UV MicroTime 200

Crossing the Border towards Deep UV – Time-resolved Microscopy of Native Fluorophores

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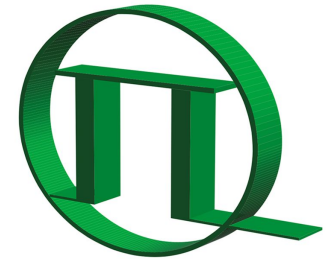

UV MicroTime 200, Webtalk 2013

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Please note...

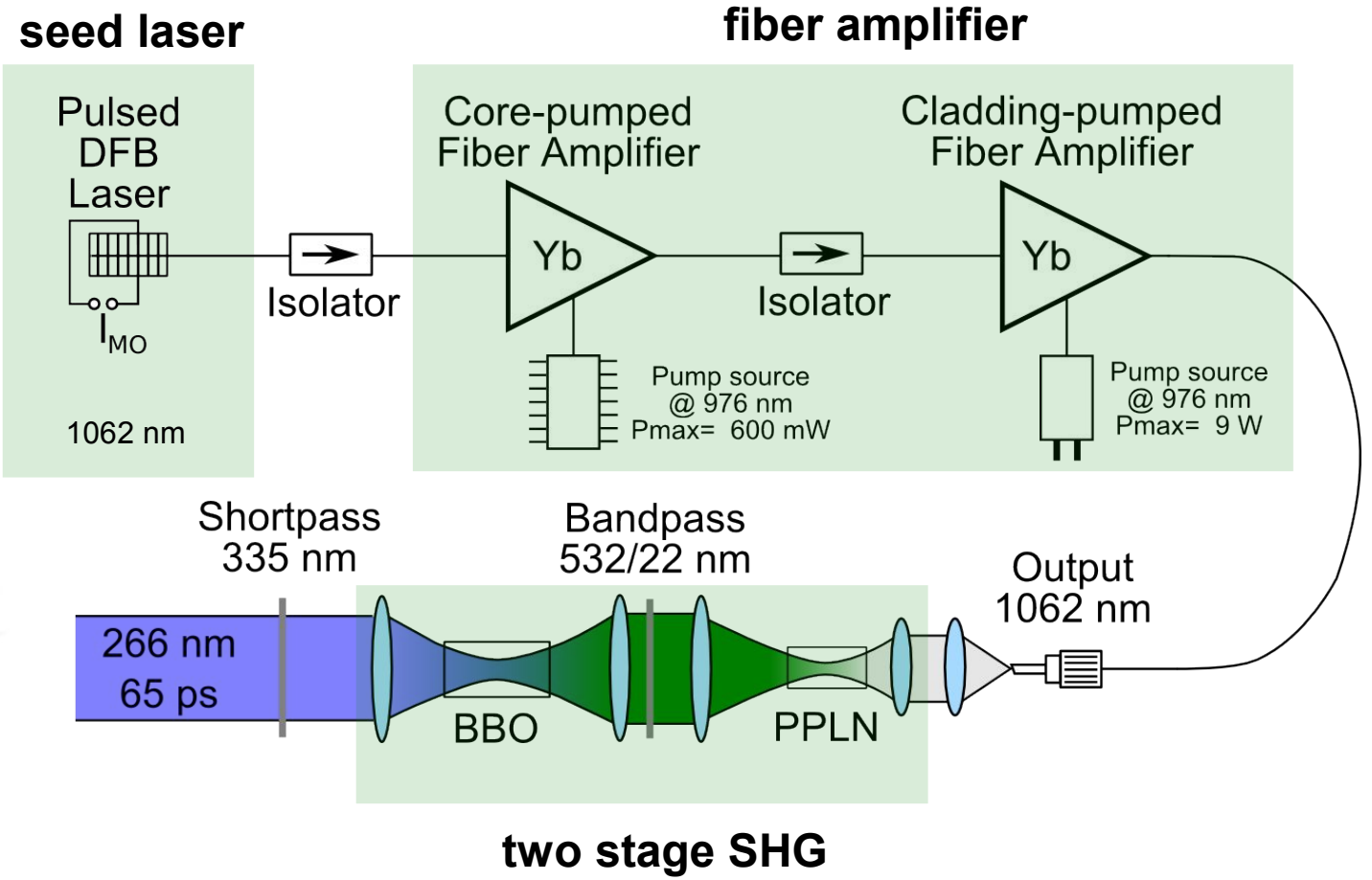
We at PicoQuant always try to present our latest findings to the scientific community as quickly as possible.

