Fast molecular tracking maps nanoscale dynamics of plasma membrane lipids

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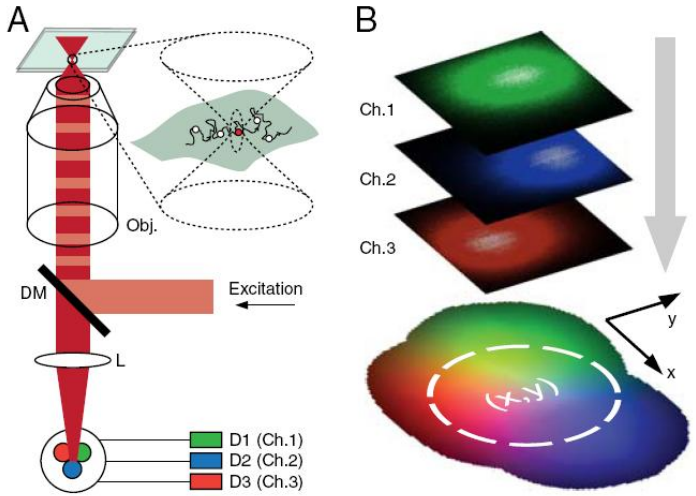
Edited by W. E. Moerner, Stanford University, Stanford, CA, and approved March 1, 2010 (received for review November 8, 2009)

We describe an optical method capable of tracking a single fluorescent molecule with a flexible choice of high spatial accuracy (~10–20 nm standard deviation or ~20–40 nm full-width-at-half-maximum) and temporal resolution (<1 ms). The fluorescence signal during individual passages of fluorescent molecules through a spot of excitation light allows the sequential localization and thus spatio-temporal tracking of the molecule if its fluorescence is collected on at least three separate point detectors arranged in close proximity. We show two-dimensional trajectories of individual, small organic dye labeled lipids diffusing in the plasma membrane of living cells and directly observe transient events of trapping on <20 nm spatial scales. The trapping is cholesterol-assisted and much more pronounced for a sphingo- than for a phosphoglycero-lipid, with average trapping times of ~15 ms and <4 ms, respectively. The results support previous STED nanoscopy measurements and suggest that, at least for nontreated cells, the transient interaction of a single lipid is confined to macromolecular dimensions. Our experimental approach demonstrates that fast molecular movements can be tracked with minimal invasion, which can reveal new important details of cellular nano-organization.

single molecule | tracking | membrane dynamics | lipid rafts



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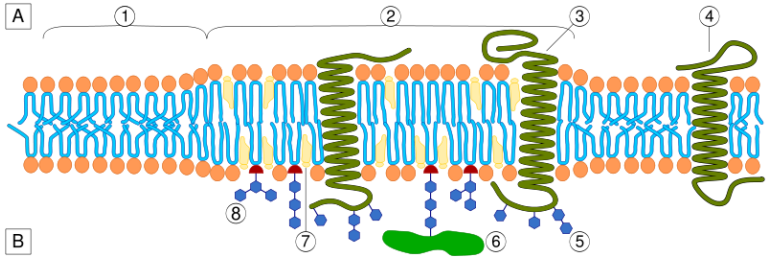
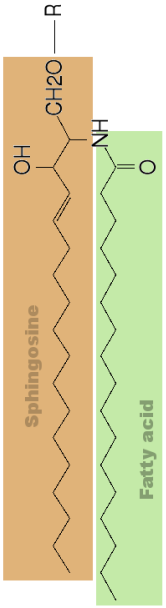
Journal Club

Lipid laft

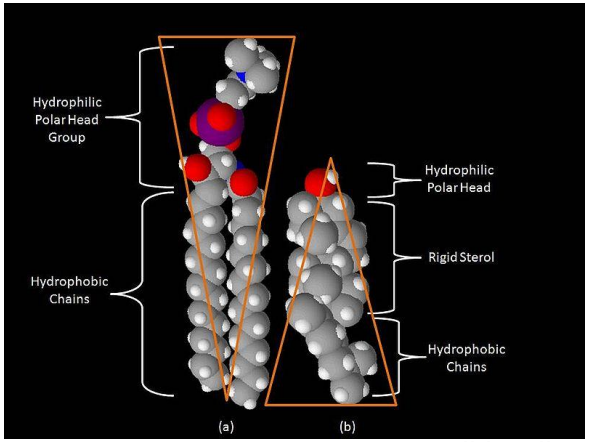
Standard
lipid bilayer

lipid laft





Wikipedia



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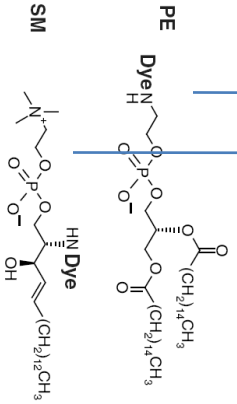


