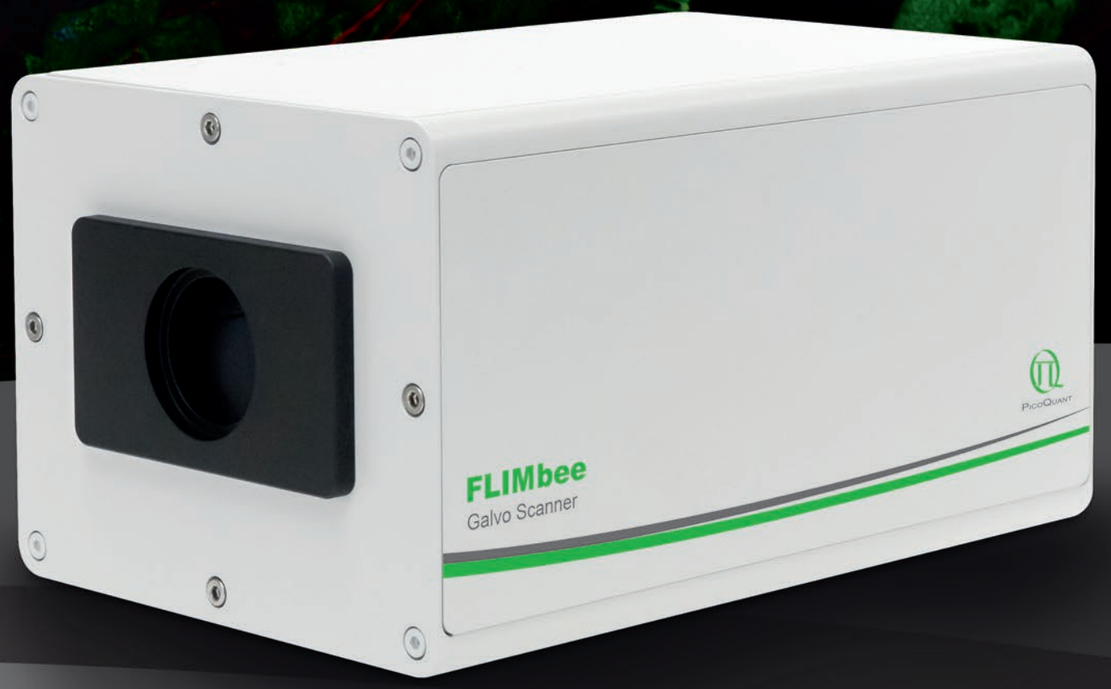
PicoQuant

FLIMbee

Galvo scanner for the MicroTime 200



PicoQuant GmbH
Rudower Chaussee 29 (IGZ)
12489 Berlin
Germany
Phone: +49 30 1208820-0
info@picoquant.com
www.picoquant.com

Published by: PicoQuant GmbH
April 2017
Editorial and production: Pink Lemon Werbeagentur
Tina Stundner, Berlin
Photos: PicoQuant GmbH

FLIMbee Scanner

Fast imaging add-on for the MicroTime 200

The FLIMbee galvo scanner provides a fast scanning option for the MicroTime 200, enabling imaging of quickly changing samples and fas processes. In combination with the novel rapidFLIM technique it is even possible to acquire several FLIM images per second. This unique approach opens up the door to exploring the exciting and dynamic processes of life in much higher detail than before.

The MicroTime 200 platform is the most flexible and extensible high-end research tool for experiments requiring single molecule sensitivity. Over the last 15 years, the platform has evolved continuously, keeping pace with the latest developments in microscopy and spectroscopy. Today, it is used in life and materials science laboratories all over the world. FLIM, STED, and FCS are only a few of the many techniques that are supported by the MicroTime 200.

Fast scanning

The FLIMbee add-on expands the MicroTime 200 platform with fast scanning capabilities. It allows recording FLIM images of 512 × 512 pixels with a speed of 5 frames per second. Such a high scan speed significantly reduce overall measurement times and can help in preventing phototoxicity and photobleaching.

Expand and adapt

The MicroTime 200 can be fitted with multiple exit ports guiding fluorescence light to external devices. These ports can be equipped with a wide range of beam splitters, filters, and free-space or fiber coupling outputs, thus allowing for maximum flexibility in choosing, for example, spectrographs or special detectors to be interfaced.

FLIM

Fluorescence lifetime imaging probes differences in excited state lifetime of labels inside cells and tissues and can reveal information about the environment such as, co-localization, ion concentrations, or pH value. Imaging fast interactions in live cells is possible with the FLIMbee add-on.

