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

by

Photonic Research Systems Ltd
Sensor: 2/3” format monochrome interline CCD sensor, peltier cooled to -18°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.
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

quenched  unquenched  quenched  unquenched  quenched  unquenched

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The next slide shows the resultant decay-weighted image when the appropriate pair of time-resolved images are divided. A pseudo-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.
N.B. the background ‘noise’ is due to the division of pixel values close to zero.

Notice how the lifetime ratios are clear even where the source signal is very weak. The low noise of the Imagex NanoCCD gives excellent dynamic range.

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

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