CREST OPTICS

X-Light™ V2TP
Confocal Imager

VCS
Super-resolution module

Installation and
User's Guide
V1.9

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CREST OPTICS X-Light V2^{TP} –Installation and user guide

1.0 X-Light V2^{TP} hardware

1.1 X-Light V2^{TP} description

CREST X-Light V2^{TP} is a high throughput full spectrum spinning disk confocal imager with super-resolution add-on module capability. It can be attached to all major inverted epi-fluorescence microscopes allowing full spectrum (360nm-700 nm) confocal imaging.

Detailed Specification:

• Easy plug-in spinning disk box

• Full compatibility with Crest VCS (Video Confocal Super-resolution) module for 3D resolution enhancement

• Microscope side-port adapter mounting for original C-mount adapter

• Single hole pattern spinning disk or double hole pattern spinning disk, on same physical disk

- High density hole patterns, with Nipkow design

• Anti Reflection coated spinning disk for highest throughput on the market

• Fast spinning disk 15.000 rpm

• Up to 2000 frame/s (tested with CMOS High Speed Systems)

• Custom hole patterns are available for dedicated applications (90-120 days delivery), from receipt of customer specification/pattern geometrical description

• Excitation Gimbal mount for easy alignment on custom microscope setup and for best S/N

• LED and Laser excitation mounts for high efficiency coupling

• Motorized Dichroic five positions Filter Wheel

• Standard eight positions motorized Emission Wheel

• Extraction tools for easy insertion and removal of both dichroic filter and emission filter

• Up to 22mm field of view for new sCMOS confocal imaging on single hole pattern disk

• Up to 12mm x 12mm field of view in double pattern configuration (for each pattern)

• CCD focal plane easy focusing without moving the CCD Camera

• Adapter for Multimode Laser or SMA-905 fiber excitation

CREST X-Light V2^{TP} can be used with most high end EMCCDs, interline or frame transfer CCD cameras, sCMOS cameras together with imaging software including Metamorph, Nis Element and MicroManager.

Fig. 1.1 CREST X-Light V2^{TP}

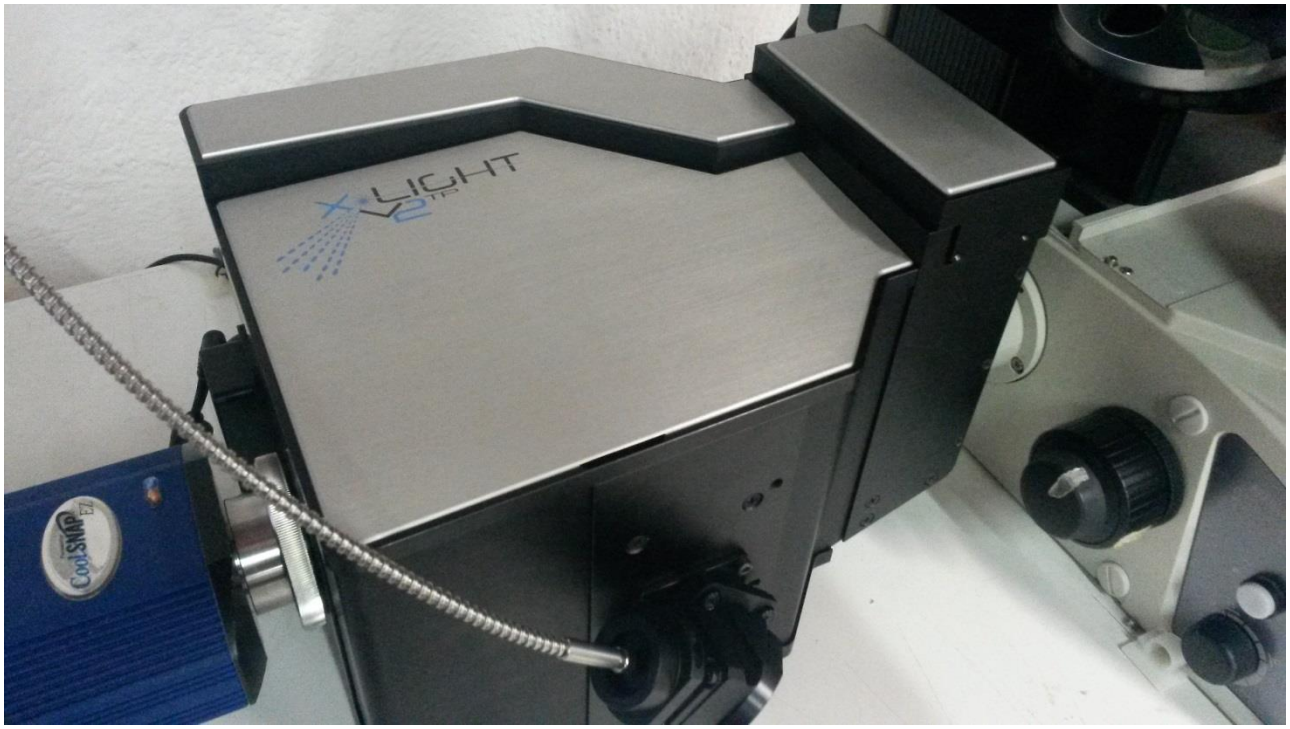


Fig 1.2 CREST X-Light V2^{TP} complete system.

1.2 CREST X-Light V2^{TP} Confocal Imager

Specifications

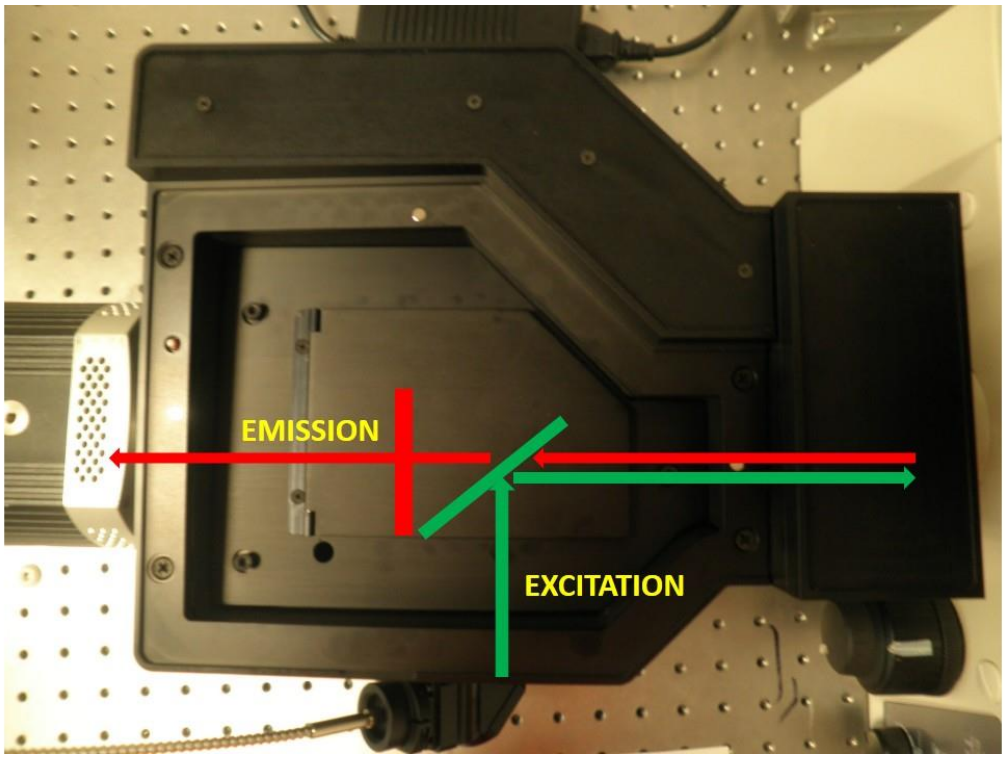
Confocal scanner	Nipkow spinning Disk (pinholes) with single and double hole pattern
Disk scan rate	> 2000 scans per second
Pinhole diameter	60 micron single pattern standard, 40-70 micron double pattern standard, custom pattern on request
Spectral transmission	360 nm – 700 nm
Z- resolution	0.7 micron (PSF); 60X PlanApo 1.4NA
Illumination source	Lumencor LEDs Systems, Laser illumination System
Internal excitation changer	On request
Internal dichroic changer	Automated 5 position wheel (up to 25.5x36 mm filters).
Internal emission changer	Automated 8 positions wheel (25mm diameter, up to 5mm thick filter).
Operation mode	Automated confocal, wide field, bright field, super-resolution with VCS module add-on (option)
Observation	CCD camera/sCMOS camera port with fixed position; manual focusing by X-Light V2^{TP} focusing hex screw system
Microscope Compatibility	Inverted fluorescence microscopes with 100% camera port. Upright Microscopes
Control	USB serial control
Software	Metamorph, Nis-Elements, MicroManager
Size	11.4 (w)x 14.2 (L) x 9.06 (h) inches 29.00 (w) x 36.00 (L) x 23.00 (h) cm
Sound noise	Background 37db working 45db
Working Temperature	15-35 °C
Storage Temperature	10-70°C
Weight	24.3 lbs /11.0Kg
Power	100-240VAC 2.5A 47-63Hz

graphic symbols:

Not dispersion this product in the environment in accordance to RAEE directive 2003/118CE

Attention

1.3 CREST X-LIGHT V2 Light Path



X-LIGHT V2^{TP} Confocal Imager Light path

Excitation Filter Wheel: The X-Light V2^{TP} has been engineered to work with LEDs and Lasers mainly.

Dichroic Filter Wheel: The quick release dichroic wheel has 5, positions and accepts commercially available **up to** 25.5x36 mm dichroic mirrors. Dichroic positions can be controlled via serial commands (software control).

Emission Filter Wheel: The quick release emission filter wheel has 8 filters positions and accepts commercially available 25mm filters (up to 5mm thickness ring mounting included).
Dichroic positions can be controlled via serial commands (software control).

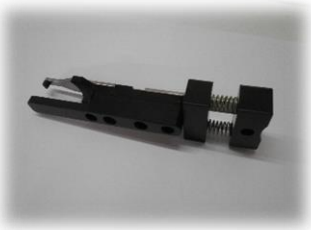
Spinning Disk: The Nipkow spinning disk can be moved in and out of the light path and confocal images and wide-field images can be obtained respectively. The spinning disk can be controlled via serial commands (software control).

Camera: A wide range of CCD/EMCCD cameras and sCMOS cameras can be attached to the confocal unit to obtain confocal, wide field or bright field images.

2.0 CREST X-LIGHT V2^{TP} Installation

2.1 CREST X-LIGHT V2^{TP} Components

1	Confocal Head	
2	Automated dichroic wheel filter holders (5)	
3	Camera C-Mount	
4	X-Light power supply	
5	Power cord	
6	USB cable	
8.1	Dichroic Filter extraction tool for automated 5 positions dichroic wheel	

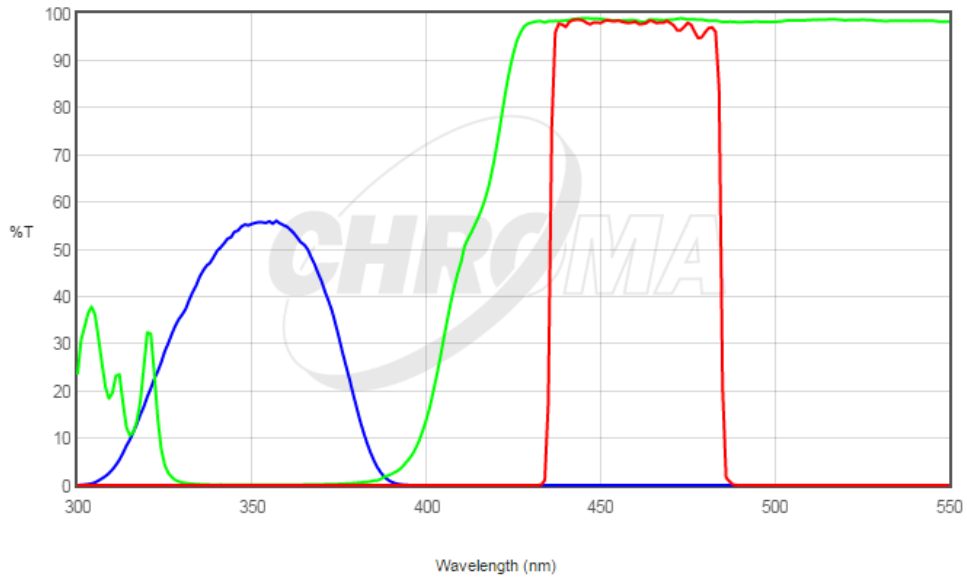
8.2	Emission Filter extraction tool for automated 8 positions filter wheel	
9	Bubble level	
10	C-mount side-port adapter ring	
11	Threaded ring (2)	

2.2 Filter wheels Assembly

2.2.1 Filter descriptions for most common applications (filters are not included)

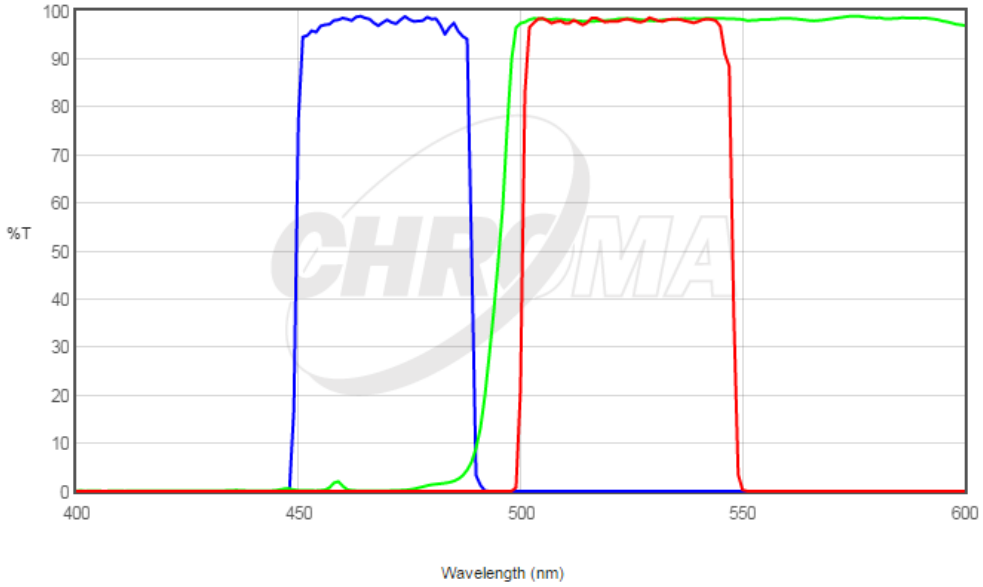
DAPI Chroma technology®

Exciter
Dichroic
Emission



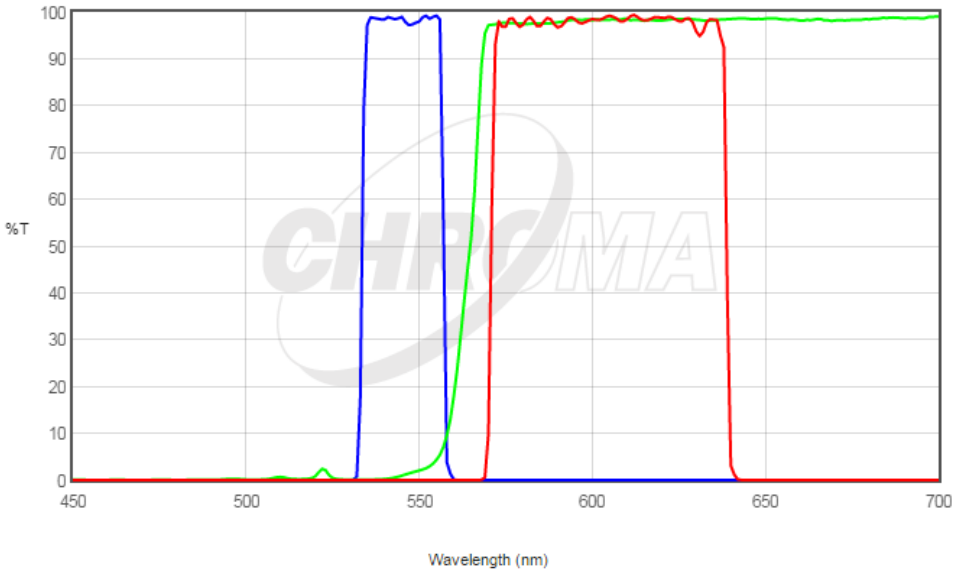
EGFP Chroma Technology®

Exciter
Dichroic
Emission



TRITC/Cy3 Chroma Technology®

Exciter
Dichroic
Emission



2.2.2 Changing emission filters and dichroic mirrors

Changing emission filters:

