High Repetition Rate Femtosecond Lightsource for CARS Microscopy

Potma, Eric O.; Boeij, Wim P. de; Pshenichnikov, Maxim S.; Wiersma, Douwe A.

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High Repetition Rate Femtosecond Lightsource for CARS Microscopy

Eric O. Potma, Wim P de Boeij, Maxim S. Pshenichnikov, and Douwe A. Wiersma
Ultrafast Laser and Spectroscopy Laboratory, Materials Science Centre, Department of Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
Fax +31-50-3634441; Phone: +31-50-3634352; e-mail. potma@chem.rug.nl

The use of femtosecond pulsed excitation in microscopy permits the application of nonlinear optical techniques to microscopic studies of biological samples. Among the these techniques, the method of coherent anti-Stokes Raman scattering (CARS) is particularly promising for biological imaging since it allows spectralty selective probing for specific biochemical species without the need for extrinsic markers.[1] In order to perform spectrally resolved CARS microscopy, a multicolour tunable coherent lightsource is required. Due to the non-linear nature of the signal generating process, the signal yield would benefit from high peak powers whereas at the sample a low dose (low average power) is wanted. Furthermore, high repetition rates would significantly enhance the image acquisition times.

We have constructed a high repetition rate femtosecond multicolour lightsource based on a cavity-dumped visible optical parametric oscillator (OPO).[2] The OPO is pumped by the second harmonic (400 nm) of a mode-locked femtosecond Ti:sapphire laser and delivers 30 fs pulsed excitation within the tuning range of 570-660 nm at variable repetition rates. The radiation of the OPO as well as the residual fundamental beam of the Ti:sapphire oscillator is used in the CARS process. The current system is capable of addressing molecular vibrations in the range of 2400-4500 cm^{-1}, which includes the C-H and O-H stretch vibrations. We have configured our microscope such that two-photon excited (TPE) fluorescence can be simultaneously detected with the CARS signal. Figure 1 displays the TPE fluorescence image of living Dictyostelium cells as well as the CARS image observed in the transmitted direction, with lasers tuned to the O-H stretch vibrational transition (3200 cm^{-1}) of a water molecule. The acquisition time of the images is only a few seconds. The dark contours in the image are ascribed to hydrophobic organelle structures such as (intra-)cellular membranes. Note that this information is complimentary to the one derived from the two-photon image. The high repetition rate lightsource enables the real-time mapping of intracellular hydrodynamics of single living cells. This is illustrative of the exciting new possibilities in studying the (dynamical) chemical compound distributions inside biological samples, especially living cells.

Fig. 1. *Simultaneously measured TPE auto-fluorescence of Dictyostelium cells (left panel) and CARS signal (right panel) using a 0.75 NA objective. Pump beam at 636 and Stokes at 800 nm, 100 μW in both beams at 800 kHz. (30 x 30 μm).*

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Optical near-field analysis of biostructures in aqueous solutions: Problems and proposals

J. Beuthan[1], C. Dressler[1], H. G. Eberle[1], C. Labouvie[2], M. Paul[2]
[1] Institute for Medical/Technical Physics and Laser medicine, Free University of Berlin
Krahmerstr. 6-10, 12207 Berlin (Germany), Tel. 0049/30/8449 2332, Fax: 0049/30/8449 2399
[2] Institute for Clinical Pharmacology and Toxicology, Free University of Berlin

The cellular mechanisms relating to the accumulation and metabolism of estrogens (e.g. 17β-Estradiol, E_2) are gaining in significance for the diagnostics and therapy of mamma carcinoma. The classical mechanism of the intracellular E_2 action is described as the regulation of DNA transcription and protein synthesis via E_2-specific receptors [1]. Investigations on the accumulation kinetics of estrogens have to be performed on vital cells in aqueous environments. The optical properties of E_2 and E_2-induced cell reactions were first analysed by confocal laser scan microscopy. However no E_2 specific intracellular fluorescence was detected. When a laser phase microscope (LPM) was used differences of the intensity profiles of the phase shifts were measured in single cells exposed to E_2 compared to control cells. Although the LPM method indicated metabolic changes on a nanoscale, the results were ambiguous since the phase shifts were influenced by refractive indices as well as the cell morphology. Cell structures can also change during metabolic processes.
In order to ascertain structure correlated data on a nanoscale AFM or SNOM measurements could be carried out. The latter would not only show the presence and location of E_2 in the cell by specific fluorescence but could also yield information regarding the mechanisms associated with the intracellular accumulation of E_2. However the time needed to complete one measurement using the SNOM leads to a significant degradation of the sample because of drying. Conversely measurements made under moist conditions often lead to artefacts and subsequent misleading results. This is probably due to the interaction of the AFM or SNOM probe with the sample [2]. A new technological approach, which has been under development in our institute for some time now, might help to minimize these problems. The main feature of this new concept is a 2D arrangement of nano light sources (Fig.1) which would allow near-field optical examinations of nanoscale objects by laser or electron beam excitation under appropiate conditions. Due to the rapid serial scanning or parallel examination of a sample the data acquisition time is significantly shorter compared to other scanning methods, thereby allowing a more accurate characterisation of dynamic accumulation and metabolic processes.

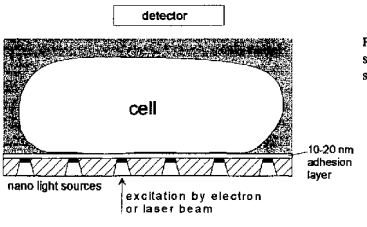

Fig 1 Scheme of a 2-dimensional array of nano light sources

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