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 for those cases, it would be beneficial to the scientific community to make our presentations or parts of presentations, that where given on conferences, available to the public. As a consequence, please understand that it might be possible that sometimes information is missing to understand all information included in a slide.

Don't hesitate to contact us in case you have any questions or need more information.

Fiber Amplified UV Laser for Pulsed Deep UV 266 nm Excitation



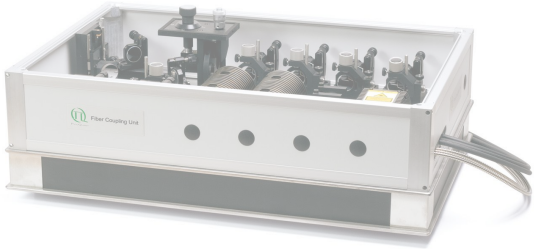
- 266 nm, < 100 ps pulses
- variable repetition rates from 1 MHz to 80 MHz

T. Schönau et al., Biomedical Optics (BIOMED), Miami, Florida, 2012

UV Configuration of the MicroTime 200

deep UV excitation
(266 nm, < 100 ps pulses with variable repetition rates from 1 MHz to 80 MHz)

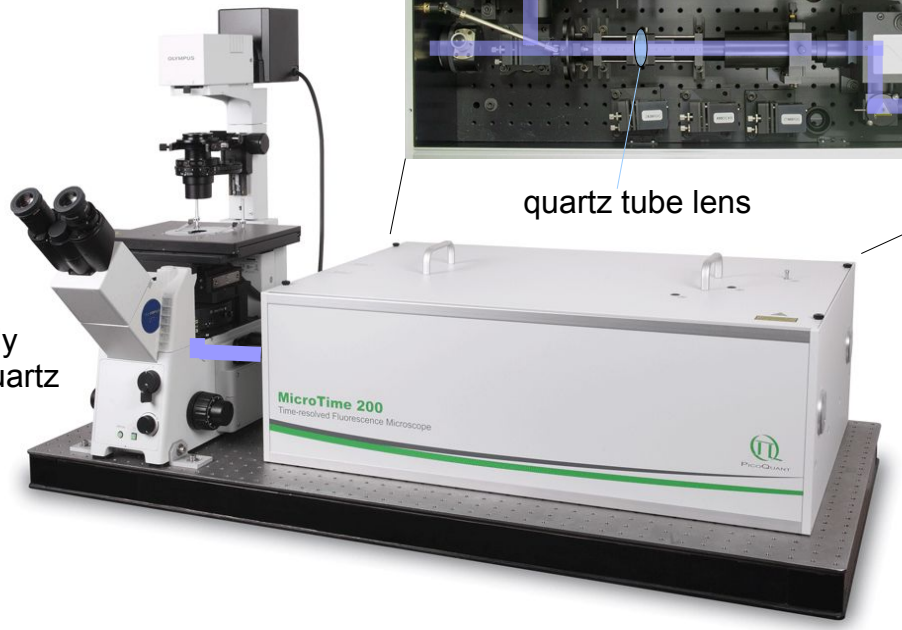
VIS excitation
(375 nm, 405 nm, ..., 640 nm)



modified main optical unit with quartz optics, for parallel VIS and UV detection

detection extension unit with ultrabialkali PMT for UV detection
(42 % @ 350 nm)

quartz tube lens



modified microscope body with glycerine immersion quartz objective for UV

UV Microscopy Applications: Intrinsic Fluorescence

Label-free detection of molecules

Measuring intrinsic fluorescence from proteins

- native occurrence of detected species
- no labeling strategy needed
- low quantum efficiencies