~20% of the plasma membrane

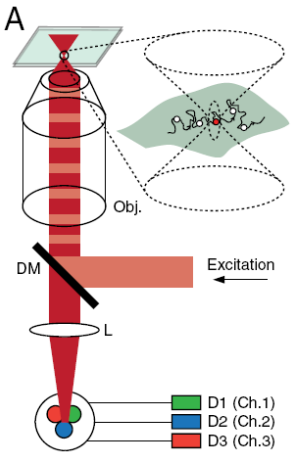
~10% of the plasma membrane

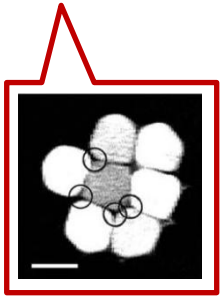
Lipid lafts are commonly defined as cholesterol- and sphingolipid-enriched membrane microdomains that function as platforms that concentrate and segregate proteins within the plane of the bilayer.

Stuided by direct imaging(hard), smFRET, FRAP, single particle tracking, FCS …

Neuropharmacology **55** 1265

Methods & Materials



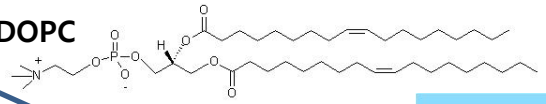


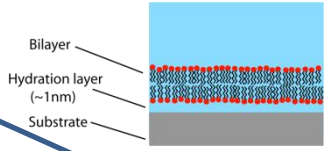
Scale bar : 125um

HeNe laser : 5~20uW 633nm (Melles Griot)
100x NA=1.4 Oil immersion (Leica)
APD (SPCM-AQR-13, Perkin elmer)
TCSPC (SPC-830, Becker&Hickl)
Router (HRT-82, Becker&Hickl)
Fiber : multimode, 100um core diameter
Closed-loop piezostage (for reference)

