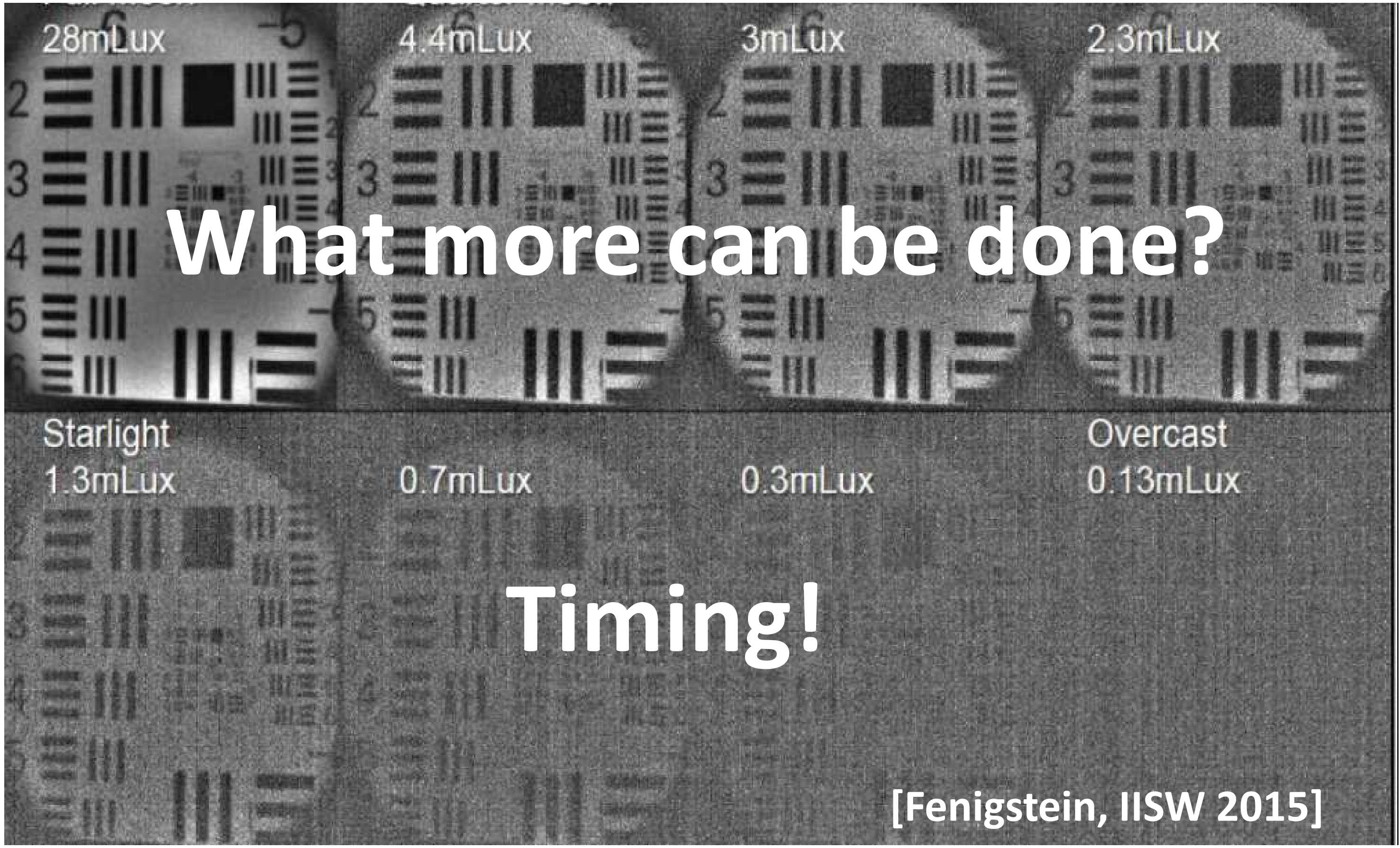
SPADs for Fluorescence Lifetime Imaging Microscopy (FLIM)

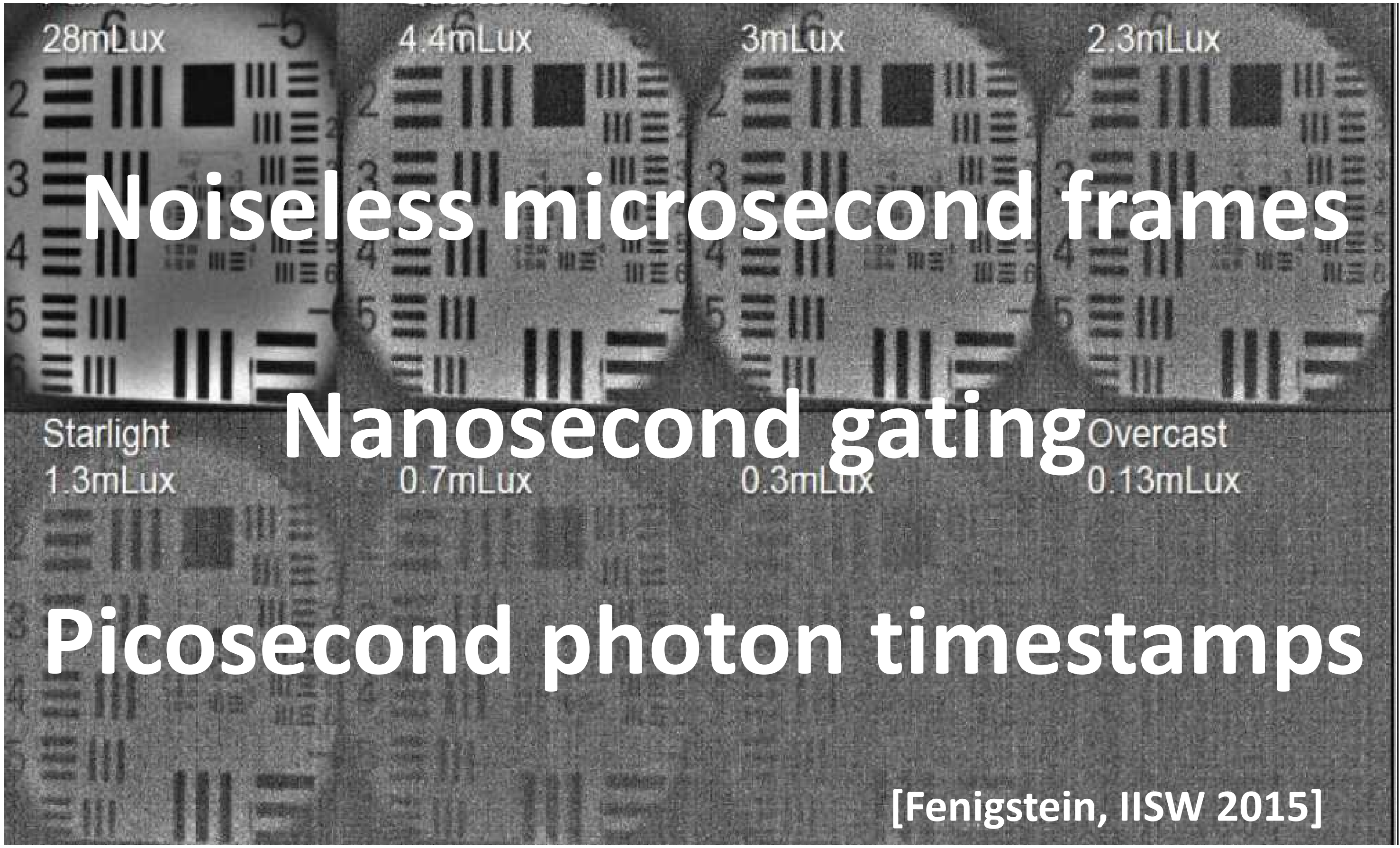
Robert K. Henderson, Istvan Gyongy,
Tarek Al Abbas, Ahmet Erdogan

THE UNIVERSITY *of* EDINBURGH
CMOS Sensors & Systems Group

Low Light Imaging

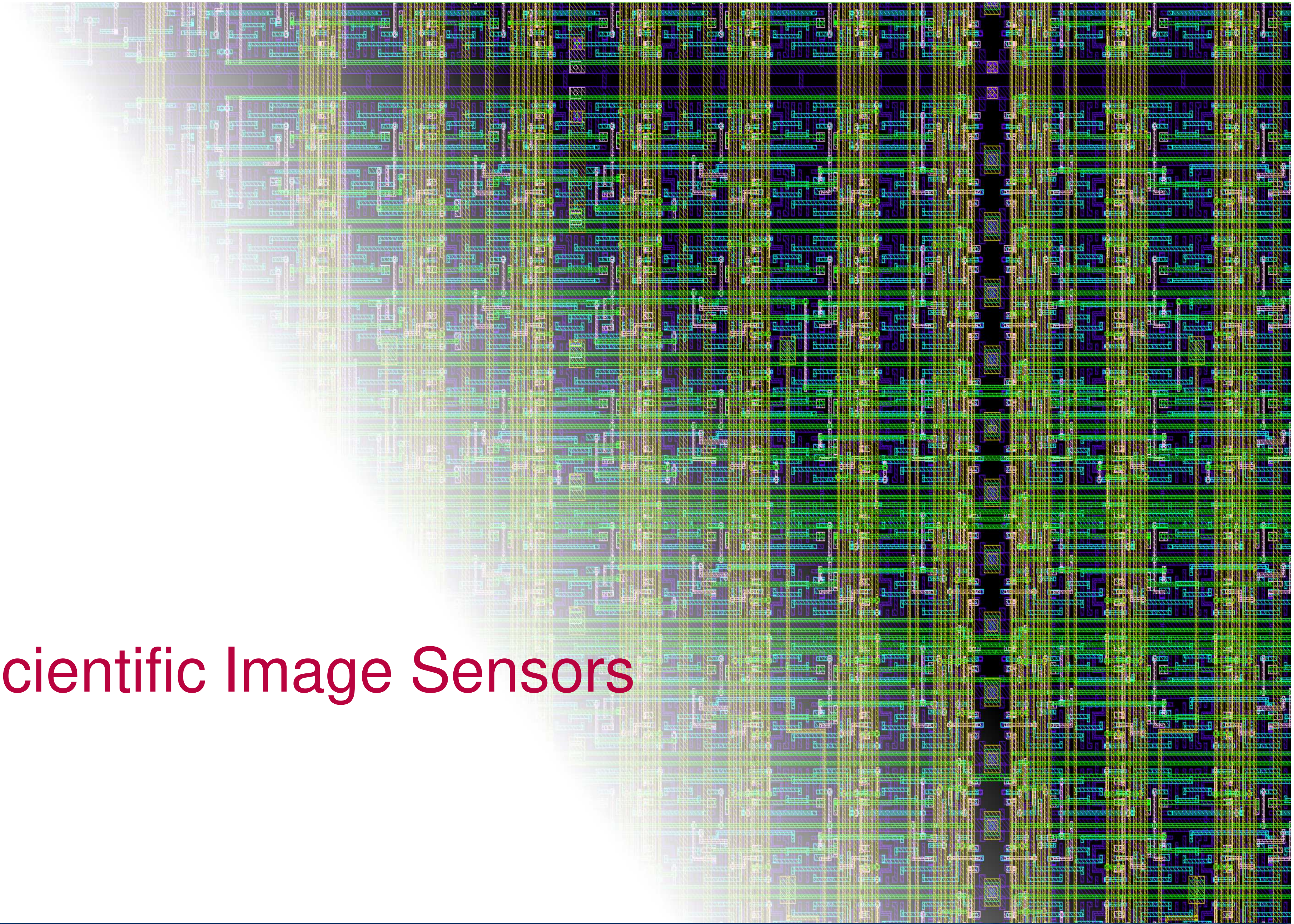


Low Light Imaging++



Content

- Scientific Image Sensors
- Fill-factor Problem
- Binary SPAD Sensors
- Digital SPAD Sensors
- Scientific SPAD Sensor Outlook

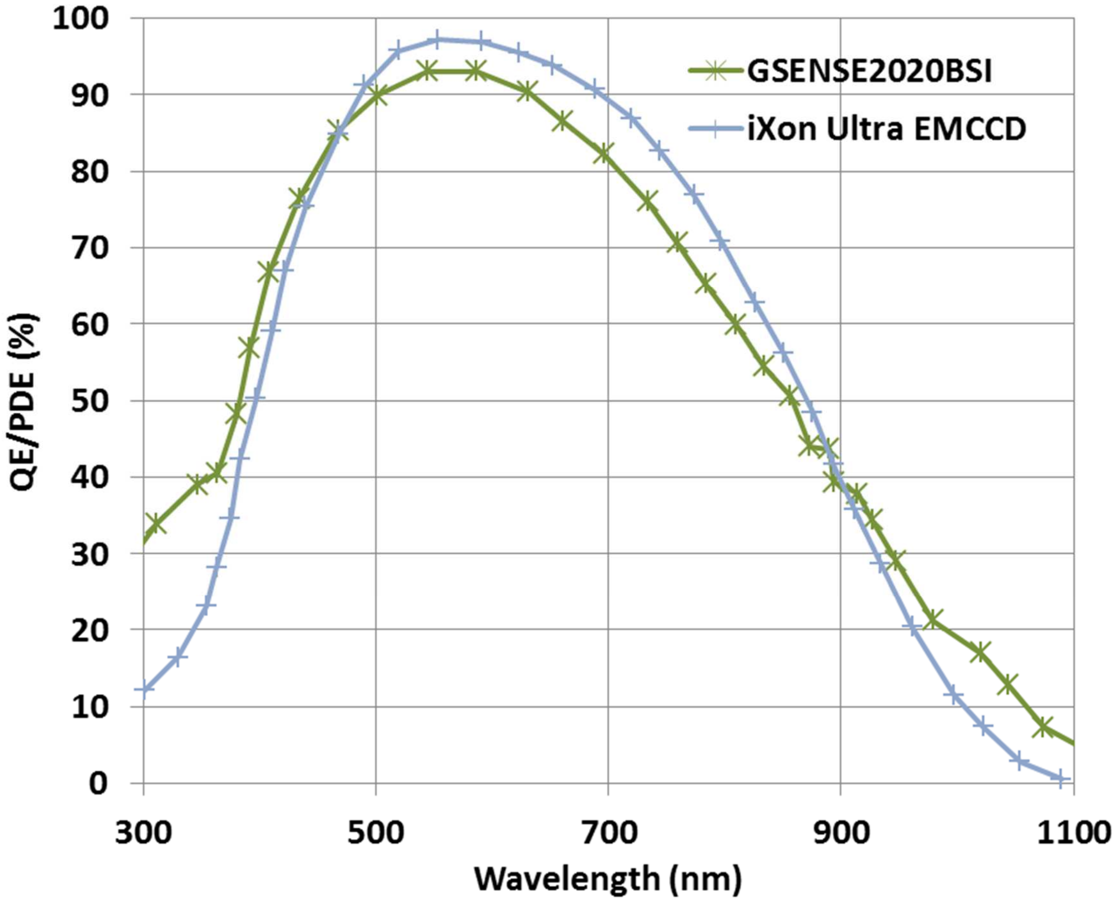


Scientific Image Sensors

Specifications

Specification	GSENSE2020BSI	iXon Ultra EMCCD
Resolution	2048 x 2048	1024 x 1024
Pixel Size	6.5µm × 6.5µm	13µm × 13µm
Full well	54ke-	80ke-
Dynamic Range	90dB	98dB (est.)
Max. SNR	47dB	49dB (est.)
Readout Noise	1.2e-	<1e- with EM gain
Dark Current	0.2e-/p/s @ -20°C	0.00025e-/p/s @ -80°C
Frame Rate	74fps	26fps
Power	1.2W	-
Peak QE	94% @ 550nm	95% @ 600nm

Quantum Efficiency (QE)



- Backside Illuminated EMCCD and scientific CMOS

Scientific SPAD Sensor

Scientific SPAD sensors promise:

1. Noiseless frame capture with typical cooling (<-60C).
2. ADC-less oversampled operation at high frame rate and low bit depth for high speed low light transient capture.
3. High time resolution (nanosecond time scale) for fluorescence lifetime/Raman, quantum correlations or ToF.

Scientific SPAD Sensor

Can a scientific SPAD sensor ever match EMCCD/sCMOS?:

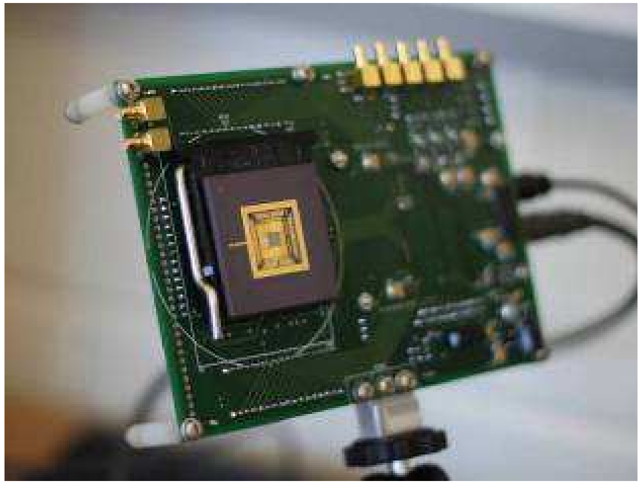
1. Pixel pitch (6-16μm) for 60x-150x objectives.
2. Resolution (>1Mpix) and fill-factor (>50%).
3. Full well capacity (>40ke-).
4. Dark current (few e-/p/s at RT).
5. Pixel defectivity of <1%.

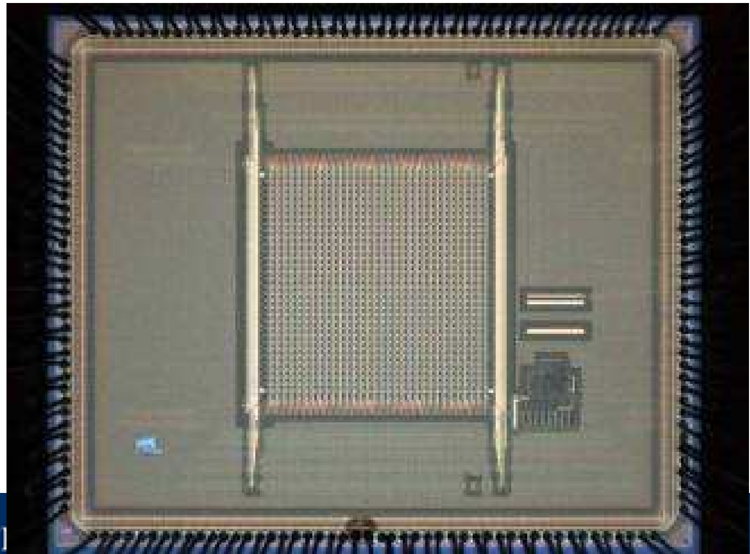
Low Fill Factor SPAD Imagers

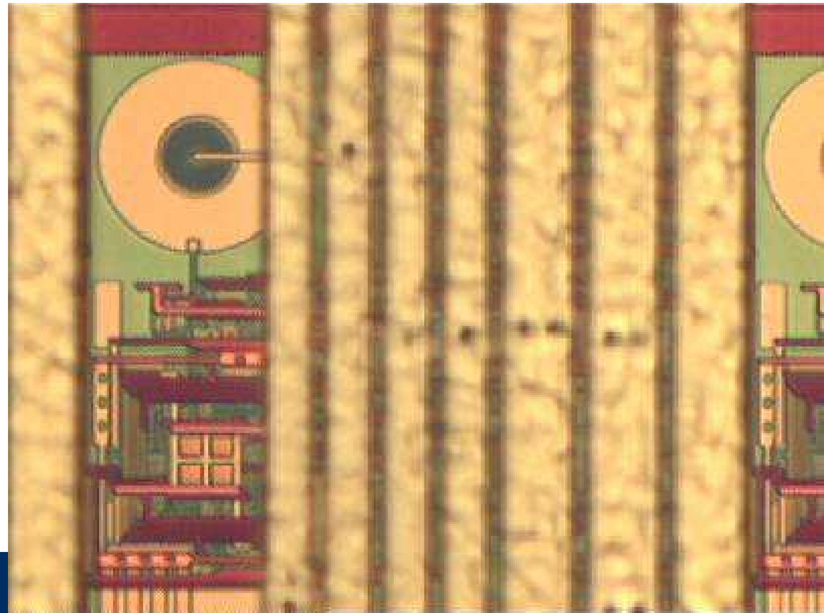
MegaFrame 32x32 Imager

32 x 32 50μm pixels each containing 7μm diameter SPAD + 50ps resolution stopwatch (50ns full scale) + 10-bit counter

Rate = 512Mphotons/s

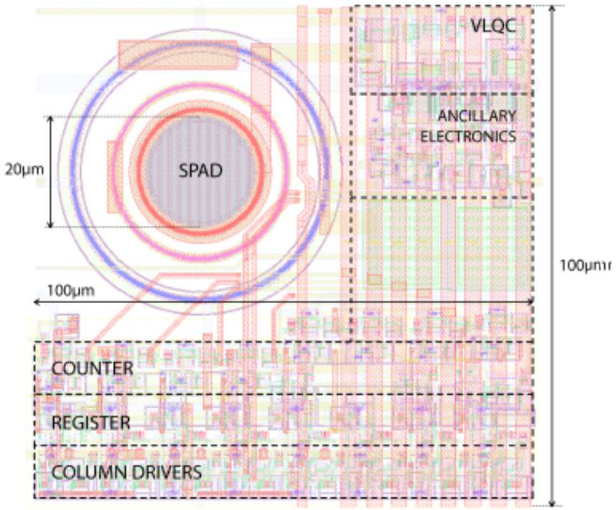




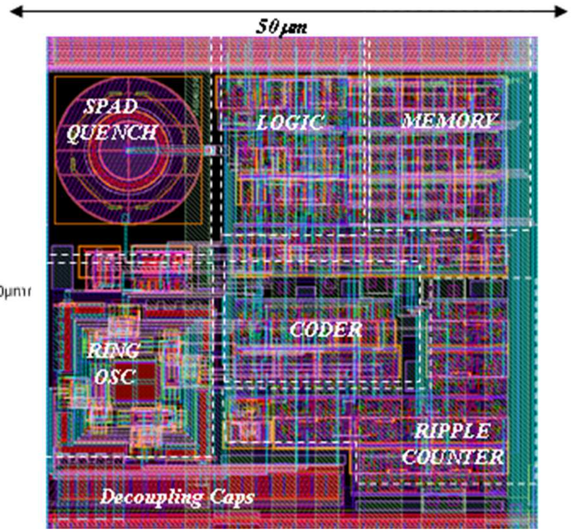


[MegaFrame EU Project]

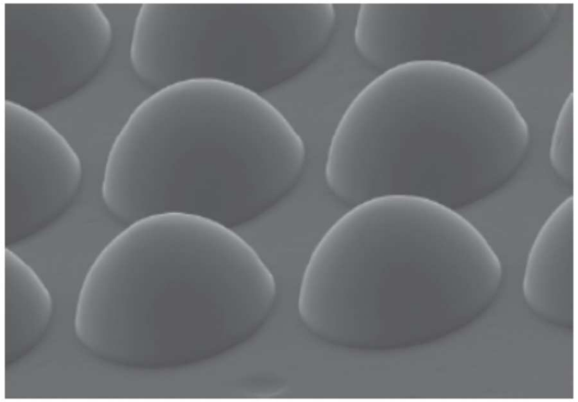
Fill-factor Problem



3% FF
[Guerrieri, 2010]



1.5% FF
[Richardson, 2009]



50µm Pitch Microlens Array
[Donati, 2007]

- First generation SPAD pixels large with low fill-factor – difficult to microlens at wafer scale.

