MicroTime 100: A Compact Microscope for FLIM, Time-Resolved Photoluminescence (TRPL) and Screening

Volker Buschmann, Peter Kapusta, Felix Koberling, Marcelle König,
Benedikt Krämer, Steffen Rüttinger, Sebastian Tannert,
Manoel Veiga, Rainer Erdmann, Uwe Ortmann

Webtalk January 2014

Please note ...

We at PicoQuant always try to present our latest findings to the scientific community as quickly as possible.

The creation of suited presentation material with in-depth information is, however, not always possible in a short time frame.

We have therefore decided that it would be beneficial to the scientific community making our presentations or parts of presentations, that were given on conferences, available to the public. As a consequence, it might be possible that information is missing to understand all information included in a slide.

Thus, please don't hesitate to contact us in case you have any questions or need more information. We hope for your understanding and looking forward to hearing from you.

Your PicoQuant team

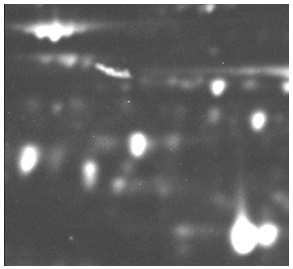
Configurations and Applications of the MicroTime 100

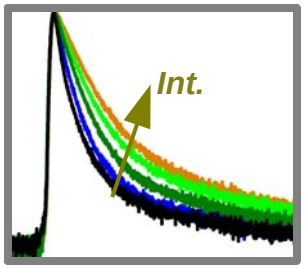
Components and configurations of the MicroTime 100

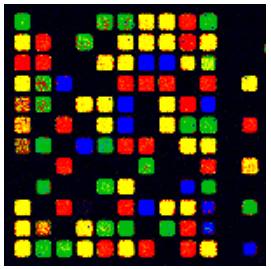
- Diode lasers for excitation
- Detector types
- Photon counting boards
- Scanning options

Applications for the MicroTime 100

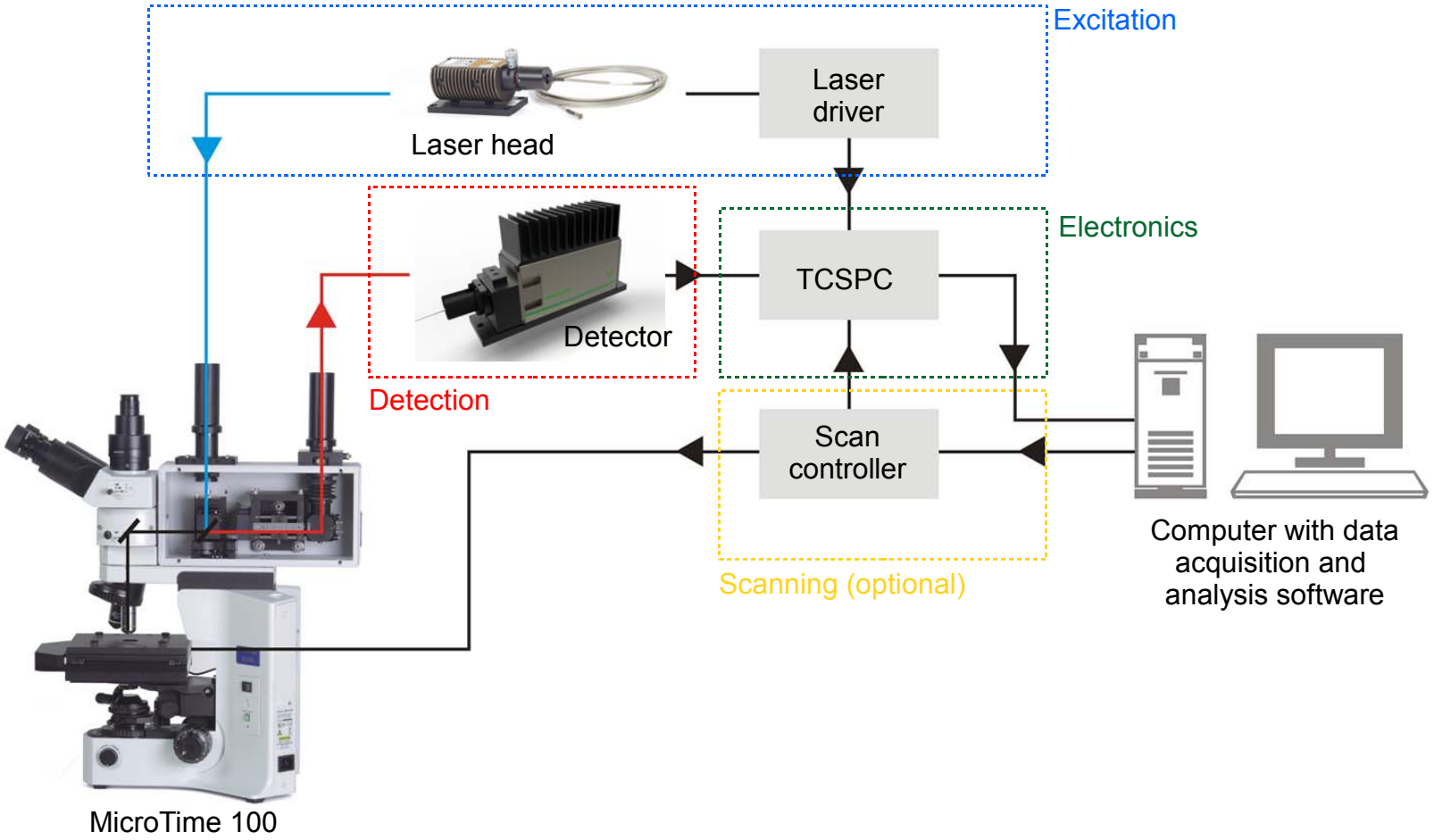
- Fluorescence Lifetime Imaging Microscopy (FLIM)
- Lifetime screening in microwell plates
- Time-resolved Photoluminescence (TRPL)





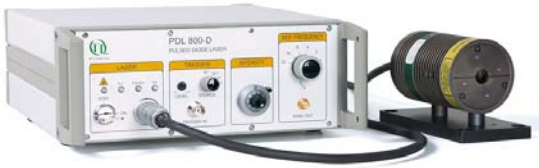


General Set-up

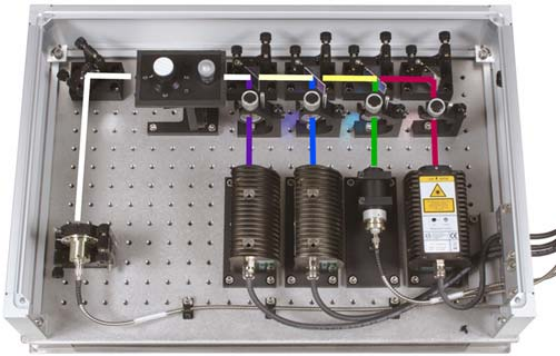


Excitation Configurations

Single pulsed diode laser



Manually controlled laser driver unit (PDL 800-D)
- For one diode laser head
- Repetition rates from 31.25 kHz to 80 MHz