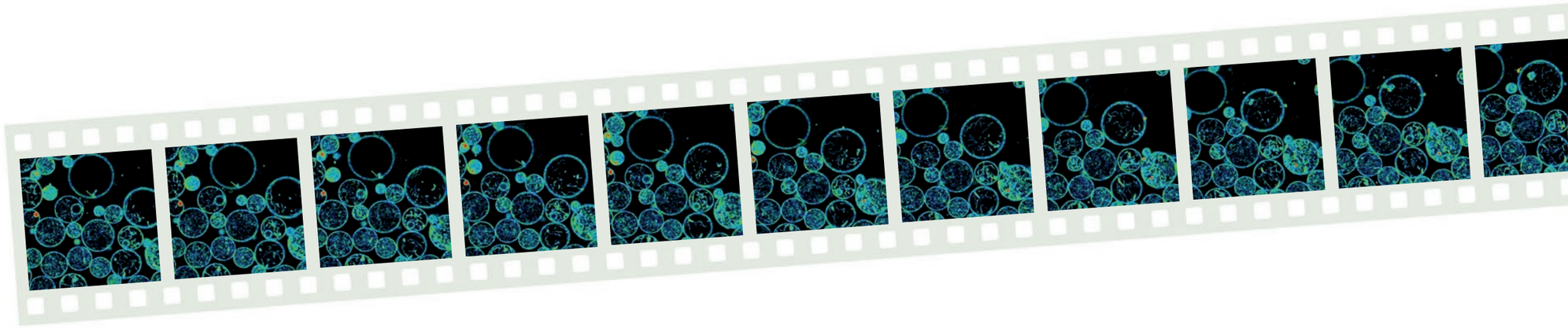
FCS

Fluorescence correlation spectroscopy is a powerful tool to study the diffusion, interaction, or binding of molecules and macromolecular complexes. By combining it with time-resolved fluorescence, artifacts can be quickly removed and data quality improved.

STED

The MicroTime 200 STED add-on provides the opportunity to study samples at spatial resolutions beyond the diffraction limit. STED can be applied to both FLIM and FCS measurements, providing a greatly increased spatial information for your samples.

FLIMbee for ...



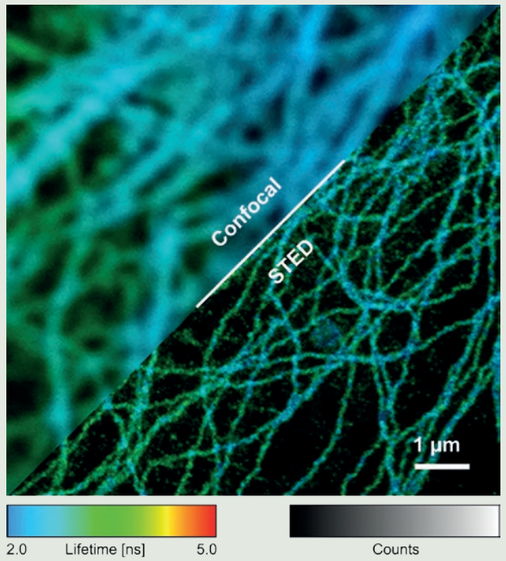
rapidFLIM

Unilamellar vesicles can be produced in different sizes ranging from giant (GUVs), to large (LUVs) and even down to small (SUVs). They are a very powerful model for investigating, e.g., the formation of microdomains or the organization of proteins in membranes. The flexibility in the composition of a vesicle membrane allows introducing specifically labeled lipids, thus increasing their importance in biophysical studies using highly sensitive lifetime measurement techniques. Both the fast processes occurring within the vesicle membranes and their high mobility require very fast lifetime imaging to prevent information loss. Thanks to the FLIMbee scanner add-on and rapidFLIM approach, multiple FLIM images per second can be acquired, making it possible to accurately observe and analyze both unilamellar vesicles as well as processes occurring in their membranes.

The series of FLIM images of Giant Unilamellar Vesicles (GUVs) shown above reveals information on vesicle movement and the fluorescence lifetime of dyes embedded in the membranes. The vesicle membranes contain the dyes rhodamine and NBD. Slight variations in fluorescence lifetime can be observed since the dye ratio is not constant over the surface. The image sequence was acquired with a frame rate of 6 images per second.

