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FLUORESCENCE MICROSCOPY

SCHOOL OF GEOGRAPHICAL SCIENCES UNIVERSITY OF BRISTOL

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INTRODUCTION

Fluorescence microcopy requires intense, specific wavelengths of light. Each wavelength will excite a fluorophore with the light they emit being captured, thus generating an image. There are a wide range of applications for fluorescence microscopy from counting populations of cells to visualising molecular interactions within a cell. In essence the fluorescence microscope will require a microscope, a light source (with power supply) and excitation filters. Some of the earliest light sources used to generate the intense light required were mercury-vapor lamps.

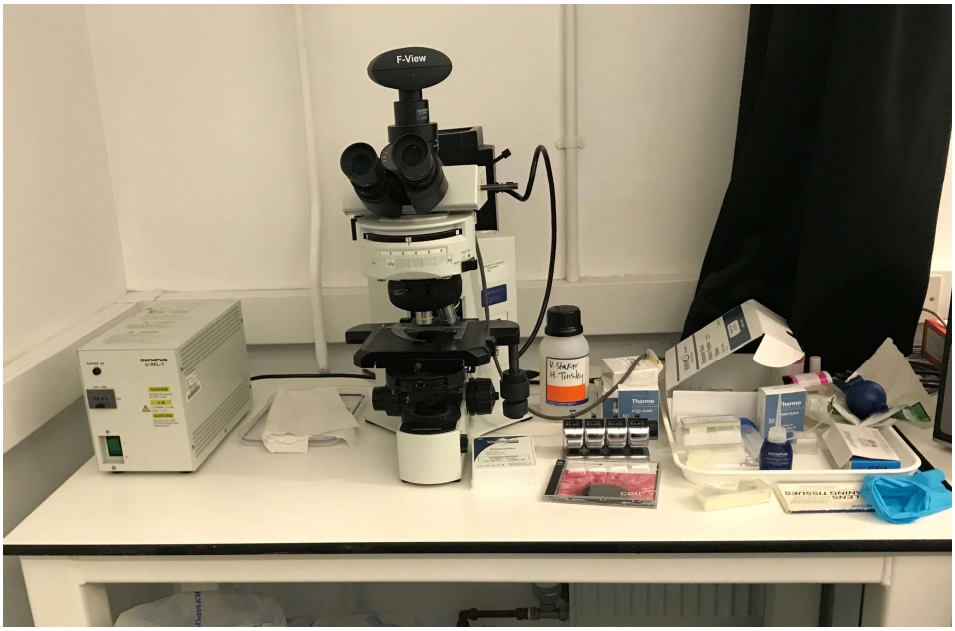

Figure 1. Olympus BX41 microscope with mercury-vapor lamp attached and power supply on the left.

MERCURY-VAPOR LAMPS

Mercury-vapor lamps are still widely used, however, in the last 15 years technology has advanced to offer a number of alternatives to this light source. One driving factor behind this search is the toxicity of mercury and the safety considerations that accompany it. In the case of a bulb explosion mercury vapor is released into the room, therefore ventilation is a mandatory requirement when placing the microscope (Figure 2).

The University of Bristol have a number of fluorescence microscopes, many still using mercury-vapor lamps. An alternative light source is the Light Emitting Diode (LED) and over two days these two technologies were compared in the lab setting. All costs and energy consumption data has been collected on site in the Department of Geographical Sciences. The energy consumption monitors used have a kWh variance of ≤ 1%.

Figure 2. Extraction of mercury vapor must be accounted for when siting the microscope.

MERCURY-VAPOR VERSUS LED

The microscope used is that shown in figures 1 & 2; an Olympus BX41. The power supply for the lamp was an Olympus U-RFL-T. The mercury-vapor bulbs used by the researchers are replaced after 200 hours of usage. This microscope was regularly used to carry out bacterial counts using DAPI, a common fluorophore, typically being on for eight hours (continuous) per working day. The LED system used in this comparison was a CoolLED pE-300^{ultra} direct fit illumination system (Figure 3). The LED system was connected to the microscope and the end user adjusted the intensity of the LED so that the bacteria were as bright and as easy to count as when using the mercury-vapor lamp. The intensity of the CoolLED unit is set per percentage point, for this procedure the LED was set at an intensity of 76% (Figure 4).

Figure 3. The pE-300^{ultra} fitted to the Olympus BX41.

The data collected for each unit aimed to highlight the costs in energy, bulbs and staff time. The LEDs from CoolLED have a minimum estimated lifespan of 25,000 hours of usage, and the costs of running both systems have been calculated using this figure (Figure 5).