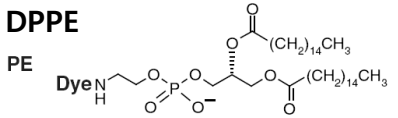
Supported Lipid bilayer

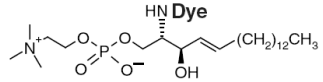
DOPC

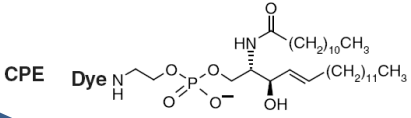


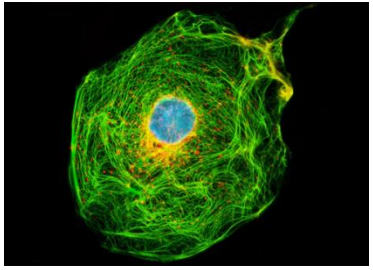


DPPE



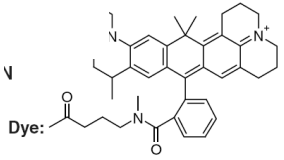




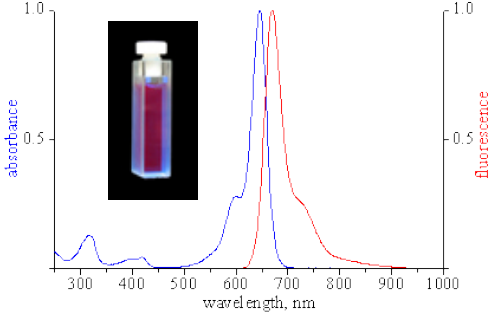


PtK2 Cell

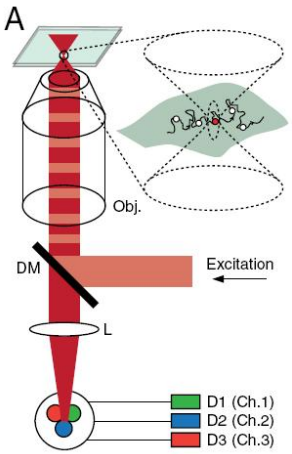
Incorporate

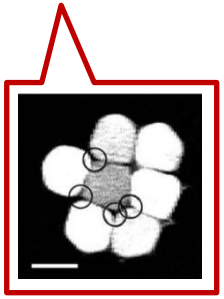


Atto647N

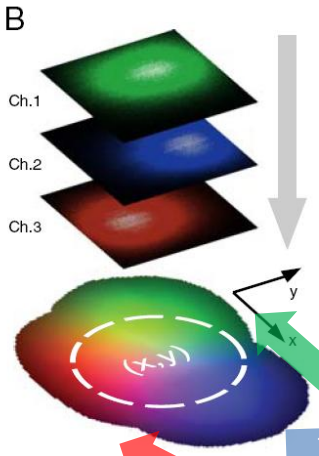


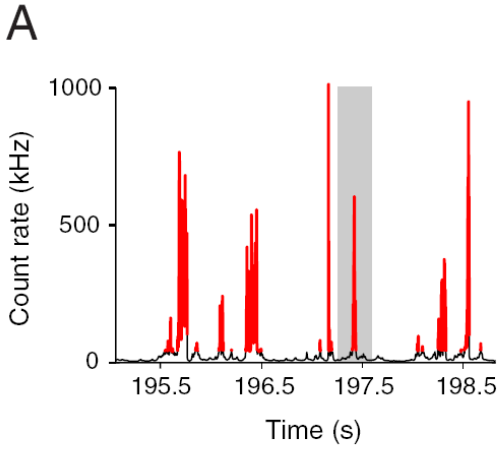
Direct Access to Biomolecular Dynamics by Fast Tracking

