Topic Introduction

Light-Emitting Diodes for Biological Microscopy

Tomokazu Sato and Venkatesh N. Murthy

Various technological advances have made imaging an increasingly useful tool in the life sciences. Imaging techniques that move away from the limitations of wide-field microscopy have allowed deeper, higher-resolution imaging of thick biological tissue. Even within wide-field microscopy, advancements such as structured and sheet illumination, as well as improvements of biological probes, have led to better visualization of cells and their subcellular structures. The illumination source for wide-field microscopy, however, has remained relatively unchanged for decades, relying mainly on xenon, mercury, and halogen lamps. Light-emitting diodes (LEDs) have existed for more than 80 years, but their spectral range and output light flux have only recently become large enough for use in biological applications. This article presents the basic information necessary to build and to use an LED-based illumination source for microscopy. It also provides some useful resources about LED advancements. Although commercial LED-based illuminators for microscopy are available, custom-built illumination systems can incorporate the latest LED chips into microscopes more quickly and inexpensively than is possible through the retail market.

INTRODUCTION

An LED is made of a combination of semiconducting materials (the so-called LED chip or die) that are doped with impurities to allow conversion of electrical energy into light through a reversible process called injection electroluminescence (Schubert 2006). The wavelength of generated light is determined by the energy bandgap at the p–n junction in the semiconductor. Various advances have been made in the growth and the choice of the semiconductor materials, allowing for brighter LEDs over a broader emission spectrum. Although there is a gap in high-efficiency LEDs within the yellow–green range, some LEDs emit in this wavelength by converting the primary emission through phosphors or semiconductor materials (Schubert 2006).

An LED package is not simply a small piece of the right mix of semiconductor materials. A major issue is the extraction efficiency, or how much of the produced light can be brought out of the LED package. Much of the light emitted from the junction is either reabsorbed on its path out to the surface or, even if it reaches the surface, reflected back inward and eventually reabsorbed. The geometry of the LED die, as well as the built-in optics, can affect how much of the light escapes. Although some LED die shapes can minimize surface reflection losses, they are not always very practical to produce. Instead, epoxy domes have traditionally been used to lessen the refractive index mismatch and reduce internal reflection. More recently, lens arrays, photonic lattice waveguides, antireflection coatings, transparent substrates, and reflectors have been built into LED chips to further enhance the efficiency of light output. LEDs typically come in one of two emission types: surface emitting or edge emitting. In surface-emitting LEDs, the light exits over the entire area of the p–n junction. The radiation pattern is typically close to Lambertian (in which intensity is proportional to the cosine of the

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angle measured from the axis perpendicular to the surface of the junction). In edge-emitting LEDs, the light is guided along in the plane of the junction and comes out from all sides of the LED in a highly directional flux. The characteristics of the optical output from LEDs are important for at least two reasons. First, the amount of useful light that can be brought to the specimen depends not only on the total amount of light emitted, but also on the spatial pattern of emission, the wavelength dependence on junction temperature and current, and individual variability (even among the same model of LEDs). A second important characteristic of an LED is its efficiency: If more energy is converted to electromagnetic radiation, the less the LED will heat up. Heat management is an important issue that will be discussed later. Currently, LEDs may convert around 5%–10% of the electrical energy to optical output; the rest will mostly end up as heat. Future advancements may help improve the percent conversion, but as of now, careful thermal management is required for high-power LEDs.

Most LED producers provide data sheets containing important spectral, mechanical, and electrical characteristics, as well as more detailed application briefs that help users integrate these LEDs into various illumination systems. Many of these application briefs delve into far more detail on the implementation of LED circuits, thermal management, mounting options, and other important issues for specific LEDs than space in this article allows; we direct the interested reader to these sources, some of which are listed at the end of this article.

COMPARING LEDs WITH OTHER ILLUMINATION SOURCES

One of the advantages of an LED over more traditional filament-based or arc-based illumination systems is that the timescale of its operation is much faster: on the order of a microsecond. There is no need to wait tens of minutes for a lamp to heat up and to reach thermal equilibrium. Because of their much faster response, LEDs do not need a high-speed shutter system (which is at least a few orders of magnitude slower) as long as the on/off control is integrated into the LED control circuitry. Even without that circuitry, LED light can be controlled from a power supply located at a distance from the experimental apparatus, thereby eliminating vibrations caused by the use of shutters or other light-blocking methods on the microscope itself. An electric circuit or power supply can control the intensity of the LED light; there is no need to swap neutral density filters or to change the gain and the contrast on a camera. Furthermore, LEDs can be synchronized with the acquisition camera to rapidly shutter the excitation light and thus to reduce phototoxicity (Nishigaki et al. 2006).

