PHOTEK Application Note

Fluorescence Lifetime Imaging Microscopy (FLIM)

Fluorescence Lifetime Imaging Microscopy is based on measuring the decay of fluorescence molecules in biological samples and other physical specimens. When a fluorophore is excited by a fast pulsed light source, it de-excites with an average lifetime, τ, typically between a few nanoseconds (ns) and a few milliseconds (ms). The initial intensity of light emitted by these excited fluorophores, I_0, decays with time t, as $I(t) = I_o e^{-t/\tau}$. A unique property of fluorescence lifetime is its immunity to the local environment. Fluorescence intensity can often be affected by the concentration of fluorophore, photo-bleaching and variations in detection efficiency. Fluorescence lifetime by contrast, is an intrinsic property of the molecule. The lifetime can be changed due to other processes, such as non-radiative energy transfer to local molecules and other physical phenomena, enabling images of these processes. Measurement applications include neural imaging, cancer cell detection, cellular function and FRET.

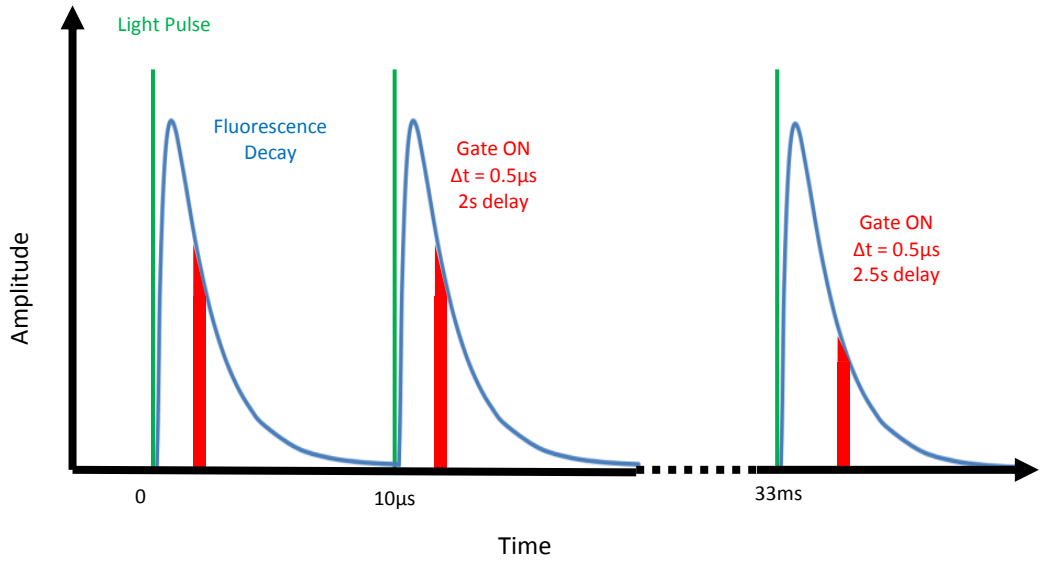

Figure 1: Fluorescence decay after excitation with a light pulse. An Image Intensifier is used to sample the decay curve as a function of time after excitation in order to measure lifetime.

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Instrumentation

In fluorescence lifetime imaging an image intensifier is used to optically gate the fluorescence signal being imaged through a microscope. To measure the decay time of the fluorescence signal a delay generator is used to "walk" an image intensifier gate through the decay of the fluorescence signal, as illustrated in Figure 2. Multiple excitations all having the same time delay from the light pulse can be integrated in a single CCD frame. By taking images over a sequence of delays the full fluorescence decay at each pixel in the image can be determined. In the example shown a 100 kHz light pulse, either a laser diode or LED, excites the fluorescence molecule which emits with a decay time of about 3 µs. For the first 33 ms the gate delay is set to 2 µs with a 0.5 µs gate window, the time during which the Image Intensifier is ON. The CCD integrates the signal from all 3300 gate periods before reading out the image. The gate pulse is then advanced to a 2.5 µs delay and a new CCD frame begins integration. This is repeated until the full fluorescence decay profile has been measured.

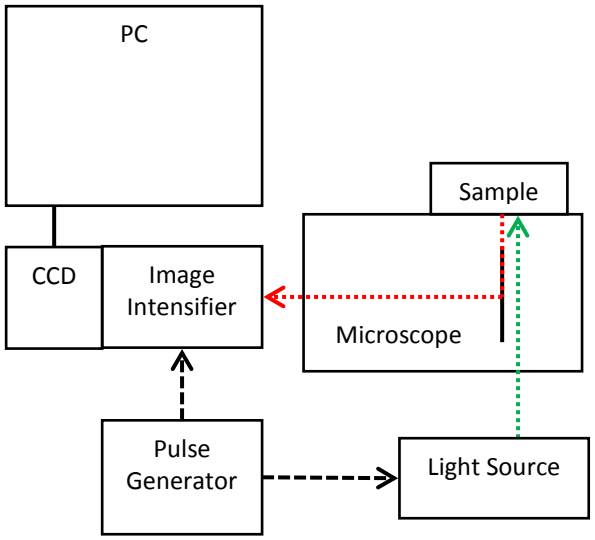

Figure 2: Typical FLIM experimental set-up. A light source excites the fluorophore in a microscope sample, fluorescence emission is imaged on the Image Intensified CCD. A pulse generator coordinates firing of the light source and controls the gating of the intensifier.

PHOTEK Recommendations

Image Intensifier- MCP118/Q/S20/P43/IFO

Power Supply - WP610

Gate Unit - GM300-3

Gate Controller and Pulse Generator - IGC2

(Option of Gated ICCD Camera - ICCD118)

Dark Box

LED Lighting

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