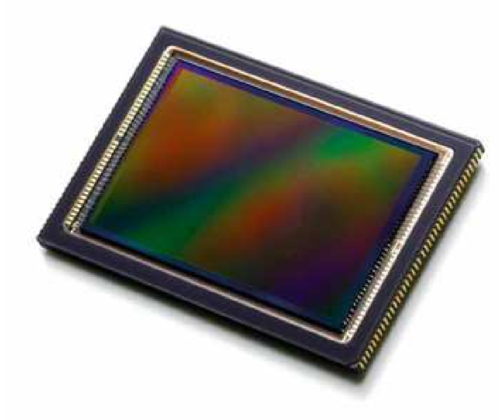
Binary SPAD Imagers

Binary SPAD sensors

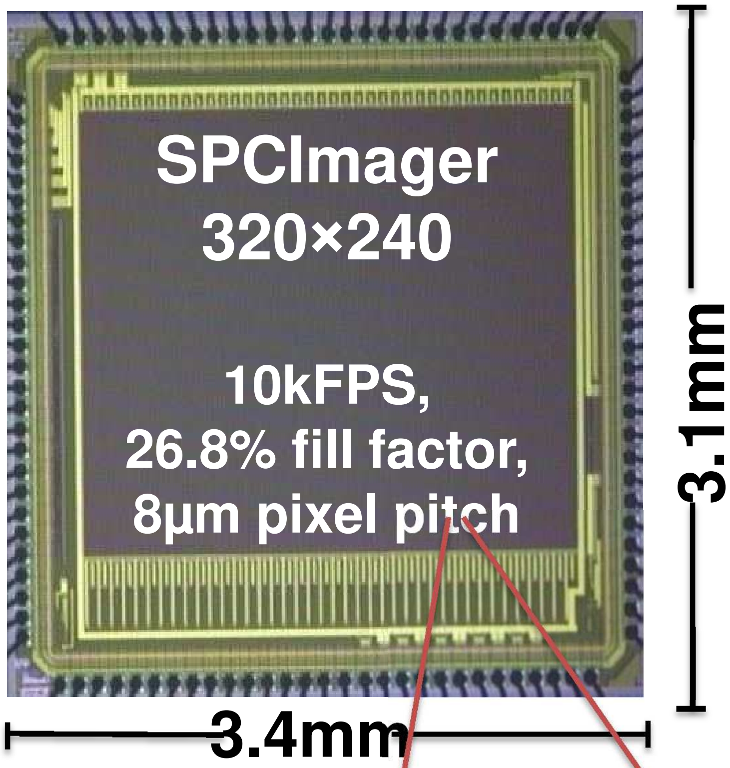
-Simple binary pixels for improved fill factor and resolution

--High speed of operation (100kFPS+)

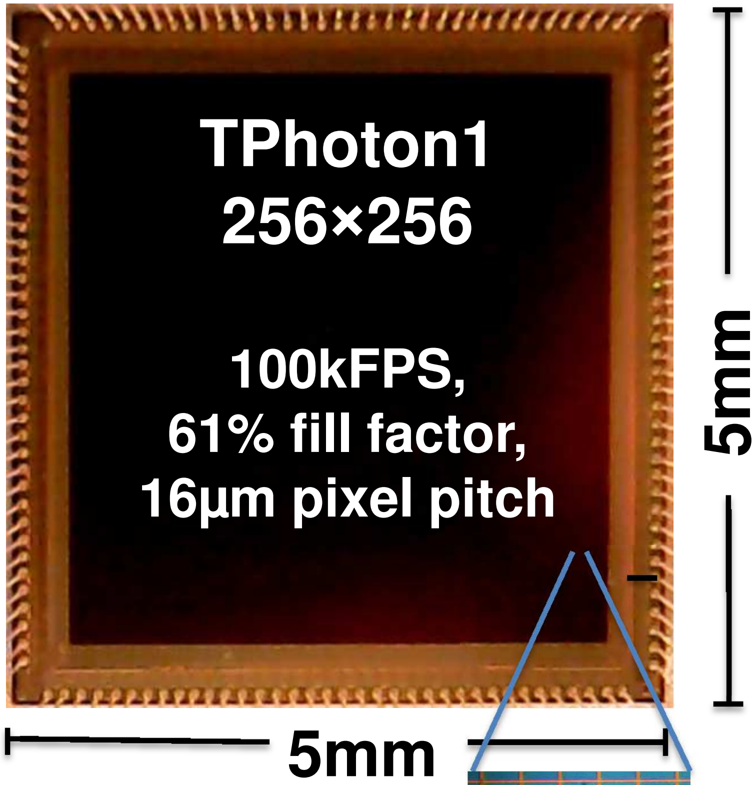
-Negligible readout noise

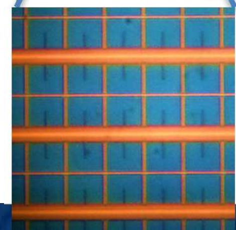


Binary SPAD image sensors

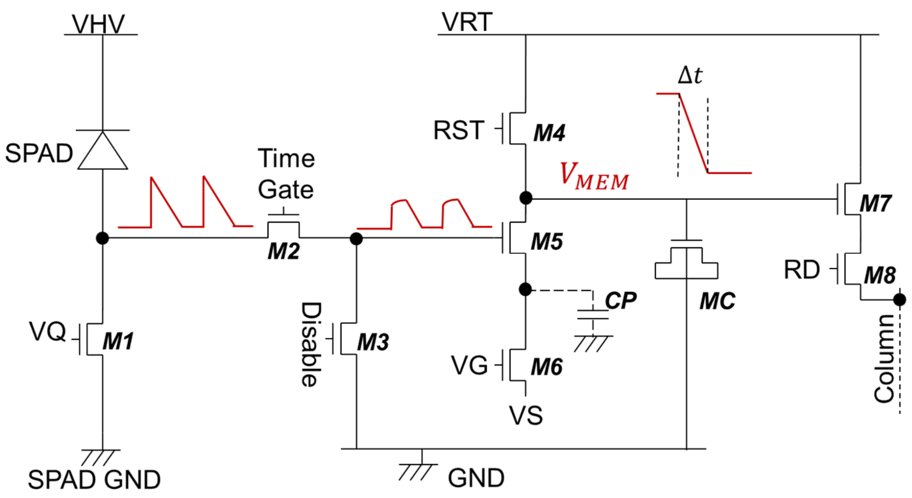


≈50% fill factor after microlensing

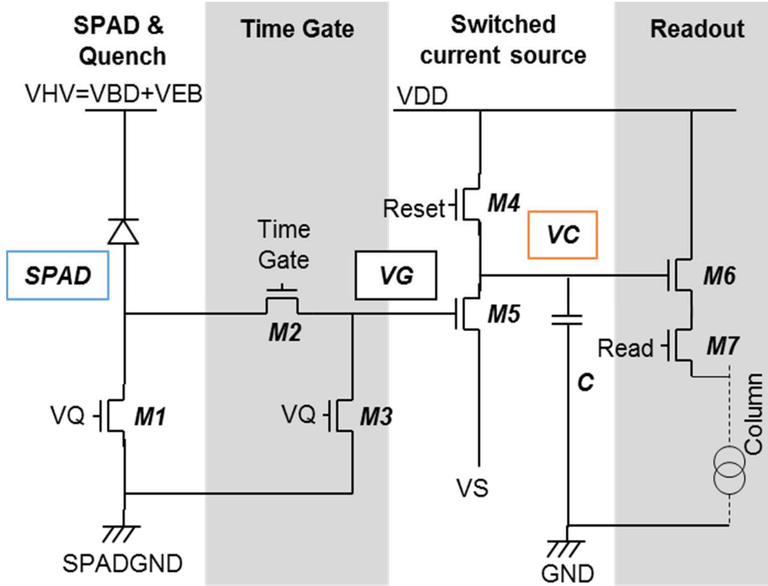




Pixel Circuits



SPCImager

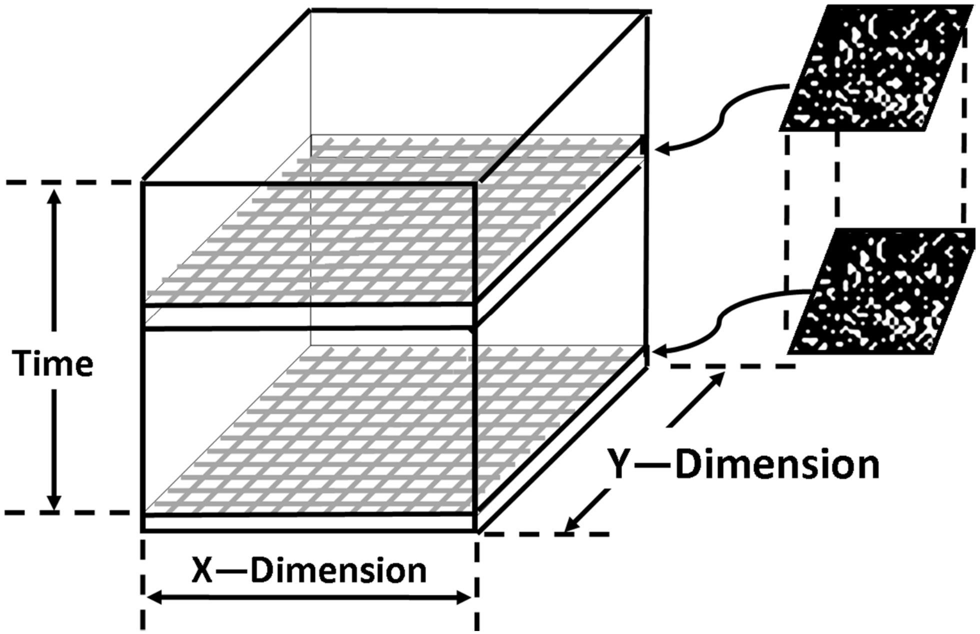


Tphoton1

- All NMOS circuits
- Gated analogue photon counting or binary mode

Raw output

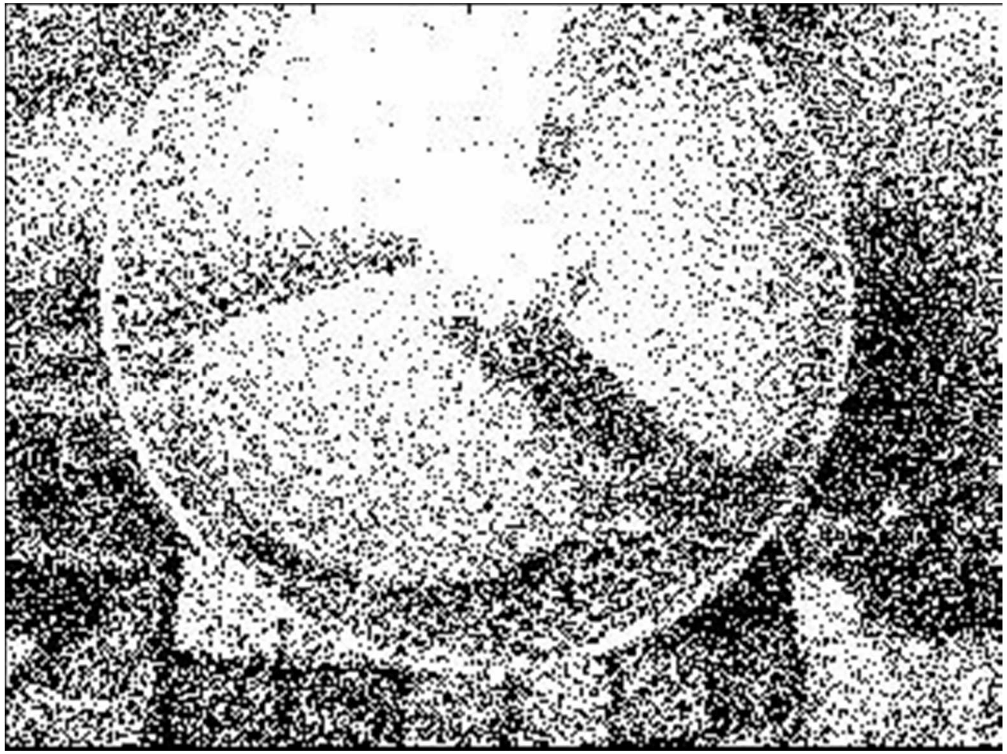
Single photon oversampled binary camera (Quanta Image Sensor)



Fossum (2005) “Gigapixel Digital Film Sensor (DFS) Proposal”

Example – Rotating fan

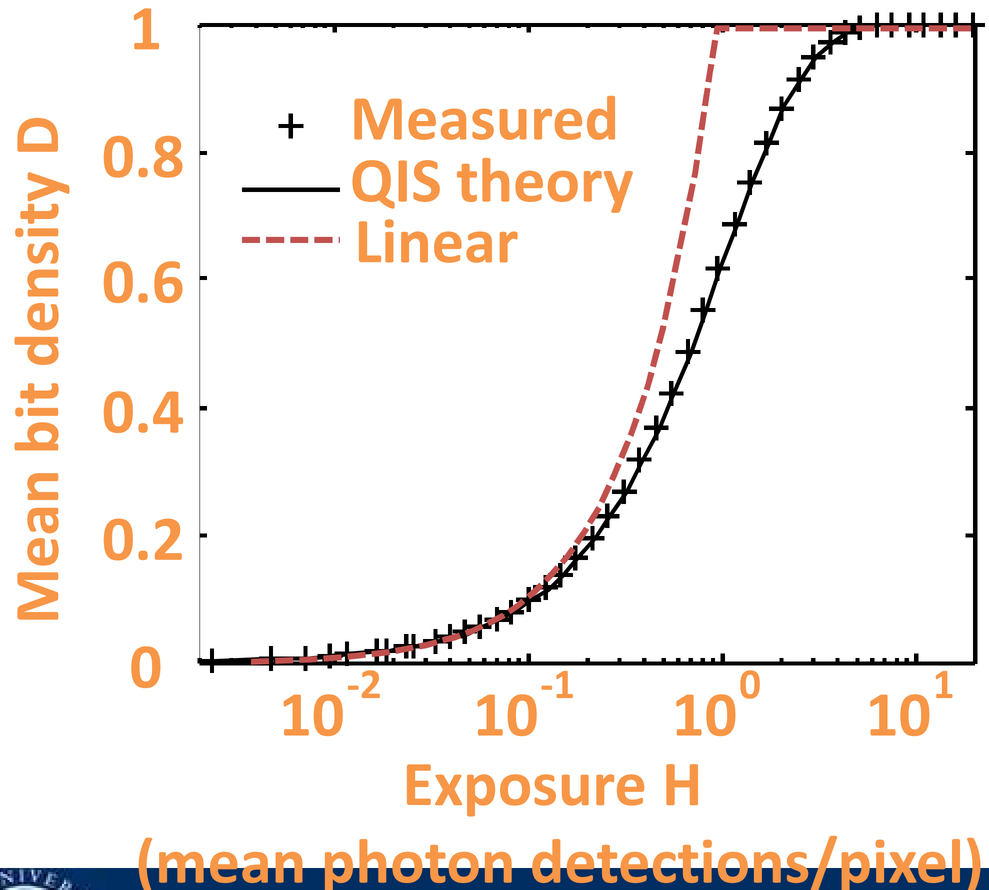
Sequence of raw bit-planes at 10kfps



(Playback at 500× slower rate)

Raw output (cont.)

Response is akin to photographic film



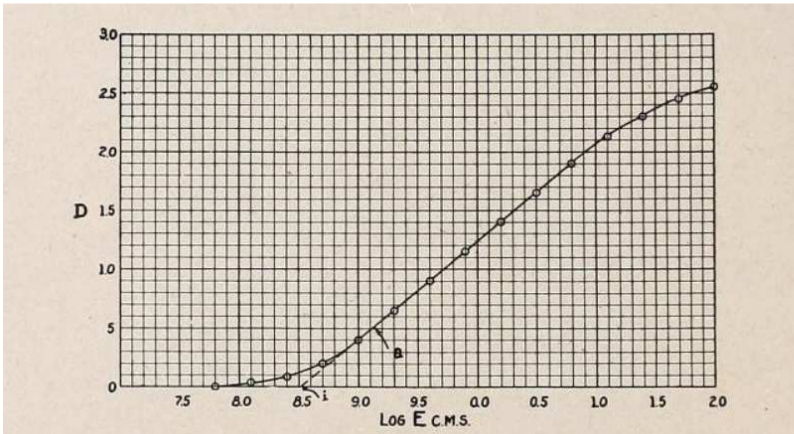
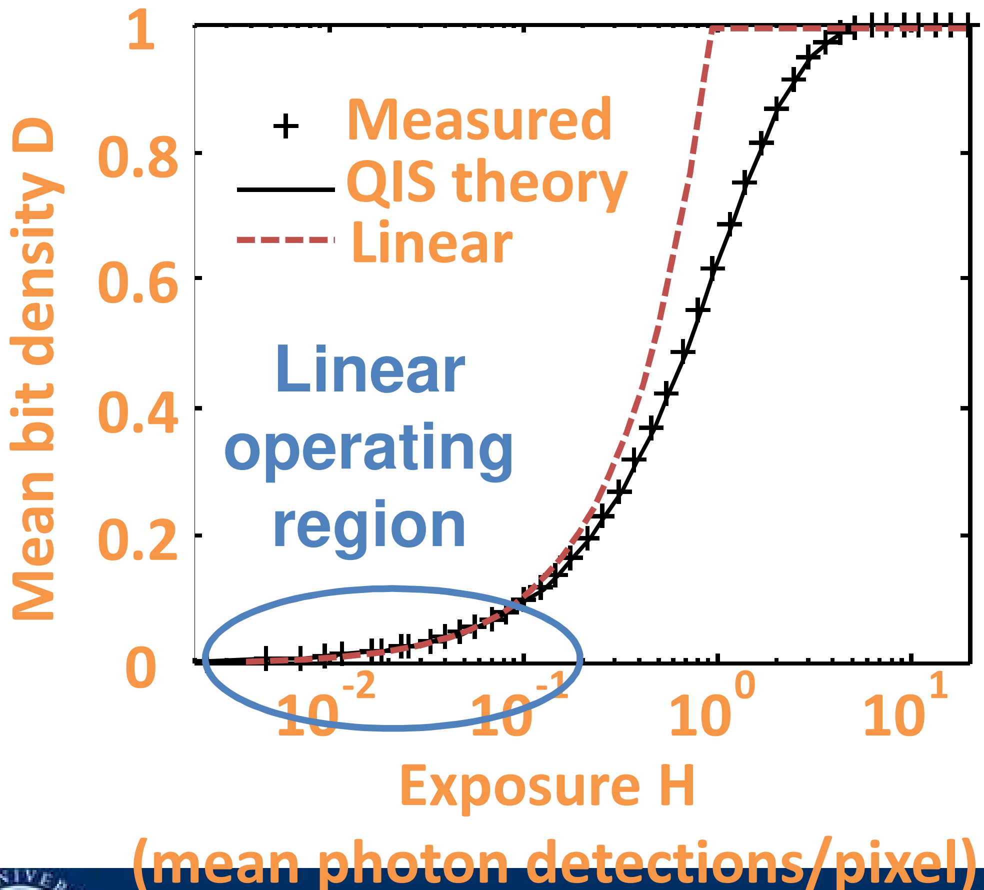


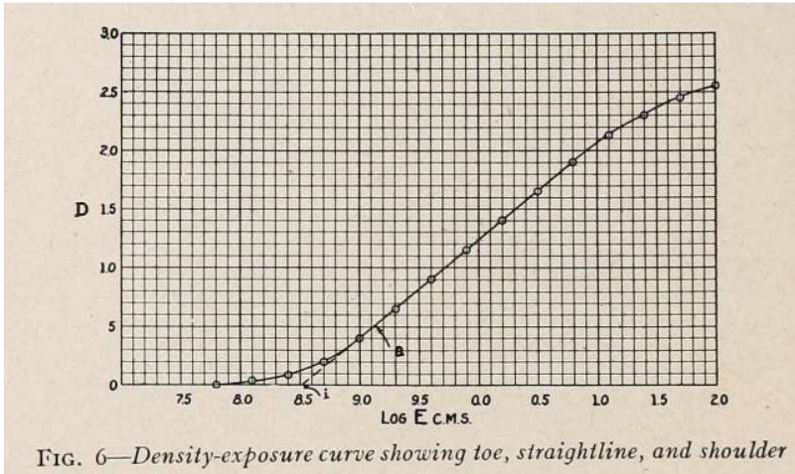
FIG. 6—*Density-exposure curve showing toe, straightline, and shoulder*

F. Hurter and V.C. Driffield, *Journal of the Society of Chem. Ind.* 1890

Raw output (cont.)