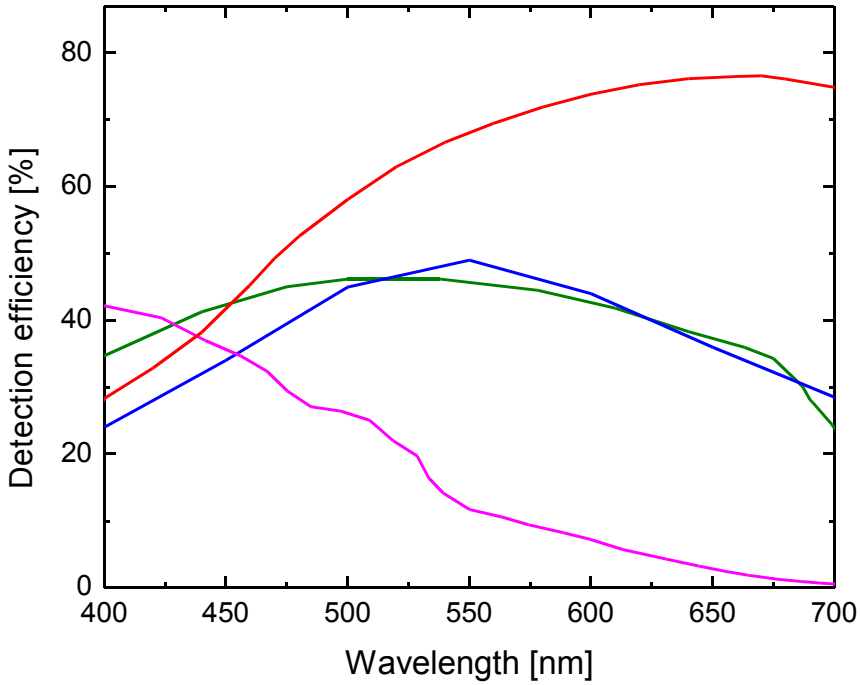


Laser Coupling Unit (LCU)
- For up to 5 laser diode heads

Software controlled laser driver unit (PDL 828 “Sepia II”)
- For up to 8 diode laser heads
- Programmable pulse patterns

Available wavelengths
- 375, 405, 440, 470, 485, 510, 530 nm (40 MHz, < 100 ps, 1-3 mW)
- 635-850 nm (80 MHz, < 100 ps, 5-10 mW)

Detection Efficiency



τ-SPAD

PMA Hybrid

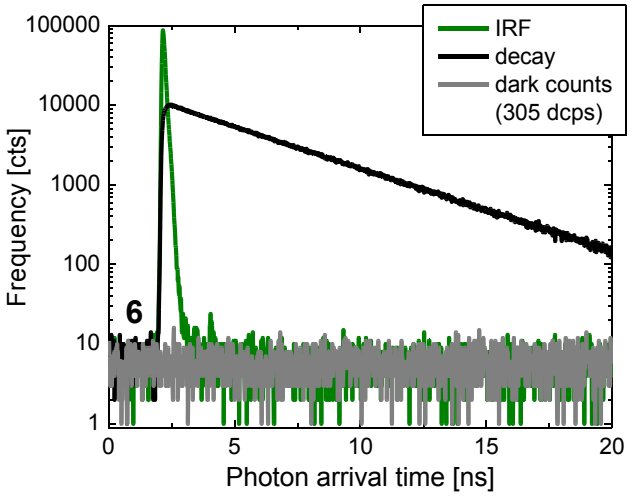
MPD SPAD

PMA (PMT with ultrabialkali cathode)

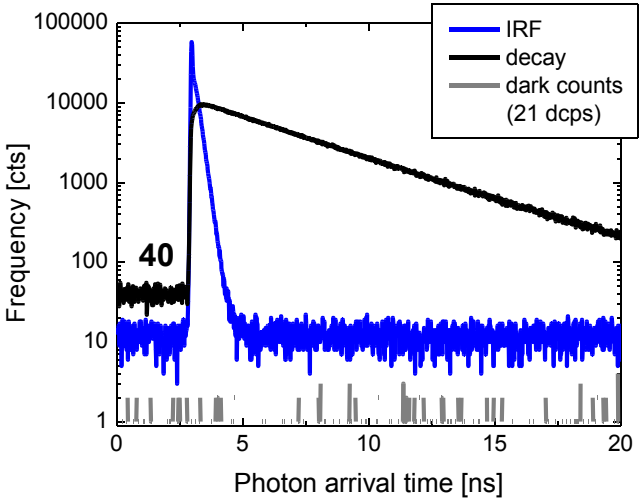
Dark Counts, IRF and Afterpulsing

IRF: backscattered excitation light, 60 s measurement with 30 kcps
Decay: aqueous ATTO 488 solution, excited with 470 nm (20 MHz), bandpass 520/40, 60 s measurement with 100 kcps
Dark counts: closed detector

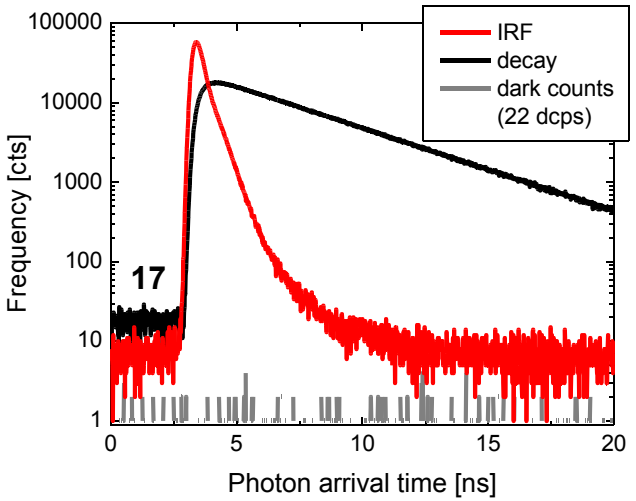
PMA Hybrid



MPD SPAD

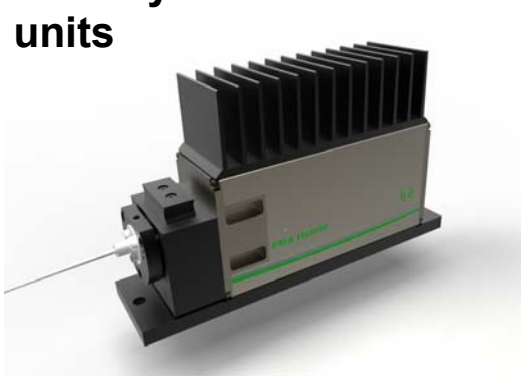


τ-SPAD



Detection Configurations

Single channel PMA or PMA Hybrid detection units



Multichannel PMA or PMA Hybrid detection unit

- For up to 4 detectors

Single channel SPAD unit

Dual channel SPAD unit

- In all detection units, standard Ø 25 mm filters can be mounted.
- In the multichannel units, 25.5 x 36 x 1 mm standard dichroic mirrors can be mounted.
- An additional filter position is available in the main optical unit of the MicroTime 100.

