Wide-Field Time-Correlated Single Photon Counting

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Abstract

We are reporting a novel method for wide-field imaging using an ultra-fast CMOS camera with a 40 kHz frame rate coupled with a 3-stage image intensifier mounted on a standard microscope. This system combines high frame rates with single photon sensitivity and temporal information. The output of the phosphor screen, consisting of single-photon events, is collected by an ultra-fast CMOS camera allowing to create a wide-field image with parallel positional and timing information of each photon.
The system is mounted on a microscope to image luminescent samples. Using a pulsed excitation source and decaying luminescent sample, the arrival times of hundreds of photons can be determined simultaneously in many pixels with microsecond resolution. We will discuss centroiding, timing of the events and the associate Fixed Pattern Noise (FPN).

Introduction

• Fluorescence related techniques have been used intensively in recent years especially in biology, medicine and biomedical fields for example in imaging, sensing and microscopy.
• Observation of the fluorescence emission can provide quantitative information about the the local environment of the fluorophore, such as the pH, polarity, ion concentration, molecular interaction and viscosity.
bullet This information can be obtained through several fluorescence features such as lifetime, position, intensity, the excitation and emission wavelength and polarization[6].
• Using the Single Photon Counting (SPC) technique for imaging has the advantages of high sensitivity, large dynamic range and very good signal-to-noise ratio. For that reason, this technique is suitable for very low light excitation condition and low dye concentration allowing to reduce the photo-toxicity and bleaching.
• Wide-field imaging allows parallel acquisition of positional information. In order to obtain lifetime information using SPC, the sample has to be excited with a pulsed source and the arrival time of every photon generated by the excitation to be measured.
• The commonly used techniques for Time-Correlated Single Photon Counting (TCSPC) include one-dimensional detectors (e.g. photomultiplier tubes or Single Photon Avalanche Photodiodes) and require the scanning of the sample. This, in addition to the electronics inherent dead time, decreases the system efficiency and the maximum frame rate. Using a two-dimensional detector can partially address the problem but such detectors are usually not sensitive enough to acquire single photon events nor to achieve fast timing (e.g. CCD-detectors) or reduce the detection ability to one photon at a time (microchannel plate with quadrant anode).

Experimental set-up

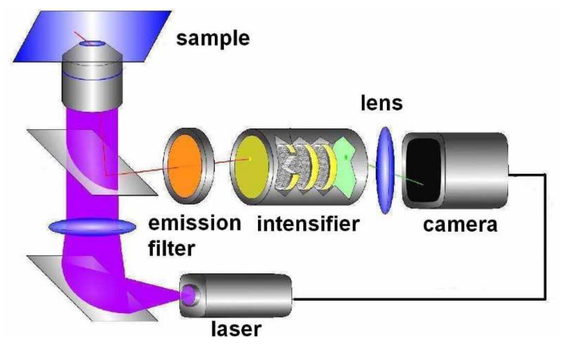


Figure 1: Schematic diagram of the set-up. The intensifier cathode will be hit by photons and the resulting photoelectrons amplified through the multichannel plate to the phosphor screen. The events on the phosphor screen are then imaged on the CMOS detector of the camera using an objective lens.

The apparatus used consists of an inverted microscope (Nikon ECLIPSE TE2000-E), coupled to a dual proximity-focused 3-stage image intensifier (Photek, UK), operating in photon counting mode (Fig. 1). The phosphor screen (P20) of the intensifier is imaged by a ultrafast camera (Fastcam-SA1, Photron, Japan) acquiring up to 100,000 frames per second with a resolution of 128×128 pixels.
Images were obtained by processing all the frames with a home-made software which thresholds the images, and detects every single photon event using a recursive algorithm; every photon detected is centroided, using different algorithms in order to reduce Fixed Pattern Noise (FPN)[4], thus providing sub-pixel resolution[1] (Fig. 3).