Response is akin to photographic film





F. Hurter and V.C. Driffield, *Journal of the Society of Chem. Ind.* 1890

Example – BPAE cell

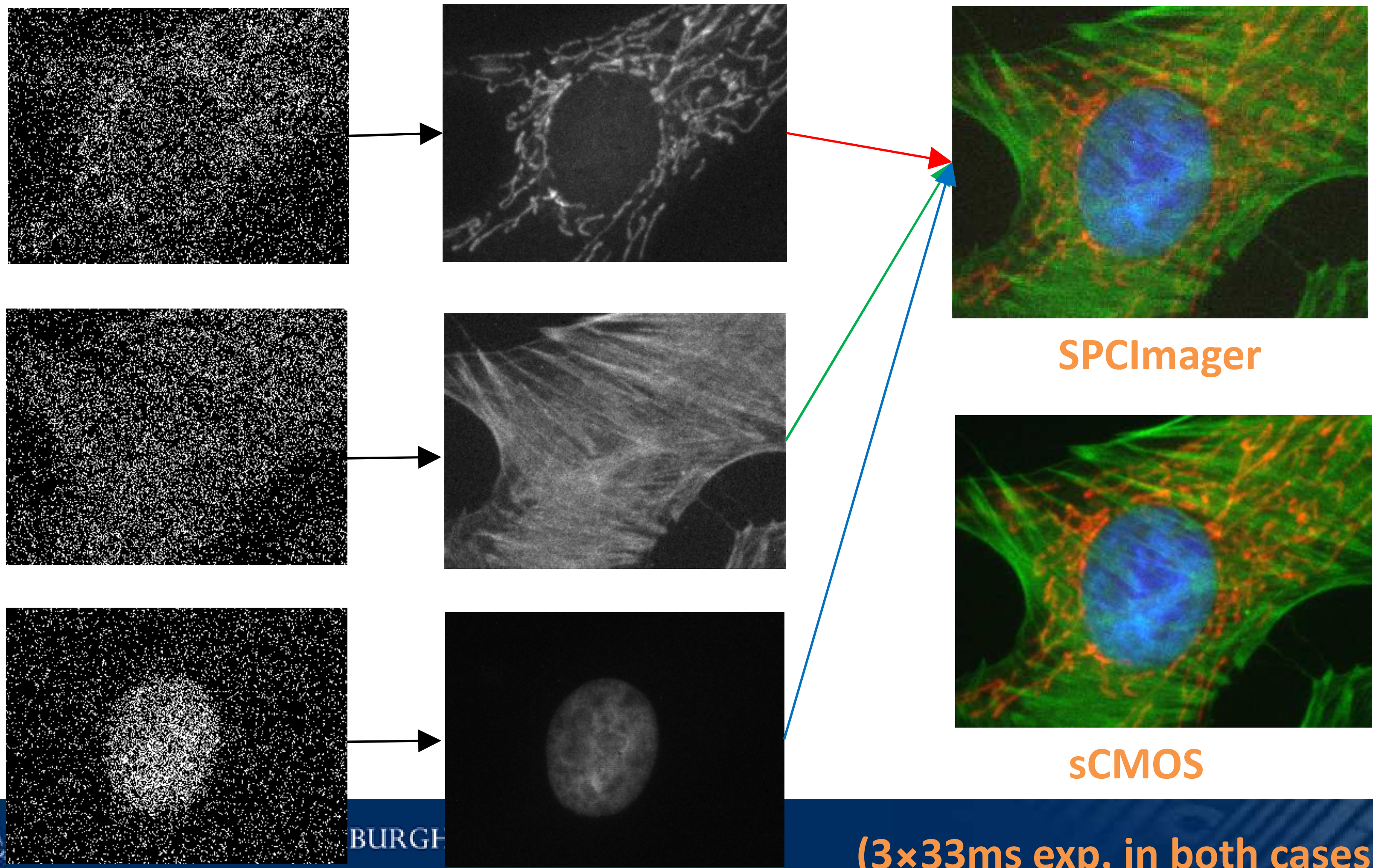
Single bit-plane (10µs exp.)

Σ 330 bit-planes (33ms exp.)

R

G

B

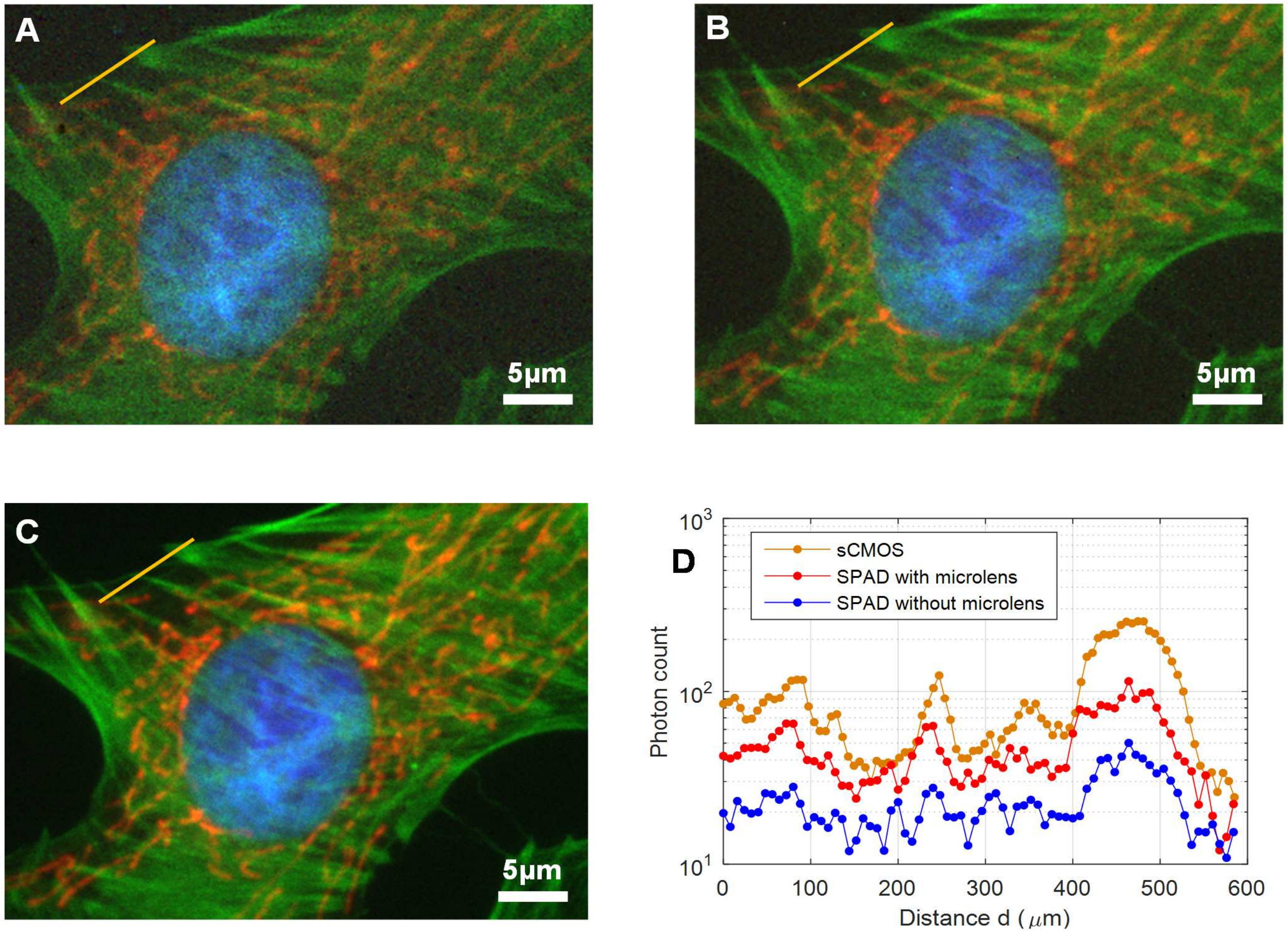


SPCImager

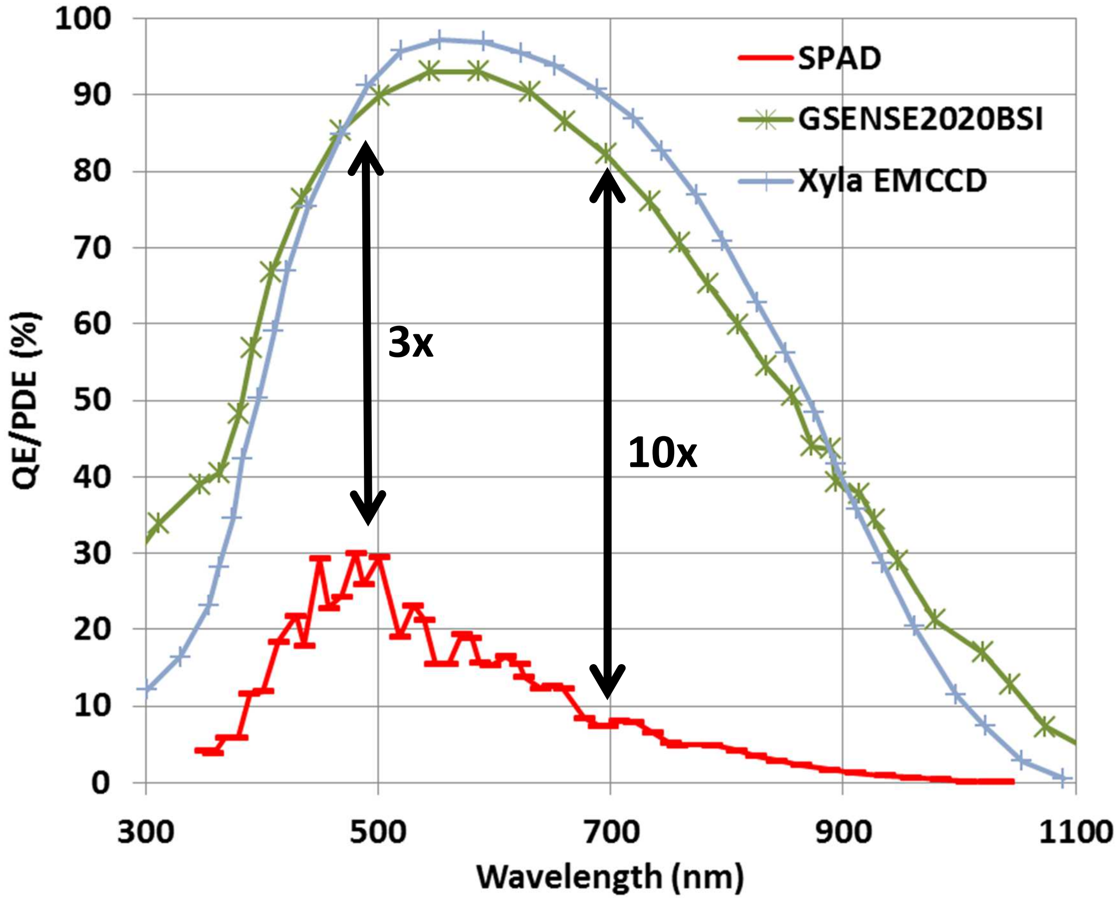
sCMOS

(3×33ms exp. in both cases)

