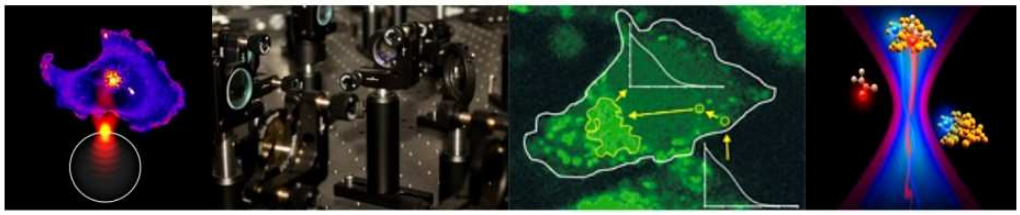
12th Carl Zeiss sponsored workshop on FCS and related methods



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12th Carl Zeiss sponsored workshop on Fluorescence Correlation Spectroscopy and related methods

12-16 October 2009, Cargese (Corsica, France)

Organizers

Hervé Rigneault, Cyril Favard, Patrick Ferrand, Jérome Wenger

Mosaic Group, Institut Fresnel, Marseille, France

Hai-Tao He, Didier Marguet

Centre d'Immunologie de Marseille Luminy, Marseille, France

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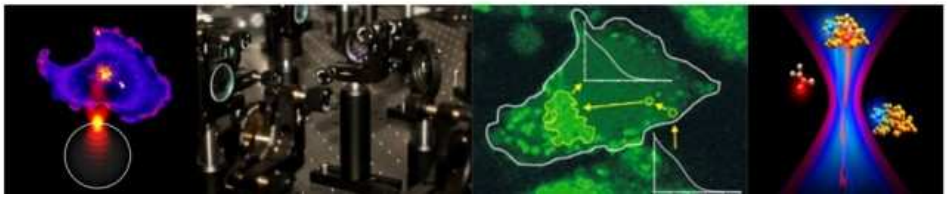
12th Carl Zeiss sponsored workshop on FCS and related methods 2009

	Monday 12 Oct	Tuesday 13 Oct	Wednesday 14 Oct	Thursday 15 Oct	Friday 16 Oct
09:00		Registrations starting 08:20			
09:20		Opening	Groves	Petrasek	Bus transfer Ajaccio airport
09:40		Rigler	Hamon	Kuhnemuth	
10:00		Langowski	Vereb	Wennmalm	
10:20		Coffee break	Coffee break	Coffee break	
10:40		Engelborghs	Enderlein	Mueller	
11:00					
11:20		Visser	Orthaus	Werner	
11:40		Wohland	Galland	Vukojevic	
12:00		Rigler	Octeau	Pfiffi	
12:20		Lunch	Lunch	Lunch	
12:40					
13:00					
13:20					
13:40					
14:00		Eggeling	Free Time	Weidtkamp-Peters	
14:20					
14:40		Jaffiol		Delon	
15:00	Bus transfer Ajaccio airport Shuttle 1	Wenger		Gall	
15:20		Coffee break		Coffee break	
15:40		Wiseman		Brock	
16:00					
16:20		Poster Session		Tramier	
16:40				Hendrix	
17:00				Closure	
17:20			Widengren		
17:40	Bus transfer Ajaccio airport Shuttle 2				
18:00			Sauer		
18:20					
18:40					
19:00			Conference Dinner		
19:20					

12th Carl Zeiss sponsored workshop on FCS and related methods



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Monday October 12th

Bus transfers from Ajaccio airport and installation in Cargese

Tuesday October 13th

08h20 : Registration desk opening

9h00-9h20 : H. Rigneault, Opening Introduction

9h20-10h00 : R. Rigler, APD-Imaging:FCS in Single Cell Kinetics

10h00-10h20 : J. Langovski, Generating mobility maps from two-dimensional fluorescence fluctuation data

10h20-10h40 : coffee break

10h40-11h20 : Y. Engelborghs, short sampling time fluorescence correlation spectroscopy reveals early oligomer formation of alpha-synuclein

11h20-11h40 : T. Visser, Global analysis of autocorrelation functions and photon counting distributions: some experimental tests involving EGFP and EGFP dimers

11h40-12h00 : T. Wohland, Advances in Fluorescence Correlation Spectroscopy: Quantitation of Biomolecular Interactions and Imaging of Membrane Dynamics

12h00-12h20 : P. Rigler, Towards single molecule enzymology of encapsulated enzymes

12h20-14h00 : lunch break

14h00-14h40 : C. Eggeling, Fluorescence Correlation Spectroscopy at the nanoscale: Revealing more details with STED microscopy

14h40-15h00 : R. Jaffiol, near-field fcs: driving biology beyond the diffraction limit

15h00-15h20 : J. Wenger, Nanophotonics to enhance FCS

15h20-15h40 : coffee break

15h40-16h20 : P. Wiseman, Recent Advances in Image Correlation Spectroscopy and its Applications in Cells

16h30-18h30 : Poster Session, *finger food & drinks kindly supported by PicoQuant*

PicoQuant

Wednesday October 14th

9h00-9h40 : J. Groves, Different acylation motifs direct multiply orthogonoal co-localization of lipid anchored proteins in live cell membranes

9h40-10h00 : Y. Hamon, Essential role for raft nanodomains in initiation of T cell receptor signalling upon antigen recognition

10h00-10h20 : G. Vereb, ligand-stimulated egfr causes lipid domain-dependent higher order aggregation and internalization of erbb2

10h20-10h40 : coffee break

10h40-11h20 : J. Enderlein, dual-focus fluorescence correlation spectroscopy

11h20-11h40 : S. Orthaus, New Methods for FCS: Fluorescence Lifetime Correlation Spectroscopy and Two-Focus FCS

11h40-12h00 : R. Galland, Multi-confocal fluorescence correlation spectroscopy for parallel multi-spot measurements in living cells

12h00-12h20 : V. Octeau, Photothermal Absorption Correlation Spectroscopy

12h20-14h00 : lunch break

14h00-17h20 : free time

17h20-18h00 : J. Widengren, transient state monitoring by fcs and related techniques

18h00-18h40 : M. Sauer, super-resolution fluorescence imaging wth standard fluorophores

19h00-21h00 : Conference Dinner, kindly supported by Carl Zeiss MicroImaging

Thursday October 15th

9h00-9h40 : Z. Petrasek, Applications of scanning FCS

9h40-10h00 : R. Kühnemuth, laser scanning microscopy with avalanche photodiodes

10h00-10h20 : S. Wennmalm, Inverse-Fluorescence Correlation Spectroscopy

10h20-10h40 : coffee break

10h40-11h20 : J. Mueller, Hetero-Species Partition Analysis Revels Binding Curve and Stoichiometry of Protein Interactions in Living Cells

11h20-11h40 : A. Werner, applications of fluorescence correlation spectroscopy in rna biochemistry

11h40-12h00 : V. Vukojevic, functional synthetic hox genes: imaging the activity and quantifying transcription factor-dna interactions in live cells

12h00-12h20 : D. Pfiffi, Improvement of Signal Strength and Photostability of Fluorophors by quenching triplet and radical states

12h20-12h40 : Drinks and finger food, kindly supported by IdQuantique

12h40-14h00 : lunch break

14h00-14h40 : S. Weidtkamp-Peters, Multiparameter Fluorescence Imaging Spectroscopy including Fluorescence Correlation Spectroscopy in living cells

14h40-15h00 : A. Delon, measuring numbers of fluorophores labeling cDNA in solution, with fluorescence correlation spectroscopy and continuous photobleaching.

15h00-15h20 : K. Gall, Concurrent Fluorescence Fluctuation and Electrophysiological recordings of the Apoptosis Inducer PorB

15h20-15h40 : coffee break

15h40-16h20 : R. Brock, Quantitative Analyses of Protein Interaction Networks

16h20-16h40 : M. Tramier, Dual color GFP and mCherry Fluorescence Lifetime Correlation Spectroscopy for protein interactions in live cells

16h40-17h00 : J. Hendrix, Cellular tunable focus FCS for the study of the molecular mechanism of LEDGF/p75 mediated chromatin tethering of HIV-1 integrase

17h00-17h10 : K. Weisshart, Concluding remarks

Friday October 16th

9h00 : shuttle bus departure to Ajaccio airport

Tuesday October 13th

APD-Imaging:FCS in Single Cell Kinetics

Rudolf Rigler

Department of Medical Biochemistry and Biophysics ,Karolinska Institutet,Stockholm and Laboratory of Biomedical Optics , EPFL, Lausanne.(rudolf.rigler@epfl.ch)

We have developed in collaboration with Carl Zeiss.Jena the use of Avalanche Photo Diodes for background free detection and imaging of single molecule processes in individual cells (1,2) I will discuss the analysis of kinetic processes in various parts of the cells including diffusion and chemical relaxation taking membrane receptors as well as production of cytoplasmic and nuclear proteins and their interaction with specific targets as examples . The use of Poisson analysis (Poisson Imaging) classical confocal FCS . and scanning FCS will be discussed.

(1)Vukojevic,V.et al. (2008) Quantitative single-molecule imaging by confocal laser scanning microscopy.PNAS ,105,18176–18181

(2) Rigler,R, (2009) FCS and Single Molecule Spectroscopy. Nobelsymposium 138 :Single Molecule Spectroscopy in Chemistry,Physics and Biology.Sånga Säby.

Dynamics of supercoiled DNA studied by FCS and computer modeling

Tomasz Wocjan[1], Jan Krieger[1], Oleg Krichevsky[2] and Jörg Langowski[1]

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[2] Physics Department, Ben-Gurion University, Beer-Sheva 84105, Israel

The organization of the genome in the cell nucleus is fundamental for cellular function: transcription and its regulation, genome duplication, epigenetic effects and chromosome folding are all intricately connected to genome architecture. On the lowest level of genome organization, DNA folding is determined by its bending and twisting properties; superhelical DNA is a well-known model system to study these properties. Recent experiments by fluorescence correlation spectroscopy (Shusterman et al., (1008) Phys Rev Lett. 100:098102) indicated a sensitivity of the monomer mean square displacements in DNA circles towards superhelicity. To explain this phenomenon, we investigated the dynamics of a single-fluorophore-labeled pUC18 plasmid through a Brownian dynamics algorithm, followed by a simulation of the fluorescence correlation spectroscopy (FCS) process. Simulations with homogeneous DNA elasticity and local straight equilibrium are not sufficient to reproduce the observed behavior. But inserting permanently bent sequences into the DNA, which favor end loop formation, caused a dependence of the calculated FCS correlation curves on superhelical density. Furthermore, our simulations allow us to take into account the orientation of the fluorophore in polarized excitation, which might explain the observed appearance of a Rouse-like regime at intermediate time scales.

SHORT SAMPLING TIME FLUORESCENCE CORRELATION SPECTROSCOPY REVEALS EARLY OLIGOMER FORMATION OF ALPHA-SYNUCLEIN

Sangeeta Nath, Jelle Hendrix, Jessika Meuvis and Yves Engelborghs

Laboratory of Biomolecular Dynamics, Department of Chemistry & *BioSCENTer*, University of Leuven, Celestijnenlaan 200 G, box 2403, B3001 Leuven, Belgium

Yves.Engelborghs@fys.kuleuven.be

Confocal microscopy not only reveals heterogeneity, when scanning cells, but also in solution. E.g. spike formation can be observed when bright objects are formed. This analysis of heterogeneity in time can be quantitated to study protein-DNA interaction [1] or complex formation [2]. Here we study the formation of early oligomers of α-synuclein by applying Fluorescence Correlation Spectroscopy (FCS). The idea is to use trace amounts (nM) of labeled protein in the presence of a large excess of unlabeled protein and follow the aggregation process by measuring the reduction in time of the diffusion coefficient of the fluorescent species. Synuclein with an engineered cysteine (A140C) was labeled with Alexa488 and was used as a fluorescent probe in trace amounts (3-4 nM) in the presence of 100 μM unlabeled α-synuclein. The combination of short sampling times and repeated measurements produce a size distribution of the oligomers. Initially, a sharp peak is obtained (diffusion coefficient 114 ± 15 μm^2/sec) corresponding to monomers. Subsequently a distinct transient population appears, followed by the gradual formation of broader sized distributions of higher oligomers. The formation of the transient intermediate and the early oligomers is accompanied by a conformational change, as visualised using FRET between the donor labeled N-terminus and the acceptor labeled cysteine at position A140C. At longer time scales, further aggregation leads to the formation of big aggregates that are moving too slow to contribute to the short term fluctuations. The overall aggregation process can be followed by the decrease of the number concentration of fast diffusing fluorescent species.

[1] Vercammen J, Maertens G, Gerard M, De Clercq E, Debyser Z, Engelborghs Y. DNA-induced polymerization of HIV-1 integrase analyzed with fluorescence fluctuation spectroscopy. J Biol Chem., 277, 38045, (2002)

[2] Buyens K, Lucas B, Raemdonck K, Braeckmans K, Vercammen J, Hendrix J,Engelborghs Y, De Smedt SC, Sanders NN. A fast and sensitive method for measuring the integrity of siRNA-carrier complexes in full human serum. J Control Release.,26, 67,(2008)

12th Carl Zeiss sponsored workshop on FCS and related methods (12-16 October 2009, Cargese, Corsica, France)

Global analysis of autocorrelation functions and photon counting distributions: some experimental tests involving EGFP and EGFP dimers

Victor Skakun[1] and Antonie Visser[2]

[1]Belarusian State University, Minsk, 220050 Belarus and [2]Microspectroscopy Centre, Wageningen University, 6703 HA Wageningen, The Netherlands

It was shown previously that photon counting histogram (PCH) analysis and dual-color fluorescence cross correlation spectroscopy (FCCS) could monitor fluorescently labeled receptor kinases at the single-molecule detection level in living plant cells (M.A. Hink et al. (2008) *Biophys. J.* **94**, 1052-1062). Both PCH and fluorescence correlation spectroscopy (FCS) analysis use the same experimental data, but each technique focuses on a different property of the signal. FCS measures the time-dependent decay of the fluorescence fluctuations yielding, for instance, molecular diffusion coefficients, while PCH analysis calculates the amplitude distribution of these fluctuations yielding the distribution of molecular brightness (average fluorescence photon count rate per molecule per second). Both methods give information about the molecular concentration. Via separate FCCS and PCH experiments some receptors were found to be present for 15-20% as homodimers, whereas no evidence was found for higher oligomeric complexes in other receptors.

Realizing that FCS and PCH make use of the same experimental fluorescence fluctuation traces it is obvious that a global analysis protocol must be developed that simultaneously recovers the relevant parameters. In this presentation such an approach is described and tested with experimental fluorescence fluctuation data of EGFP and dimeric EGFP in aqueous solution (pH 8.0).

The algorithm for PCH analysis also allows correcting for out-of-focus emission signals. This would pave the way to 'visualize' the non-ideal brightness profile in cellular and other high-refractive-index systems.

Quantitative measurements of molecular interaction in living organisms by Single Wavelength Fluorescence Cross-correlation Spectroscopy (SW-FCCS)

Thorsten Wohland
Chemistry Department, National University of Singapore, 3 Science Drive 3, 117543 Singapore

In the past biomolecular interactions have been measured mostly under in vitro conditions because of the higher accuracy and ease of measurement. However, it has become clear in the last years that the cellular environment has an important influence on these interactions and when trying to understand cellular functions these factors have to be included. For that purpose we have developed single wavelength excitation fluorescence cross-correlation spectroscopy (SW-FCCS) which allows the determination of biomolecular interactions in vivo. Because SW-FCCS uses only a single laser line for excitation it does not suffer from problems of excitation volume overlap usually encountered in dual-color FCCS. And although that leads to lower count rates per particle detected, due to the excitation of two-spectrally different fluorophores at non-ideal wavelength, it possesses a sufficiently high signal to noise ratio to allow the quantitation of biomolecular interactions in living organisms. We demonstrate how SW-FCCS can be used to measure the dissociation constants of Cdc42, a small Rho-GTPase, with different interacting molecules (N-WASP, IRSp53, IQGAP1) in live cells and zebrafish embryos and discuss the advantages and disadvantages of the method.

Towards single molecule enzymology of encapsulated enzymes

Per Rigler[1], Kai Hassler[2], Mariusz Grzelakowski[1], Ozana Onaca[1], Karolina Langowska[1], Wolfgang Meier[1]

[1]Department Chemie, Universität Base1, Switzerland and
[2] Biomedical Imaging Group, EPFL, Lausanne, Switzerland

Single molecular fluorescence spectroscopic studies of enzymes have paved the way for a better understanding of how enzymes work at the molecular scale. Here we show first results how single or few functional enzymes can be encapsulated in polymeric nanocontainers consisting of amphiphilic block copolymers of a polymethyloxazoline-polydimethylsiloxane-polymethyloxazoline (PDMS-PMOXA-PDMS) architecture. To add functionality to these nanocontainers a membrane protein (outer membrane protein F, OmpF) has been reconstituted into the polymeric membrane and a biotinylated PMOXA-PDMS-PMOXA polymer is used allowing flux of substrate and product molecules through the above mentioned membrane and to anchor the nanocontainers specifically to a planar substrate, respectively.

Previously we have shown that it is possible to measure molecular turnover of single enzymes (horse radish peroxidase) at glass surfaces previously functionalized with a biotin-derivatized polylysin-*g*-polyethyleneglycol polymer by using various fluorogenic substrates in combination with total internal fluorescence fluorescence correlation spectroscopy (TIR-FCS)[1, 2].

Polymeric nanocontainers with reconstituted OmpF have been reproducibly tethered to glass surfaces previously patterned by microcontact printing using BSA/BSA-biotin. The enzyme acid phosphatase was encapsulated in the nanoreactors and the fluorogenic substrate ELF-97 was used as a fluorescent probe in order to determine enzyme kinetic parameters using both fluorimetry and confocal fluorescence microscopy[3]. The kinetic parameters obtained from the free, the encapsulated, and the surface-tethered encapsulated enzyme are compaired. Our results show that it will be possible in a near future to study enzyme kinetics at the single molecule level inside nanoreactors. This technique could potentially be used to develop new biosensors for screening enzymatic activity at an unprecedented sensitivity.

[1.] Hassler, K., et al., Dynamic disorder in horseradish peroxidase observed with total internal reflection fluorescence correlation spectroscopy. *Optics Express* **2007,** 15, (9), 5366-5375.

[2.] Hassler, K., et al., Total internal reflection fluorescence correlation spectroscopy (TIR-FCS) with low background and high count-rate per molecule. *Optics Express* **2005,** 13, (19), 7415-7423.

[3.] Broz, P., et al., Toward intelligent nanosize bioreactors: A pH-switchable, channel-equipped, functional polymer nanocontainer. *Nano Letters* **2006,** 6, (10), 2349-2353.

Fluorescence Correlation Spectroscopy at the nanoscale: Revealing more details with STED microscopy

Christian Eggeling *, Christian Ringemann * , Veronika Müller *, Rebecca Medda *, Günter Schwarzmann **, Birka Hein *, Andreas Schönle *, Stefan W. Hell *

* *Dep. Nanobiophotonics, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany*

** *Kekulé-Institute für Organische Chemie und Biochemie, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany*

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Fluorescence Correlation Spectroscopy (FCS) is a non-invasive and very sensitive analysis technique, allowing for the disclosure of complex dynamical processes. FCS is usually combined with far-field (confocal) microscopy. However, prominent (biological) problems can often not be solved due to the limited resolution of conventional optical microscopy. For example, in a conventional optical microscope FCS requires rather low concentrations of the fluorescently labeled molecules, which has precluded its otherwise very promising application to systems where molecular concentrations of several micro-mol per liter (µM) are required. Further, cholesterol-assisted lipid interactions such as the integration into lipid nanodomains ('rafts') are considered to play a functional part in a whole range of membrane-associated processes, but their direct and non-invasive observation in living cells is impeded by the resolution limit of >200nm of a conventional far-field fluorescence microscope. Using the superior spatial resolution of stimulated emission depletion (STED) far-field microscopy [1], we demonstrate novel use of FCS at rather large fluorophore concentration [2,3] or for the direct and non-invasive detection of single diffusing lipid molecules in nanosized areas in the plasma membrane of living cells. Combining a (tunable) resolution of down to 30 nm with FCS, we obtain new details of molecular membrane dynamics. For example, unlike phosphoglycerolipids, sphingolipids or 'raft'-associated proteins are transiently (~ 10 ms) trapped on the nanoscale in cholesterol-mediated molecular [4]. These results are corroborated by single-molecule tracking experiments.