In order to change or add new emission filter sets follow the procedure below. **Please refer to section 2.3.2 for both dichroic wheel and emission wheel positioning for filter exchange.**

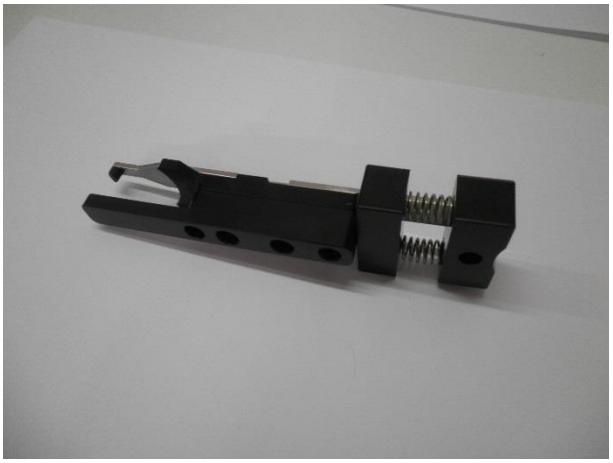
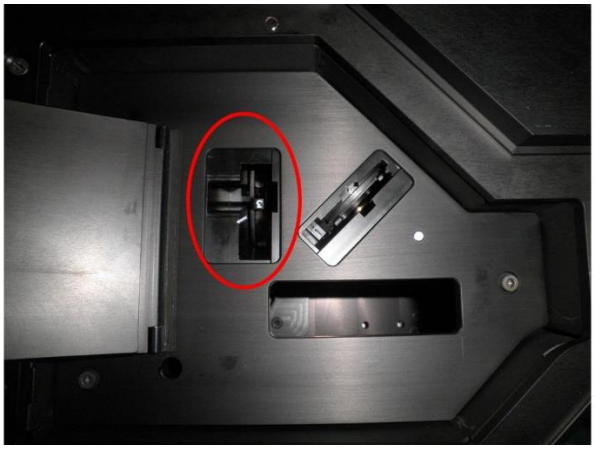


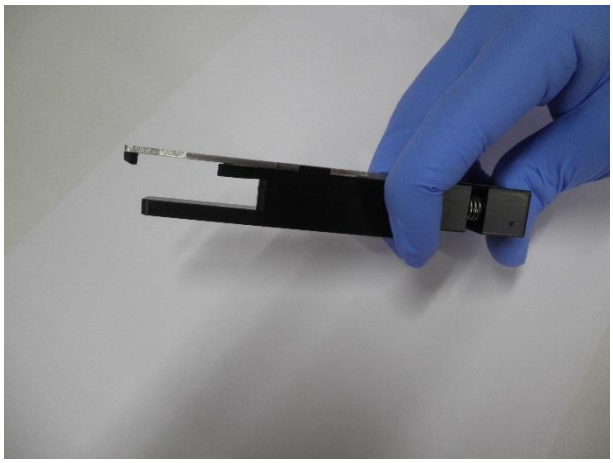
Figure: Emission Extraction tool

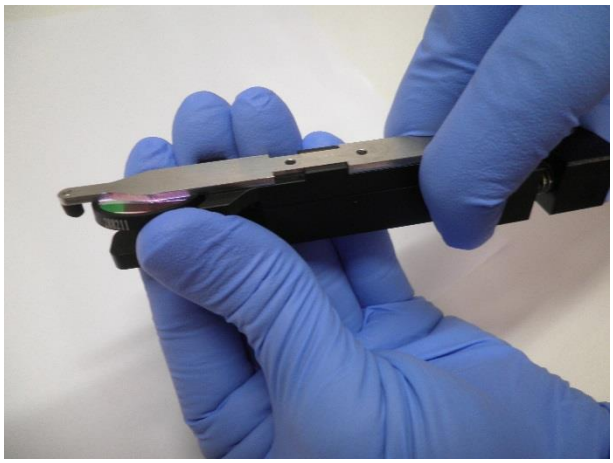
Insert the filter:

1. Locate the emission slot circled in the figure below

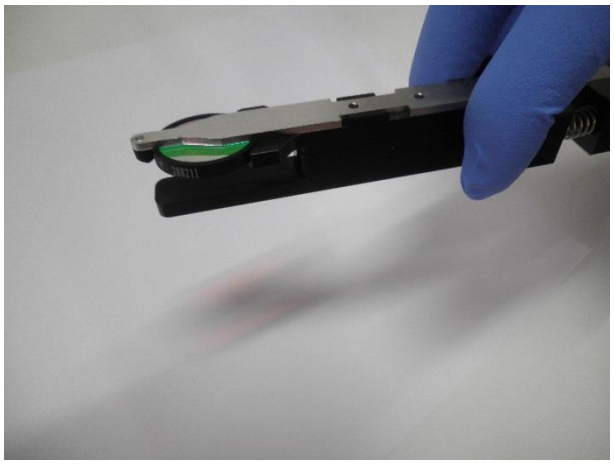


2. Push the emission tool and keeping it pushed place the emission filter in it as shown in figure below (every manufacturer has its proper way to place the filter in the optical path so follow manufacturer instructions in order to mount them correctly.)

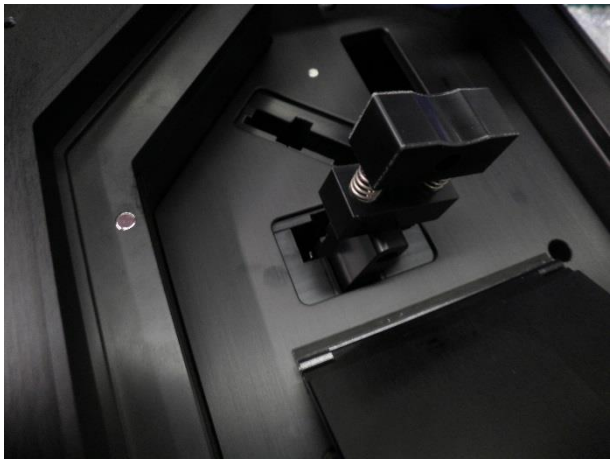




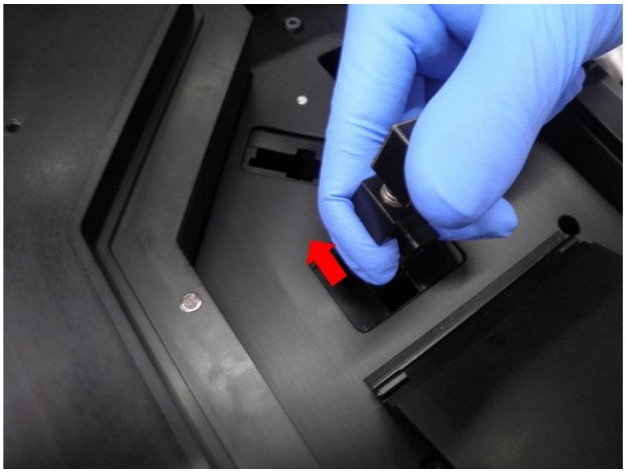
3. Release the tool in order to grab the filter in steady position



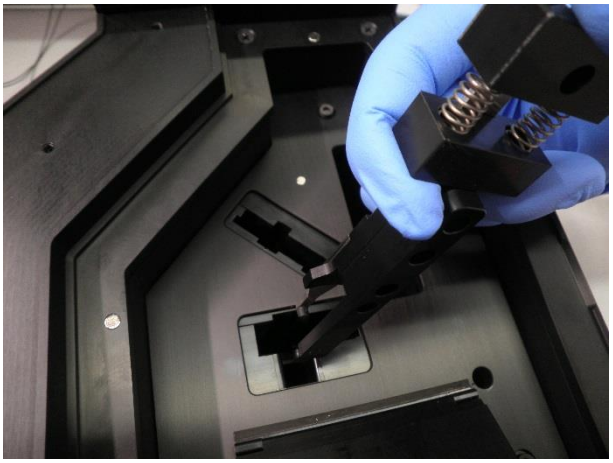
4. Insert the tool all the way down in the slot following the slot entry shape



5. Now start extracting: press the tool and move it in the direction of the red arrow below



6. Finally pull out the tool



Extract the filter (inverse insertion procedure):

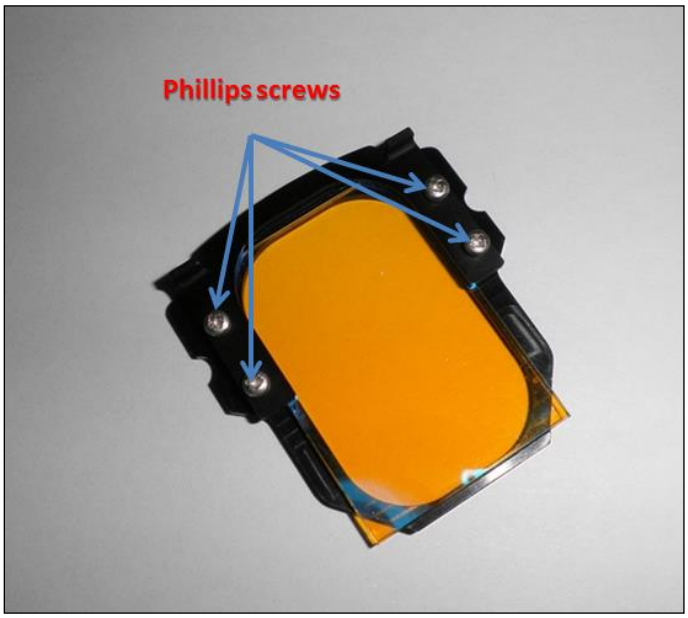
1. Push the top of the extraction tool.

2. Insert the extraction tool in the filter slot of emission wheel. Make sure the extraction tool is completely inserted against the wall indicated by the red arrow (see picture above)

3. Once in position push a little the toll toward the filter and release in order to grab the filter and extract it.

Changing dichroic filters:

Extra precaution is necessary when mounting or removing dichroic filters. Lay the dichroic filter holder flat on a flat surface so that the mounting screws are facing upwards (see figure)



Using a Phillips screw driver unscrew the filter holding plate . Now gently remove the holding plate.

It is important how the dichroic mirror is mounted on the holder. The coated surface of the mirror should be facing away from you as it sits on the flat surface. In other words the coated surface should face the light source. The coated surface has the beveled edge or on occasion the manufacturer would specify using an arrow marked at the edge.
Once the mirror is placed in the filter wheel, carefully place the holding plate in position and gently tighten the screws. Make sure not to tighten it too much since this may cause the mirror to fracture. A good way to keep check is to gently shake the filter wheels after a few turns on the screw, if you here a rattle you can tighten a bit more. Follow this until the rattle stops.

The filter are now ready for mounting on the confocal head.

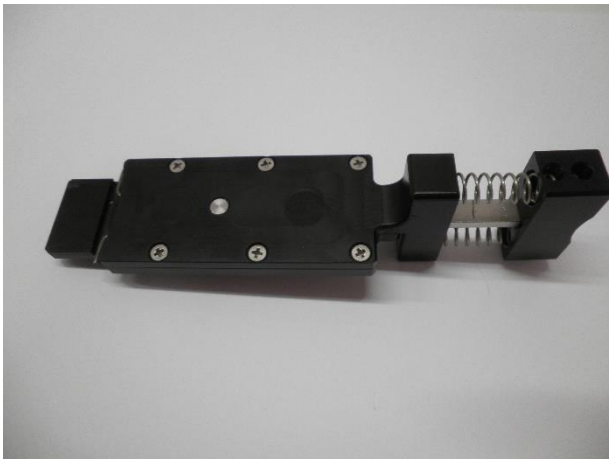
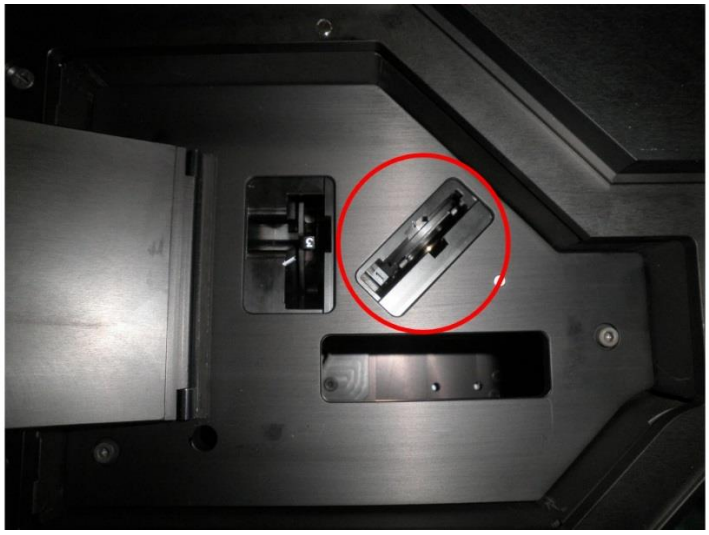


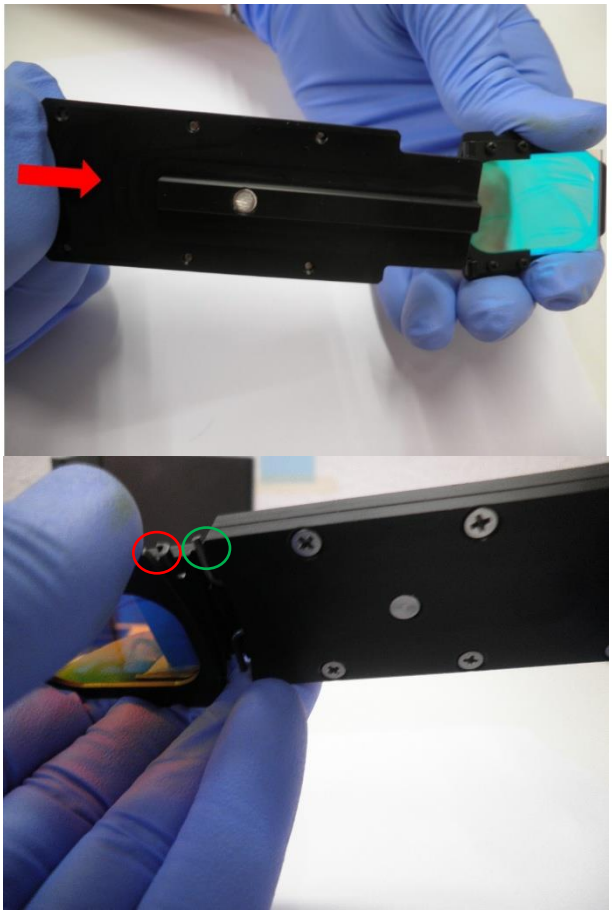
Figure: Dichroic Extraction tool

Insert the filter:

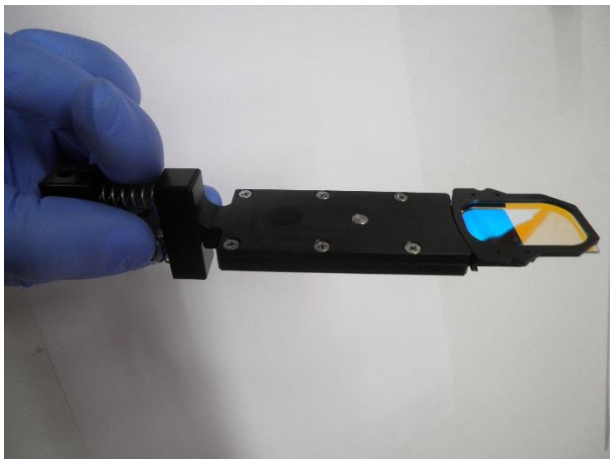
1. Locate the dichroic slot circled in the figure below



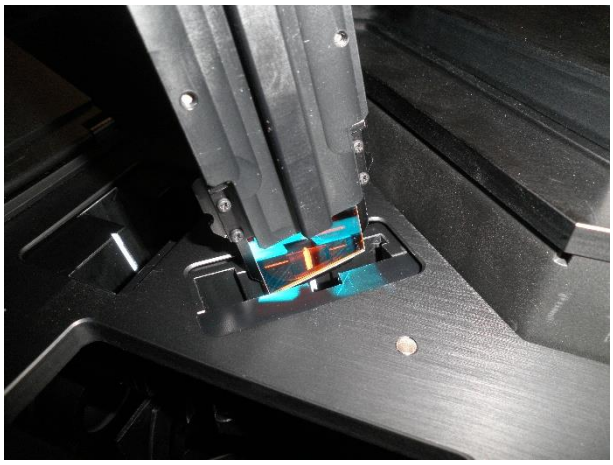
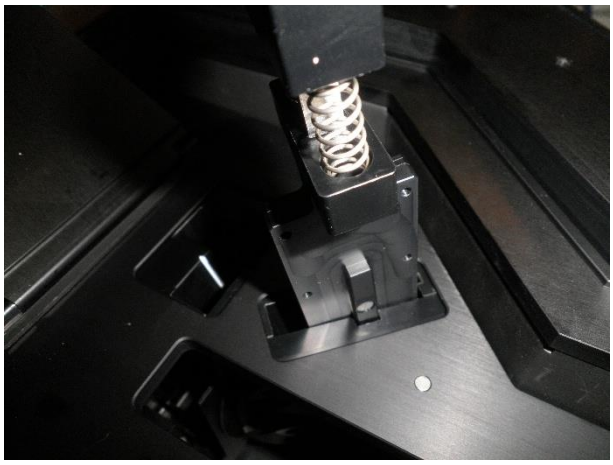
2. Push on the top of the dichroic tool and keeping it pushed place the dichroic mirror in it as shown in figure below (pay attention to mount it with the coated surface toward the excitation light).



