Research Highlight

Full Spectral Fluorescence Lifetime Imaging with the Sirona Sensor

Singular Photonics

Introduction

Time-resolved full-spectrum fluorescence lifetime microscopy (FS-FLIM) is a promising experimental technique to characterise samples ranging from semiconductors to biological tissue. Rapid FS-FLIM tissue analysis and diagnostics is a route to high-throughput histopathology applications (Williams 2021). In this Application Note we describe the work carried out by Dr. Gareth Williams and colleagues at the Centre for Inflammation Research, University of Edinburgh to develop Kronoscan, a high-speed FS-FLIM optical scanning microscope using the Sirona Single Photon Avalanche Diode (SPAD) sensor. Kronoscan builds a highly detailed FS-FLIM image composed of 512 spectral and 32 time channels per-pixel in images of up to 512x512 pixels. The primary focus of the research is lifetime-based imaging of human lung tissue to investigate lung cancer and other diseases

Materials and Methods

The Sirona sensor is a CMOS Single Photon Avalanche Diode (SPAD) line sensor with per-pixel histogramming time-to-digital converters for time-resolved multispectral imaging. Shot-noise limited sensitivity, time-correlated single photon counting (TCSPC) functionality and room temperature operation set the Sirona SPAD sensor apart from CCD device technology. Up to 1024 parallel spectral channels and 32 time bins with 50ps resolution are supported. For high-speed video-rate acquisition the chip can be configured in firmware to support smaller subsets of spectral channels and time bins (eg 2x2). Extensive triggering options are available for integration with a range of scanning systems and laser sources. A delay generator with 63 ps time resolution and 8 configurable histogram time-ranges is included.

Optical System

The Kronoscan optical microscope built around the Sirona SPAD sensor is shown in Fig. 1. A supercontinuum laser (NKT Evo HP, pulse duration <100 ps, repetition rate 20 MHz) supports up to 8 fluorescence excitation lines between 400 and 700 nm. The laser beam is passed into an achromatic scanning system which includes two orthogonal scanning mirrors, producing an XY scan at an image plane at the back aperture of the primary objective. Fluorescence returning from the sample is “descanned” through the same optical path, and separated from the excitation using an epifluorescence filter set. Dichroic filters were mounted in a 5 filter wheel allowing rapid adjustment of cut-on wavelength. The fluorescence is passed through an objective and focused onto a ~100 µm iris which acts as system pinhole. The fluorescence emission is then collimated, and directed onto a transmissive holographic grating with 600 lines/mm and focused through a second lens to produce a line on the sensor matched to the sensor pixel height.

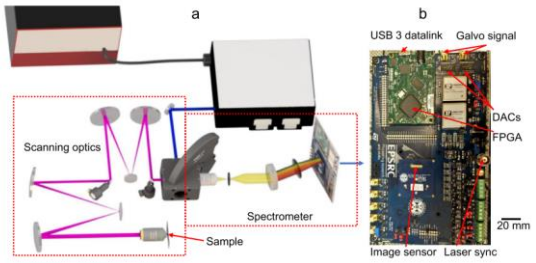

Fig. 1 Optical Scanning Microscope (Williams et al 2021)

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Sample Preparation

Ex-vivo human lung tissue section and tissue slices were obtained from a patient with non-small cell lung cancer undergoing thoracic resection surgery. The whole tissue section was approximately 6 × 12 mm and contained cancerous, margin and healthy tissue regions.

The ex-vivo tissue specimen was fixed in formaldehyde and embedded in wax with 4 µm slices cut onto slides. These samples were then used as unstained or stained with Haematoxyln and Eosin and imaged.

Fluorescence Lifetime Imaging

Full details on the lung tissue studies can be found in the journal article (Williams et al. 2021). Other samples investigated with the FS-FLIM technique included convollaria and honeybee wings.

Here we show the results of full spectral fluorescence lifetime imaging of a histology slice of lung tissue as shown in Fig. 2. In Fig. 2 **a** a Haemotoxylin and Eosin (H&E) stained histology image of lung tissue is shown with increasing cancerous (adenocarcinoma) tissue (from right to left). Highlighted areas show cancerous (1) and visibly healthier (2) regions.