Another advantage of an LED is that its emission lies within a narrow bandwidth of the spectrum, thereby minimizing unwanted light. A visible-light LED will emit very little ultraviolet (UV) or infrared (IR) radiation, thus minimizing toxicity to or heating of samples. In addition, even within the visible spectrum, because most of the light will be within a particular band, there is less concern about undesirable light bleeding through filter sets and being detected by a camera. Thus, although there may be less total emitted power compared with a more traditional illumination source, with an LED, the signal to background is often greater. With careful electronic design, LED emissions are stable and reproducible, making it much simpler to correct for background and to compare image brightness among different specimens.

LEDs are susceptible to fast noise in the controlling circuitry because there is no thermal inertia to rely on for smoothing. The noise in illumination is generally related to noise in the power source, and oscillations or ripples in light intensity can sometimes be seen. However, other illumination sources, such as arc lamps, can have fast noise as well. In general, the problem can be resolved by using well-designed circuitry and well-regulated current supplies. Because an LED's output power, peak wavelength, emission spectrum, and voltage characteristics depend heavily on the applied current and temperature of the LED, careful design of the LED control system is needed to avoid drifting of the output light. Unless the LED undergoes catastrophic failure, this change in light output is a slow drift. However, an LED with a stable driver can have significantly lower low-frequency noise compared to arc lamps (although high-frequency noise may be comparable) and therefore offers an overall improvement in signal-to-noise ratio (Rumyantsev et al. 2004).

Another light source that is increasingly being used is the laser. LEDs produce light that has far less spatial and temporal coherences than does a laser. In some applications, the laser simply cannot be replaced by an LED. However, there are instances, such as spinning-disk confocal microscopy, where LEDs may potentially replace lasers. LED use could reduce speckle noise and wash out some reflection-induced interference patterns. Cost and maintenance will also be improved significantly. However, LED brightness is still an issue, and the lack of collimation of the emitted light places restrictions on optical coupling to a fiber or the optical train that must be used to bring the light to the specimen.

LEDs are made completely out of solid-state components, so they are more robust than light sources that use filaments or highly pressurized gas. In addition, they are safer with no risk of explosions. Typically, LEDs will start to break down at $\sim 125^{\circ}\text{C}$, which places some constraints on illuminator design and in some ways makes them safer than tungsten lamps, which operate at much higher temperatures. Although LEDs can undergo catastrophic breakdown when the temperature is not controlled or when there are large current spikes from the power source, a well-designed system provides continuous light for thousands of hours with very little chance of spontaneous failure. LEDs, even when mounted on heat sinks, are usually much smaller than other light sources. Some small LEDs are powerful enough to be mounted inside a microscope (as opposed to in an external lamp-house), directly under the specimen, or even on a live animal (Moser et al. 2006; Huber et al. 2007; Lang et al. 2008). LEDs can be used in cold environments in which other illumination systems may have difficulties. Thus, the long-term costs of an LED-based system, when considering that shutters are not needed and bulb replacement is rare, tend to be much lower than other illumination sources.

CHOOSING AN LED FOR IMAGING

The following factors should be considered when choosing an LED as an illumination source for an imaging system.

- The most important is the wavelength(s) of light needed.
- Other important features are the LED's brightness, emission directivity, thermal manageability, and the size of the LED package.
- Consider the dependence of the optical power and spectra on junction temperature and drive current.
- Other important factors are the maximum allowable current, voltage–current relationship, allowable duty cycle, and other electrical specifications needed for control circuitry design.
- If possible, obtain diagrams showing the physical dimensions as well as the mounting options.

Most LED manufacturers provide detailed data sheets containing information for a typical LED package. Note, however, that because of the complicated production process, there is a lot of variability between individual LEDs of the same model (Benavides and Webb 2005). For most experiments, when using LEDs for simple epifluorescence or other less demanding applications, this variability is unimportant. Some manufacturers bin their LEDs based on brightness and spectral characteristics, but be aware that there can still be significant variability within bins. Currently available high-power LEDs can provide radiance nearing that of mercury lamps in many parts of the spectrum. If desired, the user can perform calibrations of various operating parameters such as the forward voltage, spectral output, and thermal resistance from the junction. A list of major LED manufacturers and their websites is provided at the end of this article.