Electronics Configurations

HydraHarp 400

- Up to 8 independent detector channels
- Independent sync channel
- Down to 1 ps base resolution

PicoHarp 300

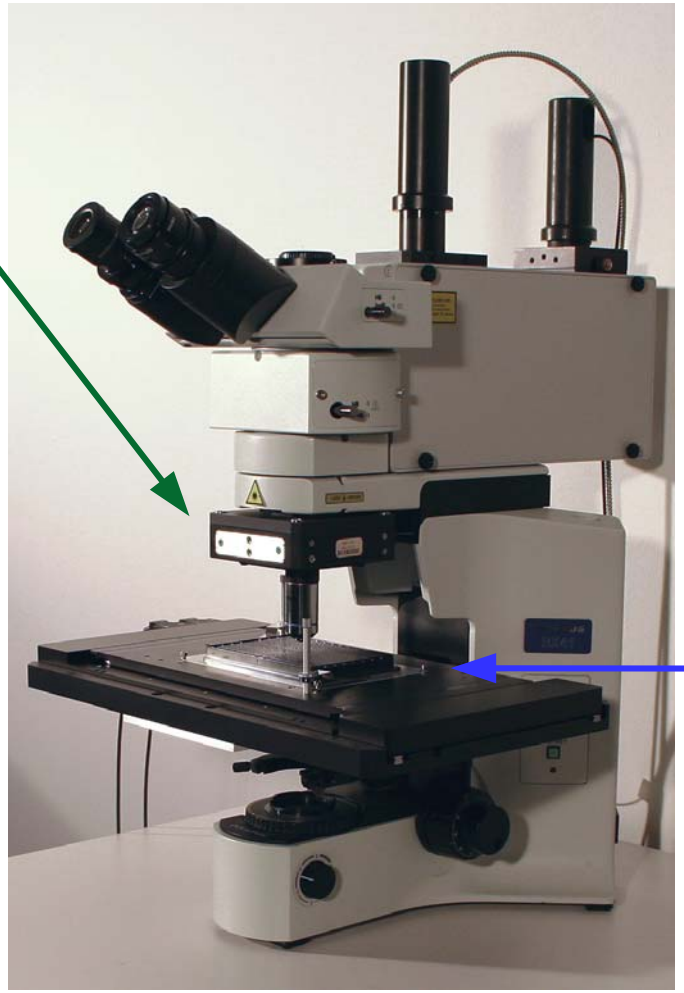
- 2 independent channels
- Down to 4 ps base resolution

TimeHarp 260

- Either 25 ps (“PICO version”) or 1 ns (“NANO version”) base resolution
- One or two independent detector channels
- Independent sync channel

Scanning Configurations

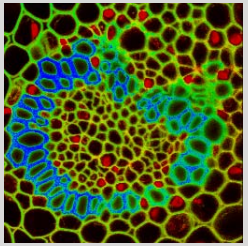
High resolution piezo scanner
- Objective scanning configuration
- Max. 80 x 80 µm effective imaging range
- Optional additional z-scanning (100 µm range)



Wide range piezo scanner
- Sample scanning configuration
- Max. 7.2 x 7.5 cm effective imaging range
- Max. 10 x 7.5 cm positioning range
- ~ 23 min/full image with 512 x 512 pixel resolution

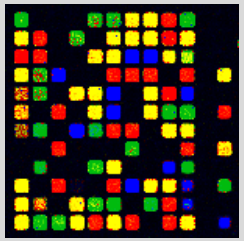
Applications for the MicroTime 100

Fluorescence Lifetime Imaging (FLIM)



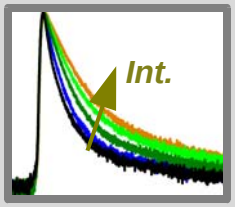
- Environmental sensing
- Binding studies via resonance energy transfer (FLIM-FRET)
- Autofluorescence studies

Lifetime based screening and quantification



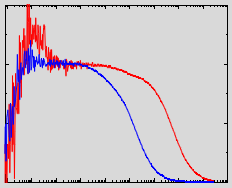
- Reaction control, e.g. multi-well based assays
- Lifetime based background removal, e.g. for gel-quantifications

Time-resolved photoluminescence studies



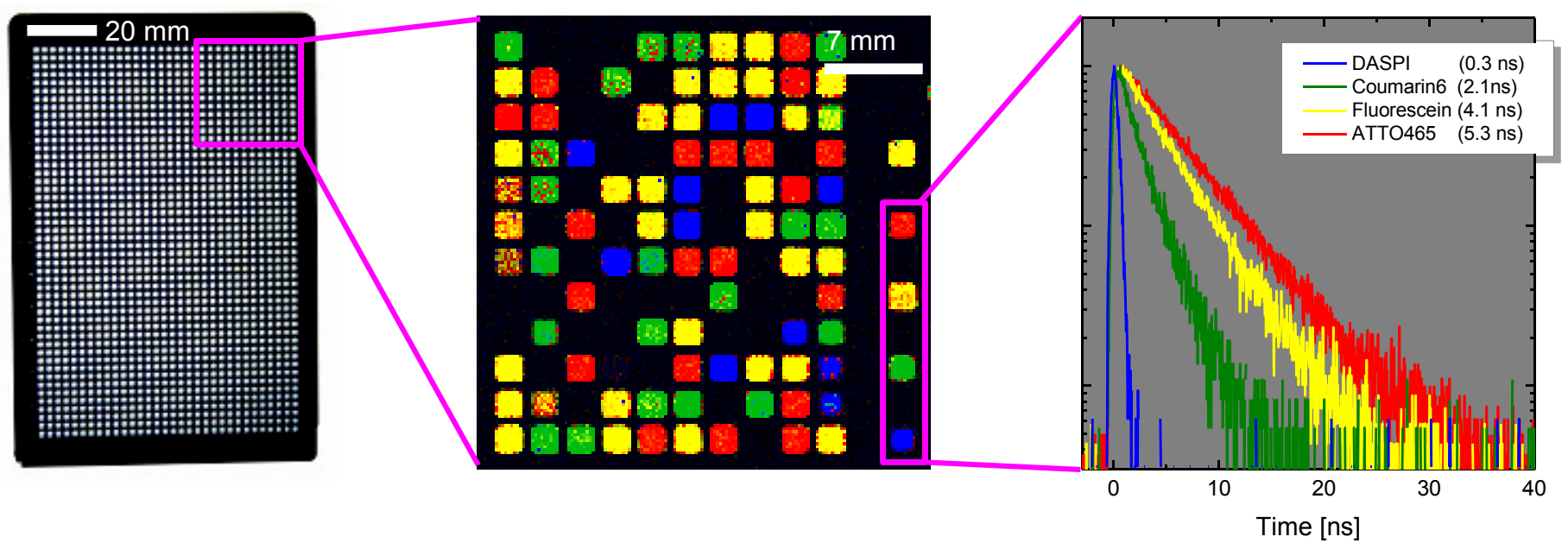
- Confocal or widefield illumination
- Intensity-dependent resolved luminescence decays
- Spatially resolved luminescence decays