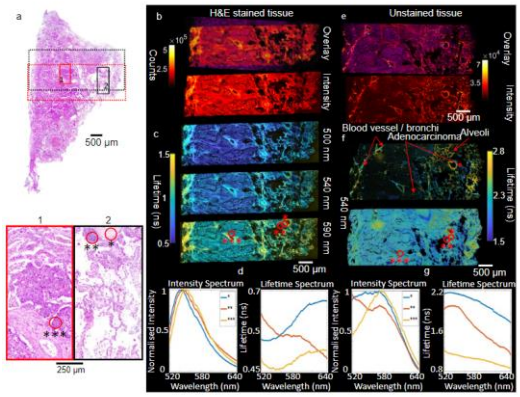

Fig. 2 Full spectral fluorescence lifetime imaging of a histology slice of lung tissue (Williams et al 2021)

Dashed areas indicate the regions imaged for the stained (red) and the equivalent unstained (black) slice. Three regions of interest are highlighted.

In Fig, 2 **b** Intensity based imaging of a stained histology slice both with and without overlay of the histology image are shown. Tiled images consist of 4 rows of 12, 256 × 256 pixel images with a field of view of 600 × 600 µm each which were taken with 1 ms exposure time per pixel. A series of images were taken and subsequently combined to form a composite image showing the complete area (~6 × 2 mm).

Fig. 2 **c** shows corresponding lifetime images at different wavelengths. Fig. 2 **d** shows Mean spectral intensity (left) and spectral lifetime (right) for the three regions of interest. Fig. 2 **e** shows Intensity imaging of an unstained slice both with (top) and without (bottom) overlay with the histology image. Images consist of 5 rows of 12, 256 × 256 pixel images, each ~600 × 600 µm, 1 ms exposure time per pixel. Fig. 2 **f** shows lifetime images at 540 nm displayed with intensity-based color saturation and an inverted color saturation. Fig. 2 **g** shows Mean spectral intensity and spectral lifetime or the three regions of interest. The data shown is from a single sample per slice with 60 FS-FLIM images per slice, taken once each. One histology image was taken.

Conclusion

The capabilities of Sirona-based FS-FLIM to acquire highly detailed spectral and temporal information were demonstrated at ~0.2 frames per second in the Kronoscan optical scanning microscope. Where full spectral lifetime information is not required spectral and temporal binning can be applied to yield video frame rates of up to 10 frames per second (at 128 × 128 pixels).

The power of FS-FLIM to fingerprint tissue fluorescence signals is clear. Optical fingerprinting of cellular types has the potential to streamline the pathology pathway. The lifetime contrast possible

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using Sirona SPAD FS-FLIM technology, illustrates a promising potential route to optical histopathology applications.

In summary, highly detailed fluorescence lifetime images were obtained of cancerous and normal tissue regions using a sensitive, versatile, and robust full spectral fluorescence lifetime imaging microscopy system based on Sirona SPADs. Capture of detailed fluorescence lifetime spectral data cubes enables multiple applications such as full spectral lifetime Förster resonant energy transfer (FRET) imaging, simultaneous fluorescence and Raman imaging - techniques that have applications throughout the life sciences.

Acknowledgements

The Engineering and Physical Sciences Research Council (EPSRC, United Kingdom) Interdisciplinary Research Collaboration (grant number EP/K03197X/1 and EP/R005257/1) is thanked for funding this work.

Ethics. All experiments using ex vivo human lung tissue were performed following a favorable ethical opinion received from the South East of Scotland Research Ethics Service REC 1 (on behalf of the National Health Service), and approved by the NHS Lothian NRS BioResource (REC ref: 13/ES/0126 and 15/ES/0094). All subjects gave written informed consent.

References

Williams, G. O. S., Williams, E., Finlayson, N., Erdogan, A. T., Wang, Q., Fernandes, S., Akram, A. R., Dhaliwal, K., Henderson, R. K., Girkin, J. M., & Bradley, M. (2021). Full spectrum fluorescence lifetime imaging with 0.5 nm spectral and 50 ps temporal resolution. Nature Communications, 12(1), 6616. https://doi.org/10.1038/s41467-021-26837-0

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