LED Microscope Illuminators

LED-based illuminators are available that have been designed to mount to specific microscope models from Olympus, Zeiss, Leica, Nikon, and other manufacturers. Thorlabs, Inc., Newport Corporation,

FRAEN, CoolLED, and Zeiss are some of the sources for these LED-based illuminators. These illuminators come fully aligned, usually mount easily onto a microscope, and are packaged with easy-to-use software and hardware controls.

BUILDING AN LED ILLUMINATION SYSTEM FOR IMAGING

Next, we introduce the concept of building your own LED illumination system. Why might this be desirable? Although some companies use proprietary technologies to produce LED units having unusual spectra, many rely on existing LED chips. Even if a new LED package comes out with highly desirable properties, these may not be integrated into commercially available LED illuminators. Specific power and spectral combinations desired by a researcher may not be available from a single company. It can be easier to repair a homemade LED illumination system if it breaks. The skills involved in building an LED illuminator for a microscope can be adapted to other uses, such as LEDs mounted on animals for in vivo applications, something that is not currently available commercially. Finally, there may not be any commercial LED microscope illuminators that are compatible with the specific microscope in the laboratory.

LED Light

LED packages emit either single-color or white light. LEDs that emit white light do so either through a suitable combination of colored LEDs or through wavelength conversion. White LEDs can be used for trans-illumination and for oblique or reflectance imaging, and the white color may be more pleasant to a viewer who is looking directly through the eyepiece of a microscope. However, white LEDs may not be optimal for these forms of imaging when used with a camera. For example, differential interference contrast (DIC) imaging of thick acute tissue slices often uses IR as opposed to visible light because IR is scattered less by tissue. One report indicates that blue LEDs provide better image quality than red LEDs for DIC imaging of microtubules (Bormuth et al. 2007), whereas another compares images of various color illumination for DIC and oblique illumination (Safronov et al. 2007). Intrinsic optical signal imaging also benefits from the use of certain colors of light. For example, blood vessels stand out more from the surface of the brain when green light is used. In addition, white LEDs are unlikely to be suitable replacements for traditional broad-spectrum light sources in fluorescence microscopy. With LEDs that emit white light by combining the emissions of several colored LEDs, the white light is not a continuous spectrum of wavelengths but has peaks of red, green, and blue and large gaps in spectral power within the other regions. Density constraints limit the number of LEDs per unit area, so for a given specific color, the amount of usable light available from a composite white LED package will be lower than that available from a single-color LED package of the appropriate wavelength. With LEDs that emit white light through wavelength conversion, a broad spectrum of light can be obtained, but the inefficiency of wavelength conversion reduces the usefulness of these LEDs. More spectral power can be obtained by combining the beam paths of single-color LEDs. Wavelength-conversion-based LEDs should be chosen only when there is no LED package available that directly produces light in the desired spectral band.

The light from a single-color LED is pseudomonochromatic, with a spectral full width at half-maximum (FWHM) that is dependent on the wavelength, the die temperature, the drive current, and whether the light was converted via a phosphor or a secondary semiconducting device. If the FWHM is >20 nm and fluorophores do not show a significant Stokes shift, then excitation filters become necessary. This somewhat broad spectrum does provide an opportunity to excite a broader range of fluorophores.

When only a single color is needed, optical coupling between the illumination source and the microscope is relatively simple. This is described below in the sections on LED optics and illumination. Multicolor imaging requires a more sophisticated optical coupling process because the most useful LEDs are pseudomonochromatic. One option is to have a housing that combines multiple LED

FIGURE 1. LED as a light source. (A) The light from LEDs of different colors is collected by a strong lens, and the beam paths are combined by appropriate dichroic filters. To minimize light loss, the distance from each LED to the output is kept as short as possible. The output light can be directly coupled to the back of a microscope lamphouse (in the investigator's experience, there is good coupling if the LED is focused to infinity by the collection lenses), or it can be focused into a light guide. (B) Comparisons of multicolor imaging. (i) Configuration requiring filter wheels for both excitation and emission filters, (ii) configuration with one excitation filter wheel, and (iii) the full multiband configuration for a traditional light source requires a color camera because multiple fluorophores will be activated at the same time. LEDs, however, can be used in LED-specific configurations in which either multiple individual excitation filters in front of each LED or a single multiband excitation filter after all of the LED lights are combined are used. Because the different colors of the LEDs can be turned on and off individually and the narrow bandwidth will typically lead to low bleed-through into the neighboring excitation band, configurations with no filter changing can be used.

