Two color FCS and TIR-FCS
FCS = Fluorescence Correlation Spectroscopy
TIR-FCS = Total Internal Reflection FCS

Matthias Geissbühler1, Marcel Leutenegger1, Dr. Iwan Maerki1, Dr. Hans Blom2, Dr. Christian Eggeling2 and Prof. Theo Lasser3

1 Laboratoire d’Optique Biomédicale, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland
2 Biomelkylär Fysik, Kungliga Tekniska Högskolan, 10691 Stockholm, Sweden
3 Max-Planck-Institut für Biophysikalische Chemie, 37077 Göttingen, Germany

We present the development and application of a dual-color setup that can be used for confocal fluorescence correlation spectroscopy (FCS), total internal reflection FCS (TIR-FCS) as well as dual color imaging. As exemplary application we performed preliminary measurements for DNA-Sequencing with a novel surface chemistry.

Abstract

Motivation

• Single molecule detection of chemical kinetics A(red)+B(blue) <-> AB
• Monitoring biological processes (eg molecular binding essays)
• in vivo observations of dynamics of molecular motors

FCS principle

1. Fluorescent system
2. Confined illumination and detection
3. Record photon emission trace
4. Calculate autocorrelation
5. Fit auto-correl.-curve with model
6. Determine system parameters (eg: total number of molecules in sampling volume, diffusion constants, triplet state parameters)

DNA Sequencing

• improved surface chemistry
• bind single DNA strand to surface
• labeling nucleotides
• observation in confocal volume

Outlook

• Advanced concepts for further confined sampling volumes
• Higher concentrations of labeled molecules
• More colors or/and lifetime separation
• More biological systems

Motivation

Total internal reflection setup (TIR)

• Dual color total internal reflection illumination of fluorescent sample
• Emitted light detected by photon detector
• Option: EMCCD for dual color imaging with limited depth of excitation (<100nm).

Confocal setup and options

• Setup can be switched to confocal illumination
• Dual color confocal FCS
• Additionally: lifetime measurements

Application

• More biological systems

DNA Sequencing

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References


Contact: Matthias Geissbühler, matthias.geissbuehler@epfl.ch, +41 21 693 77 70