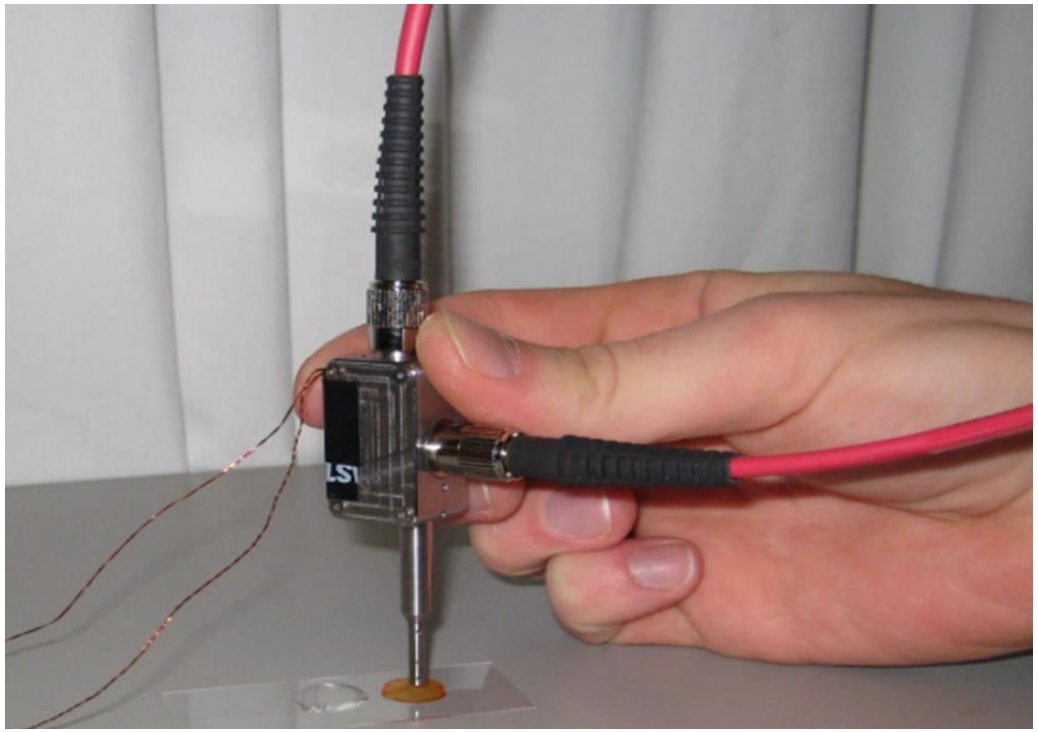
Application of Silicon Photomultipliers to Multiphoton Microscopy

Ben Hansson - Carleton Biophotonics Research Group

Motivation – Multiphoton Imaging

- **Multiphoton microscopy** allows for real time, non-invasive, chemically-specific imaging
- *In-vivo* identification of tissue from biochemical information (e.g. cancerous vs. benign)
- Requires **low-light detection**
  - Current state of art is the **photomultiplier tube (PMT)**
  - New photomultiplier: the **silicon photomultiplier (SiPM)**

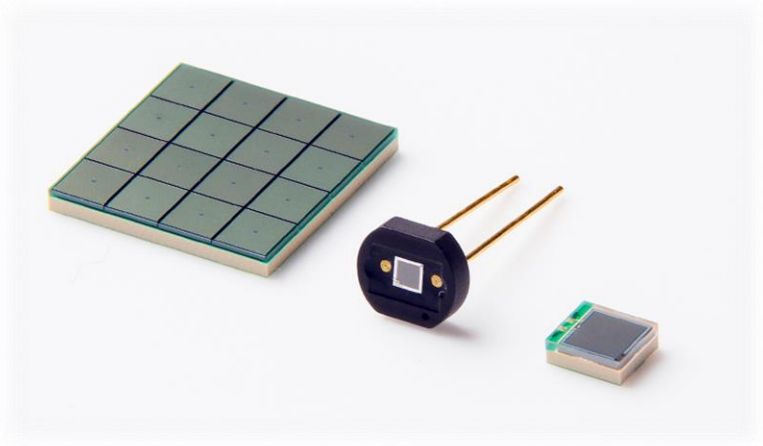


Handheld multimodal multiphoton exoscope

B. Smith, M. Naji, S. Murugkar et al, Optics Express, 21, 17161 (2013)

S. Murugkar, P. Stys and H. Anis, "System and method for multimodal CARS endoscopy", US patent 20120281211, awarded September 2014.

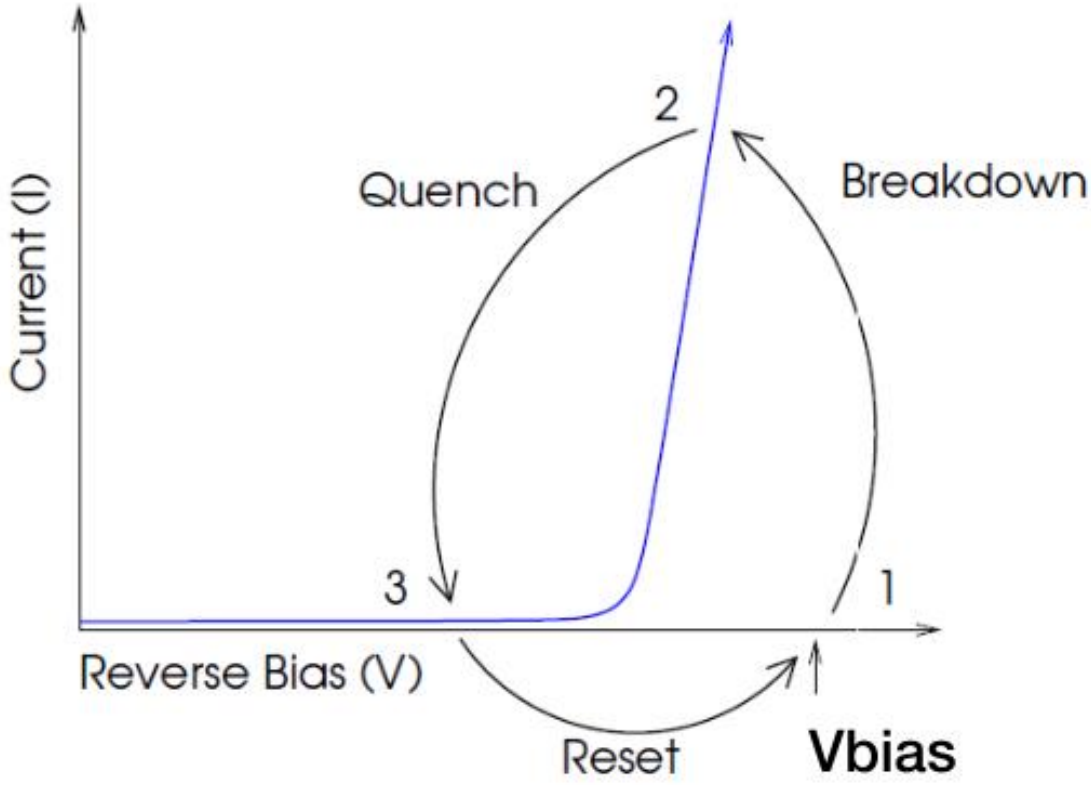
Motivation – SiPMs and PMTs Compared



	Photomultiplier Tubes	**Silicon Photomultipliers**
Gain	$10^5 - 10^7$	$10^5 - 10^7$
Bias Voltage	~ 1000 V	~ 50 V
Dark count rate	< 500 Hz	$< 1{,}000{,}000$ Hz
Ambient Light Sensitivity	Can be damaged	Immune
Price (for small area)	High	Low