lights through a combination of collection lenses and dichroic mirrors that merge the light paths, as in Figure 1A. This light can be coupled to a microscope lamphouse via direct mechanical attachment at the back of the lamphouse or through a light guide. Excitation filters may be fitted into the microscope filter cubes, or a suitable filter can be placed in front of each LED. Alternatively, light from all of the sources can be combined, resulting in a single multiband excitation. Although image quality may suffer when using this latter method caused by cross excitation of fluorophores, it is an economical option because each excitation filter costs ~\$250.

When vibration, speed, and pixel shift are not of concern, one can choose a color channel by simply rotating the microscope filter turret, which contains excitation, dichroic, and emission filters mounted in cubes. However, it is often advantageous to move different optical elements independently, and there are three alternatives: (1) a single stationary multiband dichroic and multiple excitation and emission filters that are on fast wheels; (2) a configuration in which the emission and dichroics are both multiband but with multiple excitation filter sets on a wheel; and (3) a full multiband configuration in which all of the components are fixed and multiband (Fig. 1B).

For LED-based light sources, a modified configuration with an excitation filter in front of each LED color can be used without a wheel as switching can be performed through the driving circuit that controls the LEDs. Traditionally, the full multiband configuration requires a color camera if the sample is stained with multiple fluorophores because each one would be excited and would emit at the same time. However, LED-based illumination sources permit switching between narrow bandwidth excitation light sources that correspond to only one fluorophore excitation color, provided there is enough spectral separation between the fluorophores being used. In those cases, the full multiband configuration can be used with a black-and-white camera. These LED-specific configurations are effective at avoiding pixel shift, a phenomenon in which different filters cause images as seen by a charge-coupled device (CCD) camera to shift. Correcting pixel shift requires that captured images be processed to realign the images.

LED light intensity can be controlled either via current control or by pulse-width modulation (PWM), a method in which the LED is rapidly turned on and off so that the effective brightness is reduced. Current control of LED light intensity can change the spectral properties of the emitted light even without any temperature change in the LED, although, for most imaging applications, this is not of concern. With PWM, unintentionally altering spectral properties is largely avoided, but the LED temperature can shift, which might lead to changes in the characteristic of the output light. PWM allows for greater linear control over the effective intensity. In situations in which turning the light on and off is not desirable, one should not use PWM. Note that because of the normally sublinear relationship between voltage, current, and efficiency of converting electrical power to light, control of light intensity by PWM may not be as thermally efficient for the same brightness as using the current limitation method.

Many LEDs come with built-in optics, which enhance light extraction efficiency. In addition, many companies sell optics made for collecting light from LEDs. When choosing additional optics for LEDs, consider the following.

First, consider the concept of geometric and angular losses, which involves the optical or Lagrange invariant and its related quantity, étendue. Étendue is proportional to the product of source size and divergence angle of emission. This value limits the amount of light that is available for coupling into any optical system.

Second, consider the concept of Fresnel reflection. Light passing between media having different refractive indices will experience partial reflection, even when the angle is less than the critical angle for total internal reflection. For coupling a single-color LED directly into a fiber, index matching gels or glues should be used to minimize Fresnel losses at the fiber input. The end of a polished fiber is placed flat against the smoothed flat LED surface. Some machining may be required to shape the epoxy dome so that the fiber and the LED surface are properly coupled. For optical trains of lenses, antireflection coatings can help. A good discussion on coupling LEDs to optical fibers is provided by Doric and Tubic (2004).

Homogeneous illumination of a specimen is usually achieved by Köhler illumination. Not all LEDs, however, may be able to provide perfect Köhler illumination because of their radiation pattern (although this is rarely a problem). Depending on the type of LED and the illumination collection optics used in the microscope, it may be worthwhile to use a diffuser or critical/Nelsonian illumination. In the latter method, a homogenous light source, or the end of a light guide, is imaged directly onto the sample. Many LED chips, however, do not provide homogenous light because the pattern of electrical contacts block or separate the light-emitting areas. Light guides and bent fiber optics can scramble coupled light, although the amount of light lost in either the coupling or during the transmission through the light guide might be significant enough to cause problems. Diffusers can also be used to provide light homogenization. With holographic diffusers, there is less loss of light than that from ground glass or opal diffusers.

