Two color FCS and TIR-FCS

FCS = Fluorescence Correlation Spectroscopy
TIR-FCS = Total Internal Reflection FCS

SIXTH FRAMEWORK PROGRAMME

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Abstract

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.

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

Fluorescence correlation spectroscopy (FCS)

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

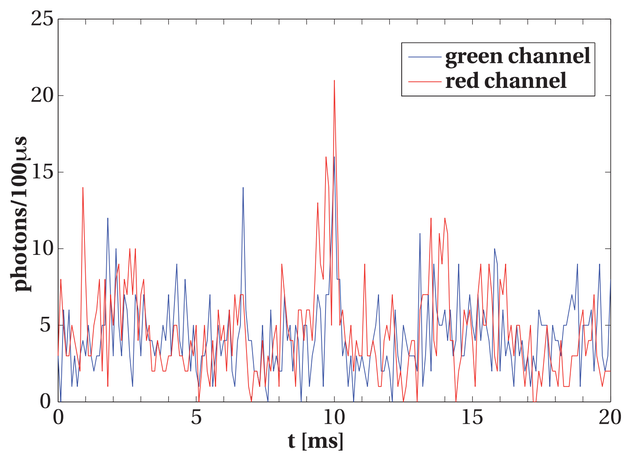


Figure 1: Dual color photon trace

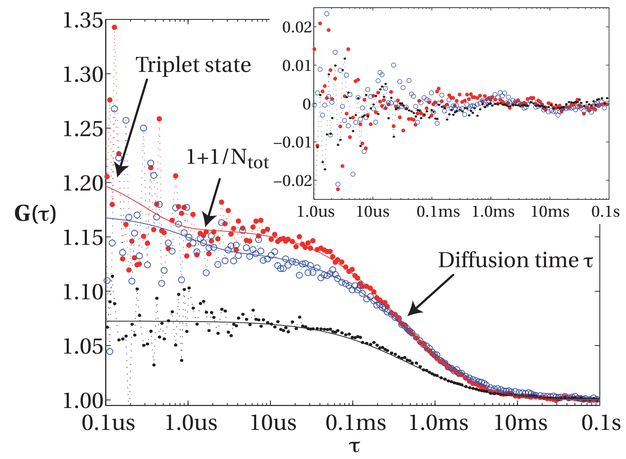
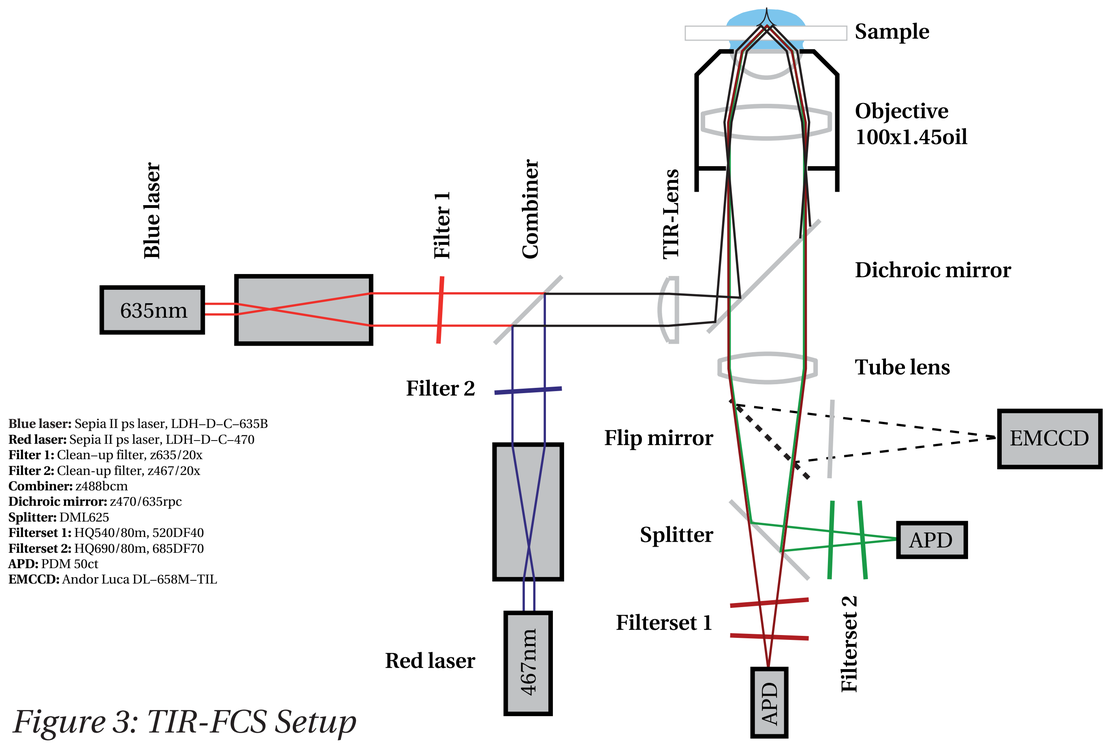