Theory – Silicon Photomultiplier

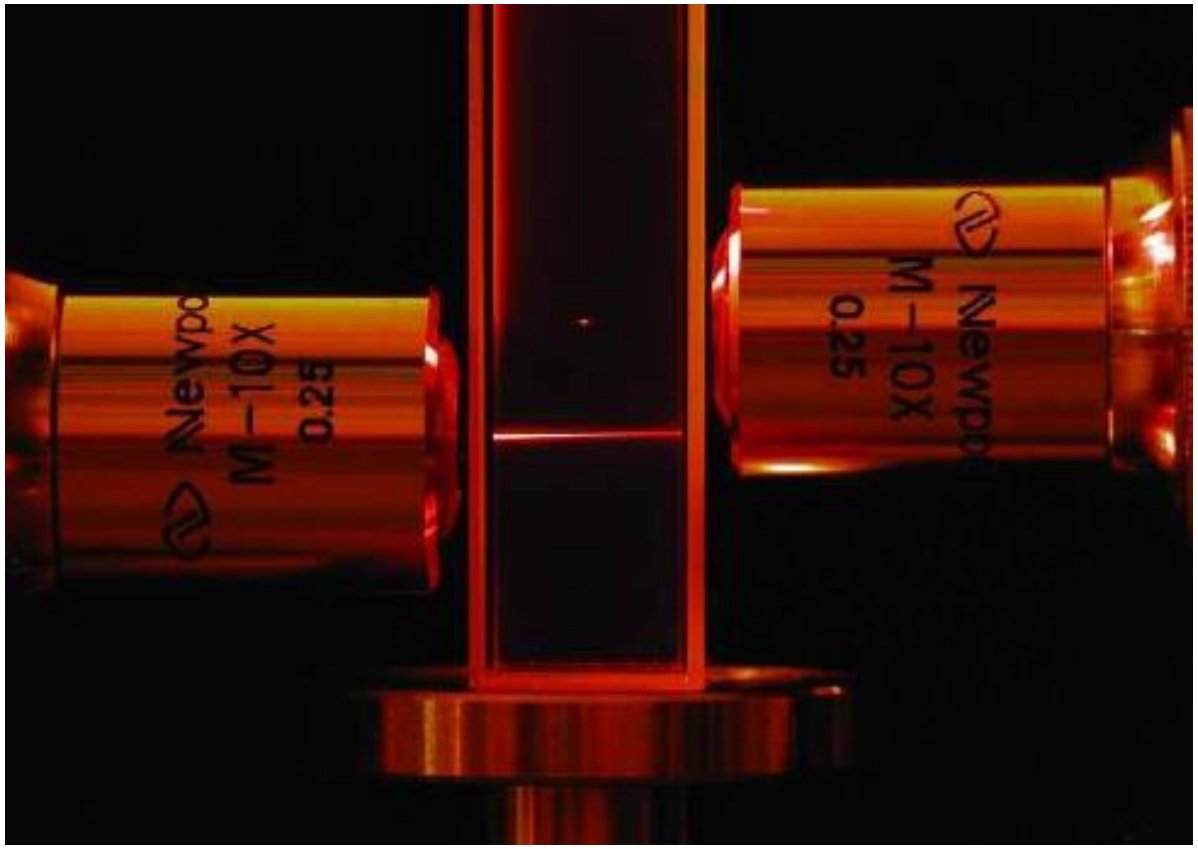
- A SiPM is composed of an array of **single-photon avalanche diodes (SPAD)**
- Each SPAD is a reverse-biased photodiode operated in **Geiger mode**, such that an incident photon triggers **avalanche current**
- Output from all SPADs are gathered in parallel at the SiPM output, to give photon flux



IV diagram of a SPAD firing (SensL)

Theory – Multiphoton Processes

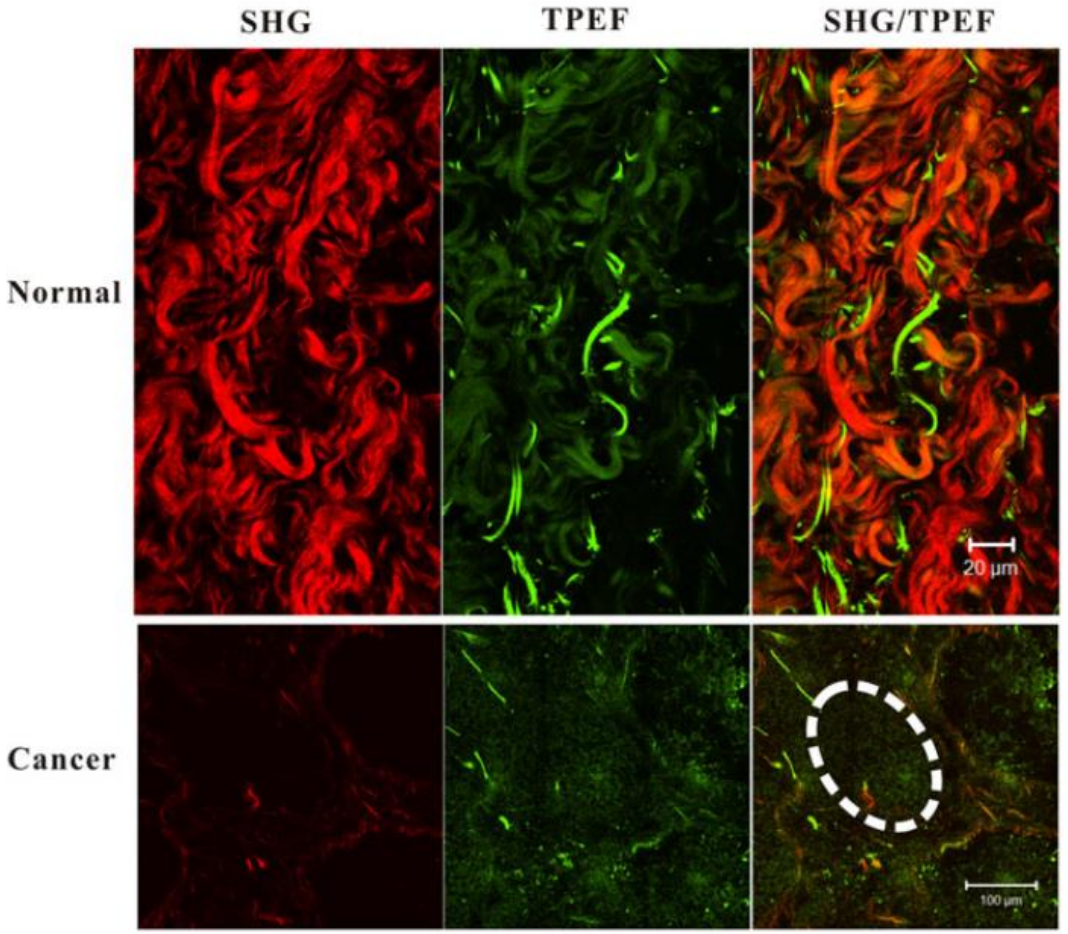
- Multiphoton/nonlinear optical processes are triggered when two or more photons are **simultaneously absorbed**
  - Requires high photon flux; necessitates use of a **mode-locked/pulsed laser**
- Signal only received from focal volume and is spectrally separated from excitation wavelength



Single vs. two-photon fluorescence (AZO Optics)

