Microscope Illumination: LEDs are the Future

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Introduction

Light microscopes in laboratories and hospitals are used for examining many different types of samples—from industrial research to life-science research and clinical screening. These procedures use conventional bright-field, differential phase contrast (DIC), and fluorescence microscopy among other techniques. In all cases, the light source on the microscope has a crucial influence on the quality of images viewed and the conclusions reached.

Microscope Illumination

For low-magnification macroscopic illumination, a single point-light source is often used. Because it is not in the optical path, the illumination is at an oblique angle. This often may be adequate, but shadowing can be a problem for applications where even illumination is required. In order to overcome shadowing and poor homogeneity, it is common to use multiple light sources or some form of ring-light positioned around the objective lens to generate an even illumination. Recently, fiber optics have been used for low-magnification illumination; light is delivered to the microscope via a fiber bundle from a remote lamp. In high-magnification light microscopy applications, the optimum method of sample illumination is by delivering the light through the optical path of the microscope itself. In this case, there are many additional parameters to consider, as described below.

Historically, illumination for samples in light microscopy has been provided by a range of conventional incandescent or discharge lamps [1]. They can be classified as: halogen, tungsten-halogen, xenon, mercury (also known as an "HBO" or a "UV Burner"), and metal-halide. In recent years, light-emitting diode (LED) illumination products have become available that offer many exciting benefits. In this article, the different types of illumination available are reviewed. The benefits and disadvantages of each type will be identified and compared with LED-based products for performance, convenience, safety, environmental effects, and operating costs.

Incandescent and Discharge Lamps

If you are using a microscope in your lab, it is likely that it will have illumination fitted using one of the conventional lamps. The most common type of microscope illuminator is the tungsten incandescent lamp. These lamps are relatively inefficient and can exhibit a shift in color temperature with time [1]. Most lamps supplied today are of the more advanced tungsten-halogen or quartz-halogen type. These generate light across the visible spectrum, but much of the energy is dissipated as heat in the infrared (IR) region, and there is little illumination below 400 nm. They are rarely used for fluorescence microscopy, which needs higher intensities. Xenon lamps exhibit a flatter intensity across the spectrum than halogen, which makes them more suitable for quantitative analysis.

For high-magnification and fluorescence microscopy, the high-pressure mercury vapor arc-discharge lamp is most common. These are significantly more powerful than other forms of lamp, but their intensity varies across the spectrum. They are hampered by poor spatial homogeneity because of their complex construction and alignment requirements. Replacement bulbs can be difficult to align, leading to uneven illumination over the microscope's field of view. Bulb lifetime is a few hundred hours.

The metal halide lamp is an enhanced version of the high-pressure mercury lamp. These more expensive bulbs provide better illumination stability and have a longer bulb lifetime. However, they still suffer from degradation in performance during the bulb lifetime (around 2,000 hours). Because they produce significant amounts of UV light, a liquid light-guide used to deliver light from the illumination unit to the microscope ages and needs to be replaced regularly [2].

Benefits. All these conventional lamps have the benefit that they produce a broad spectrum of white light that can be used for many applications. In fluorescence microscopy, the user simply changes the microscope filter cube to match the fluorophore being used; common fluorophores used are DAPI, GFP, FITC, etc. [3].

Disadvantages. Conventional lamps have a number of significant drawbacks. They generate unwanted heat, are inefficient, and require the regular replacement of bulbs. Mercury-based lamps can be dangerous for the user because they generate considerable UV light, which can damage the eye. Conventional lamps produce UV light, which is typically not required for illuminating the sample. In fact, this UV light can bleach samples, killing live tissue cells and reducing the amount of time that a sample can be examined. Although it is rare, these bulbs can explode and release toxic mercury vapor into the laboratory air.

A period of warm-up and cool-down is required before and after use, which is inconvenient. As a result, these lamps are often kept switched on all day in order to be available when required. Because they are inefficient, energy is wasted and unwanted heat is generated.

The bulbs have a limited operating life (typically measured in hundreds of hours) and degrade in performance over that period [1]. Intensity from a conventional lamp decreases through its life (Figure 1). Most lamps are quoted with a lifetime to 50% of original intensity. This means that the illumination of a sample varies dramatically through time. Any quantitative or comparative measurements cannot be done reliably unless a new bulb is used on every occasion.