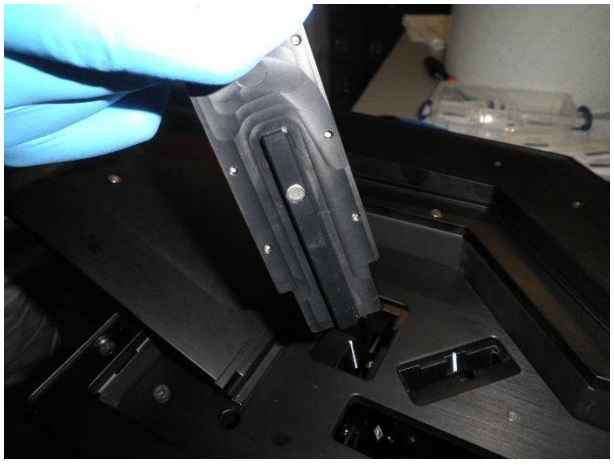
3. Release the tool in order to grab the filter in steady position. The two metal hooks (green circle above) must grab the two corresponding hooks on dichroic holder (red circle above)



4. Insert the tool all the way down in the slot following the slot entry shape

5. Finally, push on the top of the tool again and extract it. The dichroic is correctly positioned into the wheel.



<u>In order to extract a filter follow the inverse procedure:</u>

Insert the tool with the button on top pressed, once in position all the way down release the button and extract the filter

2.3 Power supply for X-Light V2^{TP} and connectors

Check that the plug-socket 110/220Vac has got the earth plate.

Attach the connector of power supply to the X-Light main power connector.

Attach one end of the USB communication cable to the X-LIGHT unit and the other end to a USB port of the computer.

2.3.1 Cleaning on Xlight V2^{TP} System

The only optical components to be cleaned are the dichroic filters mounted on dichroic wheel and the emission filters.

2.3.2 Wheels positioning for filters extraction

The positioning of the wheels for insertion and extraction of the filters must be done using Hyperterminal (or whatever terminal to send serial commands) in the following way:

C1m,…,C5m to load dichroic mirrors

B1m,…,B8m to load emission filters

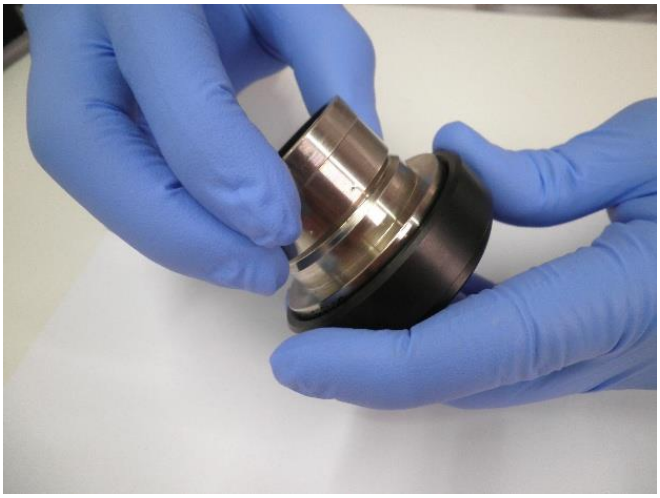
2.3.3 Laser safety interlock

As the top panel is removed, laser source power is cut off if connected to the X-light V2^{TP} laser safety interlock

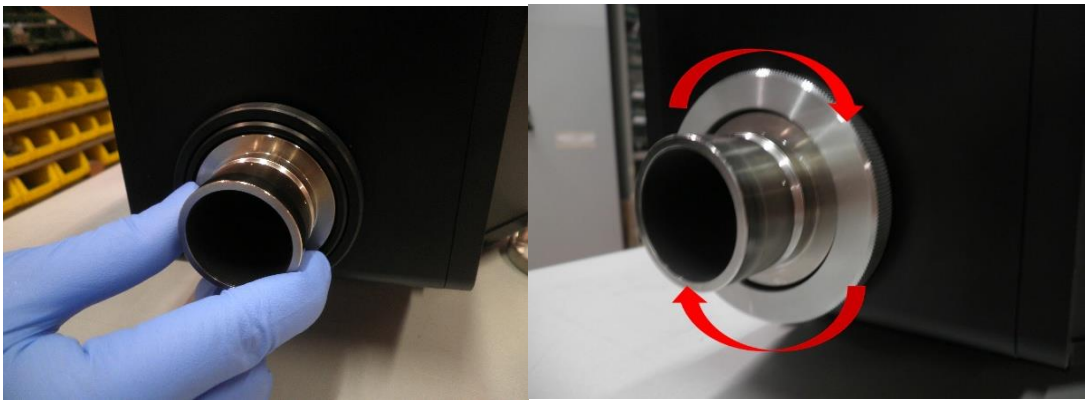
2.4 Attaching X-LIGHT V2^{TP} to the microscope

You will need the specific microscope brand side-port C-mount (1x) adapter in order to attach the X-LIGHT V2^{TP}. (see figure; Nikon adapter example).

Mount the side port-adapter on the C-Mount side-port ring

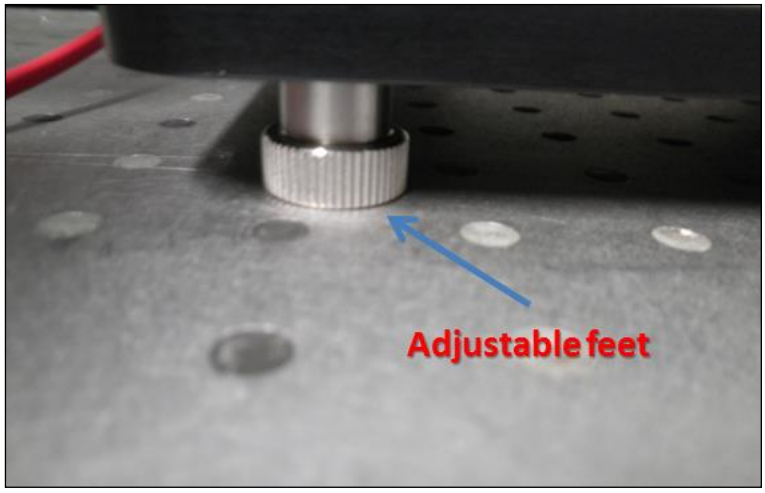


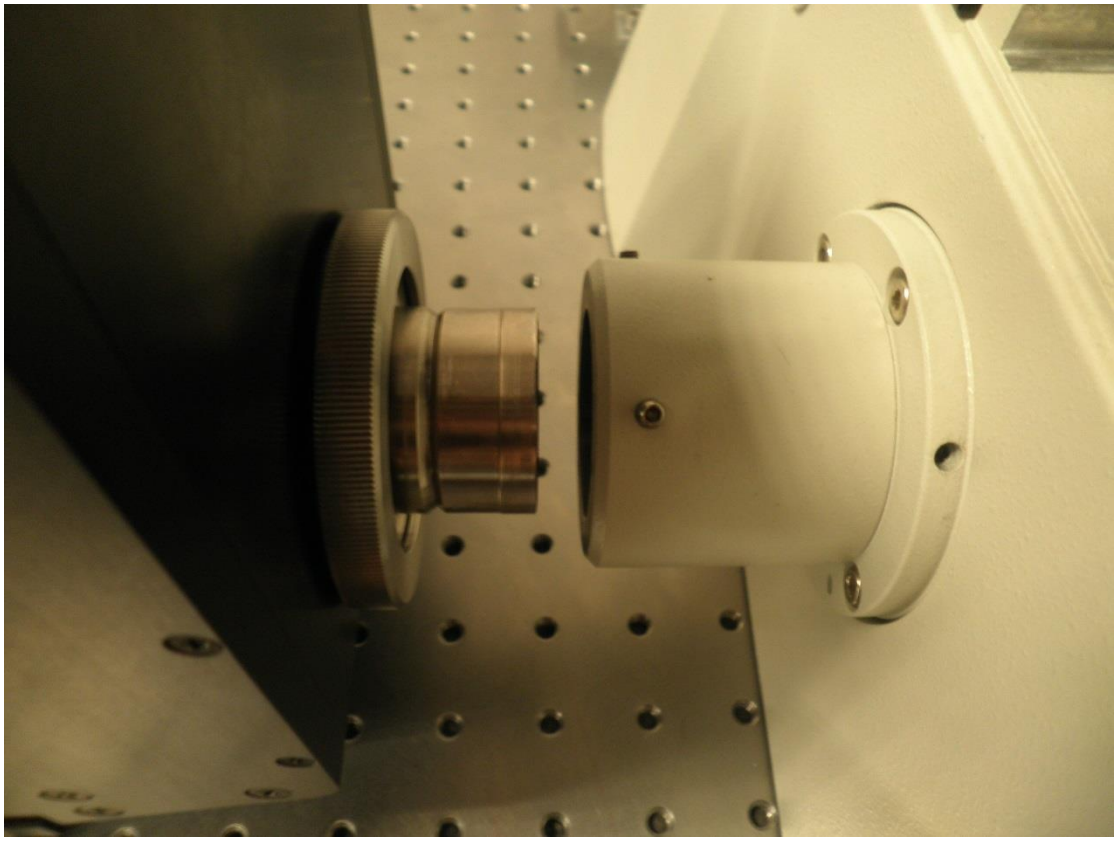
Place the adapter in its housing (disk box side) and the screw the threaded ring to block it in position



Mount the Confocal head to the side port (camera port) of the microscope. It is important that the confocal head is parallel to the side port of the microscope. This is achieved by leveling the confocal head adjustable feet. Place the bubble level provided, on the stage of the microscope and note the position of the bubble. Now place the bubble on the top flat surface of the confocal head and adjust the feet until the bubble

position is the same as it was when placed on the stage of the microscope. Now tighten microscope side port holding screws firmly.

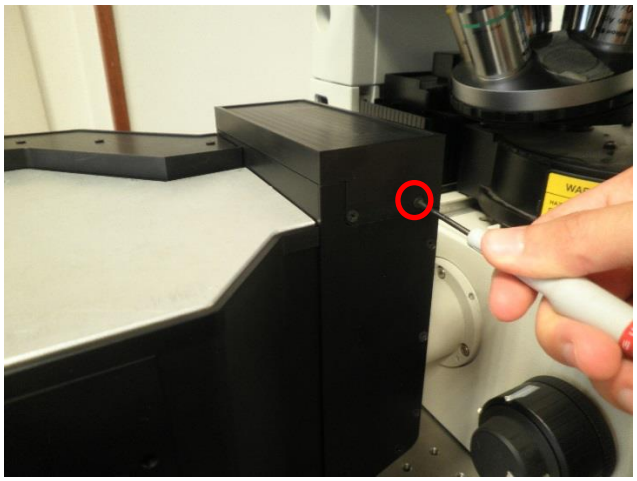


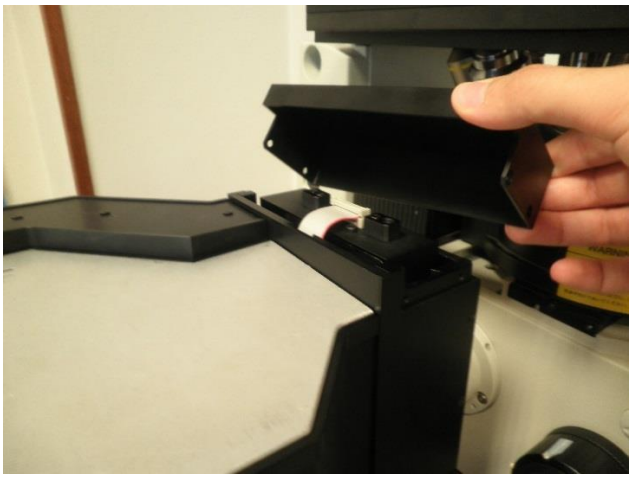


2.5 Spinning disk box Plug IN/Plug OUT

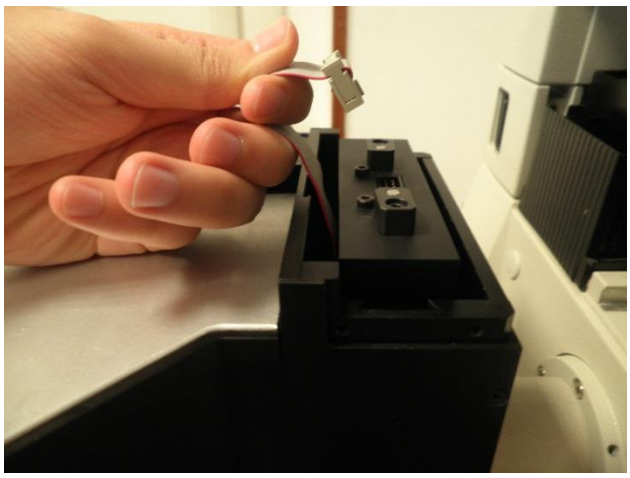
The following procedure will allow to easily switch between different spinning disk boxes you may have. **Be sure that the system is disconnected from the power.**

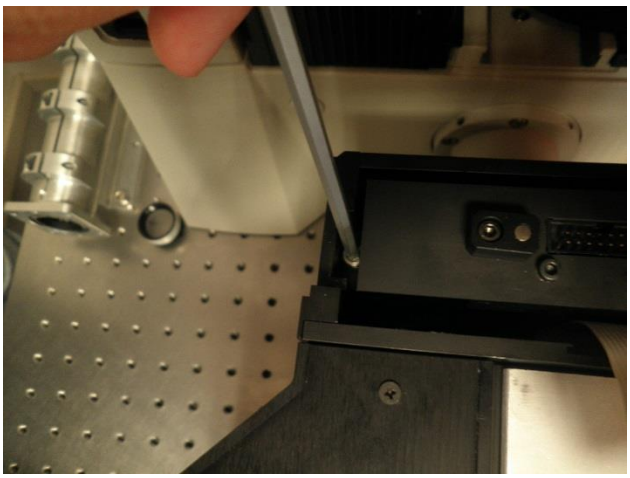
Unscrew the four screws on the top of the spinning box: two for each side and remove the cover