Theory – TPEF, SHG, and CARS

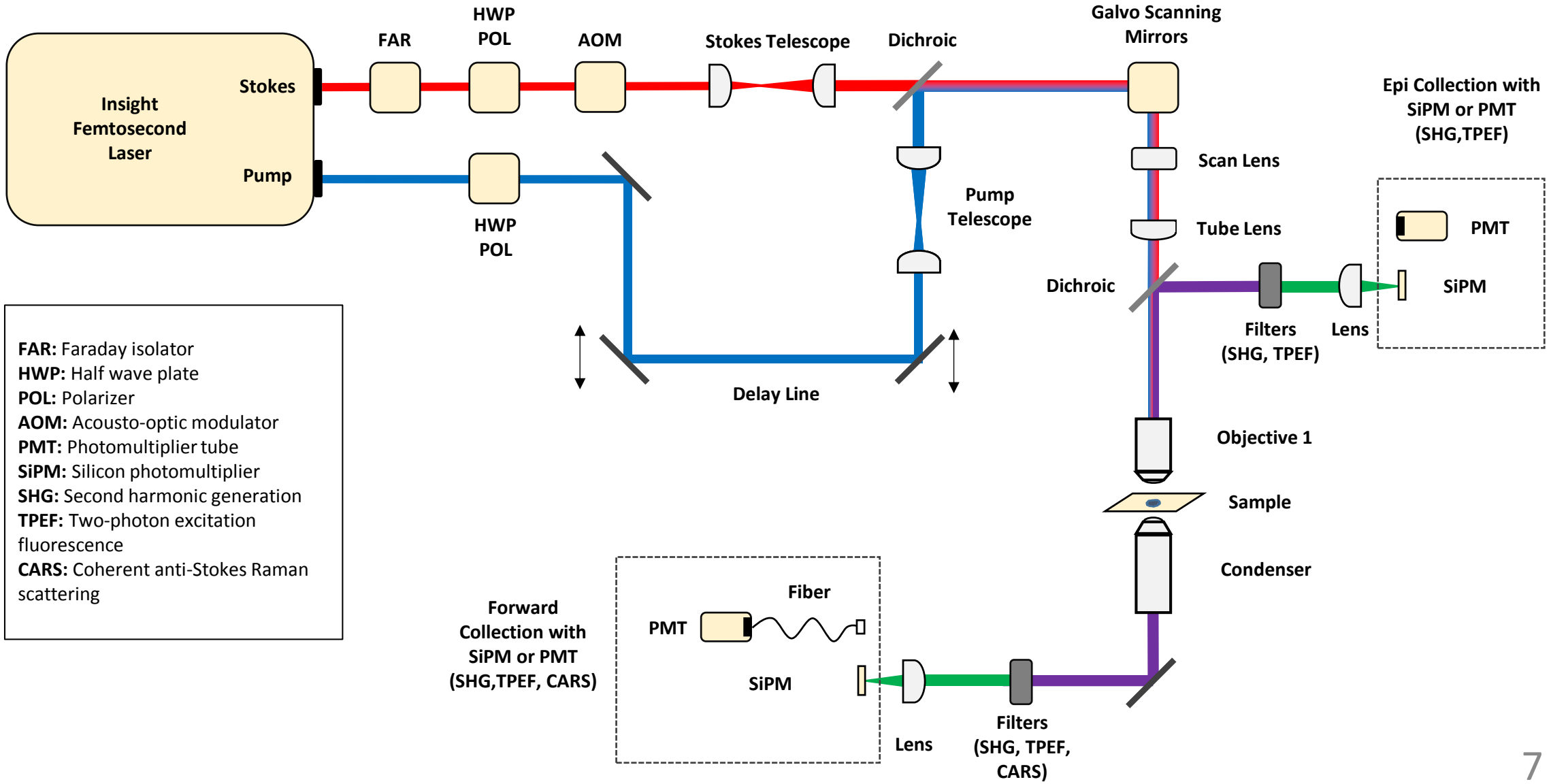
- Two-photon excitation fluorescence (TPEF)
  - Autoflourescent molecules
- Second harmonic generation (SHG)
  - Noncentrosymmetric structures e.g. collagen, muscle myosin
- Coherent anti-Stokes Raman scattering (CARS)
  - Enhancement of Raman scattering



SHG and TPEF used to distinguish between normal and cancerous oesophagus tissue

Journal of Microscopy, Vol. 253, Issue 1 2014, pp. 79–82

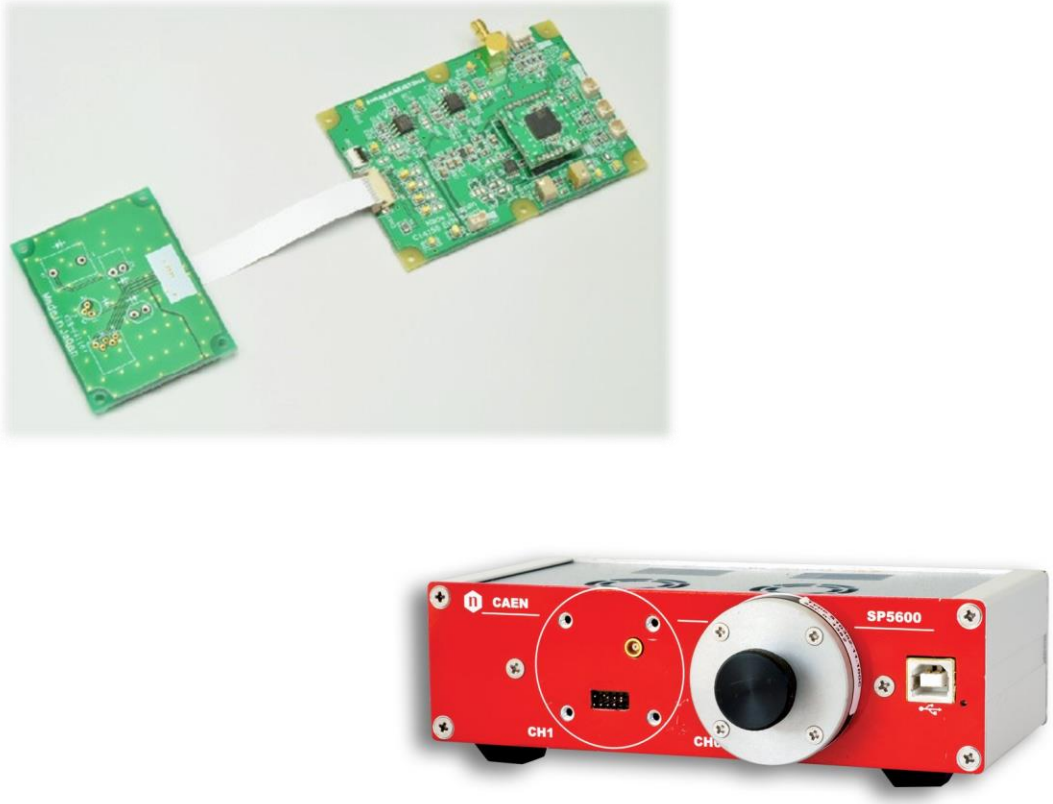
Apparatus – Multiphoton Microscope



FAR: Faraday isolator
HWP: Half wave plate
POL: Polarizer
AOM: Acousto-optic modulator
PMT: Photomultiplier tube
SiPM: Silicon photomultiplier
SHG: Second harmonic generation
TPEF: Two-photon excitation fluorescence
CARS: Coherent anti-Stokes Raman scattering