Figure 2: Auto- and cross-correlation and fits of dsDNA [1] Circles: green autocorrelation; bold points: Red auto-correlation; dots:green-red cross-correlation. Inset: fit residuals $G_{fit}/G(\tau)$-1. N_{tot} = No. of molecules

Optical Setup (TIR-FCS)



Blue laser: Sepia II ps laser, LDH–D–C–635B
Red laser: Sepia II ps laser, LDH–D–C–470
Filter 1: Clean–up filter, z635/20x
Filter 2: Clean-up filter, z467/20x
Combiner: z488bcm
Dichroic mirror: z470/635rpc
Splitter: DML625
Filterset 1: HQ540/80m, 520DF40
Filterset 2: HQ690/80m, 685DF70
APD: PDM 50ct
EMCCD: Andor Luca DL–658M–TIL

Figure 3: TIR-FCS Setup

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 exciation (<100nm).

Confocal setup and options

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

Application

DNA Sequencing

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

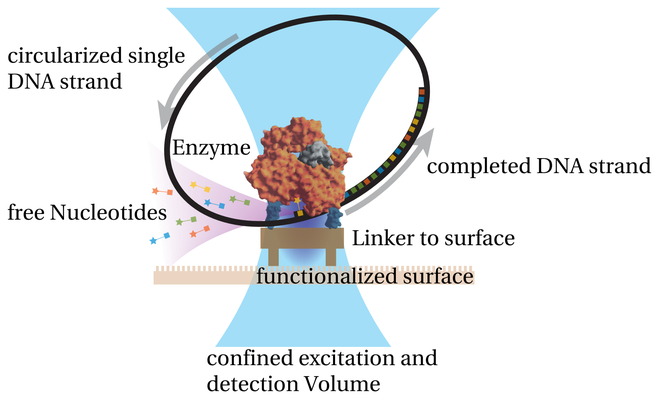


Figure 4: DNA Sequencing method

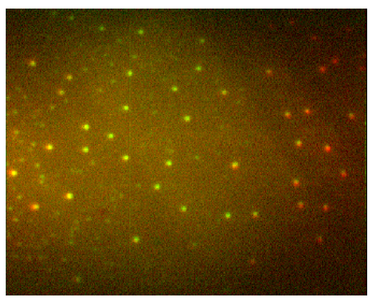


Figure 5: Image of dual colored spots after several minutes of nucleotide-incorporation

Outlook

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

References

[1] *M. Leutenegger et al. (2006) "Dual-color total internal reflection fluorescence cross-correlation spectroscopy" – JBO Letters – Vol. 11(4)*

[2] *D. Axelrod (2001) "Total internal reflection fluorescence microscopy in cell biology" – Traffic (Oxford, UK) Vol. 2, 764-774*

[3] *K. Hassler et al. (2005) "Total internal reflection fluorescence correlation spectroscopy (TIR-FCS) with low background and high count-rate per molecule" – Opt.Exp. Vol. 14, 7415-7423*

[4] *R. Rigler et al. (1993) "Fluorescence correlation spectroscopy with high count rate and low background - Analysis of translational diffusion" – Eur. Biophys. J. Vol. 22, 169-175*

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