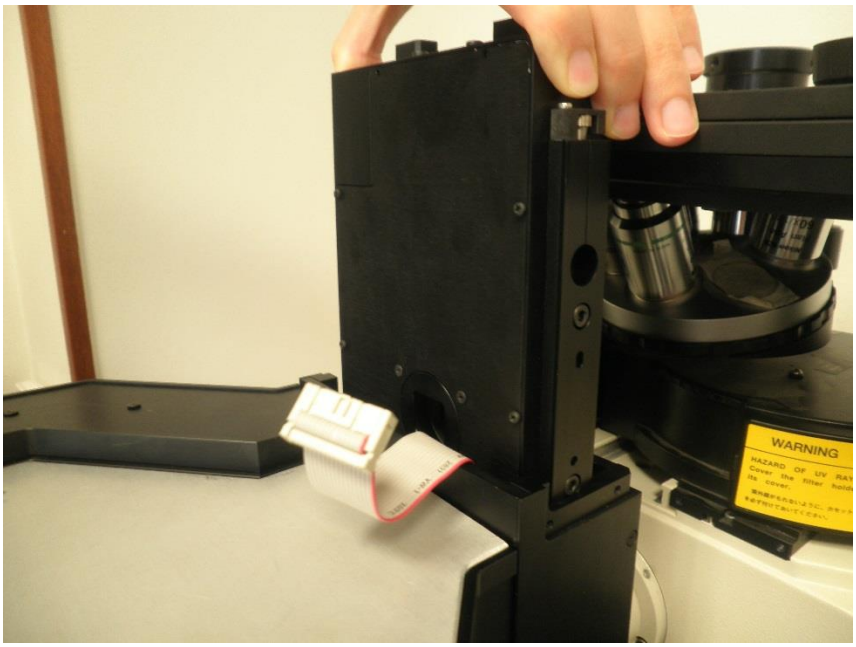


Disconnect the flat cable and unscrew the two hex screws that keep the disk blocked in the housing



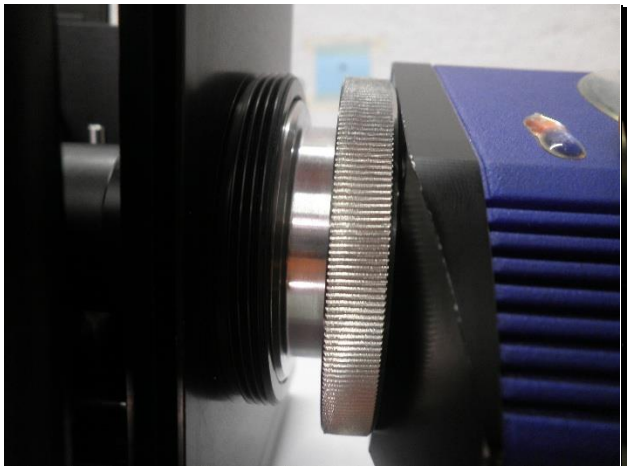


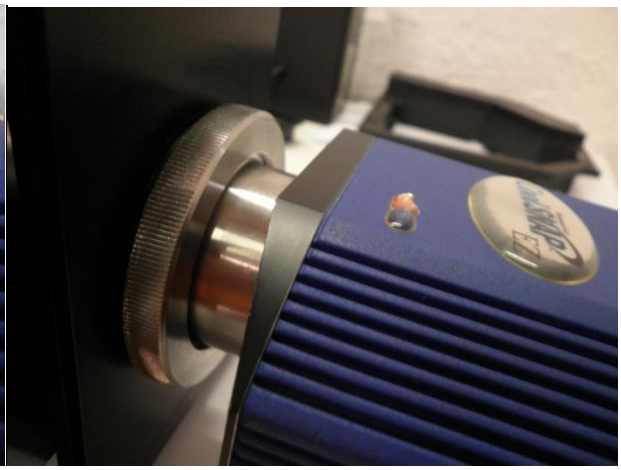
You can finally lift the spinning disk box



2.6 Attaching the camera

A range of CCD/sCMOS cameras can be used with the X-LIGHT V2^{TP}. Mount the CCD/sCMOS to the C-mount confocal head adapter. You will need to mount the c-mount and the ring at the same time (see pictures). Secure the camera in its final position by blocking it with the threaded ring (see pictures).





2.7 X-LIGHT alignment

Focusing the pinholes

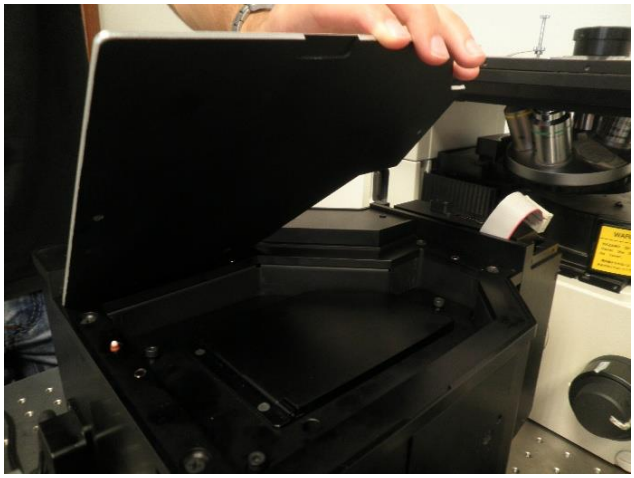
The spinning disk is in a plane conjugate to the image plane. As it spins, it scans the microscopic fluorescent image point by point in real-time. It is essential that the CCD/sCMOS cameras be precisely focused on the image plane (the pinholes) of the spinning disk in order to obtain a sharp image.

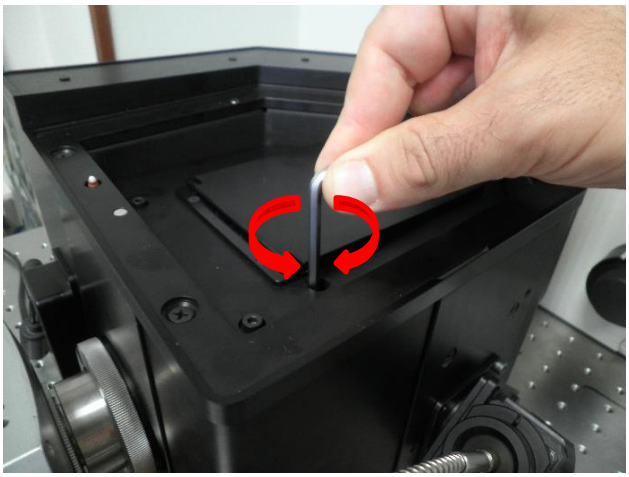
Turn on the bright field lamp of the microscope and send the light to the side port of the microscope. You don't need a sample on the microscope. Using your imaging software do the following:

(1) Select the GFP filter set in the light path
(2) Move spinning disk into light path (confocal mode)
(3) Stop the disk from spinning
(4) Switch the camera into live mode using the imaging software
(5) Adjust the exposure time to 50 ms and then adjust the bright field lamp power until a non-saturated image of the pinholes can be observed
(6) The pinholes should be close to focus. The idea is to adjust the X-Light V2^{TP} focusing lens (see picture) until the pinholes are in focus and the image is sharp.

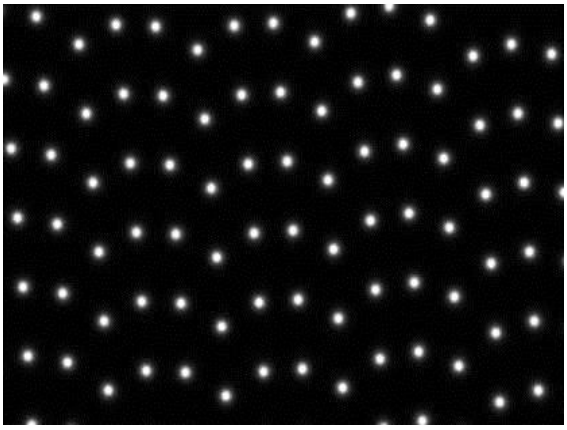
Remove the cover

Rotate the hex key to focus

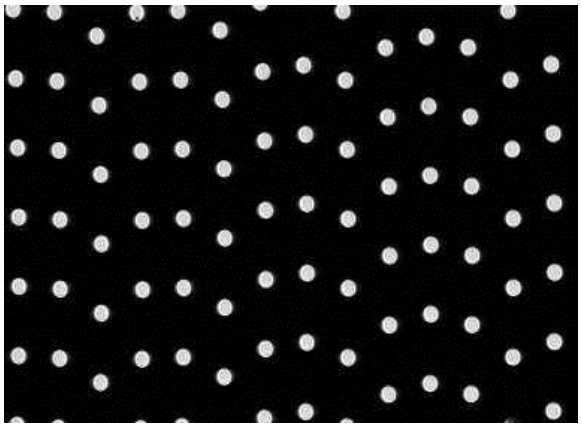




Pinholes not in focus



Pinholes in focus



When mounting the confocal head be sure it is levelled on the same microscope plane

WHENEVER YOU ARE USING UV ILLUMINATION OR LASER SOURCE IS ABSOLUTELY NEEDED TO PROTECT YOUR EYES BEFORE TO DO THIS OPERATION. PLEASE WEAR PROTECTIVE GOOGLES.

Adjusting the excitation light path

The X-Light excitation adapter is pre-aligned by factory. Nevertheless some further fine adjustments may be required in order to optimize illumination efficiency.

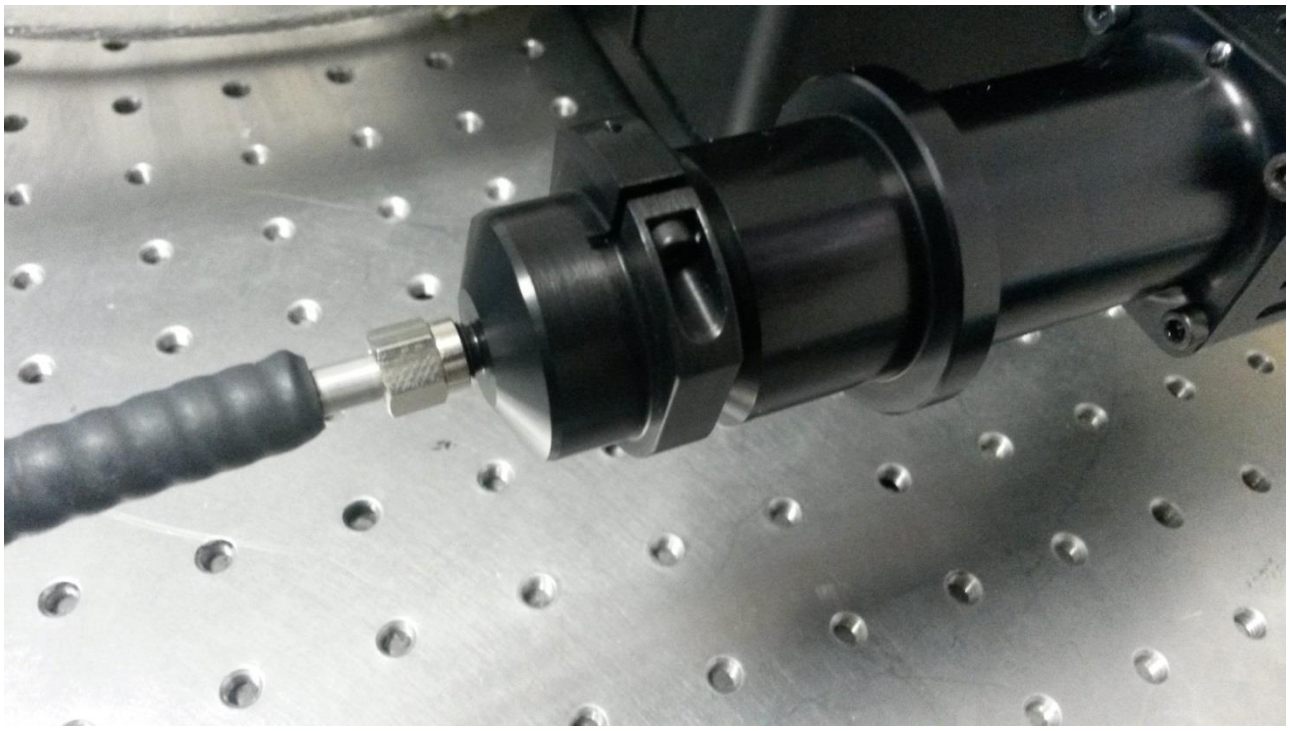
For this purpose we introduced angular and XY adjustments.

Fig 1.2 CREST X-Light V2 excitation SMA adapter.

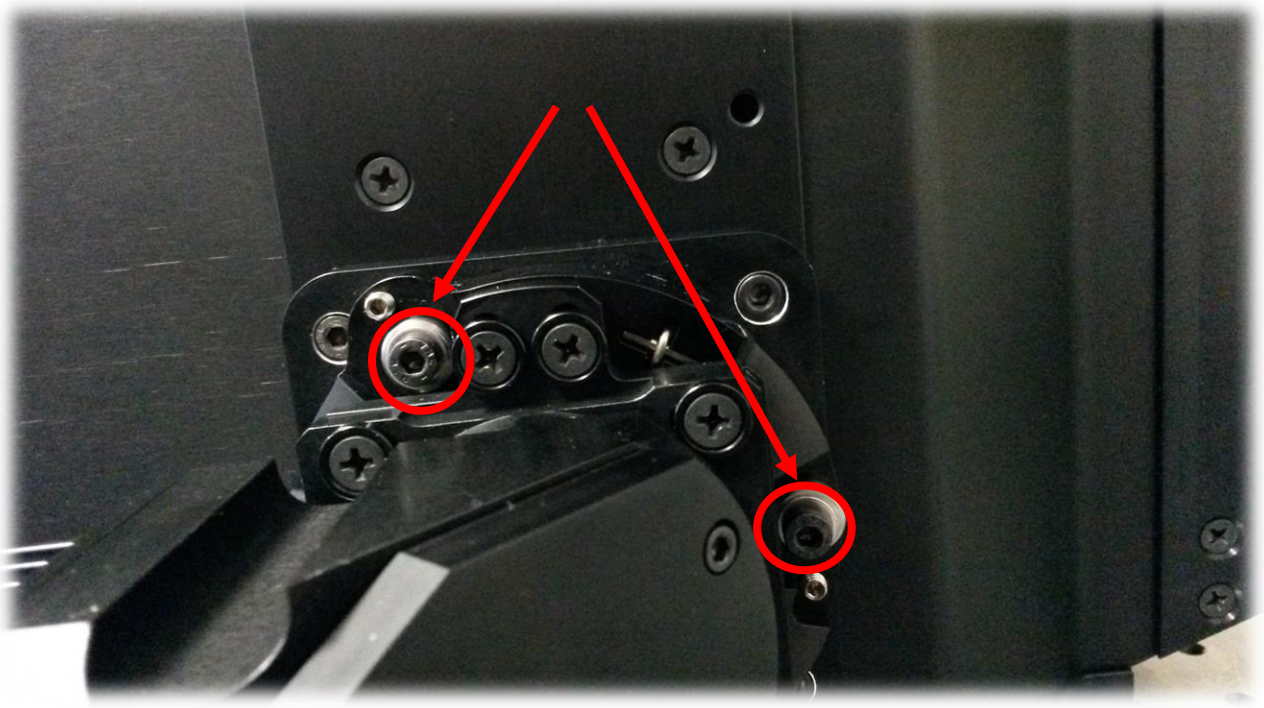
ALIGNMENT PROCEDURE:

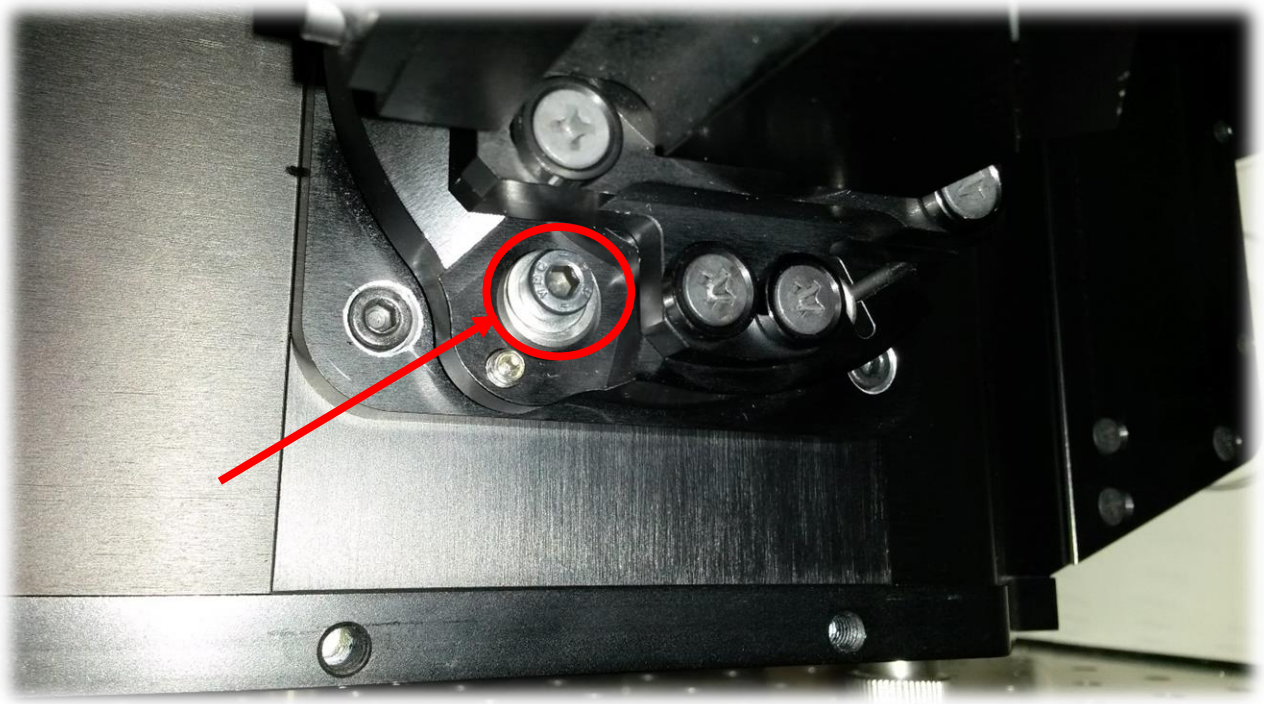
Excitation angle

1. Connect your excitation source, to the excitation SMA adapter

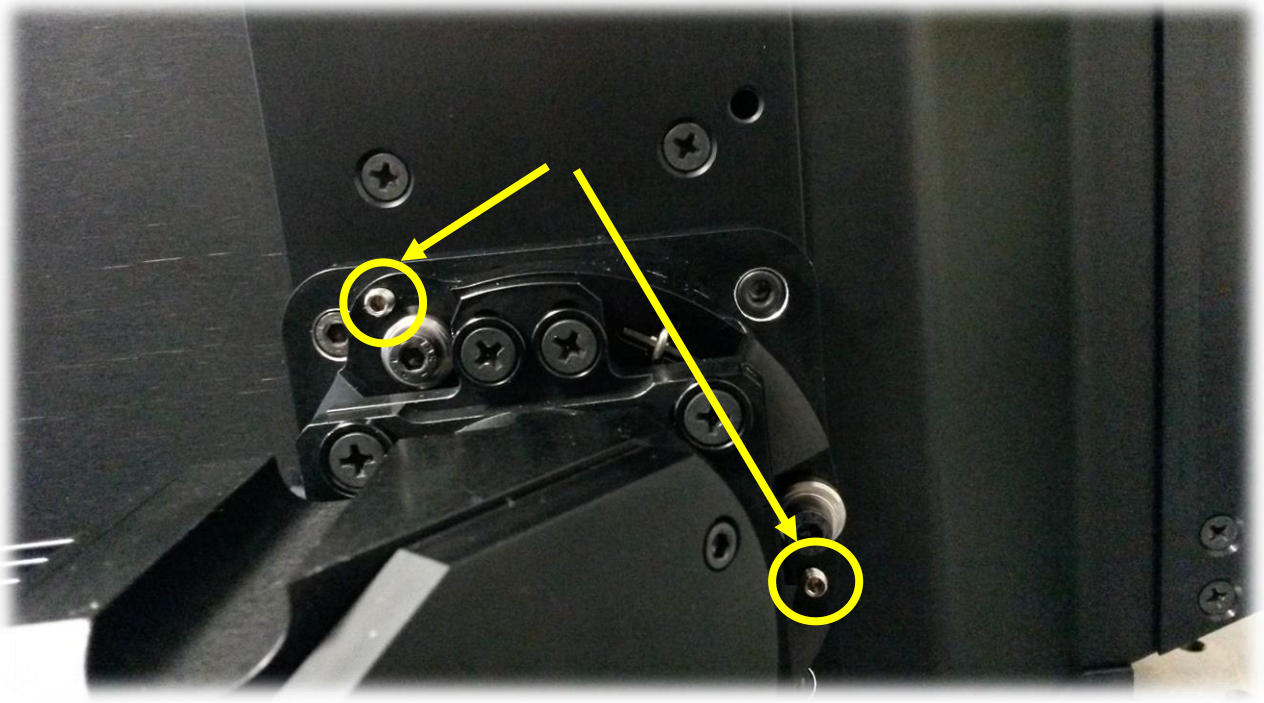


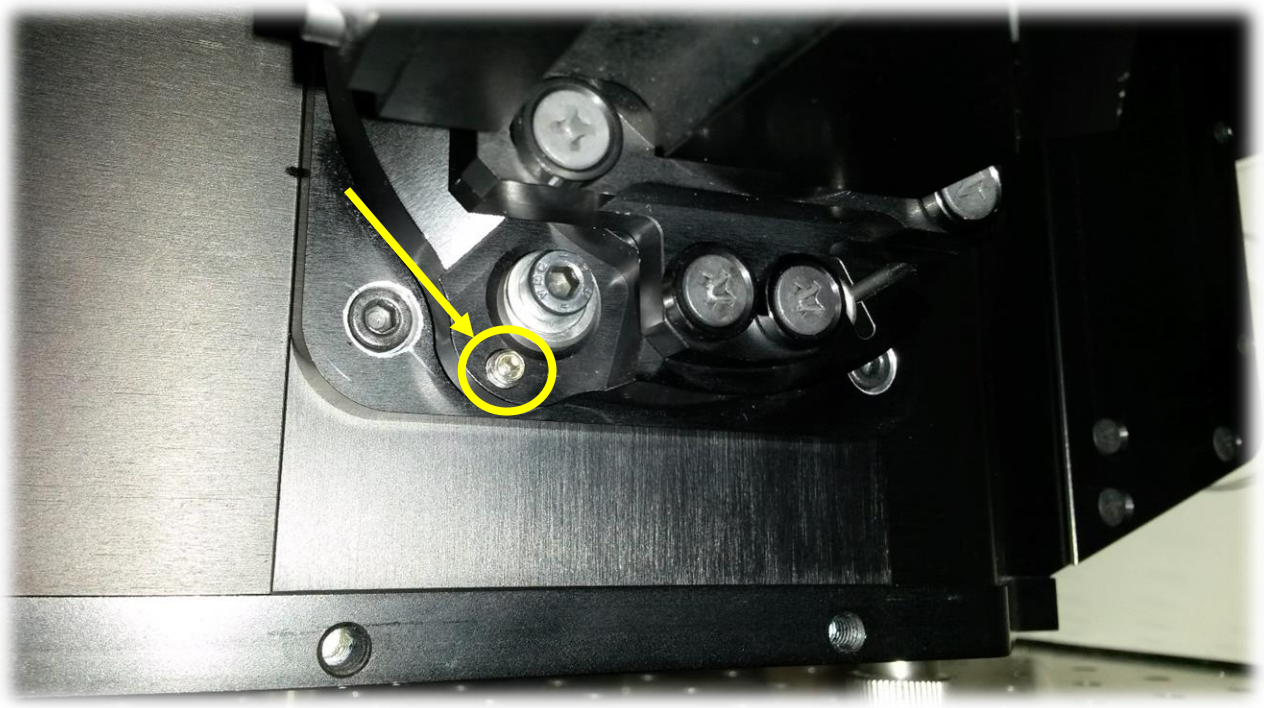
2. Unloose the three hexagonal screws circled in red (see picture)

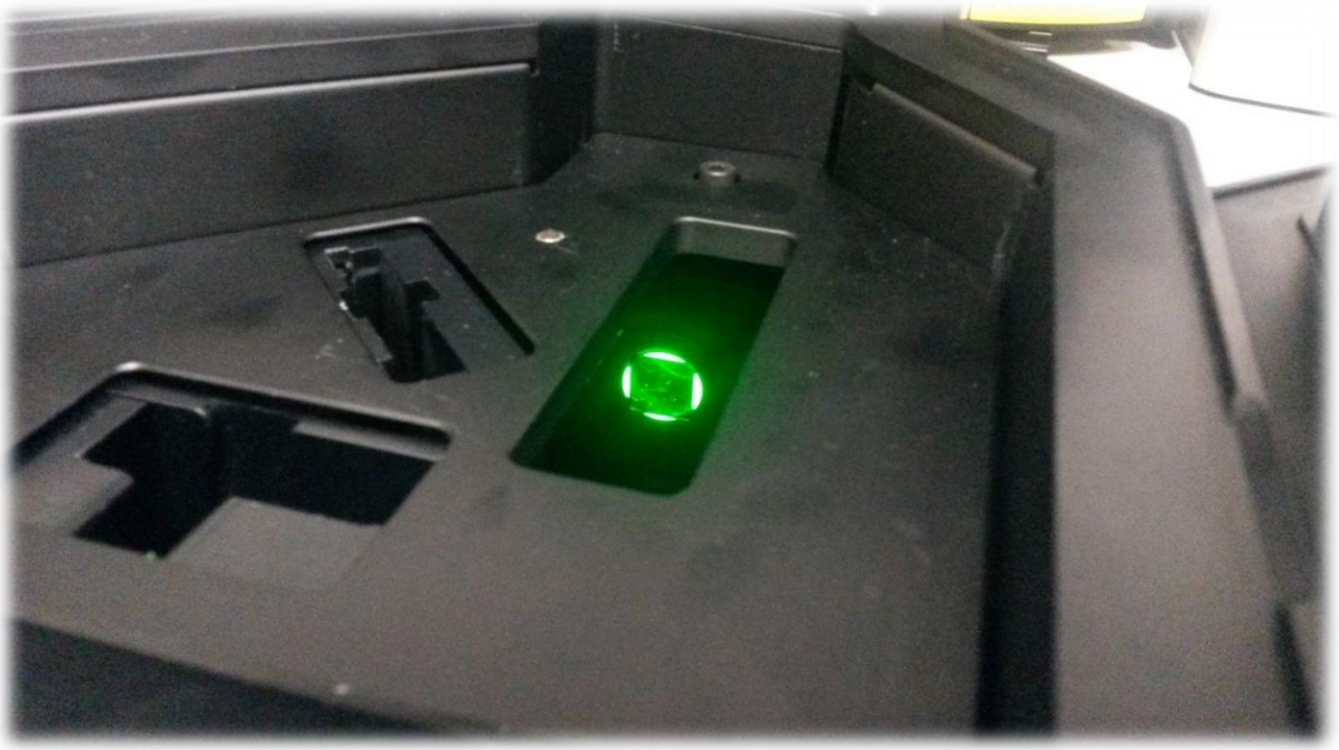




3. Adjust the three rods circled in yellow (see picture) in sequence until you can center the illumination spot on the spinning disk box window





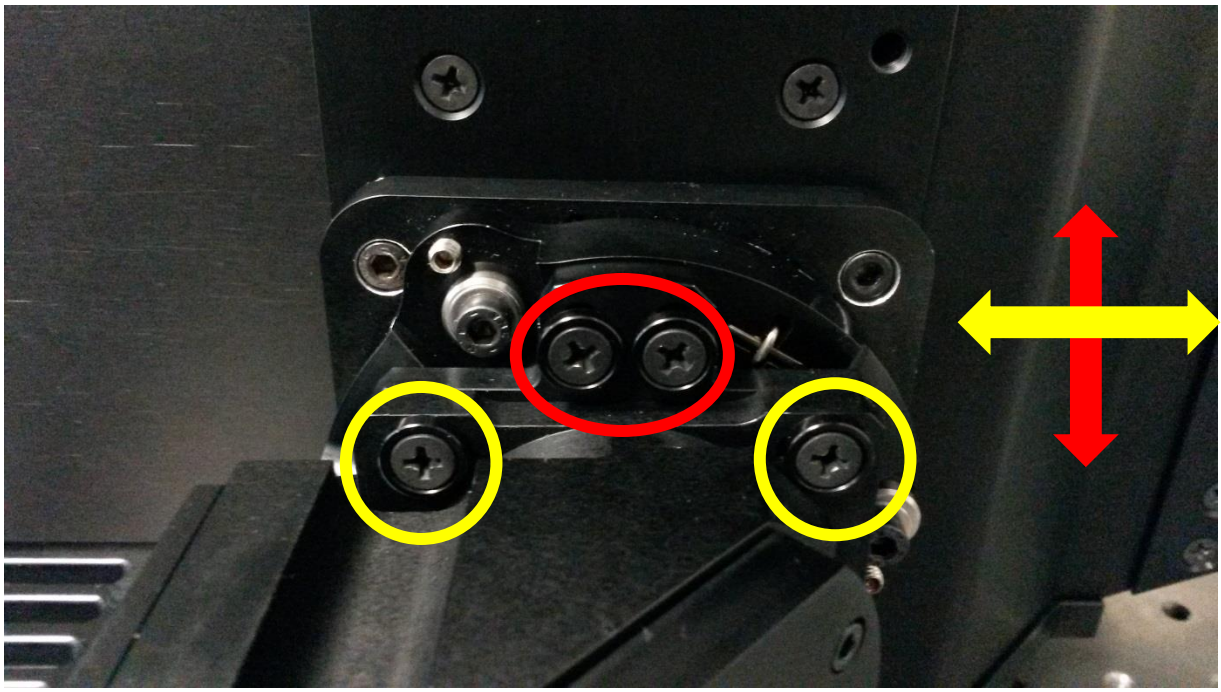


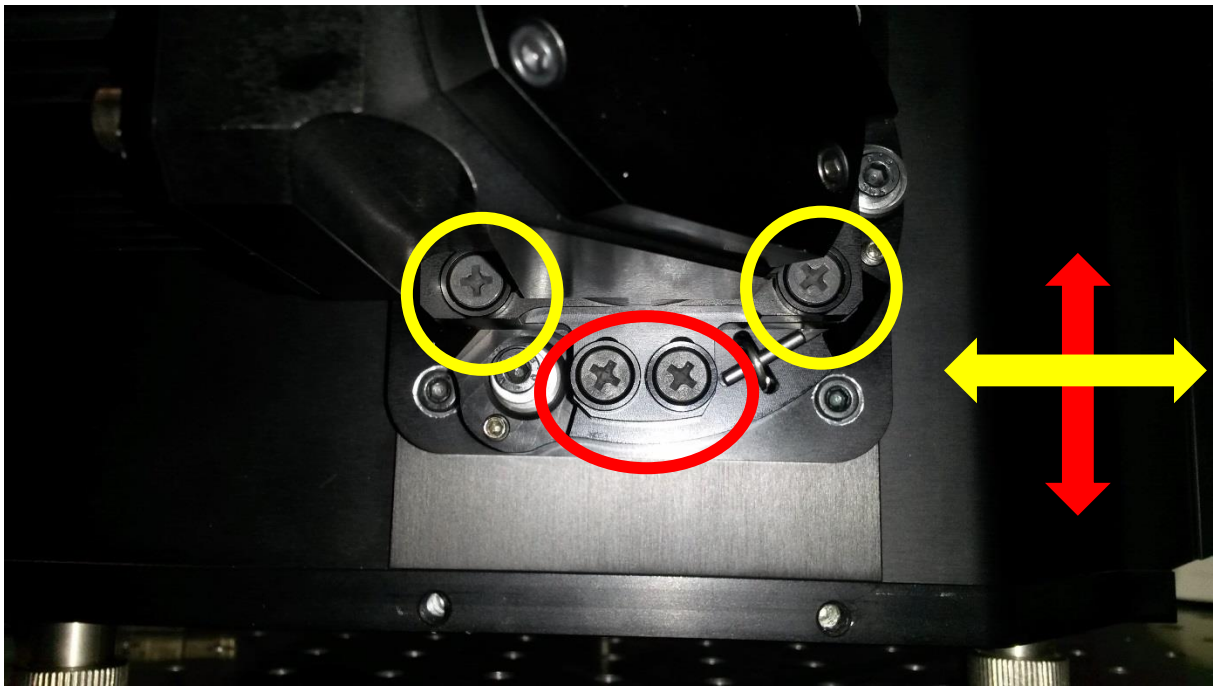
4. Tighten hexagonal screws in sequence to fix the gimbal mount excitation position. Make sure your light path stays aligned during this step.

