Optimizing illumination for microscopy and measurement

Barbara Foster

For over 50 years, arc lamps have been the solution to low-light-level applications in microscopy. However, arc illuminators have been inflexible and often challenging to use. Until very recently, they could operate only at a single intensity, were difficult to align accurately, and were often spectrally limited. Furthermore, if bulb life was not properly monitored, the bulbs occasionally exploded. While modern lamp housings are designed to contain the debris from the explosion, as anyone who has been near a detonation can verify, it is a heart-pounding experience.

The system developed by **Opti Quip** (Highland Mills, NY) solves many of these problems. By combining a new stabilized power supply, a long-term stabilizer (LTS), and versatile lamp housing (*Figure 1*), the technology stabilizes the arc in the short and long term, offers intensity control over a wide range, and provides a choice of arc lamps to best match an application. This results in greatly increased bulb life, more consistent measurement, and the ability to individualize and memorize settings optimized to each experimental protocol.

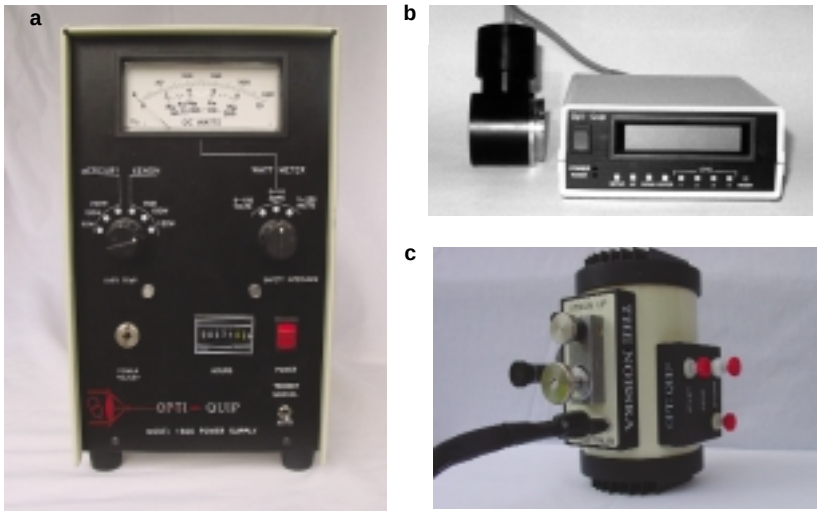

Figure 1 *a) The model 1600 power supply, b) LTS, and c) the Nobska lamp housing.*

Arcs versus incandescent light sources

The dramatic growth in fluorescence microscopy has driven a massive movement from conventional incandescent halogen lamps to arc lamps. According to a number of research studies, fluorescence is the most rapidly growing area in light and confocal microscopy. Approximately 80% of all microscopists involved in cell biology and neuroscience depend on this technique,[1,2] as do a surprising 20% of microscopists working in materials sciences.[3,4]

Because of its specificity and sensitivity, fluorescence provides answers not available with other microscopy approaches. However, the intensity of the typical fluorescent signal is 1/10,000 that of the ambient background. As a result, this technique requires the extra power afforded by arc sources. *Figure 2* contrasts relative intensities of three common arc sources. The mercury arc (HBO) produces a powerful but spiky spectrum in the visible. Well known for its 405-, 436-, and 560-nm peaks, mercury is routinely used for excitation of common fluorophores such as fluorescein isothiocyanate (FITC) and rhodamine.

In comparison, the xenon arc (XBO) presents a continuous spectrum from the infrared, where it exhibits a number of strong peaks through the visible area and well into the UV, well beyond the mercury output. An ozone-free version of this bulb reaches 220 nm, while the regular version extends to 150 nm. In comparison, the incandescent halogen spectrum runs along the baseline.

Combining the mercury and xenon into one arc source (Figure 2, shaded line) significantly improves both the spectral options and intensities over the visible range. Especially noteworthy for the semiconductor industry are three new spikes at 305, 315, and 336 nm.

Arc lamps are not just used for fluorescence. Many low-light-level situations would benefit from substituting arc sources for conventional halogen sources. For example, arc lamps are increasingly found in materials laboratories involved in polarized light analyses as well as those investigating complex, multilayer systems such as the polymer/metallic circuitry/base material sandwiches found in ordinary devices such as inkjet heads.

