

Edge 3D Microscope

Powering on the system:

In order to use the edge 3D microscope, you must be trained by one of the CSIF staff. If you are the first session of the day, you will need to power on the entire system as follows:

1. Mercury arc lamp - flip the red toggle switch up on the lamp power supply located on the left side of the microscope. Then move the trigger to manual to spark the lamp.

Note: Please check log sheet before turning on mercury bulb - these bulbs are under pressure and they must be *off* for 45 minutes before you can safely turn them on again. They must also be *on* for 30 minutes before being turned off. DO NOT use the microscope for 10 minutes and then shut everything off. Notify one of the CSIF personnel and they will turn the bulb off when it is safe to do so.

2. Turn on the power for the control box - flip the white switch to on.
3. Turn on the control box - located on the right side of the microscope, turn the dial on top of the box to turn on the transmitted light and the z-axis focus control motor.

To turn off equipment

1. Follow the above instructions backwards (step 3 to step 1).
2. Turn off cameras by setting command dials to L and IR remote control to L.

Make sure that the equipment is turned off at the end of your session if no-one is around or it is after hours. The mercury arc lamp lifetime is only 200.

Viewing specimen through eyepieces:

Each eyepiece has a polarizing filter which provides optimal left / right extinction for 3D viewing. Therefore you must use both eyepieces when viewing your specimen. Be sure to adjust the interocular distance to allow for maximum comfort.

Lenses on the Edge microscope

10x, 0.45 NA

20x, 0.75 NA

40x, 0.70 NA

***All of the objectives are dry / air objectives

Focussing

1. There are coarse and fine focus knobs on both the right and left sides of the microscope base. These knobs are linked by a common shaft and therefore move in unison. Do not turn one knob in the opposite direction of the other.
2. The right hand coarse focus knob operates a variable speed motor, which moves the stage at a rate that increases the farther the knob is turned. The stage's travel range during motorized operation has upper and lower limits which stop the motor from continuing past that point. Do not continue to manually focus past the cut-off point or you may damage the switch mechanism.
3. The distance moved along the z-axis can be measured using the dial on the left focus knob. Each full knob rotation moves the stage 20 microns, with each mark on the dial corresponding to a 0.1 micron stage movement.

Fluorescent illumination

1. Turn off the transmitted light source, or if you wish to use the motorized focus, turn the four individual light controls to zero.
2. Move the slider (on black bar ~5 inches in front of mercury arc lamp) out of its blocking position to allow the light from the mercury arc lamp to reach your specimen. Pull the slider to the right to allow 100% of the light to reach your specimen. Push the slider to the left for a neutral density filter.
3. There are two additional neutral density filters in back of the slider (ND2 and ND4) which you can use if your sample is very bright.
4. Filters - the microscope is equipped with filters for FITC, rhodamine, and a triple dichroic (DAPI/FITC/TRITC). They are located in the slider below the eyepieces. FITC is on the left, rhodamine is in the middle, and the triple dichroic on the right. To see brightfield, CAREFULLY pull slider out, making sure that you don't get fingerprints on any of the glass filters.

Brightfield illumination

1. Move the slider back to the middle position to block the light from the mercury arc lamp.
2. Turn on the control box using the master control dial on its top, which controls the brightness for all four lamps. This must be set to ten to allow the individual lamps to reach their maximum intensity.
3. It is recommended that you experiment with many different settings for each individual lamp. This will allow you to find the highest contrast and depth of field for your specimen.
4. Mirror control - The knob at the front center of the microscope adjusts the height of the pyramidal mirror inside the base, which creates viewing conditions ranging from brightfield to near-darkfield. The numbers on the knob

- represent the relative height of the mirror, with the triangle being the default.
5. 3D viewing or photographing 3D pairs - it is important to keep the right and left lamps at equal settings. In order to increase contrast and depth of field, it may be necessary to turn down the two bottom lamps while keeping the other two on full (or vice versa).
 6. Single picture 3D anaglyphs - left and right lamps may be set at slightly different levels to balance the colored elements of the image.
 7. 2D viewing - image quality may be improved by turning off one or more of the four lamps.
 8. Filter selectors:
(* indicates default setting)