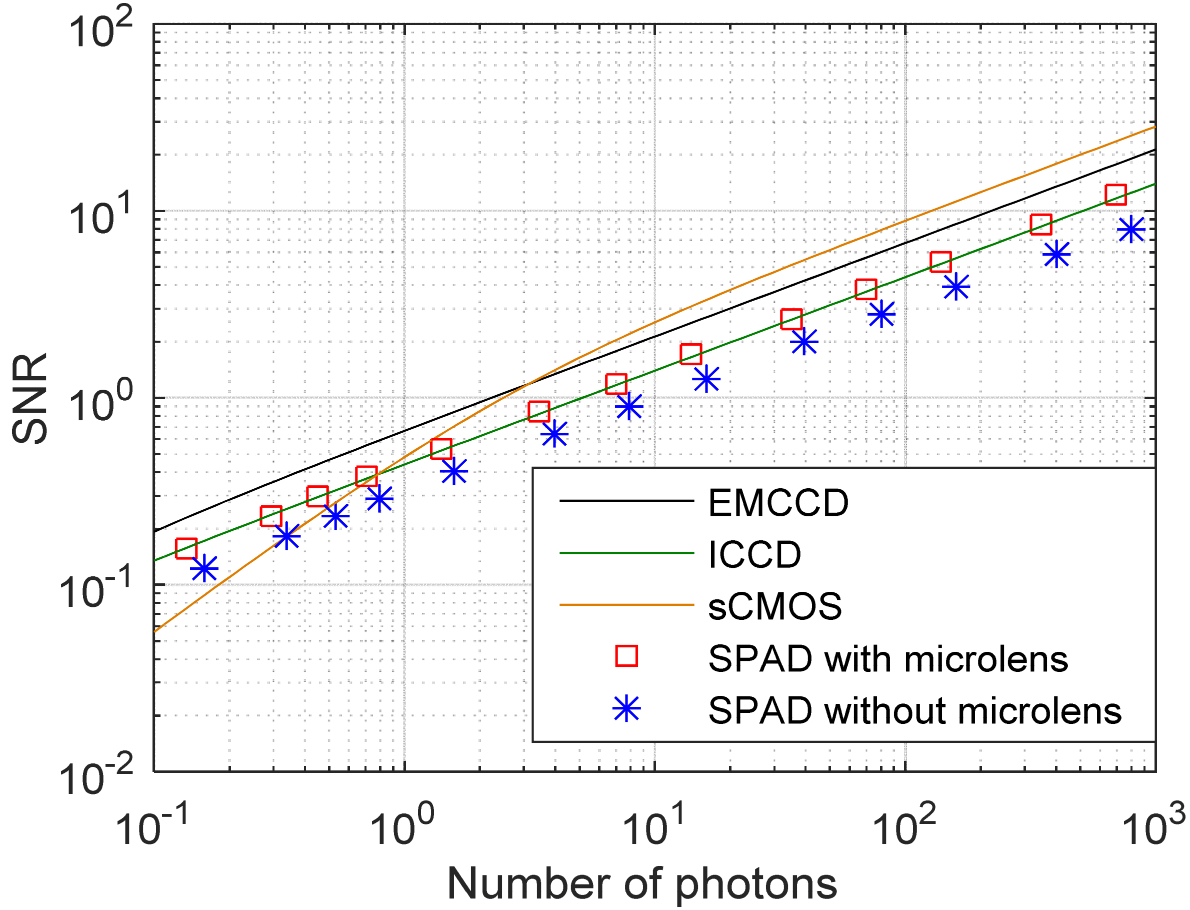
Sensitivity Comparison



PDE/QE Comparison

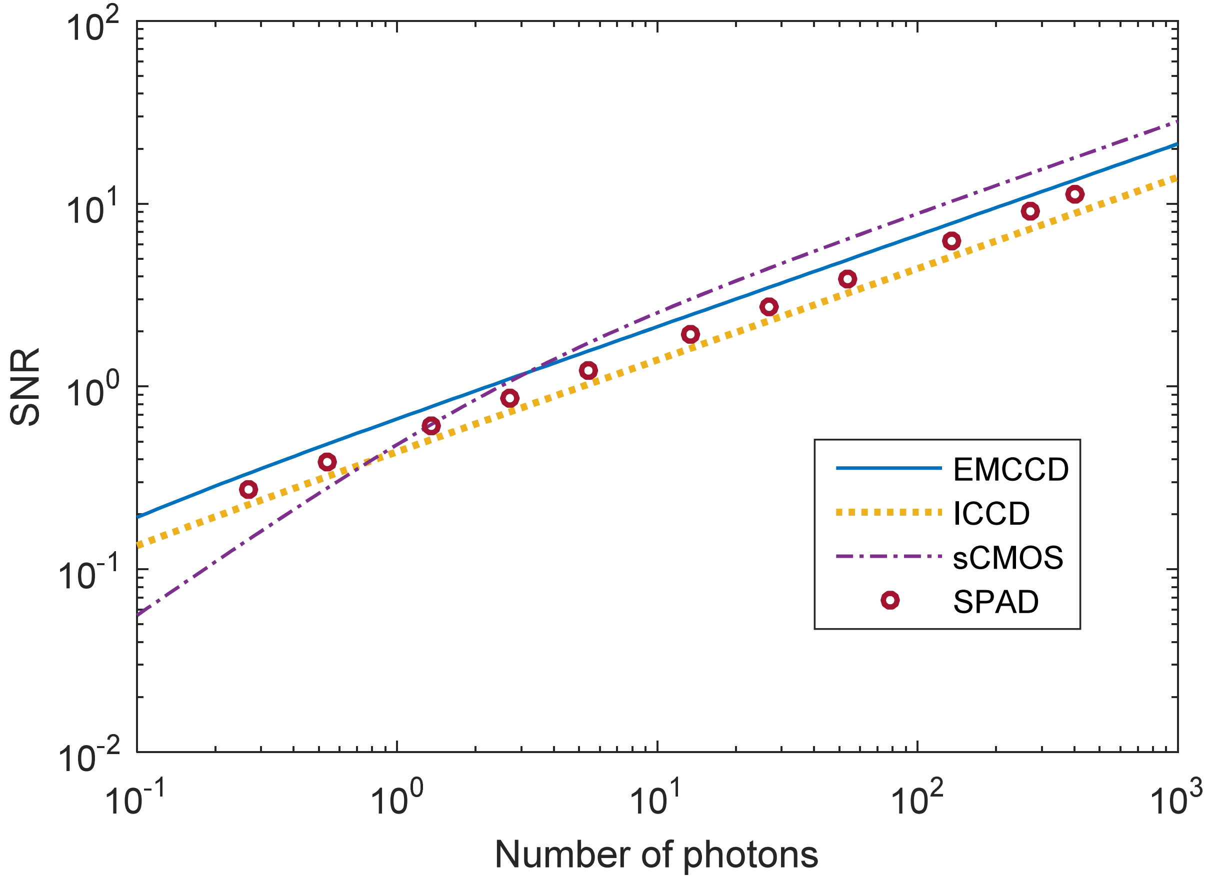


SPCimager SNR Comparison



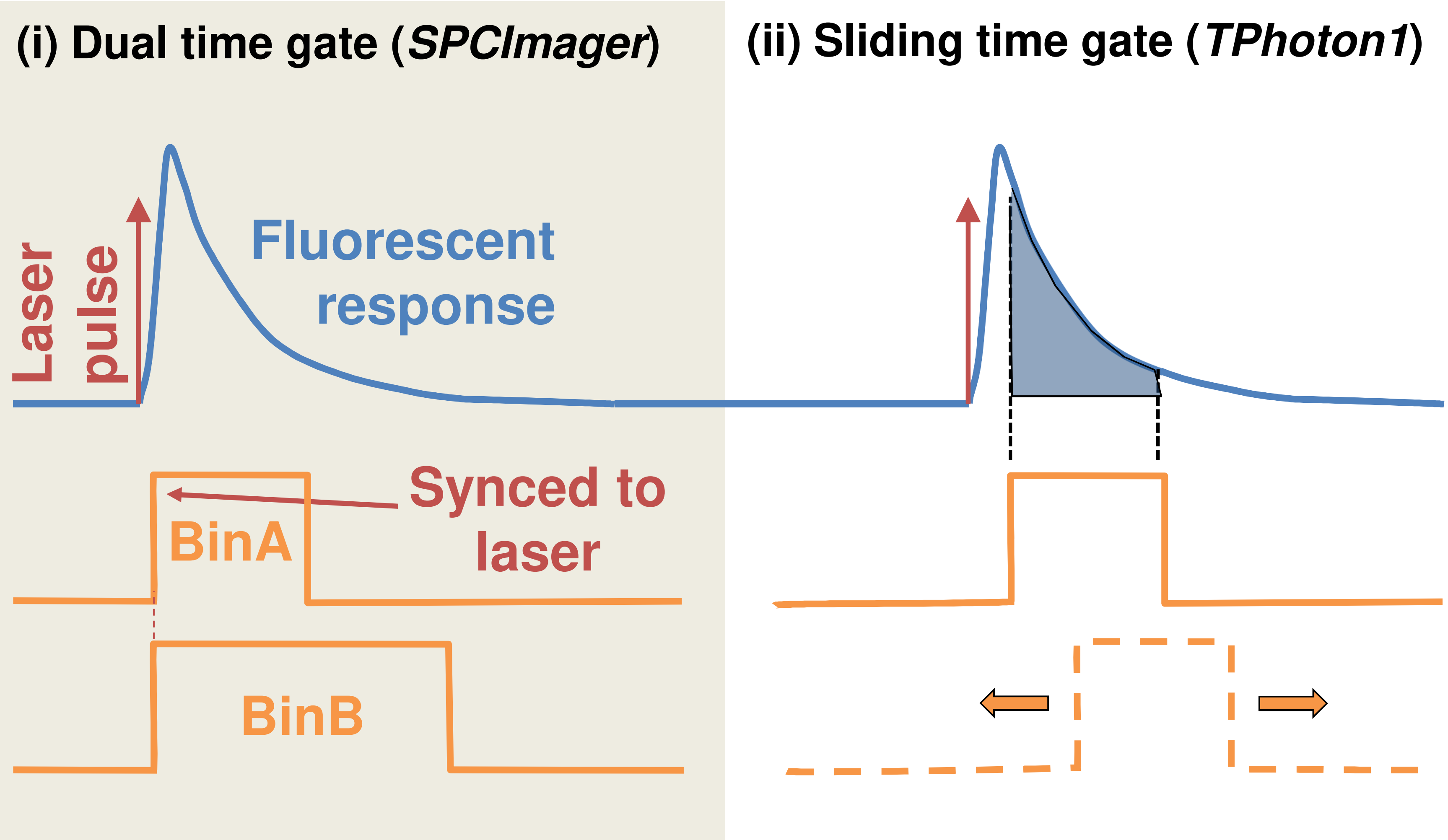
- Microlensed SPCimager

Tphoton1 SNR Comparison



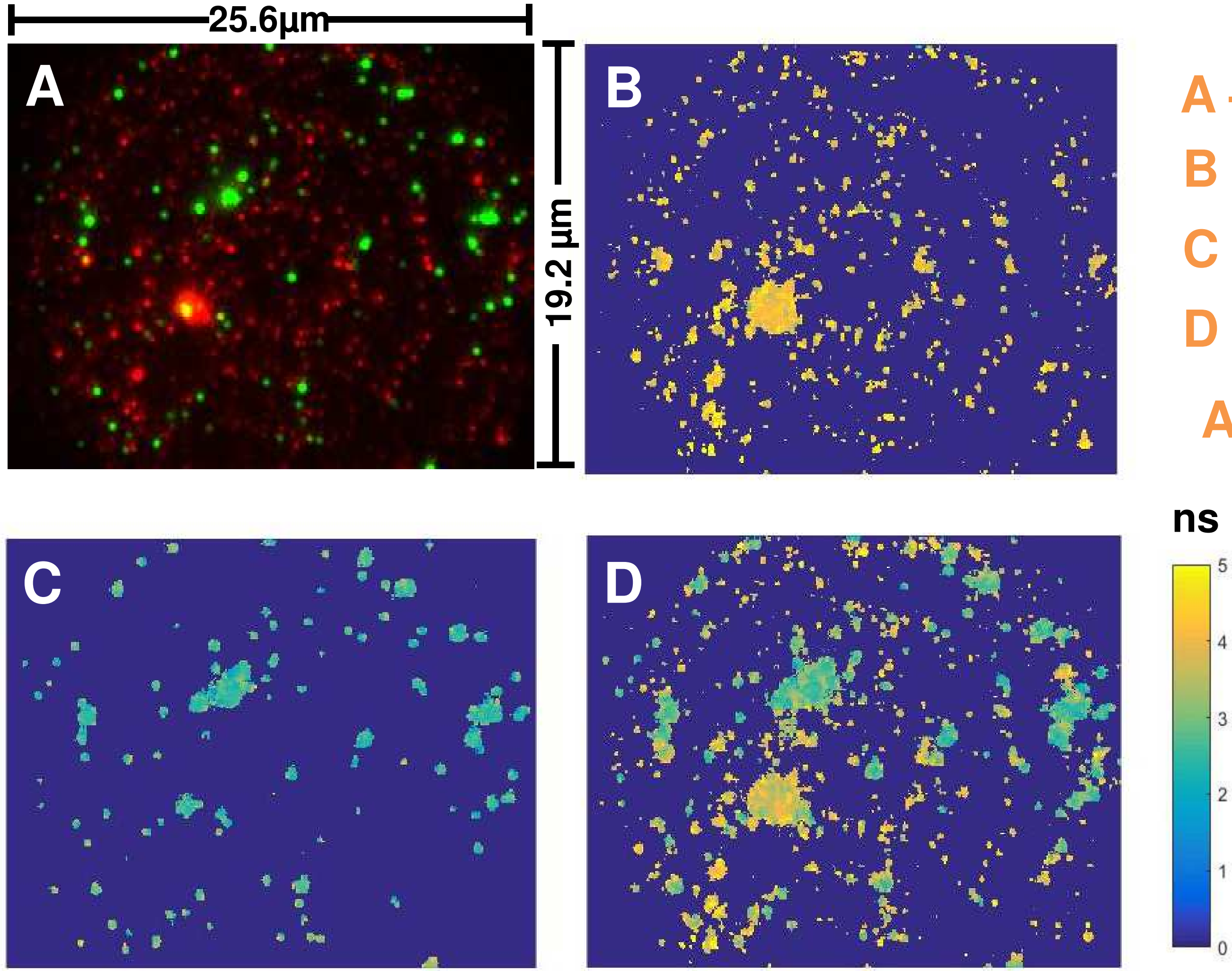
Tphoton1 SNR comparison

Time-gated lifetime estimation



Dual time gate example: R&G beads

Excitation: 405nm pulsed laser @ 5MHz

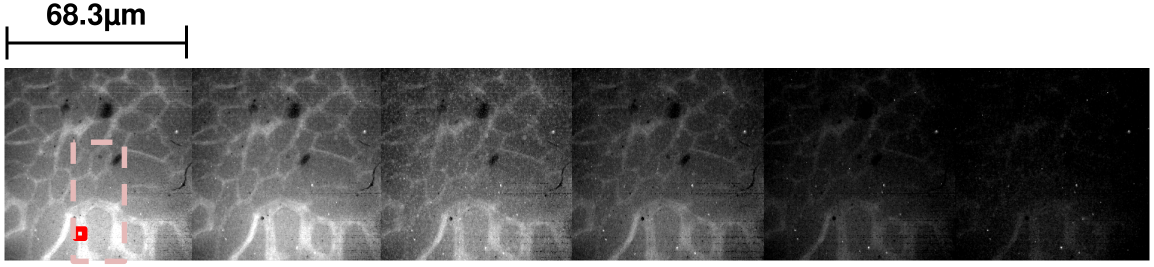


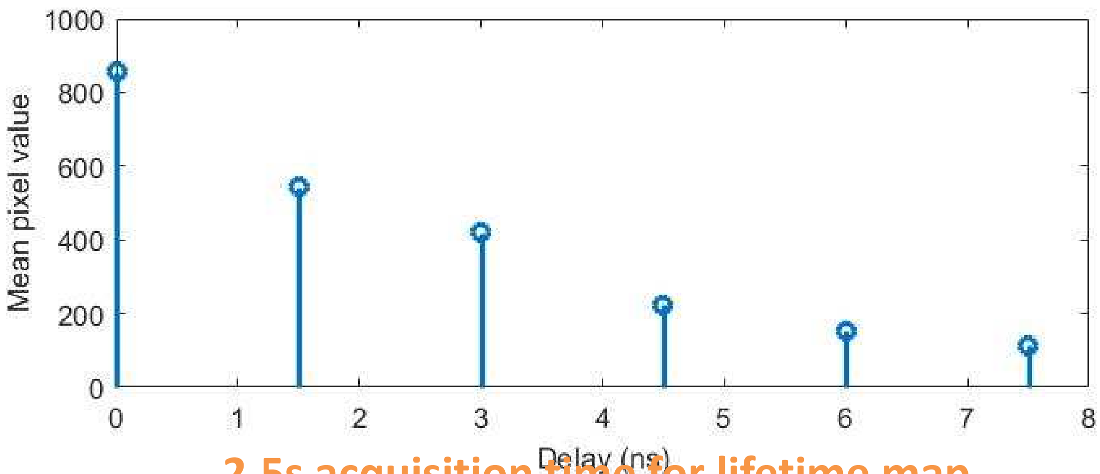
A – Intensity (R+G)
B – Lifetime (R)
C – Lifetime (G)
D – Lifetime

Acquisition time: 2s

Time gate sweep example: Convallaria

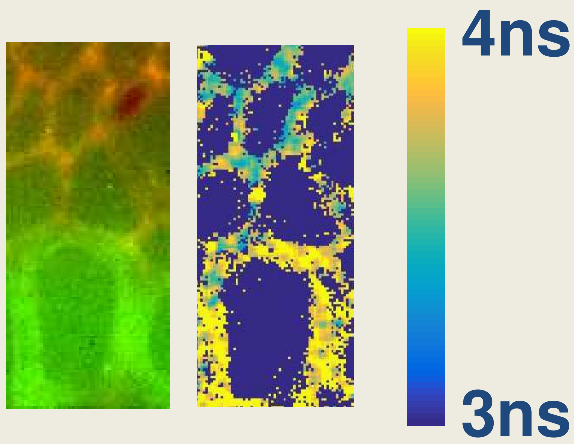
SPAD image frame at different delay settings
(15ns time gate, 250ms acquisition time per image)



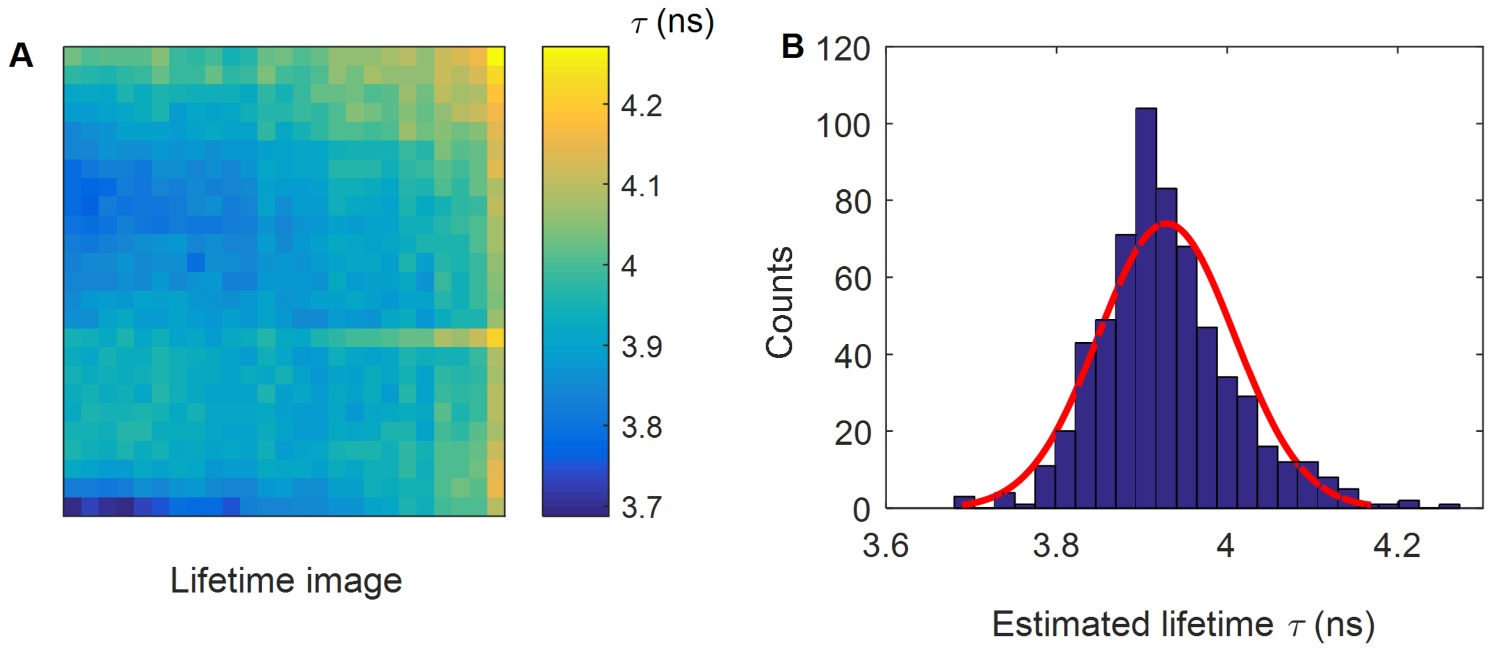


2.5s acquisition time for lifetime map
(when based on 10 image frames)

Intensity (R+G)
vs. lifetime



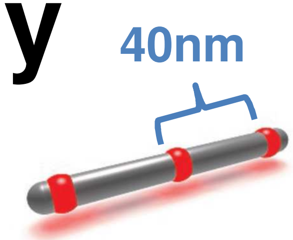
Lifetime Uniformity

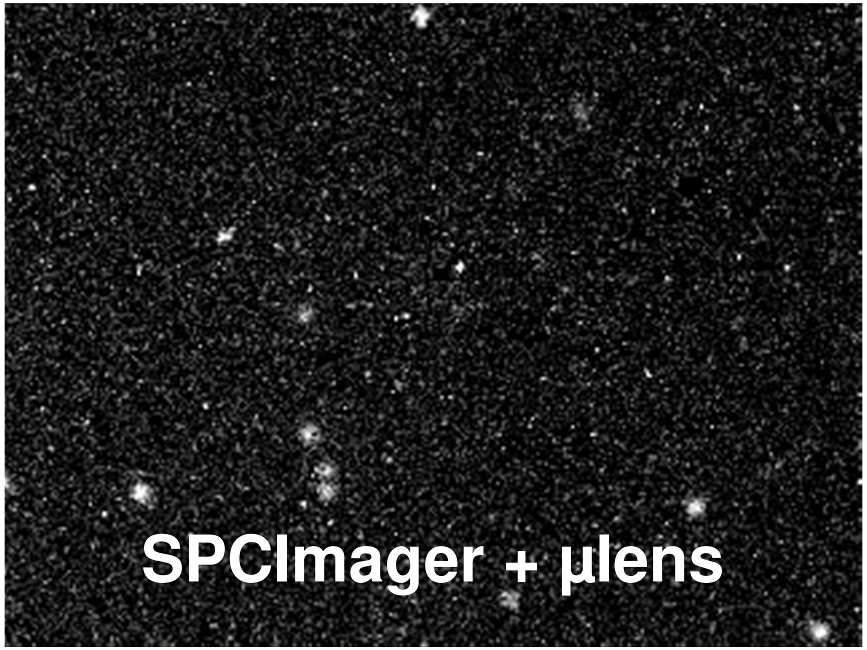


Fluorescein sample: lifetime obtained by moving a 15ns time gate in 1ns steps and composing 10 image frames, from 10000, 10 μs bit-plane exposures each, using 10×10 pixel binning

Single Molecule Localisation Microscopy

GATTA PAINT 40G (ATTO542) nanoruler



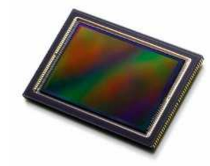


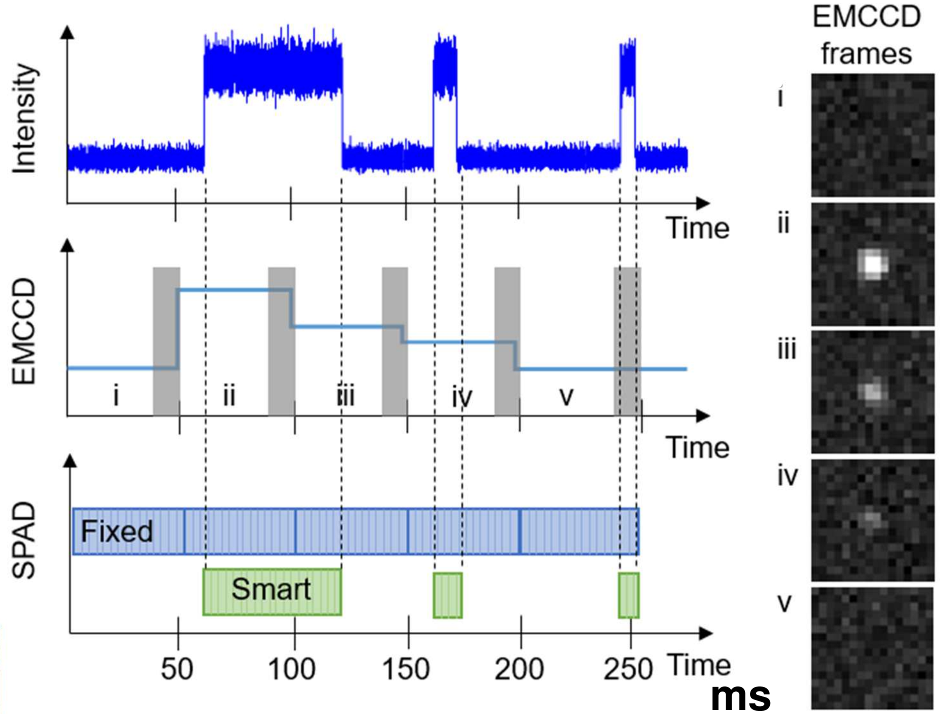
625 bit-planes (62.5ms exposure per image frame)

Single Molecule Localisation Microscopy

The camera of choice is typically an EMCCD: **high quantum efficiency** (QE×FF≈90%), and effectively **no read or dark noise**.

SPAD: lower inherent QE (QE×FF<25%), but **higher frame rates**, **continuous capture,** no read noise and **no excess noise factor**.