[1] S.W. Hell "Far-Field Optical Nanoscopy", Science, 316, 1153, (2007).
[2] L. Kastrup et al. "Fluorescence Fluctuation Spectroscopy in Subdiffraction Focal Volumes", PRL, 94, 178104, (2005)
[3] C. Eggeling et al. "Reversible photoswitching enables single-molecule fluorescence fluctuation spectroscopy at high molecular concentration", MRT, 70, 1003, (2007)
[4] C. Eggeling et al. "Direct observation of the nanoscale dynamics of membrane lipids in a living cell", Nature, 457, 1159, (2009)

NEAR-FIELD FCS: DRIVING BIOLOGY BEYOND THE DIFFRACTION LIMIT

Winckler P, Jaffiol R, Plain J and Royer P

Université de Technologie de Troyes, Institut Charles Delaunay FRE CNRS 2848,
Laboratoire de Nanotechnologie et d'Instrumentation Optique, LRC CEA,
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For single molecule detection in solution, the issue is to get the probability of finding molecule in the observation volume significantly inferior to unity. This can be achieved through diluting the dye of interest or limiting the detection to a very small volume. Classical fluorescence correlation spectrosocpy (FCS), based on confocal detection, presents two important limitations for in vivo studies. First, its requires to work with a nanomolar concentration of molecules, very far from the biologically relevant concentration (i.e μM, or higher). On the other hand, the background signal such as autofluorescence, is still often important. In order to overcome the limitations of classical FCS, we propose a new approach to reduce the detection volume based on a non radiative energy transfer. We present two applications of our technique to make FCS and fluorescence imaging at the nanoscale, see Fig. 1.

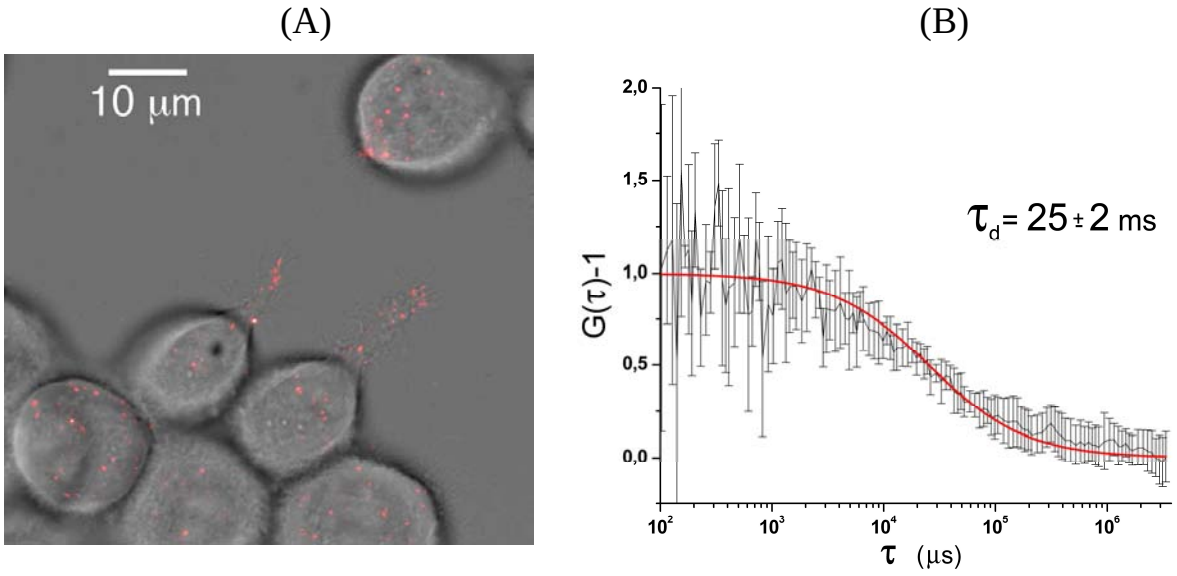

Fig. 1. (A) Picture of adhesion areas between cells and substrate. Superposition of two widefield images, one DIC picture in grey scale and one fluorescence picture in red scale. (B) Typical autocorrelation curve recorded on an cell adhesion area. Red curve, fit according to a pure 2-D diffusion of molecules.

Nanophotonic structures to enhance single molecule fluorescence detection

J. Wenger,[1] D. Gérard,[1] H. Aouani,[1] H. Rigneault,[1] E. Devaux,[2] T.W. Ebbesen,[2] and S. Blair[3]

[1] Institut Fresnel, CNRS, Aix-Marseille Université, Marseille, France

[2] ISIS, Université Louis Pasteur, CNRS, 8 allée G. Monge, 67000 Strasbourg, France

[3] Electrical and Computer Engineering Department, University of Utah, Salt Lake City, USA

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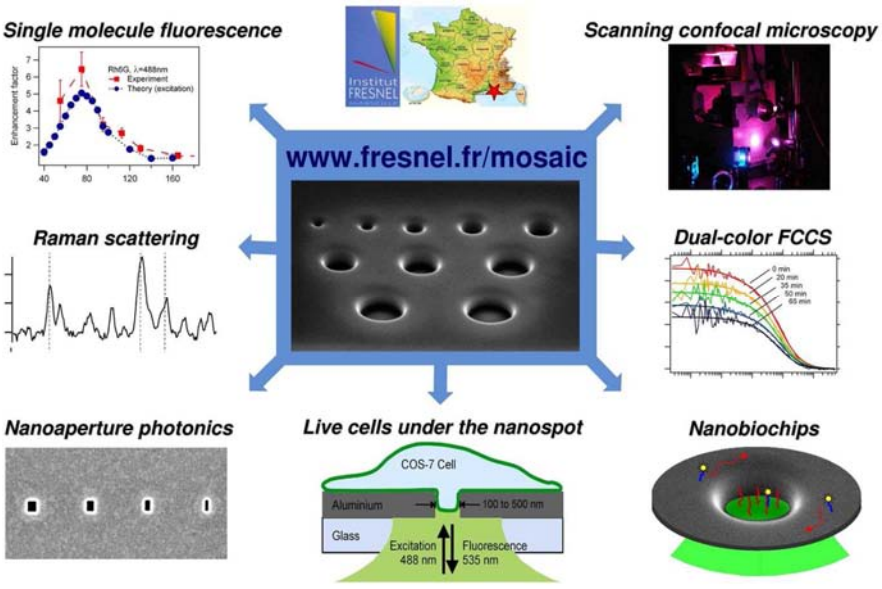
The ability to produce nanometric devices with a resolution down to a few nanometers opens the way for promising applications in nano- and bio-photonics. Such devices allow to locally confine the light, inducing large local electromagnetic fields.

In this contribution, we will focus on two fundamental questions that bear practical applications, and we will show how nanophotonic structures bring innovative answers :

- How to detect one single molecule in a highly concentrated (µM) solution ?
- How to enhance the optical signal detected per molecule ?

As an illustration, we will focus on the use of nanometric apertures milled in a metallic film and dielectric (polystyrene) microspheres. Both structures are shown to enhance the fluorescence emission of single molecules, with an enhancement factor up to 25 as compared to the emission rate in open solution [1]. Besides, the observation volume can be up to three orders of magnitude smaller than the diffraction limit in optics, enabling efficient enzymatic reactions monitoring at micromolar concentrations for FCS or FCCS.

The optimised fluorescence enhancement offers a gain in signal to noise ratio of about one order of magnitude, corresponding to a 100-fold reduction of the FCS experiment duration. This evidences the feasibility of FCS analysis with fast integration times of about one second, opening the way to the monitoring biochemical reactions at reduced time scales [2].



[1] P.-F. Lenne, et al, *Fluorescence fluctuations analysis in nanoapertures: physical concepts and biological applications*, HistoChem. Cell. Biol. **130**, 795-805 (2008).

[2] J. Wenger, et al, *Nanoaperture-Enhanced Signal-to-Noise Ratio in Fluorescence Correlation Spectroscopy*, Anal. Chem. **81**, 834-839 (2009).

Recent Advances in Image Correlation Spectroscopy and its Applications in Cells

Paul W. Wiseman *,

* *McGill University*

Departments of Physics & Chemistry

Montreal, QC Canada

paul.wiseman@mcgill.ca

This seminar will provide an introduction for the non-expert to various new methods of image correlation spectroscopy (ICS) and their application for measurements of protein transport and interactions in cells and neurons. An overview of the history and background theory of these fluctuation microscopy based methods will be presented along with a description of the microscopy instrumentation and computational resources required to perform such measurements. I will specifically cover more recent developments of ICS including spatio-temporal ICS (STICS), reciprocal or k-space ICS (kICS), and raster-scan ICS (RICS). The STICS portion will focus on its application to measuring vector maps of flows of adhesion and migration related proteins (actin, alpha-actinin, paxillin, integrins, talin) in living cells and discuss how two color cross-correlation variants can be used to measure interactions via co-transport. The kICS part will discuss how this method can avoid complications of complex photophysics of the fluorophore probe and even be used to extract information from probe blinking when quantum dot nanoparticles are used [1].

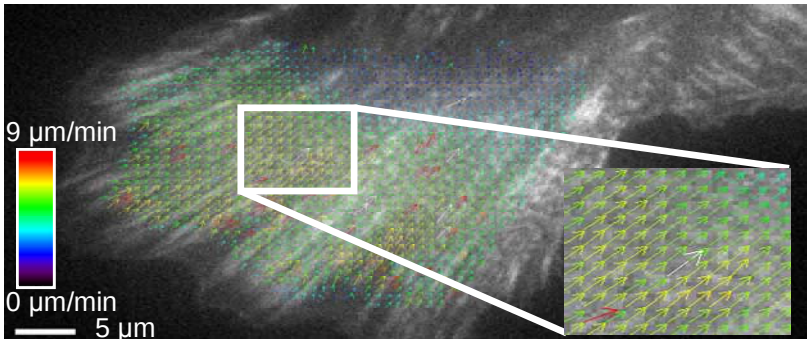


Fig. 1. Velocity map of retrograde transport of alpha-actinin/EGFP in an MEF cell. Measured by TIRF microscopy and STICS analysis.

[1] D. L. Kolin, P. W. Wiseman. Advances in image correlation spectroscopy: measuring number densities, aggregation states, and dynamics of fluorescently-labeled macromolecules in cells. Cell Biochem. Biophys. 49: 141-164 (2007)

Wednesday October 14th

Different acylation motifs direct multiply orthogonoal co-localization of lipid anchored proteins in live cell membranes

J.T. Groves*, M.B. Forstner*, B.F. Lillemeier**, and M.M. Davis**

* *Howard Hughes Medical Institute*

University of California Berkeley and Lawrence Berkeley National Laboratory

** *Stanford University*

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A number of chemically distinct genetically-encoded acylation modifications link soluble protein domains to cell membranes *in vivo*. It is widely speculated that these provide targeting mechanisms that direct protein organization in the membrane. Here, we use fluorescence cross correlation spectroscopy to probe interactions among all combinatorial pairs of four common lipid anchoring motifs expressed in live primary T cells as fluorescent protein fusions. All homogeneous anchor combinations lead to strong co-localization of the anchored proteins into small mobile clusters on the membrane surface. In contrast, results from heterogeneous anchor pairings reveal mutual avoidance among each of the three cytoplasmic membrane leaflet anchors examined (palmityl:palmityl:myristoyl from Lck, myristoyl from Src, and gerenyl-gerenyl from RhoA). The extracellular leaflet GPI anchor co-localizes with the Lck anchor while avoiding the other anchors types. Both cholesterol depletion and actin cytoskeleton disruption interfere with anchor-mediated clustering. These results indicate that protein acylation can direct co-localization into at least three orthogonally composed small dynamic clusters on the membrane surface. Additionally, at least one of these cluster types exhibits trans-bilayer structure.

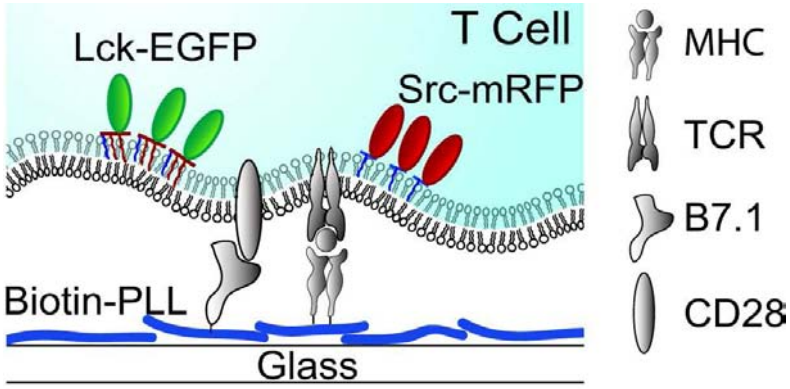

Fig. 1. A small combinatorial library of different genetically encoded lipid anchor sequences fused to red or green fluorescent proteins is used to explore anchor co-localization in the membranes of live primary T cells.

Essential role for raft nanodomains in initiation of T cell receptor signalling upon antigen recognition

Hamon Yannick, Bernard Anne-Marie, Salles Audrey, Hawchar Omar, Phelipot Marie-Claire, Guo Xiao-Jun, Sergé Arnauld, Mailfert Sébastien, Marguet Didier and He Hai-Tao

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Case 906, 13288 Marseille Cedex 9, France

Contact: hamon@ciml.univ-mrs.fr

[1] Hamon, Y., et al. Immun., Endoc. & Metab. Agents in Med. Chem. 2008, **8**, p. 358-65.
[2] Wawrezinieck, L., et al. Biophys J, 2005. **89**(6): p. 4029-42.
[3] Lenne, P.F., et al., EMBO J, 2006. **25**(14): p. 3245-56.
[4] Lasserre, R., et al., Nat Chem Biol, 2008. **4**(9): p. 538-47.

LIGAND-STIMULATED EGFR CAUSES LIPID DOMAIN-DEPENDENT HIGHER ORDER AGGREGATION AND INTERNALIZATION OF ERBB2

László Ujlaky-Nagy, Árpád Szöőr, János Szöllősi, György Vereb

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Members of the ErbB receptor tyrosine kinase family, ErbB1 (EGFR), ErbB2, ErbB3 and ErbB4, form homo- and heterodimers or higher oligomers with each other which promote cell survival and proliferation [1]. Generally, overexpression of the ligandless ErbB2 is thought to drive homodimer formation in the membrane of tumor cells. However, ErbB2 can also act as a heterodimerizing partner for other family members, and serve as a signal amplifier for their ligand-bound forms, which may have clinical relevance in tumors expressing multiple ErbBs [2]. We have used fluorescence correlation spectroscopy to investigate molecular events of ErbB1-ErbB2 transactivation in single living SKBR3 breast tumor cells. ErbB2 was labeled with A488 Fabs. Fluorescence fluctuation traces were taken from single submicron sized spots in the upper membrane of cells and used to construct fluorescence autocorrelation spectra. These were fitted using a model accounting for triplet state, diffusion of dissociated Fabs, as well as cytoplasmic and membrane (lateral, 2D) diffusion of ErbB2 molecules or their aggregates. Correlation spectra were obtained every 2.5 min for □1 hour. After 20 min, half of the samples were stimulated with 50 nM EGF to detect time-dependent changes of ErbB2 diffusion. Controls showed a time dependent fluctuation of membrane diffusion times in the 120-200 ms range (182±9 ms, mean±SEM). This was paralleled with internalization and recycling to the surface of receptors as measured by flow cytometry. Membrane diffusion of ErbB2 was divided into at least two components: a faster component characterizing monomers or smaller aggregates, and a slower component related to higher oligomers or larger clusters. The corresponding diffusion times were 94±3 ms and 360±18 ms. Co-diffusion of ErbB2 with ErbB2 upon labeling with a mixture of A488 and A647 fluorophors was detected by fluorescence cross-correlation spectroscopy (FCCS) mainly for the slower component indicating that the faster component might be biased towards monomers. Responses to EGF stimulation were vastly heterogeneous in timing, but manifested unanimously in an increase of characteristic average membrane diffusion time to maximally 550±119 ms. The average onset of response was 5.5 min post stimulus and slowed-down diffusion was maintained for ~30 min. EGF-induced transactivation did not change the faster component (100±19 ms), but lead to the increased diffusion times of the slower one (from 360 ms to 764±66 ms), at the same time decreasing its fractional proportion from 0.55 to 0.2, while the fast component increased from 0.37 to 0.65. This may be an indirect indication that larger aggregates are needed for internalization and the receptors are recycled as monomers or smaller clusters afterwards. Consequences of the increased ErbB1-ErbB2 interaction could be detected by microscopic fluorescence resonance energy transfer as well as from increased ErbB1 and ErbB2 phosphorylation in Western blots and were decreased by extraction of membrane cholesterol with methyl-ß-cyclodextrine. This treatment also accelerated the diffusion time of ErbB2 to 83±16 ms, indicating that the slower component (higher aggregates) corresponds to microscopic lipid rafts. Given the low expression of ErbB1 in SKBR3 cells, the mechanism of ErbB1-ErbB2 transactivation can be interpreted as ErbB1 serving as signal antennae that upon ligand binding recruit ErbB2 to form increasing size activated aggregates on lipid raft platforms that eventually get internalized.

[1] Y. Yarden, M. X. Sliwkowski, Nat Rev Mol Cell Biol. 2 (2001) 127-137.

[2] A. Citri., Y. Yarden, Nat Rev Mol Cell Biol. 7 (2006) 505-516.

DUAL-FOCUS FLUORESCENCE CORRELATION SPECTROSCOPY

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Several decades ago, Magde, Elson and Webb invented an ingenuous method for measuring diffusion coefficients of fluorescent molecules: Fluorescence Correlation Spectroscopy (FCS) [1]. However, over the last couple of years, an increasing number of publications have demonstrated the sensitivity of FCS measurements for even smallest changes in experimental conditions such as cover slip thickness variation, refractive index mismatch, or laser beam characteristics [2, 3]. One of the most disturbing observations was the dependence of FCS on excitation intensity due to optical saturation of fluorescence [4]. This makes even comparative measurements problematic, because the photophysics and thus optical saturation properties of fluorescence labels often change when they are chemically bound to a target molecule.
Recently, we have introduced modification of conventional FCS, which is called dual-focus FCS (2fFCS), that circumvents all the above mentioned problems [5]. The achievable accuracy of the method was shown to be better than 5 % in absolute value [6]. Meanwhile, the method was used for systematically determining absolute diffusion coefficients of reference dyes across the visible spectrum [7, 8]. In the presentation, the method is described in detail, and various applications of 2fFCS for protein biophysics as well as colloidal science will be given.

References

[1] Magde, D., E. Elson, and W.W. Webb, Thermodynamic fluctuations in a reacting system - measurement by fluorescence correlation spectroscopy. Phys. Rev. Lett., 1972. 29: p. 705-708.
[2] Enderlein, J., et al., Performance of fluorescence correlation spectroscopy for measuring diffusion and concentration. ChemPhysChem 6, 2324-2336 (2005).
[3] Enderlein, J., et al., Art and Artefacts of Fluorescence Correlation Spectroscopy. Current Pharmaceutical Biotechnology 5, 155-161 (2004).
[4] Gregor, I., D. Patra, and J. Enderlein, Optical Saturation in Fluorescence Correlation Spectroscopy under Continuous-Wave and Pulsed Excitation. ChemPhysChem 6, 164-170 (2005).
[5] Dertinger, T., et al., Two-Focus Fluorescence Correlation Spectroscopy: A New Tool for Accurate and Absolute Diffusion Measurements. ChemPhysChem 8, 433-443 (2007).
[6] Dertinger, T., et al., The optics and performance of dual-focus fluorescence correlation spectroscopy. Opt. Express 16, 14535-14368 (2008).
[7] Müller, C.B., et al., Precise measurement of diffusion by multi-color dual-focus fluorescence correlation spectroscopy. Eur. Phys. Lett 83, 46001 (2008).
[8] Loman, A., et al., Comparison of optical saturation effects in conventional and dual-focus fluorescence correlation spectroscopy. Chem. Phys. Lett. 459, 18-21 (2008).

NEW METHODS FOR FCS: FLUORESCENCE LIFETIME CORRELATION SPECTROSCOPY AND TWO-FOCUS FCS

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Ultrasensitive fluorescence detection and spectroscopy is important in many fields of fundamental research as well as chemo- and bioanalytical applications. In recent years, technical improvements in photodetector sensitivity, microscope objective optics, and laser light sources have enhanced the capabilities for the detection of single molecules. This technique allows to visualize variations from molecule to molecule which would be hidden performing ensemble measurements. Today, time-resolved measurements permit to follow fluorescence dynamics of single molecules starting in the sub-nanosecond range up to fluctuations in the second range and beyond. By exploiting the full information content of such a multi-dimensional measurement, classical intensity based analysis schemes like FCS in confocal microscopy can be significantly improved by sorting and weighting the detected photons. We will present actual instrumentation and discuss recent applications:

- **Fluorescence Lifetime Cross Correlation Spectroscopy** (FLCCS):
  Fluorescence cross correlation spectroscopy (FCCS) is a superior tool to detect binding between two molecules, each marked with a different fluorophore, in liquid environment. Concentrations ranging from pM to µM can be investigated. Only bound molecules moving together through the femtoliter detection volume contribute to the cross correlation amplitude and are quantified.
  However, artefacts like spectral bleed through are compromising the detection sensitivity of bound molecules. To overcome this limitation, the combination of FCCS with fluorescence lifetime measurements allows to suppress bleed through as well as common parasitic contributions like Raman scattering and detector afterpulsing [1].

