CARS (COHERENT ANTI-STOKES RAMAN SCATTERING) CORRELATION SPECTROSCOPY

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The ready availability of ultrafast laser sources has recently led to the development of different microscopical techniques employing non-linear optical effects. Most prominent among these methods is two photon fluorescence microscopy. It allows the generation of three dimensional images without the necessity of using a detection pinhole such as in confocal fluorescence microscopy. Fluorescence techniques however rely on the presence of a strongly fluorescing chromophore. In most samples these chromophores do not occur naturally. Either common dyes or mutants of the Green Fluorescent Protein (GFP) have therefore to be introduced by staining or by using cloning techniques. While the high achievable staining selectivity has contributed significantly to the success of fluorescence microscopy in cell biology, some problems persist. These include the phototoxicity of many dyes, their bleaching, and not at least the need to overcome a strong background signal from autofluorescence of the sample.

Coherent Anti-Stokes Raman Scattering (CARS) has been presented as a novel microscopic technique with vibrational contrast [1]. In this case the resonant excitation of molecular vibrations is exploited for selective imaging and the necessity for staining is removed. Recently, we showed that CARS microscopy can also be used for correlation spectroscopy [2]. The principle of the method is similar to correlation spectroscopy using confocal fluorescence microscopy and two-photon microscopy, two methods which have lately raised much interest. For CARS correlation spectroscopy, CARS signal fluctuations caused by the diffusion of small particles through a fixed focal volume are recorded. With sufficient sensitivity for the observation of particles with less than 100nm in diameter, CARS correlation spectroscopy offers the important advantage of monitoring unlabelled species. CARS correlation spectroscopy therefore has the promise to yield new insights in to aggregation phenomena of proteins which are of importance in a wide variety of diseases. We will discuss the general setup of the experiment and present the theory necessary for the interpretation of the experimental data. Examples for the determination of particle diameters and microscopic viscosities will as well be presented.