Apparatus – SiPMs and Modules

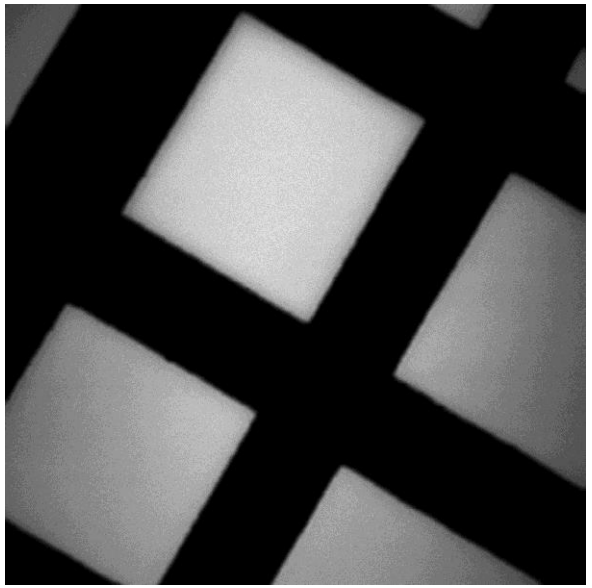
- We used Hamamatsu S13360 1.3x1.3, 3x3, and 6x6 mm^2 SiPMs
- Initial experiments used a CAEN educational SiPM power supply and amplification (PSAU) module
- Most recent experiments have used a Hamamatsu pole zero cancellation SiPM testing board

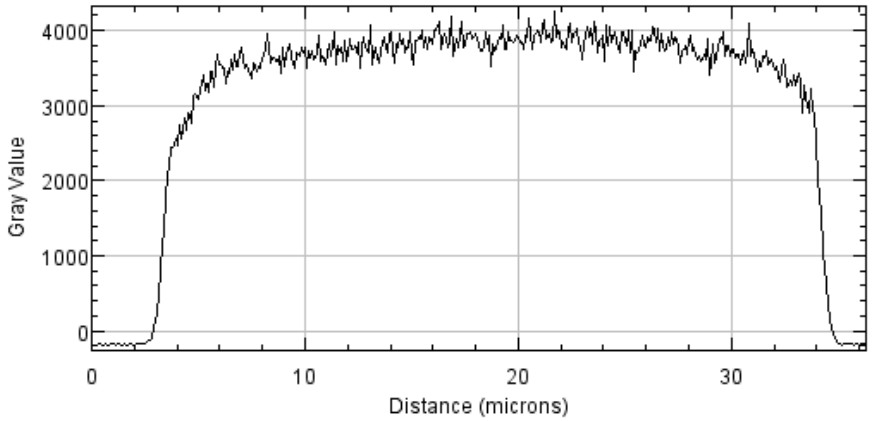


Hamamatsu (top) and CAEN (bottom) SiPM modules

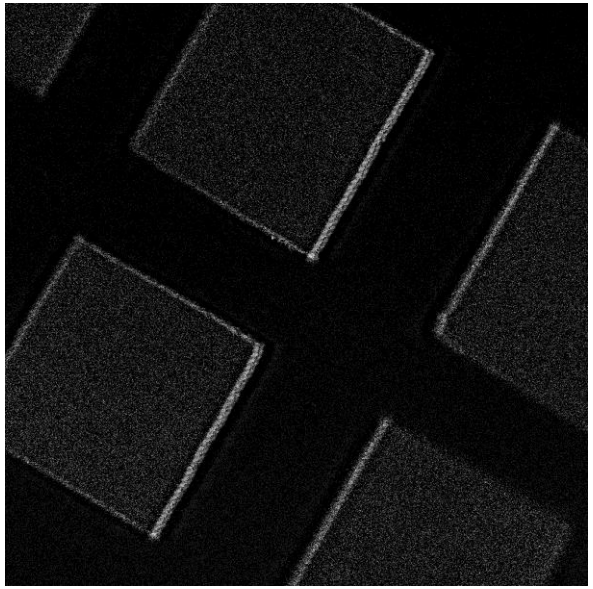
Results – CARS of Oil-Immersed Grid (CAEN)

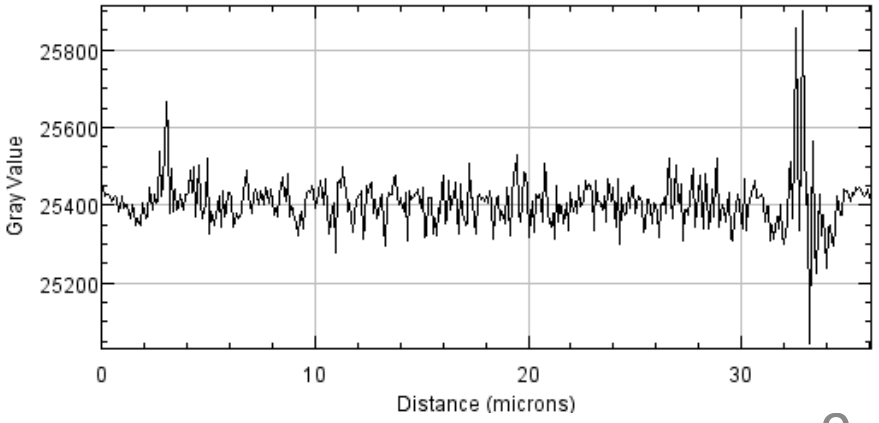
CARS oil grid with **PMT**
Dynamic range of ~4000 counts





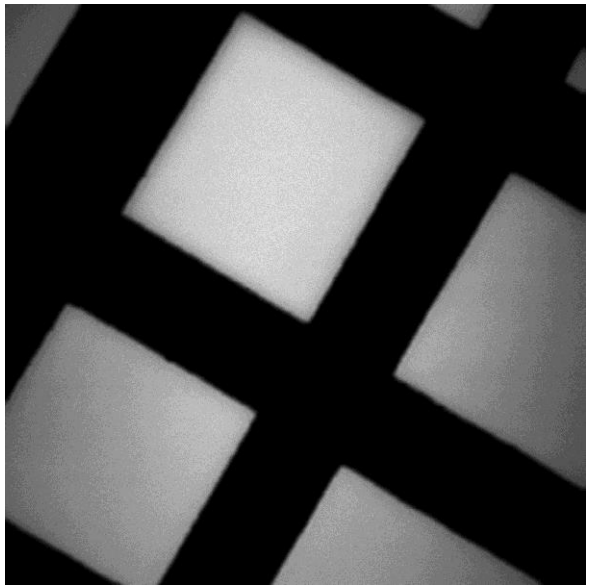
CARS oil grid with 1.3x1.3 mm^2 **SiPM** in **CAEN** module
Dynamic range of ~50 counts

