

THE BIOMEDICAL SCIENTIST



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**Microscope illumination:
the LED revolution**

Microscope illumination: the LED revolution

Light-emitting diode products are set to replace conventional discharge and incandescent lamps to provide more simple, cost-effective and environmentally-friendly alternatives. Here, James Beacher summarises current microscope illumination and looks at LED developments.

The use of microscopes in hospitals and laboratories covers a very wide spectrum of applications, from the identification of fungal mycelium in a clinic to the screening of cervical preparations for premalignant change in cytopathology, and the identification of acid alcohol-fast bacilli (AAFB) in microbiology. Such procedures make use of different microscopy techniques including conventional brightfield (transmitted light), phase contrast (DIC) and fluorescence techniques.

Historically, illumination has been provided by a range of conventional incandescent or discharge lamps. They can be classified as halogen (also 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 and offer many exciting benefits.

In this article, the different types of illumination available are reviewed, their benefits and disadvantages identified, and

consideration given to the convenience, safety, environmental and reduced operating costs of new LED-based products.

INCANDESCENT AND DISCHARGE LAMPS

If you use a microscope in your laboratory it is likely that it will have illumination fitted using one of the common lamp types summarised below. Probably the most common type of microscope illuminator overall is the halogen lamp. This is suitable only for transmitted light applications due to its low intensity. It is also relatively inefficient and can exhibit a shift in colour temperature with time.

For fluorescence microscopy, the high-pressure mercury vapour arc-discharge lamp is most common. This is significantly more powerful than other forms of lamp but its intensity varies across the light spectrum. It is hampered by poor spatial homogeneity due to its complex construction and alignment requirements. Therefore, replacement bulbs can be difficult to align

and this leads to uneven illumination over the microscope's field of view. In addition, bulb lifetime is just a few hundred hours.

The metal-halide lamp is an enhanced version of the high-pressure mercury lamp. It provides more stable illumination and has a longer bulb lifetime. It does, however, suffer from degradation in performance during the bulb lifetime (around 2000 hours) and is more expensive to purchase. As it produces ultraviolet (UV) light, the liquid light-guide used to deliver light from the unit to the microscope also needs to be replaced regularly.

A less-common option for fluorescence is the xenon lamp. This exhibits a flatter intensity across the light spectrum, which makes it more suitable for quantitative analysis.

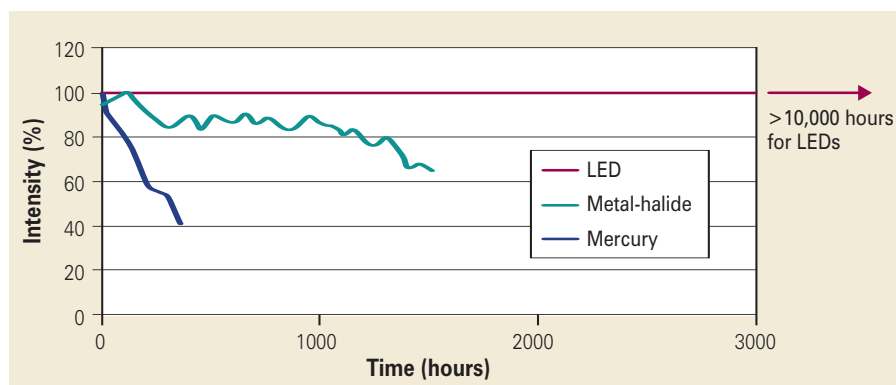
BENEFITS AND DISADVANTAGES OF CONVENTIONAL LAMPS

The conventional lamps referred to above have the benefit that they produce a broad spectrum of white light that can be used for most applications. In fluorescence microscopy, the user simply changes the microscope filter cube to match the stain (eg auramine or acridine) and then has the appropriate illumination configuration.

However, conventional lamps suffer various significant drawbacks. They generate unwanted heat, are inefficient and require regular bulb replacement. In addition, many of these lamp types also use mercury, which is a recognised hazardous material.

A warm-up period and cool-down period is also required before and after use, which is inconvenient. As a result, they are often kept switched on throughout the day in order to be available when required. As they are inefficient, energy is wasted and unwanted heat is generated. The bulbs have a limited operating life and their performance reduces over that period (typically from a few hundred to a few thousand hours).