- **Two-Focus FCS** (2fFCS):
  Small structural changes of molecules (e.g. proteins) can be investigated in their natural environment by determining the diffusion coefficient. The necessary accuracy for measuring the molecular hydrodynamic radius down to some ångström is met with Two-Focus FCS.
  Two orthogonally polarized laser beams pulsed in an alternating fashion (Pulsed-Interleaved Excitation, PIE) are used to generate a robust dual foci geometry with a well known focal distance. This intrinsic length scale refines diffusion studies in solution and allows to overcome various uncertainties formerly relying on the size and shape of the confocal volume in single focus FCS. It also dramatically improves the accuracy of determining absolute diffusion coefficients [2].

References:

[1] P. Kapusta, M. Wahl, A. Benda, M. Hof, J. Enderlein, “Fluorescence Lifetime Correlation Spectroscopy”, *J. Fluorescence*, **17**, 043-048 (2007)
[2] T. Dertinger, A. Loman, B. Ewers, C. B. Müller, B. Krämer, J. Enderlein, “The Optics and Performance of Dual-Focus Fluorescence Correlation Spectroscopy”, *Optics Express*, **16**, 14353-14368 (2008)

Multi-confocal fluorescence correlation spectroscopy for parallel multi-spot measurements in living cells

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Fluorescence correlation spectroscopy (FCS), a method based on the statistical analysis of the fluctuations in the fluorescence signal collected in a small volume, provides a highly sensitive tool for studying concentrations, mobility and interactions dynamics of molecules in living cells. However, FCS measurements are usually limited to a single confocal volume, so that information can only be obtained from one position at a time. To investigate the dynamics of cellular process, there is great interest in simultaneously monitoring several locations within the cell. This requires developing parallel multi-spot strategies for both excitation and detection that would allow flexible spatial configuration of the spots location. We implemented a multi-confocal FCS setup to study cellular response to thermal stress. Multiple excitation spots are created using a spatial light modulator (SLM) that displays programmable phase gratings to diffract the incoming laser beam. During an experiment, one can then choose freely the number and position of the excitation spots by simply pointing at interesting regions of the cell in study. Parallel detection is performed using an Electron-multiplied CCD camera (EM-CCD). Each pixel of the camera acts as a standard pinhole, enabling confocal detection in parallel. Although CCD cameras have a rather low frame rate due to sequential readout, applying a special readout mode makes it is possible to reach a time resolution compatible with the study of molecular mobility in cells. Performance and limitations of our multiplexed confocal strategy will be presented and compared with standard FCS setups.

Photothermal Absorption Correlation Spectroscopy

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Fluorescence Correlation Spectroscopy suffers from artifacts originating from the poor fluorophore photophysics: photobleaching, blinking, and saturation. To circumvent these limitations we present here a new correlation method called Photothermal Absorption Correlation Spectroscopy (PhACS) which relies on the absorption properties of tiny nano-objects. PhACS is based on the photothermal heterodyne detection technique and measures akin FCS, the time correlation function of the detected signals. Due to the exceptional photostability of gold nanoparticles, PhACS has the advantage to give access to extremely slow dynamics. Applications of this technique to the precise determination of the hydrodynamic sizes of different functionalized gold nanoparticles are presented, highlighting the potential of this method.

TRANSIENT STATE MONITORING BY FCS AND RELATED TECHNIQUES

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FCS offers a straightforward means to extract information from the population dynamics of transient dark states, generating fluorescence fluctuations superimposed on those due to translational diffusion of the fluorescent molecules into and out of the detection volume. Here, two different approaches for transient state monitoring by FCS will be presented.

First, we will show how FCS can be used to monitor local proton exchange. The approach was used to investigate local proton exchange at membrane surfaces, providing evidence for a proton collecting antenna mechanism, where the membrane itself can increase the effective cross section of proton uptake for fluorophores and membrane proteins [1,2]. The monitoring approach is also applicable to locally monitoring the exchange of other ions, for instance the biologically important exchange of calcium ions.

Second, FCS provides a convenient way to monitor photo-induced, long-lived transient dark states of fluorophores, such as triplet states, and states generated by photo-isomerization and photo-induced charge transfer. Photo-induced transient dark states are exhibited by practically all common fluorophores. Generally, their lifetimes are in the μs to ms time range typically rendering these states highly environment sensitive. This sensitivity has however, to date only been sparsely exploited due to methodological constraints. As a complement to FCS, we recently presented a concept based on modulation of the excitation light to extract information about these states [3], which utilizes both the environmental sensitivity of these states and the sensitivity of fluorescence-based detection. Triplet state images of liposomes with different internal environments were generated by a standard CLSM, resolving local environmental differences, not clearly distinguishable via other fluorescence parameters [4]. The concept can provide several new, useful and independent fluorescence-based parameters in biomolecular imaging. A similar modulation of the excitation intensity can also be used in Fluorescence Correlation Spectroscopy (FCS) measurements [5], to suppress background and triplet state populations, and to reduce cross-talk in spectral cross-correlation measurements [6].

[1] Brändén M, Sandén T, Brzezinski P, Widengren J "Localized proton microcircuits at the biological membrane-water interface" *Proc. Natl. Acad. Sci.* 103(52), 19766-19770 (2006)
[2] Öjemyr L, Sandén T, Widengren J, Brzezinski P "Lateral Proton Transfer between the Membrane and a Membrane Protein" *Biochemistry*, 48(10), 2173-2179 (2009)

[3] Sandén T, Persson G, Thyberg P, Blom H, Widengren J "Monitoring kinetics of highly environment-sensitive states of fluorescent molecules by modulated excitation and time-averaged fluorescence intensity recording" *Anal. Chem.* 79(9), 3330-3341 (2007)

[4] Sandén T, Persson G and Widengren J "Transient state imaging for microenvironmental monitoring by laser scanning microscopy" *Anal. Chem.* . 80(24), 9589-9596 (2008)

[5] Persson G, Thyberg P, Widengren J "Modulated Fluorescence Correlation Spectroscopy with complete time range information" *Biophys J.* 94, 977-985 (2008)

[6] Persson G, Sandén T, Sandberg A, Widengren J "Fluorescence cross-correlation spectroscopy of a pH-sensitive ratiometric dye for molecular proton exchange studies" *PCCP* 11(21), 4410-4418 (2009)

SUPER-RESOLUTION FLUORESCENCE IMAGING WITH STANDARD FLUOROPHORES

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Fluorescence microscopy has become an essential tool in biological and biomedical sciences for imaging of living cells and tissues. In contrast to other imaging techniques, such as electron microscopy, fluorescence microscopy offers an advantage because it enables imaging even of dynamic processes in living cells. The weakness, however, has been the fact that the spatial resolution was limited to ~ 200 nm in the imaging plane for more than 100 years [1]. Only recently methods emerged that enable subdiffraction resolution imaging based on photoswitchable or photoactivatable fluorescent proteins and organic fluorophores [2]. Currently, most super-resolution approaches control fluorescence emission of fluorophores by modulating the transition of fluorophores between a fluorescent (on) and non-fluorescent (off) state. However, super-resolution fluorescence imaging with commercially available small organic fluorophores remains challenging.

Here we introduce a novel and facile method that enables super-resolution imaging with standard Alexa Fluor and ATTO dyes. We demonstrate that these frequently used fluorophores which span the visible wavelength range from 480 to 700 nm can be switched reversibly between an on- and off-state under similar experimental conditions. The recipe we developed comprises solely the addition of thiol-containing reducing agents such as ß-mercaptoethylamine (MEA), dithiothreitol (DTT), or glutathione (GSH) which efficiently populate a relatively stable non-fluorescent state. Since oxygen does not have to be removed and reducing agents such as glutathione are present in the cytoplasm of cells in the µM to mM range the method we describe is very relevant for super-resolution imaging with molecular–scale resolution (~ 20 nm) even in living cells [3].

[1] E. Abbe, *Arch Mikrosk Anat 9*, 413 (1873).
[2] M. Heilemann, P. Dedecker, J. Hofkens, M. Sauer, *Laser and Photonics Review 3*, 180 (2009).
[3] M. Heilemann, S. van de Linde, A. Mukherjee, M. Sauer, *Angew Chem Int Ed*. (2009).

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Applications of scanning FCS

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Scanning FCS (sFCS) is a modification of standard FCS where the probed volume is moved across the sample in a defined way during the measurement. This is usually realized in a confocal laser scanning microscope by scanning the beam in linear or circular fashion. Introduction of scanning into the FCS measurements is motivated by different reasons, all of which aim to reduce or remove particular limitations of standard FCS. These limitations include the problems encountered with slowly moving molecules, such as poor statistical accuracy or photobleaching, optical distortions of the measurement volume affecting the spatial calibration and the precision of measurements, slow fluctuations of membranes disturbing the measurements on molecules diffusing within the membrane, etc. Focusing on a different problem then leads to different sFCS implementations. Here we present applications of sFCS employing circular scan paths with large and small radii. While the former is applied when improvement of statistical accuracy and reduction of photobleaching is desirable, the latter is particularly suitable in situations where robust measurements of diffusion coefficients in the presence of optical distortions are the main goal.

LASER SCANNING MICROSCOPY WITH AVALANCHE PHOTODIODES

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Conventional laser scanning microscopes (LSMs) equipped with photomultiplier detection usually lack the sensitivity required for successful analysis of single molecule fluorescence or to perform sensitive fluorescence correlation spectroscopy (FCS). Most modern LSMs offer access to a descanned fluorescence signal to attach external detectors, e.g. single photon sensitive avalanche photodiodes (APDs). In this presentation the performance of such a system will be compared to other confocal setups. To that end a procedure based on FCS to quantitatively assess the overall sensitivity of the instruments has been developed and will be demonstrated.

High sensitivity as provided by APDs allows for multiparameter fluorescence detection (MFD). MFD is a technique that simultaneously records all information observable in a fluorescence experiment, i.e. polarization, color, photon arrival time and spatial origin. This is particularly useful in single molecule studies, where sequential measurements of different properties are affected by temporal variations in heterogeneous or unstable samples, but also in low light level investigations where the number of available photons is very limited. In MFD a single raw data set delivers fluorescence decay histograms, anisotropy decay histograms, intensity ratios and time traces or fluorescence correlation curves for individual molecules or sub-ensembles with spatial resolution close to the diffraction limit.

We will show first results using biological and nonbiological samples to demonstrate the performance of the extended LSM. For that purpose also the analysis and presentation of multiparameter data will be discussed.

Inverse- Fluorescence Correlation Spectroscopy

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Using Fluorescence Correlation Spectroscopy (FCS) for analysis of biomolecules requires labelling. An alternative approach that circumvents the need for labelling is inverse-FCS (iFCS), where the signal from a medium surrounding the particles of interest is analyzed, as opposed to a signal from the particles themselves. As unlabeled particles traverse the detection volume, part of the medium will be displaced whereby fluorescence fluctuations are generated. This allows for analysis of non-labelled particles or potentially biomolecules. We have tested iFCS by measuring on unlabeled polystyrene beads of 800, 400, 200 and 100 nm diameter, in a surrounding medium of highly concentrated alexa 488-fluorophores. Our aim is now to enhance the sensitivity of iFCS and thereby allow for analysis of unlabelled biomolecules of ≤ 10 nm diameter.

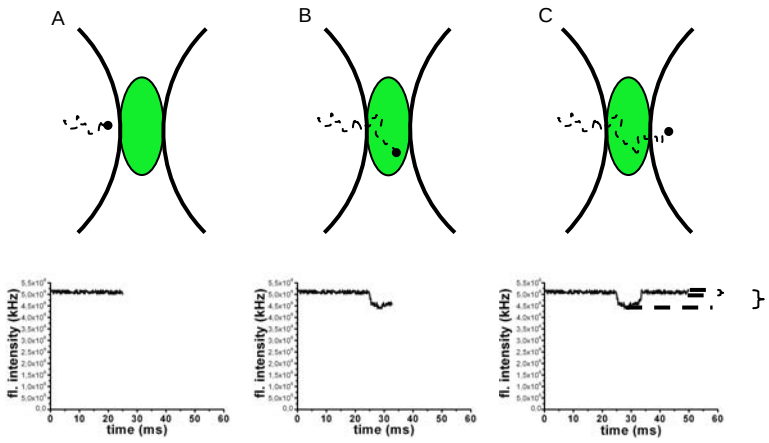

Fig. 1. Cartoon showing the principle of inverse-FCS: A) An unperturbed, high, fluorescence signal is measured when the detection volume is absent from particles. B) The signal is reduced upon the entrance of a particle. C) The fluorescence signal is restored as the particle leaves the detection volume.

Hetero-Species Partition Analysis Revels Binding Curve and Stoichiometry of Protein Interactions in Living Cells

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Measuring the binding curve and stoichiometry of protein complexes in living cells is a prerequisite for quantitative modeling of cellular processes. Dual-color fluorescence fluctuation spectroscopy provides a general framework for detecting protein interactions. However, quantitative characterization of protein hetero-interactions remains a difficult task. To address this challenge we introduce hetero-species partition (HSP) analysis for measuring protein hetero-interactions of the type D + nA ↔ DAn. HSP directly identifies the hetero-interacting species from the sample mixture and determines the binding curve and stoichiometry in the cellular environment. The method is applied to measure the ligand-dependent binding curve of the nuclear receptor retinoic X receptor to the coactivator transcription intermediate factor 2. The binding stoichiometry of this protein system has not been directly measured yet. A previous study using protein fragments observed a higher binding stoichiometry than biologically expected. We address this difference in stoichiometry by measuring the binding curves of the full-length proteins in living cells. This study provides proof-of-principle experiments that illustrate the potential of HSP as a general and robust analysis tool for the quantitative characterization of protein hetero-interactions in living cells.

APPLICATIONS OF FLUORESCENCE CORRELATION SPECTROSCOPY IN RNA BIOCHEMISTRY

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An *in vitro* assay based on fluorescence correlation spectroscopy (FCS) is presented which allows to investigate the kinetic behavior of human Dicer by different diffusion coefficients of product and substrate. To investigate the linearity of the enzyme assay, FCS data of defined solutions of two mobility species were analysed by different fitting routines, fitting autocorrelation curves individually and globally. The optimised data analysis approach allowed to monitor product formation with high accuracy.
As RNA does not emit fluorescence in the visible spectrum by itself, chemical or indirect labeling is required to detect RNA by a fluorescence signal. The suitability of a sulforhodamine B binding RNA aptamer to monitor RNA dynamics at the single molecule level was tested. Additionally, molecular size and oligomerization state of the fluorophore-RNA complexes could be characterized by FCS, FCCS and SAXS [1].

[1] Arne Werner, Petr V. Konarev, Dmitri I. Svergun and Ulrich Hahn, 'Characterisation of a fluorophore binding RNA aptamer by Fluorescence Correlation Spectroscopy and Small Angle X-Ray Scattering', Analytical Biochemistry, 389, 52-62 (2009)

FUNCTIONAL SYNTHETIC HOX GENES: IMAGING THE ACTIVITY AND QUANTIFYING TRANSCRIPTION FACTOR-DNA INTERACTIONS IN LIVE CELLS

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Homeotic genes encode transcription factors that specify body segments along the embryonic anteroposterior axis [1]. Now, 26 years after the discovery of the Homeodomain (HD), the molecular mechanisms underlying Hox-mediated transcription remain ill-defined and we still do not understand how transcription factors, and Hox proteins in particular, find their specific target sequences in a large eukaryotic genome. In order to address this question, we have designed synthetic *Sex Combs Reduced* (*Scr*) genes and investigated their function genetically and quantitatively using advanced fluorescence imaging and Fluorescence Correlation Spectroscopy, techniques with single-molecule sensitivity [2,3]. We were able to visualize and quantify physiologically relevant levels of Scr-HD molecules in live cells, characterize their dynamics and interactions with nuclear DNA in different nuclear compartments, discern specific from non-specific DNA–Scr-HD interactions, determine their corresponding dissociation constants (K_d) and study the kinetics of these interactions in live cells.

[1] Gehring W.J. (1987). Homeo boxes in the study of development. Science 236, 1245-1252.
[2] Vukojević V, Heidkamp M, Ming Y, Johansson B, Terenius L, Rigler R. "Quantitative single-molecule imaging by Confocal Laser Scanning Microscopy", Proc Natl Acad Sci USA, 105, 18176-18181, (2008).
[3] Vukojević V, Pramanik A, Yakovleva T, Rigler R, Terenius L,Balkanin G. "Study of molecular events in cells by fluorescence correlation spectroscopy" Cell Mol Life Sci 62, 535–550, (2005).

Improvement of Signal Strength and Photostability of Fluorophors by quenching triplet and radical states

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In single molecule spectroscopy the signal strength and photostability of the fluorophore is often limited by the population of long-living triplet states and photoionisation due to the high excitation power.

In this study, we focused on the effects of triplet and radical state quenchers on the photophysical processes of Rhodamine 123. Fluorescence Correlation Spectroscopy and photobleaching experiments allow us to investigate the timescales of the different excited-state reactions of the fluorophore and give new insights in the triplet, radical and photobleaching kinetics of the dye. Adjustment of the quencher's redox properties allows addressing special electron transfer pathways of the flurophore in the singlet, triplet and radical state and provides a new way of reducing dark states of the dye via electron transfer.

By using novel quenchers with low lying triplet energies the triplet state population of rhodamine is quenched efficiently with rate constants in the range of $2.5 - 3.6 \times 10^9$ M^{-1} s^{-1}. Furthermore, the quencher shows a remarkable increase of the reduction rate from the radical dark state of Rhodamine 123. Molecules with both, triplet and radical quenching properties, increase the dye brightness in comparison to a pure triplet quencher by a factor of five.

Multiparameter Fluorescence Imaging Spectroscopy including Fluorescence Correlation Spectroscopy in living cells

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The main goal of molecular cell biology is to identify and monitor the interacting network of proteins and other molecules in a cell with respect to different conditions to understand the biological processes on a molecular level.
Fluorescence microscopy and spectroscopy are sensitive and non invasive methods to gain insight into processes going on in living cells but most approaches in advanced fluorescence microscopy techniques focus only on single aspects of these processes, e.g. protein interaction, dynamic behaviour of proteins or structure, size and composition of molecular complexes.
In contrast Multiparameter Fluorescence Imaging Spectroscopy (MFIS) is a holistic approach used to monitor simultaneously a variety of fluorescence parameters in confocal fluorescence microscopy. As the photons are registered one by one, MFIS allows for fully parallel recording of Fluorescence Correlation/ Cross Correlation Spectroscopy (FCS/ FCCS), fluorescence lifetime and pixel/ image information over time periods of hours with picoseconds accuracy.
The multidimensional analysis of correlated changes of several parameters measured by FRET, FCS, fluorescence lifetime and anisotropy increases the robustness of the analysis significantly. The economic use of photon information allows one especially in live cell studies to keep the expression levels of fluorescent fusion proteins as low as possible (down to the single-molecule level).
Therefore in contrast to conventional FCS measurements taken from regions of interest in a cell the MFIS approach provides information on concentrations and mobilities of proteins or other molecules by correlating the fluorescence signal in each single pixel of an MFIS image.

[1] Weidtkamp-Peters, S., S. Felekyan, A. Bleckmann, R. Simon, W. Becker, R. Kühnemuth and C. A. M. Seidel. "Multiparameter fluorescence image spectroscopy to study molecular interactions." Photochem Photobiol Sci **8**(4): 470-80 (2009).

MEASURING NUMBERS OF FLUOROPHORES LABELING cDNA IN SOLUTION, WITH FLUORESCENCE CORRELATION SPECTROSCOPY AND CONTINUOUS PHOTOBLEACHING.

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We developed a Total Internal Reflection Fluorescence Microscopy experiment in order to detect very small amount of cDNA strand labeled with Alexa647 fluorophores. However the interpretation of this kind of measurement needs to accurately characterize the labeling of the cDNA strand, i.e. to know precisely the number of fluorescent bases per cDNA strand and their brightness.
In the present study we present different approaches that aim at determining the brightness and the number of Alexa647 molecules labeling the C bases of two sequences of cDNA, corresponding to two transcripts of different sizes, a short and a long transcript (123 and 306 base long, with resp. 45 and 74 dCTP deoxynucleotides). In each case, the dCTP-Alexa647 labeled bases have been incorporated during reverse-transcription.