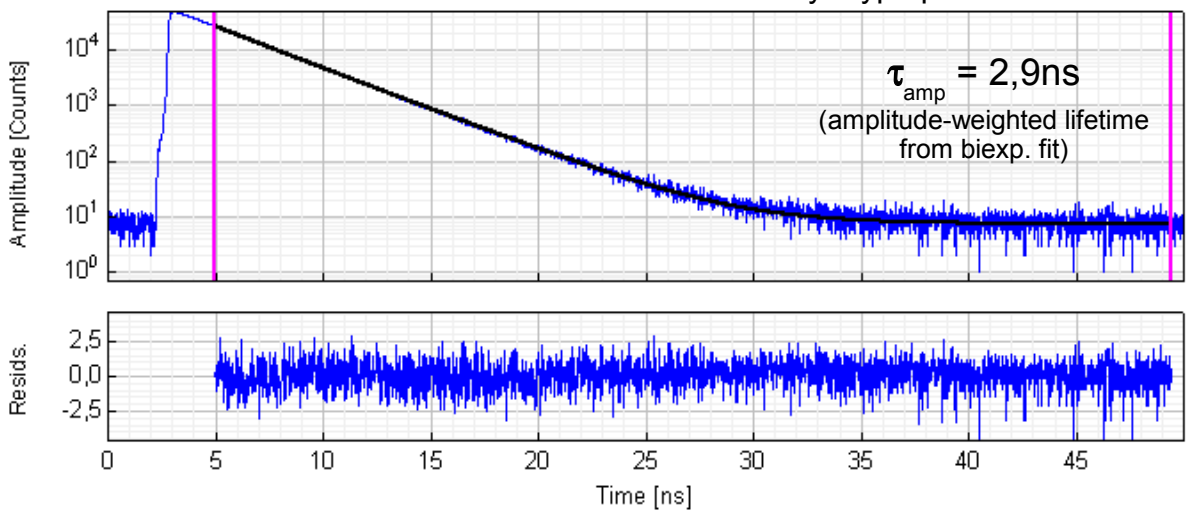
Natural fluorophores

- excitation with 266 nm
  → aromatic amino acids in proteins:
  - tryptophan (e.g. NATA)
  - tyrosine
  - (phenylalanine)
- excitation with 355 nm
  - enzyme cofactors NADH/NADPH
  - collagen, elastin

Excitation of several natural fluorophores in biological samples

- identification with the aid of fluorescence lifetimes

N-acetyl-tryptophanamide **NATA**

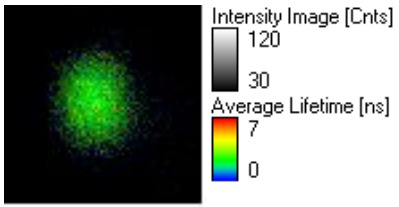


- Excitation: 266 nm (PicoQuant), 20 MHz
- Quartz objective 40x, NA 1.25, glycerin
- Optical filters: Z266RDC, longpass 300 nm
- Detection: ultrabialkali PMT

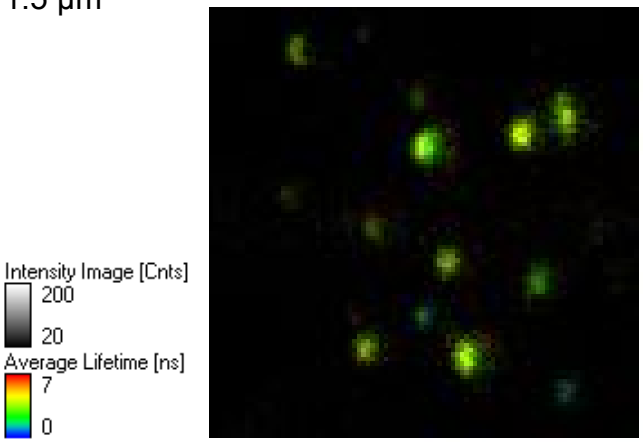
UV MicroTime 200: Label-free FLIM

266 nm grants access to the intrinsic fluorescence of tryptophan-containing proteins