Conventional lamps also produce UV light, which often is not required to study the sample. This UV light can bleach stained samples and also kill live cells, which reduces



Comparison of stability and lifetime of different light sources.

the amount of time that they can be examined.

Intensity from a conventional lamp decreases through its life. Most lamps are quoted with a lifetime to 50% of original intensity, which means that illumination varies dramatically over time. Thus, quantitative or comparative measurements are not possible unless a new bulb is used on every occasion.

LIGHT-EMITTING DIODE ILLUMINATION

With the introduction of LED-based microscopy illumination, almost all of the disadvantages of conventional incandescent and discharge lamps are overcome. Light-emitting diodes are solid-state semiconductor devices that emit light directly without using a bulb. Previously used only as indicators, LEDs are now sufficiently intense to be used to illuminate. Their potential in consumer applications is making them ubiquitous in markets such as the automotive industry and in lighting for buildings.

Light-emitting diodes can be up to five times more efficient than a conventional incandescent or discharge lamp. As a result, considerable development has been carried out, both by the LED-chip manufacturers to increase brightness and by the end-product manufacturer to engineer specialised packaging, thermal management and optics dedicated to end-user applications. Together, these improvements have now resulted in LED illumination systems that provide greater intensity than incandescent lamps in regions of the spectrum important for microscopy.

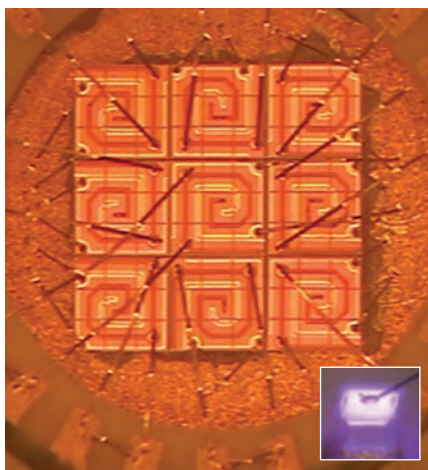
As intensity has increased, the additional benefits offered by LEDs have become very attractive. These are instant on/off capability, long lifetime, low running costs, greater efficiency and less heat generated.

BENEFITS AND DISADVANTAGES OF LIGHT-EMITTING DIODES

When one considers that the LED can be switched on/off instantly, the amount of actual 'on' time in a day can be measured in a few hours, and thus intensity remains broadly the same over its lifetime, with 10,000 or even



With instant on/off capability and 0–100% intensity adjustment, LED illumination offers convenience and control. No warm-up or cool-down time saves on energy costs.



An array of LEDs for increased performance, with inset, a single LED die showing the brightness achievable. Intensity on the surface of an LED can be as much as 70 W/cm².

50,000 hours now being offered, so LED illuminator lifetime can now be expected to be 10 or even 20 years! This amounts to a significant financial saving against the cost of purchase, alignment and disposal of the equivalent conventional bulbs required over this period.

As LEDs produce narrow wavebands of light, consideration must be given to the correct filter cube and stain excitation region. Optically misaligned or poorly performing filters can reduce performance considerably. The LED intensity can be greater than mercury lamps (100 W) in the blue and red excitation regions but are weaker than mercury in the green excitation (red emission) region.

As LEDs are more efficient, less energy is used and less heat needs to be dissipated, which can be an important issue if the microscope is being used in close or cramped conditions. They exhibit greater stability and repeatability, which makes comparative tests, or tests against a reference sample, more reliable. With their instant on/off capability, LEDs can act as a shutter, saving additional costs on the microscope.

The LED can produce white light with a fixed colour 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 LEDs with red, green and blue wavelengths (as a television picture is generated). Furthermore, the ability to tune the colour is possible.

Applications for illumination requiring a single LED wavelength are perfect for clinical screening. Fluorescence microscopy lends itself to illumination by a specific colour. In fact, auramine, acridine and fluorescein isothiocyanate (FITC) can all be illuminated using a single 470 nm LED light source, and there are now LED colours for most common fluorophores used for all fluorescence applications.

ENVIRONMENTAL CONSIDERATIONS

There are three main areas where microscope illumination is affected by environmental considerations: 1) health and safety considerations relating to eye damage from UV radiation, 2) the safe use and disposal of mercury products, and 3) 'green' aspects of reduced energy consumption.