Fluorescence Correlation (FCS) studies

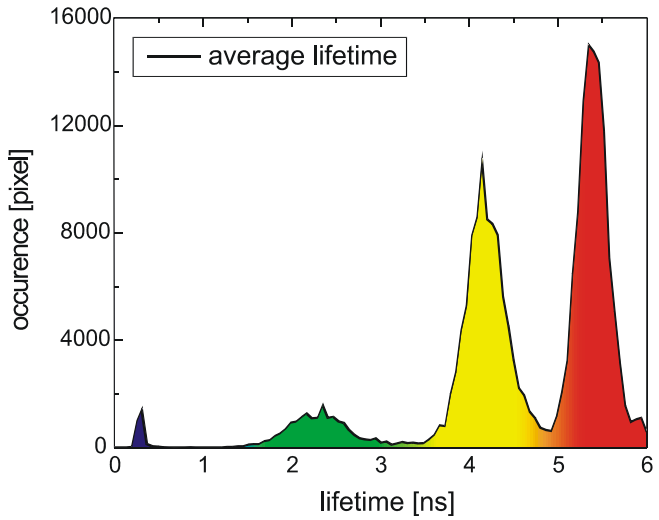


- Binding studies via auto- and crosscorrelation
- Size determinations

Application 1: Lifetime-Multiplexing in Microwell Plate Imaging

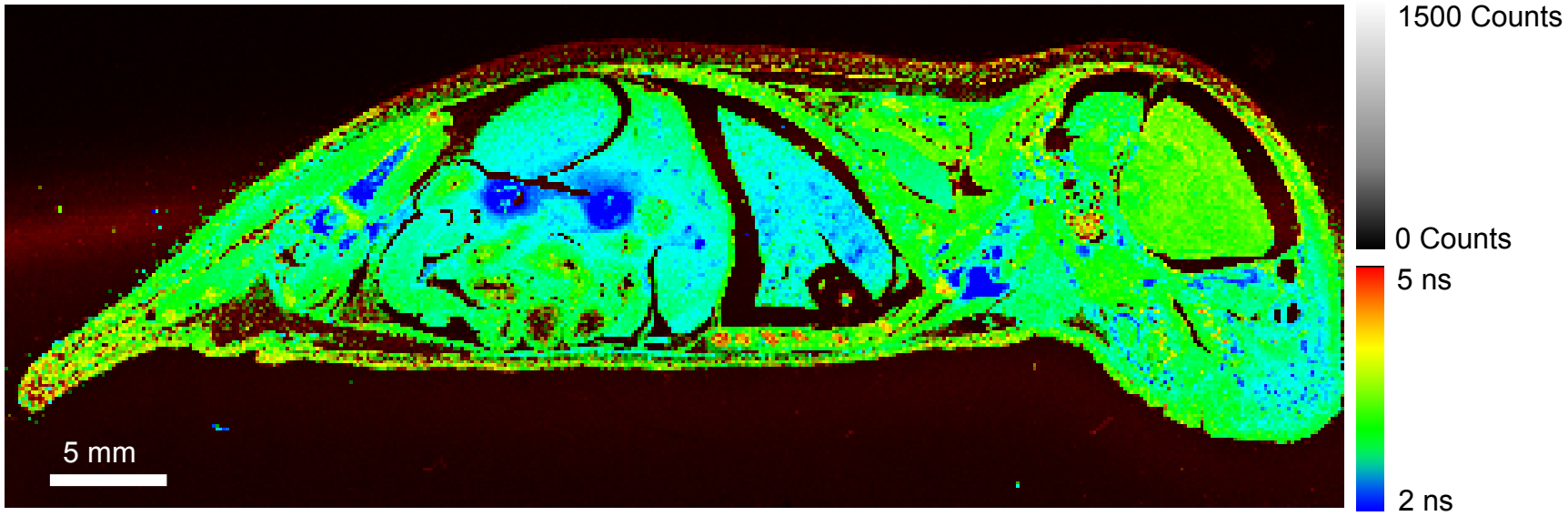


Lifetime histogram summarizing the extracted lifetimes per pixel based on a single exponential tailfit.



→ Lifetime-multiplexing results in reliable fluorophore identification in each microwell plate.

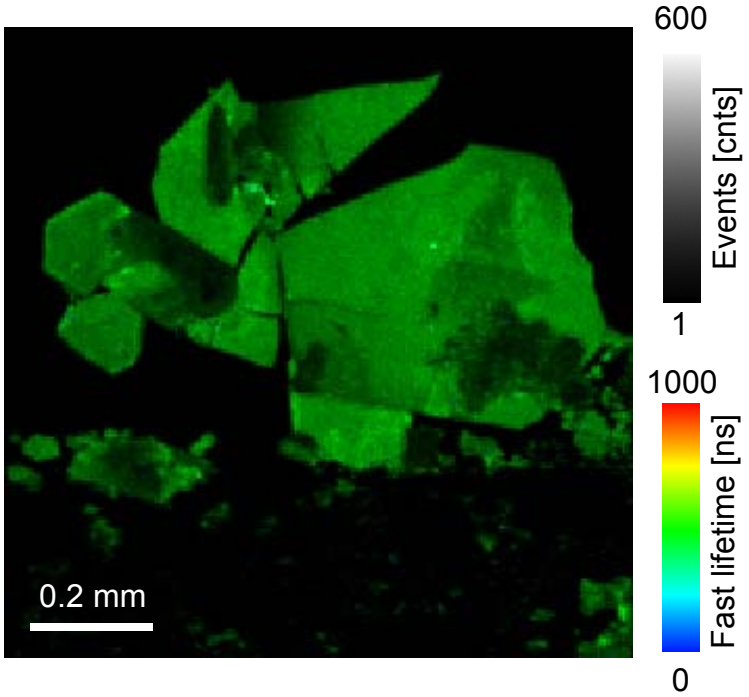
Application 2: Autofluorescence Screening of Larger Tissues

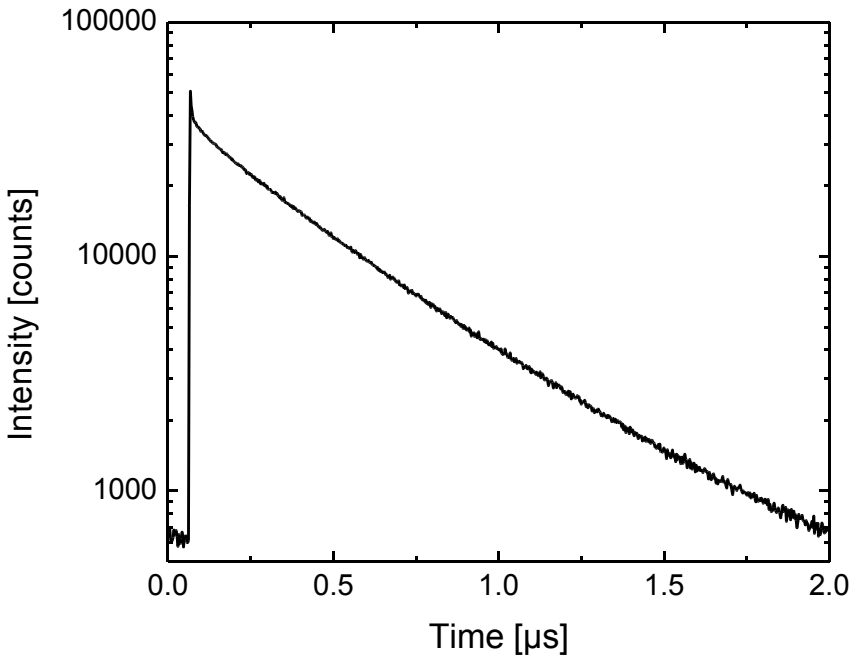


Sample: unstained mouse embryo
Data acquired with: MicroTime 100, equipped with a widerange scanner
l_{exc} = 485 nm, 20 MHz
l_{det} = 530 nm - 550 nm
Hybrid-PMA detector
Pixel time: 5 ms
Aquisition time: 22 min
800x300 pixel, 2x2 binning

→ FLIM can also be applied for measuring autofluorescence in larger specimen, if the sample is rasterscanned over the confocal spot.

