Available online at www.sciencedirect.com
ScienceDirect
Ultramicroscopy 108 (2008) 523–528
ultramicroscopy
www.elsevier.com/locate/ultramic

Cell imaging by coherent backscattering microscopy using frequency-shifted optical feedback in a microchip laser

O. Hugon[a,*], I.A. Paun[b], C. Ricard[c], B. van der Sanden[d], E. Lacot[a], O. Jacquin[a], A. Witomski[a]

[a]Laboratoire de Spectrométrie Physique, Université Joseph Fourier, Grenoble, France
[b]Faculty of Applied Sciences, Physics Department, University Politehnica of Bucharest, Romania
[c]Inserm, U594, Neuroimagerie fonctionnelle et métabolique, Grenoble, France
[d]Inserm U647, Rayonnement Synchrotron et Recherche Médicale, Grenoble, France

Received 9 January 2007; received in revised form 16 July 2007; accepted 1 August 2007

Abstract

In this work, we present a new development of the laser optical feedback imaging technique for imaging biological structures with a high resolution. The first results obtained on human red blood cells and mice cerebral and muscular tissues slices are shown. The performances of the system and its future developments are also discussed.

PACS: 42.25.Kb; 42.60.Rn; 42.62.Be; 87.64.−t

Keywords: Miscellaneous instruments; Microscopic methods; Specifically for biological specimens

1. Introduction

The coherent re-injection of light in a laser cavity can be at the origin of a modification of the laser behavior. Often considered as a parasitic phenomenon, it can for example drive the laser in a chaotic regime [1]. When it is correctly controlled, the optical re-injection in a laser can, however, be used in order to improve its characteristics, such as the lindwidth narrowing [2], or for metrology purpose [3–8], thus conferring on the laser the role of source and detector at the same time. Since 1963, this principle was employed in an autodyne configuration to measure distances or speeds [9,10]. More recently, a heterodyne imagery technique called LOFI (laser optical feedback imaging) was developed [11]. The characteristics of this new method of imagery are particularly interesting for the biomedical applications. The used laser emits a radiation of a few milliwatts at 1.064 μm that suit well for biological tissues observation. Coherent detection with the great sensitivity conferred by the dynamical amplification performed by the used laser makes it possible to obtain images in scattering media, without need for contrast agent [12]. In addition, the TEM_{00} mode of the laser plays the role of a spatial filter during the detection stage, like the pinhole of a confocal microscope, which allows the localization of the signal in the three dimensions of space [13]. Lastly, the simplicity of the instrumentation and its ease of use make this system easy to adapt on any type of microscope.

2. Principle

The principle of the LOFI imagery method is as follows: the beam of a class B laser (i.e. which shows relaxation oscillations of the intensity, such as a laser diode, a microchip laser or a fiber laser) is frequency shifted by a quantity Δf before being focused on a target. The photons, which are backscattered by the target, will spontaneously come back into the laser cavity, in accordance with the reverse path principle. The number of these photons depends on opto-geometrical parameters of the target (absorption and diffusion coefficients, index contrasts,

*Corresponding author. Tel.: +33 4 76 51 47 49; fax: +33 4 76 63 54 95.
E-mail address: olivier.hugon@ujf-grenoble.fr (O. Hugon).

0304-3991/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ultramic.2007.08.009

interfaces orientations etc.). On the return path, the photons are once again frequency shifted by Δf. After a round trip, the re-injected photons are thus frequency shifted by $2\Delta f$ compared to the natural cavity frequency of the laser. The interference between intra-cavity light and the re-injected light causes in the cavity an optical beating at frequency $2\Delta f$ with an amplitude depending on the quantity of re-injected photons while its phase is governed by the optical path length between the laser and the target. Amplitude and phase of the beating are measured by taking a fraction of the output intensity of the laser which will be analyzed by means of a photodiode connected to a lock-in amplifier. This configuration has several advantages compared with traditional heterodyne interferometers. First of all, the system is self-aligned by construction since there is no external reference arm. Consequently, the greater part of the laser power is sent towards the target. Moreover, the signal can be amplified by several orders of magnitude by the dynamics of the laser. Maximal gain is obtained when the shift frequency is resonant with the relaxation frequency of the laser. One thus obtains shot-noise limited method of detection which is very easy to implement [14].