FLIM with streptavidin-coated beads (Ø 500 nm, 1 streptavidin = 24 tryptophans), immobilized on quartz coverslips

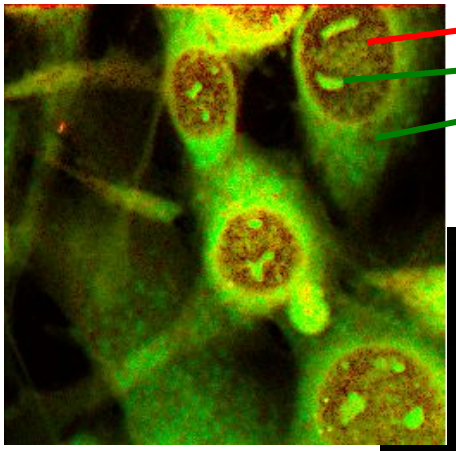


1.5 x 1.5 µm

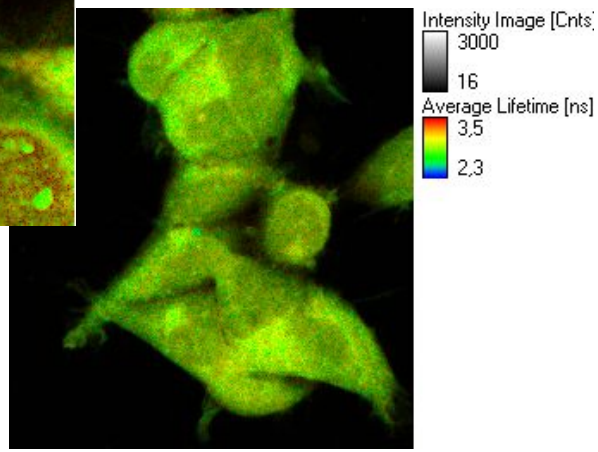


13 x 13 µm

Label-free FLIM with biological cells → aromatic amino acids (mainly tryptophans) within the proteins become visible



3T3-L1 cells, fixed (methanol) 80 x 80 µm



HEK293 cells, fixed (paraformaldehyde) 73 x 79 µm

- Excitation: 266 nm (PicoQuant), 20 MHz, < 100 ps pulses
- Quartz objective, 40x, NA 0.6, glycerine
- Optical filters: Z266RDC, longpass 300 nm
- Detection: Ultrabialkali PMT (42 % @ 350 nm)