Two kinds of experiments have been performed and combined: continuous photobleaching and Fluorescence Correlation Spectroscopy (together with the factorial cumulant analysis method).
Continuous photobleaching measurement was realized in exciting small quantities of cDNA strand confined in micrometric well under continuous illumination. As a result we show that there is almost no interactions between fluorophores on the same cDNA strand and that the photobleaching cross section of Alexa incorporated in cDNA strand is about half that of free Alexa in aqueous solution.
On the other side, Fluorescence Correlation Spectroscopy measurement enable us to measure the concentration, the diffusion constant and especially the brightness of the labeled cDNA strand and the free Alexa. We thus observe that the cDNA strand brightness is about twice the brightness of free Alexa providing information about the interplay between the radiative, non radiative and photobleaching decay rate in relation with their photobleaching cross section.

We then studied the brightness and concentration evolution of labeled cDNA strand during the solution photobleaching. Factorial cumulant analysis of the fluorescent fluctuations put into evidence the fact that the brightness of cDNA strands is not uniform, due to the distribution of the number of Alexa647 fluorophores labeling cDNA. We thus propose that the number of fluorescent labels per cDNA follows a Poisson distribution, with a mean value of about 2 for the long cDNA strands.

Concurrent Fluorescence Fluctuation and Electrophysiological recordings of the Apoptosis Inducer PorB

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A detailed understanding of the molecular mechanisms of bacterial infections is crucial for the development of successful strategies against antibiotic resistance. The porin PorB of *Neisseria gonorrhoeae* or *Neisseria meningitidis* is an intriguing bacterial factor owing to its ability to translocate from the outer bacterial membrane into host cell membranes where it modulates the infection process. After prolonged infection of epithelial cells with pathogenic Neisseria species programmed cell death is induced. The underlying mechanism includes the translocation of the porin into the inner mitochondrial membrane which leads to a complete breakdown of the membrane potential, release of cytochrome c and subsequent proteolytic activation of caspase 3, a main 'executioner' molecule for apoptosis.

In order to gain new insights into the individual properties of PorB from *Neisseria gonorrhoeae* we used a Ionovation Bilayer Explorer to determine the diffusion constant and the molecular brightness of this protein in a lipid bilayer and in the surrounding buffer while simultaneously observing channel activities under a controlled membrane potential. The diffusion constant was used to determine the oligomeric state of electrically active PorB in the bilayer.

FIDA data directly and hydrodynamic calculations with the results derived from FCS experiments support the homotrimeric structure of the membrane-bound PorB and give a trimer radius of approx. 4 nm. With the innovative concept of the Ionovation Bilayer Explorer, the electrophysiological activity can directly be correlated to this oligomeric state of PorB.

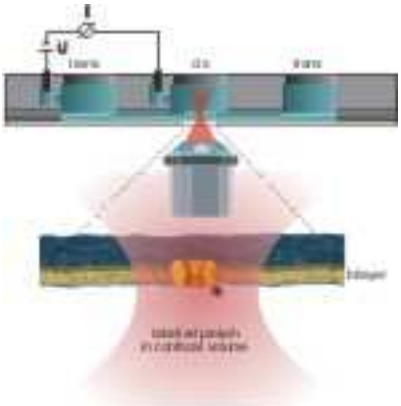

Fig. 1. Schematic diagram of the Ionovation Bilayer Slide. Electrophysiological measurements and confocal observation can be conducted simultaneously.

Fig. 2. Detection unit of the Ionovation Bilayer Explorer (Photoart) with a fully automated liquid handling and perfusion unit on a standard inverted microscope.

Quantitative Analyses of Protein Interaction Networks

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Quantitative analyses still are a major challenge in cell biology. In order to understand how an extracellular signal is translated into a cellular response, quantitative knowledge on the number of signaling molecules inside a cell, the fraction of molecules that actually participate in signal transduction and the temporal and spatial organization of the signaling machinery is required.

Here, we present a combination of fluorescence correlation spectroscopy (FCS) in cell lysates and in intact cells, confocal laser scanning microscopy and quantitative Western Blots to address this problem. We focused our analyses on interactions of the transmembrane scaffolding protein Linker for the Activation of T cells (LAT) and down-stream signaling proteins interacting with LAT. These complexes form upon activation of T cell receptors in T lymphocytes and are linked to the initiation of an immune response.

For the quantification of intracellular protein expression we developed a protocol in which transiently expressed fusion proteins with fluorescent proteins serve as an internal standard. In cell lysates, fusion proteins are quantitated with FCS and the relative amount of fusion protein and endogenous protein determined by Western Blots. Furthermore, we account for different folding efficiencies of individual fluorescent protein fusion proteins. This protocol overcomes the requirement for the purification of recombinant proteins associated with standard quantitative Western Blots.

In cells expressing a LAT-GFP construct and a red fluorescent mCherry-fusion protein of a down-stream signaling protein next to the non-fluorescent endogenous proteins, stimulation-dependent interactions were quantitated through a combination of confocal laser scanning microscopy using single photon detectors for fluorescence detection, fluorescence correlation spectroscopy and cross-correlation spectroscopy. Owing to the sensitivity of the detection methods, interactions could be quantitated for cells expressing only low levels of fusion proteins, thereby avoiding potential artifacts associated with the overexpression of fusion proteins. Through integration of optical tweezers, contacts of T cells with antigen-presenting cells could be established in a highly controlled fashion, enabling these measurements with precise timing control for preselected cells.

In summary, the results present an answer to the experimental challenges associated with quantitative intracellular analyses. This approach critically depends on the ability of FCS to determine absolute concentrations of fluorescent molecules, both in vitro and in cells.

DUAL COLOR GFP AND MCHERRY FLUORESCENCE LIFETIME CORRELATION SPECTROSCOPY FOR PROTEIN INTERACTIONS IN LIVE CELLS

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The combination of TCSPC-FLIM and FCS (FLCS) [1, 2] takes advantage of time resolved detection to differentiate between two different FCS contributions. Each fluorescent component coming from the sample (e.g. green, red, non correlated background…) can be resolved on the basis of their lifetime signatures. The time delay measured for each photon (which gives the fluorescence decay for each species excited with a pulsed laser) combined with the "macroscopic" arrival time constitutes the basis of the FLCS approach and is potentially a very useful tool in biology.

When performing FCCS experiments, one must take care of a known artifact that corrupts cross-correlation data: the cross-talk effect [3]. In the case of using green/red fluorophores (e.g. EGFP and mCherry) some photons coming from the EGFP emission are counted in the red detector, and hence the cross-correlation function treats them as if green and red photons were actually correlated, hence they contribute to the calculation of the cross-correlation curve. A way of getting rid of the bleed-through is the so-called switching method [4, 5]. In this method two alternating laser beams are controlled by an AOM, the synchronized correlation signals in both detectors are calculated as cross-correlation functions with no cross-talk.

Here, we have used another way based on FLCS to overcome the above referred problem. By using the scanning confocal time-resolved microscope Picoquant MicroTime 200 (Picoquant, Germany), a blue pulsed laser at 470 nm and a continuous green laser at 561 nm are directly focused onto live cells expressing GFP and mCherry fused proteins. The time trace corresponding to the green detector contains only the information related to the correlated photons coming from the GFP (excited with the pulsed laser and therefore with a decay form). The time trace corresponding to the red detector contains both: uncorrelated photons coming from mCherry, and correlated photons (fluorescence decay) coming from the GFP. Using FLCCS, we apply the decay filter to the data coming from the green detector and next, we apply the background filter to the red detector; this way, only "green decay" photons and red un-correlated photons will be used in the final cross-correlation analysis. We demonstrate our approach by using cells expressing GFP and mCherry separately or in tandem as negative and positive control, respectively. First results to monitor interaction of nuclear pore proteins during mitosis using this methodology will be also presented.

[1] Böhmer M, Wahl M, Rahn H-J, Erdmann R, Enderlein J. "Time-resolved fluorescence correlation spectroscopy", Chem Phys Lett, 353:439-445, (2002).

[2] Kapusta P, Wahl M, Benda A, Hof M, Enderlein J. "Fluorescence lifetime correlation spectroscopy", J Fluoresc, 17:43-48, (2007).

[3] Bacia K, Schwille P. "Practical guidelines for dual-color fluorescence crosscorrelation spectroscopy", Nat Protoc, 2:2842-2856, (2007).

[4] Müller BK, Zaychikov E, Bräuchle C, Lamb DC. "Pulsed interleaved excitation", Biophys J, 89:3508-3522, (2005).

[5] Takahashi Y, Nishimura J, Suzuki A, Ishibashi K, Kinjo M, Miyawaki A. "Crosstalk-free fluorescence cross-correlation spectroscopy by the switching method", Cell Struct Funct, 33:143-150, (2008).

Cellular tunable focus FCS for studying the molecular mechanism of LEDGF/p75 mediated chromatin tethering of HIV-1 integrase

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Human transcriptional co-activator LEDGF/p75 is hijacked by HIV-1 integrase (IN) during the replication of HIV. Little is still known about the molecular complex of these two proteins in the living cell. In this work we first studied the cellular chromatin interaction of eGFP-tagged LEDGF/p75 with tunable-focus fluorescence correlation spectroscopy (TF-FCS) and show that LEDGF/p75 is in equilibrium between a free Brownian motion and a very slow movement on the chromatin. Being dependent on the size of the laser focus, this slow movement represents a continuous association-dissociation-reassociation process that is governed by diffusion. Concentration-dependent continuous photobleaching measurements (CP) furthermore revealed the existence of high-affinity chromatin binding sites. Next, we co-expressed mRFP-tagged IN and confirmed its intracellular interaction with LEDGF/p75 by fluorescence cross-correlation spectroscopy (FCCS). Interestingly, CP and fluorescence recovery after photobleaching (FRAP) indicated that the affinity of this complex for chromatin is exceptionally high. By two-photon fluorescence lifetime imaging (2P-FLIM) we verified if the cellular stoichiometry was altered when the proteins were expressed together. We believe that this work is useful for the understanding and targeting of HIV-replication.

A DUAL SPOT LASER SCANNING FCS SYSTEM

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Fluorescence Correlation Spectroscopy (FCS) is a powerful for cell biology since it allows to quantify a wide range of phenomena such as photophysical, photochemical, interaction, diffusion, and transport property of specifically labelled molecules. In order to overcome the inherent drawbacks of local measurements (bleaching due to the continuous exposure to the excitation, absence of control of drift, etc.), many variants of FCS have been proposed that use a scanned observation volume. Beyond the spatial information, they usually provide also a better flexibility in terms of timescale, a lower bleaching, and a better statistics [1]. We have developed a fluorescence multipurpose system made by the combination of two fully independent custom confocal laser scanning microscopes. Thus, two excitation spots can be generated independently within the three dimensions of the sample, each one being associated to a detection channel. The system was designed in order to provide the highest versatility for a multimodal analysis [2]. A complete protocol to control the location, size and shape of the measurement volumes has been developed, as well as a method which allows monitoring independently the excitation and collection efficiency distribution. The system can operate either two simultaneous independent single spot measurements (including imaging, FCS, Photon Counting Histogram, scanning FCS, etc.) or one real-time cross-analysis between two distant locations.

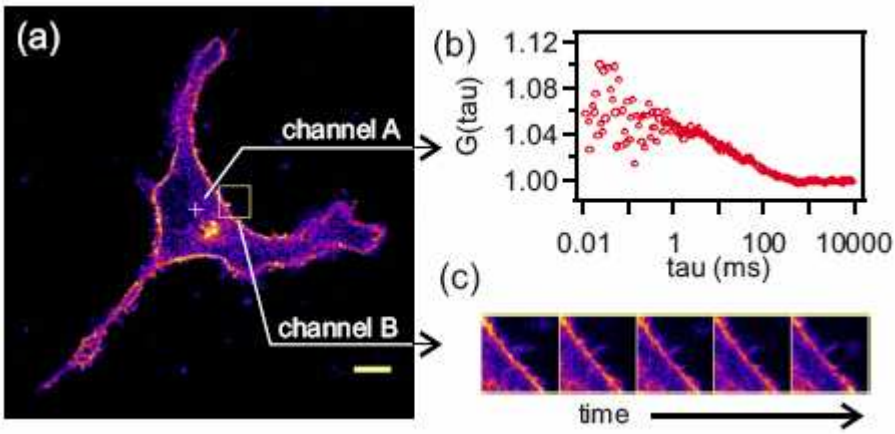

Fig. 1. Example of dual spot measurement on a COS7 cell. From ref. 2.

This project was funded by the French Agence Nationale de la Recherche under contract ANR-05-BLAN-0337-02 and Region PACA.

[1] For a review, see D. L. Kolin, P. W. Wiseman, Cell Biochem. Biophys., 49, 141 (2007).
[2] P. Ferrand, M. Pianta, A. Kress, A. Aillaud, H. Rigneault, D. Marguet, "A versatile dual spot laser scanning confocal microscopy system for advanced fluorescence correlation spectroscopy analysis in living cell", Rev. Sci. Instrum. 80, 083702 (2009).

POSTERS

Portable FCS setups with latex microspheres

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We demonstrate the use of dielectric microspheres as cost-effective tools to develop efficient and portable setups for Fluorescence Correlation Spectroscopy (FCS). Two implementations are discussed : (i) with a low numerical aperture lens to form a disposable microscope objective, and (ii) with an etched optical fiber to form an endoscope. Both designs have a sensitivity high enough to detect the fluorescence fluctuations from standard single molecules.

First, we explore the combination of a latex microsphere with a low NA lens to form a high performance optical system, enabling the detection of single molecules. Viable FCS experiments at concentrations 1-1000 nM with different objectives costing less than $40 are demonstrated [1]. This offers an alternative to the conventional microscope objectives.

Second, we report the use of an optical fiber combined with a latex microsphere to perform FCS analysis. The sensitivity of the technique is demonstrated at the single molecule level thanks to a photonic nanojet effect [2]. This offers new opportunities for reducing the bulky microscope setup and extending FCS to remote or in vivo applications.

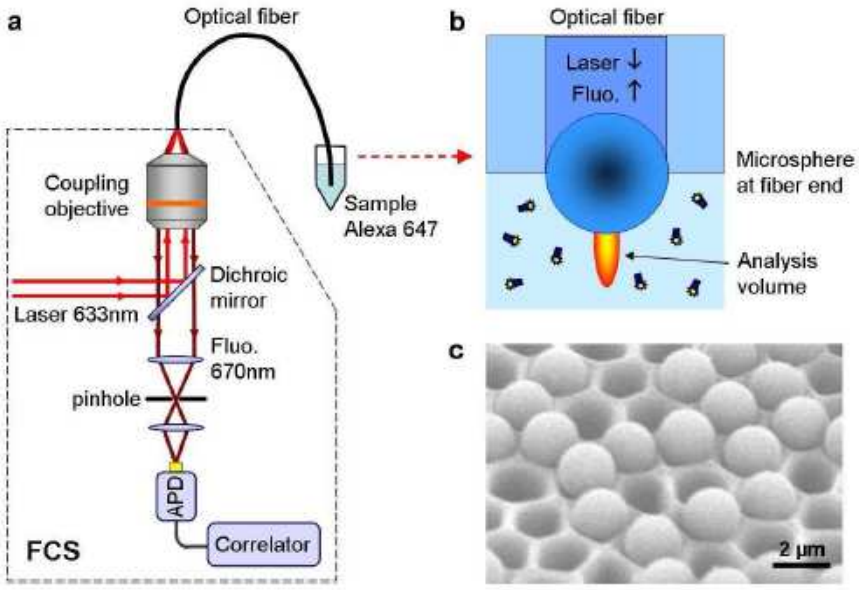

Fig. 1. (a) experimental setup, (b) end of the optical fiber with the microsphere, (c) electron microscope image of the optical fiber bundle etched with polystyrene microspheres.

[1] Wenger J., Gérard D., Aouani H., Rigneault H., *Disposable Microscope Objective Lenses for Fluorescence Correlation Spectroscopy Using Latex Microspheres*, Anal. Chem. **80**, 6800-6804 (2008).

[2] H. Aouani, et al, *Optical-fiber-microsphere for remote fluorescence correlation spectroscopy*, submitted (2009).

FCCS in yeast: accounting for problems in live cell measurements

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The aim of my project is to establish Fluorescence Cross-Correlation Spectroscopy in budding yeast (S. Cerevisiae). More specifically, proteins involved in the asymmetric construction of the spindle pole body during cell division are under investigation.
It has been shown that a number of different proteins (Kar 9, Bim1, Cdc28 and others) may play a role in this asymmetric localization, however their exact interplay is not known. By labeling the proteins pairwise with GFP and mCherry and quantifying their interactions using FCCS we hope to get a better understanding of the procedure. Our preliminary results (and data from the literature) have shown that such experiments are feasible but difficult.
My work focuses on the experimental problems encountered when measuring in vivo.
The small cell volume limits the pool of available fluorescent proteins and causes visible photobleaching, resulting in short possible measuring times.
Cellular autofluorescence as well as possible cell damage due to high laser power limit the signal-to-noise ratio in live cells.
We seek to develop methods granting semi-automatic determination of correlation curves. This would enable us to significantly increase the amount of collected data and therefore improve the statistics to gain more reliable results.

Systematic biomarker discovery of glioma

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ABSTRACT

Systems biology is the study of an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions which give rise to life. Instead of analyzing individual components or aspects of the organism, systems biologists focus on all the components and the interactions among them, all as part of one system using computer-based or special algorithms-based analysis. Here we introduce a novel method for predicting genome-wide condition-specific subcellular locations of human proteins using only limited and condition-unspecified known locations. With systems biological analysis of glioma, the key target genes which involved in the control of the tumoregenesis process can be estimated. After systemic molecular biological experiments for target genes with immunohistochemical staining, FCCS, Olink system, western blotting, siRNA and growth/proliferation inhibition assay technique to determine protein binding sites on DNA and molecular imaging, the gene regulatory mechanism can be investigated and validated functionally based on key mechanisms. We discover that the relocated but still interacting PSPN and GFRa4 in endoplasmic reticulum do not interact with ret proto-oncogene (RET), whereas the three proteins are highly interact to each other in plasma membrane in normal brain tissues. The novel relocation of each gene discovered here can be a biomarker in glioma.

FLUORESCENCE CORRELATION SPECTROSCOPY ON NANO-FAKIR SURFACES WITH TWO-PHOTON EXCITATION

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Single biomolecule behaviour can reveal crucial information about processes not accessible by ensemble measurements. It thus represents a real biotechnological challenge. Common optical microscopy approaches require pico- to nano-molar concentrations in order to isolate an individual molecule in the observation volume. However, biologically relevant conditions often involve micromolar concentrations. These impose a drastic reduction of the conventional observation volume by at least three orders of magnitude. This confinement is also crucial for mapping subwavelength heterogeneities in cells which play an important role in many biological processes.

We propose an original approach that couples Fluorescence Correlation Spectroscopy (FCS), powerful tool to retrieve essential information on single molecular behaviour, and nanostructured plasmonic substrates with strong field enhancements and confinements at their surface. These electromagnetic singularities at nanometer scale, called "hotspots", are the result of the unique optical properties of surface plasmons. They provide an elegant way for studying single-molecule dynamics at high concentrations by reducing dramatically the excitation volume and enhancing the fluorophore signal by several orders of magnitude.

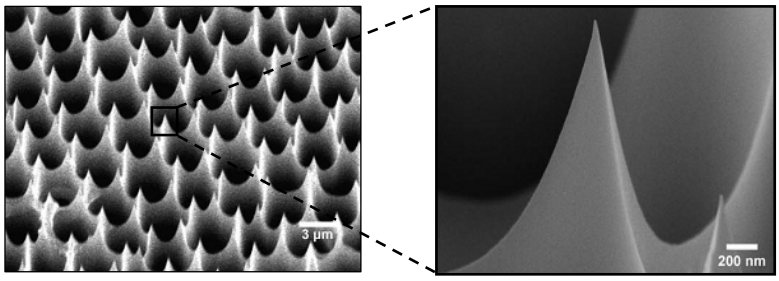

Fig. 1 Scanning electron micrographs of a part of the nanotip array

The nanostructured substrates are obtained by electrochemistry of an optical fibre bundle covered by a metallic thin film (see Figure 1) [1]. This technique is versatile and highly reproducible. It permits to create "nano-fakir surfaces" which cover a wide range of surface topographies to tune and tailor the hotspots density, localisation and size.

We present two-photon FCS results on these nano-fakir substrates using fluorescent nanobeads. We show *i*) a dramatic reduction of the observation volume and *ii*) the ability to perform at high concentration measurements, which is promising for biological applications.

[1] V. Guieu, F. Lagugné-Labarthet, L. Servant et al., "Ultrasharp Optical-Fiber Nanoprobe Array for Raman Local-Enhancement Imaging" Small, **4**, 96-99 (2008).