Application 3: Phosphorescence Lifetime Imaging





Sample: RuBiPy (Tris(bipyridine) ruthenium(II) chloride) crystals
Data acquired with: MicroTime 100
λ_{Exc}: 375 nm, 0.5 MHz
UPlanSApo 10x, NA 0.4
Time/pixel: 0.9 ms
Image size: 1 x 1 mm (200 x 200 pixel)
Total recording time: 120 s
Emission filter: HQ500LP
Detector: Hybrid PMT

TimeHarp 260 PICO, long range mode

- Max. time range > s
- Dead time < 2.5 ns
- Minimum channel width 2.5 ns

→ MicroTime 100 enables for phosphorescence lifetime imaging.

Protein Content Determination in Proteomics

Cell

↓

Protein extract

- Typically 10.000-20.000 different proteins
- Typical abundances: pg/ml
  → 50 mg/ml
  → 10 orders of magnitude

↓

Dye labeling

- Labeling at the Lys residues
- Typical labels: Cy2, Cy3, Cy5
  → max. 3 different labels can be applied to one gel

↓

2D gel electrophoresis

↓

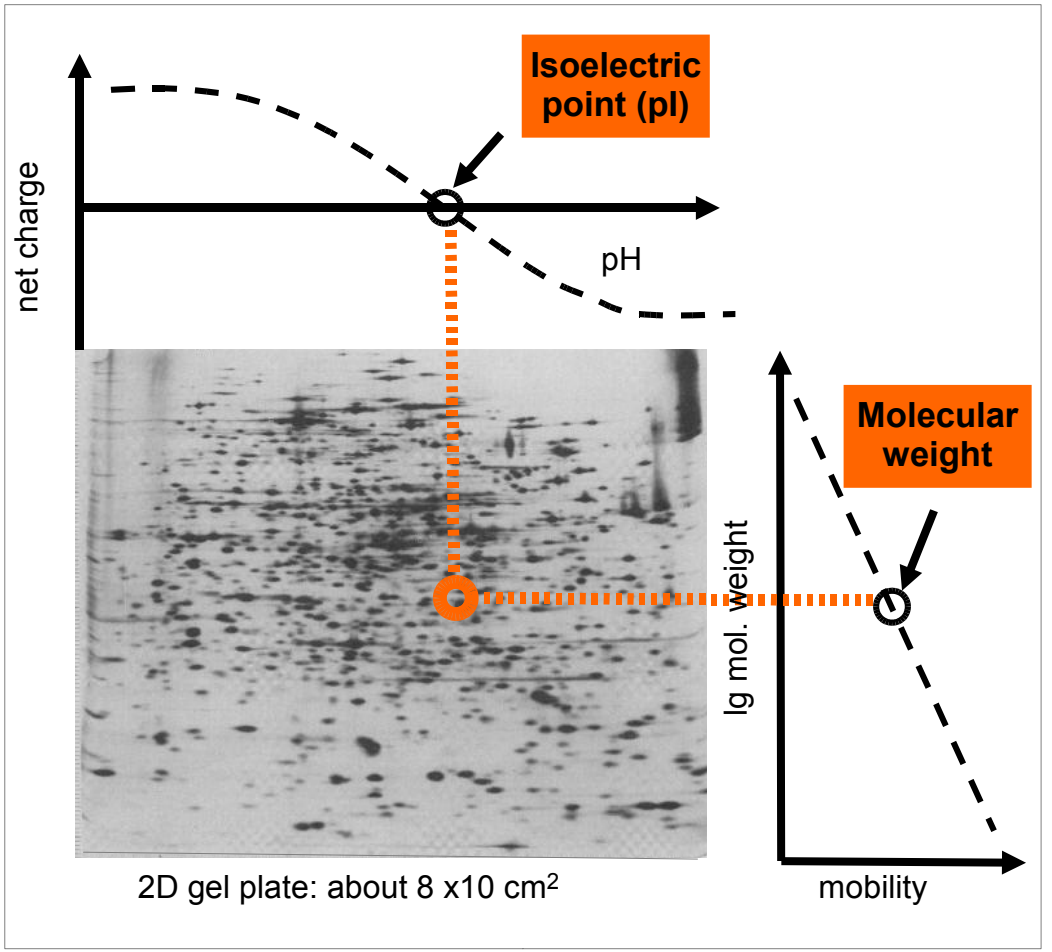
Fluorescence imaging

- Resolution of modern scanners: **4 orders of magnitude**
- Limitations are intrinsic background fluorescence, scatter and detector noise (analogue PMT)

↓

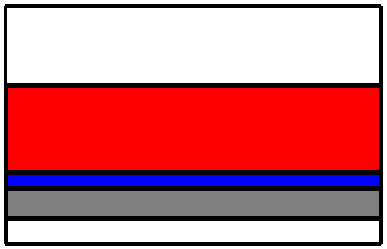
Data analysis

Protein separation via a 2D gel



Application 4: Protein Content Determination in Proteomics
Pulsed Excitation for Background Identification

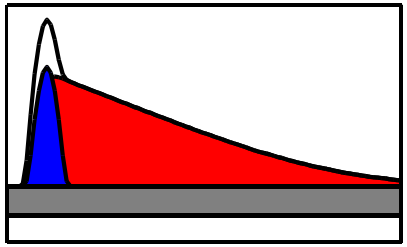
Standard (cw) excitation



t [ns]

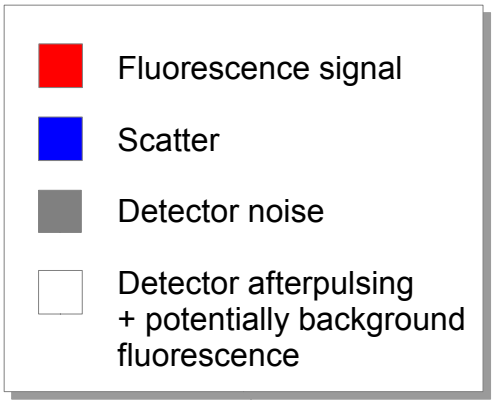
- The detected intensity signal is the sum of many contributions.

Pulsed excitation



t [ns]

- Different signal contributions can be identified
- Requires time-resolved data recording
- Multiexponential data fitting

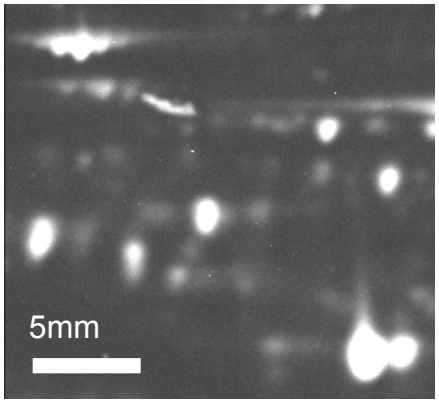


Application 4: Protein Content Determination in Proteomics
Lifetime Based Decomposition

Fitting model:

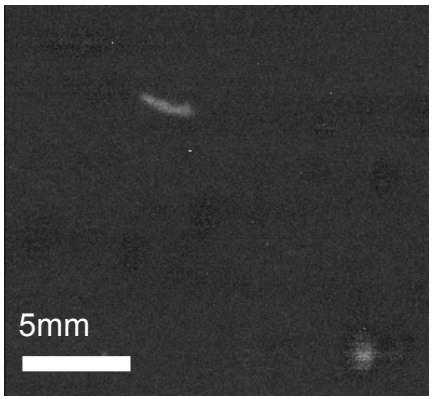
Multi-exponential decay: $I(t) = \sum_{i=1}^{n} A_i \, e^{-\frac{t}{\tau_i}}$

