UWM RESEARCH FOUNDATION

Spectral Resolution Add-on for Two Photon Microscope

(OTT ID 1105-1242)

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Problems

Typical two-photon microscopes scramble dynamic spectral information during data acquisition

- Samples are scanned with a laser in a **serial**, i.e. point by point, fashion which limits real-time imaging of dynamic processes.
- Beam splitters and filters, used in these microscopes to separate signal from different fluorescent molecules**, have to be changed after each serial scan**, and therefore spectral information about dynamic molecular complexes is **scrambled** before all the spectral information is acquired.
- Spectral bleed-through, which is the crossing of signal from one fluorescent molecule into the detection channel designated for another, occurs when beam splitters are used to separate signal from different fluorescent molecules.
- Because of spectral bleed-through, fluorescent molecules have to be chosen that **do not** spectrally overlap, which severely **limits the range** of fluorescent molecules which may be employed.

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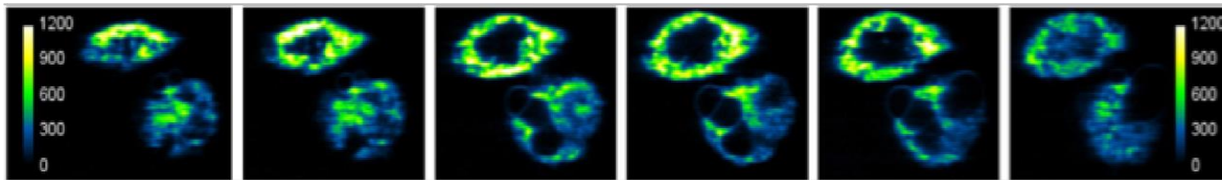
Technical Solutions

We present a module upgrade which separates fluorescence signal obtained from multiple locations in a sample into hundreds of wavelength channels simultaneously



Fluorescence intensity maps of Chinese Hamster Ovary cells expressing rhodopsin labeled with SCFP3A.

- ❑ Fluorescence emission from a sample is projected through a transmission grating onto an electron-multiplying CCD (EM-CCD) camera with single photon sensitivity. Different wavelength channels are all detected **at the same time**
- ❑ Because the detector is a 2D array, multiple points in a sample can be excited and imaged **in parallel**, allowing for a two order of magnitude increase in speed and/or sensitivity

This module is specifically designed to deliver real-time, spectrally resolved analysis of single molecules and molecular complexes and their spatial distribution in living cells

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Add-on Device (Specifications)

- **Microscope**-Module can be directly coupled to left or right side ports on a wide range of microscopes sold by any of the major manufacturers.
- **Detector**-Either the Andor iXon X3 or iXon Ultra 897 EMCCD can used for detection of emitted fluorescence
- **Laser**-Can be coupled to any femtosecond pulsed free-space laser emitting above 680 nm
- **Computer-**Compatible with any standard PC running either Windows 7 or Windows 10. A National Instruments PCIE DAQ Card must be installed on the computer

Applications

- Live Cell Imaging
- 3D Imaging
- Deep Tissue Imaging
- Long Term Imaging without compromised tissue viability
- Whole Organ or Slice Imaging
- Many more

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Intellectual Property and Licensing

- **US and European issued patents**
  US7,973,927, US8,094,304, US9,103,721, US8,982,206 and EP20120782796 validated in Belgium, France, Germany, United Kingdom, and Ireland

Current Status

- This technology can be sold separately or as an add-on attachment with new microscope
- Prof. Raicu's lab also offers this technology on service basis along with operating systems
- Technology is available for licensing under exclusive or non-exclusive terms

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Additional services provided

- Characterizing the effect of ligands on protein-protein interactions, particularly on association of membrane receptors using 2D FIF method.
  - The method is based on 2D FIF, published by Stoneman et al in in Nature Methods in 2019

- Screening of chemical compounds based on their ability to bind to the drug target (i.e., cell cultures expressing receptors of interest)

- Cost of service will depend on type of sample and sample size, and is based on an hourly fee for the imaging facility and technician/scientist time

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In Summary

- The Raicu lab has developed a two-photon microscope add-on device that increases both resolution and data acquisition speed.
- The add-on design is complete and several have been manufactured. Operating software has also been written and is available.
- The technology has been used in a number of quantitative FRET imaging publications where the binding stoichiometry and geometry of protein complexes has been determined

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