PHOTO

- | | |
|------|---|
| L | Extinguishes the right-side lamps for taking the left picture of a 3D pair (brightfield). |
| *ALL | All four lamps on for observation or for taking 2D photos or single picture 3D anaglyphs. |
| R | Extinguishes the left-side lamps for taking the right picture of a 3D pair (brightfield). |

ANAGLYPH 3D

- | | |
|------|---|
| IN | Inserts magenta filters into the left light channel and green filters into the right light channel for taking single-picture 3D anaglyphs, or for employing the anaglyph 3D viewing method. |
| *OUT | Select for normal 3D viewing, 2D viewing, and 3D-pair-photography. |
| TV | video |

DENSITY

- | | |
|-----|---|
| 1 | Inserts the lighter of 2 neutral density filters. |
| OUT | No filter inserted. |
| 2 | Inserts the darker of 2 neutral density filters. |

FILTERS

- | | |
|-----|--|
| G | Inserts a green filter for use in B & W photography. |
| OUT | No filter inserted. Select when photographing with tungsten-balanced film. |
| B | Inserts a blue correction filter for photographing with color daylight film; can also be used to improve color balance during observation. |

Please see the laminated card for a visual reference of the settings.

Photography

Film:

Each user will be responsible for bringing film for his/her 3D or 2D pictures. To load film, slide down the camera back latch (on the left side of the camera) to open the camera back. Then align the edge of the film leader with the orange mark on the camera and close the camera back until it snaps shut. The film will then advance to frame one automatically. Make sure that the frame counter display shows one. If not, the film needs to be reloaded.

After the last frame is exposed, the camera will automatically rewind the film. When the film is completely rewound, the film cartridge symbol in the lower left of the LCD panel will blink. If it is blinking, open the camera back and remove the film cartridge. To rewind the film before reaching the last frame, press the film rewind button on the back of the camera (it has two underlined arrows next to a circle with a dot in the middle).

Film rewind is normally set to the silent mode. Pressing the film rewind button will switch to high-speed rewind mode.

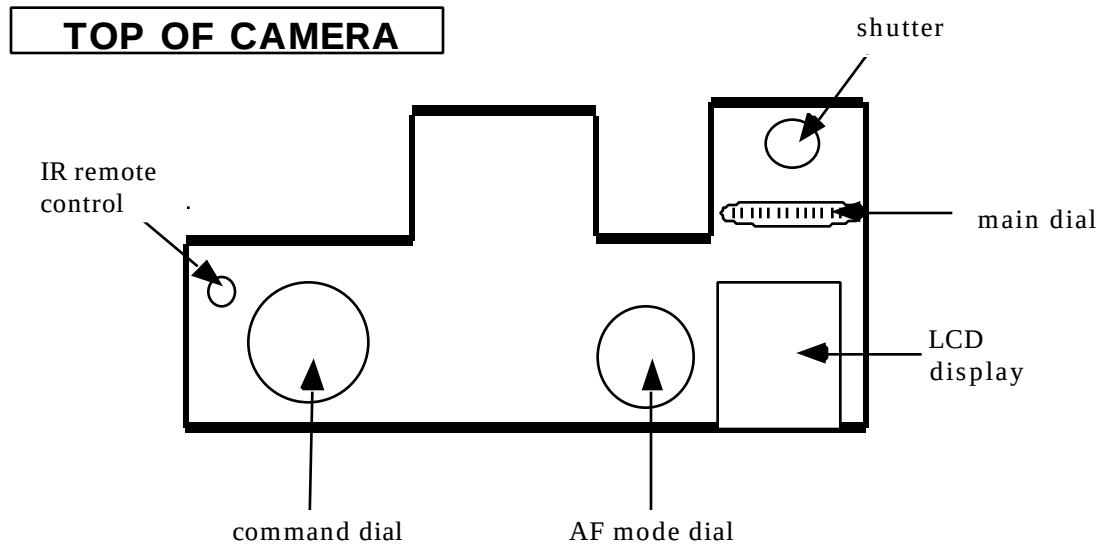
Direct output to camera(s)

There is a black lever immediately below the eyepieces which must be adjusted depending on whether you will be using the eyepieces or the single camera. When this lever is pointing toward you, the light will be directed to the eyepieces. When the lever is to the left, the light will be directed to the single camera. In addition, there is a silver plunger above the eyepieces which must be adjusted. When the plunger is up, the light is directed to the dual cameras. If it is down, the light will go to the eyepieces.