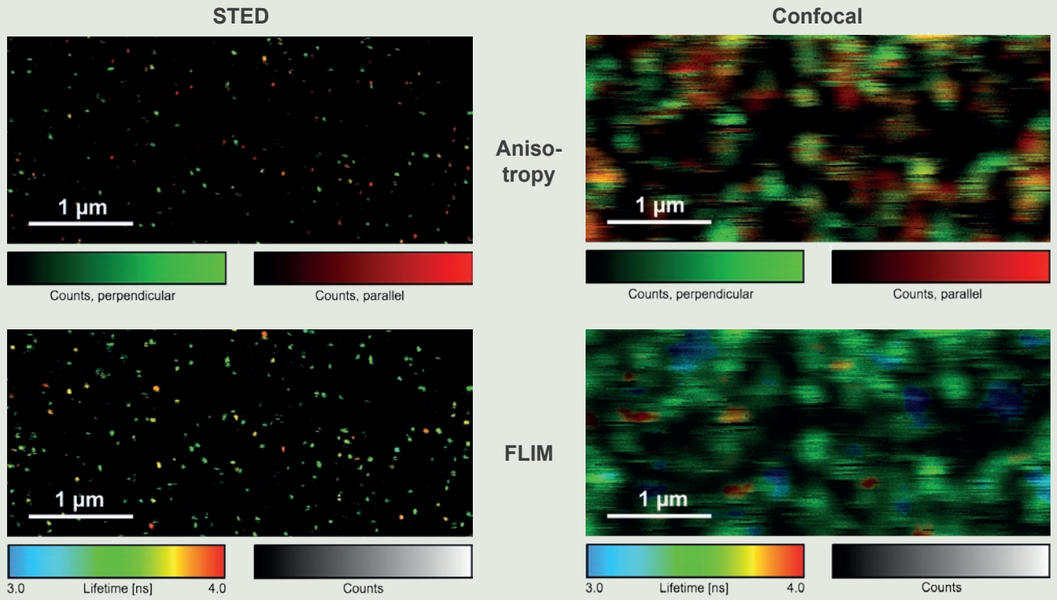
STED

Super-resolution STED imaging is now a well established tool in molecular biology. Single molecules, cells, and tissue samples can be investigated using a wide variety of parameters, like emission color, polarisation, and fluorescence lifetime. With the MicroTime 200 STED, super-resolution FLIM images can be easily recorded while the high scan speed of the FLIMbee helps reducing phototoxicity and bleaching during STED imaging.
The example shows an image of the microtubule network in an adherent cell. The higher resolution of STED allows studying the interaction within the network in much more detail.



Single molecule imaging

Detecting the emission of single molecules is important in biochemistry, drug development, and fundamental research. Single molecule sensitive systems aim at minimizing the number of optical elements to maximize light throughput, which is why piezo scanning systems were commonly used. With the FLIMbee add-on, the MicroTime 200 retains its outstanding single molecule sensitivity, but now with a much higher scanning speed. The images shown in this example were obtained from single ATTO 655 molecules bound to a glass coverslip, imaged under STED and confocal conditions with polarization- and time-resolved data acquisition. By using Pulsed Interleaved Excitation (PIE), STED and confocal data can be acquired quasi simultaneously, making the analysis of blinking and bleaching of single molecules straightforward.