LED Illumination

With LED-based microscopy illumination, almost all of the disadvantages of conventional incandescent and discharge lamps can be overcome. LEDs are solid-state semiconductor

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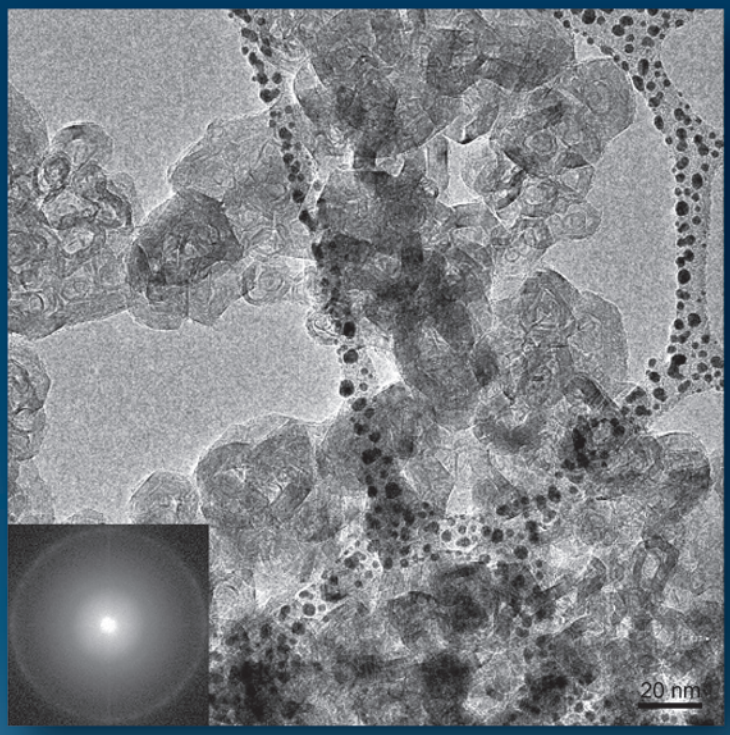

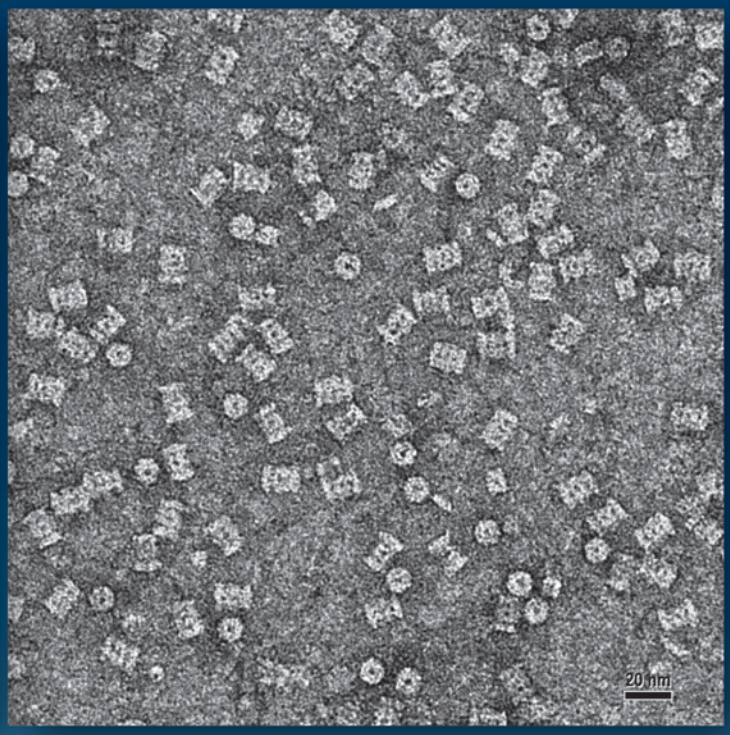

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Left image: Graphite fringes demonstrating resolution to Nyquist limit; imaged on Gatan K2Base™ camera at 14.5kx nominal TEM magnification and 200 kV.