Raw image



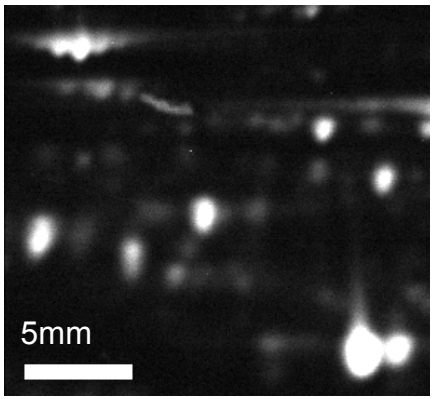
I(τ = 0.18 ns) + I(τ = 5.00 ns)

Scatter + autofluorescence



I(τ = 1.07 ns)

Protein staining



τ_1 = 0.18 ns (scatter)

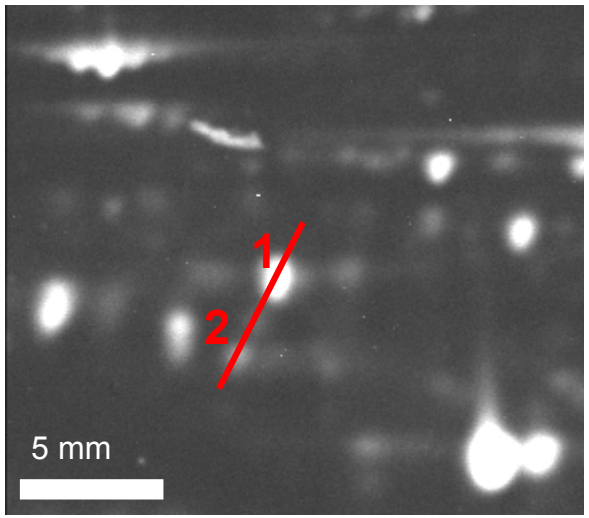
τ_2 = 1.07 ns ← Cy2 label

τ_3 = 5.00 ns (autofluorescence)

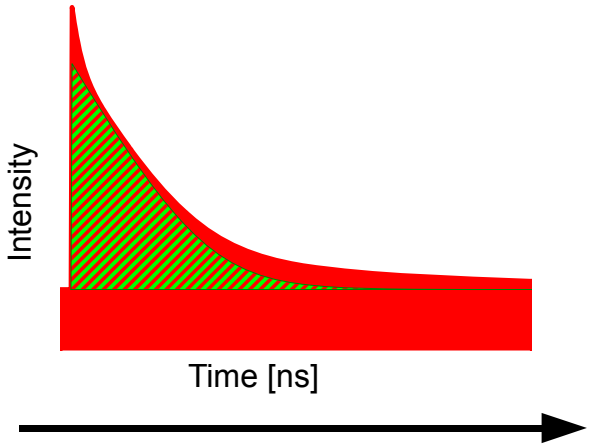
In collaboration with Åsa Wheelock, Karolinska Institute, Stockholm, Sweden

Application 4: Protein Content Determination in Proteomics
Improved Signal to Background Ratio

