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Fluorescent Imaging of pH with Optical Sensors Using Time Domain Dual Lifetime Referencing

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Abstract:

We present a referenced scheme for fluorescence intensity measurements that is useful for imaging applications. It is based on the conversion of the fluorescence intensity information into a time-dependent parameter. A phosphorescent dye is added in the form of ~10-µm particles to the sample containing the pH-sensitive fluorescent indicator. Both the reference dye and the pH probe are excited simultaneously by a blue LED, and an overall luminescence is measured. In the time-resolved imaging method presented here, two images taken at different time gates were recorded using a CCD camera. The first image is recorded during excitation and reflects the luminescence signal of both the fluorophore (pH) and the phosphor(reference). The second image, which is measured after a certain delay (after switching off the light source), is solely caused by the long-lived phosphorescent dye. Because the intensity of the fluorophore contains the information on pH, whereas phosphorescence is pH independent, the ratio of the images displays a referenced intensity distribution that reflects the pH at each picture element (pixel). The scheme is useful for LED light sources and CCD cameras that can be gated with square pulses in the microsecond range. The fundamentals and potential of this new method, to which we refer as *time domain dual lifetime referencing* (t-DLR), are demonstrated.