Right image: 20S proteasome negatively stained by uranyl formate showing 1k x 1k region of interest; imaged on Gatan K2Base™ camera at 9.6kx nominal TEM magnification and 200 kV. Sample courtesy Dr. Yifan Cheng, University of California, San Francisco.

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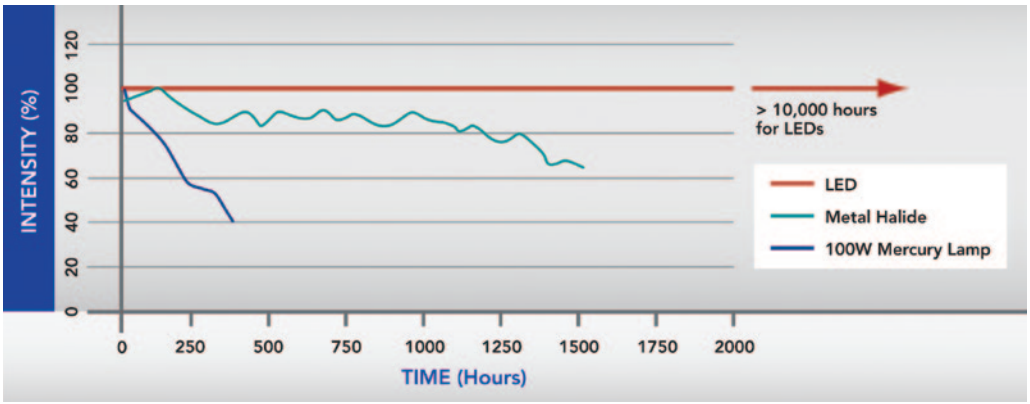

Figure 1: Comparison of lifetime and intensity for microscope illumination sources. LEDs last significantly longer and consume less power [4].

devices that emit light directly without using a bulb [5]. Although previously used only as indicator lights, LEDs are now sufficiently intense to be used for illumination. Their potential in consumer applications is making them more common in markets such as automotive products and lighting for buildings. In fact, LEDs can be up to six times more efficient than a conventional incandescent or discharge lamp.

As a result, considerable development has been carried out by the LED-chip manufacturers to increase brightness and by the end-product manufacturer to engineer specialized packaging, thermal management, and optics dedicated to end-user application. Together these improvements now offer LED illumination systems that provide greater intensity than incandescent lamps in the regions of the spectrum important for microscopy. By actively cooling the LEDs even greater intensity and stability can be achieved [6]. In addition to increased intensity, LEDs offer instant on/off, long lifetime, low running costs, greater efficiency, and less heat generation. There are significant performance benefits that make use of the light source simpler and more convenient. When one considers that LEDs can be switched on and off instantly, the amount of actual "on" (viewing or imaging) time in a day could be less than one hour. Furthermore, LED intensity remains broadly the same over its entire life [7]. Lifetimes of 10,000, or even 50,000 hours, are now commercially available. This means an LED illuminator can be expected to be usable for 15 to 20 years—the lifetime of the microscope itself. So a significant financial saving can be made against the cost of purchase, replacement, alignment, and disposal of the equivalent conventional bulbs that would have been required over that period.

Fluorescence, semiconductor, and art restoration are all applications where the ability to change the color balance is a noticeable benefit of LEDs. As white light from LEDs can be made up from distinct wavebands, color balancing to provide a high color-rendering index (CRI) can be achieved for true color rendering. LED illumination can allow the user to switch from "white" light to near monochromatic light, thus removing the tendency for chromatic aberration and improving contrast.

When light is delivered through the episcopic port, consideration must be given to the optical filters that are used [3]. Misaligned or poorly performing filters can reduce performance considerably. Also, LED intensity can be greater than 100-watt mercury lamps in the blue and red excitation regions, but it is weaker than mercury in the green excitation region [1]. Arrays of LEDs can produce lamp intensities as high as 70 W/cm^2 (Figure 2). The introduction of higher-performing sputter-coated filters can be beneficial where more intensity is desired.

Because LEDs are more efficient, less energy is used. There is less heat dissipated, and this can be an important issue if the microscopes are used in close or cramped conditions. They exhibit greater stability and repeatability, which makes comparative tests, or tests against a reference sample, more reliable. Also, with the instant on/off capability and intensity control, LEDs can perform the functions of a shutter and a neutral density (ND) filter, which can save on costs (Figure 3).

