Detection of Auramine O stained *Mycobacterium tuberculosis* by means of transmitted light LED fluorescence – a new approach.

Transmitted light microscopy on sputum samples is the most widely diffused method to diagnose pulmonary tuberculosis in low-income countries. However, this method is complex and has low sensitivity compared with culture, while the more-sensitive fluorescence microscopy method is a standard diagnostic tool in high-income countries.

The present application note describes a new device for fluorescence microscopy, which can be employed on small microscopes at an affordable price. Furthermore it can be adapted on existing microscopes. Battery operation is also possible.

Key terms: light-emitting diode, transmitted light fluorescence

Introduction

The *Mycobacterium* genus includes pathogens responsible for severe human diseases such as *Mycobacterium tuberculosis* - the agent of tuberculosis (TB), and *Mycobacterium leprae* - the agent of leprosy.

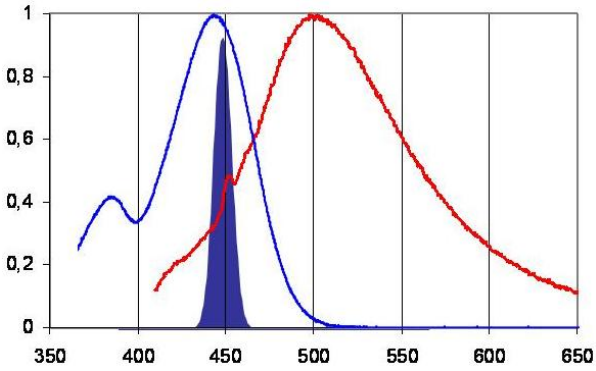
The rod-shaped *Mycobacterium tuberculosis* is a non-motile bacteria about 3 to 4 um in length and less than 0.5 um in width.

The species are classified as acid-fast bacteria and a widely diffused staining method is the Ziehl-Neelsen, where bacilli are observed in transmitted light. Illumination is typically provided by the built-in light source (mainly halogen lamp) or even sunlight reflected by a mirror.

The relative complexity of this method and the need of high magnification lenses are major drawbacks, while fluorescence microscopy is credited with improved sensitivity. The identification of mycobacteria with the fluorescent dye Auramine O is due to the affinity of the fluorochrome to mycolic acid in the cell walls. Recent investigations also indicate a potential affinity to the bacteria DNA. Auramine O is excited by blue light and emits in the region from ~500nm to ~650nm (green-yellow-red).

Fluorescence microscopy displays some important advantages:

1. High contrast fluorescence images allow easier detection of bacteria.
2. The use of low to medium power lenses (typically 10x, 20x, and 40x) permits a larger field of view than conventional microscopy, where typically a 100x lens is used.
3. The fluorochrome staining method is simpler than the Ziehl-Neelsen method.



LEDs emit an extremely efficient and narrow spectrum only in the desired bandwidth, thus producing excellent signal-to-noise ratio.

Royal Blue LED | Auramine excitation | Auramine emission

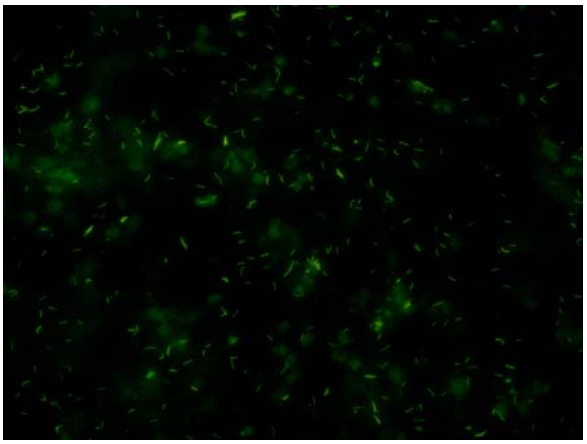
A potential shortcoming of fluorescence microscopy is the risk of false-positive results: all acid-fast organisms can be stained by Auramine O, including some parasites. Furthermore inorganic objects may incorporate the fluorochrome too.

FluoLED: the transmitted light fluorescence module

A unique, proprietary illumination system has been developed to utilize high-power solid-state (LED) light sources to replace the mercury arc-lamps used in traditional epi-fluorescence microscopy.

The LED fluorescence module is designed to attach to a standard bright field microscope and is used in transmission mode. Bright field microscopy capability is not affected since the halogen white light function remains intact.

Fraen FluoLED modules on standard microscopes from Carl Zeiss (left) and Olympus (right).



The mycobacteria will appear as bright luminous rods on a dark background. A potassium permanganate counterstain helps prevent non-specific fluorescence.

Results

Transmitted-light fluorescence microscopy proved to be a valuable technology for observation of Auramine O stained *Micobacterium tuberculosis*.

The use of a high power solid-state (LED) source enabled increased performance, significantly increased light source lifetime, reduction of initial costs and operating costs, and reduction of maintenance and heat production. And, a rechargeable battery pack permits diagnosis in remote areas and during power failures.

Main FluoLED product benefits are:

- Light source lifetime: typically 30000 hours, thus allowing many years of operation and cost savings.
- No need of any special alignment procedure.
- Variable light control for adjustment of illumination intensity.
- No warm-up time required for the light source.
- Allows transmitted light observation without removing the fluorescence module.
- Battery pack option for field operation.

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