Compact Optical Setup for Wide-field Fluorescence Lifetime Imaging

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A compact optical setup for real-time, wide-field fluorescence lifetime imaging (FLIM) in the nano- to micro-second range was developed. The system is based on a fast lock-in imager and a modulated diode laser, and yields 2D maps of fluorescent lifetimes with nanosecond resolution. The prototype is considerably cheaper and easier to operate than commercial FLIM systems, opening the door to novel applications of fluorescence lifetime imaging.

Fluorescence lifetime imaging microscopy (FLIM) has been applied since the early 1990s for the mapping of molecular environment parameters (pH, ion concentration, oxygen content) and protein interactions in living cells, tissues and model organisms. More recently, the use and value of FLIM in medical diagnostics, histology and high-throughput pharmacological compound screening has been demonstrated. Despite its intrinsic value, the adoption of FLIM outside specialized research labs has been limited by the cost of the required instrumentation and by the expertise necessary for its maintenance and operation.

Solid-state image sensing with in-pixel demodulation, on the other hand, is a well-known technology for 3D imaging (TOF, time-of-flight). The existing products are applied with success in markets such as industrial metrology, robotics and safety. The technology also has large potential in the fields of life science and medical diagnostics, which, however, are still largely unexploited. The potential of lock-in pixels for fluorescence lifetime measurements was previously demonstrated by CSEM and others [1, 2]. These demonstrations, however, relied on expensive external components (microscope, external light source) and, therefore, partially shared the limitations of existing solutions.

Figure 1: Optical setup for wide-field FLIM, allowing a 2D map of fluorescence lifetimes with nanosecond resolution.

To promote a wide-spread application of FLIM outside of academic and research laboratories, a demonstrator incorporating all the necessary hardware components in a single, compact and cost-effective setup was developed within the Nano-Tera project FlusiTex (Figure 1). The system consists of a modulated solid-state light source (laser diode), a CMOS lock-in imager, optical components, electronics and software interfaces. The lifetime measurement is carried in the frequency domain (phase fluorometry). In this method, the fluorescence lifetime is obtained from measurements of the phase lag and demodulation of the emission as compared to the excitation light.

The setup was tested with various fluorescent dyes (rhodamine, dichlorofluorescein) in solution as well as in thin films. A 2D map of the fluorescence lifetime in the low nanosecond range could be obtained (Figure 2). The system was also tested with ruthenium-doped thin films used for oxygen sensing, with lifetimes in the microsecond range.

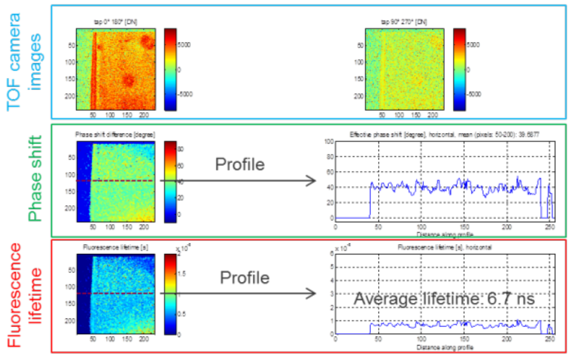

Figure 2: Fluorescent lifetime distribution of a fluorescein/rhodamine mixture (4:1 molar ratio). The sample was provided by EMPA.

The obtained results show that the combination of a fast lock-in imager with novel solid-state light sources has the potential to bridge the technological divide that limits the use of lifetime imaging in relevant areas that could benefit from its application.

As a next step, a more integrated prototype will be developed. The camera electronics will be redesigned based on a stacked PCB approach, including a base board, a FPGA processing module and a sensor head PCB. The camera will allow control of the modulation signal frequency, amplitude and offset of the illumination. The optical interface will also be further miniaturized. The complete, integrated FLIM prototype will be made available to application partners for characterization and testing.

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[1] A. Esposito, *et al.*, Opt. Exp. 13 (2005), pp. 9812-9821

[2] L.-E. Bonjour, *et al.*, in Sensors, IEEE (2011), pp. 724-727