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LED illumination for sperm analysis from Prior Scientific

Introduction

In this article we describe the use of the Prior L100 LED illumination system to accurately distinguish between live and dead cells. Such discrimination is common across a broad range of disciplines in the biosciences and medicine. In the case described here, the L100 LED illumination system from Prior Scientific was integrated into Microptic's SCA sperm analysis system. This system provides Microptic with excellent excitation of the relevant fluorophores whilst being both compatible with a broad range of microscopes and easily integrated with Microptic's software.

Distinguishing between live and dead cells

Accurately distinguishing between live and dead cells, and working out the relative proportions of each within a population, is a common requirement in the life sciences, from toxicology to biomedical research to microbiology. In a high throughput system where thousands or millions of cells must be characterised within a limited period of time precise discrimination may prove challenging. Further complications may be posed by the presence of artefacts, such as elements of the growth medium or substrate, within the sample. Fluorescent microscopy utilising two different dyes is a frequently used method, which both discriminates well and is fast enough to be used in high throughput systems.

Microptic's Sperm Analysis System

Microptic, a biomedical company based in Spain, designs, manufactures, and installs analysis systems, which can be used for analysis of both human and animal spermatozoa. A system known as the Sperm Class Analyser (SCA) can be used to characterise various characteristics of the cells, including both the absolute and relative amounts of living and dead sperm inside a given sample .

The SCA does this via fluorescence microscopy, which provides a more accurate description of the viable sperm: non viable sperm ratio of the sample than simply observing sperm cells (many of which may be dead) or moving cells (which may substantially underestimate the number of viable cells). Two dyes – Propidium iodide and Trihydrochloride trihydrate – are applied to the sample. The latter binds to DNA but cannot penetrate cell membranes, whilst the former, whilst also binding to DNA, can penetrate cell membranes. Propidium iodide, when optically excited, emits light in the red region of the spectrum; whilst Trihydrochloride trihydrate emits light in the blue region (Fig 1). Thus, dead cells (or at least, cells with severely compromised membranes) will glow red when excited, whilst living cells will be blue. It is then a simple matter for Microptic's software to automatically count the relative abundances of these cells and generate a report.

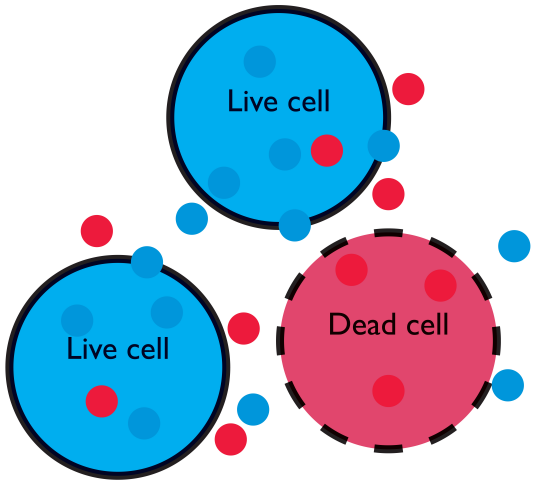

Figure 1 – Propidium iodide, which glows red when excited, cannot pass through living cell membranes but can pass through dead cell membranes. Therefore, dead cells will appear to be red when viewed under fluorescence microscopy.

By contrast, Trihydrochloride trihydrate, which glows blue, can pass through cell membranes; thus living cells will appear blue when excited.

Mircoptic also uses this system to assess sperm motility as well. Usually, this is done using phase contrast techniques, but when the sample is not pure – for example, extended with milk or egg products, which is very common in samples from animals – fluorescence imaging provides a more accurate method of locating the sperm cells and tracking their movements to provide an accurate measurement of sperm movement. Overall the system allows easy visualisation of sperm cells and a reliable indication of their status (Fig. 2). To work well, the system requires illumination to successfully excite the fluorescent dyes.