XY adjustment

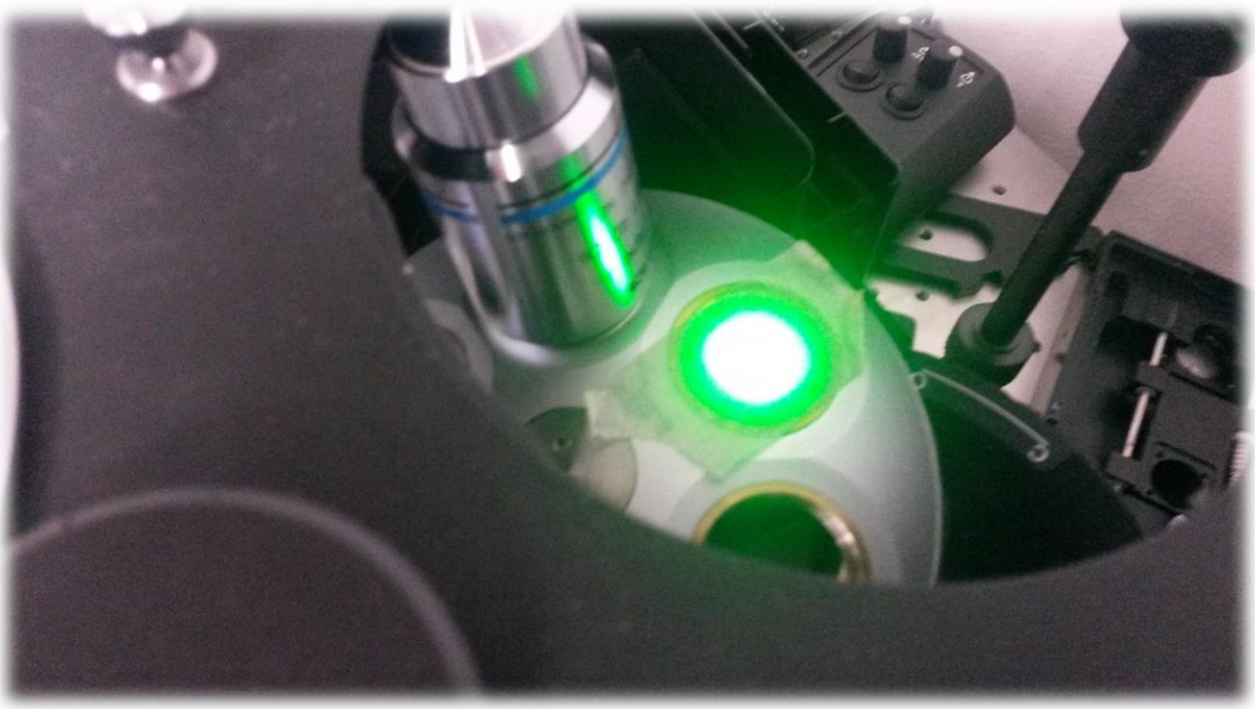
Additionally, verify the beam spot alignment at the entrance of the objective through the XY Gimbal adjustment.

1. **You have to adjust one axis at a time.** You have 4 screws for **X axis** (circled in red) and 4 screws for the **Y axis** (circled in yellow). The red and yellow arrows show the corresponding adjustment directions. See pictures below





2. Adjust Gimbal overall position <XY> until you center the spot at the entrance of the objective



3. Tighten XY adjustment screws to fix the gimbal mount excitation position.

N.B. An iteration of both angle adjustment and XY adjustment could be needed to optimize alignment

2.8 Maintenance

The system doesn't need maintenance.
In case of malfunctioning the system shall be shipped to the supplier to be checked by our authorized engineers. Not being permitted the opening of the system and the access to its internal components, the system hasn't got risk for the user.

2.9 Installing X-Light Drivers

Connect the USB cable to the X-Light V2^{TP} unit and to the computer. Wait that the system discovers the new hardware. When the 'Found New Hardware Wizard' comes up select the folder "X32-X64 bit Windows" on the CD supplied with X-Light and install the drivers.

You can refer to the internet page **http://www.ftdichip.com/FTDrivers.htm** to get latest version of the drivers and for support.

3.0 X-Light V2^{TP} options

3.1 VCS – Video Confocal Super-resolution

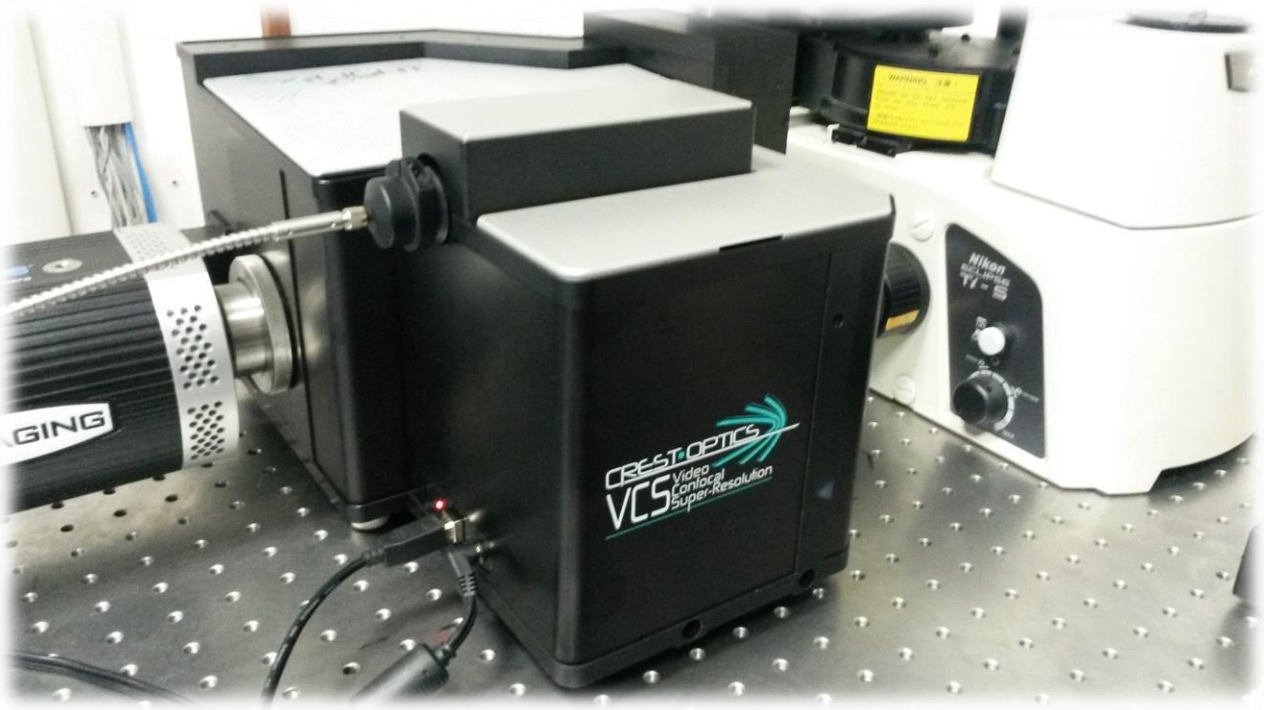


Fig 1.2 CREST X-Light V2^{TP} with VCS complete system.

3.1.1 Crest VCS description

Crest VCS is an add-on module for X-Light V2^{TP} resolution enhancement.

Specifications

VCS scanner	Glass mask with double square grid hole pattern
Pattern specifications	• 13um diameter, 78um pitch (grid) 13um diameter, 78um pitch (slits)
Spectral transmission	360 nm – 700 nm
Illumination source	Lumencor LEDs Systems, Laser illumination System

Observation	EMCCD cameras or sCMOS camera port with fixed position; camera pixel ≤ 6.5um for higher sampling
Microscope Compatibility	Inverted fluorescence microscopes with 100% camera port
Control	USB serial control
Software	Metamorph, Nis-Elements
Power*	15V Vdc 1.5A, 47-63Hz

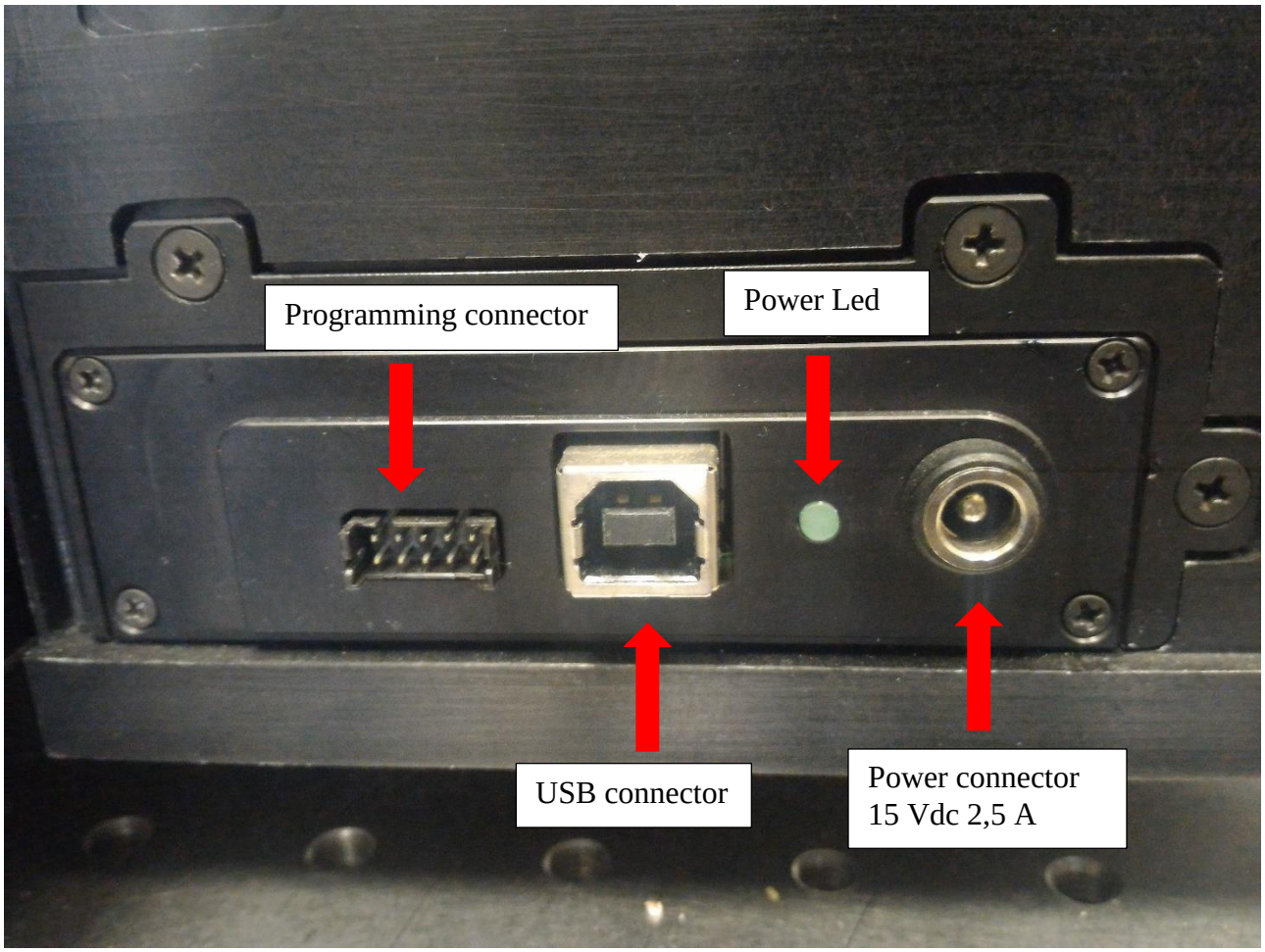
*If you use external power, specifics are: Vin 110-240 Vac, Vout 15Vdc, 2.5A

Key features:

- Bypass mode for wide-field illumination and acquisition
- Motorized control for VCS pattern focusing and color correction
- Piezo motors system for structured illumination scan
- Two patterns on the same physical glass mask
- Motorized mask positioning from VCS mode to wide-field and confocal mode
- Excitation mount for easy alignment
- LED and Laser excitation mounts for high efficiency coupling
- Adapter for SMA-905 fiber excitation

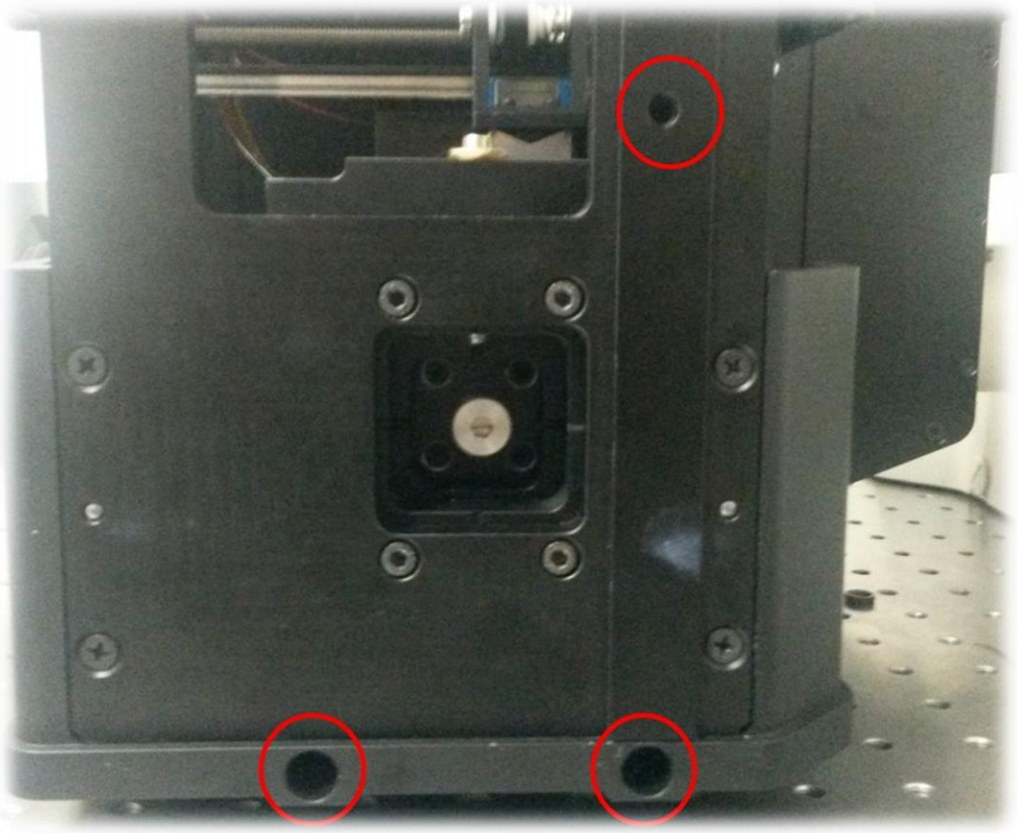
3.1.2 VCS Connectors:

To power the unit just insert the connector.
The power Led will flash at the start and during the whole hardware initialization procedure