Mounting and Heat-Sinking LEDs

Thermal management is an important consideration when building an LED illuminator. The electrical power required to run a high-power LED far exceeds the emitted optical power. The rest of the energy is converted to heat, and depending on the LED used, this amount could easily exceed 50 W. Many LED data sheets show the maximum allowable temperature for the LED junction as well as the maximum current at a given LED temperature or ambient room temperature. The heat sink should be designed to ensure that the LED does not burn out. Although there are theoretical methods for estimating the necessary characteristics of the heat sink, these do not account for convection or the efficiency of heat spreading within the heat sink. There are ways of measuring or estimating the LED junction temperature by measuring the temperature of the LED package or the temperature dependence of the forward voltage, and some LED packages have built-in temperature readout lines. The websites of many LED producers (e.g., OSRAM Opto Semiconductors, Luminus Devices, Inc., and Philips Lumileds Lighting Company) have application notes and data sheets that discuss the details of heat-sink selection, temperature measurement, and mechanical attachment methods of LEDs to other surfaces.

The heat generated by an LED must be effectively dissipated using heat sinks. It is not enough to simply place the LEDs onto a heat sink; the two must be glued together with a thermal adhesive designed for the task. Effective heat transfer also requires that there are no air gaps between the LED and heat-sink surfaces. One option is to use screws to attach the LED to the heat sink after having placed thermal grease (and possibly an electrically isolating, thermally conductive mica sheet) between the LED and the heat sink. For some LEDs, another option is soldering the LED and heat sink together. A thermally conductive adhesive pad or glue can also be used, although without some method for applying pressure to the area of contact, thermal transfer is compromised. Refer to the LED manufacturer's website, or contact them about acceptable attachment methods.

In general, heat-sink efficiency is a function of the material used; its total surface area, shape and surface finish; flow of fluid over the heat sink; and the ambient room and fluid temperatures. Most heat sinks are made of aluminum, although more expensive and efficient ones are made of copper or an alloy. Typically, a larger surface area means greater efficiency. Although finned heat sinks are less efficient per unit of surface area than flat heat sinks (as a result of less radiative heat loss and less convection), they are more efficient when considering the overall footprint. The temperature around the heat sink affects how much (or the rate at which) heat can be dissipated. A fan greatly improves heat-sink efficiency because heat is transferred to the ambient environment by convection. Many heat-sink fans generate sufficiently small vibrations so that experiments are not affected. If a heat-sink fan cannot be used, orient the heat sink vertically so that rising warm air creates convection through the fins. Low-temperature circulating fluids may also help.

LED Electronics

The simplest solution for powering an LED is to connect it to an alternating-current to direct-current converter, although this method is fraught with problems and is not recommended. There is no control over light intensity. Nonisolating transformers are susceptible to spikes in power. Importantly, because temperature is unregulated, thermal runaway can occur when as an LED heats up, the forward voltage drops, leading to an increase in drive current, which further heats the LED. This could lead to catastrophic failure of the LED. There is also no electronic control for turning the light on and off, which could be a minor inconvenience or a critical flaw depending on experimental requirements. A voltage source with a resistor in series is a simple way to limit current but can waste power through the resistor, and in the end, the current, not the voltage, is the primary determinant of optical output.

The simplest functional solution for powering and controlling an LED is a high-quality, regulated, adjustable current supply. This will at least permit some control for intensity, put a cap on current, and allow for some on/off switching (either manual or perhaps through digital inputs depending on the power supply), all for as little as \$200. Alternatively, many companies now offer drivers for LEDs, a simple and cost-effective choice. Homemade control circuits can be built for <\$50, but much time can be lost designing and building the circuitry. Potentiometers could be used in a circuit for light intensity control,

FIGURE 2. A high-intensity Luxeon V LED driving circuitry and timing. (A) A simple electronic circuit that can be used to drive a single blue LED is shown. Its output intensity is adjusted by a potentiometer; the light can be turned on and off by a low current TTL input. Note that the specific resistors should be optimized for the specific drive current required by the LED. (B) An LED driven by a high square wave input follows the signal closely. The speed of a typical LED array is clearly illustrated by its response to a voltage step. The driver circuit was that shown in A. (Reproduced from Albeanu et al. 2008.)

and transistors could be used for switching on and off the light either as part of an experiment or to shut down an LED before it reaches critical temperature. The use of op-amps or feedback from photo-diodes can further stabilize light output. Note that the circuitry may have a time constant slower than that of the LED itself, but again, this can be limited to the order of microseconds. Figure 2A illustrates a simple circuit for driving a Philips Luxeon V blue LED. The light intensity is adjusted by a potentiometer, and the light can be turned on and off by a low-current 5-V transistor–transistor-logic (TTL) input (Albeanu et al. 2008). Figure 2B shows the square wave voltage input and the resulting intensity of the LED light. The time constant is on the order of 10 msec in this simple but suboptimal circuit.