Sample courtesy of Astrid Tannert, University of Leipzig, Germany

UV MicroTime 200: FCS

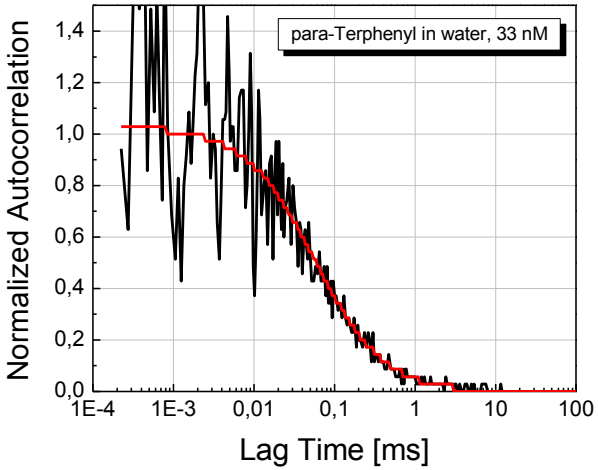
Benchmark: FCS in the deep UV

No commercially supported application

Dyes with high quantum efficiency in the UV: laser dyes as single emitters

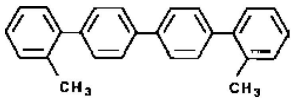
para-Terphenyl

Φ = 0.93

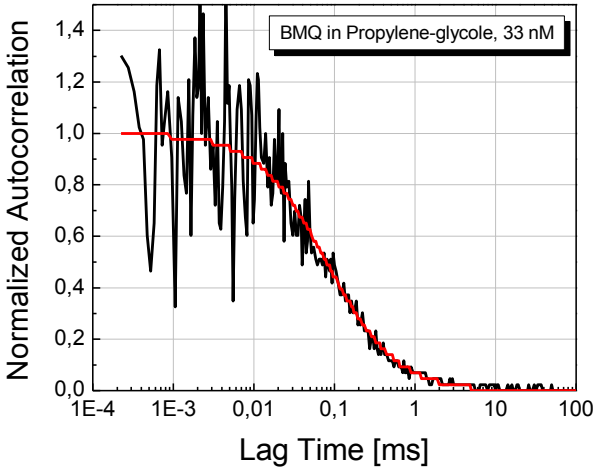


Diffusion time: τ(diff) = 57 µs
Molecular brightness: 510 Hz per molecule
Ca. 220 µW, 10 min data acquisition

BMQ (2.2'''-Dimethyl-p-quaterphenyl)



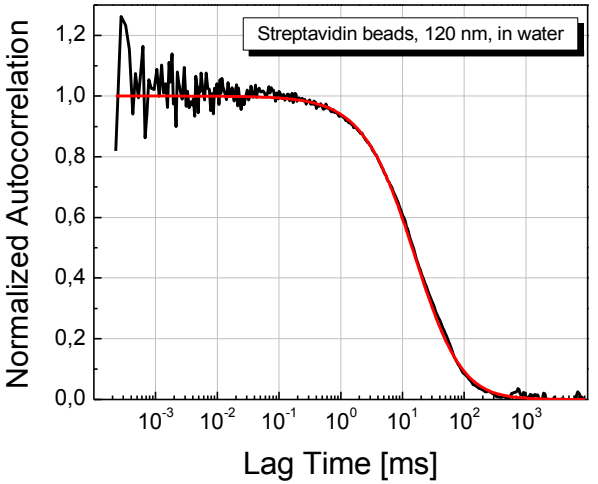
Φ = 0.89



Diffusion time: τ(diff) = 79 µs
Molecular brightness: 460 Hz per molecule
Ca. 280 µW, 15 min data acquisition

Streptavidin-coated beads (Ø 120 nm)

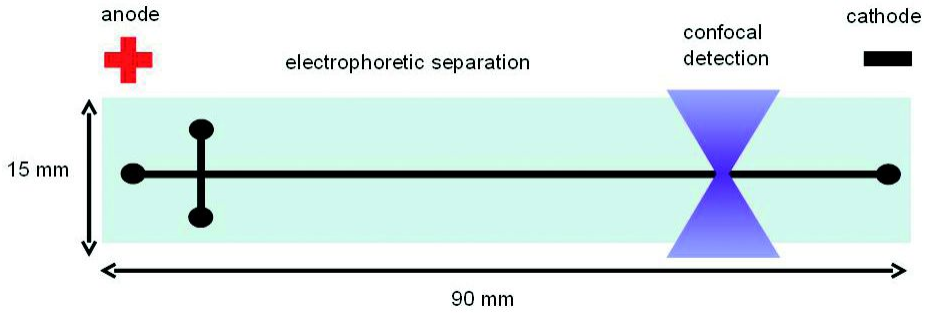
Approx. 1000 streptavidin per bead
(1 streptavidin = 24 tryptophans)



Diffusion time: τ(diff) = 16 ms
Molecular brightness: 1.2 kHz per bead
Ca. 200 µW, 5 min data acquisition