LEDs can produce white light with a fixed color temperature. This can be achieved using either a blue LED with a phosphor overlay to shift the light (as used in most consumer products) or by combining individual LEDs with red, green and blue wavelengths (as in the way a TV picture is generated). The ability to tune the color is possible [7].

Applications. Microscopy applications requiring illumination at only one or two discrete wavelengths lend themselves to LED illumination. A simple LED unit is highly cost-effective in these circumstances, although care must be taken to ensure that optical homogenising elements are used so that the LEDs and any artifacts are not imaged on the sample. Fluorescence microscopy lends itself to illumination by a specific color. In fact, many of the common clinical screening stains such as auramine, acridine, and FITC (fluorescein isothiocyanate) can all be illuminated using a single 470-nm LED light source. There are now LED colors available for most

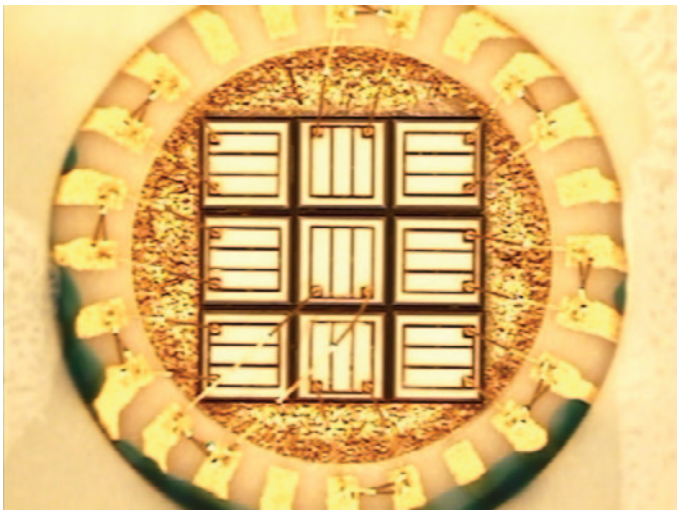

Figure 2: LEDs can be combined in arrays for superior performance. Intensity can be as high as 70 W/cm^2 on the LED surface.

Figure 3: LEDs are easily controlled from a control pad. Instant On/Off and intensity control make shutters and ND filters redundant.

common fluorophore stains. For more complex applications such as dual staining of live cells and FRET [8], the absence of UV, fast-switching, and temporal stability are highly attractive.

Environmental Considerations

There are three main areas where the type of microscope illumination used is affected by environmental considerations: (a) health and safety relating to eye damage from UV radiation, (b) the safe use and disposal of mercury products, and (c) energy consumption. UV light is known to damage the human eye. Broad-spectrum incandescent or discharge lamps generate mostly unwanted UV light, which needs to be filtered out. These lamps are safe when fitted to microscopes that have a UV filter installed to reduce this risk. Care must be taken when operating the lamp if no filters are in place or when it is not fitted to the microscope. Because LEDs only generate specific wavelengths of light, only those LEDs intended to illuminate in the UV region can cause UV damage.

Where mercury is used, consideration must be given to the risk of a bulb exploding and releasing mercury vapor into the air. Health and safety requirements for mercury are becoming ever more onerous because it must now be treated as a hazardous material. There are also strict rules for the safe disposal of spent mercury bulbs.

Laboratories are being encouraged to reduce their energy consumption. With the higher efficiencies of LEDs, about six times less energy is consumed in comparable usage. Because many conventional lamps are left on all day to overcome warm-up requirements, the actual reduction in energy by using instant on/off LEDs can be significant.

Conclusions

Conventional incandescent and discharge lamp illumination offers the benefit of broadband illumination. This reduces the need to ensure that the optical filters are correctly matched to the light source being used. A single light source can satisfy most microscopy applications. However, these lamps are inconvenient to use and their bulbs require regular replacement.

LEDs are set to replace incandescent and discharge lamps because they offer better characteristics in illumination intensity, lifetime, wavelength specificity, and ease-of-use. The environmental and safety benefits of LEDs are also persuasive. No mercury is used, and there are no replacement and disposal issues to address. Lower power consumption satisfies the ever-increasing demand for a "greener" laboratory.

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