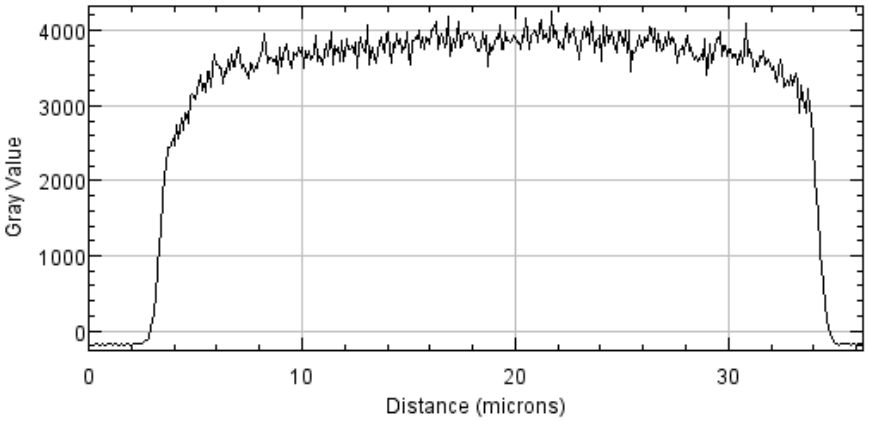




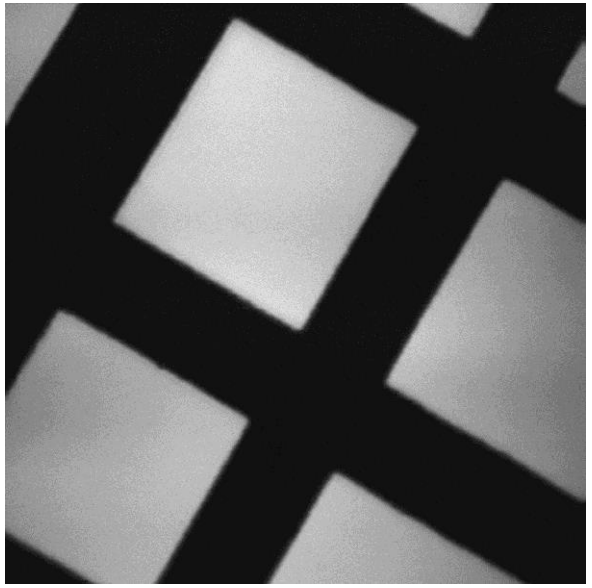
Results – CARS of Oil-Immersed Grid (PZC)

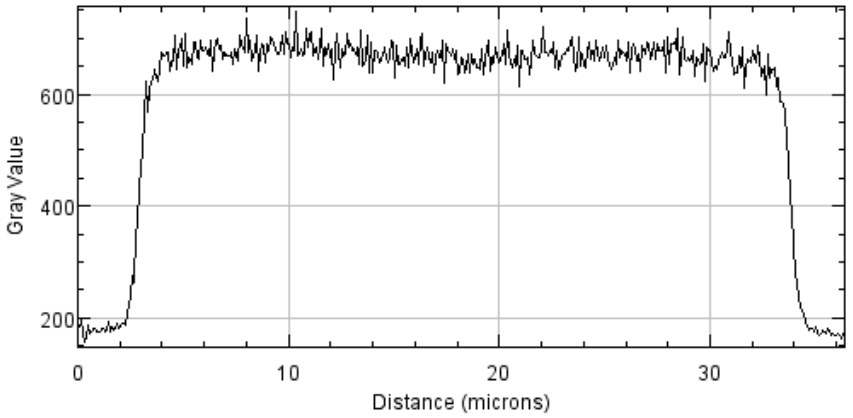
CARS oil grid with **PMT**
Dynamic range of ~4000 counts





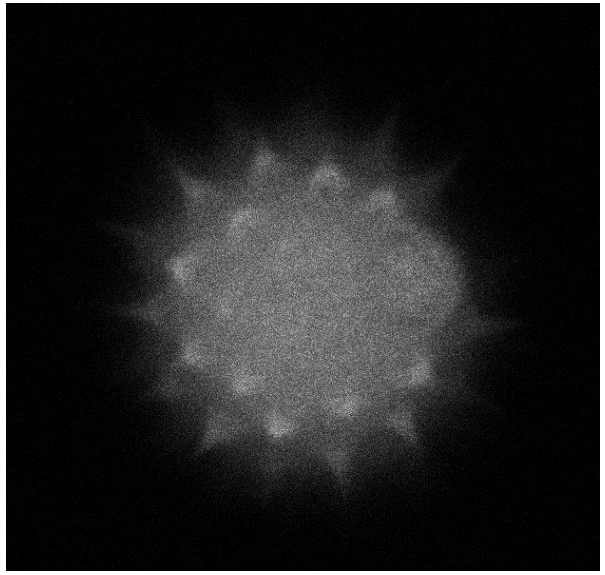
CARS oil grid with 3x3 mm^2 **SiPM** in **PZC** module
Dynamic range of ~500 counts

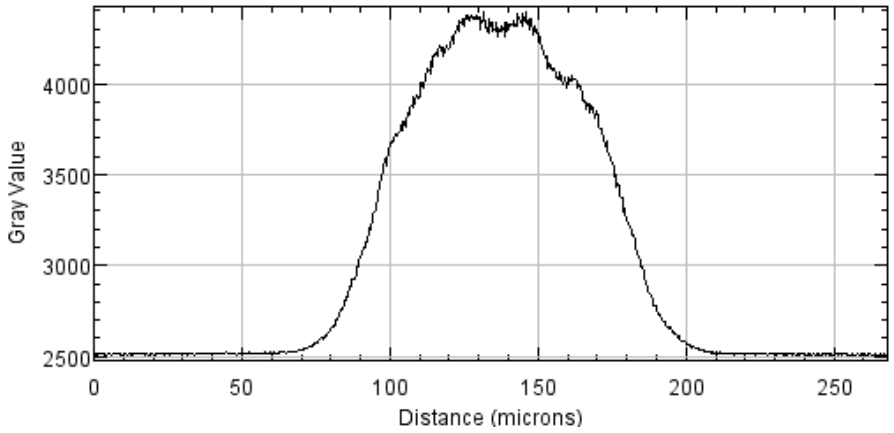




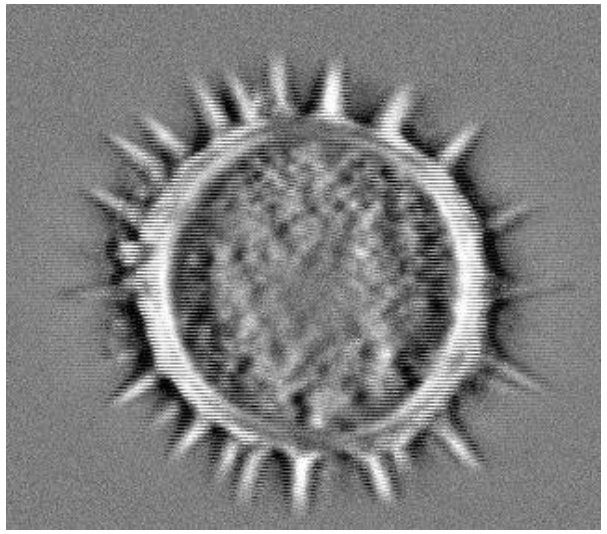
Results – TPEF of Pollen Grain

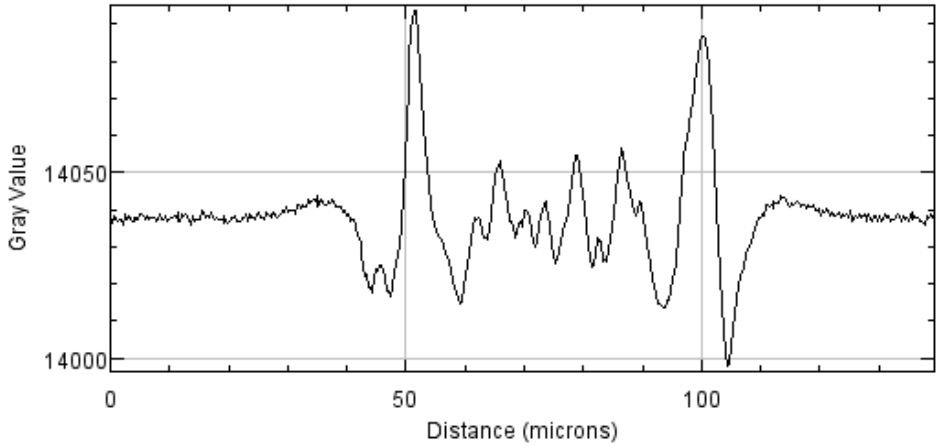
TPEF of pollen grain with **PMT** Dynamic range of ~1800 counts