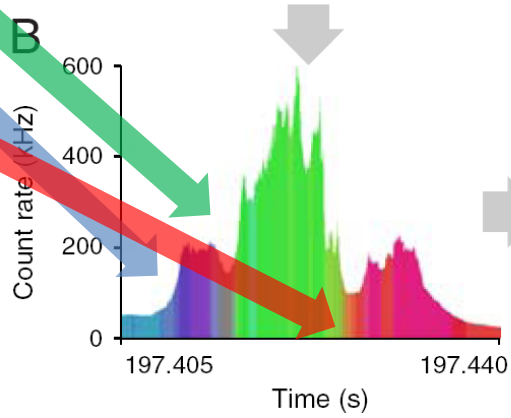


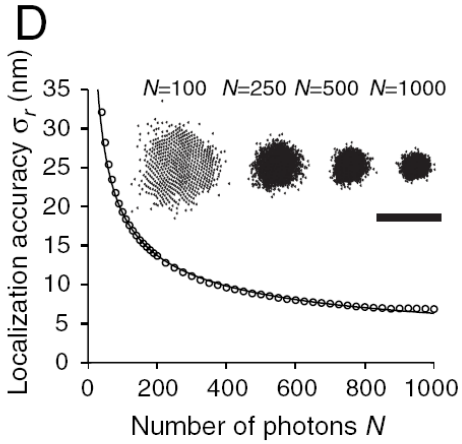


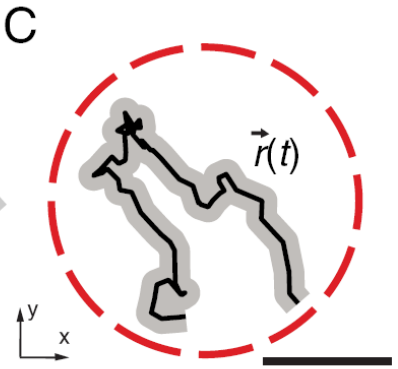
o : candidates for the fiber bundle



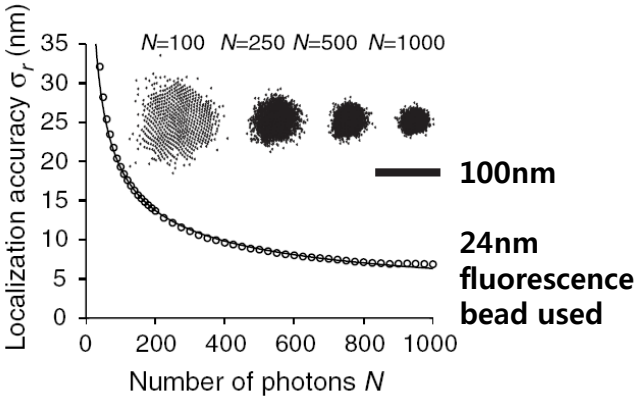


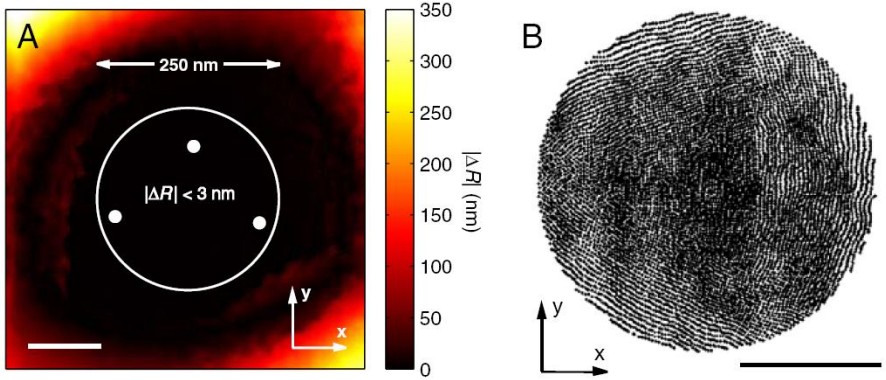


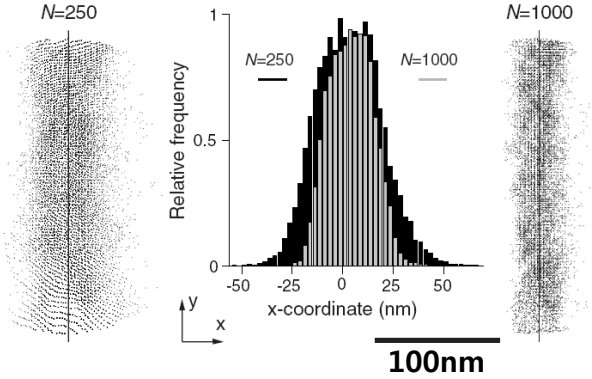


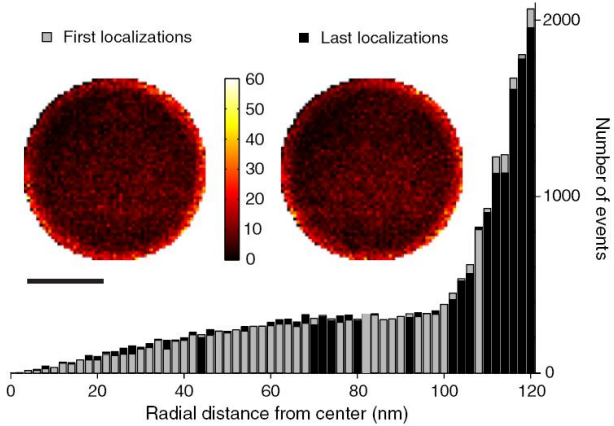


Localization Performance



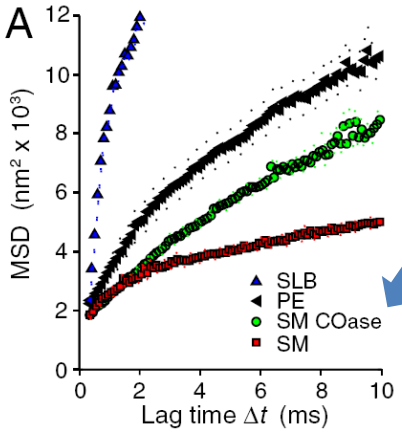






Photostability of the fluorophore

Analysis of Lipid Trajectory



Mean Square Displacement

Cholesterol oxidase

From Wikipedia, the free encyclopedia

In enzymology, a **cholesterol oxidase** (EC 1.1.3.6) is an enzyme that catalyzes the chemical reaction

cholesterol + O_2 ⇌ cholest-4-en-3-one + H_2O_2

Analysis of Lipid Trajectory

Cumulant Probability

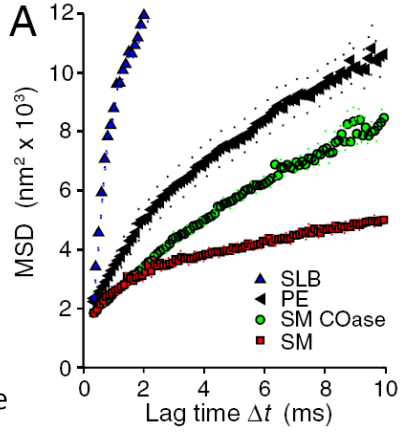
P(Δr², Δt) : probability that a molecule will be found within a circle of radius Δr from its starting point after time Δt