Arc lamps also provide a hidden benefit over incandescent lamps. With incandescent systems, lowering voltage to decrease intensity shifts color temperature, which casts a yellow pall on the image. The ideal solution is to control intensity by inserting neutral density filters. However, that solution is not always practical or effective.

continued

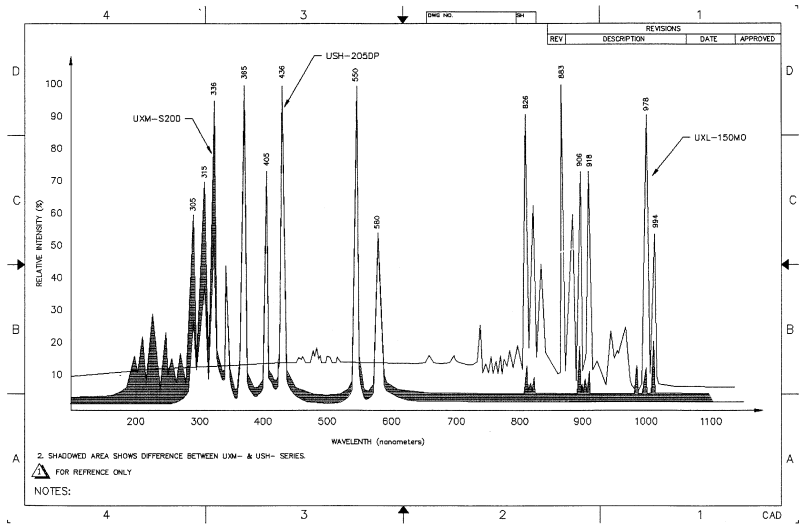

Figure 2 *Relative intensities of HBO, XBO, and combined XBO/HBO arcs. In comparison, the spectrum for an incandescent halogen lamp would run along the baseline. (Courtesy of* ***Ushio America, Inc.,*** *Cerritos, CA.)*

In contrast, arc lamps maintain color fidelity over a wide voltage range; thus lowering the voltage has no effect on the color in the image. However, until recently, commercially available arc lamps lacked that capability—they were either on or off.

Stabilized intensity: A power supply problem

Biologists have been a key driving force for the development of fluorescence microscopy and the resulting popularity of arc lamps. Their efforts followed the typical scientific progression from observation to description to measurement. At this last stage, predictable, stabilized intensity is a necessity since any variation has a direct impact on intensity-based quantification such as calcium ratiometry, fluorescence resonance emission transfer (FRET), fluorescence recovery after photobleaching (FRAP), or even simple optical density measurement. Since segmenting features for measurement also depends on gray scale and, therefore, intensity, even simple dimensional measurements such as area and perimeter can be affected.

The advent of both 3-D deconvolution and image montage has added further support for consistent intensities. The more constant the background from one optical slice to another, the better the deconvolution and the crisper the resulting image. Similarly, the more constant the background from one image tile to another, the better the resulting montage.

Stability has several components. For the practicing microscopist, the most visual test is the amount of flicker seen in the arc during alignment.* Stabilized power supplies control flicker over the short term in minutes or hours. Power supplies of this type have been available since the early 1930s and it is rare in today's laboratory not to find stabilization, either within the power supply or at least in the electrical line to the microscope. However, this approach to stabilization has two

*Caution: Looking directly at the arc can cause irreversible retinal damage. Flicker can be observed by placing a white card on the stage in lieu of a sample, removing an objective, and allowing the resulting image to fall on the card. Neutral density filters can be inserted if the image is still too bright. Alternatively, the lamp housing can be removed and the image of the arc projected directly on a wall. Even in these cases, the observer should take care not to look at the very bright image for too long a period.

limitations—the inability to adjust intensity and the inability to monitor and correct intensity long term over the life of the bulb.

Producing consistent intensity over the long term is a thorny issue. As the bulb ages, the electrodes that form the arc erode, decreasing intensity over time. Secondly, residual electrode ionization products coat the inner surface of the envelope, making it dark.

A two-step process solves these short- and long-term problems. The model 1600 power supply controls short-term flicker while the long-term stabilizer provides both infinitely variable intensity adjustment and long-term intensity stability. When the microscopist chooses the appropriate intensity for the experimental situation, that sampled value is memorized and stored by onboard smart technology in the LTS. The sensor monitors the output of the arc 2000 times per second, compares it to the stored intensity, then signals the power supply to adjust the voltage to maintain the chosen intensity. A conventional power supply may experience 8–10% variation, a fluctuation that is not typically visible in straight imaging mode. However, this new approach drops the variation to ±0.05%, optimizing the illumination for measurement and deconvolution.

Flexibility for multiple fluorochromes

Fluorescence microscopes are expensive, often requiring budget support from multiple users. For most of the new variable-intensity arc systems, consistently setting intensity for each new user and/or each fluorochrome or experimental procedure is a challenge. However, the LTS can store chosen intensities for up to four fluorochromes or protocols. These values can then be set either manually or via computer control through an RS232 connection.

The system's flexibility and rapid response provides another benefit. Using controls on the stabilizer, the lamp can be turned down for observation, reducing phototoxicity and photobleaching, then reset to maximum intensity within 25 msec for shorter imaging exposures.