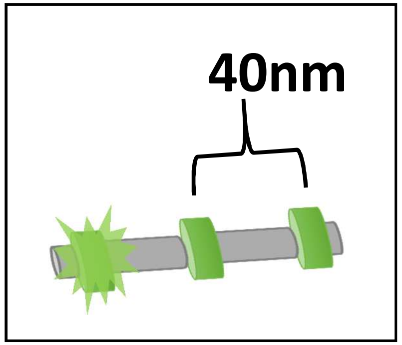




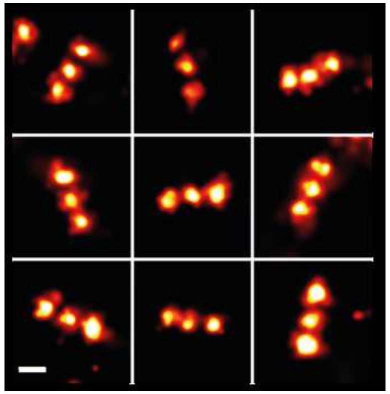
High SPAD frame rate can be exploited for background suppression (“smart aggregation”)

I. Gyongy et al., *Scientific Reports* 2016

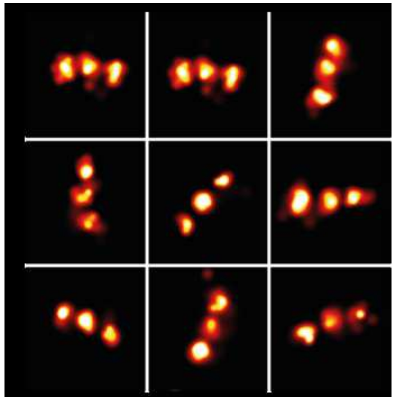
SMLM (dSTORM) example: nanoruler



GATTA-PAINT HiRes 40G nanoruler



EMCCD (FWHM* ≈ 32nm)



SPCImager (FWHM* ≈ 28nm)

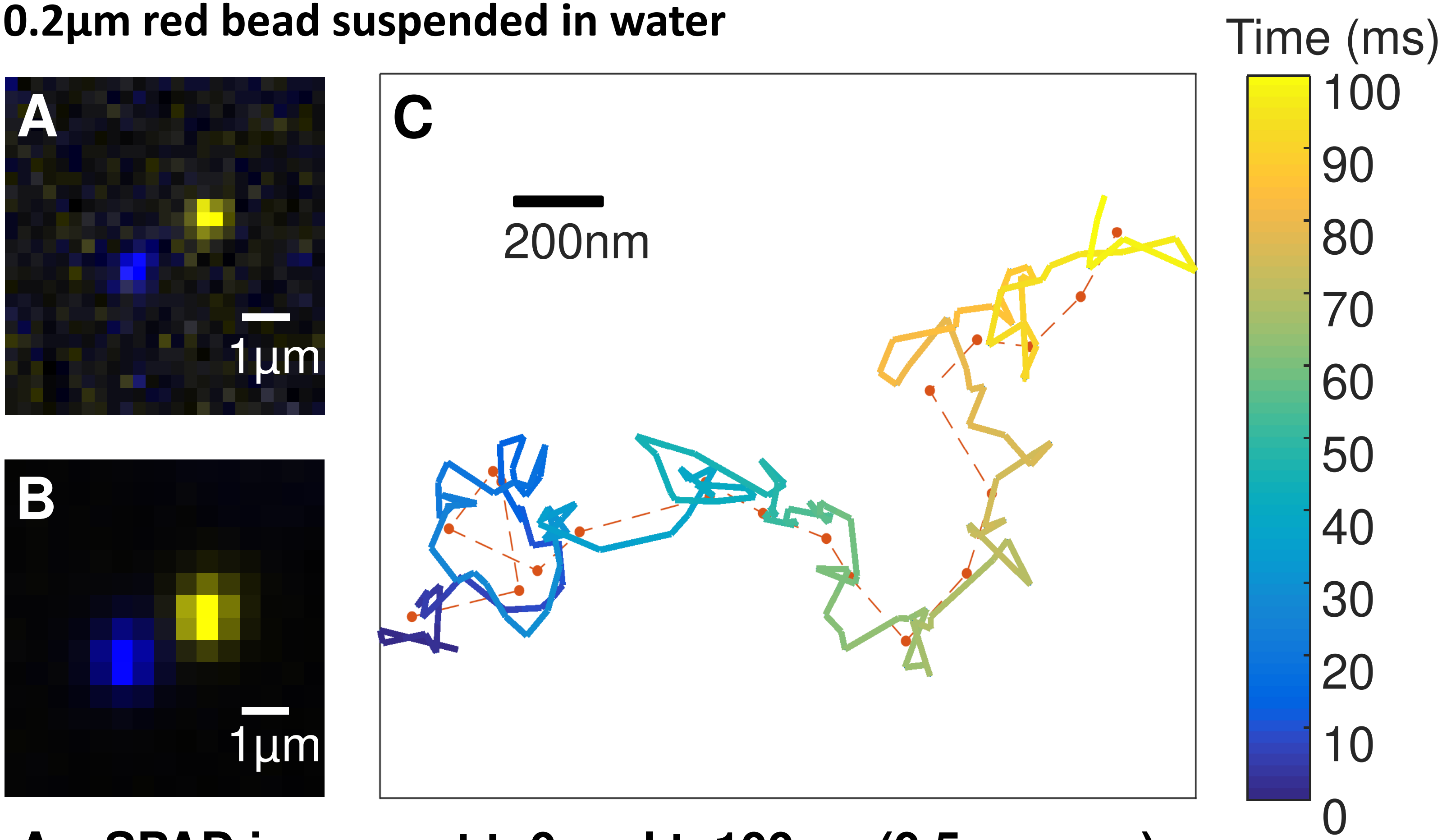
*Mean FWHM of individual markers in super-resolution image

I. Gyongy et al., *Optics Express* 2018

Comparable localisation performance to EMCCD

Particle tracking

0.2μm red bead suspended in water



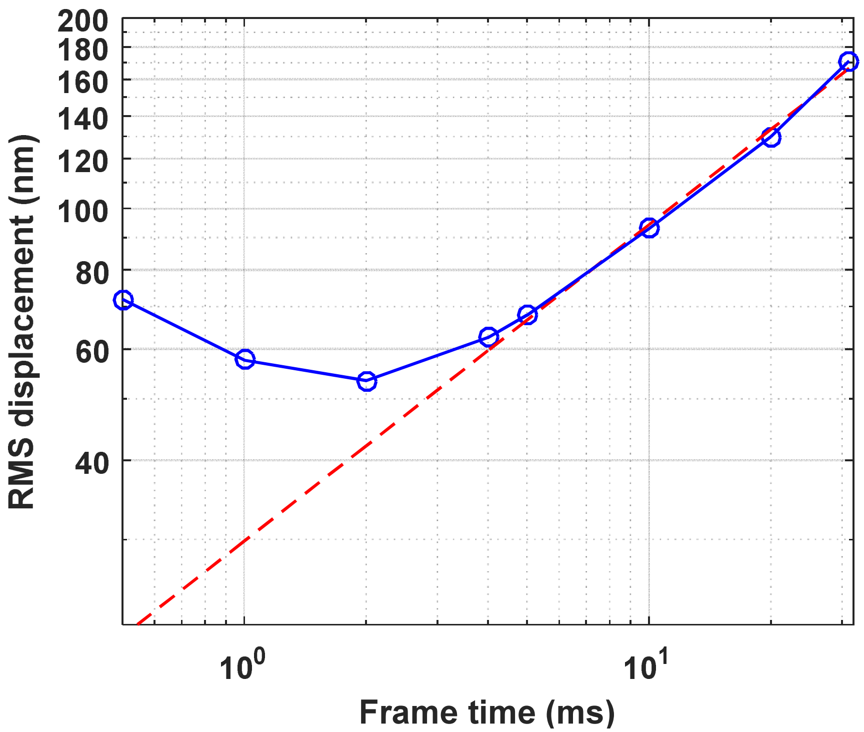
A – SPAD images at t=0 and t=100ms (0.5ms exp.)

B – sCMOS images at t=0 and t=100ms (5ms exp.)

C – Bead trajectory as captured using SPAD (full line, 2kFPS) and sCMOS (dashed line, 200FPS)

Brownian motion – experimental results

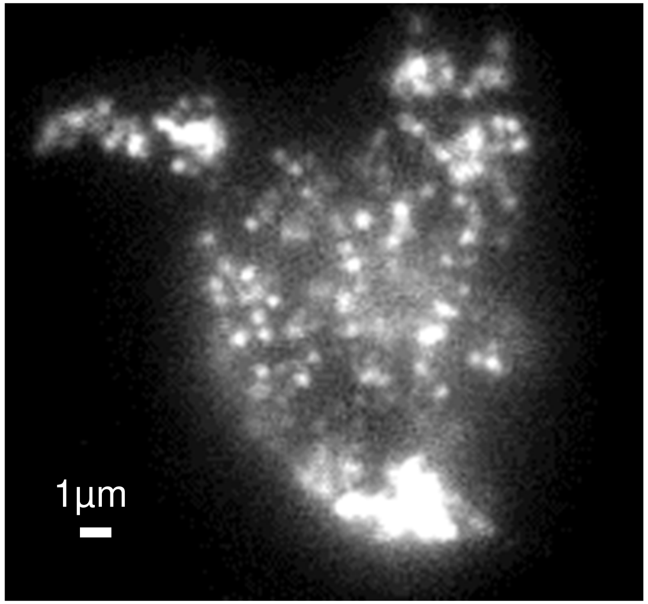
0.5µm red beads in a 50:50 mixture of glycerol and water



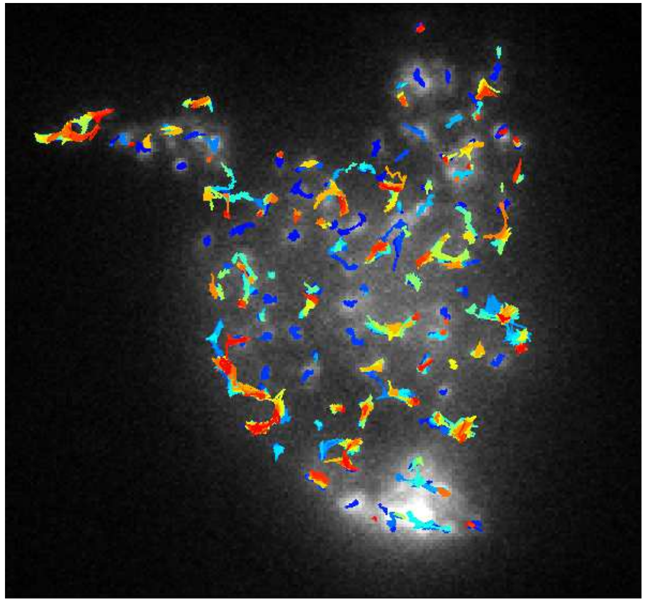
Set frame rate post capture to optimal value

Live cell vesicle tracking

Secretory (Pheochromocytoma) cell with vescicles labeled using soluble cargo Neuropeptide Y (NPY) fused to mCherry



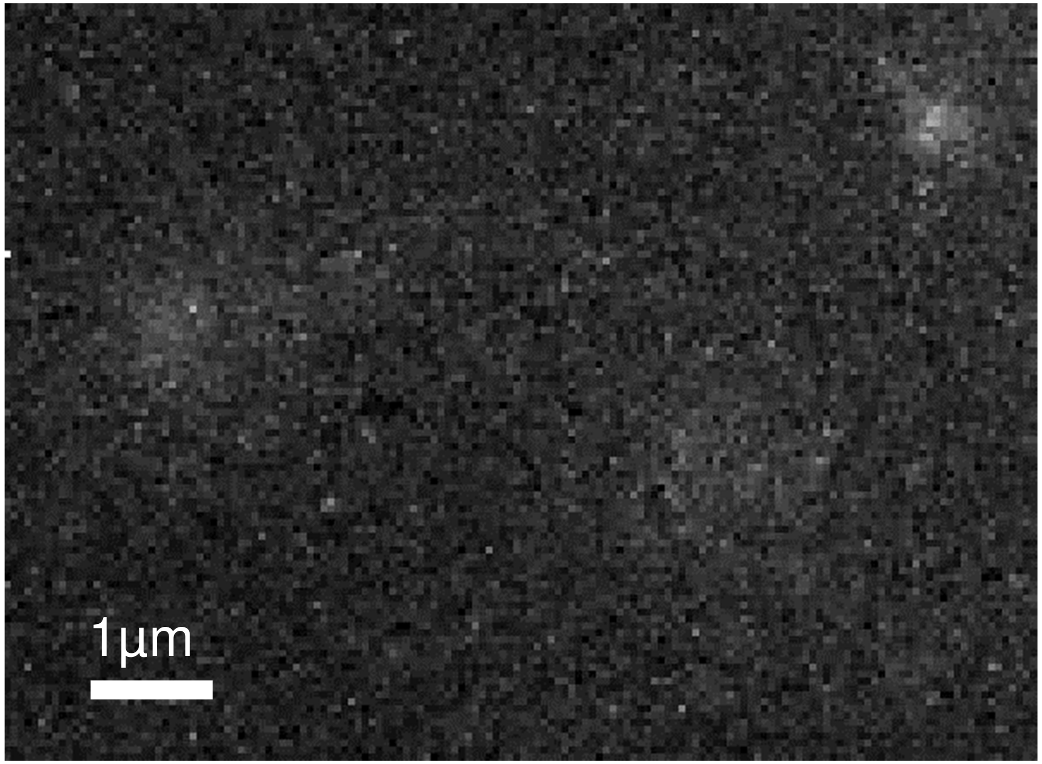
EMCCD – 55ms frame time (time lapse)



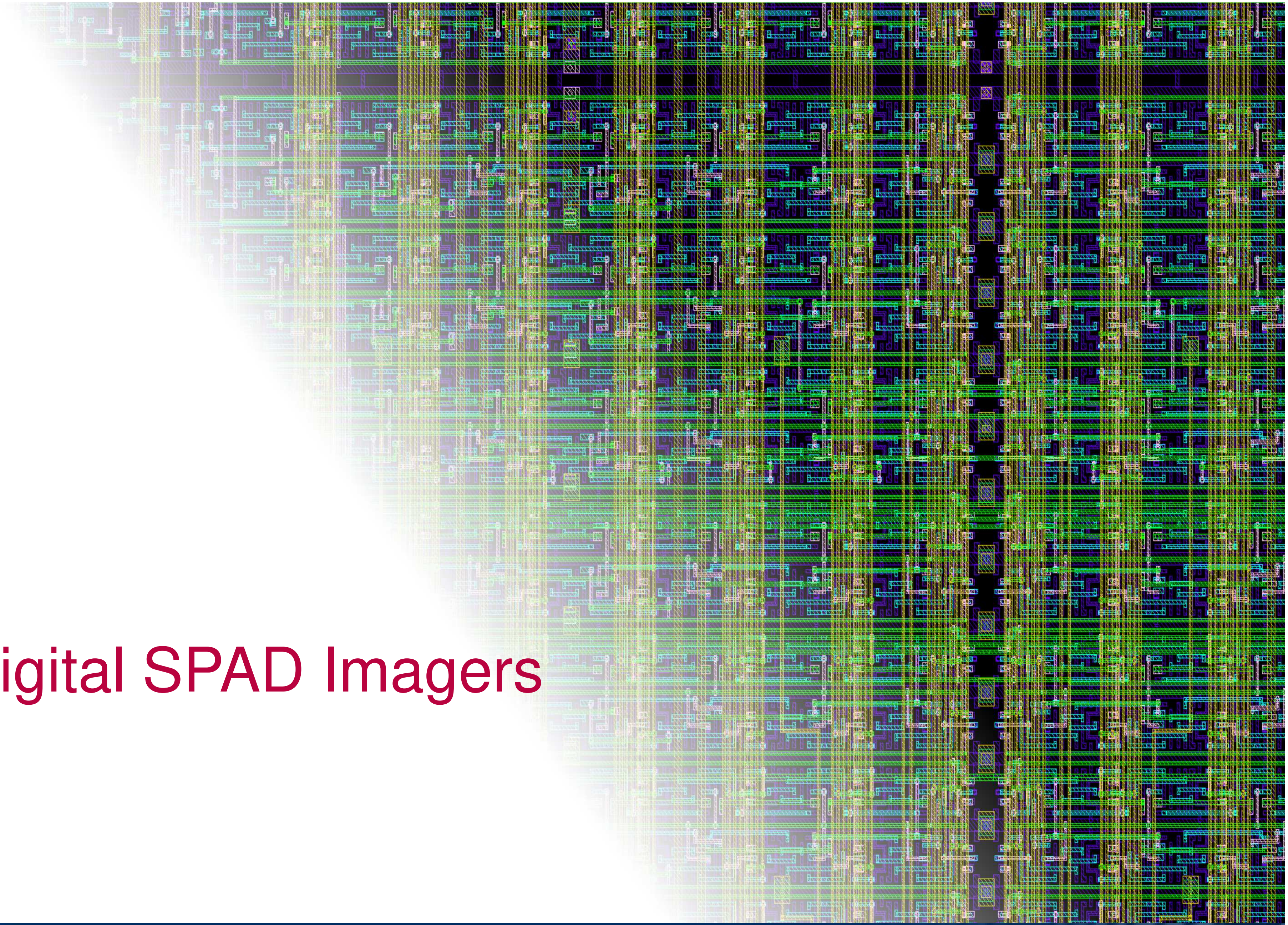
Extracted tracks

Live cell vesicle tracking

Secretory (Pheochromocytoma) cell: close-up



SPAD – 55ms (150×110 pixel ROI is shown)



Digital SPAD Imagers

TCSPC Histogramming In Pixel

A 16.5 Giga Events/s 1024 × 8 SPAD Line Sensor with per-pixel Zoomable 50ps-6.4ns/bin Histogramming TDC

Ahmet T. Erdogan[1], Richard Walker[1], Neil Finlayson[1], Nikola Krstajić[1,2], Gareth O.S. Williams[2], Robert K. Henderson[1]

1 – CMOS Sensors and Systems Group, University of Edinburgh, UK

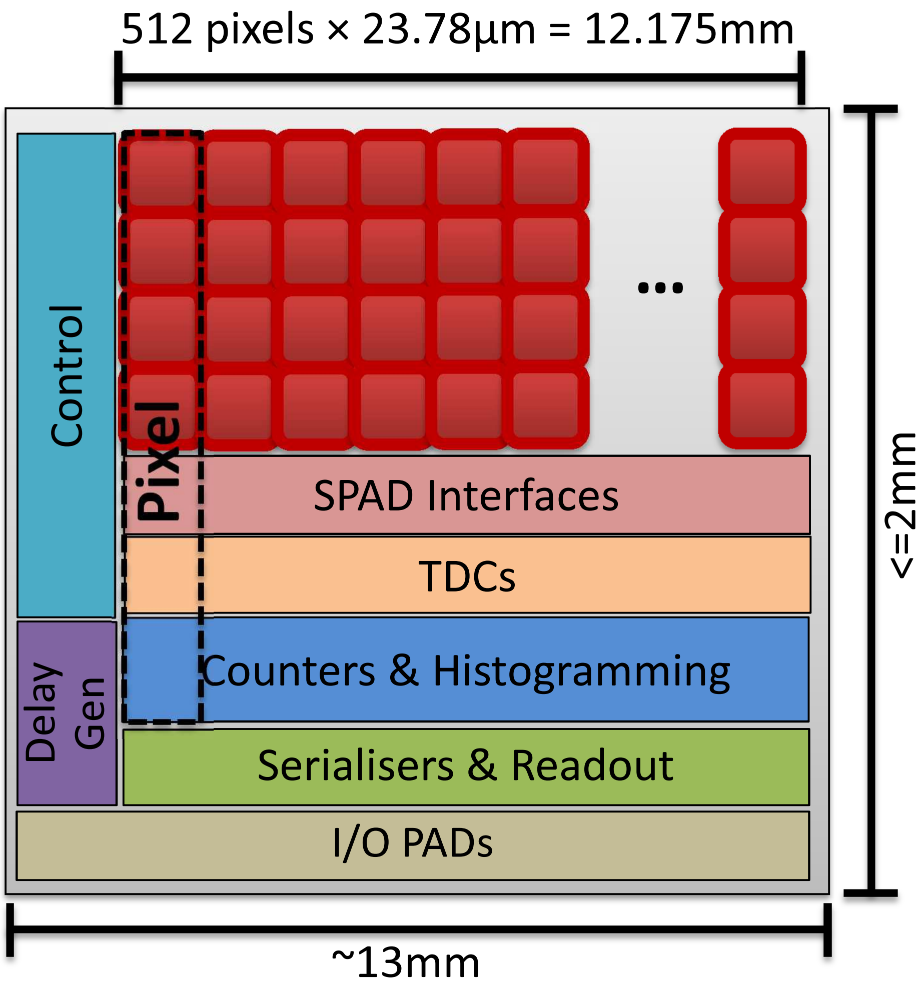
2 – EPSRC IRC Hub in Optical Molecular Sensing & Imaging, Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, UK

Abstract

A 1024 × 8 single photon avalanche diode (SPAD) based line sensor for time resolved spectroscopy is implemented in 0.13 μm imaging CMOS with 23.78 μm pixel pitch at 49.31% fill factor. The line sensor can operate in single photon counting (SPC) mode (65 giga-events/s), time-correlated single photon counting (TCSPC) mode (194 million events/s) or histogramming mode (16.5 giga-events/s), increasing the count rate up to 85 times compared to TCSPC operation. This performance is enabled by a 512 channel histogramming TDC with 50ps-6.4ns/bin zoomable time resolution. **Keywords:** CMOS, SPAD, TCSPC, Histogramming, Time-resolved spectroscopy.