Not only to the **mean** but to the **full probability information**

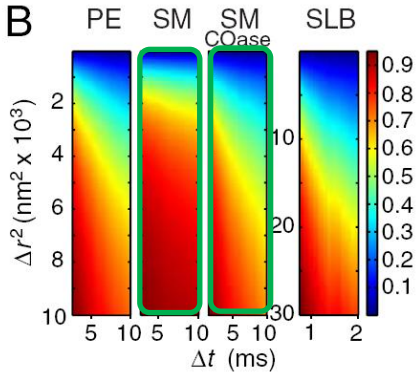
Directional flow
Lateral heterogeneities
Lateral constraints
.
.
.

Multi-component mobility

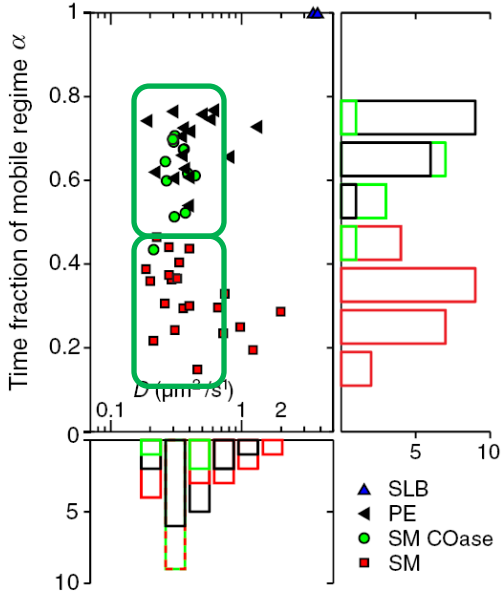
Biophys. J. **73** 1073



Mean Square Displacement



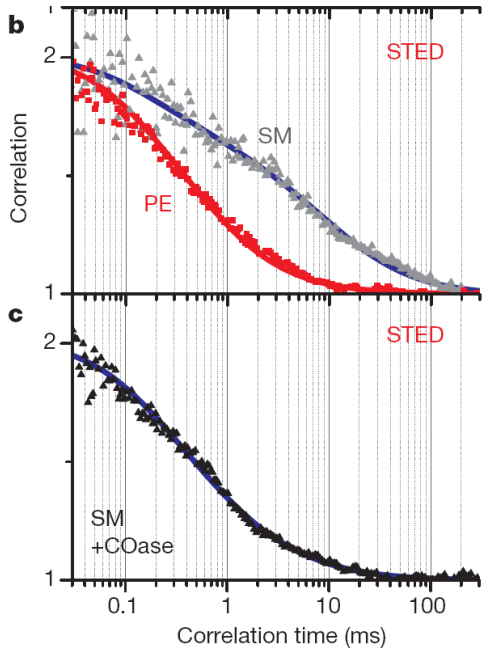
Cumulant Probability

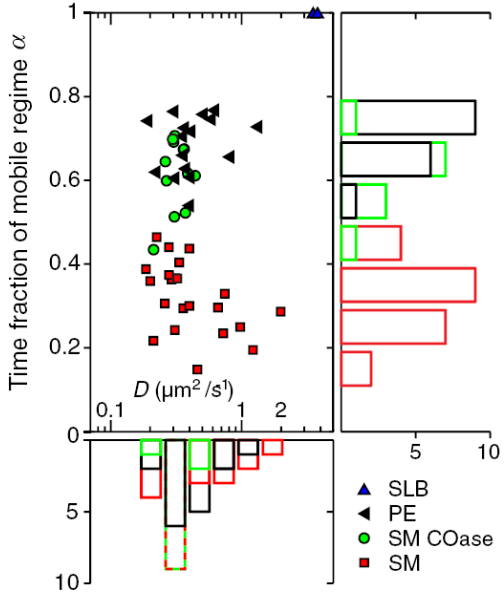


$$P(\Delta r^2, \Delta t) = \alpha\left[1 - \exp\left(\frac{-\Delta r^2}{4\sigma_r^2 + 4D\Delta t}\right)\right] + (1-\alpha)\left[1 - \exp\left(\frac{-\Delta r^2}{4\tilde{r}_{\text{trap}}^2}\right)\right]$$