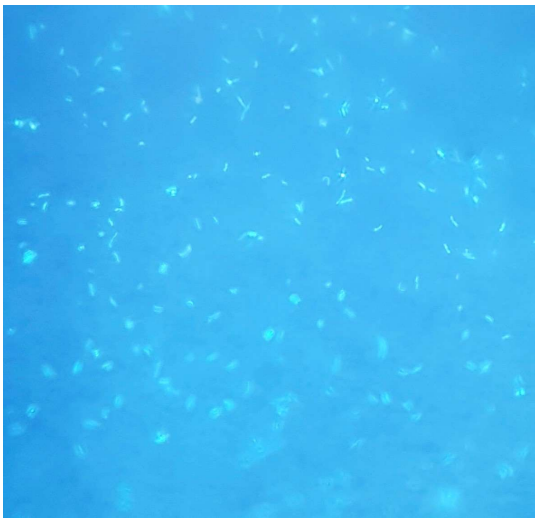

Figure 4. Down the lens, DAPI labelled bacteria

Cost	Mercury-Vapor Lamp	LED. CoolLED pE-300^{ultra}
A. Bulbs used in 25,000 hours	**125 Bulbs**	**None**
B. Cost of bulbs used in 25,000 hours	£250 each (bulb + installation): **£31,250**	**£0.**
C. Time for Microscope Alignment per bulb	30 minutes per bulb, 125 bulbs **62 hours 30 minutes**	**Not Applicable**
D. Energy Consumed in 25,000 hours usage	0.114 kWh/h **2,850 kWh**	0.010 kWh/h **250 kWh**
E. Electricity cost of 25,000 hours (9.7p/kWh)	**£276.45**	**£24.25**
F. Warm Up Time	**20 minutes**	**Not Applicable (instant ON/OFF)**
G. Warm Up Time Energy Consumption	0.117 kWh/h x 20 minutes **0.039 kWh**	**Not Applicable (instant ON/OFF)**
H. Warm Up Time for 8 Hours of usage per bulb	**24 Hours (per 200 hours/bulb)**	**Not Applicable (instant ON/OFF)**
I. Cost of unit being usable for 8 hours (adding warm up time of 20 minutes)	Warm up energy + 8 hours usage energy + bulb cost ((£250/200 hours per bulb) x 8.33) **£10.50**	8 hours energy usage **£0.00776**
J. Cost of 6 hours of viewing samples in the 8 hours the unit is on for.	Cost is the same due to time for warm up and cool down **£10.50**	6 hours energy usage (off for 2 hours) **£0.00582**
K. Cool Down Time	**30 minutes**	**Not Applicable (instant ON/OFF)**
J. Total Cost Over 25,000 Hours of usage	(25,000 hours/8.33 hours) X Cost of unit being usable for 8 hours) **£31,512.61 (£1.26/hour)**	(25,000 hours X energy consumption in use per hour **£24.25 (£0.00097/hr)**

Figure 4. Mercury-vapor lamp versus the CoolLED pE-300^{ultra} LED system.

From the data collected we can see that the cost in bulbs constitutes the majority of the running costs associated with using the mercury-vapor lamp. However, it must also be noted that when in use (section D in figure 5) the CoolLED unit uses 8.77% of the electricity that the mercury-vapor lamp uses. If we compared total costs over the 25,000 hours (section J in figure 5) the CoolLED unit costs less than 0.08% of what it costs to run the mercury vapor bulb. If we were to consider that using the LED system is instant ON/OFF then the costs would be even lower as during the 8.33 hours the mercury-vapor lamp is on, the LED unit would only be on for 6 hours.

What must also be considered when using mercury-vapor lamps is the cost to the University in staff time; **62.5 hours** of time would be spent realigning the microscope versus zero hours over the same time period for the LED system.

The only cost not included in this study is the disposal of the bulbs. This is a cost that is paid for by the University but will vary depending on demand and volume. The cost is estimated to be £5.50 for the first item and then £5.50 per additional kilo this is based on a scheduled collection, additional charges would be incurred outside of the arranged schedule. Even without this cost to consider, over 25,000 hours the CoolLED unit would use less than 0.08% to run compared to costs of the mercury-vapor lamp.

COSTS OF OWNERSHIP AND REPLACEMENT

By using the data in Figure 5, we can estimate two costs, ownership and replacement. If a researcher were to consider buying a fluorescence microscope and light source for this application, the cost of ownership would be as follows. In the case of a mercury-vapor lamp and power supply, the cost to buy is estimated at £3,000. This if used for 25,000 hours in eight hour shifts would cost £31,512.61 to run. Therefore the total cost of ownership over 25,000 hours would be **£34,512.61**. The cost of buying the CoolLED pE-300^{ultra} is £3995, the cost to run it over 25,000 hours is £24.25. Therefore the total cost of ownership would be **£4,019.25**. Spending the extra £995 to buy the LED light source instead of the mercury-vapor lamp would save an end user over £30,000 in costs.

If looking at replacement, the CoolLED saves £1.25903 per hour of usage. If we divide the cost of the CoolLED unit and its energy consumption during a 25,000 hour period by the hourly saving it is calculated that the CoolLED unit pays for itself in 3,192.34 hours of usage.

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For further information on this study or lab sustainability please contact office@greenlightlabs.co.uk