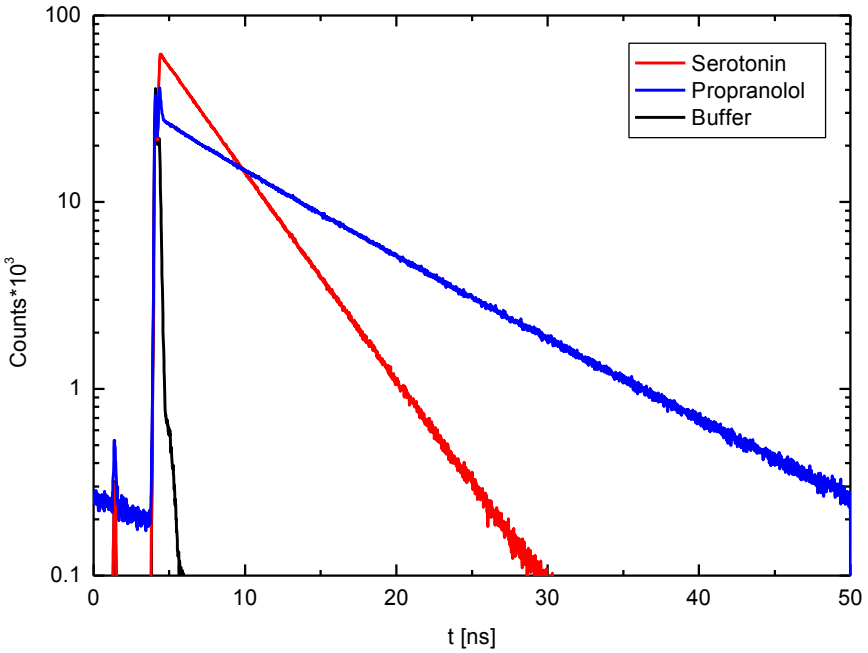
- Excitation: 266 nm (PicoQuant), 20 MHz, < 100 ps pulses
- Quartz objective, 40x, NA 0.6, glycerine
- Optical filters: Z266RDC, longpass 300 nm
- Detection: Ultrabialkali PMT (42 % @ 350 nm)

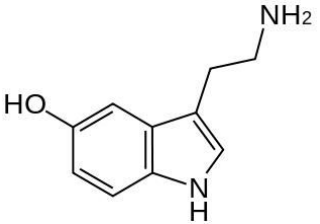
Microchip Electrophoretic Separation and Label Free Detection of Aromatic Analytes



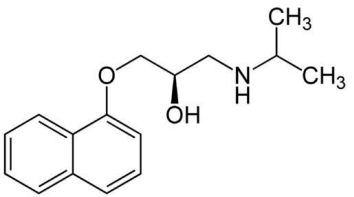
Substances in the microchannel (20 x 50 micron) are separated in the electric field and detected with a confocal microscope set-up.

Fluorescence decay curves are gathered on-the-fly and average lifetimes can be determined for each substance separately.