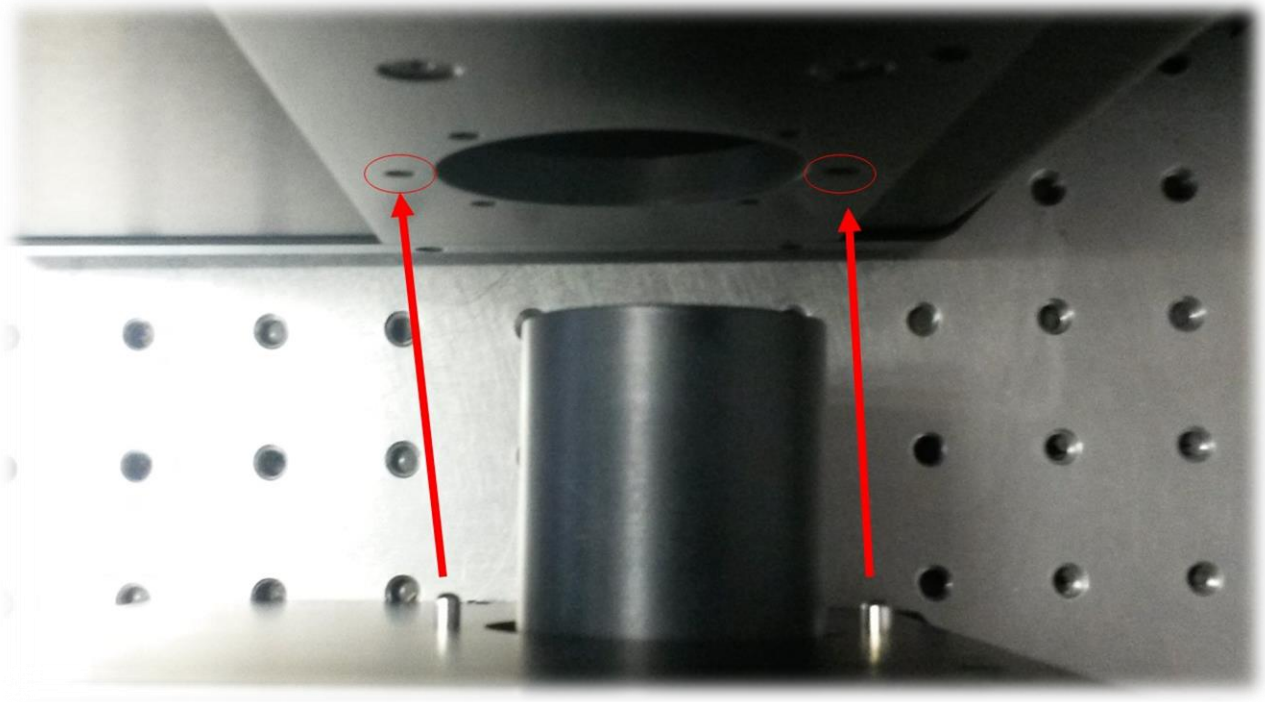


3.1.3 Alignment procedure:

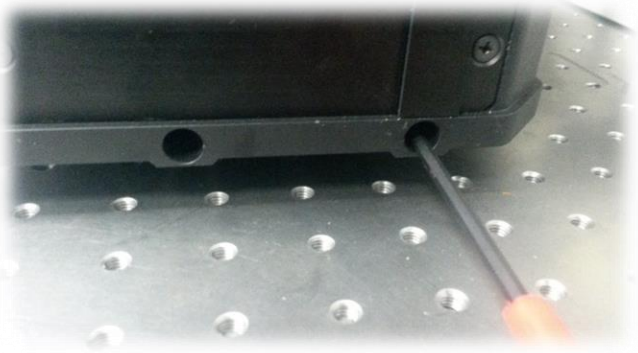
VCS module has three hexagonal captive screws that allow the connection to the X-light V2. Screws are already mounted inside the module (see picture below)



VCS module is also provided with two pins. During the VCS module connection, before screwing, be sure to insert the pins in the corresponding holes on the X-light V2 side (see below).



Once inserted you can screw



VCS module has four major degrees of freedom to accomplish the alignment:

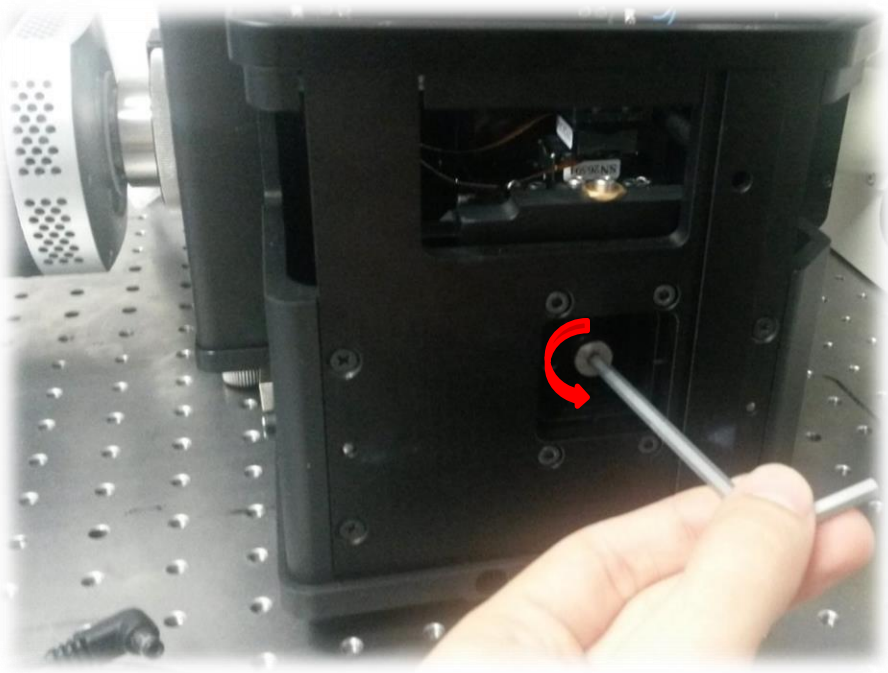
- Traslational degree in X and Y with respect to the X-light V2
- Angular degree in sagittal and tagential plane with center of rotation coincident with the center of the dichroic mirror

The following procedure shows how to perform the alignment:

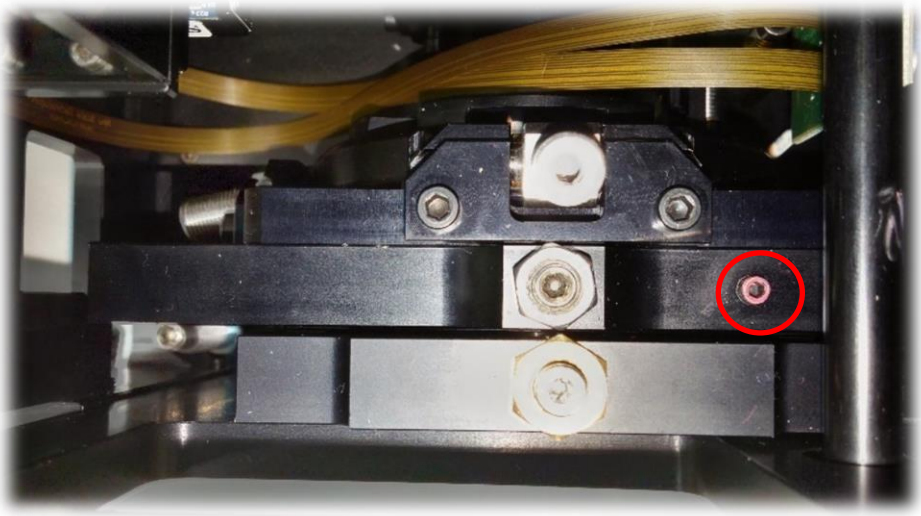
1. Remove the X-light V2 top and verify to have a clear visibility of the spinning box window

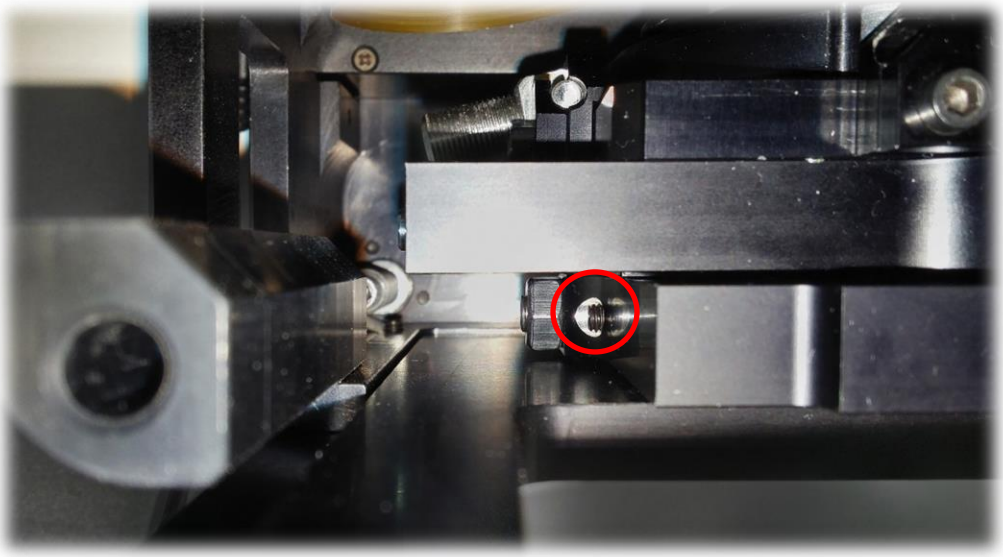
2. Remove the VCS lateral panels. They are held by magnets.

1. Loosen the rear hexagonal screw you see in picture below. **A quarter of one complete turn is required.**



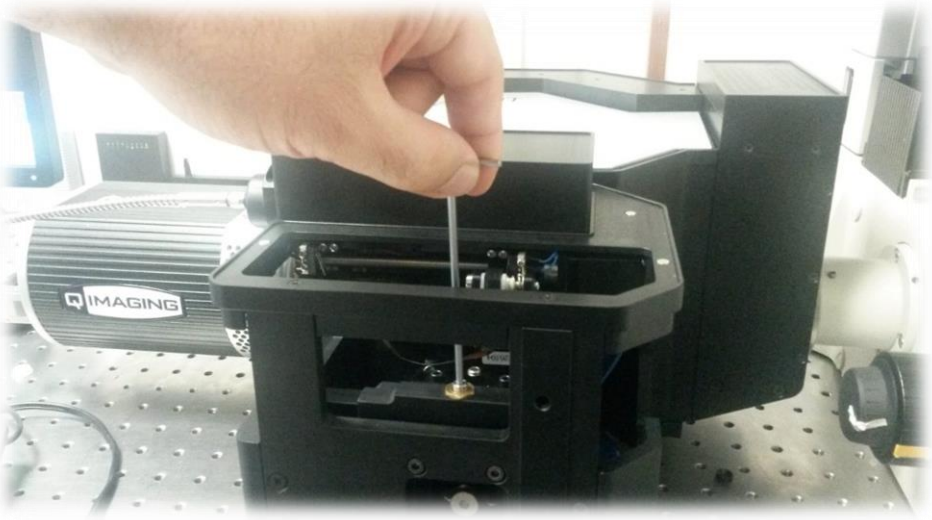
2. Loosen the 2 little grub screws (see picture below circled in red). No more than a complete turn is required. Do not turn more than a complete turn.



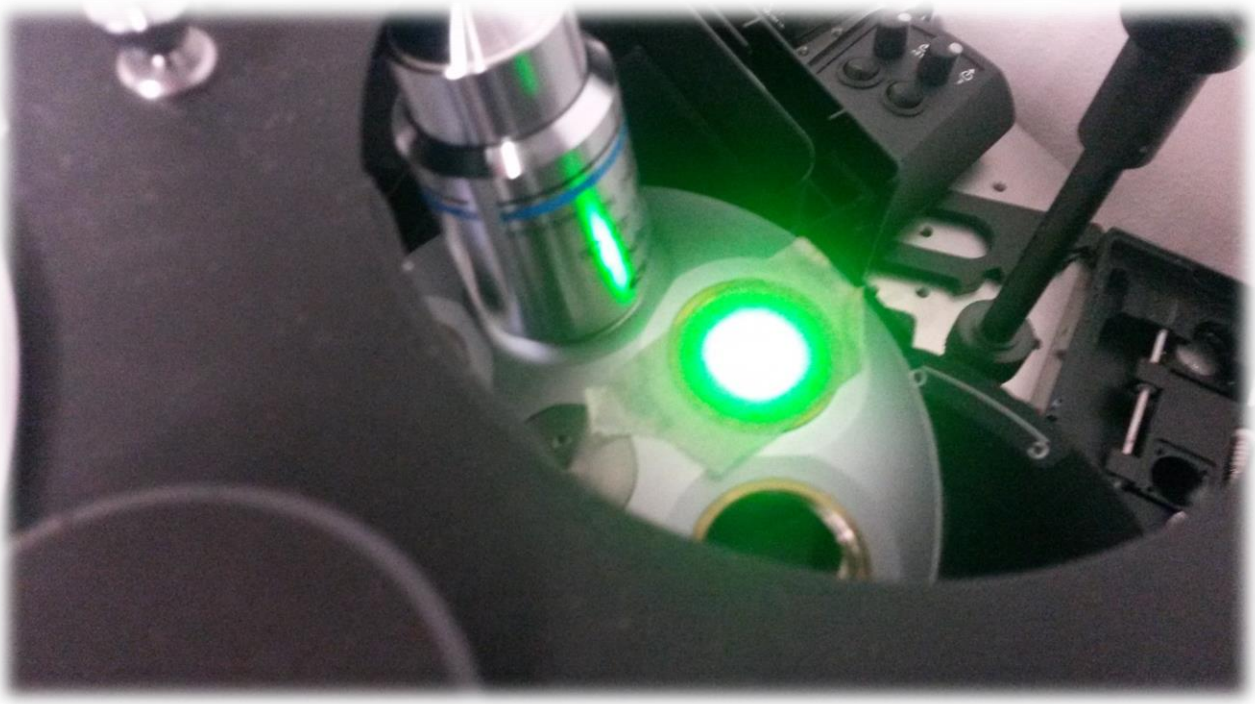


XY adjustment:

2. The hexagonal screws for X e Y adjustament are respectively shown in the two pictures below



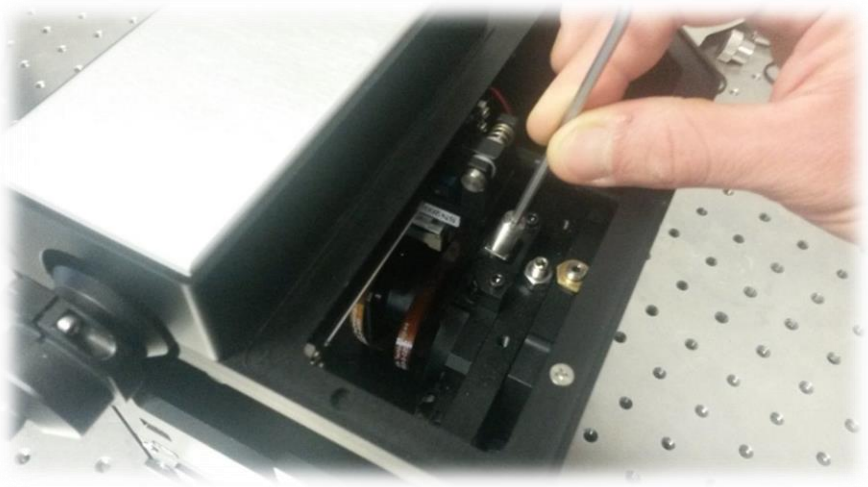
3. By the XY adjustament try to center the beam at the objective entrance