Free Brownian Motion **Hindered Motion**

Results, and a comparison with STED-FCS





	$\alpha_{STED\text{-}FCS}$	$\alpha_{fasttracking}$
PE	1	0.7
SM	0.36	0.3
SM+COase	0.85	0.65

	D (μm²/s)	α	r_{trap} (nm)	r^*_{trap} (nm)	$\bar{t}_{trap}$ (ms)
SLB	3.6	1	-	-	-
PE	0.4	0.7	11	3	3
SM	0.35	0.3	6	3	17
SM+COase	0.3	0.65	9	3	3

Conclusion

High spatial localization accuracy (10-20nm)
Temporal resolution (0.5ms)

Applicable to lifetime, color, polarization anisotropy …

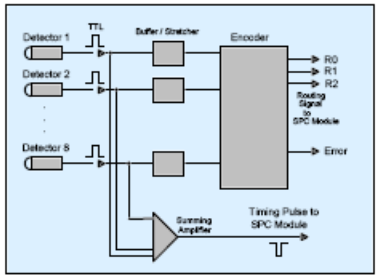
Disadvantage is the limited observation area (~250nm)
-> limiting the length of the trajectories observed

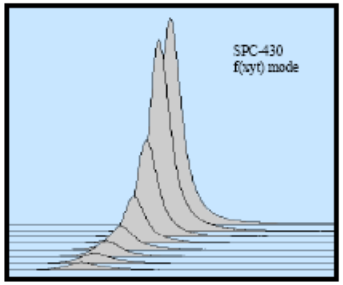
HRT-82

8 Channel TCSPC-Router for APD Modules

- Connects up to eight separate APD modules to one bh TCSPC module
- Simultaneous measurement in all detector channels
- Applicable with SPCM-AQR Modules and other TTL Output Detectors
- Count Rate > 3 MHz

The HRT-82 module is used to connect up to eight individual avalanche photodiode (APD) detectors to one of the time-correlated single photon counting modules SPC-xx0. The photons from the individual detectors are routed into different curves in the SPC memory. Thus the measurement yields a separate decay function for each of the detectors. Typical applications are fluorescence depolarisation measurements or simultaneous decay measurements at different waveleghts.





HRT-82

Specification

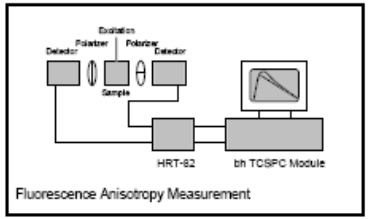
Input Polarity	positive
Input Voltage	TTL, 1.2 V to 5 V
Input Threshold	adjustable from 0.1 V to 2 V
Input Impedance	50 Ω
Input Pulse Duration	8 ns to 60 ns
Input Connectors	SMA
Timing Output Polarity	negative
Timing Output Voltage (2.5 V Input)	120 mV or 60 mV into 50 Ω (Jumper)
Timing Output Impedance	50 Ω
Timing Output Connector	50 Ohm, SMA
Delay Difference between Channels	max. 60 ps per Channel
Routing-Signal	TTL 3 bit + Error Signal
Routing Signal Connector	15 pin Sub-D/HD
Power Supply	+5V, -5V, via Sub-D Connector from SPC Module
Dimensions	120mm × 95mm × 34mm

Output Voltage Configuration

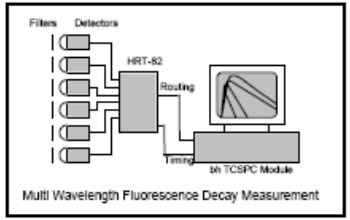
Vout = 120 ... 150 mV mV (SPC-x30)

Vout = 50 ... 60 mV (SPC-x00)

Applications



Fluorescence Anisotropy Measurement



Multi Wavelength Fluorescence Decay Measurement

Also available: HRT-41 4 Channel and HRT-81 8 Channel Routers for PMTs and MCPs. Please see individual data sheets.