Ultraviolet light is known to damage the human eye. Broad-spectrum incandescent or discharge lamps generate a lot of 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. As LEDs only generate the desired wavelengths of light, only those with specified illumination in the UV region can cause UV damage.

Where mercury is used, consideration must be given to risk of the bulb exploding, and the release of mercury vapour into the air. Health and safety requirements for mercury can be onerous as it is a recognised 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 offered by LEDs, up to five times less energy is consumed during comparable usage. As many conventional lamps are left on all day to overcome the warm-up requirements, the actual reduction in energy by using instant on/off LEDs can be significant.

DEDICATED AND GREEN

Conventional incandescent and discharge-lamp illumination offers the benefit of broadband illumination. This reduces the need to consider optical filtering and a single light source can satisfy most microscopy applications. However, they are inconvenient to use and require regular bulb replacement.

Light-emitting diodes are set to replace incandescent and discharge lamps due to their improved intensity, long lifetime and ease of use; however, greater consideration must be given when selecting wavelengths and optical filters to optimise performance. For many defined applications, such as the use of auramine, acridine, Calcofluor and FITC, dedicated LED illumination offers only benefits.

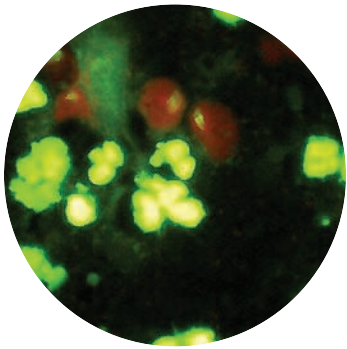
The environmental and safety benefits of LEDs are also persuasive. No mercury is used and no replacement and disposal issues need to be addressed. Finally, lower power consumption satisfies the ever-increasing demand for a 'greener' laboratory.

Further information is available from the author, Jim Beacher, CoolLED, CIL House, Charlton Road, Andover, Hants SP10 3JL (tel +44 [0]7887 750496, email jim.beacher@cooled.com).

Replace your Mercury lamp with a pE-100

Select the single wavelength pE-100 to match your fluorophore and test

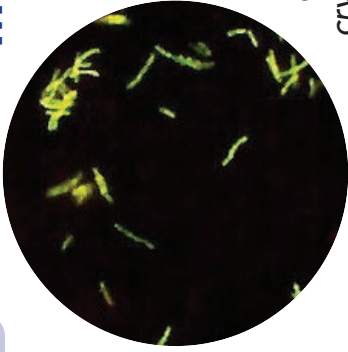
Examples of tests using the CoolLED pE-100



BACTERIOLOGY

A typical test would be for *Trichomonas vaginalis*.

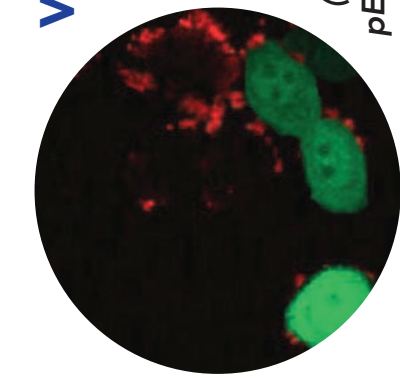
For testing with **Acridine Orange** select a pE-100 at 470nm



MYCOBACTERIA

A typical test would be for tuberculosis (TB), and other acid fast bacilli (AFBs).

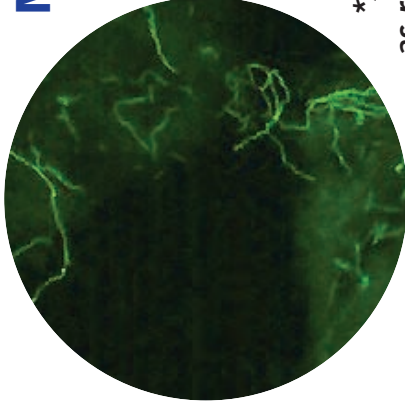
For testing with **Auramine** stains select a pE-100 at 470nm. (Similar staining can also be used for *cryptosporidium*)



VIROLOGY

A typical test would be for RSV or parainfluenza.

For testing with **Fluorescein Isothiocyanate (FITC)** select a pE-100 at 470nm



MYCOLOGY

A typical test would be for fungal mycelium/elements.

For testing with **Calcofluor White** select a pE-100 at 400nm*

*Also available at 365nm as required



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