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Multispectral Fast FLIM based on Compressive Sensing Single-Pixel Camera for Imaging of Biological Dynamic Processes

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Abstract: A fast fluorescence lifetime imaging microscopy (FLIM) system using compress sensing and a single-pixel camera enables real-time imaging of biological dynamics. Dual detection paths provide high-speed (20 fps) or multispectral measurements, enhancing biomedical analysis. © 2025 The Author(s)

1. Introduction

Fluorescence lifetime imaging microscopy (FLIM) is widely used in biomedicine to probe cellular interactions by detecting variations in fluorescence lifetime [1]. The resulting spatial map of lifetimes helps to localize changes within a sample. To capture real-time dynamics, recent FLIM advancements focus on high throughput, fast acquisition, and high frame rates. Compressive sensing (CS) with a single-pixel camera (SPC) scheme enhances light collection and reduces acquisition time while maintaining the temporal resolution needed for fluorescence decay recording [2]. Additionally, CS-SPC systems can be adapted for multispectral imaging using detector arrays and a spectrometer, improving measurement specificity by capturing fluorescence spectra alongside lifetime data [3, 4]. We propose a high throughput FLIM system based on a CS-SPC scheme that can exploit two different detection paths. The first path contains a single silicon photomultiplier (SiPM) detection module enabling FLIM measurements while in the second path light is dispersed by a spectrometer and focused on a 16-channels array of SiPM detectors enabling multispectral FLIM measurements. Thanks to the high efficiency of the SiPM technology along with the parallelization of multichannel detection, we were able to reach acquisition rate up to 20 fps. These acquisition speeds are compatible with the possibility to follow in real time the dynamics of most of biological processes opening for more accurate and specific analysis for new biomedical studies.

2. Setup and measurement reconstruction

Figure 1 reports a schematic of our setup. As light source, we use a mode-locked supercontinuum laser (SC-450, Fianium Inc.) with a repetition rate of 40 MHz and a pulse duration of few tens of picoseconds. The excitation wavelength for fluorescence is selected through a band pass filter (F1) with a bandwidth of 10 nm. A digital micromirror device (DMD) (V-7000, ViaLUX GmbH) acts as a spatial light modulator (SLM) to project the light patterns for SPC acquisition on the sample. Fluorescence light from the sample is filtered by a dichroic mirror (DM) and a long pass filter (F2). A beam splitter (BS) directs the light to two different collection paths. The first one ends with a CMOS camera (C13440-20CU, Hamamatsu) to acquire high-resolution intensity images of the sample. The second path furthers divides in two paths, that can be selected with a flip mirror (FM). In the first one, light is focused on a single SiPM detector. In the second one, light is coupled to a spectrometer (Kymera 193i, Andor) that disperses it over a 16-channels SiPM array detector. The bandwidth of each spectral channel is 7 nm full width at half maximum (FWHM). The SiPMs used have an active area of 1.3 mm $\times$ 1.3 mm, a time resolution

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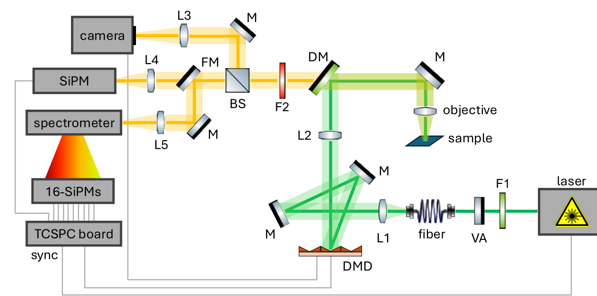

Fig. 1. Scheme of the setup. F: filter. VA: variable attenuator. L: lens. M: mirror. DM: dichroic mirror. BS: beam splitter. FM: flip mirror.

of <100 ps and a dead time of 4 ns [5]. In both cases the detectors are connected to a multichannel timing systems (Multiharp MH160, Picoquant GmbH and a prototype of Lynx, Tediel Srl) along with the laser, for time correlated single photon counting (TCSPC) measurements. The DMD is connected to the camera and the timing system for synchronization of the SPC measurement.

In SPC measurements, instead of recording every pixel individually, we project a set of light patterns onto the sample and measure the resulting light intensity [6]. These patterns come from a scrambled Hadamard (SH) matrix. The full set is composed by $N = 1024$ patterns, each one with 32×32 pixels. The SPC approach allows also to perform compress sensing (CS) [7]. We can project just $M < N$ patterns, still being able to reconstruct the original image, employing minimization algorithms. In this work we project $M = 256$ patterns, achieving a compression ratio ($CR = 1 - M/N$) of 75%. The final result is a 32×32 pixels image with temporal resolution (and spectral resolution too in the case of the 16-channels SiPM acquisition).

