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
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
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

- Computer with data acquisition and analysis software
- Laser head
- TCSPC
- Laser driver
- Scan controller
- Detector
- Scanning (optional)
- Excitation
- MicroTime 100
Excitation Configurations

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)

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

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

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

Single pulsed diode laser

Manually controlled laser driver unit (PDL 800-D)
Detection Efficiency

Detection efficiency [%] vs. Wavelength [nm]

- 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
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
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
$\lambda_{\text{exc}} = 485 \text{ nm}, 20 \text{ MHz}$
$\lambda_{\text{det}} = 530 \text{ nm} - 550 \text{ nm}$
Hybrid-PMA detector
Pixel time: 5 ms
Acquisition 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
\( \lambda_{\text{Exc}} \): 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

→ MicroTime 100 enables for phosphorescence lifetime imaging.

**TimeHarp 260 PICO, long range mode**
- Max. time range > s
- Dead time < 2.5 ns
- Minimum channel width 2.5 ns
Protein Content Determination in Proteomics

Cell → Protein extract → Dye labeling → 2D gel electrophoresis → Fluorescence imaging → Data analysis

- Typically 10,000-20,000 different proteins
- Typical abundances: pg/ml → 50 mg/ml → 10 orders of magnitude

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

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

Protein separation via a 2D gel

- Isoelectric point (pI)
- Molecular weight
- 2D gel plate: about 8 x 10 cm²
Application 4: Protein Content Determination in Proteomics
Pulsed Excitation for Background Identification

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

- 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: \( I(t) = \sum_{i=1}^{n} A_i e^{-\frac{t}{\tau_i}} \)

\( \tau_1 = 0.18 \text{ ns (scatter)} \)
\( \tau_2 = 1.07 \text{ ns} \leftarrow \text{Cy2 label} \)
\( \tau_3 = 5.00 \text{ ns (autofluorescence)} \)

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

Intensity [counts]

Distance [mm]

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

Before activation step

Intensity image

Lifetime image

After activation step

Count rate (top) and lifetime (bottom) distributions

$\lambda_{\text{exc}} = 635 \text{ nm, 40 MHz}$

$\lambda_{\text{det}} = 814 - 870 \text{ nm}$

$\tau$-SPAD detector

Collaboration with Christian Kraft, University of Jena, Germany
Application 5: TRPL for Semiconductor Analysis
Device Architecture Characterization

- $\lambda_{\text{exc}} = 635 \text{ nm, } 40 \text{ MHz}$
- $\lambda_{\text{det}} = 814 \text{ nm - 870 nm}$
- $\tau$-SPAD-Detector

Collaboration with Christian Kraft, University of Jena, Germany

$\tau_{(\text{ROI 1})} = 0.13 \text{ ns}$
$\tau_{(\text{ROI 2})} = 0.4 \text{ ns}$

Lifetime increases from air interface to the $pn$-junction
Bright areas don't have necessarily the longest lifetime
Application 5: TRPL for Semiconductor Analysis

Excitation Intensity Dependence

The lifetimes of semiconductor materials depend on excitation intensity.

Sample: GaAs-based Quantum Well

Adapted MicroTime 100

- $I_{\text{exc}} = 635 \text{ nm}, 40 \text{ MHz}$
- $I_{\text{det}} = > 664 \text{ nm}$
- 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

Further Information

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

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