

How to use the CoolLED Light Source for Fluorescence Microscopy

Nikon imaging scopes in ISB 360 use the **pE-300^{white} CoolLED*** LED light system to provide a broad spectrum of illumination, allowing excitation of many different fluorescent stains. Using the Manual Control Pod, you can control three independent light channels

STEP 1. Find the CoolLED Control Pod



Control Pod is on the bench between the microscope and computer

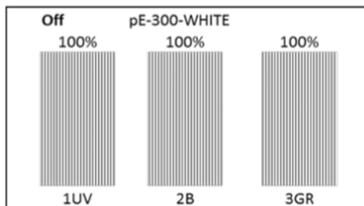
STEP 2. Turn System ON

Quickly tap the round **ON/OFF** Button - lower left face of controller

(Caution: **QUICK TAP ONLY** If you press longer, the system changes to a different menu)

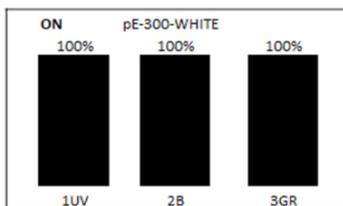


Power Button



OFF

Display screen helps you work with the system. "OFF" shown in tiny letters (top left) and horizontal bars are grey when powered down



ON

Bars darken when illumination is ON keep all the wavelengths selected ON.

To control when light illuminates your stains, you can use the manual shutter on the microscope (if Micromanager software is not yet open).

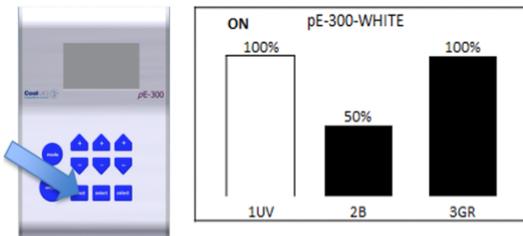
Once Micromanager Software is opened, we must instead use a software-controlled automated shutter to control when light reaches our samples.

STEP 3: Selecting a Fluorescence Excitation Wavelength:

Use the Filter Wheel under the microscope objective lens turret to control the color of light you use to excite fluorescent stains (Instead of using the control pod)

Although different excitation wavelengths can be independently turned on / off using the square “Select” buttons on the control pod, **we will instead use special filters installed in our scopes** to control the wavelengths of light that are able to reach and excite our fluorescent stains. These same filters also control the wavelength of light emitted from our samples to reach our eyes at the level of the oculars (eye pieces).**

Note: If you press one of the “Select” buttons, you will disable one of the wavelength controls. For example, here a UV light indicator shows a white bar indicating that the UV is turned off. If you want to use UV just press the “Select” button under the first bar to turn it back on.



To enable **Blue Fluorescence** (Emission), the 1st bar (“1UV”) controls **UV Excitation Light**.

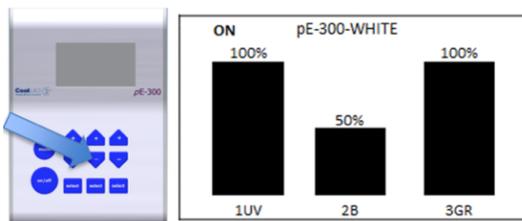
To enable **Green Fluorescence** (Emission), the 2nd Bar (“2B”) is for **Blue excitation Light**.

To enable **Red Fluorescence** (Emission), a 3rd bar (“3GR”) is for **Green Excitation Light** ***

STEP 4: Controlling Light Intensity (Optional)

You can control the intensity of light that you shine onto your samples, by using the Up / Down Arrows under the bar corresponding to the color of light you want to control.

One reason to reduce light intensity is if you’re trying to reduce emission signal loss in your sample, that will occur due to photobleaching. (However, the best way to prevent bleaching is to keep the light OFF the sample until you need to use it)



Here, to reduce the risk of photobleaching Green fluorescence, the blue “Down Arrow” below the 2nd bar on the control Pod (“2B”) has been used to reduce **Blue Excitation** light intensity down to 50%,

* More pE-300^{white} COOLED capabilities are detailed at: <https://www.cooled.com/products/pe-300white/>

** You can see the list of our ISB360 Nikon filter cubes at: <https://wahoo.cns.umass.edu/node/287>

*** Actually, “3GR” is for exciting samples using red, yellow, and green light.

(However, we will block all but the green excitation wavelength by using the “G” filter on the microscope’s filter wheel.)