With LEDs that require very high currents, it may be necessary to use high-power dissipation potentiometers (it may not be easy to vary their resistance), and even the use of a Darlington pair transistor may not be enough if the current drive of the TTL input is too low. There is not necessarily one simple circuit design that could be easily adapted to all LEDs currently on the market because of the highly variable electrical ratings and the voltage–current relationships of the devices. Also, in the investigators' experience, switching very high current devices on and off can produce substantial artifacts on electrophysiological recordings. These artifacts can be reduced by electrically isolating the electrophysiological recording area or by using long light guides or fiber optics to transmit the light to the microscope from a source placed some distance away.

LED-Based Illuminators

Uncoupling the LED illuminator and the microscope can be beneficial, especially if light loss through beam splitters is undesirable, dichroics cannot be used, one wants to bypass the other coupling optics in a microscope, or one desires oblique illumination. The LED light will move from the illuminator(s) through fiber-optic cables or liquid light guides to some collection optics that focus the light onto the target. The end of the light guide will generally be outfitted with a lens to either spread or focus the light to the appropriate distance and spot. (Such an illuminator can also be used for nonimaging microscopes such as surgery scopes.) For cell culture and slice imaging, it might be possible to use an LED module that fits under the sample stage of an upright microscope, eliminating the need for light guides. A microscope-mounted LED might not work for experiments in which a bulky condenser is aligned for some form of contrast imaging. As another alternative, the LED illuminator can be coupled to the microscope through one of the lamphousing ports or via a removable filter, field stop, or aperture stop port (Albeanu et al. 2008). This type of coupling can be performed either with a fiber into which the different LED light is coupled (Fig. 3A) or from a homemade lamphouse that collects LED light of different wavelengths into one path and then emits it (Fig. 3B). For multicolor LED sources, the size of the illuminator will most likely constrain the coupling options to either light guides or through the rear lamphouse port. Finally, it is worth noting that the low-noise characteristics of

FIGURE 3. Two methods for coupling an LED-based illuminator to a microscope. (A) An optical fiber is placed on the surface of an LED to direct light into a small box consisting of a focusing lens and mirror that is inserted into a side port on the microscope. (B) A homemade lamp consisting of an LED, a heat sink, a tube, and a lens is coupled to the lamphousing port in the back of the microscope. (Reproduced from Albeanu et al. 2008.)

LEDs (Herman et al. 2001; Rumyantsev et al. 2004; Salzberg et al. 2005) compared to arc lamps make them useful for imaging voltage-sensitive dyes, which typically exhibit low modulation of signals for physiological voltage fluctuations (Grinvald and Hildesheim 2004).

ADVANCED USES FOR LEDs

LEDs can be used for more than simply replacing the light source of a commercial microscope. Technical advances continually provide new opportunities for using LEDs. One example is patterned illumination either for advanced imaging techniques that rely on patterned illumination or for patterned stimulation of neurons (Fukano et al. 2004; Wang et al. 2007; Poher et al. 2008). One very simple way to create a patterned LED illuminator with the desired wavelength is to purchase a digital light processing (DLP) projector and to swap its light source with a suitable LED. Most higher-end projectors use halogen or metal halide bulbs and may include complicated systems to ensure that

the unit is functioning properly. In these projectors, after replacing the bulb with an LED, it may be necessary to trick the projector into thinking that the bulb is still present, which may be performed through a combination of electronic and optical controls. Most projectors that use broad-spectrum bulbs have color filters and wheels that will have to be removed to optimize the projector for use with an LED. However, many of the cheapest projectors, costing \$500 or less, are easier to modify. They are often LED-based, although the pre-installed LEDs may not be optimal for imaging or neuronal stimulation. A simple solution is to move the original LEDs away from the system by extending the connecting wires, thus maintaining the electrical characteristics. Another point to note is that almost all DLP projectors will have timing issues because a single light chip is used to sequentially create multiple repeated red, green, blue, and white (brightness) frames within one frame of a video signal. The technical aspects of adapting a DLP projector for use as a pattern illuminator are beyond the scope of this article. We simply wish to point out that LEDs can provide an additional source of temporal and intensity control, with much lower noise than arc lamps.