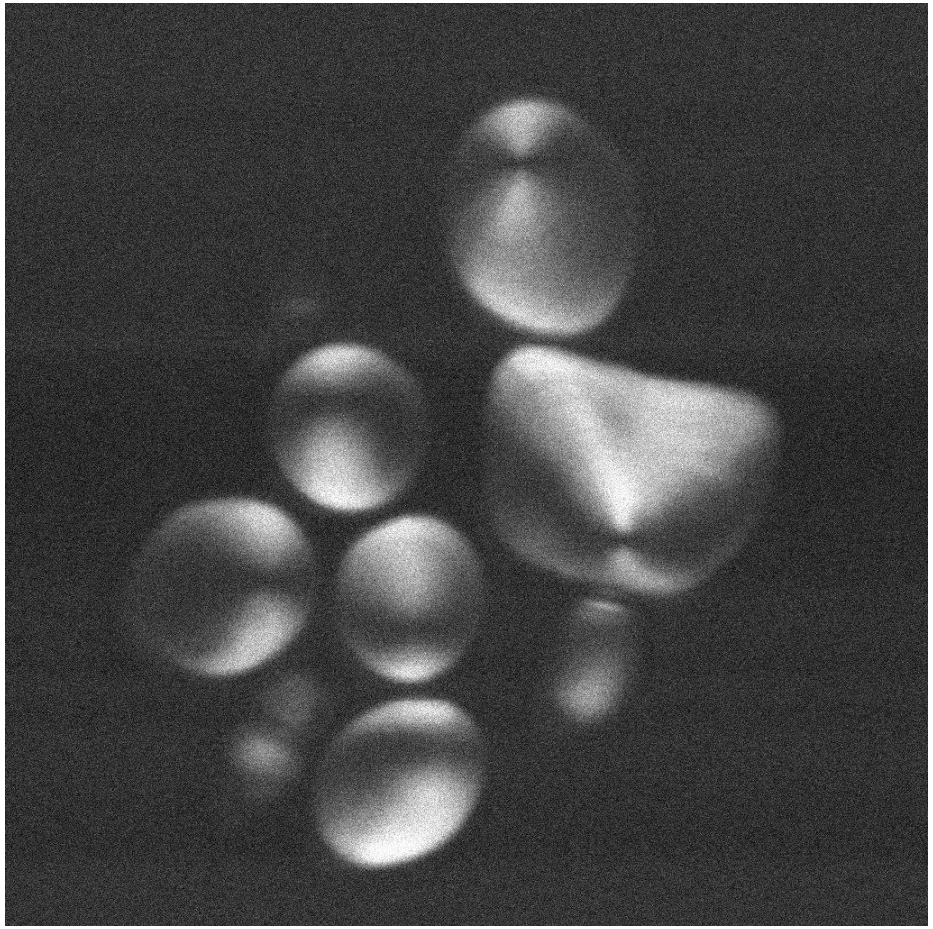


TPEF of different pollen grain with 6x6 mm^2 **SiPM** in **CAEN** module Dynamic range of ~100 counts

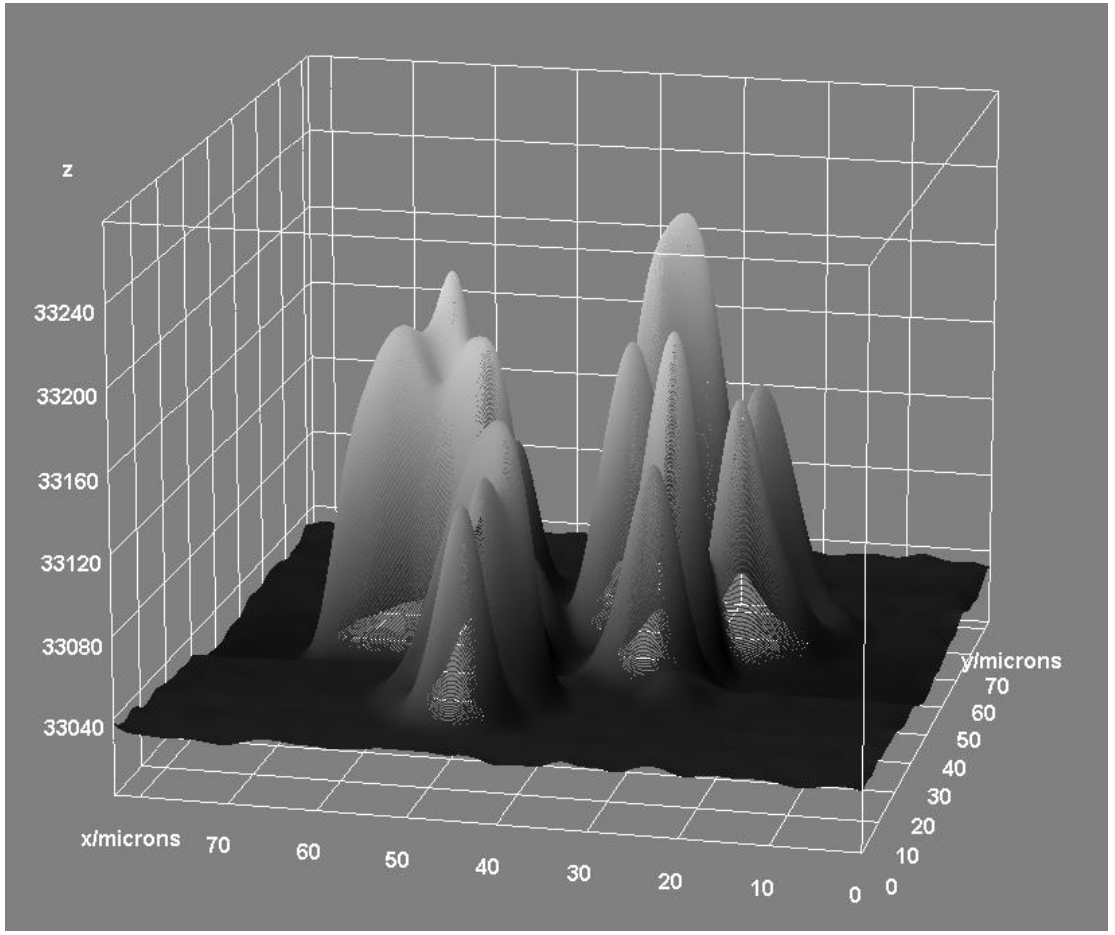




Results – SHG of Starch Granules



SHG of starch granules with 3x3 mm^2 SiPM in PZC module



3D surface plot with counts in z axis, dynamic range of ~250 counts

Conclusions

- SiPMs can be used to capture multiphoton images, for TPEF, SHG, and CARS
- Dynamic range/contrast good, but ~ order of magnitude lower than PMT
  - Cooling of the SiPM would reduce dark count – comparable to PMT
- Silicon photomultipliers are a promising alternative for PMTs in multiphoton microscopy

Acknowledgements

- **Dr. Sangeeta Murugkar**
- Dr. Balazs Nyiri
- Dr. Sami Qutob
- Dr. Vinita Chauhan
- Bradford Behr
- Connor Mcnairn
- Duale Ahmed
- Harry Allen
- Mike Antunes
- Nadine Adam
- Olivia Raiche-Tanner
- Penka Matanska
- Philippe Gravelle

Health Canada Santé Canada

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tornado spectral systems

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Thank you!