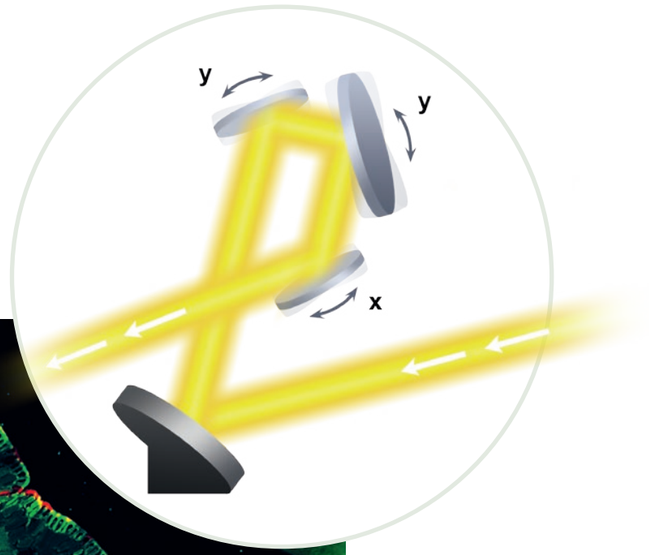


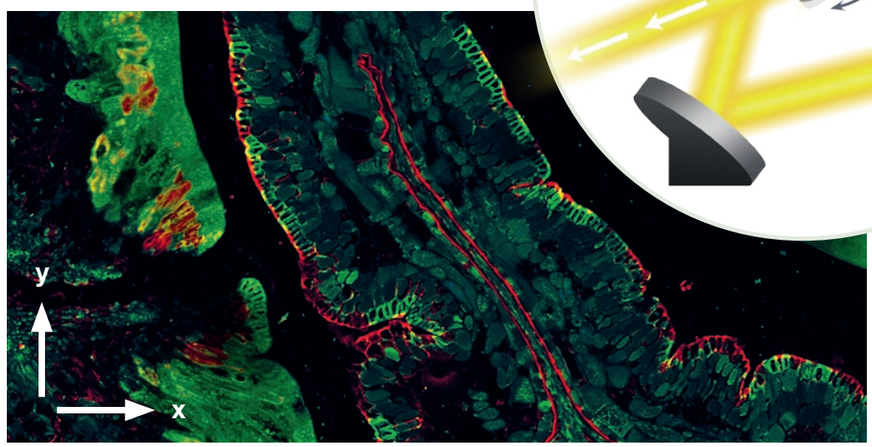
Scanning Technologies

The great versatility of the MicroTime 200 platform is complemented by the FLIMbee galvo scanner which can provide scanning speeds ranging from very slow to fast while maintaining high precision. This high degree of flexibility in speed allows for applications ranging from Phosphorescence Lifetime Imaging (PLIM) to fast fluorescence lifetime measurements using rapidFLIM. Furthermore, with its high precision and sensitivity, the FLIMbee scanner is optimally suited for super-resolution microscopy via STED, enabling imaging down to the single molecule level.
A MicroTime 200 equipped with a FLIMbee scanner is a good choice for Single Molecule Detection (SMD) methods such as spFRET, PIE-FRET, (STED-)FCS, FLCS, FLCCS, dual-focus FCS (2fFCS), and even anisotropy measurements. Additionally, Two-Photon Excitation (TPE) with descanned and non-descanned detection is possible.

The core of the FLIMbee galvo scanner consists of three high precision oscillating mirrors with excellent linearity, repeatability and low drift. The two y-axis galvo mirrors ensure that the laser beam is stationary at the entrance of the objective. This mirror configuration minimizes vignetting of the image field and ensures a constant focal volume over a wide scan range. The FLIMbee scanner provides a minimal pixel size of 10 nm when using a 100x objective.

Use of the standard piezo scanner is recommended for applications requiring light from the UV (255 to 400 nm) and NIR (1100 to 1400 nm) spectral regions or when pixel sizes smaller than 10 nm are desired.





FLIMbee Specifications*

Optical system
- Suitable for wavelengths ranging from 405 to 1064 nm
- Compatible with two-photon excitation
- Laser blanking for reduced photobleaching (with LDH-D-C and LDH-P-C series lasers)
- STED resolution below 50 nm
- Suited for imaging (FLIM, FRET, STED) and correlation spectroscopy (FCS, FCCS, FLCS)

Objectives
- Various high-end objectives available (oil/water immersion, air-spaced, IR/UV enhanced, TIRF, or long working distance)
- Objective turret
- Software field-of-view calibration for custom objectives

Scanning
- Image size ranging from 10 × 10 to 2048 × 2048 pixel
- Maximum field-of-view: 250 × 250 µm (60x objective)
- Up to 2.6 kHz line frequency (bi-directional scanning), 5.2 FPS @ 512 × 512 pixel
- Optional z-axis control e.g., for z-stacks (piezo-based, up to 100 µm)
- Pixel dwell times from 0.5 µs up to 1 s

Software
- Automated measurement modes such as time-lapse, z-stack, or multi-point
- Step-by-step field-of-view calibration
- Intuitive hardware control features
- User-profiles for individual hardware settings

	FLIMbee	Piezo-Scanning
FLIM imaging	250 × 250 µm (60x objective) 1.5 x 1.5 mm (10x objective)	80 × 80 µm (independent of objective magnification)
STED-imaging	40 × 40 µm (100x objective)	80 × 80 µm
Minimal pixel size	10 nm (100x objective)	1 nm (independent of objective magnification)
Shortest pixel dwell time	0.5 µs	0.2 ms
Wavelengths range	from 405 up to 1064 nm	from 266 to 1400 nm

*For MicroTime 200 specifications, please refer to the respective brochure.