Technical method

Small quartz halogen lamps for immunofluorescence microscopy

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Mercury arc lamps, such as the Wotan HBO 200 W and HBO 50 W/AC, are usually fitted to fluorescence microscopes, although for work with fluorescein conjugates tungsten halogen sources have been recommended by, for example, Heimer and Taylor (1972), who described a dark ground instrument with a 150 watt lamp. The epi-illumination microscope, which has now superseded other types, is more efficient for fluorescence excitation and thus increases the potential value of tungsten halogen lamps. In this type of instrument the microscope objective serves both to illuminate the specimen and to collect light emitted by the specimen. Therefore a combination of a low magnification eyepiece ($\times 4$ or 5) and a high magnification, high numerical aperture objective is preferred, although some dry objectives may have a working distance and a depth of field insufficient for work with specimens mounted in buffered aqueous glycerol. Immersion objectives corrected for this medium are not available.

It is not generally appreciated that for high efficiency the dimensions of the light source should not exceed those of the field of view. The area of specimen visible in an objective of moderate magnification, say $\times 25$, may be no larger than 0.6 mm in diameter. Consequently, lamps such as the HBO 100 (arc size 0.25×0.25 mm) and HBO 50 W/3 (0.2×0.35 mm) are to be preferred to the HBO 50 W/AC (0.3×1.0 mm) in common use and the HBO 200 W (0.6×2.0 mm). Because most quartz halogen lamps at their rated voltages run at similar filament temperatures, 3200 to 3400°K , all have similar light outputs per unit area of filament surface and thus differ from arc lamps. We have used for several years for antinuclear factor tests a 12 volt 100 watt quartz halogen lamp in place of the HBO 50 W/AC in our Leitz Ortholux lamphouse 100Z. Substitution by the smallest quartz halogen lamps we could find,

the Thorn M29 10 watt (filament 1.27×0.71 mm) or M30 20 watt (1.83×0.95 mm), made no visible difference to the fluorescence of our specimens. In our particular instrument the sole gain was that the small power requirement of these lamps allowed us to use a less expensive power supply. Low-wattage bulbs are often used as 'built-in' light sources for conventional microscopy; an elaborate lamphouse with quartz lenses is not required. With suitable design it should therefore be possible at lower cost to make a more efficient light collecting optical system than is possible with the larger quartz halogen sources.

Method of lamp alignment and filter selection for fluorescein conjugates

We found a semisilvered surface, such as the grid of a counting chamber, makes a satisfactory specimen slide for adjustment purposes. With the grid lines in focus the filament image was focused and centred, then defocused slightly to illuminate the field of view uniformly. Filament lamps have a higher red and a lower blue/violet light output than mercury sources; a pale yellow exciter filter (eg, Schott GG450 or 470) was not required for quartz halogen lamps. With the appropriate Leitz mirror and barrier filter (No. 3) the Balzer FITC exciter filter gave a red filament image. Addition of a blue Chance LP1 filter 4.5 mm thick to the exciter filter removed all red light, leaving a dim green background. This filter combination was selected for the lowest magnification objective in routine use, $\times 25$. With still lower magnifications the background was brighter. However, as the silvered slide presented an unusually severe test to the filter system, no difficulty was encountered in normal use.

Reference

Heimer, G. W., and Taylor, C. E. D. (1972). Improved immunofluorescence obtained with a tungsten halogen lamp in a modified inverted microscope. *Journal of Clinical Pathology*, **25**, 88-92.

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