Single Molecule Spectroscopy – Instruction Notes

Keywords: fluorescence, Förster resonance energy transfer (FRET), single molecule spectroscopy, confocal microscopy, evanescent field

I. GOALS OF THE EXPERIMENT

Single molecule spectroscopy has become a common tool to study biological systems. The possibility of revealing individual occurrences usually hidden by bulk solutions is one of the most important advantages of this technique. In bulk solutions, an average will be taken over all molecules, regardless of their different states and properties. Thus, rare events will remain undetected and falsified conclusions may be made. This is why it is crucial to consider the individual behaviour of each molecule. In this experiment of the practical course a fluorescent DNA sample will be meas- ured on a single molecule level. The students will learn how to biochemically immobilise the molecules in a flowchamber as well as the safe and careful handling of a home-build total internal reflection microscope (TIRFM) and thus gaining optimal insights into the current research opportunities in the field of biophysics.

II. LEARNING CONTENT

- Single molecule spectroscopy
- Förster Resonance Energy Transfer (FRET)
- Working with a fluorescence microscope technique
- Total internal reflection fluorescence microscopy (TIRFM, Snells law, evanescent field)
- Energy transfer and Jablonski-Diagramms
- Quantum mechanical solution for weak coupling regime (Fermi's golden rule)
- Biochemical experiments in physics (Immobilisation of biomolecules, fluorophore labels)
- Statistical interpretation of measurement data

III. PROCEDURE

FIRST LAB SESSION

- Sample preparation: biochemically immobilise the molecules in a previously prepared flowchamber
- Adjustment of the TIRFM and alignment of the optical setup
- Measurement of FRET with the given software

SECOND LAB SESSION

- Evaluation of FRET data
- Statistical analysis of the obtained results

- Calculation and determination of the different label distances

IV. REFERENCES:

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