

General Instructions for Alignment of an Arc Lamp Illuminator on an Fluorescence Microscope

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Introduction.

In a multi-user environment each user inherits the microscope in an unknown state left by the previous user. Additionally, placing and removing covers can brush knobs and change settings. For critical work each user needs to know how to align the arc lamp to provide even, reproducible illumination. While it may seem daunting and mysterious at first, anyone who can program a VCR can certainly follow these instructions. Just be careful to understand the overview and familiarize yourself with the location of the controls before starting. If unrecoverable problems are encountered, please shut down the arc lamp and get more experienced help.

General Description of Arc Lamp Illuminator Optics.

The usual arrangement is to have the arc lamp placed at the focus of the collector lens. This placement gives the optimal light gathering for any particular diameter of collector lens. Rays leaving the arc source are thus gathered and roughly collimated (roughly, since the arc is not a point source) and leave the collector as ~parallel rays. There is also usually a spherical reflector placed behind the arc lamp on the same axis as the arc and collector lens; this reflector, when properly focused and aligned, reflects a good deal of the light leaving the back side of the arc lamp forward, creating an inverted image of the arc just beside the actual arc. Light from this reflection image is also gathered by the collector lens and sent to the microscope, increasing the illuminating power. There is usually a second lens that works with the collector to bring the parallel rays of light to a focus, eventually at the back focus of the objective lens (which serves the dual purpose of illumination condenser and imaging lens). Since the conjugate focal length of the condensing system (lenses that re-image the light collected by the collector lens) is usually much longer than the collector focal length, a magnified image of the arc is produced at the back focus of the objective. Light leaving from a point at the focal length will leave the objective parallel. And since there is a 3-d image (of the arc plasma), not a "point", at the back focal point, some rays leaving the objective will be parallel, some diverging, and some converging; but the general effect is to yield roughly parallel illumination that evenly covers the field of view with the excitation light.

Special Notes about Arc Lamps: Read and Heed!

Always wear safety glasses when handling arc lamps from a package, or anytime the lamphousing cover is open - as for replacement. Xenon lamps have a high pressure even when cold and dropping, or stress, can cause violent explosion! While Hg arc lamps are not hyperbaric at room temperature, don't take chances, especially with a hot lamp.

Never ignite an arc lamp with the illuminator cover removed! Most systems have interlocks to prevent this, but just don't do it.

Never open an illuminator and handle a mercury arc lamp while hot. Allow 30 min or more to cool. While hot, there is a high pressure in a mercury arc lamp and small stresses can cause explosion.

There are special "objectives" that allow viewing the objective back focal point for aligning the arc lamp and optics, or sometimes special ports that can be pushed into the beam to provide equivalent viewing of the arc alignment. Lacking such features, remove an objective and place a piece of white paper at the stage position (where a slide would be placed). When any steps below specify to "focus the arc", or "align the arc image", it is referring to the image viewed at the back focus of the objective (or equivalent).

You should NOT be looking at a "white light" image since this would be unfiltered light and could be very harmful to your eyes! While glass optics remove much energy at and below 365nm, the mercury arc is such a powerful source much energy remains! Be sure to use a green or blue (NOT UV!) filter sets to protect your eyes. Since the arc has less power in the blue, and the eye is less sensitive to blue, blue can be used if the green is overpowering to view. The Field Diaphragm, if one is provided in the epi-illumination system, can be stopped down to get a better view of the arc image.

If an image of the arc, even a poor one, is not visible when you start, use extreme caution. First (and quickly) find and focus the forward image; then bring the reflected image into alignment. Do not allow the reflected image to fall upon the electrodes for very long since the electrodes can overheat causing lamp stress and explosion. Try to quickly find and identify forward and reflected images and keep them visible (therefore not falling on an electrode and hidden).

These are general instructions. Arc-lamp illuminator systems vary in detail. Some have knobs for adjustment (typical of Nikon illuminators) and some have hex-socket screws and require inserting hex wrenches into little holes (common on Zeiss). Most will provide for some form of adjustment as described below. Sometimes the knobs are labeled, sometimes there is a cryptic image/icon to show the function. ***If at all possible, find the manual and confirm the function of each adjustment control!***

Practical Alignment Tasks.

This is the short and easy part once you know the game plan and what not to do!

The forward image and the reflected image must be aligned sequentially. The "Z" axis is the optic axis of the illumination system.

- ?? Turn on the arc lamp (ignite if not automatic) and warm up for about 5 min.
- ?? Select the blue epi-fluorescence "cube" position.
- ?? Obtain an image of the arc at the back focus of the microscope objective using a special viewer in an objective position, or remove an objective and project onto white paper at the level of the stage.
- ?? First the reflected image is displaced so that you can clearly identify the forward image, and the reflected image is not obstructing the center of the field or falling on an electrode. The reflector controls are typically toward the rear of the arc lamp housing. Turn one of them and look for movement of an arc image. Both may need moving to find the image, and focus may be necessary. Leave the reflected image off to one side of the field.
- ?? The forward image is focused by using the collector lens focus knob to optimally focus the image of the arc. The collector focus control is typically at the very front of the lamp housing near the output light port.
- ?? Next, using the two knobs that move the arc (the actual lamp) in the X,Y plane, place the arc just off-center in the field of view. The forward image is now aligned.
- ?? Use the reflector X,Y controls (again, typically toward the rear) to swing the reflected image into the field of view and place it just off-center opposite the forward image.
- ?? Finally use the reflector focus knob to optimally focus the reflected arc image.
- ?? The forward and reflected arc images should be side-by-side, but not overlapping, at the center of the field. Some re-iteration of the alignment sequence may be necessary.
- ?? Do not "defocus" at this point; this is the optimal adjustment and should give bright edge-to-edge fluorescence illumination.

Note: Some microscopes have a Condenser Aperture Diaphragm and Field diaphragm in the epi-illumination path. These are adjusted just as for Kohler Illumination: the CAD is imaged in the back focal plane of the objective and this is the diaphragm you may see when aligning the arc images. If there is a constricting aperture around the arc images, look for CAD controls and center; then open the diaphragm. If there is a bounding aperture of the illuminated field when you observe a sample through the system, this is the Field Diaphragm, and it should be centered and opened to allow full-field viewing.