LEDs can also be used to optically stimulate awake and moving animals. Although this may not be an imaging development per se, recent advances in optogenetic techniques for controlling neurons with light (Boyden et al. 2005) have opened the doors for research using animals with head-mounted illumination sources (Huber et al. 2007). Without LEDs, these techniques would be unthinkable because the size, thermal management, control of timing, and spectral broadness of more traditional light sources would make rodent-mounted light sources highly impractical. Small LEDs mounted onto an animal may not need a heat sink if the duration of illumination pulses is short enough. The circuitry to control an animal-mounted LED can be quite small and simple and, thus, can be integrated into the mounting apparatus.

CONCLUSION

Recent advances in LED technology have made LEDs both practical and valuable in biological microscopy. As a wider array of spectra from high-power LEDs becomes available, LEDs will be able to replace even mercury lamps and will be able to expand the use of all of the available fluorophores. Further enhancements in LED efficiency will simplify heat management, making it possible to use smaller heat sinks. Further reductions in the size of LED illumination modules could be useful for building smaller imaging systems or for mounting onto small animals. One exciting recent advance is in the field of organic and polymer LEDs, which are now used to make display systems and can be particularly bright. By having LED chips that provide pixel-by-pixel control, structured illumination may be possible without the need for external light patterning technologies.

RELATED INFORMATION

General Background

<http://www.ecse.rpi.edu/~schubert/Light-Emitting-Diodes-dot-org/> The light-emitting diodes website, maintained by the research group of E. Fred Schubert at Rensselaer Polytechnic Institute.
<http://www.olympusmicro.com/primer/lightandcolor/ledsintro.html> LED information from Olympus.
<http://zeiss-campus.magnet.fsu.edu/articles/lightsources/leds.html> LED information from Zeiss.

Application Notes and Design Considerations

<http://catalog.osram-os.com/catalogue/catalogue.do?favOid=000000000001fab4000100b7&act=showBookmark> OSRAM Opto Semiconductors.
<http://www.doriclenses.com/lire/39.html> Doric Lenses, Inc.
<http://www.lunaraccents.com/> Lunar Accents Design.
<http://www.philipslumileds.com/docs/docs.cfm?docType=4> Philips Lumileds Lighting Company.

Latest Information

<http://www.ledsmagazine.com/> LEDs Magazine.
<http://www.photonics.com/> Photonics Media, Laurin Publishing Company.

Major LED Producers and Distributors

<http://www.acriche.com/en/> Seoul Semiconductor, Inc.
<http://www.cree.com> Cree.
<http://www.epitex.com/> Epitex, Inc.
<http://lsgc.com/> Lamina Ceramics, Inc.
<http://www.lumex.com/> Lumex, Inc.
<http://www.luminus.com/> Luminus Devices, Inc.
<http://www.tech-led.com/> Marubeni America Corporation.
<http://www.nichia.com/> Nichia Corporation.
<http://www.osram-os.com/> OSRAM Opto Semiconductors.
<http://www.philipslumileds.com/> Philips Lumileds Lighting Company.
<http://www.roithner-laser.com/> Roithner Lasertechnik.
<http://www.toyoda-gosei.com/> TOYODA GOSEI Co., Ltd.

The investigators have experience using LEDs from Philips Lumileds Lighting Company, Luminus Devices, Enfis, and Roithner Lasertechnik for imaging various biological probes such as green fluorescent protein and its cousins, Oregon Green BAPTA, rhodamine derivatives, various Alexa dyes, as well as for channelrhodopsin-2 stimulation, and in all applications, optical power has been sufficient.

Optics and Optomechanics

Lens tubing systems and microscope adapters were obtained from Thorlabs, Inc. (Newton, NJ). Other parts were obtained from Thorlabs, Inc., Edmund Optics (Barrington, NJ), Newport Corporation (Irvine, CA), and LINOS Photonics GmbH & Co. (Munich, Germany).

Filters

Filters were obtained from Semrock (Rochester, NY), Chroma Technology Corp. (Rockingham, VT), and Omega Optical, Inc. (<https://www.omegafilters.com/>), as well as the previously mentioned optical components suppliers.

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