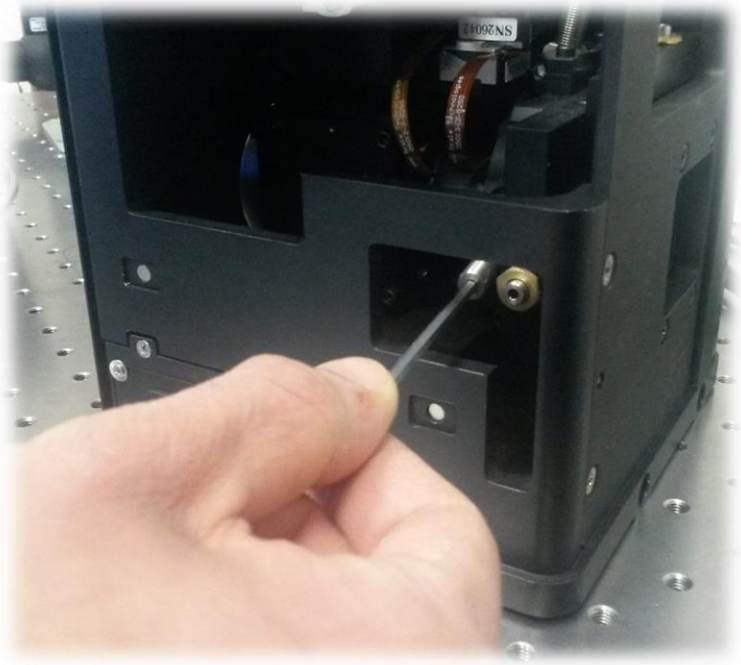
Angle adjustment

3. The two angles are adjustable by the two screws you see in picture below.

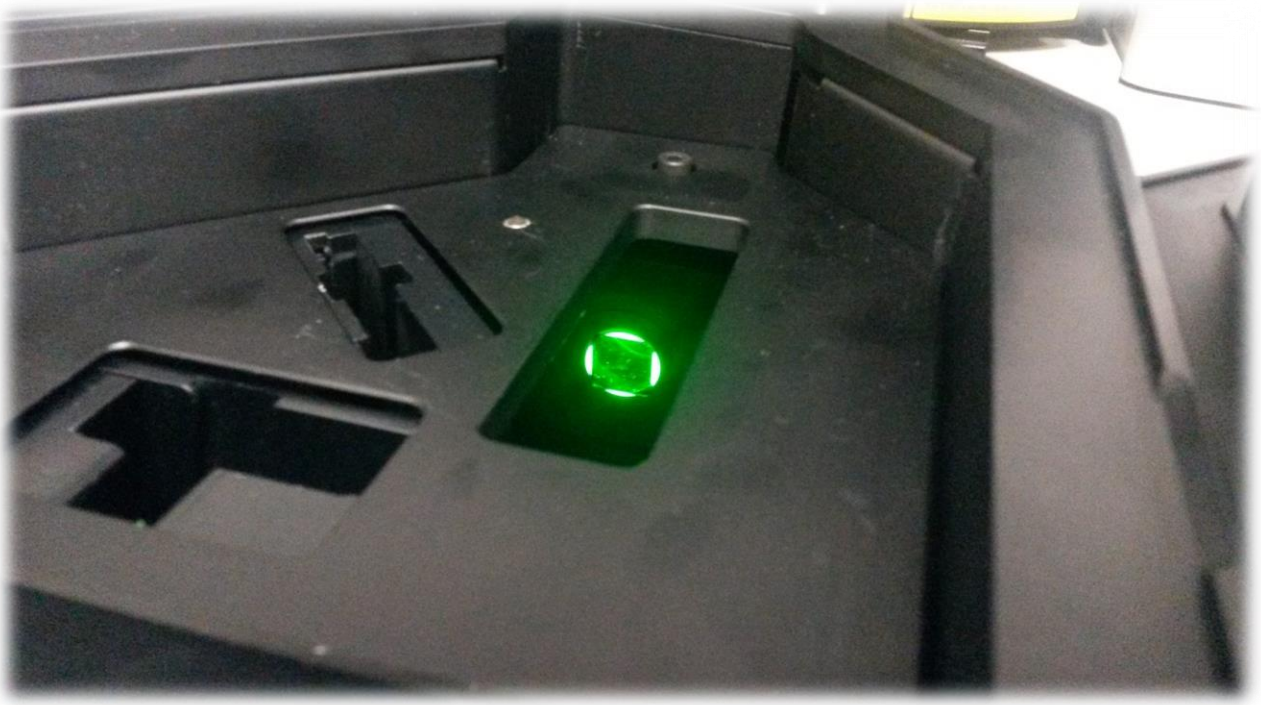
Sagittal plane angle



Tangential plane angle

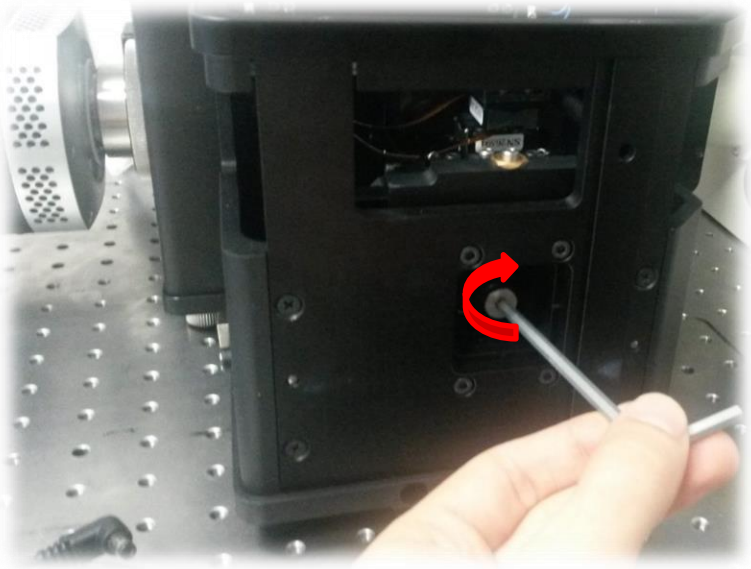


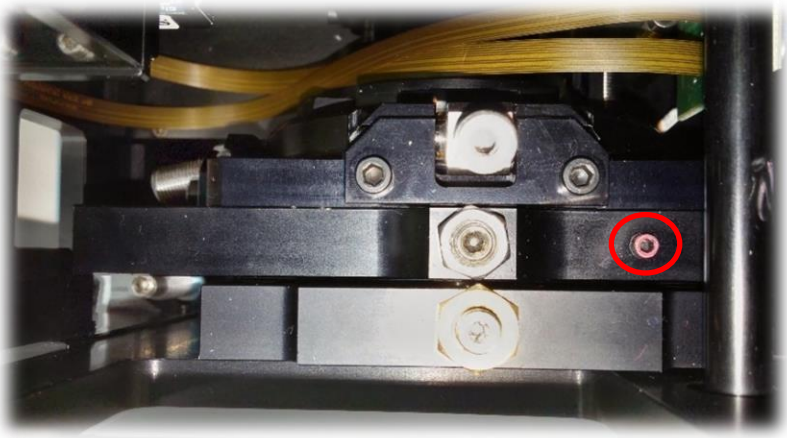
4. By angle adjustament try to center the beam on the spinning box window (see figure below)

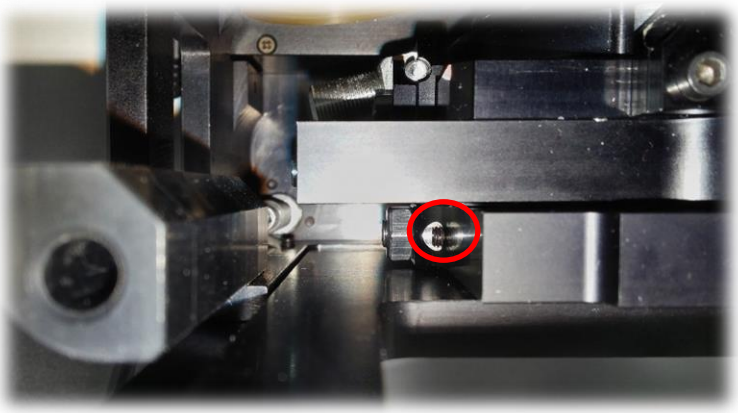


5. If at the end of the procedure the spot at the entrance of the objective has been decentered, iterate the procedure.

When the illumination is aligned, tighten back the rear hexagonal screw and the 2 little grub screws

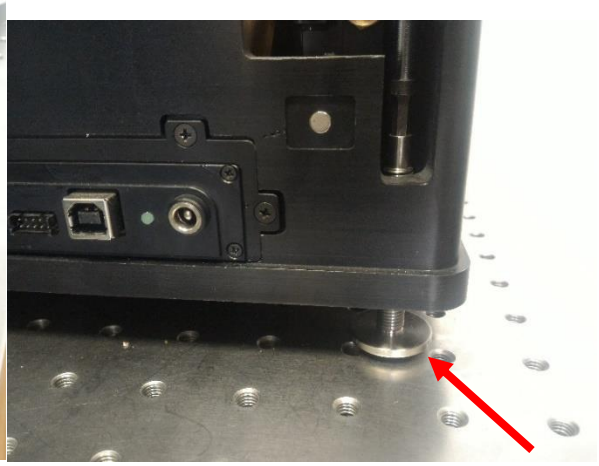






Supporting foot for VCS balancing

VCS module is equipped with a balancing foot which must be propped against the table by screwing a hexagonal screw inside the unit (see pictures below). The hex key that can be used is the long 5mm hex key shipped in the tool box



3.1.4 VCS Mask replacement

VCS module will be shipped with a mask that is separate from the unit and with the following specifications

1. Double pattern mask:
    a. 13um pinholes 78um pitch (grid)
    b. 13um pinholes 78um pitch (slits)

Replacement procedure

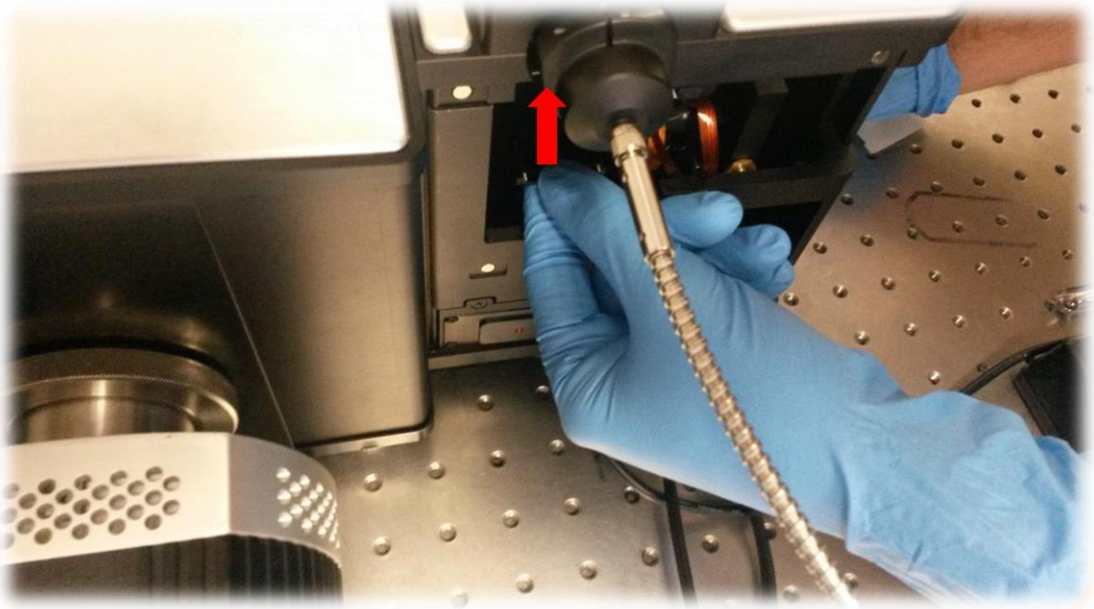
Use gloves for the entire procedure

Insertion:

1. Put the piezo motors group in the fourth position (command “D3”, maintanance mode)
2. The glass mask has an arrow on the side. It must be inserted with the arrow pointing toward the light source.

Extraction:

1. Put the piezo motors group in the fourth position (command “D3”, maintanance mode)
2. Gently push the mask upwards on the outer side. A little lift is necessary to disengage the mask from the mounting



3. Pull out the mask

4.0 X-light command sets

X-light V2^{TP}

Regardless of the controlling application, the settings for the COM port used to connect to the X-LIGHT V2^{TP} are as follows: **9600 baud, 8 data bits, 1 stop bit, No parity, and no flow control**.

Devices

The X-LIGHT has three individual devices that can be actuated to affect the light path. Each device has a single capital letter name (Device ID), which is used to address the individual device with the commands that are described later in this manual. The number of allowable positions differs for different types of devices and they are all listed in the chart below.

X-LIGHT V2^{TP}

DEVICE	DEVICE ID	Valid Positions	Homes To:
Dichroic Wheel	C	1, 2, 3, 4, 5	Position 1
Confocal Disk Slider	D	0,1,2	0=Disk Out 1=Disk position 70um 2=Disk position 40um
Confocal Disk Motor	N	0,1	0= Disk Off (not spinning) 1= Disk On
Autom. Emission Wheel	B	1,2,3,4,5,6,7,8	Position 1
Setting Command	Command	Valid Position	Homes To:
Select baud rate	W	0,1,2,3,4,5,6	Setting baud rate 0=9600 baud (default) 1=19200 baud (no set) 2=38400 baud(no set) 3=57600 baud(no set) 4=115200 baud (*Note) 5=128000 baud(no set) 6=256000 baud(no set)
Rehome All Devices	H		C1,D0,N0
Response Activation	R	0,1	0=no response sent to host (default) 1=responses sent to host

Note: the baud rate pos. 4 is only for Xlight unit 20Krpm .

When the X-LIGHT is first turned on all the devices are initialized to their “Home Position” listed in the chart above. The “H”, Home command can also be used to return all the devices to these positions.

When you switch-on the power supply the self-test of all functions are running.

Command Conventions

Regardless of their origin, all commands must adhere to the following conventions:

1) All commands must be terminated with a carriage return <CR>. The X-LIGHT II will not even attempt to recognize any characters sent on the serial port until it receives a <CR>.

2) Syntax errors in commands are known by receiving a plain <CR> response (no command echoed).

3) When the entire command string (all strings preceding the <CR>) has been processed the entire command string is echoed back to the PC host. The exceptions are the lower case commands. Lower case commands signify the command requests information from the X-LIGHT. The responses to these commands append the requested information to the original command. Erroneous commands, either invalid Device ID or invalid parameters, are simply ignored but are echoed back to the host as they were sent; in any case the board answer with a CR.

Command Formats

In the following command descriptions the **Format** describes how the command should look as an individual command. **Parameters** describe any characters that must accompany the command or are returned with the echo of the command. The **Return Value** describes how the echo of the individual command will look. The **Example** illustrates the sending of the command and its subsequent echo using actual parameters.

DEVICE ID – Move Device (C , D , N)

Format	<device ID><position><CR>
Parameters	<device ID> ID assigned to a device (see table on page 1)
	<position> Desired position or state
Return Value	<device ID><position><CR>
End of action execution:	<device ID><position><CR>

Examples D1<CR> → D1<CR> Disk 40um

C2<CR> → C2<CR> → Dichroic Wheel 2

D2<CR> → D2<CR> → Disk 70um

Macro available:

BxCx<CR>

Examples B2C3<CR> → B2C3<CR> Dichroic Wheel 2 Emission Wheel 3

Available on version > 2.4.4

If any device not responding the system send: <device>0<CR>

Examples **B2**<CR> → B0<CR> Dichroic Wheel is damaged

H – Rehome all devices

Format	H<CR>
Parameters	NULL
Return Value	setting: HB1C1D0N0<CR>
Examples	H<CR> → HB1C1D0N0<CR>

R – Response Activation: Turn on/off responses to Host

Format	R<active><CR>
Parameters	<active> 0 = no response sent to host (no echo) 1 = responses sent to host
Return Value	If <active> = 0, no response If <active> = 1, R1<CR>
Examples	R0<CR> → R1<CR> → R1<CR> this command generate always a response to the host.

q – Query State of All Devices

Format	q<CR>
Return Value	q <B'N'><C'N'><D'N'><N'N'> <CR>
Examples	q<CR> → B1C1D1N1<CR>

r – Read Current Position of Individual Device

Format	r<device ID><CR>	
Parameters	<device ID>	ID assigned to a device (see table on page 1)
	<position>	Current position or state
Return Value	r<device ID><position><CR>	
Examples	rC<CR> → rC1<CR>	The dichroic filter wheel is in position 1.
	rD<CR> → D1<CR>	Disk 40um
	rB<CR> → B1<CR>	emission wheel1
	rN<CR> → N1<CR>	Disk on

v – Read Firmware Version

Format	v<CR>	
Parameters	<version>	version of touch screen firmware
Return Value	v< version ><CR>	
Examples	v<CR> → Ver. 2.0.1.<CR>	
	The firmware version is reported as Ver. 2.0.1.	