

A solid-state time-gated luminescence microscope with UV LED excitation and EM-CCD detection

Russell Connally and James Piper

Department of Physics, Division of Information and Communications Science, MQ Photonics, Macquarie University, Sydney, NSW 2109, Australia.

E-mail: rconnall@ics.mq.edu.au

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Many naturally occurring materials are autofluorescent and this property can reduce the discriminative ability of fluorescence methods, sometimes to the point where they cannot be usefully applied. Shifting from the spectral to the temporal domain, it is possible to discriminate fluorophores on the basis of their fluorescence decay lifetime. Luminophores with sufficiently long lifetimes can be discriminated from short lived autofluorescence using time-gated luminescence (TGL). This technique relies upon the application of a brief excitation pulse, followed by a resolving period to permit short lived autofluorescence to decay, after which detection is enabled to capture persistent emission. A high power UV light emitting diode (LED) was mounted in the filter capsule of an Olympus BX51 microscope to serve as the excitation source. The microscope was fitted with an Andor DV885 electron multiplying CCD camera

(EM-CCD) with the trigger-input synchronized to UV LED operation. *Giardia lamblia* cysts labeled with the europium chelate BHHST were analyzed against an autofluorescent

background with the TGL microscope. The EM-CCD camera captured useful TGL images in real-time with a single exposure cycle. With (4x) frame averaging, images acquired in TGL mode showed a 30-fold improvement in SNR compared with conventional fluorescence microscopy. The line

profile shown above the images in Figure 1 illustrates graphically the reduction in background that was achieved in TGL mode. SNR was improved from 1 to 4.6 when the instrument was operated in TGL mode but without EM gain enabled. SNR was improved by a factor of 28 over frame (A) when EM gain of the camera was increased to 576 in TGL mode (frame C).

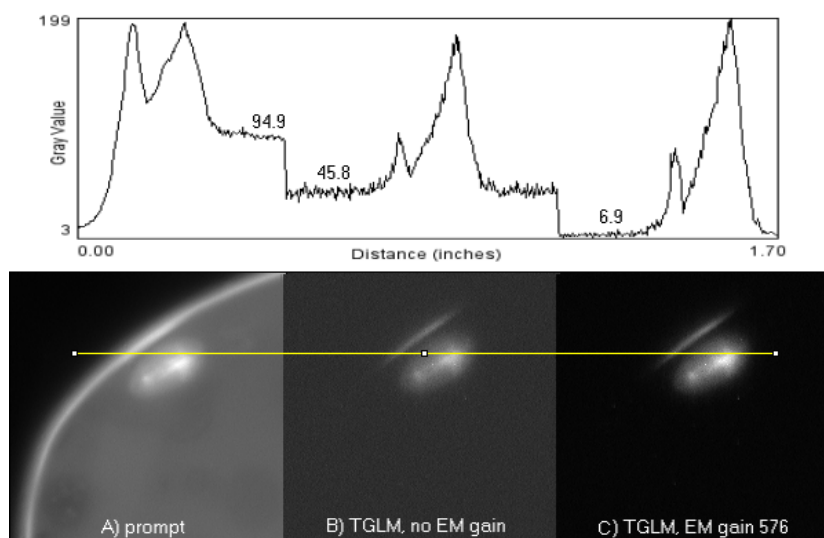


Figure 1: A BHHST labeled *Giardia* cyst suspended in an aqueous solution of UV excitable dye (DMACA) imaged in conventional epifluorescence mode (A), in TGL mode without the assistance of EM-gain (B) and in TGL mode with EM-gain enabled (C).