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Published in:

Conference Digest. 2000 Conference on Lasers and Electro-Optics Europe, 2000

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2000

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Potma, E. O., Boeij, W. P. D., & Wiersma, D. A. (2000). Nonlinear coherent four-wave-mixing in optical microscopy. In *Conference Digest. 2000 Conference on Lasers and Electro-Optics Europe, 2000* (pp. 178-178). University of Groningen, The Zernike Institute for Advanced Materials.

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08.30-10.30 CWC – Lasers in Medicine I
 Presider: T.G. Papazoglou, F.O.R.T.H. – I.E.S.L., Crete, GREECE

CLIO

08.30 CWC1

METHOD FOR REAL TIME COLOUR DOPPLER OPTICAL COHERENCE TOMOGRAPHY

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Colour Doppler optical coherence tomography (CDOCT) has recently shown great promise in two-dimensional, high-spatial-resolution (on the order of tens of microns) tomographic velocity mapping of blood in living tissue.¹ CDOCT is based on optical interference in a scanning Michelson interferometer under broadband illumination. When a scattering object is in motion, it generates a signal in the detection circuit, an interferogram, the envelope of which gives the spatial reflectivity, and the frequency of which is proportional to the sum of the object's velocity and that of the reference arm scanner. Conventionally, post-processing of the entire interferogram is performed, requiring a large amount of computation, which precludes the real time operation of CDOCT necessary to provide motion-artefact-free images *in vivo*. Another major problem with the conventional approach concerns the bandwidth of the detection electronics.² For high dynamic range (sensitivity), the detection bandwidth should be as narrow as possible. However, the need to measure rapid and variable flows present in the vast majority of blood vessels requires a wider detection bandwidth to accommodate the variable Doppler frequency. Thus, either the dynamic range or the velocity range must be compromised.

In this paper, we present a novel detection scheme, based on an electronic phase-locked loop (PLL), that circumvents these problems. The key property of the PLL is its ability to establish and maintain phase-lock to the interferogram's periodic signal over a wide range of frequency and amplitude variations. Importantly, our PLL tracks in real-time both the interferogram frequency (velocity) the interferogram envelope (reflectivity) and, through frequency tracking, selects an optimally narrow detection bandwidth. Furthermore, for a given sample period, only the values of the frequency and reflectivity must be stored on computer, representing a massive reduction in stored data compared to the conventional approach.

In order to test our detection scheme, we implemented a CDOCT system in bulk optics. Using this system, we performed cross-sectional imaging of diluted milk flowing through a glass pipe of 0.58 mm bore tilted at 76° with respect to the incident near-infrared radiation. Figures 1(a) and 1(b) show simultaneous mapping of milk reflectivity and velocity, respectively. The system exhibits high dynamic range, 98 dB, as well as large velocity range, -20 to +20 mm/s. Fig. 1(c) shows the velocity profile across the sample's centroid (solid line). The good fit to a parabola (dashed line) is evidence that the velocity map correctly reproduces the laminar flow of the milk solution.

The application of a PLL detection technique to CDOCT provides the best prospects reported to date for real-time, *in vivo* tomographic imaging of blood flow velocity and, simultaneously, tissue morphology in the absence of motion artefact.

¹ Z. Chen et al., "Noninvasive imaging of *in vivo* blood flow velocity using optical Doppler tomography", *Opt. Lett.* **22**, 1119 (1997).

² M. Kulkarni et al., "Velocity-estimation accuracy and frame-rate limitations in color Doppler optical coherence tomography", *Opt. Lett.* **23**, 1057 (1998).

08.45 CWC2

Nonlinear coherent four-wave-mixing in optical microscopy

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The use of nonlinear optical techniques in microscopy has intensified the versatility of optical methods to explore biological samples at microscopic resolution. In addition to multiphoton excited fluorescence, coherent four-wave-mixing (FWM) optical methods like third harmonic generation (THG)¹ and coherent anti-Stokes Raman scattering (CARS)², have been adopted in microscope configurations. The application of coherent FWM techniques with high spatial resolution has paved the way for the possible implementation of a broad spectrum of spectroscopic tools in microscopy.

Opposed to multi-photon excited fluorescence, which is an incoherent process, FWM methods yield coherent signals. It is therefore expected that the spatial distribution of coherent emission near the focal volume may significantly differ from its incoherent counterpart. Consequently, in order to define resolution criteria for FWM microscopes, insight into the spatial organization of the coherent emission field is desirable.

In this contribution we present a detailed theoretical analysis of the imaging properties of coherent nonlinear microscopes. We have developed a model that allows calculation of the generation and propagation of coherent signals under high numerical aperture (NA) conditions without invoking the slowly varying envelope approximation. Based on calculations of coherent anti-Stokes Raman scattering (CARS) signals it is shown that diffraction effects play a prominent role in the spatial distribution of the coherent signal intensity. It is emphasized that, contrary to fluorescence microscopy, the detected signal is not a straightforward convolution of a point spread function (PSF) and the object but is shaped by the complex interplay of object size and coherent build-up dynamics.

Fig. 1. Intensity plot of CARS emission field near the focal volume of a NA = 0.9 air objective (a) and the corresponding illumination volume (b)

- 1 Y. Barad, H. Eisenberg, M. Horowitz and Y. Silberberg, *Appl. Phys. Lett.* **70** (1997) 922
- 2 A. Zumbusch, G.R. Holtom and X.S. Xie, *Phys. Rev. Lett.* **82** (1999) 4142