Nanosecond Fluorescence Lifetime Imaging using a directly gated interline transfer CCD detector

by

Photonic Research Systems Ltd

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Experimental Details

- *Sensor: 2/3” format monochrome interline CCD sensor, peltier cooled to -18^{o}C*

- *Light Source: Diode pumped, frequency tripled solid-state pulsed laser with active Q-Switching. Pulse repetition rate was 5kHz, wavelength 355nm, pulse width at half maximum was 1.2 nsec, laser jitter was <1nsec.*

- *Samples: Two solutions of fluorescein were prepared one having twice the emission intensity of the other. The brighter sample was then quenched with potassium iodide until its intensity matched that of the lower intensity sample*

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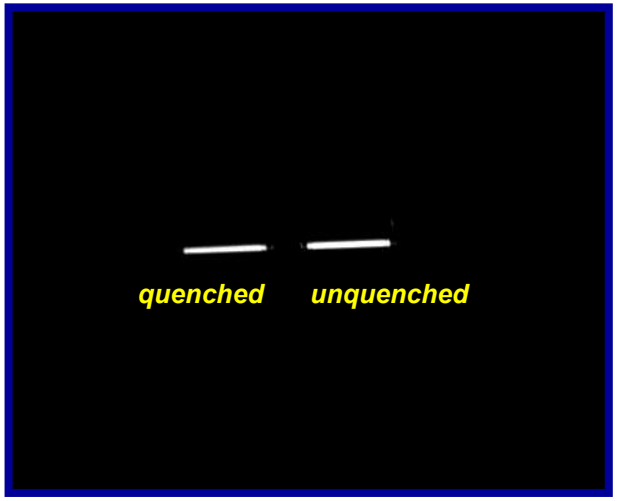
Cuvettes of fluorescein illuminated by pulsed UV laser

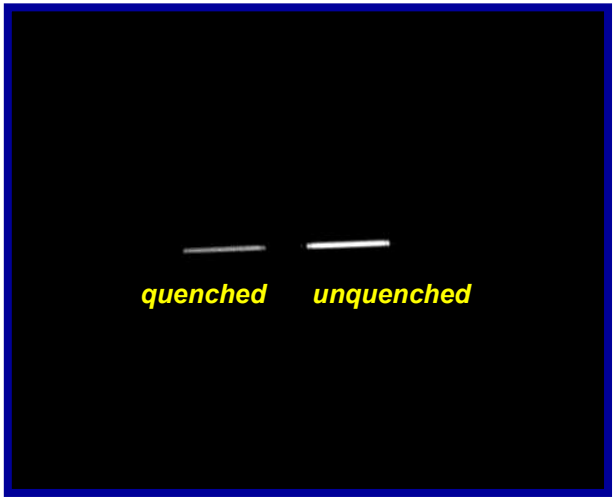
One cuvette holds fluorescein in buffer (4ns) while the other has a sample of twice the concentration but quenched with potassium iodide to match the dilute sample (2ns). This image was taken with the *Imagex* camera in steady state mode under laser illumination and with some ambient light.

Steady-State Fluorescence image of Fluorescein samples with no background light

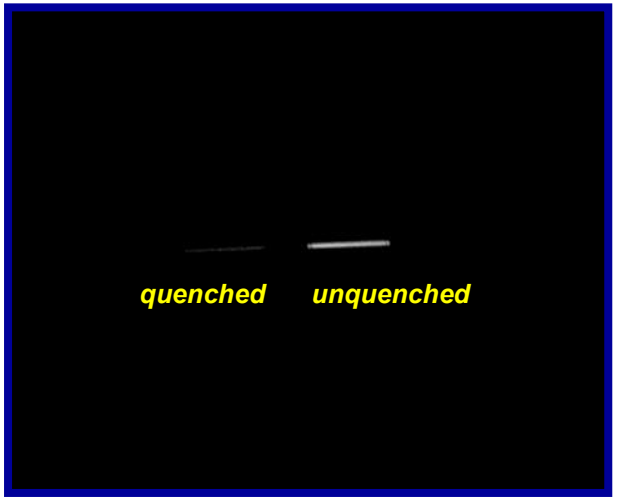
Time-Resolved images of Fluorescein Samples