We highlight that the acquisition path with the single SiPM has a more efficient light collection allowing to reach the acquisition rate of 20 fps but without spectral information. On the other side, the 16-channels SiPM array has the advantage of providing also spectral information, in addition to the time resolved measurement, at the cost of longer acquisition times because of the less efficient light collection due to the losses introduced by the spectrometer.

3. Results

We show two example measurements with each detection path of our FLIM system.

The first one is on *Chlamydomonas reinhardtii*, a single-cell alga. We excite the chlorophyll with a 630 nm excitation light (fluorescence emission peak at 680 nm) and we want to monitor the lifetime changes while the algae are moving. Since we are interested only in time resolved measurements, we use the single SiPM to achieve the maximum acquisition rate possible. We project each of the 256 patterns for 195 µs, for a total acquisition time of approximately 50 ms per frame (measurements are repeated sequentially). This results in a acquisition rate of 20 fps. In Figure 2 we show the FLIM maps of three frames of the resulting video with the corresponding camera images. A possible application of these measurements is the investigation of phototaxis, a phenomenon where an organism moves in response to a light stimulus. Thanks to the time resolved measurement we could observe the change of lifetime of the chlorophyll emission during phototaxis.

The second one is a measurement of a mixture of two different fluorescent beads, emitting at different wavelengths. Hence, we perform the measurement with the 16-channels SiPM, to appreciate the different emission spectra. Due to the lower collection efficiency, we increase the projection time of each pattern to 300 µs, obtaining an acquisition rate of 13 fps. In Figure 3, we present one frame of the acquisition. We select two regions of the image for which we show the spectrum and the time decay, to demonstrate the multidimensionality of the dataset. Moreover, we also report the multispectral image and the FLIM map of the sample. Multispectral FLIM images by using also a 16-channel PMT for parallel spectral readout will be presented.

4. Conclusion

The proposed fast FLIM system successfully integrates two distinct detection paths, offering flexibility in data acquisition. The single SiPM path ensures high light collection efficiency and rapid frame rates (up to 20 fps), making it ideal for real-time imaging of dynamic biological processes. In contrast, the spectrometer-coupled 16-channels SiPM array provides valuable multispectral information at the cost of longer acquisition times (about 10 fps) due to reduced light collection efficiency.

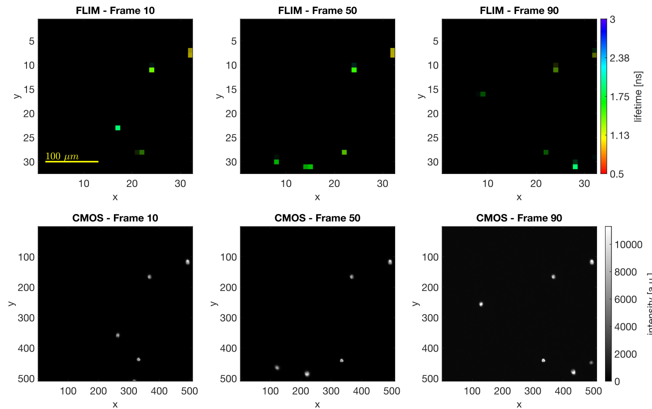

Fig. 2. Three frames of the *Chlamydomonas reinhardtii* measurement. In the top row we report the FLIM images and in the bottom one the corresponding CMOS camera frames.

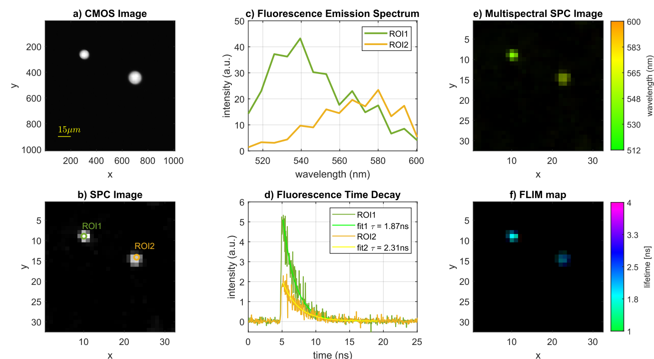

Fig. 3. One frame of the measurement with beads sample. Two regions of interest (ROIs), for which we plot the spectrum at the time-resolved decay, are selected in correspondence of the two beads.

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