP unit and the controller. If this is problem then ASI can provide a special shielded cable.

TTL Control of the CRISP focus lock

TTL input function (TTL X=9)

In some instances it may be desirable to be able to turn on/off the CRISP lock using a TTL signal. Imaging software that does not support CRISP explicitly may support controller TTL I/O which can provide a simple method to synchronize the autofocus function with other imaging functions.

The MS2000 TTL Input can be programmed for several functions. Use TTL X=9 to select the CRISP control function. (See the TTL command section in the MS2000 Programming manual for more details.)

Use ASI Console, the @ button interface, or explicit serial commands to calibrate and set the CRISP system to the READY state (R on the LCD display). Once in the READY state, and with TTL X=9 selected, a logic HIGH on the TTL IN BNC connector will engage the CRISP lock. The lock will be maintained until the TTL IN signal goes to a logic LOW, at which point the system will unlock and return to the READY state.

TTL output function (TTL Y=11, 12)

You can also use TTL control to determine when the system is in focus. Use TTL Y=11 to have the controller send a TTL output pulse after any commanded move, providing CRISP is in the 'F' state. The controller will wait for the CRISP 'F' state after the move is completed before sending a pulse. Firmware version 9.2i+, the controller STATUS ('B' / 'N') will follow both the stage move and the CRISP transition to the ‘F’ stage to get the 'N' state when TTL Y=11 is set.

The TTL output can be used to directly monitor if CRISP is in the 'F' state or not using TTL Y=12. TTL OUT0 is high when CRISP is 'F' state, low otherwise.

Serial polling of the CRISP focus lock

The TTL control discussed above can eliminate polling delays, but sometimes it is easier to implement serial polling. When XY stage motion and focus lock all need to be coordinated with image capture, the TTL Y=11 function will also enable the global controller busy state to include a successful focus condition before the 'N' Not Busy state is allowed. Polling the controller for busy status using / can provide a simple and efficient method of knowing if all stage axes and focus are ready. In the TTL Y=11 mode, there are a few pathological conditions that can develop. If a move is executed but no focus system is activated, then the ‘B’ state will remain indefinitely. The set-up command TTL Y=11, will reset the status to 'N' regardless of the stage or focus status. Initiating a move will change the status to 'B' where it will remain until both the move is completed and CRISP focus state is 'F', in-focus.

For any other TTL Y=n state (n |= 11) the global status command will only respond to the XY stage busy status. If knowledge of both XY stage and CRISP functionality are desired, the individual axes status command RB X, RB Y, and the CRISP state LK X? can all be used to poll for the desired information.

Computer Control of the CRISP System

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

- Command:AFLIM (AL) 2016/02/23 19:18
- Command:EXTRA (EX) 2016/02/23 19:06
- Command:LOCK (LK) 2016/02/23 20:02
- Command:LOCKRG (LR) 2016/02/23 19:42
- Command:RTIME (RT) 2016/02/22 19:30
- Command:TTL 2017/07/26 13:03
- Command:UNLOCK (UL) 2016/02/23 19:58

Serial Command Cheatsheet

The only deprecated command is KADC which has been replaced with LR T.

AFLIM Y? and AFLIM Y=# (shortcut AL) have been left out of the table because you should read and set the LED intensity with UL X? and UL X=# instead. The AFLIM command is not recommended for this purpose. Also it's possible to use AFLIM X=# to set the AGC but it is not recommended, use the LK M=# command instead.

On TG-1000 don't forget to prefix the card address to the command. For instance, if the card address is 2, use 2UL X=100 to set the LED Intensity to 100%.

CRISP Serial Commands			
Property	**Set**	**Get**	**Notes**
CRISP State	LK F=#	LK X?	Also shown on the LCD
Log Cal State	LK F=72	LK X?	Calibration Step 1
Dither State	LK F=102	LK X?	Calibration Step 2
Set Gain State	LK F=67	LK X?	Calibration Step 3
Set Offset State	LK F=111	LK X?	Reset focus offset
Get Focus Curve	LK F=97	LK X?	MS-2000 or Tiger v3.53 firmware required
Lock State	LK F=83	LK X?	Focus Lock
Unlock State	UL	LK X?	Unlock Focus
LED Intensity	UL X=#	UL X?	Set as a percentage (0-100)
Objective NA	LR Y=#	LR Y?	Objective numerical aperture
Loop Gain	LR T=#	LR T?	Gain applied to signal
Number of Averages	RT F=#	RT F?	Focus variation reduction
Update Rate (ms)	UL Y=#	UL Y?	Previously “Number of Skips”
Lock Range (mm)	LR Z=#	LR Z?	Max travel range limit
In Focus Range (mm)	AL Z=#	AL Z?	Focus lock sensitivity
Lock KI	EXTRA Z=#	EXTRA Z?	Focus lock KI Z value
SNR	-	EXTRA Y?	Signal to noise ratio
Sum	-	LK T?	Also shown on the LCD
Error Number	-	LK Y?	Also shown on the LCD

Lock Offset	LK Z=#	LK Z?	Set during calibration
Focus Axis	UL F=#	UL F?	Select the focus axis
Knob Speed	UL Z=#	UL Z?	Knob speed during focus lock
LogAmp_AGC	LK M=#	LK M?	Set automatically during logAmp calibration step, also queried by AL X?
Calibration Range	LR F=#	LR F?	Move distance during dither calibration step
Calibration Gain	LR X=#	LR X?	Use Loop Gain instead

Setting the `Objective NA` also sets the `Calibration Range` and `In Focus Range (mm)` settings.

Accessing Diagnotistics over Serial

The text normally shown on bottom line of the LCD display is accessible by sending the serial command `[Addr#]EXTRA X?` and parsing the response. The response contains the current state, sum, and error number. This is handy especially on Tiger controllers where there is no LCD display.

crisp, manual

1) These aberrations are increasingly more severe with larger back aperture of the objective, i.e. the relative NA per magnification.

2) The “PSL” is a C60-SHORT-PORT with slot for dichroic and glued-in 75mm lens, which is placed right below the objective.

3) Note that this is actually different from the `Err` signal computed during operation

4) Note this is really the system magnification; for CRISP use in MIM systems often the tube lens for CRISP is 100mm or 75mm and hence this M might be different from the objective's nameplate magnification. However for DCMS-type applications M would almost always be the nameplate magnification.