3. Experimental set-up and performances

Fig. 1 shows a schematic of the experiment. In this study, we used a diode-pumped Nd^{3+}:YAG microchip laser with an 800 μm thick cavity which emits a radiation of a few milliwatts at the wavelength of 1.064 μm. Its relaxation frequency is around 1 MHz and the gain can reach 60 dB at this frequency. The frequency shifter is constituted by two acousto-optic deflectors, respectively operating at 81.5 MHz (order +1) and 81.5 MHz$-\Delta f$ (order -1), so that the overall frequency shift is Δf. This frequency shift is controlled by a function generator (Stanford Research, model DS345). A two-axis galvanometric mirror scanner (Cambridge Technology, model 6220) makes it possible to move the beam on the surface of the sample to obtain an

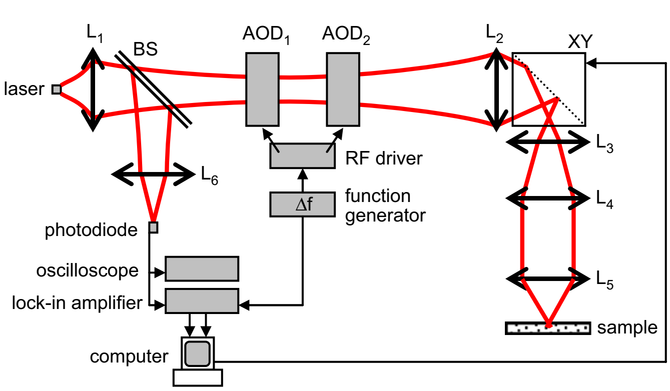

Fig. 1. Description of the experimental set-up: L_1, collimating lens; BS, beam splitter; AOD_1, acousto-optic deflector at 81.5 MHz (order +1); AOD_2, acousto-optic deflector at 81.5 MHz$-\Delta f$ (order -1); L_2, adaptation lens; *XY*, two-axis galvanometric mirror scanner; L_3, scan lens; L_4, field lens; L_5, microscope objective; and L_6, focusing lens.

image. The beam is injected into the microscope (Zeiss Axio Imager M1) via the camera port and is focused on the sample by the objective (Zeiss A-Plan 40x/0.65). Because of the great sensitivity of the system with respect to any back-reflection, all the lenses (except for the microscope objective) are anti-reflection coated for the working wavelength: 1.064 μm. A small fraction of the output intensity of the laser is sent on a Si-photodiode with a beam sampler.

The power spectrum of the laser is monitored with a digital oscilloscope (Fig. 2) in order to tune the shift frequency in connection with the relaxation frequency of the laser, which permits to adjust the gain of the system. That also makes it possible to supervise the re-injection level in order not to saturate the laser. The amplitude and phase of the interferometric signal are provided by a lock-in amplifier operating at $2\Delta f$ (Stanford Research, model SR844) and digitized by a 12 bits data acquisition board (National Instruments, model PCI-MIO-16E-1). Home-made software has in charge to drive the scanner and to synchronously acquire the data in order to obtain the images.

The LOFI images have a typical size of 256 × 256 pixels and are obtained in 4 s approximately. The acquisition time is limited by the dynamics of the used laser, in particular by the population inversion lifetime. Assuming a TEM_{00} Gaussian beam, the transverse resolution is fixed by the beam waist after the microscope objective

$$\omega_0 = \frac{\lambda}{\pi \arcsin(\mathrm{NA})}, \tag{1}$$

where λ is the wavelength and NA is the numerical aperture of the microscope objective. A typical value for the transverse resolution is 0.5 μm for a 40 × objective with a 0.65 numerical aperture. The depth of field has been experimentally determined by moving the upper surface of a microscope slide around the beam waist position with a piezoelectric translation stage. Fig. 3 shows the amplitude of the detected signal versus the distance δ to the beam waist. The full width at half maximum is 12 μm for our set-up, which corresponds to a pinhole diameter of 3.7 μm in a classical confocal set-up working at the same wavelength.

4. Results

We carried out experiments on several types of biological samples which we observed simultaneously in bright field microscopy and in LOFI for comparison. In this paper, only the amplitude images are presented. The phase images are much more difficult to obtain because they are strongly influenced by the thermal drifts and mechanical vibrations. The equipment used for these experiments did not enable us to obtain satisfying phase images. The first images show a single red blood cell (Fig. 4) or a stacked pile of coagulated red blood cells (Fig. 5). The LOFI images clearly reveal the concave shape of the cell. This relief effect is explained by the sensitivity of the system to the interfaces