Full correlation FCS (fcFCS) to investigate the bulk polymerization of styrene

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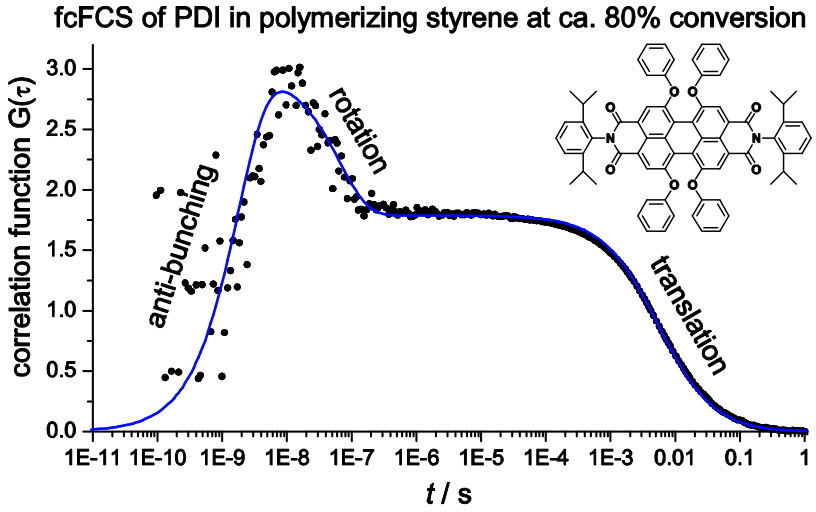
Radical polymerization represents a challenging topic for basic kinetic research. Proceeding from dilute to semi-dilute and finally concentrated polymer solutions, the mobility of monomers, polymer chains and other solutes becomes more and more restricted. This restriction starts to dominate polymerization kinetics as soon as polymerization or termination rates become diffusion-controlled. In previous work, we have concentrated on studying the decrease of translational diffusion coefficients of single perylendiimide (PDI) molecules by fluorescence correlation spectroscopy (FCS) and wide-field fluorescence microscopy (WFM).[1]

Here, we present our latest results on rotational and translational motion of different sized PDIs during bulk polymerization of styrene. Rotational diffusion coefficients in dilute solutions were determined by fluorescence anisotropy measurements. When rotational diffusion times clearly exceed the fluorescence lifetime (ca. 5 ns for the PDI used), a situation which is reached at some point during increasing monomer-to-polymer conversion, this method cannot be applied anymore. However, in this case, rotational diffusion can be observed by fcFCS with correlations down to nanoseconds.[2,3] The crosscorrelation curves show antibunching, rotational and translational motion. Triplet contributions do not appear due to the low ISC rate of the PDI. With fcFCS we were able to follow rotational motion of the probing dye over an extended range of monomer-to-polymer conversion. Thus, a direct comparison between rotational and translational motion, both observed by the same fcFCS experiment, is possible. This knowledge will be used to gain insight into changes in mobility during radical polymerization and how they depend on the polymerization conditions.



References

[1] D. Wöll, H. Uji-i, T. Schnitzler, J. Hotta, P. Dedecker, A. Herrmann, F. C. De Schryver, K. Müllen, J. Hofkens, *Angew. Chem. Int. Ed.* **2008,** *47*, 783.

[2] M. Wahl, H.-J. Rahn, I. Gregor, R. Erdmann, J. Enderlein, *Rev. Sci. Instr.* **2007**, *78*, 033106.

[3] S. Felekyan, R. Kühnemuth, V. Kudryavtsev, C. Sandhagen, W. Becker, C. A. M. Seidel, *Rev. Sci. Instr.* **2005**, *76*, 083104.

Latex microspheres to enhance fluorescence correlation spectroscopy beyond the diffraction limit

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We propose a simple and low-cost method to simultaneously decrease the confocal observation volume and enhance the fluorescence brightness [1]. A micron-sized dielectric sphere is set in the focus of a high numerical aperture objective to further focus the excitation beam and enhance the fluorescence collection (Figure 1).

When a dielectric microsphere is illuminated by a tightly focused Gaussian beam, it over-focuses light in a focal spot whose dimensions are sub-wavelength *in the three directions of space* [2]. In order to experimentally investigate this effect at the single-molecule scale, we use fluorescence correlation spectroscopy. We show that in the vicinity of a 2µm polystyrene sphere, the collected fluorescence signal from Alexa-Fluor 647 molecules is enhanced by a factor of 5. This factor stems from a simultaneous enhancement of the local excitation intensity and of the collection efficiency of the setup [3]. Compared to confocal microscopy with high numerical aperture, we also monitor a detection volume reduction of one order of magnitude below the diffraction limit.

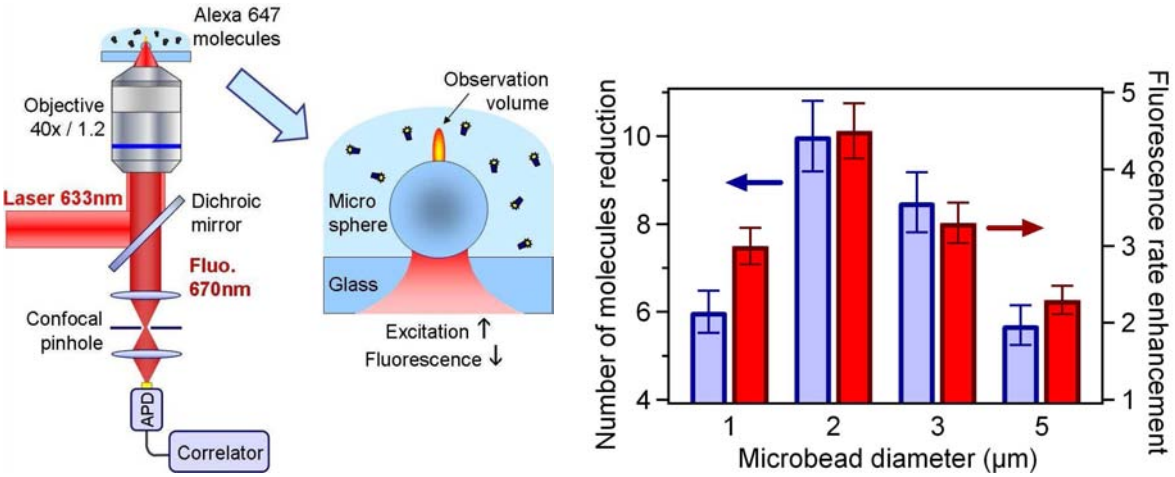

Fig. 1: Left: Schematic view of the experimental setup. Right: observation volume reduction and fluorescence rate enhancement versus microsphere diameter.

[1] D. Gerard, et al, "*Strong electromagnetic confinement near dielectric microspheres to enhance single-molecule fluorescence,*" Opt. Express **16**, 15297 (2008).
[2] A. Devilez, et al, "*Three-dimensional subwavelength confinement of light with dielectric microspheres,*" Opt. Express **17**, 2089 (2009).
[3] Gérard D., et al, *Efficient excitation and collection of single molecule fluorescence close to a dielectric microsphere*, J. Opt. Soc. Am. B **26**, 1473-1478 (2009).

FCS STUDY OF ATP DIFFUSION IN CARDIOMYOCYTES AND ISOLATED MITOCHONDRIA

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The aim of this research project is to investigate the intracellular diffusion and metabolic microcompartmentation of ATP by using fluorescence correlation spectroscopy (FCS). While the mechanisms of ATP synthesis in mitochondria and the biochemistry of MgATPases are well understood, the question of how ATP diffuses from mitochondria to all sites of ATP consumption is still a subject of debates [1]. A precise description of the ATP diffusion process within cardiomyocytes is clearly needed for a correct understanding of the mechanisms of regulation of mitochondrial respiration and energy fluxes in the cells *in vivo*.
The method of FCS was used here to study the diffusion kinetics of the ATP fluorescent analogue ATP-Alexa 647 in experimental systems with increased complexity: first in solution, then in isolated mitochondria and finally in permeabilised cardiomyocytes.
The autocorrelation curve in solution was successfully fit by the one-component Brownian diffusion model (D_{diff} 240 μ^2/s). However, pronounced multicomponent autocorrelation was revealed both in isolated mitochondria and cardiomyocytes (Fig.1). At least three characteristic ranges of autocorrelation were identified using a multicomponent free diffusion model and their relative amplitudes were related to the physiological state of the cells.
Tested separately, the anomalous diffusion model with two components was also able to fit the curves but it did yield "sub-" and "super-" diffusion factors, that were difficult to interpret.

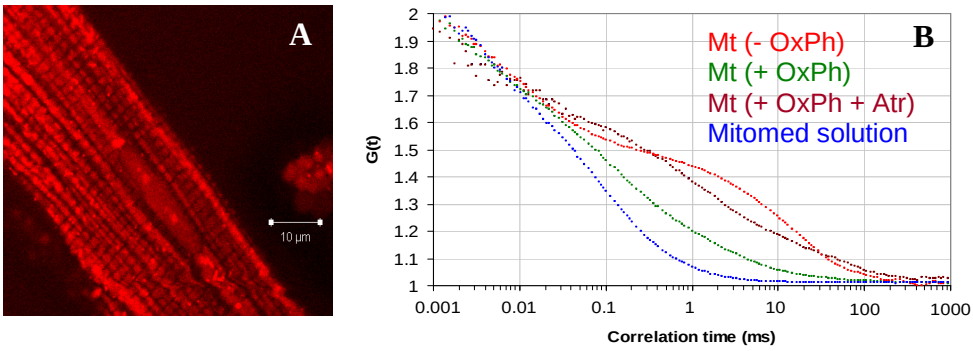

Fig. 1. Permeabilised cardiomyocyte incubated with the fluorescent ATP analogue (**A**) and normalized autocorrelation curves measured under different conditions (**B**). OxPh - oxidative phosphorylation, Atr – atractyloside, Mt – mitochondrion, Scale bar - 10 µm.

The relative contribution of correlation components was found to be sensitive to the disorganisation of the ordered structure of the cardiomyocyte, the functional state of the mitochondria and the presence of respiration inhibitors thus indicating the existence of ATP pools with different restricted diffusion properties.

[1] Saks V., Beraud N., and T. Wallimann "Metabolic compartmentation – a system level property of muscle cells", Int. J. Mol. Sci., 9, 751-767, (2008).

Measuring FRET via Acceptor triplet states.

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Fluorescence resonance energy transfer (FRET) offers a versatile means to monitor intra- and intermolecular distances in biomolecular research. The FRET signal can be recorded by several independent parameters, typically via the donor lifetime or the ratio of the donor and acceptor fluorescence intensities. Here we present a new method for determining FRET efficiencies based on the monitoring of the triplet state population of the acceptor dyes. From FCS measurements, it is shown how the triplet state population of the acceptor dye can be used to distinguish between donor-acceptor pairs of different separation lengths. We show how the approach can offer a unique sensitivity at low FRET efficiencies and a good signal-to-noise-ratio for high FRET efficiencies, altogether covering a very broad range of donor-acceptor distances. The approach can be realized based on a relatively simple instrumentation. The readout of the acceptor triplet state populations can be performed by FCS or by modulated excitation in time or space. The latter allows triplet state imaging by a standard LSM to be performed, and also opens for a wide field readout in a massive parallell fashion [1,2]."

[1] Tor Sandén, Gustav Persson, Per Thyberg, Hans Blom, Jerker Widengren “Monitoring Kinetics of Highly Environment Sensitive States of Fluorescent Molecules by Modulated Excitation and Time-Averaged Fluorescence Intensity Recording, Anal. Chem., 79, 3330, 2007.

[2] Tor Sandén, Gustav Persson, Jerker Widengren, “Transient State Imaging for Microenvironmental Monitoring by Laser Scanning Microscopy”, Anal. Chem., 80, 9569, 2008.

SATURATION SPECTROSCOPY WITH A FCS SETUP: ARTIFACTS RELATED TO THE SIZE VARIATION OF THE OBSERVATION VOLUME

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Saturation Spectroscopy combined with Fluorescence Correlation Spectroscopy (FCS) constitute an important method to characterize photophysical parameters of fluorescent molecules. However, experimental observations show an anomalous decrease of the brightness (i.e. the count rate per molecules) at high excitation regime, typically when the laser intensity I_e is higher than the saturation intensity I_s of the molecules. The fundamental reason of this decrease of the brightness is actually related to a size increase of the observation volume with the excitation power, as shown in Fig 1. Although several recent researches have explored the influence of the fluorescence saturation in FCS [1,2], no accurate evaluation of the size variation of the observation volume, and FCS parameters such as the diffusion time and the number of molecule, was investigated. In this work, we propose to compare experimental FCS studies with a quantitative theoretical model to characterize the influence of the size modification of the observation volume on fluorescence saturation spectroscopy.

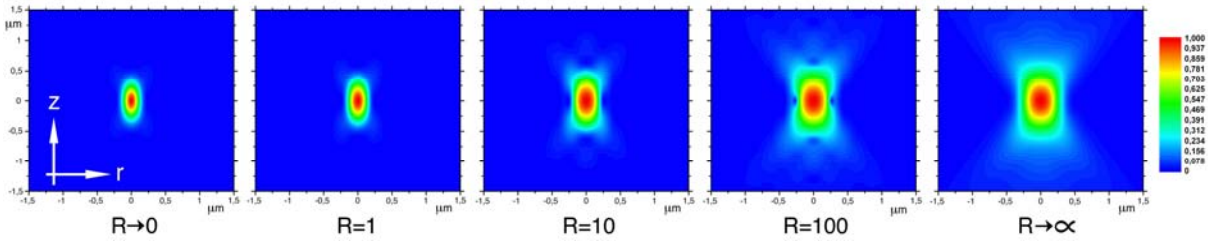

Fig. 1. Calculated observation volume for different values of the laser excitation (a scalar approach was used, as describe in [3]). R is the ratio of the laser excitation I_e to the saturation intensity I_s, $R = I_e / I_s$.

[1] A. Nagy, J. Wu, K. M. Berland "Characterizing observation volumes and the role of excitation saturation in one-photon fluorescence fluctuation spectroscopy", J. of Biomedical Optics, 10 (4), 044015, (2005).
[2] I. Gregor, D. Patra, J. Enderlein "Optical saturation in FCS under continuous wave and pused excitation", ChemPhysChem, 6, 164-170, (2005).
[3] R. Jaffiol, Y. Blancquaert, A. Delon, J. Derouard "Spatial fluorescence cross-correlation spectroscopy", Appl Opt, 45 (6), 1225-1235, (2006).

ANALYSIS OF MEMBRANE-BINDING PROTEIN DIFFUSION USING MULTIPOINT TOTAL INTERNAL REFLECTION FLUORESCENCE CORRELATION SPECTROSCOPY (MP-TIR-FCS)

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Fluorescence correlation spectroscopy (FCS) is a unique method to determine the diffusional properties and number of fluorescent molecules in the aqueous condition. The method has single-molecule sensitivity by monitoring the fluctuations of fluorescence intensity in a very tiny detection area. In spite of their wide range of applications, FCS measurements are restricted to monitoring at only one volume element defined by both a focused laser beam and a pinhole. To overcome this restriction, we developed a multipoint FCS system which was based on an objective-type total internal reflection-FCS (TIR-FCS) (1).

Intercellular signaling pathways are initiated by the binding of a ligand to its receptor on the cell surface to regulate cell activities. Therefore, studying the formation and separation of molecular complexes on the cell membrane is also necessary for understanding of cellular function as well. To analyze the diffusion properties of membrane bound proteins in very thin structures such the plasma membrane, we combined FCS with total internal reflection fluorescence microscopy (TIR-FCS). Furthermore, we constructed multipoint TIR-FCS (M-TIR-FCS) in order to elucidate the structure of cell membranes and dynamics of membrane-bound molecules in live cells (2).

However, the area of the measurement is restricted to only the surface, that is, the plasma membrane. Therefore, the next purpose of our research is further development of a multipoint FCS system that has three-dimensional capacities and can analyze the molecular complexes and molecular interactions at any point in a living cell.

References

(1) Ohsugi Y., Saito, K., Tamura M. and Kinjo M. “Lateral mobility of membrane-binding proteins in living cells measured by total internal reflection fluorescence correlation spectroscopy”, *Biophys J*, 91, 3456-3464, (2006).

(2) Ohsugi Y, Kinjo M. “Multipoint fluorescence correlation spectroscopy with total internal reflection fluorescence microscope.” *J Biomed Opt*.;14(1):014030. (2009)

Immunostimulatory CpG-DNA studied by FCS and AFM

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Toll-like receptors (TLR) are responsible for the immediate response of the innate immune system against invading pathogens. TLR recognize evolutionary conserved microbial patterns such as glycolipids and bacterial DNA. We focus on TLR9, which binds unmethylated CpG-DNA, which is ten times more common in bacterial DNA than in human DNA. Synthetic oligodeoxynucleotides (ODN) that stimulate TLR9 have been developed and are currently being used in human clinical trials for cancer therapy and as vaccine adjuvant. These immunostimulatory ODN can be divided in three classes that have a different stimulatory effect. A-class ODN stimulates interferon (IFN) production; while B-class ODN activates B-cells, and C-class ODN does both. The secondary structure of ODN in these three classes is different. A-class has poly-G ends that self-associate into quadruplex structures via Hoogsteen base pairing, and contains a palindromic sequence. B-class is linear and does not contain a palindrome. C-class contains the same palindromic sequence as A-class, but lacks the poly-G tails. [1, 2]

Recently, we showed that TLR9 activation by class B CpG-DNA involves a ligand-induced conformational change in the pre-formed dimeric TLR9 receptor [3]. Still unresolved, however, is how the three different classes of CpG-DNA elicit different immune responses.

We have investigated two different ODN classes using fluorescence correlation spectroscopy and atomic force microscopy. Our AFM images of A-class CpG-DNA confirm the nanoparticle structures that were seen by Costa et al. [4], while B-class does not form these structures. The FCS experiments also indicate higher-order structure formation of A-class. The diffusion coefficients derived from our experiments are compared to literature values and theoretical predictions based on either a rigid rod model for A-class, or a globular model for B-class.

The difference in higher order structure of the different classes of CpG-ODN most probably contributes to the difference in immune response.

[1] Hartmann, G.; Krieg, A. M. J. Immunol. 2000, 164, 944-952.
[2] Krug, A.; Rothenfusser, S.; Hornung, V.; Jahrsdörfer, B.; Blackwell, S.; Ballas, Z. K.; Endres, S.; Krieg, A. M.; Hartmann, G. Eur. J. Immunol. 2001, 31, 2154-2163.
[3] Latz, E.; Verma, A.; Visintin, A.; Gong, M.; Sirois, C. M.; Klein, D. C. G.; Monks, B. G.; McKnight, C. J.; Lamphier, M. S.; Duprex, W. P.; Espevik, T.; Golenbock, D. T. Nature Immunology 2007, 8, 772-779.
[4] Costa, L. T.; Kerkmann, M.; Hartmann, G.; Endres, S.; Bisch, P. M.; Heckl, W. M.; Thalhammer, S. Biochem. Biophys. Res. Commun. 2004, 313, 1065-1072.