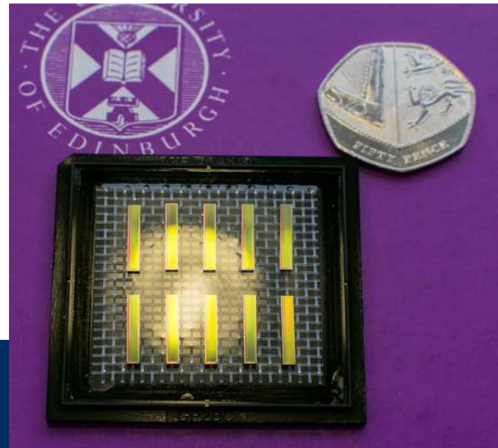
also be configured from 1 to 128 time-events per bin under the control of the Histogram decoder (Fig. 5). Together with a 50 ps resolution on-chip delay generator, this feature allows positioning and zooming of the histogram window to the spectral peak. In SPC mode, the first 4 histogram bins are used in chain mode creating two independent time gated 20-bit counters (SPCA and SPCB) allowing rapid fluorescence lifetime estimation. Simultaneous readout and detection is supported to achieve 100% temporal aperture ratio. Optical throughputs of 65 giga-events/s in SPC mode, 194 million events/s in TCSPC mode and 16.5 giga-events/s in histogramming mode are achieved. Histogramming mode increases the count rate up to 85 times compared to TCSPC

Rall Line Sensor

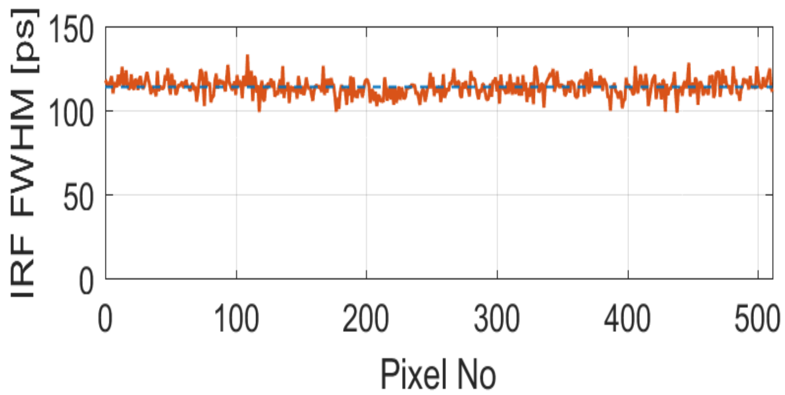


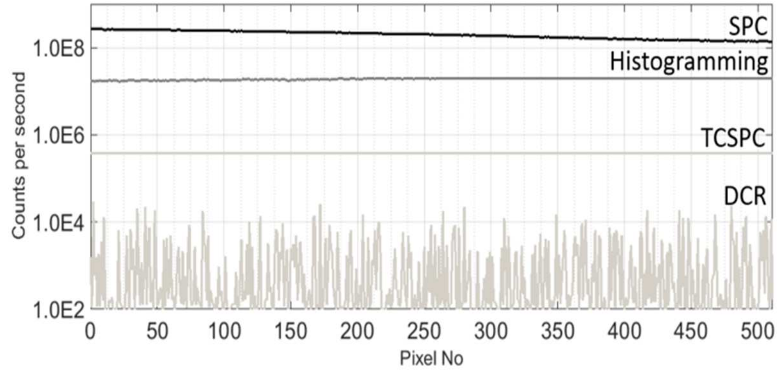
Key:
Detector (SPAD)

- 512 pixel 'line' sensor.
- 49.3% fill-factor
- Two SPAD variants:
  - Blue SPAD (450nm-550nm)
  - Red SPAD (600nm-900nm)
- Zooming TDC per pixel (50ps - 6.4ns/bin)
- On-chip, 32 bin histogram generation.
- 1Mlines/s readout rate



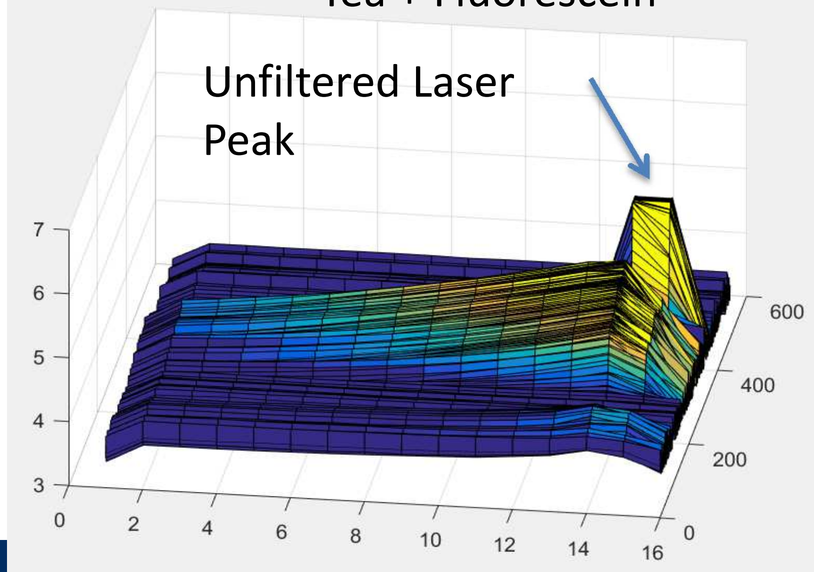
Ra-II Characterisation



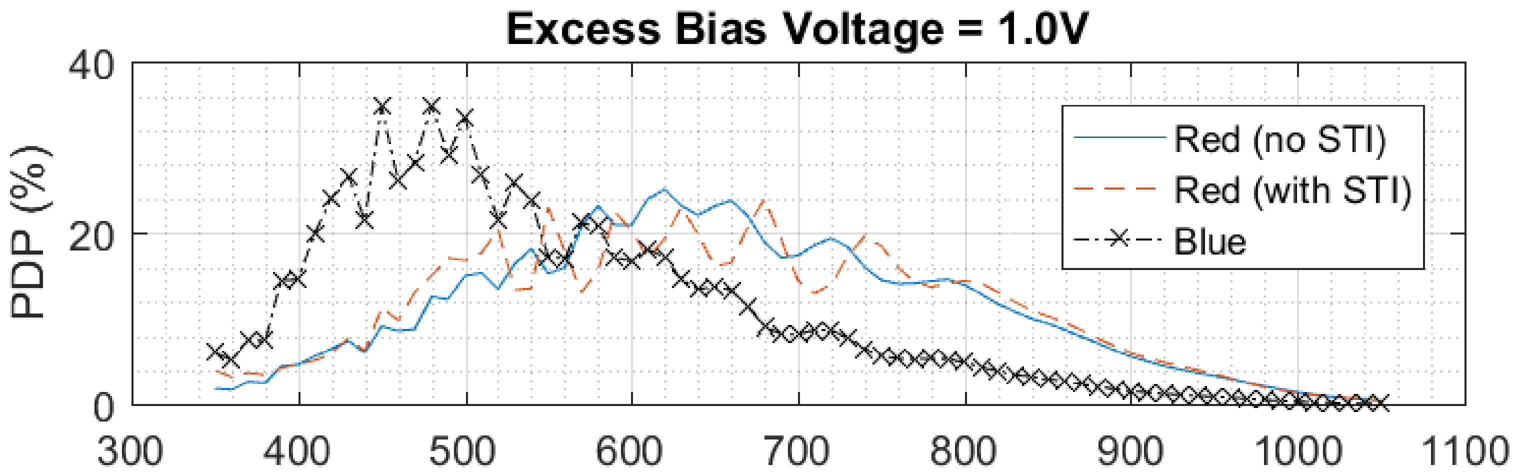


- Excellent time resolution (114ps) and uniformity
- Up to 100x dynamic range extension by on-chip histogramming.
- 300 klines/s readout

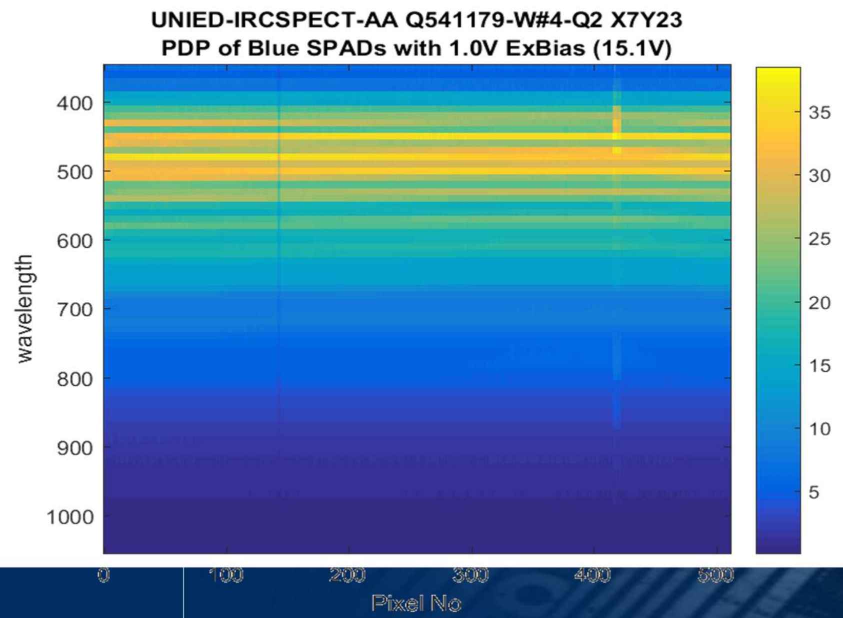
Tea + Fluorescein



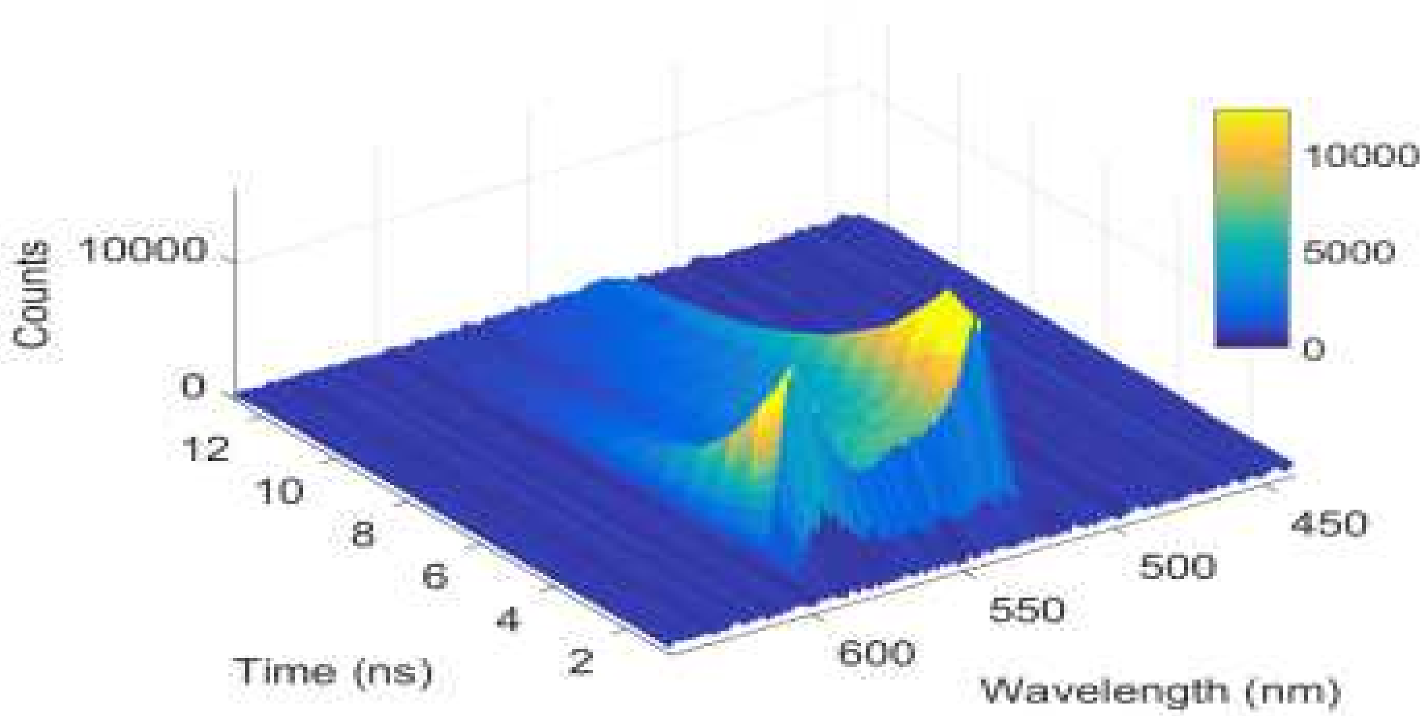
SPAD Spectral Response



- Two wavelength ranges (for Fluorescence and Raman)
- Some etaloning in CMOS FSI stack

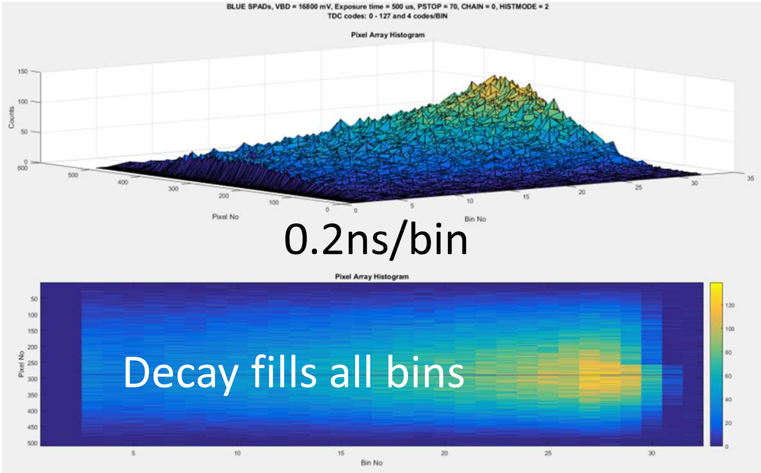


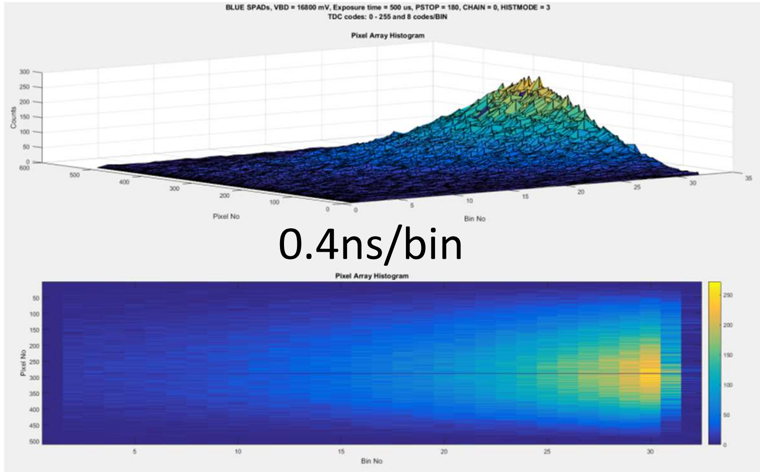
Spectroscopic Measurements

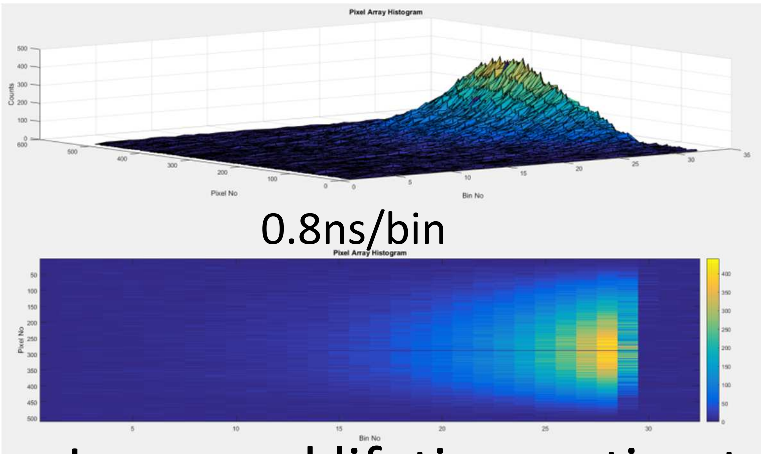


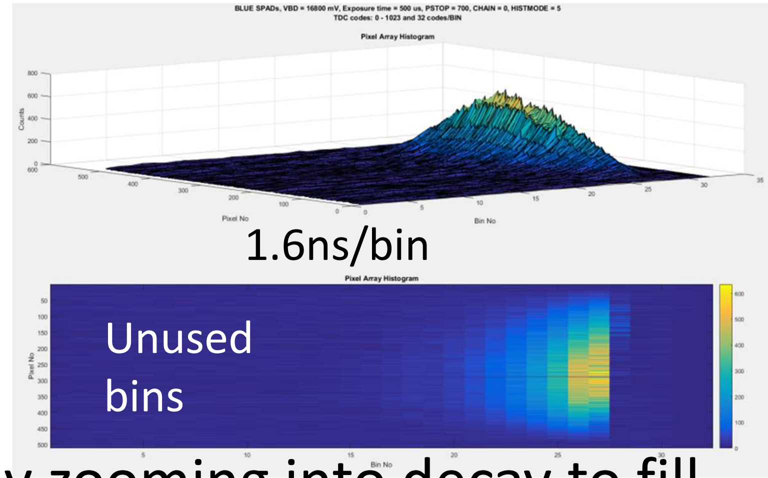
- Measurement of Ra-II with custom spectrograph
- Spectral FRET-FLIM at unprecedented rates (Fluorescein-Rhodamine)

Histogram Zooming

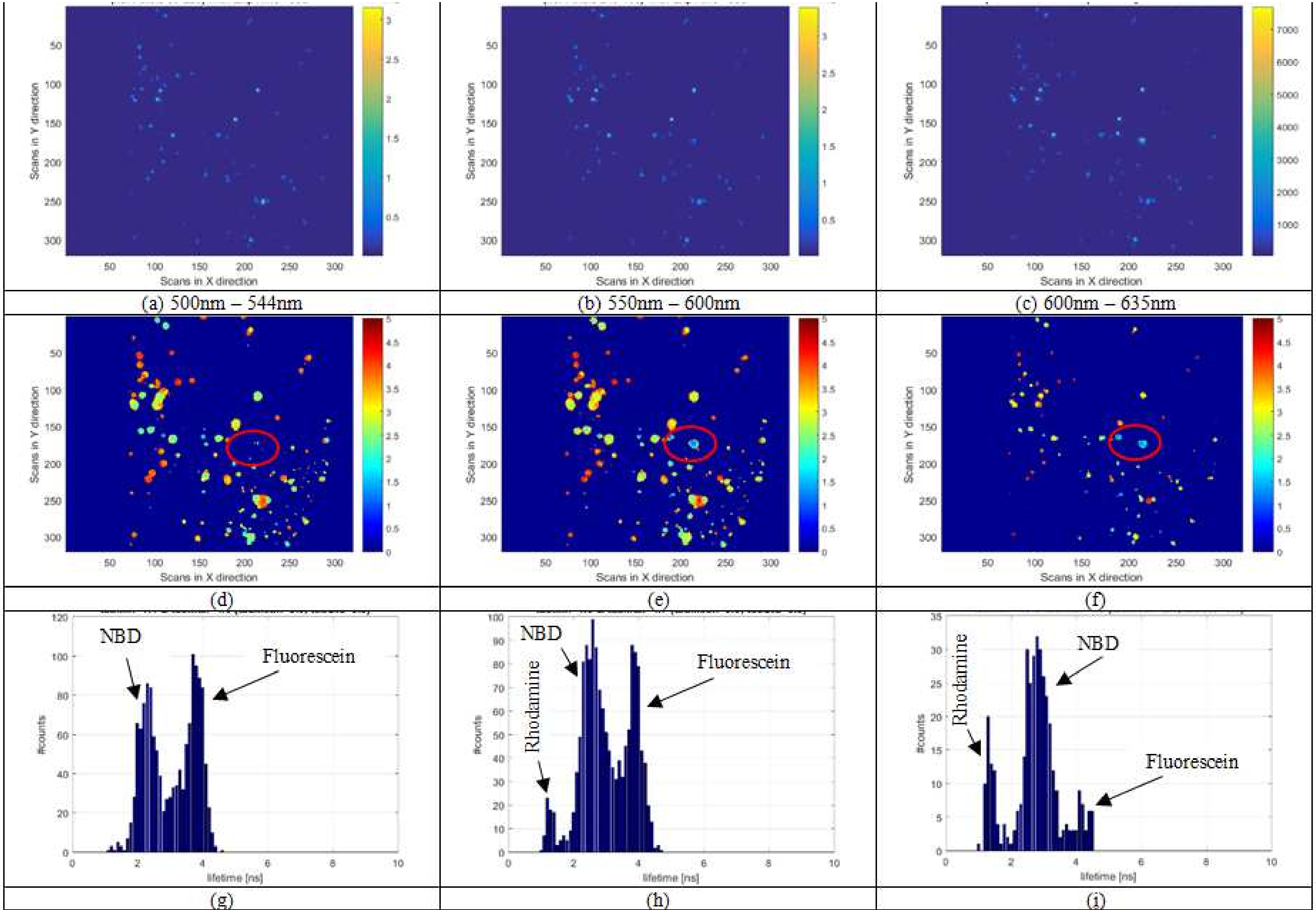








Improved lifetime estimates by zooming into decay to fill all 32 histogram bins.