Serotonin



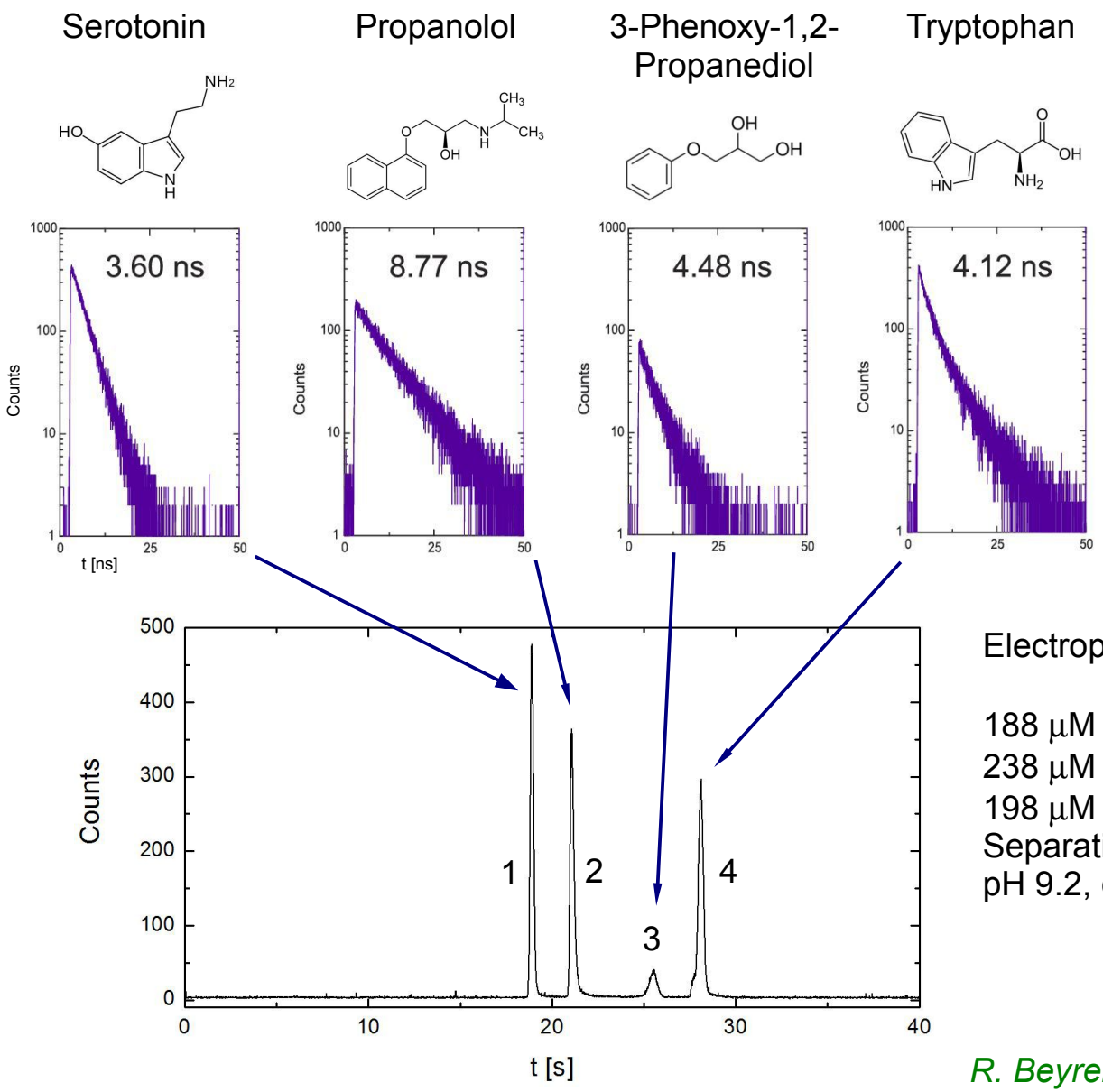
Propanolol

Time-resolved fluorescence decays of **serotonin** and **propanolol** (concentrations 250 µM in buffer, Integration time 60 s).

Max. 5 % deviation in lifetime determinations when compared to measurements in a mixture.

R. Beyreiss et al., Electrophoresis 2011, 32, 3108-3114

Electropheretic Separation of Small Aromatics



- Excitation: 266 nm (Cougar, Time Bandwidth), 20 MHz, 10 ps pulses
- Quartz Objective, 40x, NA 0.8
- Optical filters: z266RDC, bandpass 350/50, DUG11x (285-365 nm)
- Detector: super-bialkali PMT

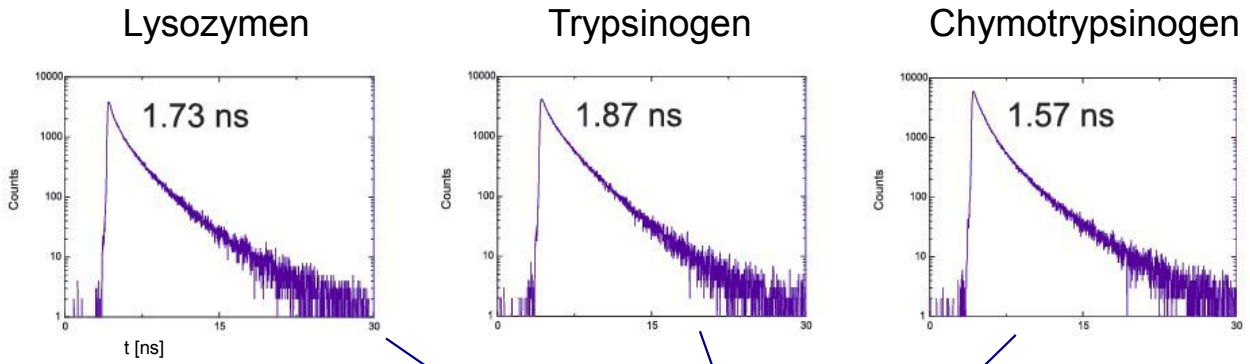
Fluorescence decays

Electropherogram:

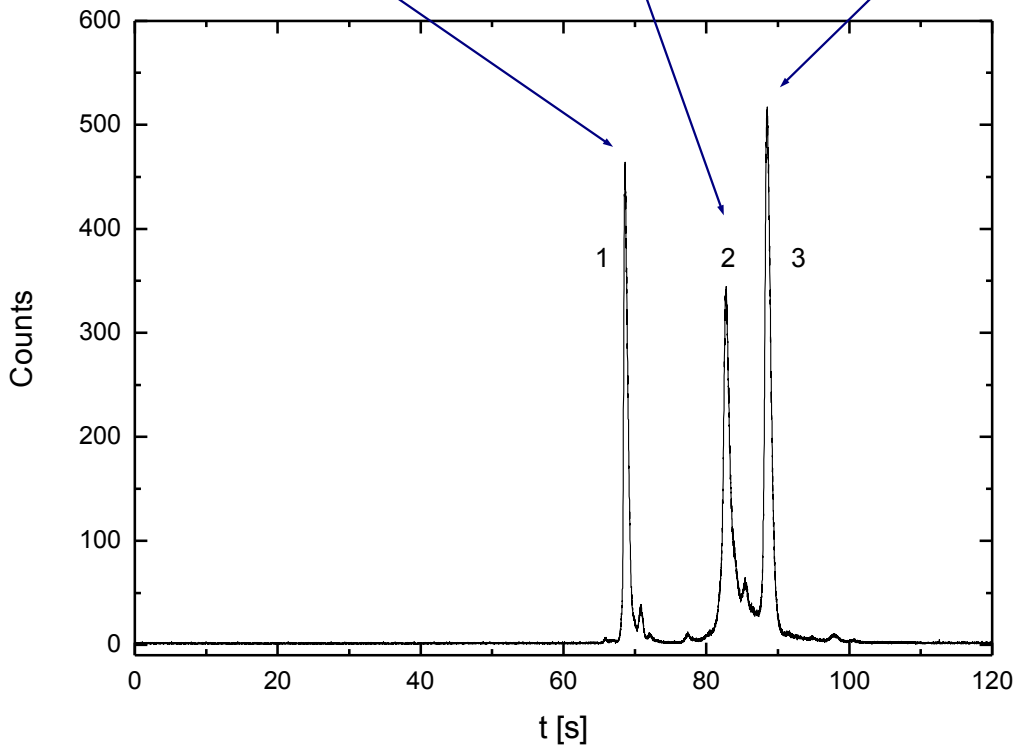
188 µM serotonin (1), 135 µM propanolol (2),
238 µM 3-phenoxy-1,2-propanediol (3) and
198 µM tryptophan (4).
Separation Buffer: 5 mM borate,
pH 9.2, effective separation length 5.0 cm

R. Beyreiss et al., Electrophoresis 2011, 32, 3108-3114

Electrophoretic Separation of Proteins



Fluorescence Decays



Electropherogram

23.3. µM lysozyme (1), 13.9 µM trypsinogen (2) and 13.0 µM chymotrypsinogen (3).
Separation buffer: 40 mM phosphate, pH 3.0
Microfluidic Channels were coated with 0.01 % w/v hydroxypropylmethylcellulose.
Effective separation length: 4.5 cm

- Excitation: 266 nm (Cougar, Time Bandwidth) 20 MHz, 10 ps pulses, 5nJ
- Quartz Objective, 40x, NA 0.8
- Optical filters: z266RDC, bandpass 350/50, DUG11x (285-365 nm)
- Detector: super-bialkali PMT

R. Beyreiss et al., Electrophoresis 2011, 32, 3108-3114

Further Informations

See specifications on our website or in the brochure.

Check our website for training courses on FLIM, FCS and Time-Correlated Single Photon Counting!

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

For further info on:

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- ➔*Possible configurations*
- ➔*Pricing information*

please contact PicoQuant at info@picoquant.com