Observation of rotational diffusion of GFP-tagged MHC molecule in living cells with polarized FCS

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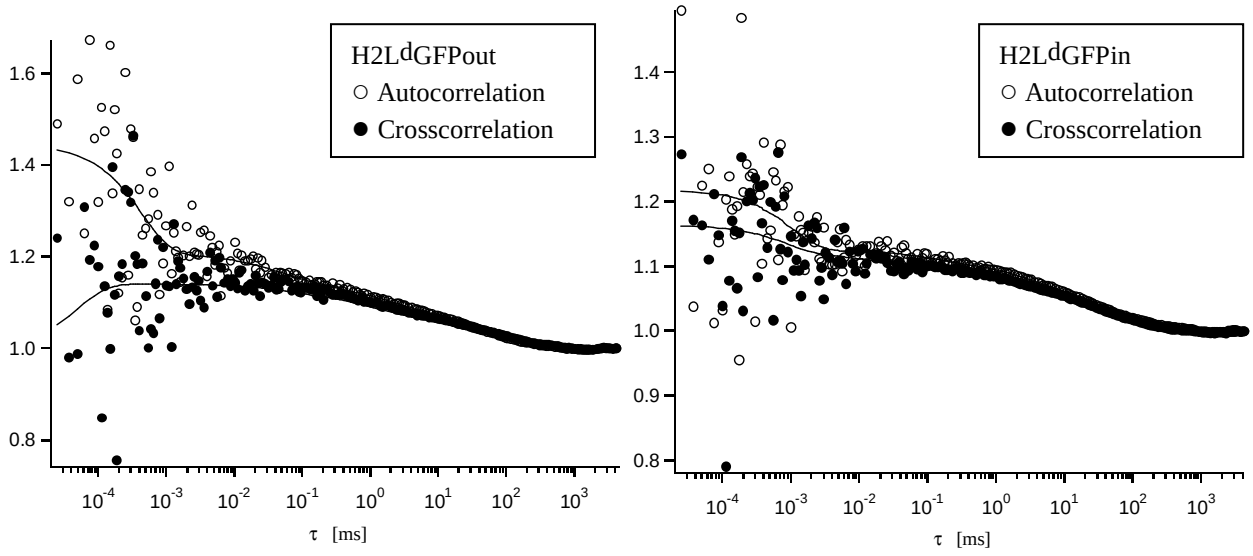
The MHC (major histocompatibility complex) – encoded class I and class II membrane glycoproteins are involved in the complex procedure of recognising a viral invasion. This process involves presenting an antigen on cell's surface via MHC membrane protein [1]. Despite of intensive research, the recognition process of antigen loaded MHC molecules via T-Cell receptors (TCR) is poorly understood. The question remains to which extend the peptide-MHC molecules act together in order to trigger the TCR signalling.
FCS polarisation methods are expected to give information on the formation and size of molecular clusters in living cells. In this work we have implemented a polarized FCS technique, in order to explore MHC clusters formation. The rotational diffusion of GFP-tagged $H2L^d$ protein $H2L^d$GFPin and $H2L^d$GFPout expressed in COS7 cells is compared. The $H2L^d$GFPin has the GFP attached to its cytoplasmic tail and is considered to be very flexible in its motions. The $H2L^d$GFPout has its GFP attached within the amino acid sequence of the $H2L^d$ protein and is considered to be rather rigid, which makes is accessible to polarized measurements [2]. Our results show that polarized FCS is able to discriminate the rotational dynamics behavior of these two systems.



Auto (○)- and crosscorrelation (●) of $H2L^d$GFPout (right) and $H2L^d$GFPin (left). On short time scales of several hundreds ns the anticorrelation of $H2L^d$GFPin is less pronounced than for $H2L^d$GFPout. The data have been recorded with circular polarized excitation and cross-correlated x, y detection channels.

[1]. R. N. Germain, *Cell*, Vol. 76, 287-299 (1994)
[2]. J. V. Rocheleau, M. Edidin, and D. W. Piston, *Biophysical Journal*, Vol. 84, 4078–4086, (2003)

The influence of chitosan structure on the intracellular trafficking and dissociation of chitosan-pDNA polyplexes studied by FCS.

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Chitosan is a biodegradable material obtained by partial or full de-acetylation of a marine biopolymer, chitin. As a cationic and biodegradable polysaccharide presenting a low cytotoxicity, chitosan is a promising nonviral gene delivery vector. Previous studies have shown that although more than 85% of a population of cells internalizes chitosan-plasmid DNA polyplexes, transfection efficiency is strongly dependent on chitosan intrinsic properties. Modifying chitosan molecular weight, degree of acetylation, structure, functionality, as well as chitosan/DNA ratio leads to the formation of polyplexes of different structures [1]. These structures might be internalized by the cells via different uptake mechanisms which may ultimately result in varied transfection efficiencies [2, 3]. Hence studying the intracellular traffic of chitosan-pDNA polyplexes as a function of the intrinsic properties of chitosan might help to improve chitosan/DNA gene delivery system.

Fluorescence Correlation Spectroscopy FCS combined with Confocal Laser Scanning Microscopy CLSM allows detection of single free and partially bound particles into a very small and carefully chosen cellular volume. Hence CLSM was used to observe chitosan-pDNA polyplexes intracellularly, while FCS (LSM 510 Meta Confocor 2, Carl Zeiss, Germany) enabled us to study dissociation of the polyplexes by measuring the apparent concentrations and diffusion times of polyplexes inside the cells. In order to identify the critical steps impeding gene transfer, this study focused on two different linear and fully deacetylated chitosan oligomers leading to high and low transfection efficacy in HEK 293 cells. Chitosan with degree of polymerisation DPn 31 and 88 were used, and chitosan DPn31 had 4 times higher transfection efficacy than chitosan DPn88. Cells were incubated with chitosan labeled with alexa fluor 647 and the oligomers were either free or complexed with pDNA.

The experimental autocorrelation curve was fitted to a two-component model. The shorter diffusion times of alexa fluor 647 labeled chitosan-pDNA polyplexes seem to indicate the presence of free chitosan both inside cells cytoplasm and nuclei. Bound DPn 31 chitosan tends to accumulate into cells cytoplasm while bound DPn88 seems to concentrate into cells nuclei 24 hours post transfection. Labeling DNA instead of chitosan might help to get more insights regarding the mechanisms of transport of polyplexes inside the cells.

[1] Reitan et al., “Characterizing DNA condensation by structurally different chitosans of variable gene transfer efficacy”, Biomacromolecules, 10(6), 1508, (2009).

[2] Issa et al., “Targeted gene delivery with trisaccharide-substituted chitosan oligomers in vitro and after lung dministration in vivo”, Journal of controlled release, 115, 103, (2006).

[3] Strand et al., “Tailoring of Chitosans for Gene Delivery: Novel Self-Branched Glycosylated Chitosan Oligomers with Improved Functional Properties”, Biomacromolecules 9(11), 3268, (2008)

Selective compartmentalization of the death receptor Fas

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The Fas receptor-ligand system is known as one of the key regulators of apoptosis and is particularly important for the maintenance of lymphocyte homeostasis. The apoptosis signaling of Fas is triggered through ligation by its ligand (FasL), either as membrane-associated FasL on FasL-expressing cells or as cross-linked soluble FasL. Following ligand engagement, Fas rapidly recruits Fas-associated death domain protein (FADD) and procaspase-8 to form the death-inducing signaling complex (DISC), which leads to activation of a caspase cascade and ultimately cell death. Although the nature of molecular events downstream of the DISC formation is rather well documented, eth knowledge of initial events, particularly at the plasma membrane level, governing the formation of this complex remains poorly defined.

To understand the molecular mechanisms that might govern the fate of Fas receptors at the cell surface upon FasL engagement, we first investigated the role of potential post-translational lipid modifications of the Fas cytoplasmic domain. We established the FCS diffusion laws [1] of Fas under different experimental conditions. We demonstrate that Fas is palmitoylated and that this post-translational modification of the receptor is essential for the redistribution of Fas into lipid rafts and its association to ezrin and actin cytoskeleton [2].

Moreover, studies have indicated endocytosis as a means for coordinating the type, extent, and timing of signals of surface receptors. This is the case for Fas, of which efficient cell death signal requires its internalization and this process involves the interaction between Fas and its lipidic environment including its targeting by palmitoylation to the cholesterol-, glycosphingolipid-rich membrane microdomains (rafts). A glycosphingolipid has an oligosaccharide headgroup linked to a ceramide backbone. Many glycosphingolipids are involved in the sorting and trafficking of proteins and lipids. Recently, a common motif with glycosphingolipid-binding specificity has been identified in proteins that share little sequence homology. We report the identification of such a motif in Fas and its role in the selection of the internalization route of the receptor, which defines its mode of signal [3].

[1] Lenne PF*****, Wawrezinieck L*****, Conchonaud F, Wurtz O, Boned A, Guo XJ, Rigneault H, He HT, Marguet D Dynamic molecular confinement in the plasma membrane by microdomains and the cytoskeleton meshwork **EMBO J** *25*:3245-56 (2006)

[2] Chakrabandhu K, Hérincs Z, Huault S, Dost B, Peng L, Conchonaud F, Marguet D, He HT, Hueber AO Palmitoylation is required for efficient Fas cell death signaling **EMBO J** *26*:209-20 (2007)

[3] Chakrabandhu K, Huault S, Garmy N, Fantini J, Stebe E, Mailfert S, Marguet D, Hueber AO The extracellular glycosphingolipid-binding motif of Fas defines its internalization route, mode and outcome of signals upon activation by ligand **Cell Death Differ** *15*:1824-37 (2008)

TIME-RESOLVED CONFOCAL FLUORESCENCE MICROSCOPY: A GENERALIZED APPROACH ENABLES NEW DIRECTIONS FOR FLIM, FRET AND FCS

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Today, fluorescence dynamics in single molecules can be followed on timescales from sub-nanoseconds to seconds and even beyond with a universal approach of time-resolved measurement. The underlying technique (Time-Tagged Time-Resolved (TTTR) Recording) allows one to simultaneously record timing and fluorescence intensity information, both spectrally and spatially, on a single photon basis. We apply photon sorting and weighting schemes determined from the nanosecond photon arrival times to extend and improve single-molecule fluorescence methodologies which up to now commonly utilize only intensity-based analysis, namely FCS and FRET.

In Fluorescence Lifetime Correlation Spectroscopy (FLCS) photon weighting provides superior suppression of common parasitic contributions, e.g., Raman scattering and detector after-pulsing. Beyond this improvement of traditional FCS, FLCS also offers the possibility to accurately determine diffusion properties of different species only requiring that the species differ in their fluorescence lifetimes, i.e., the need for multicolor labeling is eliminated [1].

In 2 Focus FCS (2fFCS) the nanosecond timing information is used to identify the spatial origin of the photons by combining Pulsed Interleaved Excitation (PIE) with time-gated detection [2]. By using two orthogonally polarized and delayed pulsed laser diodes it is possible to design a robust dual–foci geometry where the well known focal distance define an intrinsic length scale to study diffusion in solution. This enables one to overcome various uncertainties and limitations of single-focus FCS that arise because one must rely on knowledge of the size and shape of the confocal volume. Thereby, 2fFCS dramatically improves the accuracy of measuring absolute diffusion coefficients.

In addition to this, Pulsed Interleaved Excitation can be used to identify artifacts and sub-populations in single-pair FRET measurements. Nanosecond time-resolved detection offers a complementary approach to donor/acceptor intensity based methods for calculating FRET efficiencies via quenching of the donor lifetime. We compare fluorescence intensity- and lifetime-based techniques for extracting FRET efficiencies in RNA folding studies of a single tertiary binding motif, the GAAA tetraloop and its receptor.

[1] Benda A., Hof. M., Wahl M., Patting M., Erdmann R., Kapusta P., Rev. Sci. Instr., Vol.76, 033106 (2005)
[2] Dertinger Th., Pacheco V., von der Hocht I., Hartmann R., Gregor I., Enderlein J, ChemPhysChem, Vol.8, p.433 (2007)

Investigations of membrane order effects protein diffusion using phase-sensitive probes and fluorescence correlation spectroscopy

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Membrane lipid microdomains – lipid rafts – are believed to be important structures in the organization of cellular signaling events at the plasma membrane. They are thought to be highly-ordered membrane domains enriched in saturated phospholipids, sphingolipids and cholesterol [1]. We have developed advanced fluorescence microscopy methods, used together with the order-sensitive membrane probes Laurdan and di-4-ANEPPDHQ to image order distributions in live cells. These include two-photon and confocal microscopy, multi-channel total internal reflection fluorescence (TIRF) microscopy and fluorescence lifetime imaging (FLIM) using time-correlated single photon counting (TCSPC) [2]. We mainly focus on the T cell immunological synapse in which lipid rafts have been proposed to have an important regulatory role and we detect a distal ring of high membrane order at the synapse peripheral region [3].

The membrane protein LAT (linker for activation of T cells) has been shown to be raft associated and critical for T cell activation. Using fluorescent fusion constructs and mutants of LAT, we investigate the correlation between LAT diffusion, membrane order and the underlying actin cytoskeleton. This is done using a combination of quantitative order imaging and simultaneous fluorescence correlation spectroscopy measurements of LAT acquired using TCSPC.

Receptor-ligand interaction studies in plants by FCS, FRAP and FLIM

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Plants possess at the tip of the shoot and the root meristems that harbor pluripotent stem cells from which all cells of the plant body derive. Intercellular signaling processes mediated by small peptide ligands play important roles in the necessary dynamic but also tight regulation of the transition from stem cell fate to differentiation. The stem cells in the *Arabidopsis thaliana* shoot apical meristem express and secrete CLAVATA3 (CLV3*)* dodecapeptide and this signal is transmitted by the membrane localized leucine rich repeat (LRR) receptor kinase CLAVATA1 (CLV1) to the subjacent organizing cells. This signaling eventually leads to a negative feedback loop adjusting stem cell homeostasis in the shoot. A direct binding of CLV3 to the ectodomain of CLV1 was shown using radio labeled ligand and CLV1 extracellular domain [1]. Recently we discovered, based on genetic evidence, that a similar regulation, consisting of a CLV3-related peptide (CLE40) and a membrane localized receptor kinase (ACR4), also exists in the root meristem [2]. We therefore want to proof and analyze ligand-receptor interaction and putative complex formation in transiently expressing tobacco leaves and *Arabidopsis*. We are currently establishing experimental setups using fluorescently labeled receptors and ligands for these analyses using fluorescent correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP) and fluorescent lifetime imaging (FLIM) techniques.

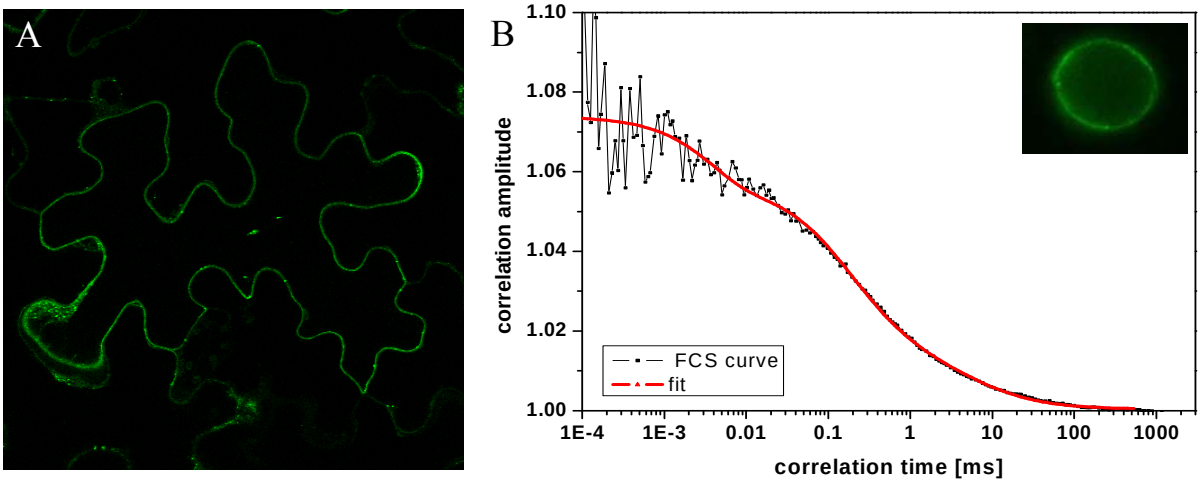

Fig. 1. A) CLV1-GFP expressing tobacco leaf cell **B)** FCS point measurement on protoplasted CLV1-GFP expressing tobacco leaf cell (inset shows protoplast used for measurement).

[1] Ogawa, M, Shinohara H, Sakagami Y, Matsubayashi Y "Arabidopsis CLV3 peptide directly binds CLV1 ectodomain" Science, 319, 294, (2008).
[2] Stahl Y, Wink RH, Ingram GC, Simon R "A Signaling Module Controlling the Stem Cell Niche in Arabidopsis Root Meristems" Curr Biol, 19, 909–914, (2009).

Nucleosome structural variations characterized by single molecule FRET

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The nucleosome has a central role in the compaction of genomic DNA and the control of DNA accessibility for transcription and replication. We studied the effect of DNA sequence and selective histone acetylation on the structure, stability and disassembly of the mononucleosomes. Quantitative single molecule FRET measurements between dyes attached to different parts of the nucleosome permitted us to detect the equilibrium between several subpopulations of reconstituted nucleosomes in solution. We obtained that the heterogeneity and stability of the samples are correlated with each other and influenced both by the DNA sequence and the histone acetylation. The path of the linker DNA is more sensitive to all studied effects than the DNA on the core. Intermediates of the disassembly pathway were identified and characterized.

Nanoaperture-enhanced FCS : understanding the physical origin of fluorescence enhancement

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Subwavelength apertures milled in a metallic film can significantly enhance the fluorescence emission rate of molecules diffusing inside of them [1]. This phenomenon stems from the electromagnetic enhancement of the excitation field inside the aperture, and also from modifications of the emission properties of the emitter (quantum efficiency and emission diagram). Understanding the relative weight of these effects in the total fluorescence signal is a crucial knowledge to design nanostructures for high-efficiency single-molecule analysis.

In this communication we present a comprehensive study of the fluorescence emission of dyes freely diffusing inside circular apertures milled in a 200 nm thick gold film with diameters from 80 up to 350 nm [2]. Fluorescence correlation spectroscopy experiments (Fig. 1) allow a quantitative estimation of the fluorescence emission enhancement factor at the single-molecule scale. Moreover, we present an experimental method to discriminate the excitation and emission contributions to the enhancement factor [3].

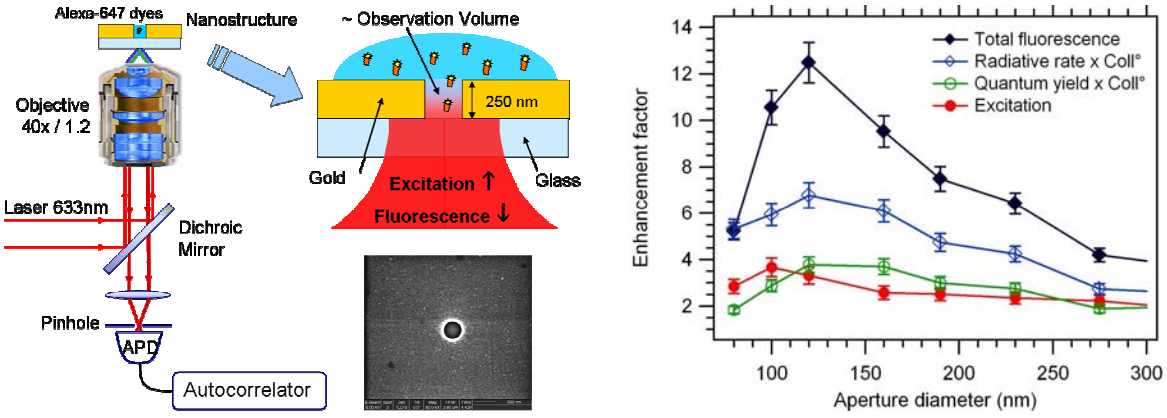

Fig. 1: (Left) Schematic view of the experimental setup: the emission from dyes diffusing in a nanoaperture is detected through a confocal microscope in epi-fluorescence configuration. (Right) Physical contributions to nanoaperture enhanced fluorescence, plotted versus the aperture diameter and normalized to the open solution case.

[1] H. Rigneault, J. Capoulade, J. Dintinger, J. Wenger, N. Bonod, E. Popov, T.W. Ebbesen, and P.-F. Lenne, *Phys. Rev. Lett*. **95**, 117401 (2005).

[2] D. Gérard, J. Wenger, N. Bonod, E. Popov, H. Rigneault, F. Mahdavi, S. Blair, J. Dintinger, and T.W. Ebbesen, *Phys. Rev. B* **77**, 045413 (2008).

[3] J. Wenger, D. Gérard, J. Dintinger, O. Mahboub, N. Bonod, E. Popov, T. W. Ebbesen, and H. Rigneault, *Opt. Express* **16**, 3008 (2008).

DISTRIBUTION OF PLASMA MEMBRANE MICROFLUIDITY AT THE SINGLE CELL LEVEL

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Fluorescence Correlation Spectroscopy (FCS) has been used to explore the plasma membrane microfluidity of multidrug-resistant cancer cells. FCS measurements together with diffusion-time distribution analysis were performed at the single cell level [1,2]. It enabled us to reveal the distribution law of the plasma membrane fluidity. The method was conducted on a large number of living cells, which allowed us to get an accurate view of plasma membrane micro-viscosity. Thus, the diffusion-time distribution obtained for MCF-7 cells is asymmetric, as shown in Fig.1. This typical distribution of diffusion-time, which is also observed for different cancer cell lines, can be clearly approximate by the well-known Gumbel distribution, Fig.1.

