Docket #: S18-388

Efficient wide-field nanosecond imaging methods using Pockels cells for low-light applications

Stanford researchers at the Kasevich Lab have developed a module that can attach to any standard optical system or sensor for wide-field, time-resolved imaging. This invention enables nanosecond image gating and modulation in low-light conditions, opening new applications for wide-field imaging such as fluorescence lifetime imaging microscopy (FLIM). This technology enables the first wide-field lifetime camera that is compatible with single-molecule microscopy.

Existing sensors for wide-field nanosecond imaging (e.g. time-of-flight cameras, SPAD arrays, and gated intensifiers) sacrifice performance and are not compatible with low-light applications in bioimaging and fluorescence microscopy. The researchers have demonstrated the use of electro-optic wide-field imaging gates for nanosecond temporal resolution with high photon collection efficiency. Their method enables wide-field FLIM in a single exposure image on any standard camera sensor. By acquiring all pixels in parallel they demonstrate 5 orders of magnitude improvement in imaging speeds compared to single photon counting. They have also demonstrated 39 MHz image modulation for fluorescence lifetime microscopy of single molecules at high frame rate. This allows FLIM applications in super-resolution microscopy and observations of single-molecule dynamics such as FRET. Prototypes have been tested on commercial microscopes.

On-going work: Researchers are continuing to improve the technique to allow larger imaging field-of-view, higher frequency operation, and improved electro-optic configurations. A compact and user-friendly module for MHz resonant image modulation is under development. The inventors have expanded upon this technology in Stanford Docket 19-424: Wide-field Resonant Electro-optic Imaging Devices and Applications .

Stage of Development:

- **Proof-of-concept** imaging studies completed
- **Prototypes tested** on two commercial microscopes

Figures:

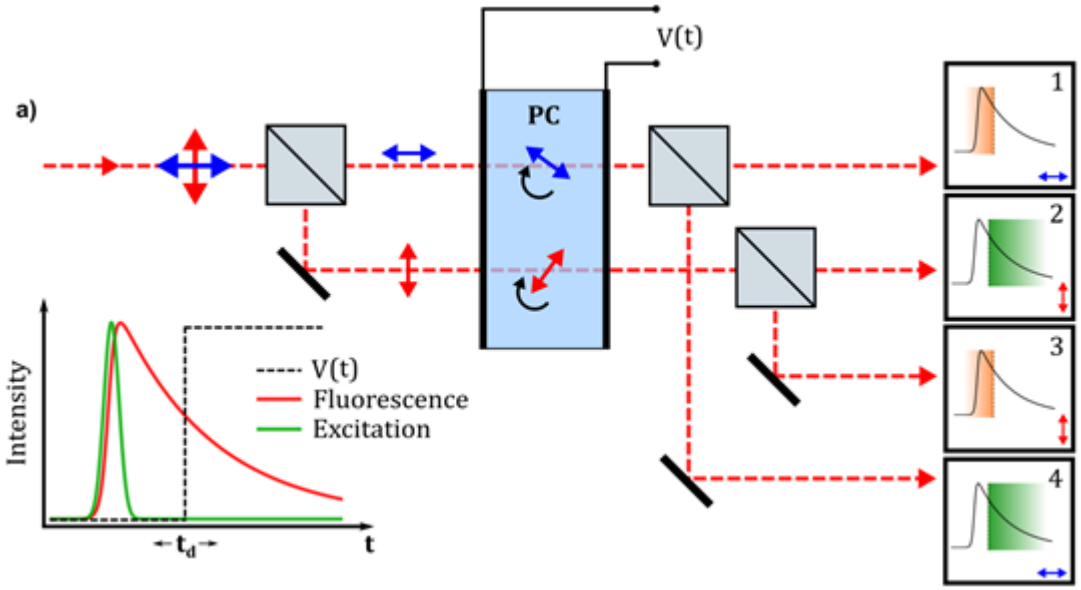


Figure description- A) Schematic of Pockels cell detection with two temporal bins. Input from an imaging system (pulsed excitation and fluorescence from a microscope is drawn) is split by two polarizing beam-splitters before and after Pockels cell. All emitted photons are captured in four spatially separated images on any standard detector. Image intensities on the sensor encode temporal information from the Pockels cell modulation. Any modulation V(t) may be applied.