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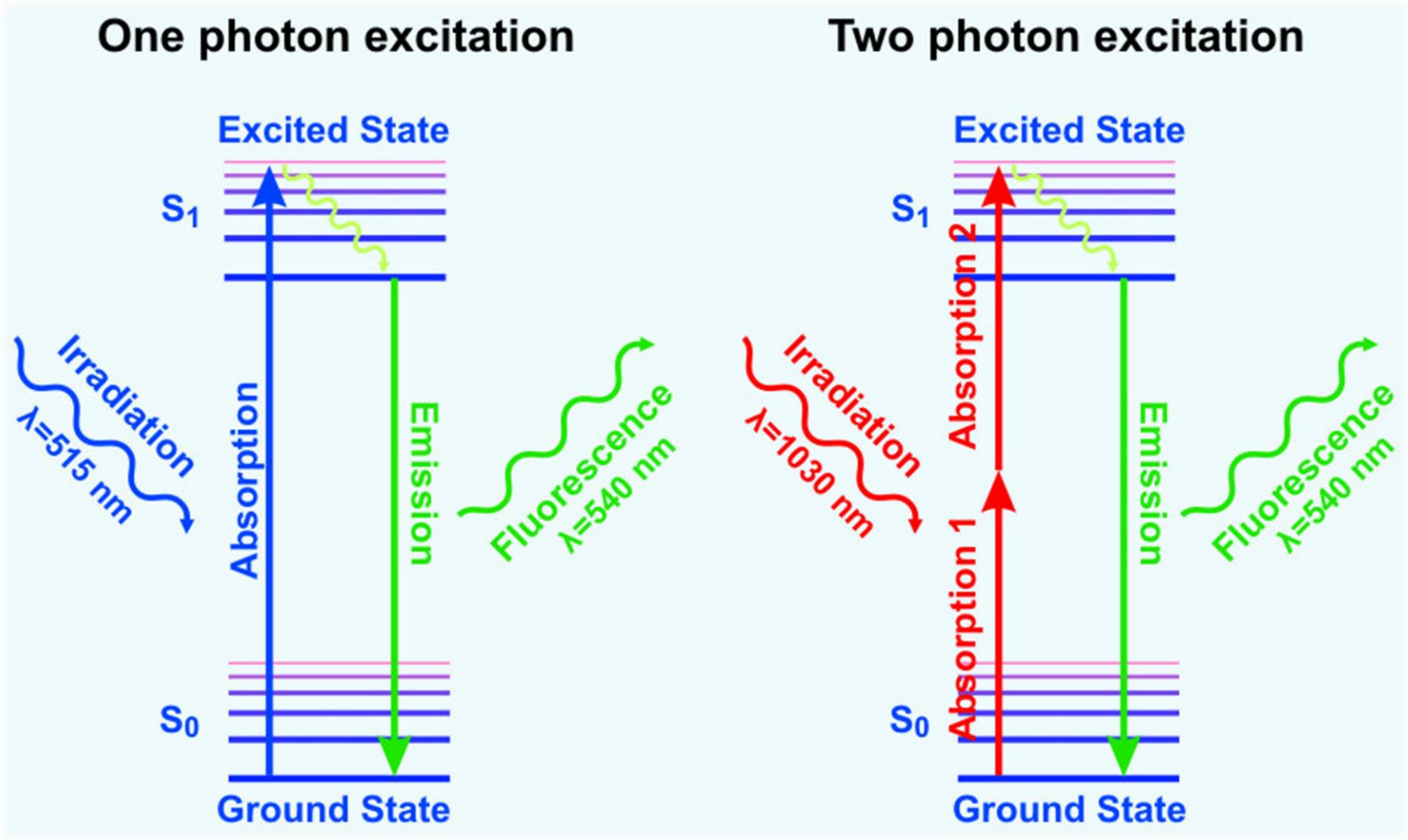
Extra Slides