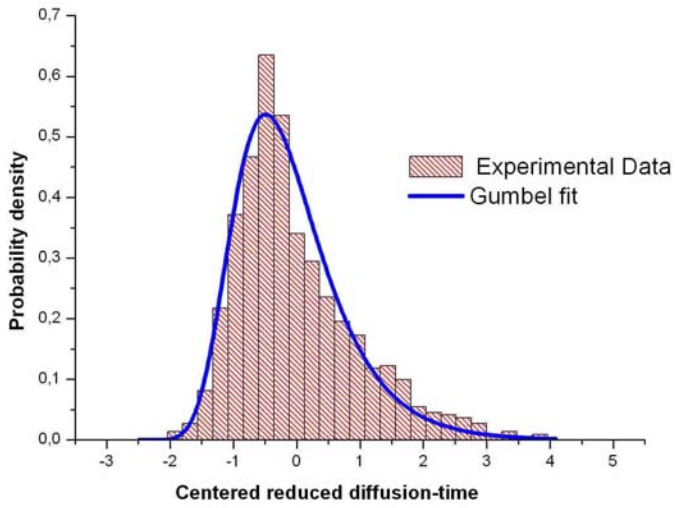

Fig. 1. Normalized diffusion-time distribution of MCF7 cell's plasma membrane (the diffusion-time distribution is centered around the mean with $< \tau_d > = 0$, and the standard deviation is normalized to unity, $\int \tau_d = 1$). This distribution was obtained from a population of 45 different cells. The blue curve correspond to a fit by the Gumbel distribution.

[1] H. Jankevics *et al.*, "Diffusion-time distribution analysis reveals characteristic ligand-dependent interaction patterns of nuclear receptor in living cells," Biochemistry 44, 11676–11683 (2005).
[2] C. Boutin *et al.*, "High heterogeneity of plasma membrane microfluidity in multidrug-resistant cancer cells" Journal of Biomedical Optics 14(3), 034030 (2009).

Beta-2a facilitated trafficking of L-type calcium channel Cav1.2 as revealed by fluorescence correlation spectroscope in insulin-secreting cells.

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*Islets Pathophysiology, Department of Clinical Science, Lund University, Malmo, 20502, Sweden.
E-mail: enming.zhang@med.lu.se

Background
Ca^{2+} signals controlled by Cav1.2 play important roles as trnsducing signals from glucose metabolism to insulin secretion in pancreatic beta islet cells. Cav1.2 trafficking between cytosol and plasma membrane, has emerged as a rapid process that can respond to abnormal extracellular environments such as quick glucose rising. In the early type-2 diabetes, the rapid response to high concentration glucose is lost in beta-cells. If the response lack is related to Cav1.2 trafficking has still not been elucidated.

Methods
Insulin-secreting INS-1 cells were cultured on glass coverslips (<0.13cm) and transfected by plasmid of Cav1.2-EGFP, pLMViβ2a-ERFP. Fluorescence correlation measurements were performed by a combined confocor3-confocal system. The fluorescence of Cav1.2 cluster was collected from NA 40x water objective and the excited laser line was 488nm and 543nm. The emission filters for Cav1.2-EGFP is BP500-530 and β2a-ERFP was BP580-610. Data analysis carried out by software that provided with Confocor3.

Results
Glucose can affect the dynamics of Cav1.2 cluster on plasma membrane. After stimulation with 20mM glucose, the molecule number (*N*) of Cav1.2 clusters on plasma membrane decreased about 40%.The diffusion time of Cav1.2 cluster delay from 400±200ms to 600±200ms.
Cav1.2 mobility on plasma membrane need facilitate with β2a subunits. Cross-correlation spectroscopy analysis shows that the simulations of fitted curve under condition both before and after glucose stimulation is match well. Interestingly, in the resting condition, the molecule number of β2a subunits is 50% more than Cav1.2 on plasma membrane.

Conclusion
High glucose decrease the concentration of Cav1.2 on plasma membrane and this effect could be regulated by β2a subunits.

An attempt to quantify tat peptide's uptake at therapeutic concentrations using Fluorescence Correlation Spectroscopy and Image Correlation Spectroscopy techniques

Egor Zindy , Benjamin Staley, Alain Pluen and David Clarke

School of Pharmacy and Pharmaceutical Sciences, Manchester University

Egor.Zindy@manchester.ac.uk

Due an apparent ability to enter cells in an energy independent manner, cell-penetrating peptides (CPPs) are increasingly being used as vectors for the delivery of macromolecules into cells. But 20 years on, their mechanism of entry is still disputed[1]. Additionally cellular uptake and delivery have been studied at relatively high concentrations (micromolar), while therapeutic doses more likely to be in the nanomolar range. Thus, we hypothesised that taking advantage of Fluorescence Correlation Spectroscopy (FCS) and Image Correlation Spectroscopy (ICS) should help to understand the delivery mechanisms of Tat peptide, the most commonly studied CPP, at therapeutic doses.

Tat-TAMRA peptide (at bulk concentrations lower than 500nM) was incubated for one hour with Caco-2 cells. Subcellular distribution was analysed with confocal microscopy revealing three localised areas -discrete, punctuate and cytoplasmic regions- sampled initially with FCS. To improve our understanding at the cytoplasmic level, ICS[2,3] was implemented to parallel and correlate FCS. ICS and FCS were applied initially to a solution of 20nm fluorescent beads of known diameter and concentration to test the influence of parameters such as image and pixel size, pixel dwell time, number of images and need for image rejection criteria. Then Tat-TAMRA peptide presence and molecular concentrations were determined in the different areas of Caco-2 cells using ICS and FCS.

Our results indicate that tat-TAMRA peptide is observed at both 250 and 500nM concentrations in all three areas sampled. The amount in cytoplasm appears to be linearly dependant with concentration. On the other hand, in the punctuate and discrete areas, the amount of Tat-TAMRA peptide did not appear to be concentration dependent.

To conclude, FCS and ICS have shown they can provide valuable information on the delivery of peptides at therapeutic levels.

References:

1. Lee, H. L.; Dubikovskaya, E. A.; Hwang, H.; Semyonov, A. N.; Wang, H.; Jones, L. R.; Twieg, R. J.; Moerner, W. E.; Wender, P. A. Journal of the American Chemical Society 2008, 130, 9364-9370.

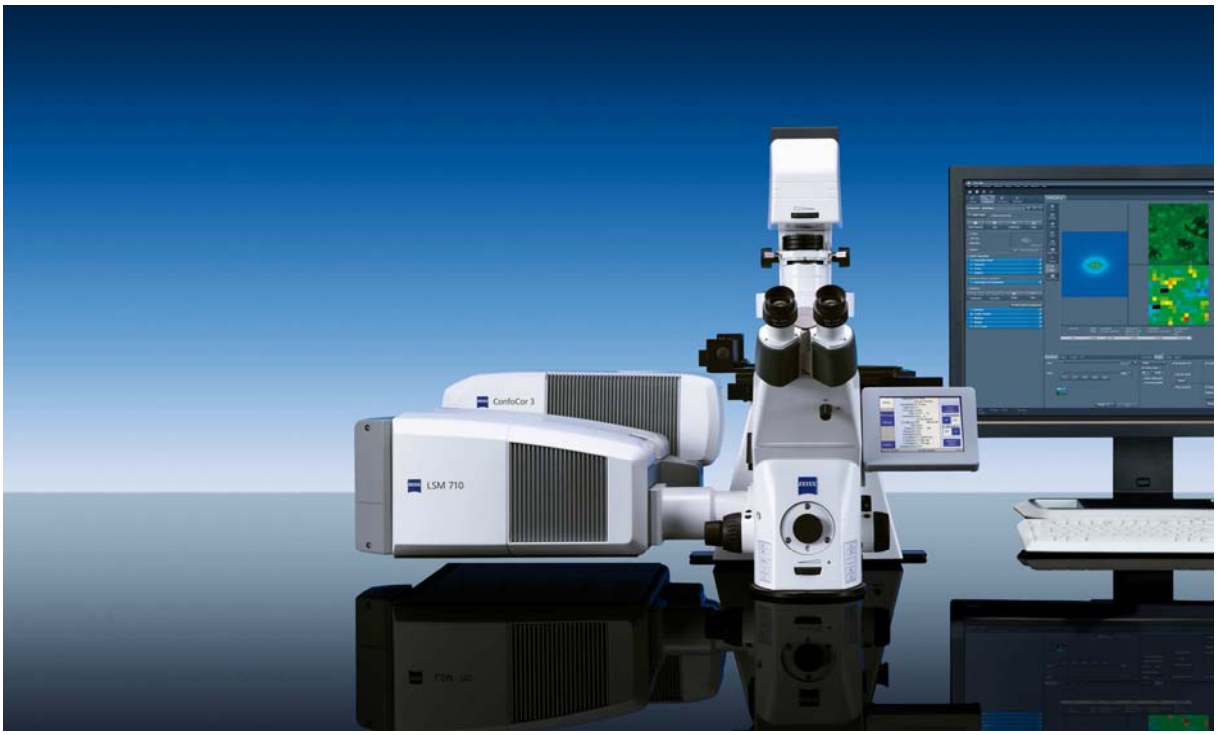
2. Kolin, D. L.; Wiseman, P. W. Cell Biochemistry and Biophysics 2007, 49, 141-164.

3. Hebert, B.; Costantino, S.; Wiseman, P. W. Biophysical Journal 2005, 88, 3601-3614.

Industrial Partners

ConfoCor 3 – Laser Scanning detection module

Bringing sensitivity to your samples!



The ConfoCor 3 was designed for highest light efficiency to analyze molecules one at a time. Outstanding features include:

- Full integration of ConfoCor functions into the LSM ZEN 710 platform for highest flexibility.
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- C-Apochromat 40x / 1.2W NIR-VIS-UV objective for highest sensitivity and excellent color correction.
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- Photon counting imaging with low background and highest sensitivity in point detection.
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- RICS for obtaining spatial information on diffusion and binding processes within your cell in one glance.
- Photo counting histogram to analyze oligomerization states of molecules.
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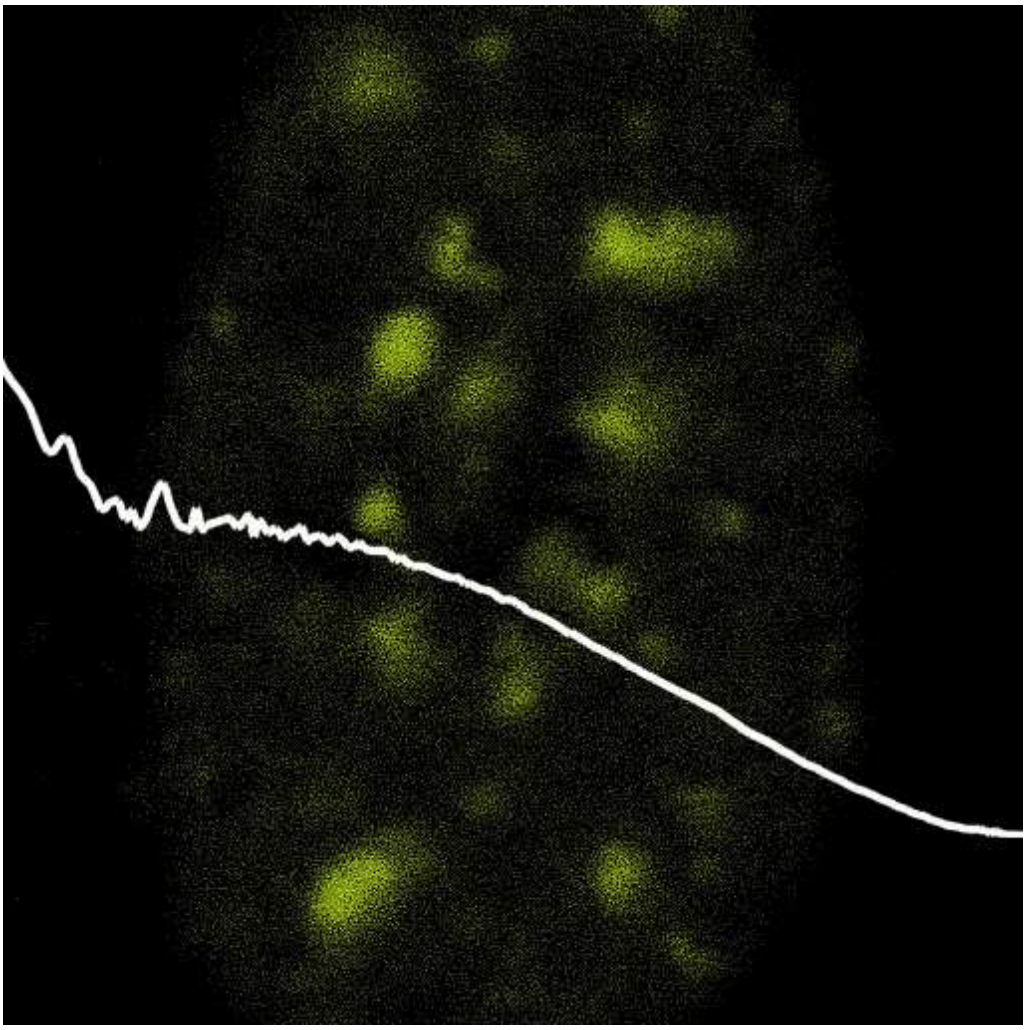
All what it takes, is to select the measurement position and than let the system perform at its best.



Specimen: HepG2 cell expressing EGFP-HP1. The protein binds chromatin. Correlation analysis shows two molecule classes having diffusion coefficients of 5.8 µm²/s (unbound) and 0.2 µm²/s (transiently bound to chromatin). Kindly provided by Karolin Klement and Peter Hemmerich, Fritz-Lipmann-Institute, Jena, Germany

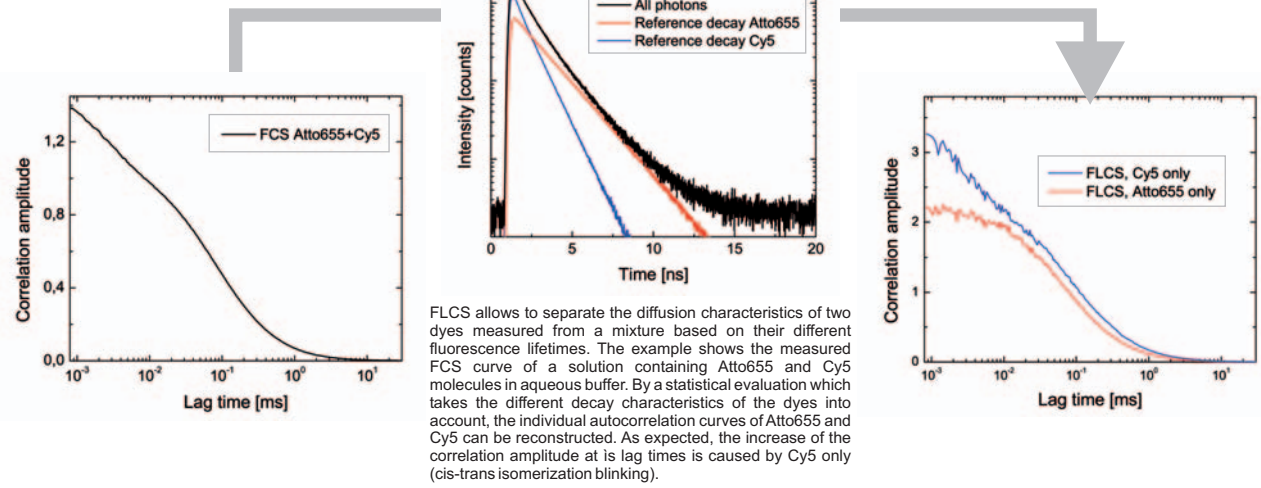
PicoQuant for FLIM, FRET & FCS

PicoQuant GmbH is a research and development company, founded in 1996 and based in the Technology Park Berlin-Adlershof, Germany. A north american subsidiary, PicoQuant Photonics North America Inc., was established in April 2008. The company is leading in the field of Single Photon Counting Applications. The product line includes pulsed light sources, photon counting instrumentation and fluorescence lifetime systems. PicoQuant offers a range of compact and easy to use picosecond diode laser systems with wavelengths from ultra-violet to infrared. PicoQuant also developed new technology for Time-Correlated Single Photon Counting (TCSCP). Combining these technologies, the outstanding range of time-resolved spectrometers was born. A variety from compact systems to high-end modular lifetime spectrometers and finally complete confocal time-resolved microscopes and upgrade kits for Laser Scanning Microscopes is available.

The combination of pulsed laers and TCSPC electronics allows new applications, such as:

Fluorescence Lifetime Correlation Spectroscopy (FLCS)

FLCS is based on a statistical separation of different intensity contributions, performed on a single photon level. It can be used to e.g. correct for detector afterpulsing without the need for crosscorrelation, to remove influence from scattered or background light or to separate the FCS contributions of two dyes measured from a mixture, based on their different fluorescence lifetimes.

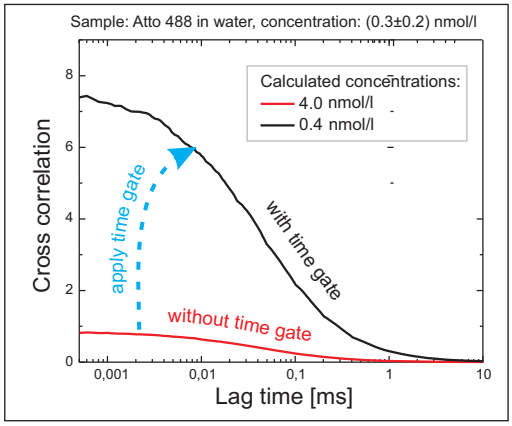


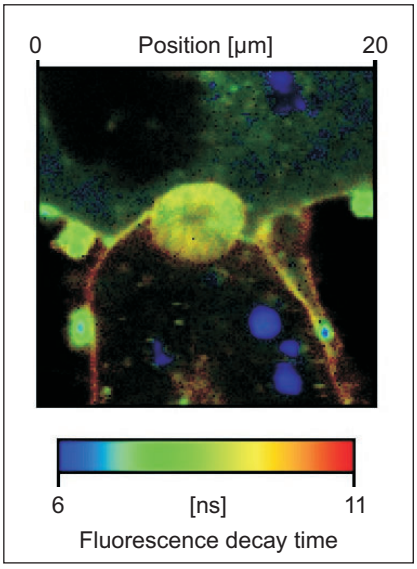
FLCS allows to separate the diffusion characteristics of two dyes measured from a mixture based on their different fluorescence lifetimes. The example shows the measured FCS curve of a solution containing Atto655 and Cy5 molecules in aqueous buffer. By a statistical evaluation which takes the different decay characteristics of the dyes into account, the individual autocorrelation curves of Atto655 and Cy5 can be reconstructed. As expected, the increase of the correlation amplitude at is lag times is caused by Cy5 only (cis-trans isomerization blinking).

An even more advanced method uses not only one, but two laterally shifted, slightly overlapping laser foci at a fixed and known distance. This measurement scheme, termed two-focus-FCS (2fFCS), is capable of measuring absolute values of diffusion coefficients without referencing against a sample with known diffusion coefficient, like it is often done in conventional FCS.

Time-gated FCS

Inserting a time-gate prior to auto-or cross-correlation analysis can be used to suppress the influence of scattered light contributions and allows e.g. a more accurate concentration measurement.





Cell biology

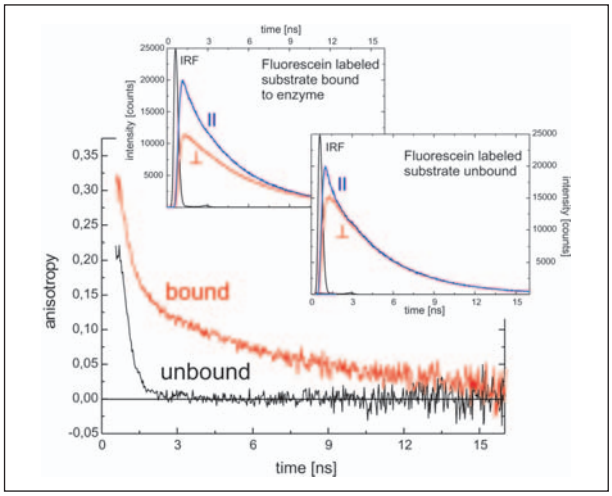
PicoQuant offers solutions to upgrade Laser Scanning Microscopes towards time-resolved measurements for Fluorescence Lifetime Imaging in cell biology

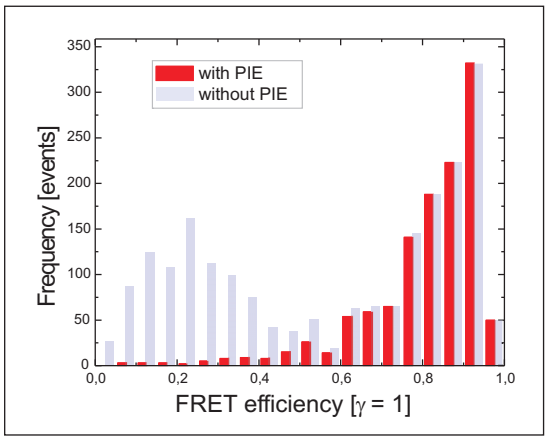
Fluorescence Lifetime Image of a part of the membrane of a living hepatocyte cell, stained with the dye NBD, whose lifetime is depending on the hydrophobicity of the environment (courtesy of Dr. Tannert, Humboldt-University Berlin, Molecular Biophysics). Every pixel shows the result of an single exponential fit to the fluorescence decay built from all photons contained in this pixel.