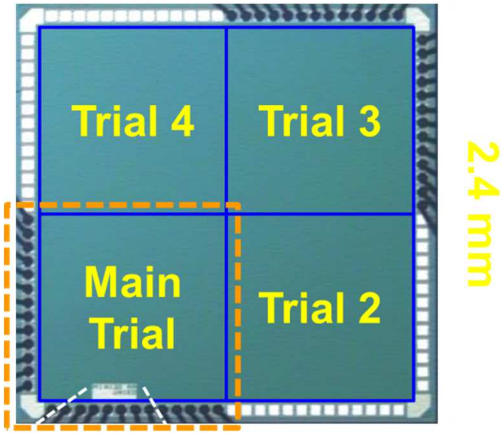
Scans in Y direction
Scans in X direction
(a) 500nm – 544nm
(b) 550nm – 600nm
(c) 600nm – 635nm
(d)
(e)
(f)
NBD
Fluorescein
Rhodamine
#counts
lifetime [ns]
(g)
(h)
(i)

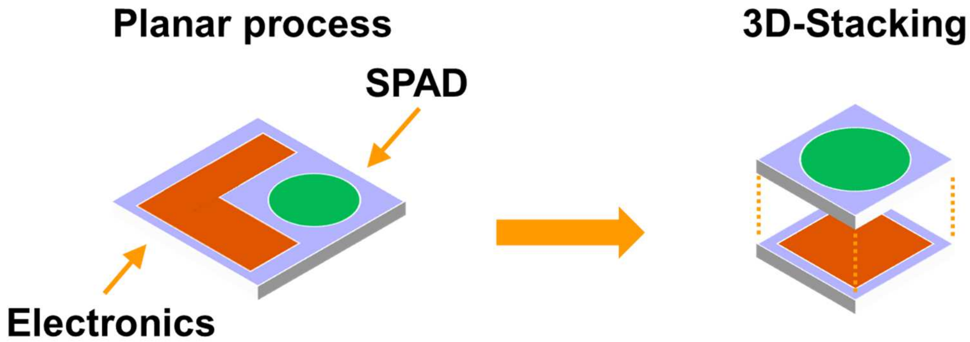
3D Stacked Digital SPAD Imagers

ENIAC POLIS Project

- 2016-2020 99MEuro project (No UK funding!)
- STMicroelectronics 3D stacked SPAD process pilot line
- 40nm/65nm stacked 3D process technology

Stacked 3D Image Sensor



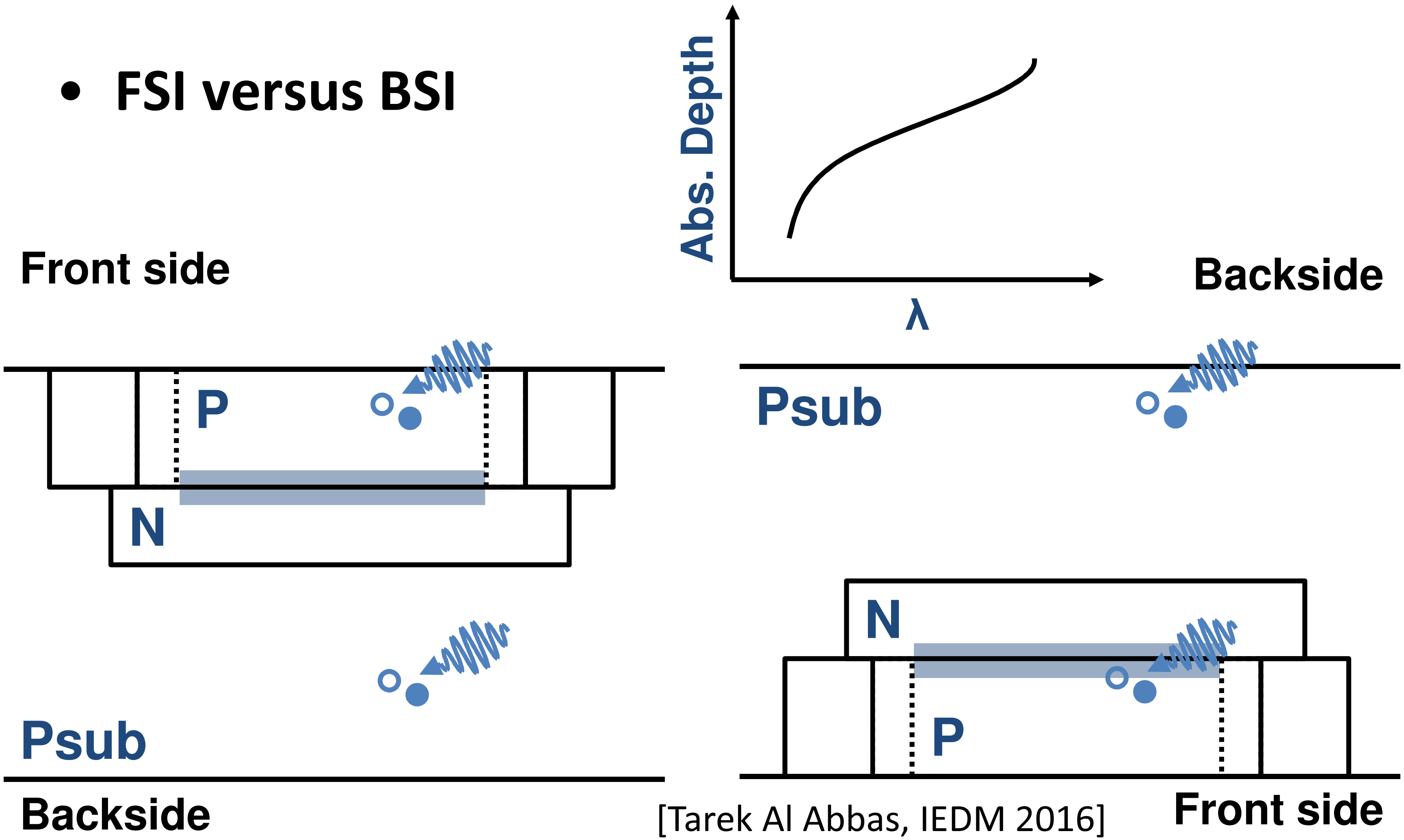


- First wafer-scale stacked SPAD image sensor
- 40nm CMOS (STMicro)
- 7.83μm pixel, 50% fill-factor, 12 bit gated pixel (4096 ke-)

[Tarek Al Abbas]

SPAD Structure

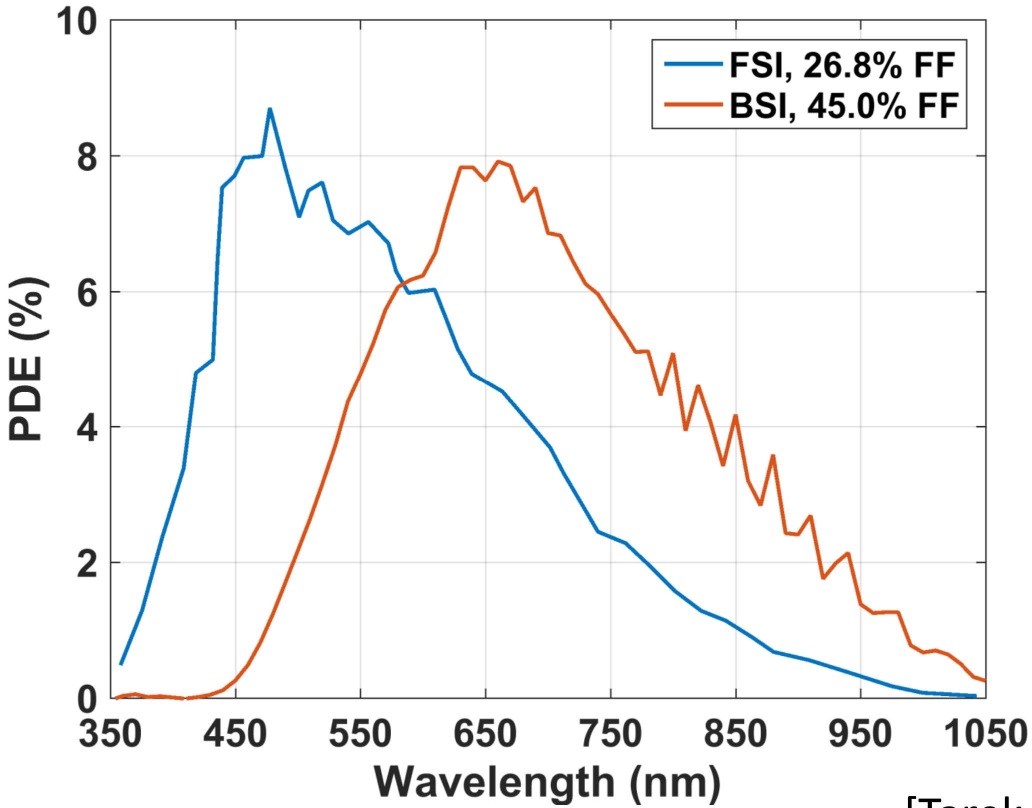
- **FSI versus BSI**



[Tarek Al Abbas, IEDM 2016]

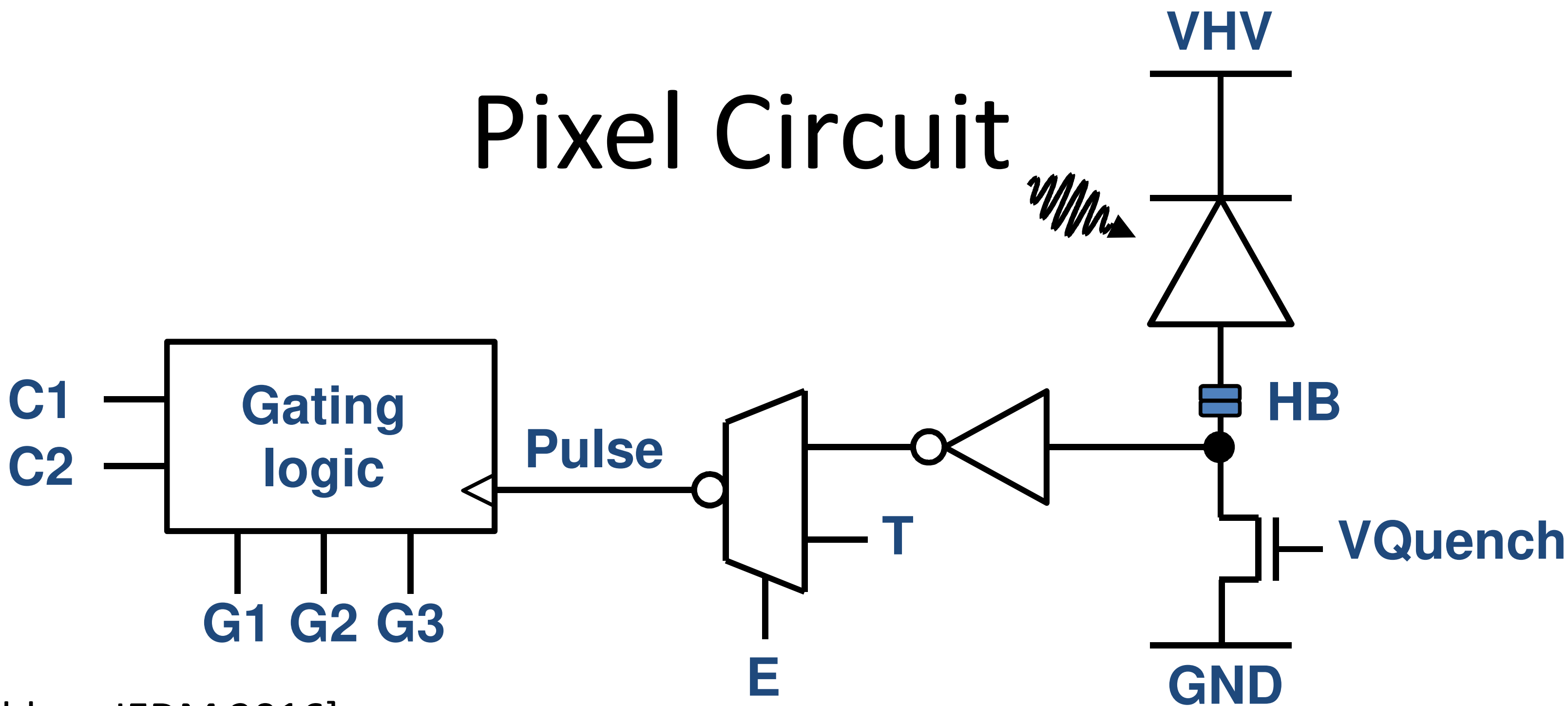
Photon Detection Efficiency

- **PDE = PDP × Fill Factor**

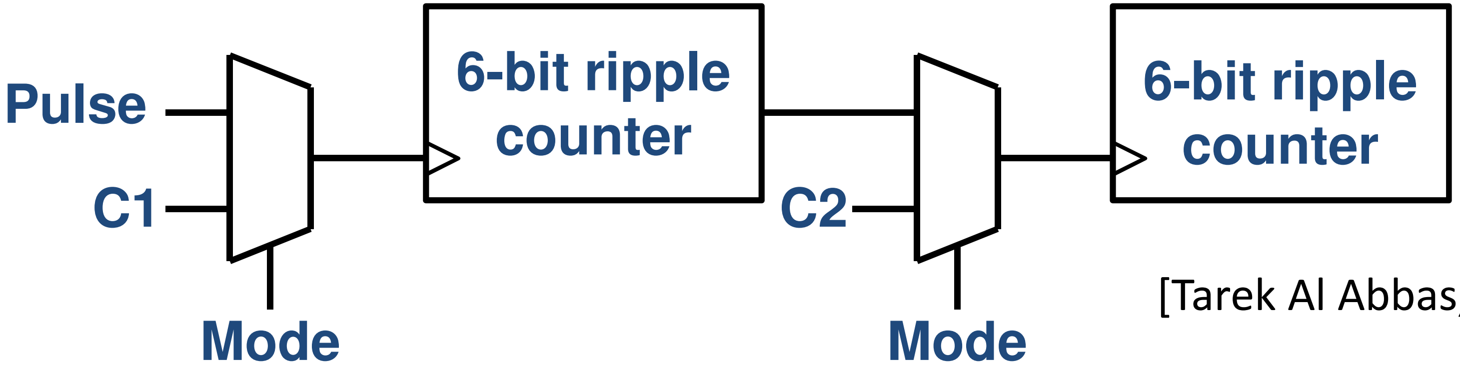


[Tarek Al Abbas, IEDM 2016]

Pixel Circuit



[Tarek Al Abbas, IEDM 2016]



[Tarek Al Abbas, IEDM 2016]

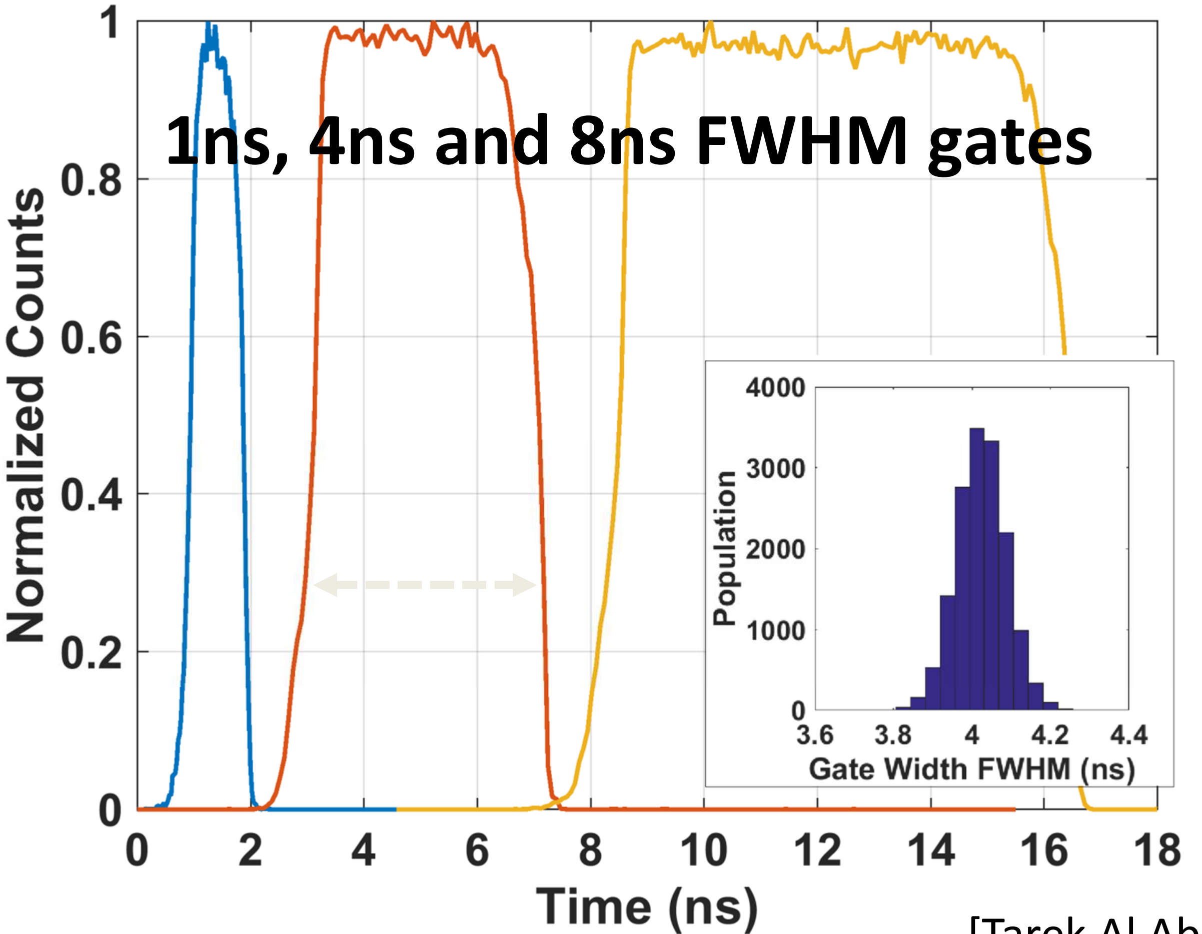
4096 Photon Full Well Capacity, 500fps

Photon Counting

Single shot grayscale intensity image (2V ExB)

[Tarek Al Abbas, IEDM 2016]

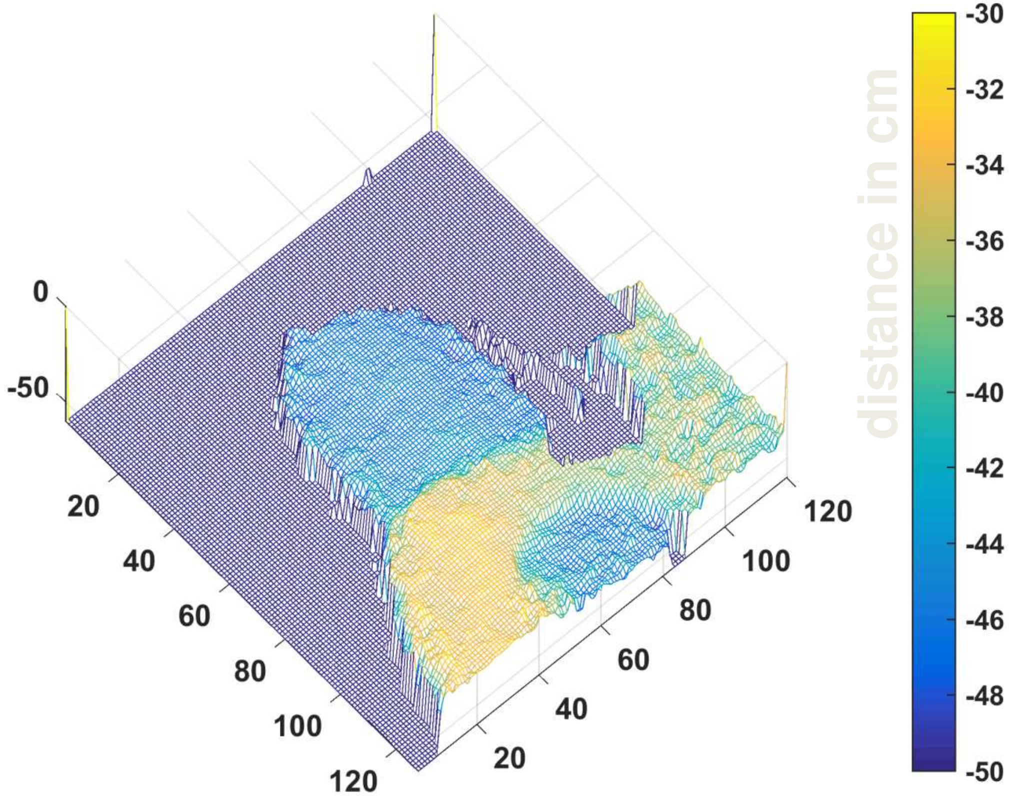
Time Gating

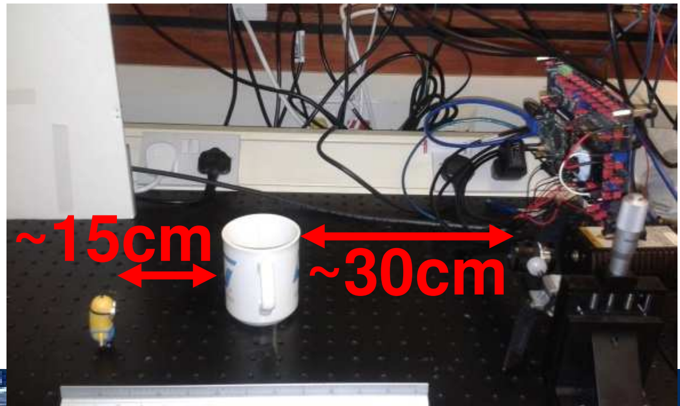


[Tarek Al Abbas, IEDM 2016]