Energy Diagram for TPEF



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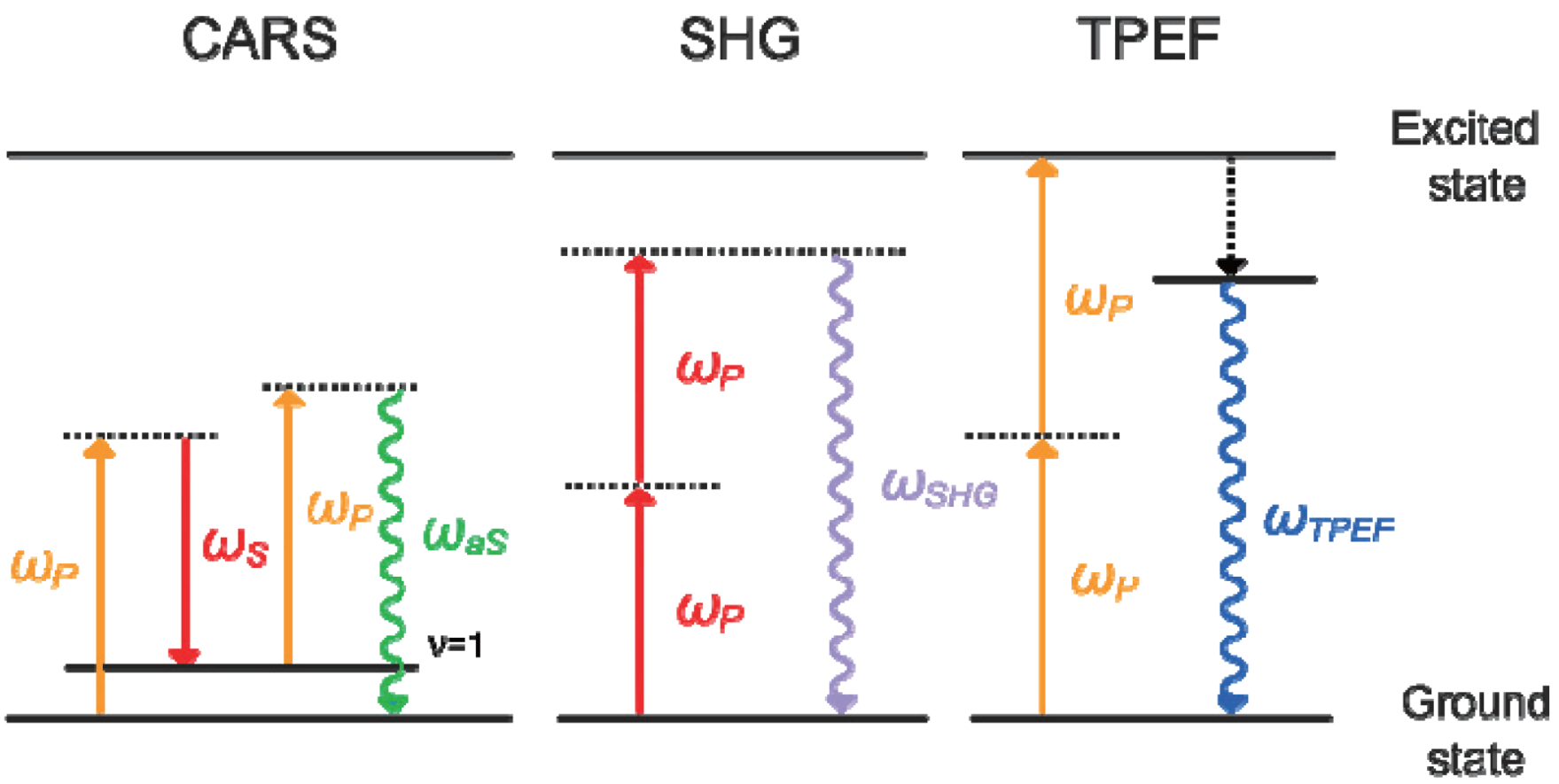
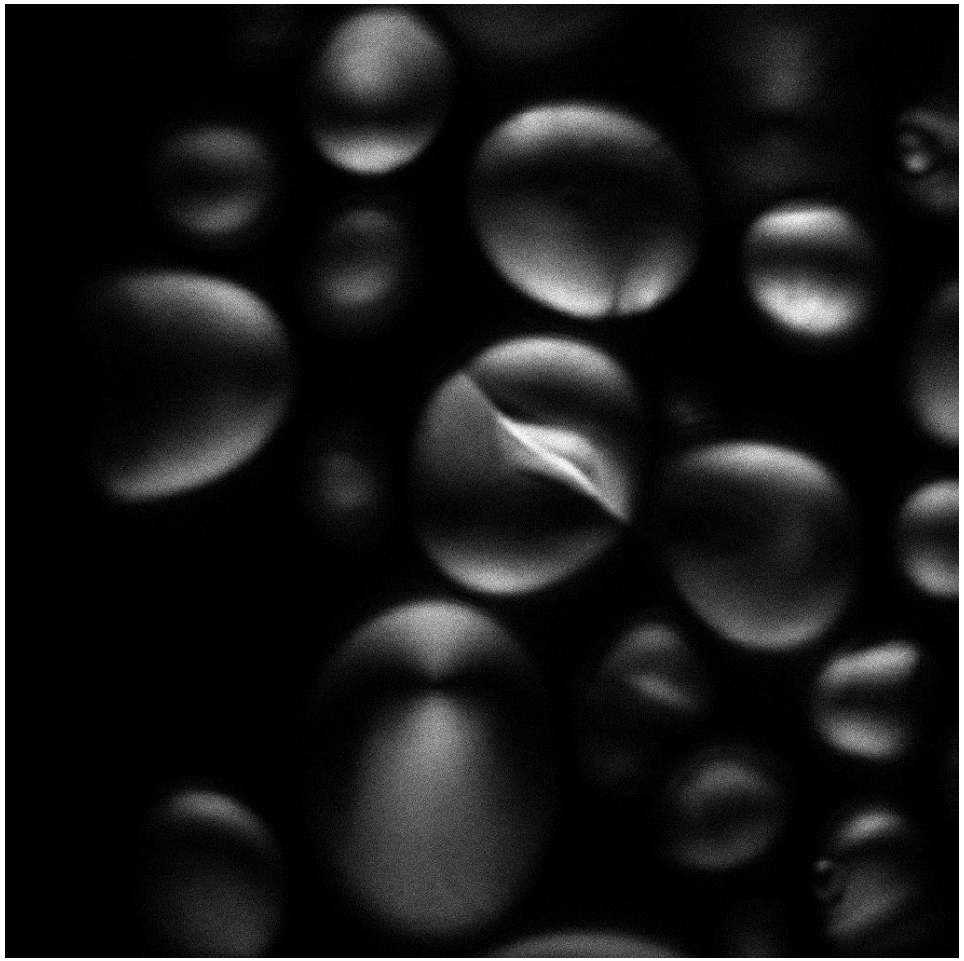
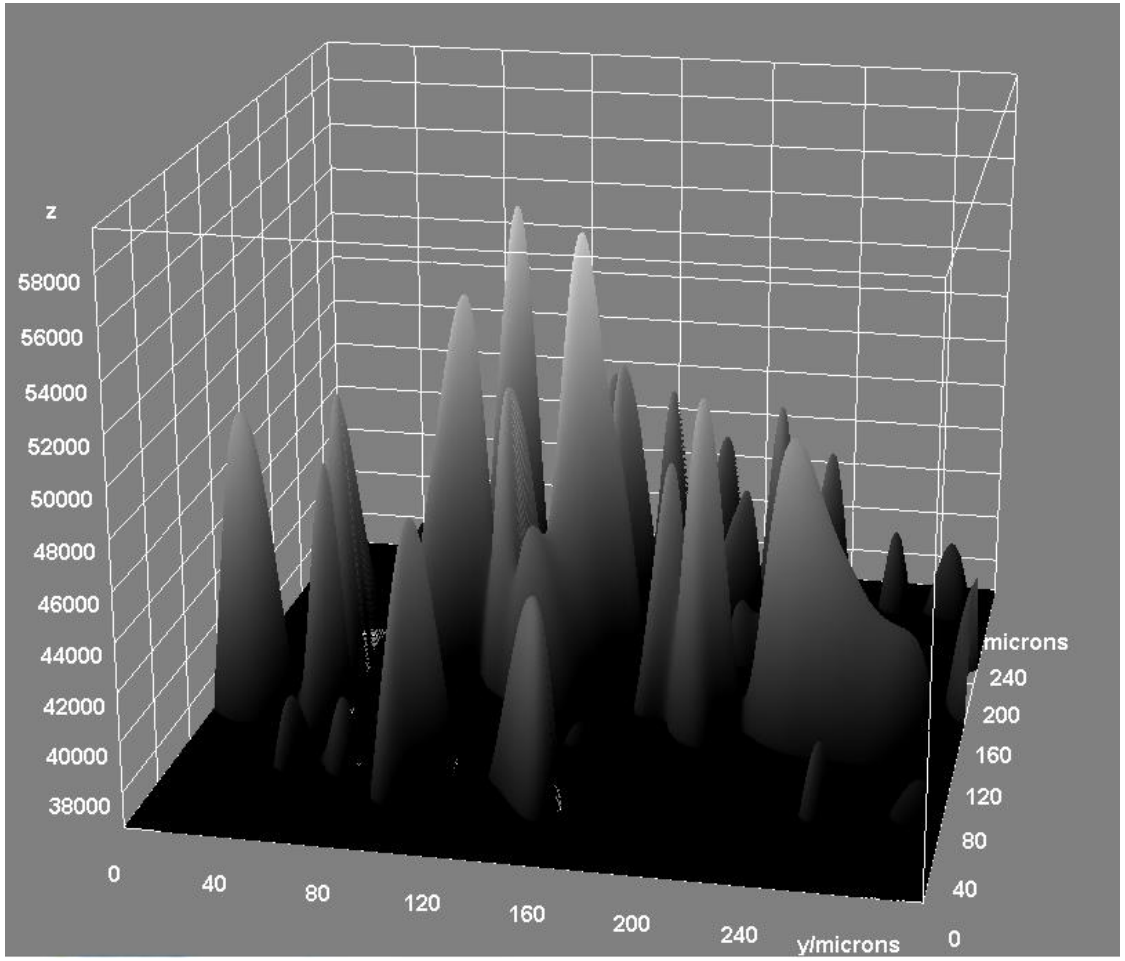
Energy Diagram for TPEF, SHG, and CARS



Healthcare **2013**, *1*(1), 64-83; https://doi.org/10.3390/healthcare1010064

SHG Image of Starch Granules with PMT





CARS Image of Pig Heart Tissue with PZC SiPM