Classification of diffusing molecules

PicoQuant systems can be used to measure fluorescence lifetime and fluorescence anisotropy.

Example of an anisotropy measurement of a fluorescein labeled substrate with and without the presence of a binding enzyme. Upon binding to the enzyme, the mobility of the substrate changes, which can be clearly seen in the anisotropy curves.





(PIE)-FRET

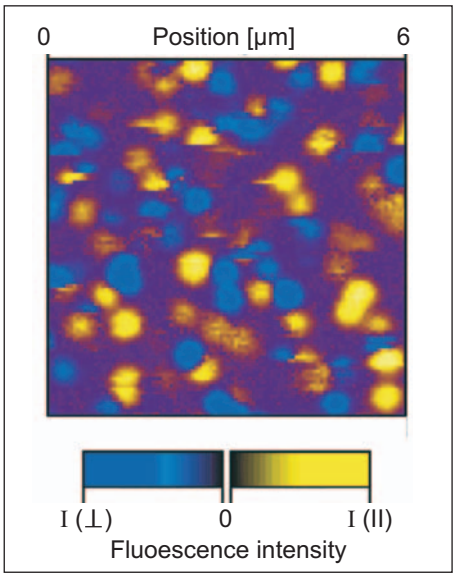
PicoQuant offers specialized systems for Förster Resonance Energy Transfer measurements using Pulsed-Interleaved Excitation (PIE).

With PIE the acceptor molecule is excited idenpendently from the donor to prove ist excistance. These measurements can e.g. be used to identify incomplete FRET pairs, which lead to additional peaks at low FRET efficiencies.

Single Molecule Spectroscopy

PicoQuant offers a complete single molecule spectroscopy microscope for Fluorescence Lifetime Imaging (FLIM) and Fluorescene Correlation Spectroscopy (FCS).

Raster-scanned image of immobilized single molecules, measured with the MicroTime 200. Polarization-resolved fluorescence of isolated, single Cy5 molecules on top of a standard glass coverslip. The collected fluorescence light was detected with two SPAD detectors. The image contains all molecules which exhibit either a predominant parallel (yellow) or perpendicular (blue) polarized emission.



Inverse time-resolved confocal microscope

- Picosecond diode lasers inside a compact fibre coupling unit for excitation, wavelengths between 375 nm and 900 nm
- Multi-channel driver for Pulsed Interleaved Excitation
- 2D and 3D high resolution piezo scanning
- Up to 4 detectors (PMTs or SPADs)
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- Advanced measurement and analysis software
- Exit ports for further extensions

MicroTime 200

Add temporal resolution to your Laser Scanning Microscope

- Picosecond diode lasers for excitation
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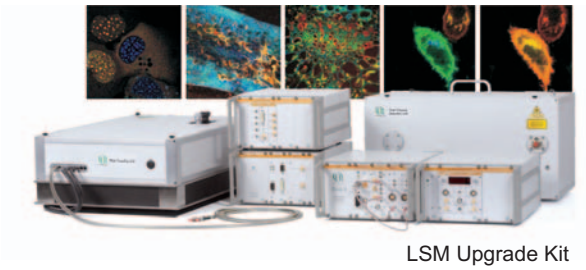
OLYMPUS (Your Vision, Our Future)	Nikon	Leica MICROSYSTEMS	ZEISS
FluoView FV1000 FluoView FV300	A1, C1, C1si	TCS SP5 TCS SP2	LSM 710 LSM 510

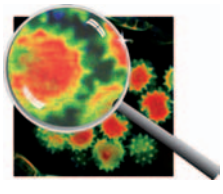


LSM Upgrade Kit

Application Notes for download - www.picoquant.com/scientific.htm

- Fluorescence (Förster) Resonance Energy Transfer (FRET)
- FRET analysis using Pulsed-Interleaved Excitation (PIE)
- Fluorescence Lifetime Imaging (FLIM)
- Two-photon excitation using the MicroTime 200
- Time-gated Fluorescence Correlation Spectoscopy
- Fluorescence Lifetime Correlation Spectroscopy (FLCS)
- FLCS analysis using the SymPhoTime
- Quantitative Fluorescence Correlation Spectroscopy
- Two-Focus Fluorescence Correlation Spectroscopy (2fFCS)

2nd Short Course on Time-Resolved Microscopy



- 16-18 February 2010, Berlin-Adlershof, Germany
- http://www.picoquant.com/_mic-course.htm
- Topics: Introduction to Microscopy, Hardware for Time-Resolved Microscopy, FCS, FLIM, FRET, Steady-State Microscopy Techniques
- Course instructors: Jörg Enderlein, Paul French, Johan Hofkens, Fred Wouters, ...
- Hands-On experimentation and lab demonstration by: Leica, Nikon, Olympus and PicoQuant

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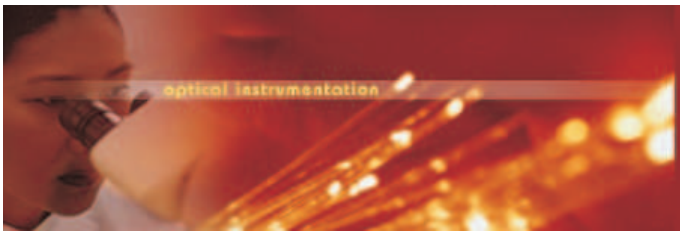

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Information Security Products

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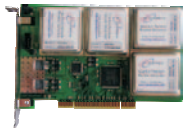

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Clavis2: high-speed encryption combined with quantum key distribution

Thanks to its world-class experience in quantum optics, *id Quantique* has developed the ultimate tool for quantum cryptography research. Whether the goal is novel protocol investigation, quantum network implementation or cryptographic study, the id3100 Clavis2 system allows quick experimental platform preparation and validation. The Clavis2 system is designed by researchers for researchers.

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id Quantique's Cerberis solution offers a radically new approach to network security, combining the sheer power of high-speed layer 2 encryption appliance (Centauris) with the unconditional security of quantum key distribution (QKD) technology.

The Centauris appliances perform high-speed encryption based on the standardized Advanced Encryption Standard (AES) (See below).

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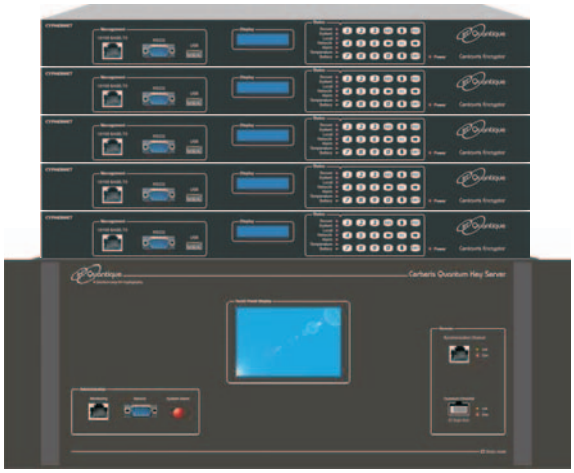

(Cerberis)

Centauris: layer 2 encryption engine

Centauris is a high performance layer 2 encryption engine. Those appliances are designed to support multi-protocol infrastructures such as ATM, SONET, Fibre channel and Ethernet. Centauris product ranges have a throughput from 2Mbps up to 10Gbps.

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Centauris is upgradable to be connected to a QKD Server in order to obtain the Cerberis solution.

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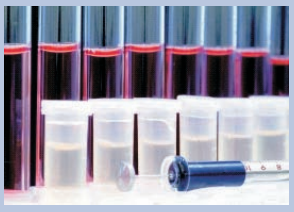

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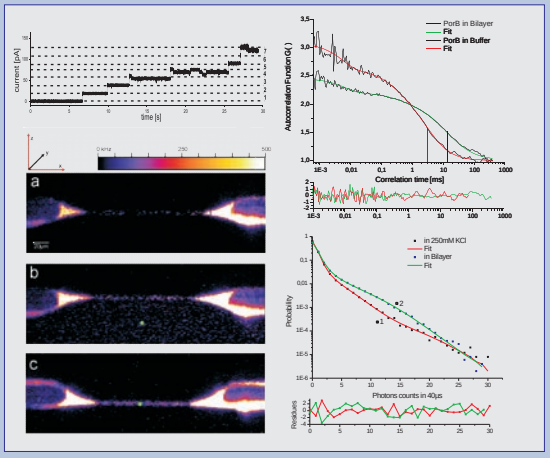
IonoVation Bilayer Explorer



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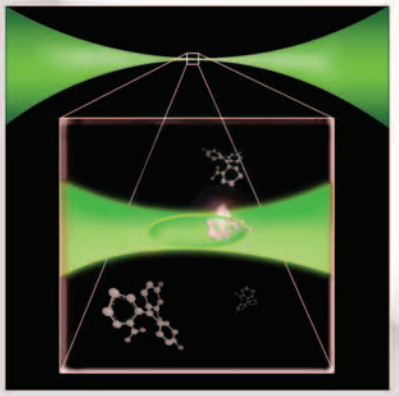
Introducing the QuantumXpert™ FCS Spectrometer

Fluorescence Correlation Spectroscopy (FCS) is a powerful single-molecule detection technique that has been costly and difficult to understand...**Until Now!**

FCS: High-sensitivity Measurements of Molecular Interactions



FCS: Single Molecule Detection Using Confocal Optics

The QuantumXpert™ is optimized for measurement of biologically and biochemically relevant particles using FCS. FCS provides information about the **molecular size**, **concentration**, and **interactions** of fluorescently-labeled particles in solution.

Use FCS to measure:

- Protein - protein interactions
- Protein - nucleic acid interactions
- Nucleic acid hybridization
- Ligand - receptor binding
- Enzyme kinetics
- Conformational changes

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Due to its **fully integrated** design, the QuantumXpert™ incorporates all optical and electronic components, eliminating the bulk, hassle, and maintenance of other FCS instruments. Its **compact design** makes the QuantumXpert™ a convenient addition to any lab.

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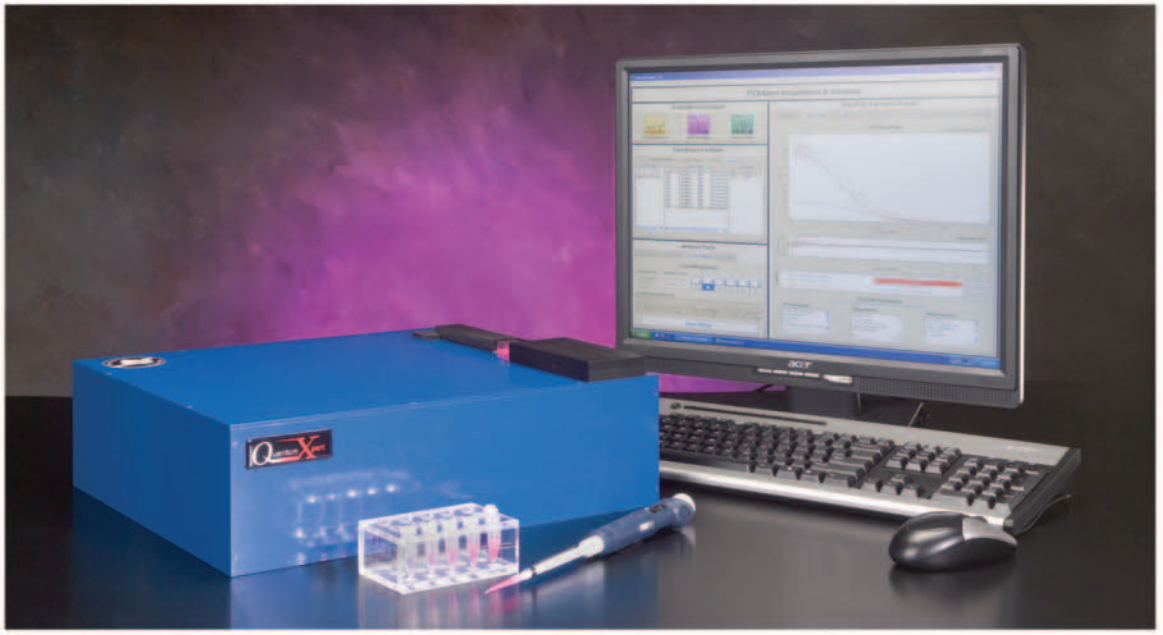
FCS no longer requires extensive technical expertise. Our FCSXpert™ software simplifies FCS data acquisition and analysis: **We put the Xpert in the Box!**



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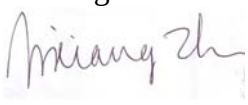
Dear Colleagues,

My name is Jixiang Zhu. Correlator.com is my trade name. My credentials include a Ph.D. in physics from City University of New York, and a research experience at Princeton University on dynamic light scattering. Using single chip programmable logic devices, I started manufacturing Flex series correlator 13 years ago. I have enjoyed working with researchers worldwide since then.

The Flex series correlator is designed specifically for FCS applications. The correlator computes multiple correlation functions simultaneously. Simultaneously, the device records photon counting histogram as well as a complete photon history at a high resolution.

Besides the correlators advertised here, I am happy to custom design a correlator for a specific application. I have a variety of technology available in house. This includes up to 32-channel correlator on one board, correlator with amplifier and discriminator. If you have any questions, please feel free to talk to me.

Best regards



Jixiang

Standard correlator, model # Flex03LQ

Specifications:

1. Single (channel AxA or AxB) correlation function, MT64 multiple tau resolution at resolutions as low as 1.56ns.
2. Dual (two auto AxA, BxB or two cross AxB, BxA) correlation function, MT32 multiple tau at resolutions as low as 1.56ns.
3. Quad (four arbitrary combination correlation functions) MT16 multiple tau at resolutions as low as 1.56ns.
4. Histogram with user selectable sample times.
5. Option to save a complete copy of photon history at a resolution as high as 640Mhz.
6. 2 channel Imaging application synchronized to a TTL pulse
7. Ready to run software and DLL compatible with Labview, C++, Visual Basic in Windows.

Low cost correlator, model # Flex02-12

Single (channel AxA or AxB) correlation function, MT64 Multiple tau resolution at 12.5ns resolution.

General Information

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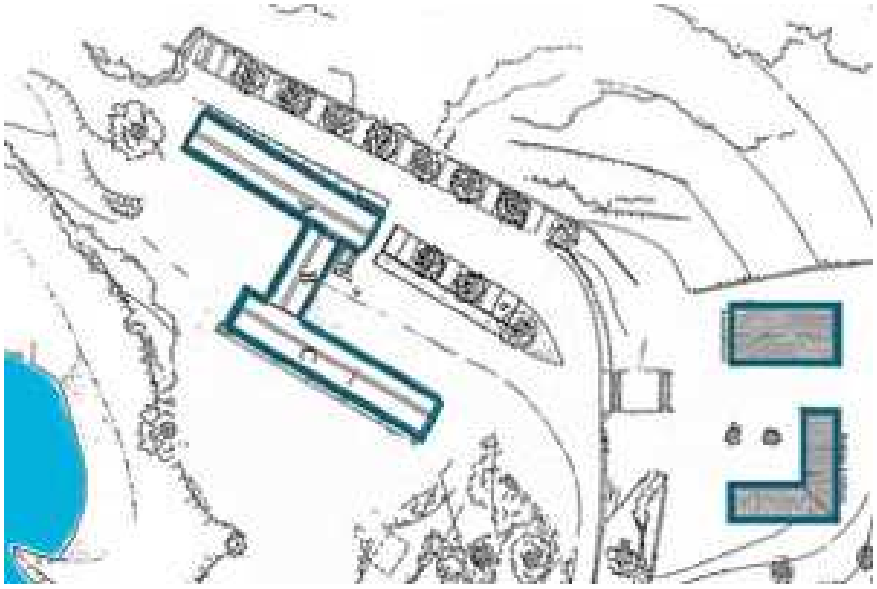
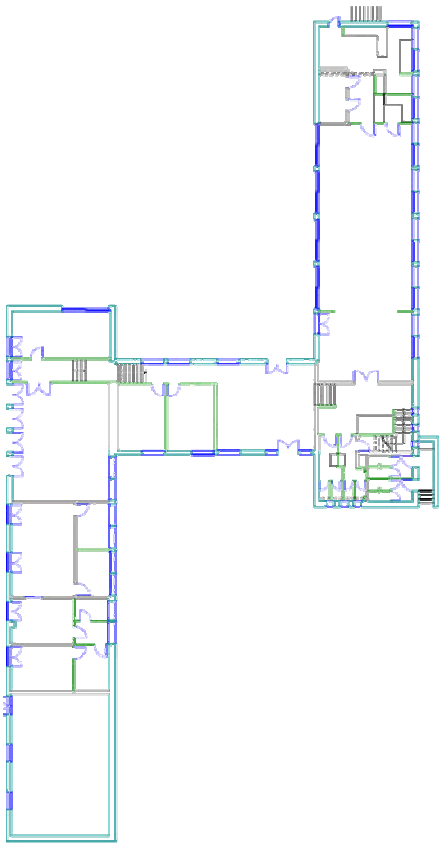
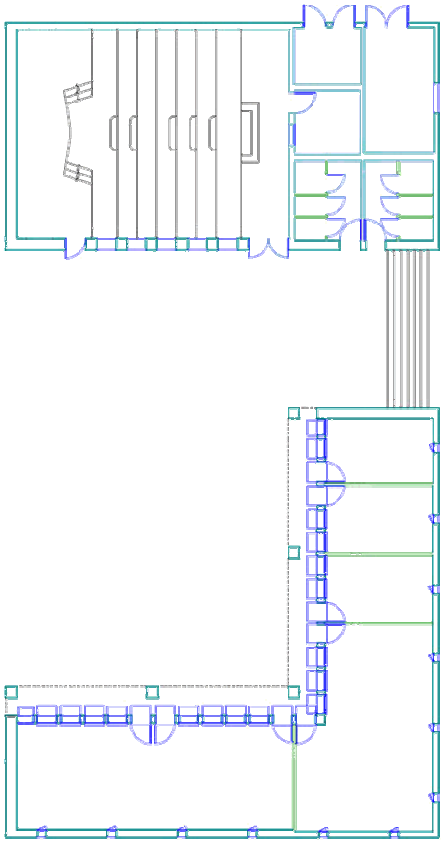
The Institute – Facilities



The Institute is split into several buildings. Wireless Internet connexion covers all these buildings. Online free access to bibliographic databases and to a broad spectrum of scientific journals is available for participants.

In the core building are located the common areas, restaurant and administration. Uphill, separate edifices host the auditorium, offices equipped with free access computers and meeting rooms for didactic needs.





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The Mosaic project

When photonics meets life

Science@Mosaic

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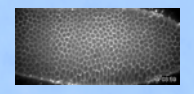
About Mosaic:

We are physicists and biologists who put effort together to unravel molecular mechanisms in the cell machinery and tissues' organization.
Our expertise in physics, photonics, instrumentation and signal processing together with the input of biologists deeply involved in our work enables innovative investigations at the Physics / Biology interface.

Biological issues:

- **Nanoscale organization and dynamics of living cell membranes and signaling processes** (CIML collaboration)

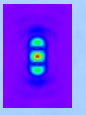


- **Cell dynamics and tissue morphogenesis** (IBDML collaboration)

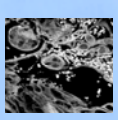
Optical and Photonic investigations:

- *Ultra sensitive microscopy and single molecule detection in bio-samples (cells, tissues, biochips)*

- *Photonics structures to enhance molecular signals and improved spatial resolution*
  - *Nanoholes in metal films*
  - *Mirrors and Microcavities*

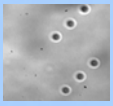


- *Improving resolution in far field microscopy*
  - *Point Spread Function (PSF) shaping with interferences*
  - *Wavefront shaping for imaging in scattering media*

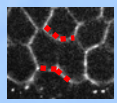


- *Non linear microscopy*
  - *Coherent Anti-stokes Raman Scattering (CARS) microscopy*
  - *Pulse shaping and polarization control for non linear imaging and spectroscopy*

- *Cell handling and manipulation with optical tweezers*



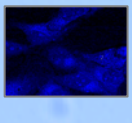
- *Laser nanodissection in live cells*



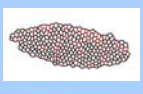
- *Microfabrication by means of the micro-stereolithography technique*

Physical issues:

- *Molecular diffusion in membranes and constrained environment*
- *Molecular imaging in cells and tissues*



- *Cell mechanics*



MOSAIC bio-partners

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