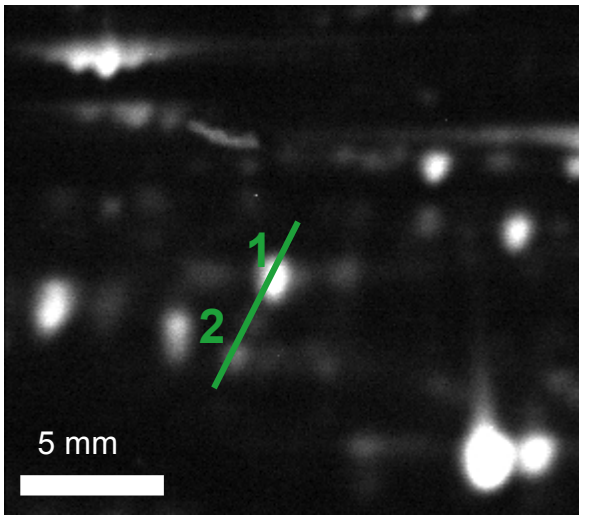
Intensity image of a 2D gel



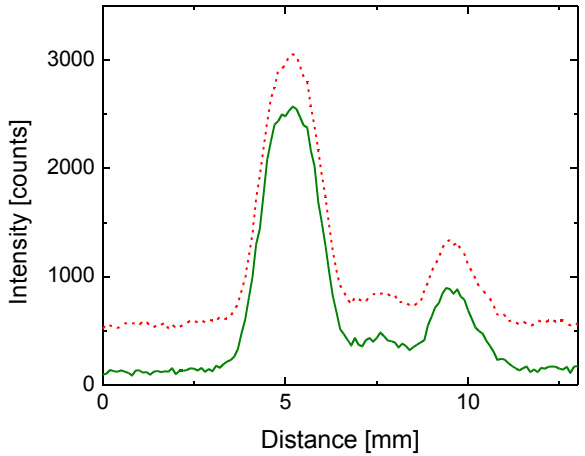
Without photon filtering

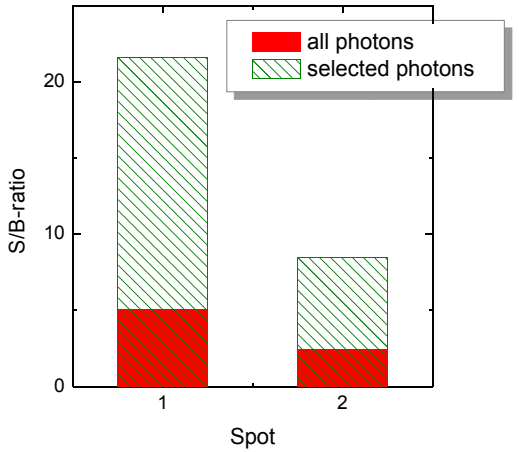


Photon filtering: only photons from the fluorescent label (green) are considered



After photon filtering

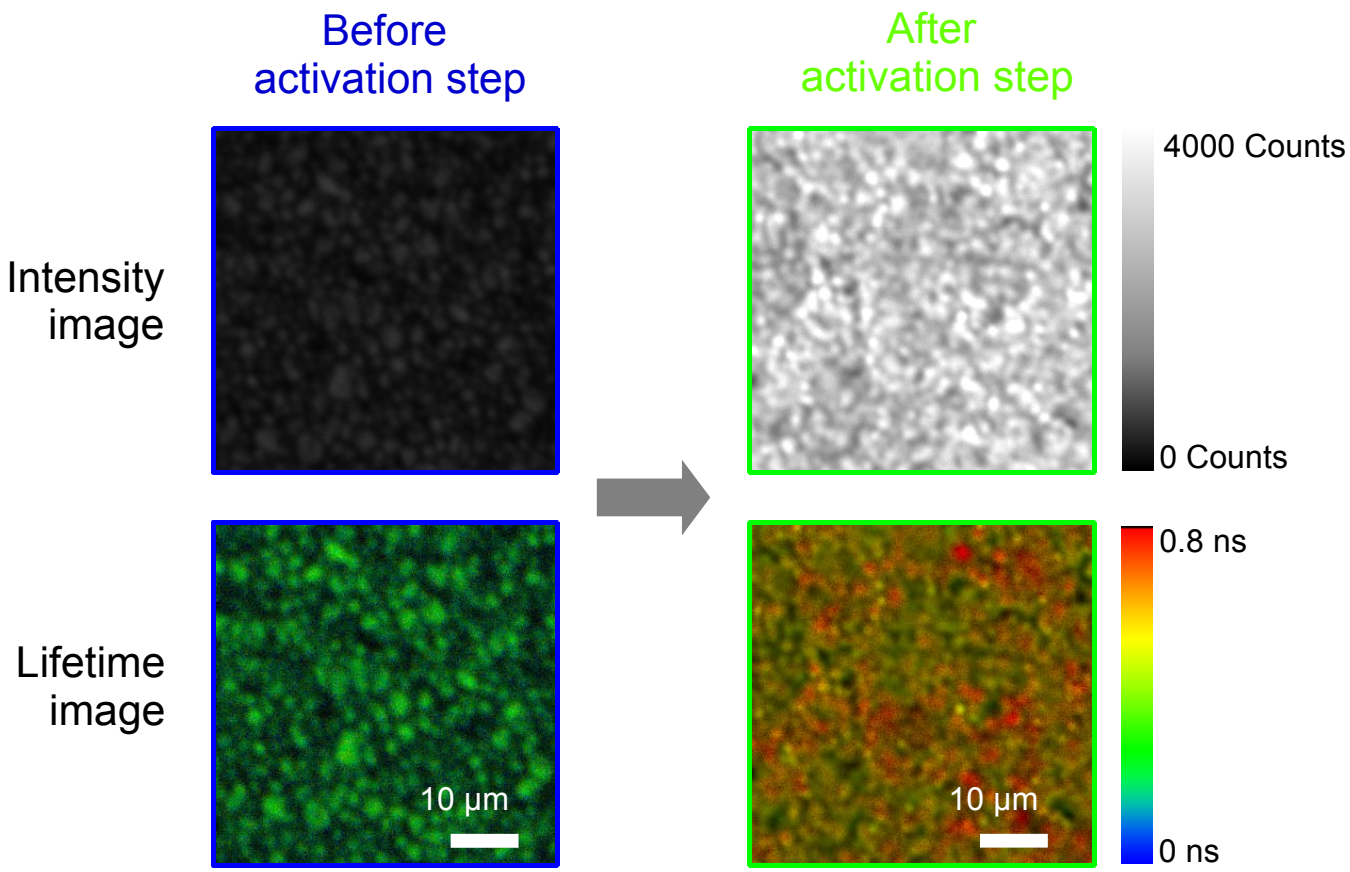


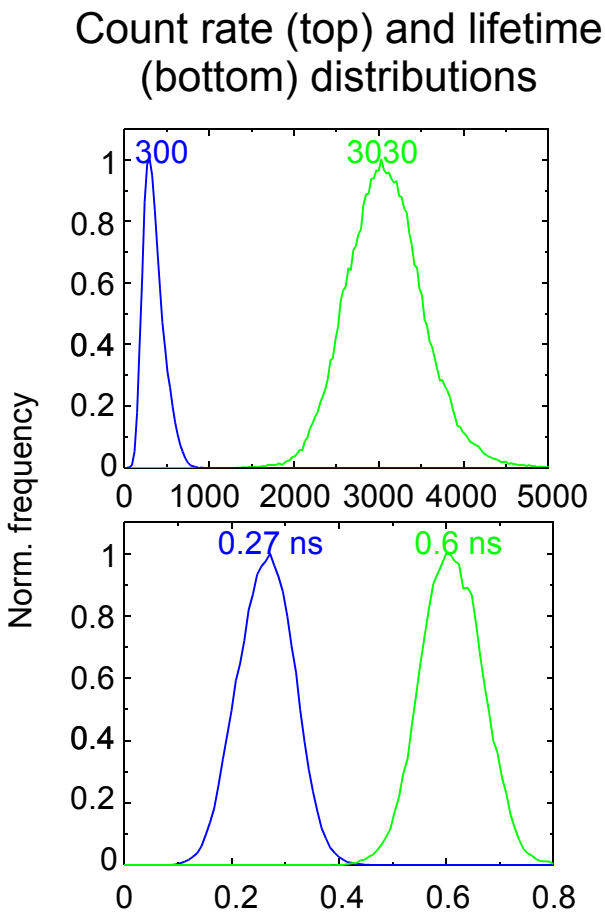


In collaboration with Åsa Wheelock, Karolinska Institute, Stockholm, Sweden

Application 5: TRPL for Semiconductor Analysis
2D Mapping for Quality Control

TRPL: Time-Resolved Photo Luminescence



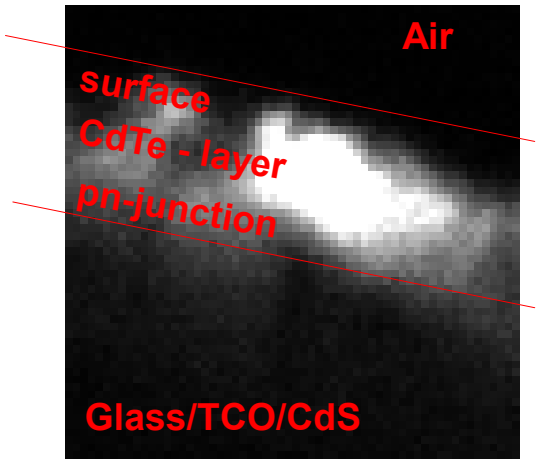


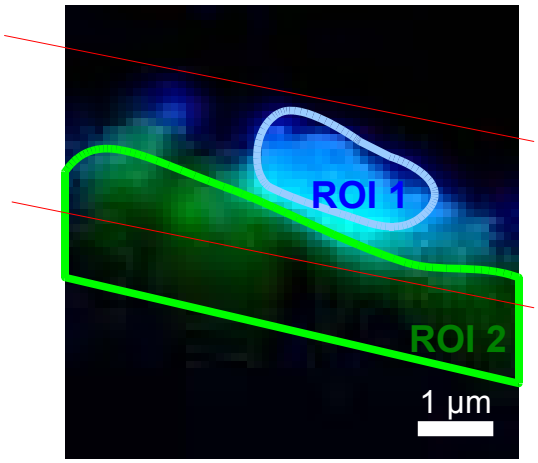
λ_{exc} = 635 nm, 40 MHz
λ_{det} = 814 - 870 nm
τ-SPAD detector

Collaboration with Christian Kraft, University of Jena, Germany
See: V. Buschmann et al., J. Appl. Spectr., 80, 449-457 (2013)

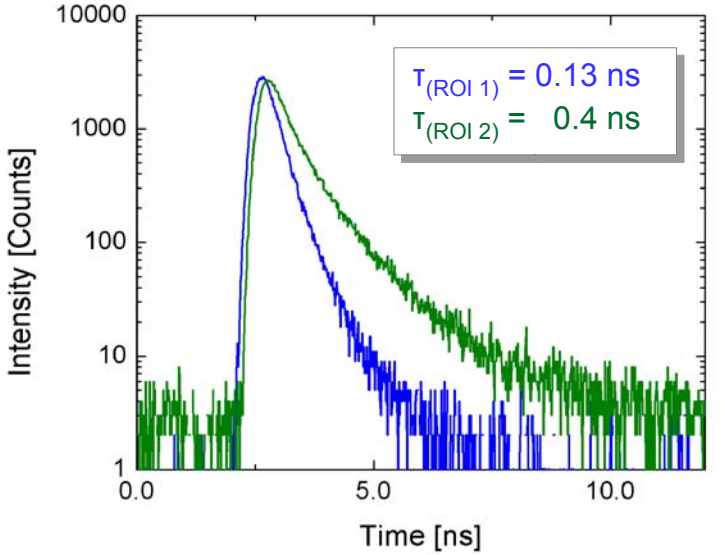
Application 5: TRPL for Semiconductor Analysis
Device Architecture Characterization

