

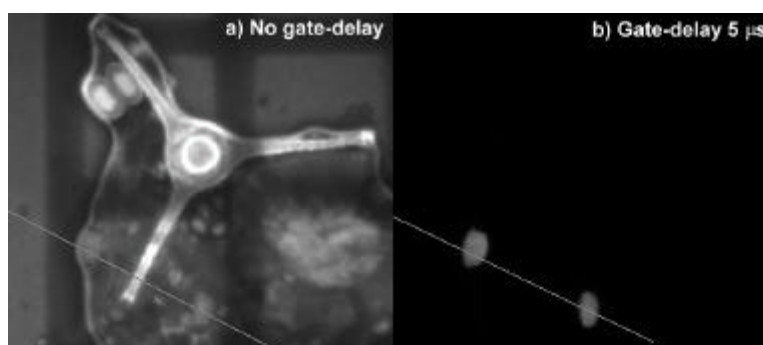
# High Intensity Solid-state UV Sources for Time-resolved Fluorescence Microscopy

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Many naturally occurring substances are intrinsically fluorescent (autofluorescent) when excited with UV or visible wavelengths. Autofluorescence emission typically spans the visible spectrum with a lifetime ( $\tau$ ) measured in nanoseconds. Time-resolved fluorescence techniques exploit the large difference in  $\tau$  that exists between typical autofluorophores and for example, lanthanide chelates (typically with europium<sup>3+</sup> or terbium<sup>3+</sup> ions). The lanthanide chelate acts to collect light from the excitation source with subsequent energy transfer to the lanthanide ion for emission. Due to the unusual electron configuration of these elements, emission occurs via a forbidden state, giving rise to a typical long-lived molecular fluorescence (300 ns). Time-resolved fluorescence microscopes that use these fluorophores require an excitation source in the UV that can be switched off instantly with little or no persistence. Short-lived autofluorescence decays rapidly once excitation has ceased whilst the longer-lived emission from the synthetic probes persists. Thus probe fluorescence can be captured devoid of background using a sensitive camera system. Excitation sources such as flashlamps are often used, however they have relatively long decay rates. We tested a new solid-state UV LED excitation source (NCCU033; Nichia Corp. Japan) rated at 100 mW continuous output centered at 365 nm. The flashlamp in our lab-built TRFM [1] was replaced by



**Fig. 1** (a) Two immunofluorescently labeled *Giardia* cysts embedded in an autofluorescent matrix of algae (line transects cysts) viewed with conventional epifluorescence and (b) using time-resolved fluorescence microscopy.

adapting the LED to fit within the filter-cube of the fluorescence microscope. The LED was pulsed at a frequency of 500 Hz, 100 ns duration at a

peak current of 792 mA. Fig. 1 (a) illustrates the poor signal-to-noise ratio (SNR) obtained using conventional fluorescence microscopy with a ratio of probe to autofluorescence intensity of 0.48:1. The S/N ratio was improved 27-fold (to 12.98:1) in time-resolved fluorescence mode (Fig. 1b) using UV LED excitation and a gate-delay of 5 ns. With similar gate-delays, the UV LED provided greatly superior SNR compared to the flashlamp.

1. Connally, R., D. Veal, and J. Piper, *High resolution detection of fluorescently labeled microorganisms in environmental samples using time-resolved fluorescence microscopy*. FEMS Microbiology Ecology, 2002. **41**: p. 239-245.