2ns delay



4ns delay



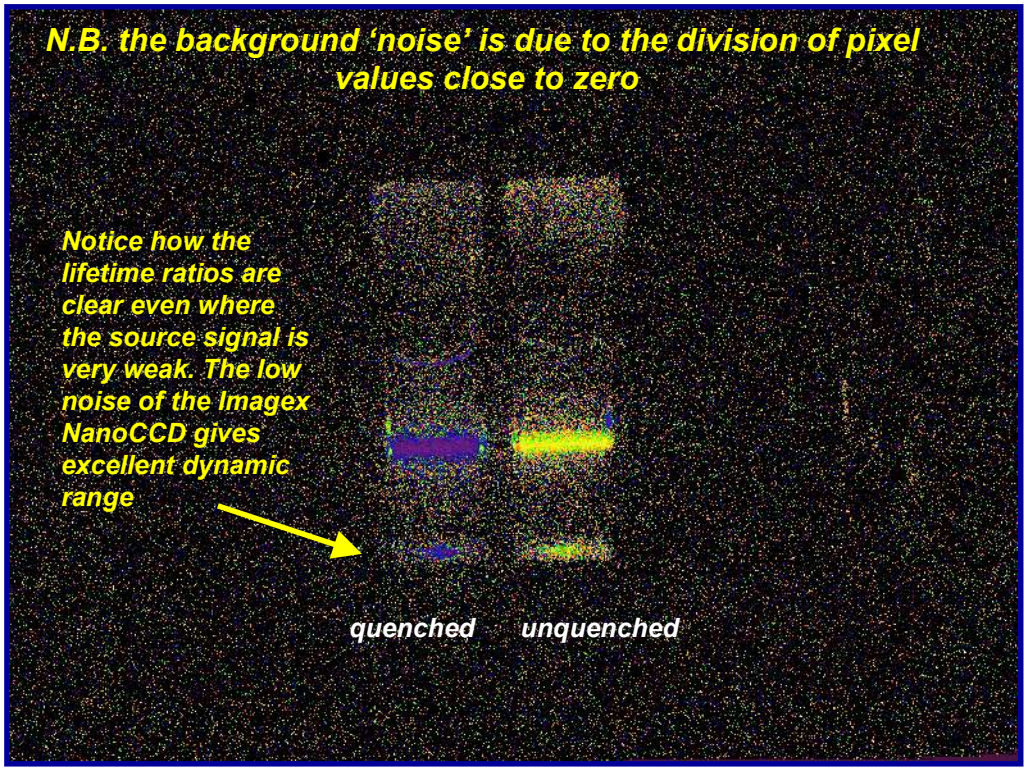
6ns delay

Delay following laser pulse

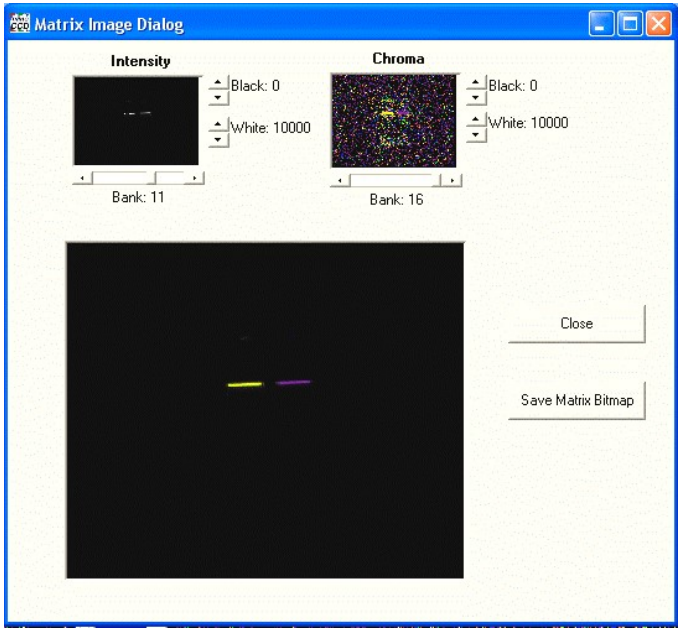
The next slide shows the resultant decay-weighted image when the appropriate pair of time-resolved images are divided. A psuedo-colour look-up table is used to show the lifetime differences.

Note the very high signal-to-noise ratio, which is evident even in the uniform colour of the pixels representing very weak signals from reflections at the meniscus and base of the cuvettes

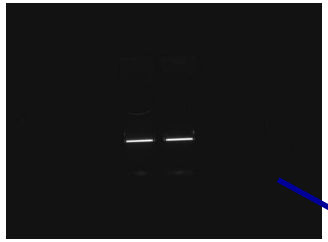
Ratio 'FLIM' image of Fluorescein Samples



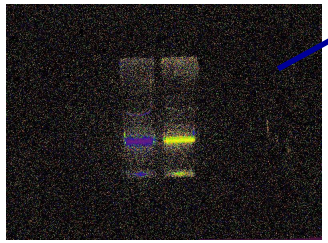
Combining Intensity and Lifetime information in a single image



The Imagex Matrix Dialog allows the user to select 2 source images and adjust their contrast settings. The images are then combined into a single 'Matrix' image



Intensity Image

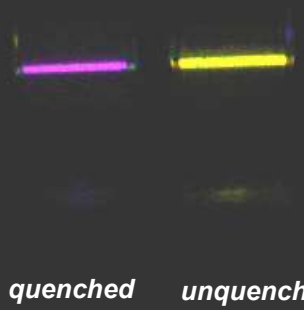


'Flim' (Ratio) Image

Intensity/FLIM Image

Intensity-Lifetime 'Matrix' image of Fluorescein Samples

Combining the Intensity and Flim Images in this way helps to suppress artifacts created by performing ratio operations on areas of very low intensity.



quenched *unquenched*

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Future Work

- *Investigation of smaller format and higher resolution CCD sensors*
- *Investigate Improved DPSS Light Source: 0.8 nsec FWHM with jitter <0.5nsec now available.*
- *Mount System on Microscope for real biological applications!*