Lower power equals longer lamp life

Traditionally, arc source power supplies have a position for "start" and "off." Lamps were ignited, allowed to come to temperature over a short warm-up period of 5–15 min, and then left on for the duration of the experimental session. Because of the high-energy surge necessary to ignite the lamp, both on/off cycling during a lunch break and between one user and another has been discouraged by the manufacturers. To avoid the thermal shock, the conventional wisdom has been to leave the lamp on, even for extended periods. Under these conditions, a standard arc bulb has a lifetime rating of approx. 200–300 hr. If the lamp is on for a traditional 40-hr work week, the bulb has to be changed and realigned every six to eight weeks.

The dramatic growth in fluorescence microscopy has driven a massive movement from conventional incandescent halogen lamps to arc lamps.

The ability to control the power lengthens bulb life and significantly reduces cost of ownership. For example, under controlled test conditions, a 100-W mercury arc (**Ushio America, Inc.**) was run at 85 W. Although rated for a 200-hr lifetime, the arc fired successfully for just under 1200 hr, at which point the test was stopped. In the context of the typical 40-hr work week discussed above, these tests suggest that bulbs only need to be changed and aligned every six to eight months.

If standard maintenance dictates replacing the bulb at its rated hourly lifetime, how will a microscopist using this new system know when to replace the bulb? With this system, the only time voltage reaches maximum is during initial firing. That surge increases as the arc ages. If the power requirements exceed maximum and the LTS senses that the power supply cannot provide the voltage necessary to reach the memorized light level, it activates a signal indicating that it is "time to replace your lamp."

For even longer life, the LTS has a special resting function. Rather than leaving the lamp in full-power position over breaks, meetings, or lunch hours, the microscopist can push a button and drop the power to 30% of maximum. On his/her return to the laboratory, the microscopist can push the button again to reset the power supply to the memorized position. Within 25 msec, the arc lamp returns to that intensity level, with no warm-up time and no flicker.

The impact of these controls in the laboratory is profound: Bulbs can last at up to five times longer, reducing replacement costs. Fewer replacements lessen both alignment time and system downtime, offering critical time savings for busy laboratories.

XBO versus HBO

Many fluorochromes as well as certain photoresists used in the semiconductor industry respond more favorably to the broader spectral distribution available from a xenon source than from the spiky peaks characteristic of mercury arcs.[5,6]

Xenon is also a better choice for materials scientists who need stronger intensity for polarized light studies. While mercury casts a greenish tint over the image, xenon produces the clear, white illumination critical for the pure-color rendition required for polarized light analyses. All too often,

however, the choice between XBO and HBO comes down to intensity; causing many microscopists to choose the more powerful mercury spikes. The model 1600 power supply and LTS can be matched with two different lamp housings and 11 different lamps, expanding the choices available to microscopists. For example, the Nobska can house either a 100-W XBO or 100-W HBO bulb. Until now, most microscopists chose the HBO to obtain sufficient light. However, a highly efficient collector lens in the Nobska housing allows use of the better color-balanced XBO. Because of the flat, spectral response, photographs can be taken with conventional daylight film, without the need for extra filtration. Digital images are brighter and exhibit truer color. A second housing is available for those rare situations that require 200-W lamps.

Conclusion

The millennium has brought a new age in arc illumination. By integrating a power supply with long-term stabilization, the **Opti Quip** system provides stability for measurement, deconvolution, and image montage as well as greater choice in intensity and spectral range. For multiple-user facilities or multiple protocols, the system offers more control over experimental parameters, memorizing intensities matched to specific fluorochromes and experimental procedures. Finally, arc illuminators offer the intensity of an XBO or HBO source coupled with short- and long-term stability and the flexibility previously enjoyed only with incandescent lighting systems.

References

1. Foster B. Cell Biology 2001 Market Research, Microscopy/Marketing & Education, Inc., Springfield, MA, Jan 2002.
2. Foster B. Neuroscience 2001 Market Research, Microscopy/Marketing & Education, Inc., Springfield, MA, Dec 2001.
3. Foster B. ISTFA 2001 Market Research, Microscopy/Marketing & Education, Inc., Springfield, MA, Dec 2001.
4. Foster B. M&M 2002 Market Research, Microscopy/Marketing & Education, Inc., Springfield, MA, Sept 2002.
5. Vivid filters for fluorescence microscopy, 8th ed. Brattleboro, VT: Omega Optical, www.Omegafilters.com.
6. The right lamp for the job. Highland Mills, NY: Opti Quip, www.Optiquip.com.

*Ms. Foster is President, **Microscopy/Marketing & Education, Inc.,** 125 Paridon St., Ste. 102, Springfield, MA 01118, U.S.A.; tel.: 413-746-6931; e-mail: bfoster@mme1.com. The author welcomes comments on her articles.*