ITOF Experiment

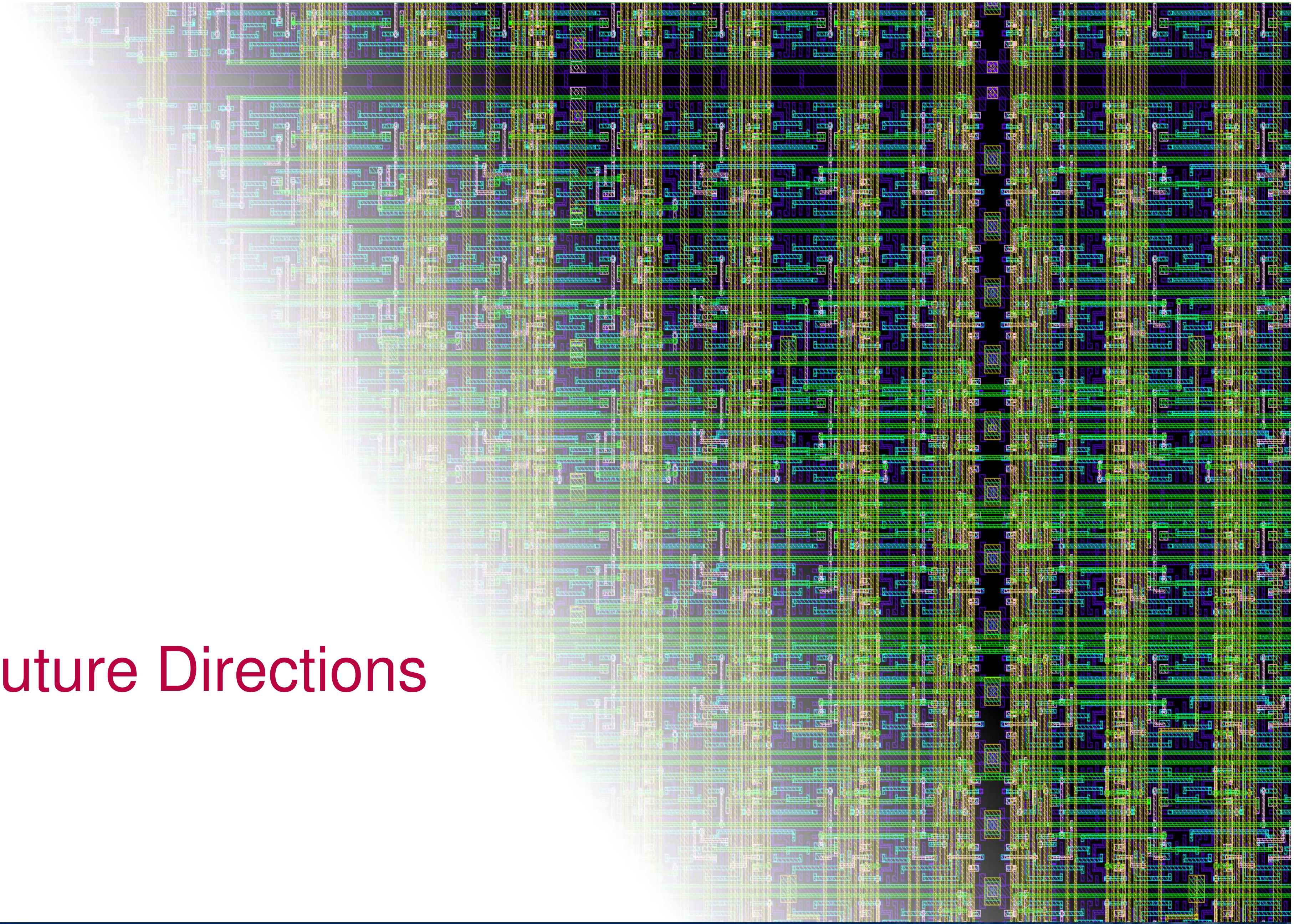
- **Indirect time of flight, 4ns pulse, 840nm**



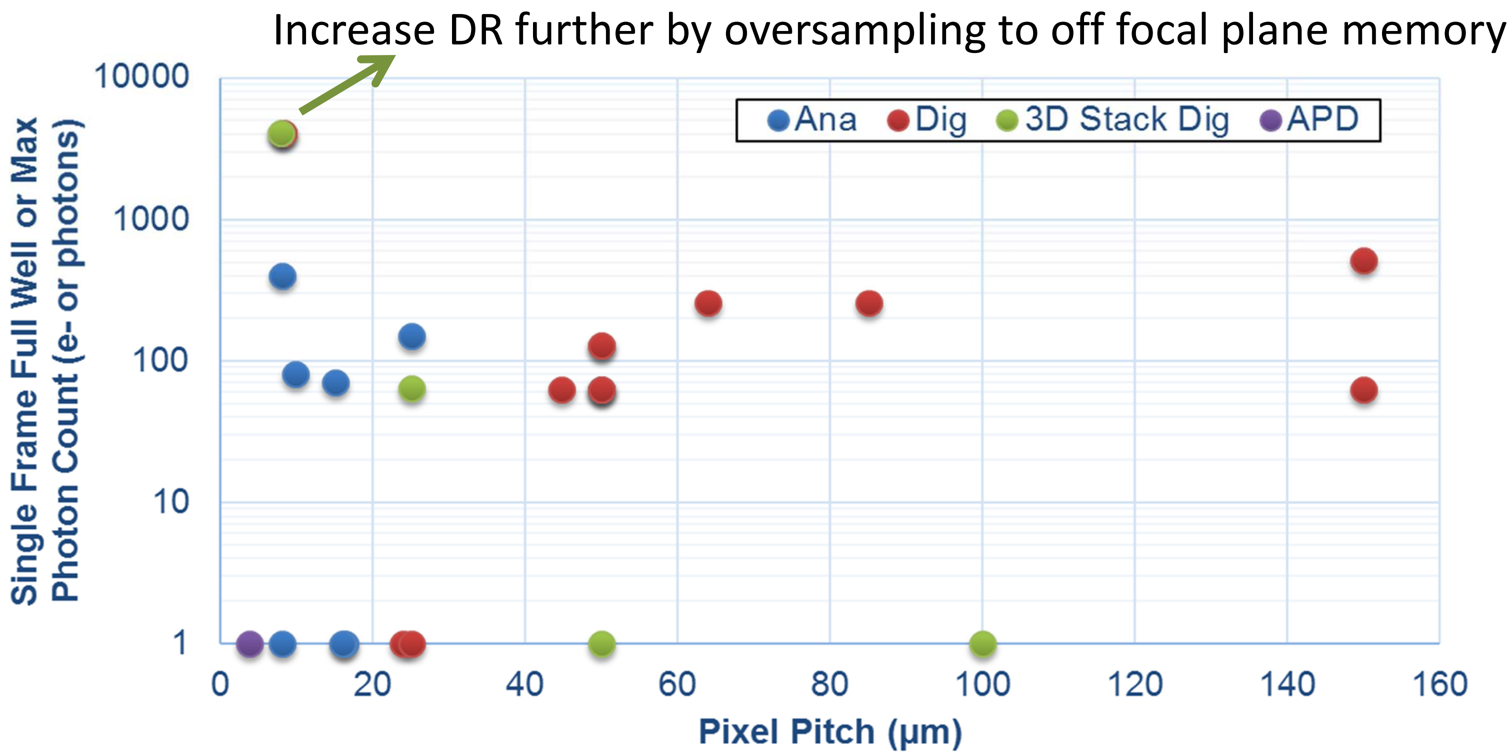


[Tarek Al Abbas, IEDM 2016]

Future Directions

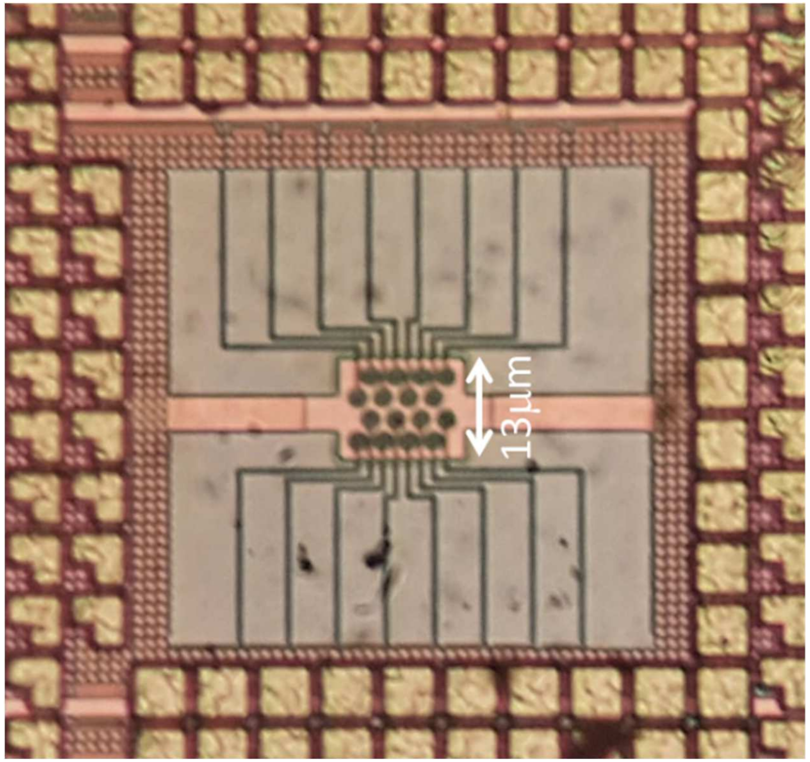


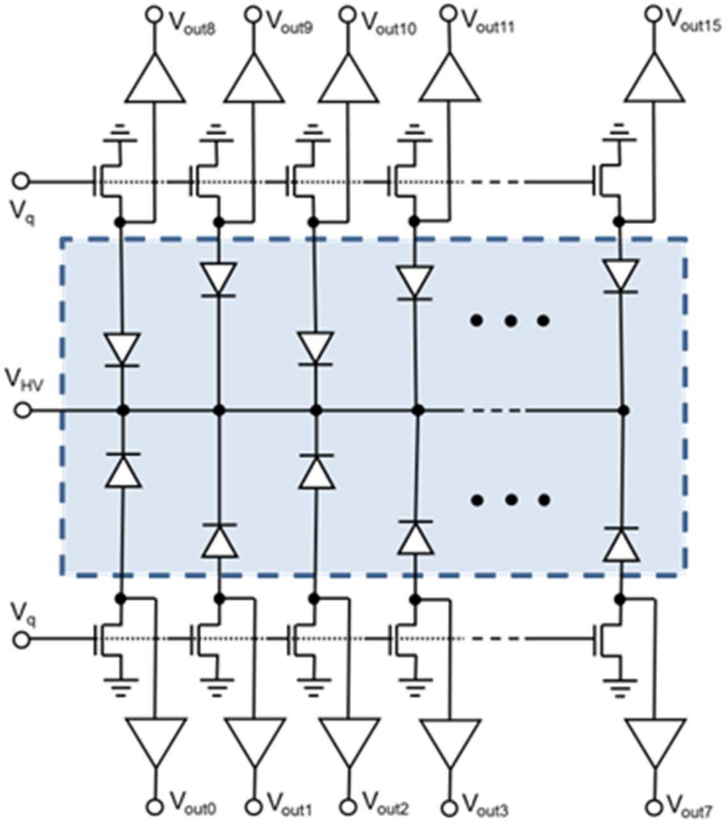
Full Well Capacity



- Some partition between on-pixel counting and oversampling is required to maintain DR.

3μm Pitch SPAD Test Structure

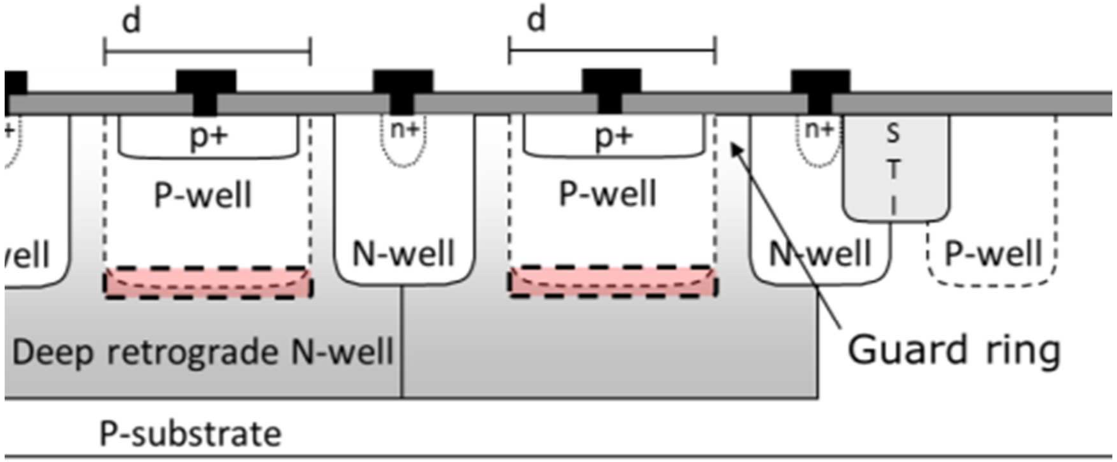


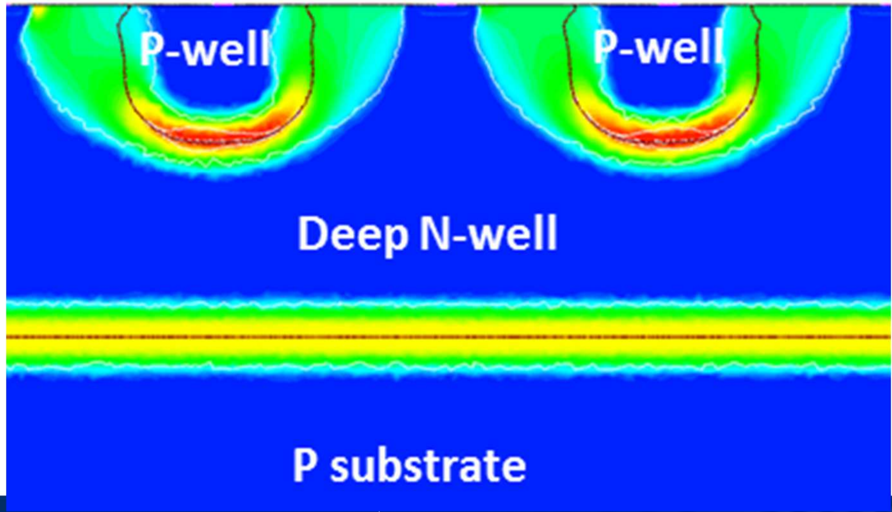


- **Demonstrates feasibility of small pitch high resolution SPAD imagers.**

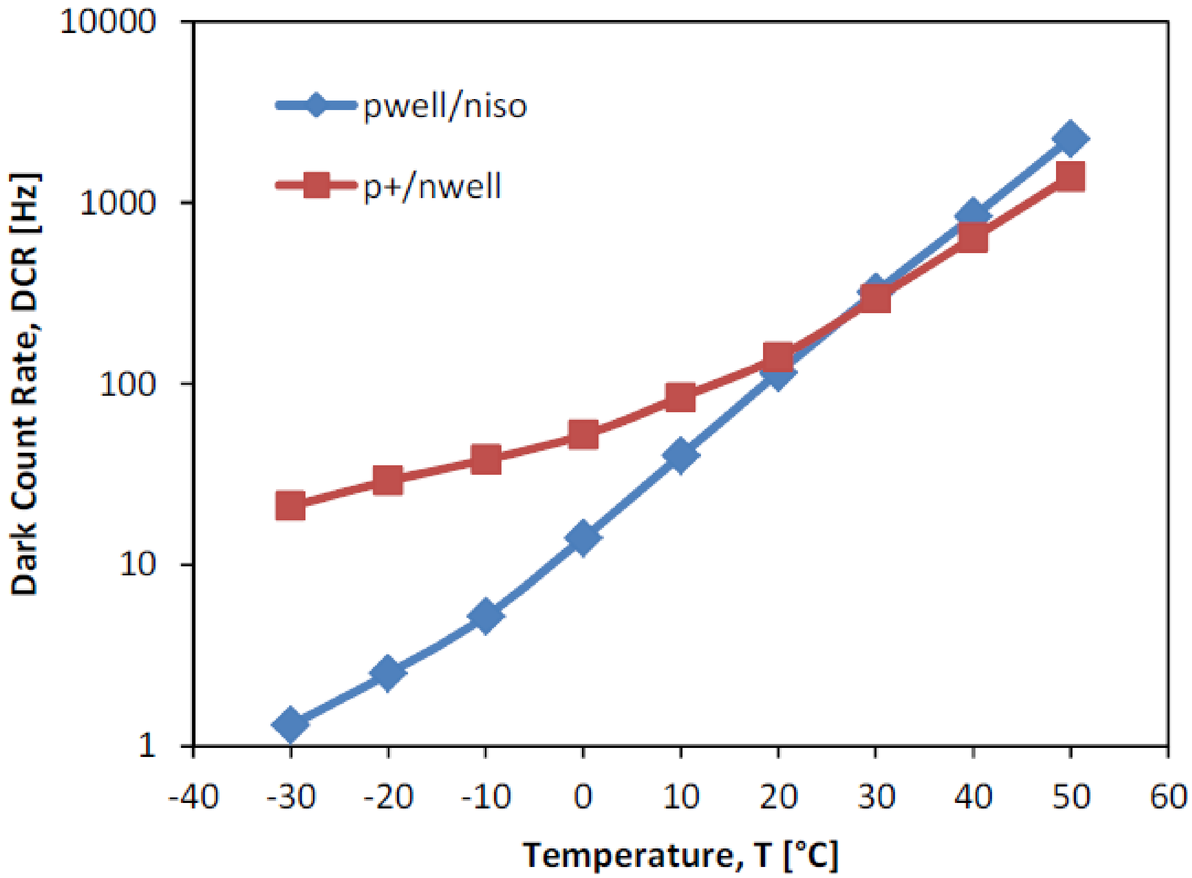
Cross Section and TCAD

- **TCAD indicates well confined high field region.**
- **VBD=15.8V not confirmed.**



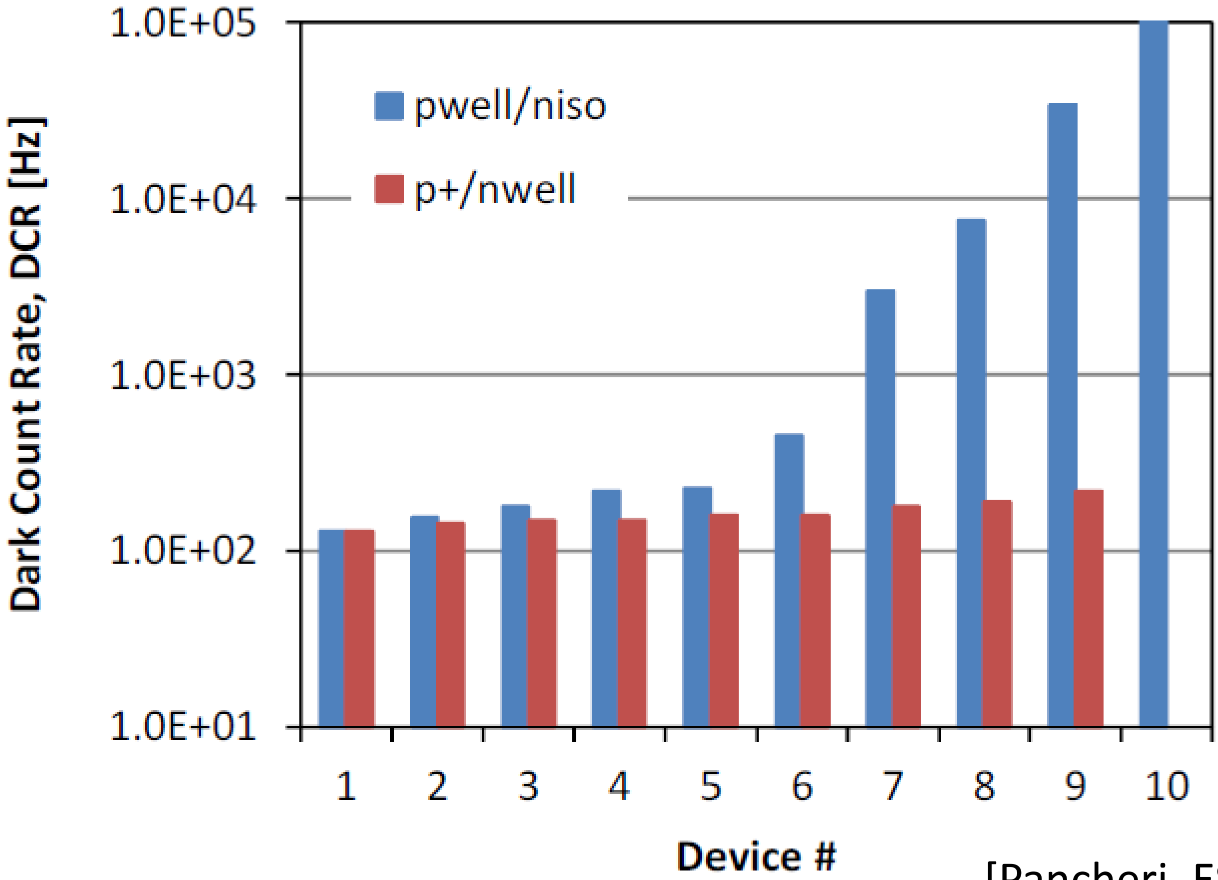


Cooling



[Pancheri, ESSDERC 2011]

DCR Distribution



[Pancheri, ESSDERC 2011]

Specifications

Specification	GSENSE2020BSI	iXon Ultra EMCCD	Scientific SPAD
Resolution	2048 x 2048	1024 x 1024	1024 x1024
Pixel Pitch	6.5µm	13µm	8µm/16µm
Full well	54ke-	80ke-	4096 (12-bit) with ~16x oversampling
Dynamic Range	90dB	98dB (est.)	1MHz/10Hz -90dB
Max. SNR	47dB	49dB (est.)	See full-well
Readout Noise	1.2e-	<1e- with EM gain	0e-
Dark Current	0.2e-/p/s @ -20°C	0.00025e-/p/s @ -80°C	10Hz/p/s @RT
Frame Rate	74fps	26fps	Easily achievable as no ADC
Power	1.2W	-	Few 100mW
Peak QE	94% @ 550nm	95% @ 600nm	~50% with BSI SPAD + microlens

Summary

- Sensitivity of SPAD arrays can approach 2-3x the best backside illuminated low-light cameras (sCMOS/EMCCD). Viable scientific SPAD image sensor still limited by SPAD PDE and defectivity.
- Post-processing to set optimal frame rate can be achieved due to noiseless frame summation for e.g. transient phenomena in microscopy, 3D motion compensation, object tracking.
- Combination of 3D stacking, pixel level counting and oversampling can address DR limitations.

Acknowledgements

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European Research Council

EPSRC Engineering and Physical Sciences Research Council

PROTEUS

ESRIC Edinburgh Super-Resolution Imaging Consortium

Thank you for listening!

Any questions?