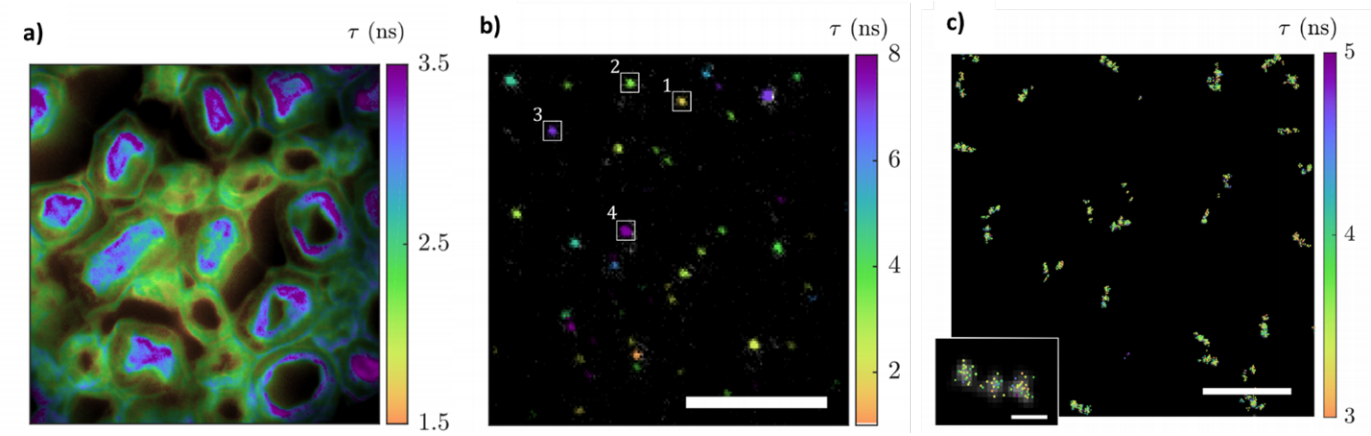


Figure description- Wide-field FLIM Examples: (a) FLIM image of a mouse kidney slice differentiating two fluorophores by lifetime (b) Single-molecule FLIM image on a

mixture of molecules having different lifetimes – dynamics for each molecule may be observed at high frame-rate (see Publications) - 10 µm scalebar (c) Combination of FLIM and single-molecule super-resolution microscopy. Lifetime is measured for each single molecule burst and plotted at the localization coordinate (1 µm scalebar, 100 nm inset)

Movie 1: Propagating action potentials. Electrical activity of a single neuron in a living fruit fly (Drosophila) using fluorescence lifetime.

Scalebar is 25 microns.

Movie 2: Propagating action potentials. Electrical activity of a single neuron in a living fruit fly (Drosophila) using fluorescence lifetime.

Scalebar is 25 microns.

Movie 3: Propagating action potentials. Electrical activity of a single neuron in a living fruit fly (Drosophila) using fluorescence lifetime.

Scalebar is 25 microns.

Applications

- Fluorescent lifetime imaging microscopy (FLIM)
- Single-molecule FLIM and FRET imaging
- Wide field lock-in detection and modulated imaging
- Imaging FRET probes such as voltage indicators
- Combination of FLIM with super-resolution and single-molecule microscopy
- Combination of FLIM with light-sheet microscopy
- Endoscopic imaging and clinical diagnostics
- LIDAR and 3D scanning
- Multi-dimensional / 'hyperspectral' imaging combining lifetime with other imaging dimensions
- Single-shot, multi-frame nanosecond imaging

- Time-to-space conversion and temporal image multiplexing with gated cavity optics
- Time-resolved charged particle detectors

Advantages

- High photon efficiency
- All-optical method
- FLIM acquisition on standard scientific cameras
- FLIM acquisition in a single-frame exposure
- Demonstrated 5 order of magnitude throughput improvement
- Single-molecule compatible
- No photon loss due to gating
- Compatible with existing imaging systems and low-cost CMOS/CCD sensors

Publications

- Adam J. Bowman et al. Wide-field fluorescence lifetime imaging of neuron spiking and subthreshold activity in vivo. *Science* 380,1270-1275 (2023).
- Bowman, Adam J. and Kasevich, Mark A. Resonant Electro-optic Imaging for Microscopy at Nanosecond Resolution arXiv (2021)
- Bowman, Adam J., Brannon B. Klopfer, Thomas Juffmann, and Mark A. Kasevich. Electro-optic imaging enables efficient wide-field fluorescence lifetime microscopy Nature Communications Published 08 October 2019.

Patents

- Published Application: 20210389244
- Published Application: 20240068871
- Issued: 11,592,393 (USA)
- Issued: 11,965,780 (USA)

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