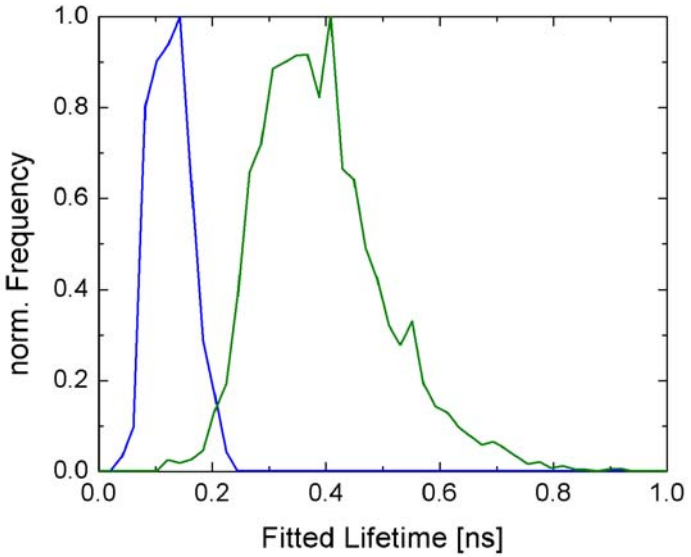
λ_{exc} = 635 nm, 40 MHz
λ_{det} = 814 nm - 870 nm
τ-SPAD-Detector





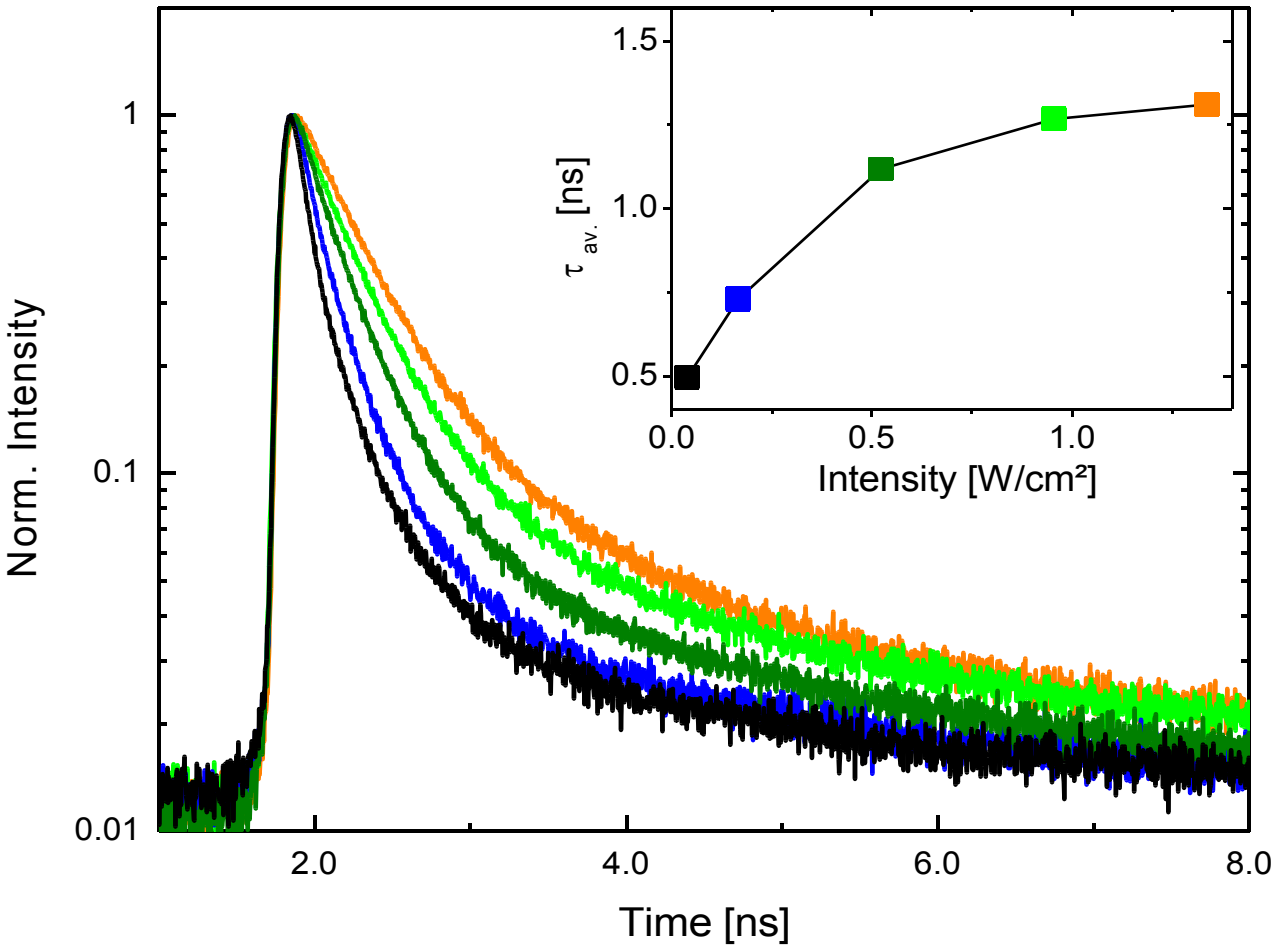
→ Lifetime increases from air interface to the *pn*-junction
→ Bright areas don't have necessarily the longest lifetime





Collaboration with Christian Kraft, University of Jena, Germany
See: C. Kraft et al., J. Appl. Phys. 113, 124510-124518 (2013)

Application 5: TRPL for Semiconductor Analysis
Excitation Intensity Dependence



Intensities:
- 1.34 µW/cm²
- 0.96 µW/cm²
- 0.52 µW/cm²
- 0.17 µW/cm²
- 0.04 µW/cm²

Sample: GaAs-based Quantum Well

Adapted MicroTime 100
λ_{exc} = 635 nm, 40 MHz
λ_{det} = > 664 nm
Spot size: ~ 100 µm
PDM 1CTC-SPAD detector

→ The lifetimes of semiconductor materials depend on excitation intensity.

Collaboration with Andrea Knigge, Ferdinand-Braun-Institut, Berlin, Germany
See: V. Buschmann et al., J. Appl. Spectr., 80, 449-457 (2013)

Further Information

See specifications on our website:
http://www.picoquant.com/products/microtime100/microtime100.htm
and the datasheet:
http://www.picoquant.com/images/uploads/downloads/microtime100.pdf

Check our website for training courses on FLIM, FCS and Time-Correlated Single Photon Counting:
http://www.picoquant.com/events/workshops-and-courses

Share your experiences with the scientific community in the PicoQuant forum at:
http://forum.picoquant.com/

Please contact PicoQuant at info@picoquant.com for further information on:

- Applications
- Possible configurations
- Prices