Improvement

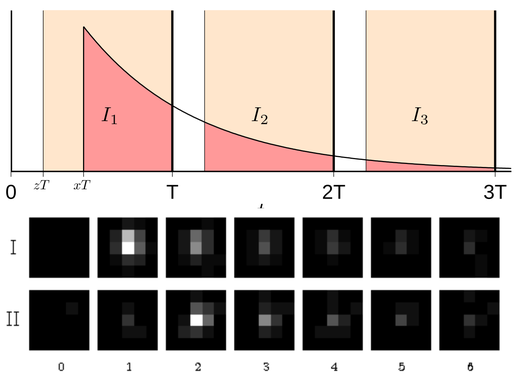
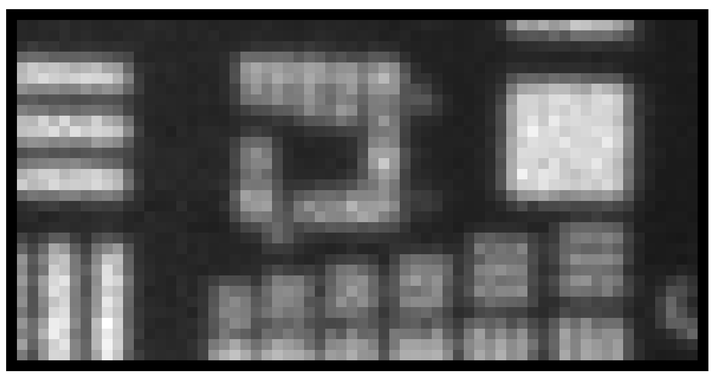


Figure 2: Each event is imaged on consecutive frame due to phosphor decay: theoretical behaviour (top) and experimental data[5] (bottom)

By exploiting the invariant phosphor decay of the image intensifier's phosphor screen it's possible to achieve sub-exposure time resolution [5]. The ratio of the intensities in consecutive frames due to the phosphor decay after the photon detection can be used to uniquely determine the photon arrival time. Fast luminescence decays in parallel in many pixels can then be measured using this method.

Results

Resolution enhancement



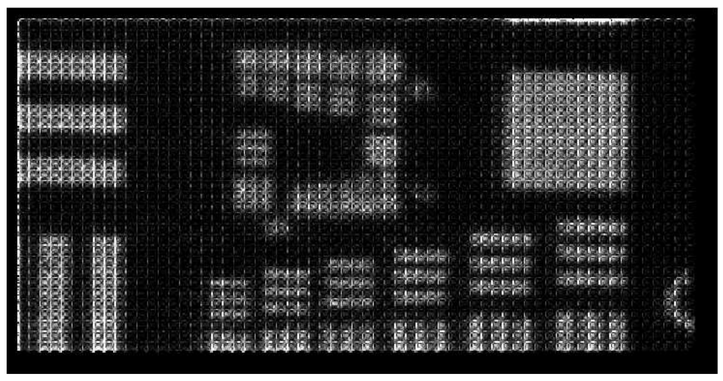


Figure 3: Average (top) and centroided (bottom) images of an USAF test pattern, obtained from 1.8 million frames (acquisition frame rate: 1000 fps,). The images clearly show a resolution improvement due to centroiding (center of gravity algorithm).

Wide-Field TCSPC

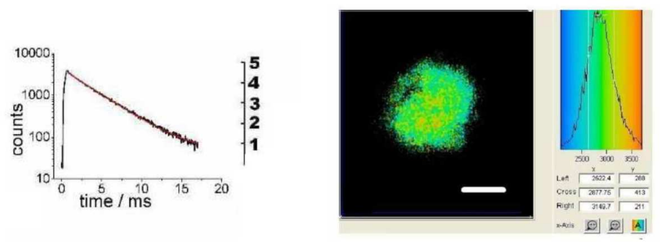


Figure 4: A Eu-POM lifetime image and lifetime histogram with a good monoexponential decay and an average lifetime of 2.9 ms. The scalebar is 100 μm (Right)[1]. A decay of Europium-containing polyoxometalate (POM) [7] nanoparticles after 256 excitation pulses averaged over the whole field of view with a time channel width of 25 μm (Left)[1]

Conclusions

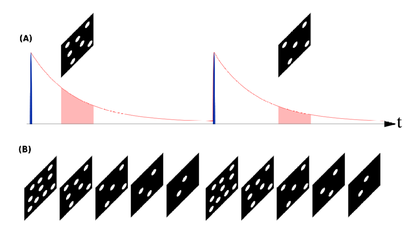


Figure 5: Schematic comparison between wide-field time-gated FLIM (A), and WiFi TCSPC (B).

The parallel acquisition on all the pixels make this method faster than traditional scanning systems and more advantageous than time-gated methods because of the most information collected (no loss of light due to the gating).

The key result of this work is the demonstration that single photons can be detected using an ultrafast camera at frame rates of 40,000 fps and more and its promising application with sub-exposure-time temporal resolution. This method combining single photon sensitivity and high frame rate can be particularly useful for fast SPC imaging. Also, the timing information connected to each photon event allows to do Time-Correlated Single Photon Counting imaging in the millisecond range.

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