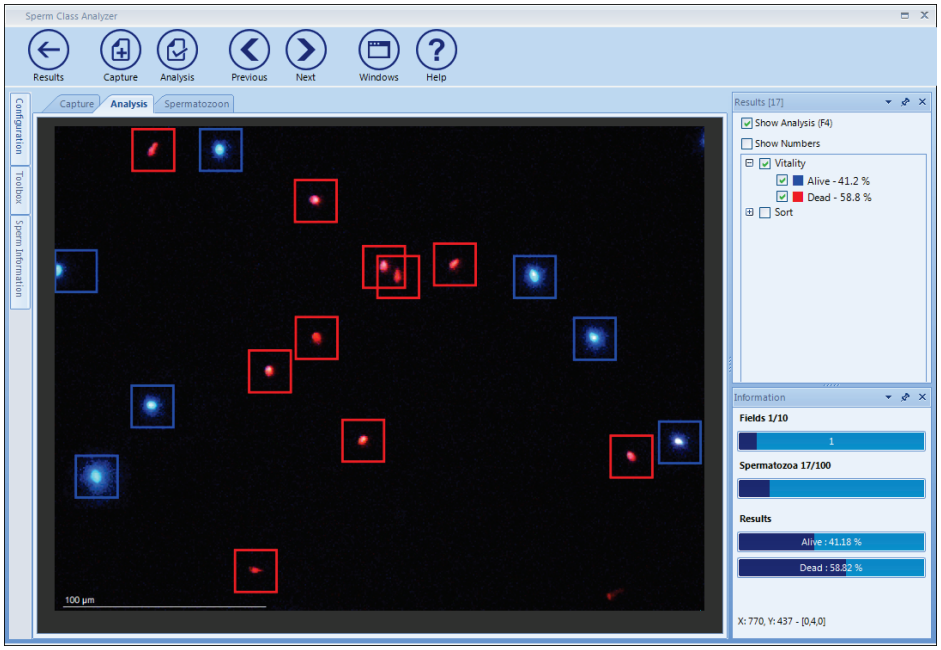

Fig. 2 – Screenshot from the SCA, courtesy of Microptic. Red indicates dead sperm, blue indicates living sperm.

Illumination for fluorescence excitation

Microptic's system utilises a Light Emitting Diode (LED) illumination device from Prior Scientific.

Prior Scientific is a British company based near Cambridge which sells a wide array of high quality microscopy equipment. In addition to control systems, motorised stages, focussing devices and other items, Prior also sells metal halide and illumination devices, as well as offering customised product manufacturing. Initially, a customised 'Brightfield LED' was used. Normally used for Brightfield, Darkfield and contrast techniques; by fitting a LED at 365 nm Prior was able to create an illumination system that precisely met Microptic's requirements for a light source for fluorescent microscopy.

Engineers from Microptic were impressed by four key features of the system. Firstly, the fact that the system could be easily adapted to fit the vast majority of modern microscopes ensured that Microptic's own system was suitable for as many end-users as possible. Secondly, the LED's ability to be controlled via software was another major advantage, allowing the whole system to be controlled from one focal point, resulting in a more streamlined and efficient user experience.

Microptic also felt that the Prior LED represented excellent value for money, providing a precisely controlled source of high quality illumination, for a reasonable outlay, which in turn made their whole system more affordable to potential customers. Finally, and perhaps most importantly, when equipped with a 365 nm LED and a 400 nm dichroic mirror, the system excellently excited the two fluorescent dyes used.

Prior's LEDs offer other advantages. The amount of heat and vibration produced is minimal, reducing disruption to imaging. They can be switched on or off instantly, meaning that no warm up or cool down time is required, and do not contain mercury, so no special disposal protocols need to be followed. Additionally, the lifespan of a Prior LED is at least 10,000 hours which, coupled with the fact it only needs to be illuminated when actually in use, means that the product can be used for extremely long periods of time without the need for part replacement. This is especially attractive for customers who no longer face the prospect of having to stop work every 200 hours to replace a bulb, as was the case with traditional mercury bulbs, with the added frustration of having to align the bulb before use.

Lumen 100-LED

Recently, Microptic have switched to purchasing a new model of LED from Prior Scientific – the 'Lumen 100-LED' (Fig. 3). Similar in many respects to the Brightfield, it has the added advantage of having its intensity altered in 1% increments, either via software or via the supplied control box. Furthermore, the product is designed specifically for fluorescence microscopy and requires no customisation. The unit can be fitted with a choice of 1 of 11 LEDs. 10 are narrow spectrum from 365 – 660 nm, whilst a final LED emits broad spectrum white light. Although this was not a concern of Mircoptic, the Lumen 100-LED also offers the user the ability to exchange LEDs – thus a whole new system need not be purchased if the user's light source requirements change.

By using such a specialised LED to produce a specific wavelength designed to excite specific fluorophores, the end user can be certain that optimum excitation of the desired dye(s) is occurring, whilst excitation of unwanted flurophores is reduced – although in many situations filters should still be used in order to ensure that only the desired flurophores are excited. By using a specific LED wavelength, Microptic

Fig.3 – Lumen 100-LED from Prior Scientific.

Conclusions

Thanks to the specificity, instant response, and long lifetime offered by LEDs, it is little surprise that many scientists are turning to use these devices, both for fluorescent microscopy, and for other techniques such as brightfield, darkfield, DIC and phase contrast techniques. Indeed, staying only within the area of distinguishing live cells from dead, there are numerous dye pairs – calcein-AM, which degrades to a fluorescent calcein emitting in the green spectrum when degraded by esterases produced by living cells, and ethidium homodimer-1, is just one example.

An LED available for the Lumen 100-LED emits at 505 nm and so would be ideal for these dyes, which are optimally excited at 494 and 517 nm respectively.

Although other illumination sources, such as metal halide bulbs, still have their place, it is likely that the number of LED systems used within microscopy will increase in the near future. With the added value provided by Prior Scientific, with decades of experience of providing equipment for optical

microscopy, an LED illumination system from Prior Scientific will be an ideal choice for many across a broad range of applications and disciplines.

Microptic can be found on the Internet at http://www.micropticsl.com/. For more on Prior's range of equipment for microscopy, including LED and metal halide illumination systems, visit www.prior.com or contact uksales@prior.com. If you have any questions or comments on this article please email jwilson@prior.com. Microptic assisted in the preparation of this article by supplying information and the image used in Figure 2.