Mounting the cameras

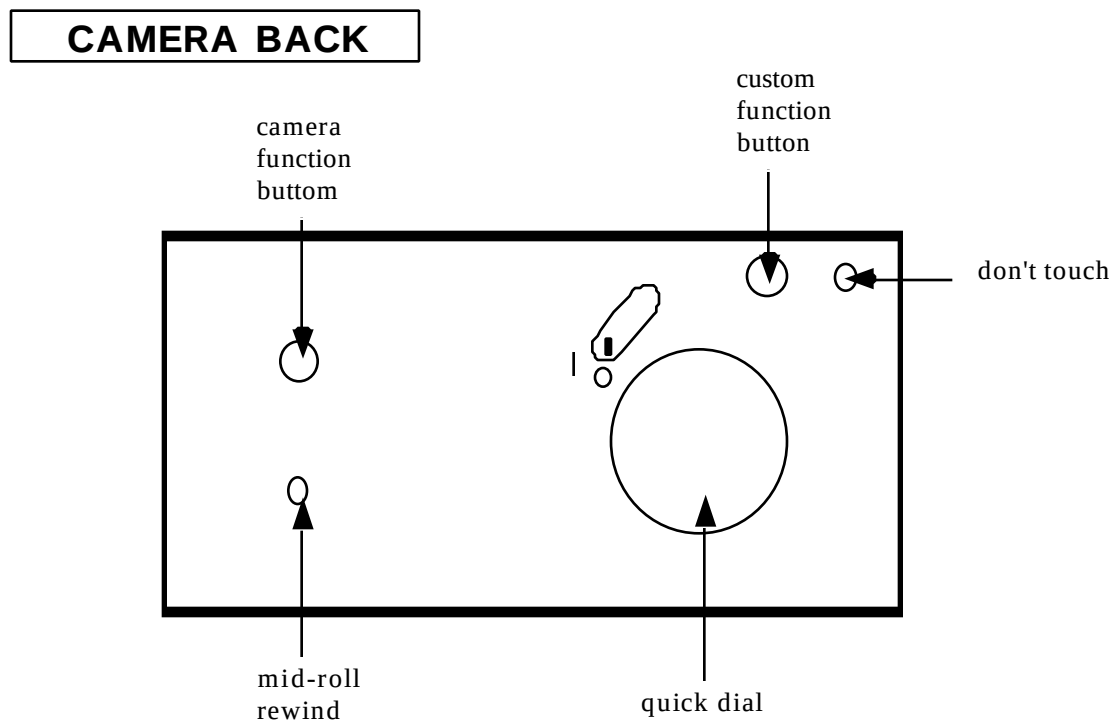
Be very careful when mounting the camera. For mounting at either of the two upper ports, hold the camera upside down and rotate a quarter turn clockwise until the red dot on the camera lines up with the red dot on the camera port. To mount at the lower port, hold the camera with the display panel facing left and rotate it clockwise a quarter turn until the red dots line up. Make sure that the cap is on the port without a camera. To remove the camera, depress the button on the left side of the lens and rotate it in the opposite direction.

Camera settings:



1) Turn on camera: Command Dial

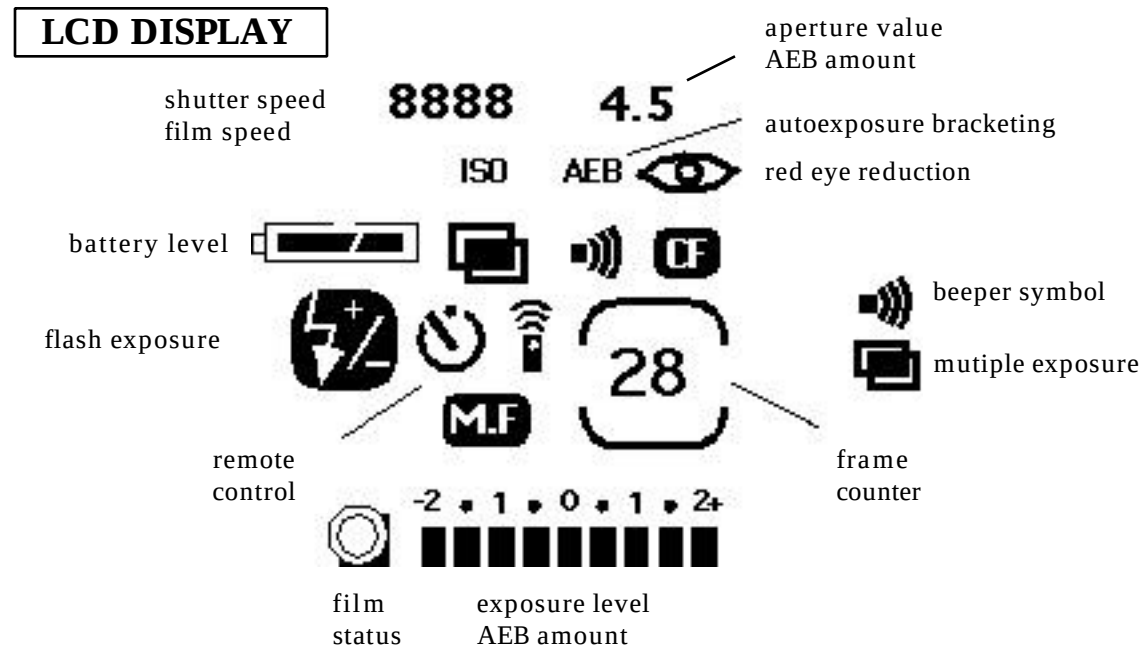
When the command dial on the camera is set to “L” it turns off and locks the camera. To release the lock, hold down the lock release button and turn the Command Dial to the desired setting.



It is recommended that you use the Manual (M) mode or the Aperture Priority AE (Av) mode. The manual setting allows you to set the desired shutter speed, while the Av mode will set this value automatically.

2) Program camera parameters

To access programming parameters, press the small black button on the camera back labelled “ISO / AEB / eye”. Each successive press will toggle you through the selections in order of setting ISO, automatic exposure bracketing, red-eye reduction, beeper sound, multiple exposures, and exposure level.



- film speed - when LCD panel registers “ISO”, turn main dial to number corresponding to the ASA of your film.

- length of exposure

In manual mode, the shutter speed can be seen at the top left of the LCD panel. The shutter speed is displayed with values representing the denominator of a fraction of a second, e.g. 4000 = (1/4000) second. Seconds are displayed as number”, e.g. 1”5 is equal to 1.5 seconds. . Turn the main dial to the right to decrease shutter speed and to the left to increase it.

The two zeros directly to the right of the shutter speed display represent the aperture. Since there is no lens attached directly to the camera, this value will always read 00.

You can change the length of exposure from -2 to +2 in half steps (f stop), where 0 is normal exposure level. First set the quick dial control switch on camera back to 1. Press the camera function button until the LCD display panel registers the exposure level indicator. The plus side of the scale indicates overexposure (more light) and the minus side indicates underexposure (less light).

Alternatively, you may program the camera to take a series of pictures (autoexposure bracketing) where the camera automatically changes the exposure level within a set range for three successive frames. Select AEB by pressing the function button on the back of the camera until AEB appears in the LCD panel. Then turn the main dial until the desired bracketing amount is set. The three bracketed shots are exposed in the order of correct exposure, underexposure, and overexposure.

Dual camera photography:

Note that when taking right **and** left dual pictures, the amount of light going to each camera will be decreased by half. For example, if normally you shoot a 5 second exposure on an immunofluorescence microscope, increase the exposure time to 10 seconds.

To synchronize the time of shutter opening of each camera, use the remote control. First press the black button above the command dial to activate the sensors. You will need to press this button every four minutes to keep the sensors active. Then set the switch on the remote control to 2, aim at the wall behind the microscope and press the button when ready to photograph a stereo pair.

When you are finished, please be sure to turn the cameras off (turn the command dial back to “L” and put the eyepiece covers back on the eyepieces).

Don't forget to take your film with you.