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Stimulated laser induced fluorescence holography for imaging fluorescent species

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ABSTRACT

In this paper pulsed digital holographic detection is coupled to the stimulated laser induced fluorescence (LIF) effect for imaging fluorescent species. A frequency tripled Q-switched Nd-YAG laser (wavelength 355 nm, pulse duration 12 ns) has been used to pump Coumarin 153 dye solved in ethanol. Simultaneously a frequency doubled pulse (532 nm) from the same laser is used to probe the solvent resulting in a gain through stimulated emission. The resulting gain of the probe beam is recorded using digital holography by blending it with a reference beam on the detector. Intensity maps were calculated from the recorded digital holograms and used to calculate the gain of the probe beam due to stimulated fluorescence emission which is coupled to the concentration of the dye. The results show that the amplification of the probe beam (532 nm) due to stimulated LIF emission is seen in the intensity maps. The gain is about 40% at a dye concentration of 0.32 g/L and decreases to be about 20% at a dye concentration of 0.04 g/L for a probe beam energy density of 0.1 mJ/cm^2. Spectroscopic measurements have been carried out to confirm the holographic results. The results show that stimulated LIF holography is a promising technique for quantitative imaging of fluorescent species.

1. Introduction

Laser induced fluorescence (LIF) is the process of the absorption of a laser beam at a particular wavelength and subsequently re-emission at longer wavelengths. LIF is used as a diagnostic technique to measure fluid flow characteristics such as temperature or species concentration [1–3] and flow dynamics [4]. It is also used as an investigation tool in combustion studies [5,6] and biomass pyrolysis [7]. Further fluorescence microscopy is widely used in many medical and biological applications [8]. Pulsed digital holography is a non-invasive and full field method suitable for recording transient events, such as propagation of mechanical waves in solids and shock waves in liquids and gases [9–13]. Using pulsed digital holography both informations about the amplitude and the phase of the probing beam can be stored in the digital holograms. Detecting compounds scattered non-elastically with holography is a rather new development that requires tuning the wavelength of the laser to the scattered compound of interest. Many physical phenomena can be considered. Previously holographic detection has been applied to coherent anti-stokes Raman scattering (CARS) [14] and harmonic imaging [15,16]. These two approaches rely on the fact that a wavelength shifted replica of the incoming beam is produced through a coherent process in the material under study. A hologram may then be detected at this wavelength by tuning the wavelength of the laser to the required wavelength. As holography is a coherent process it cannot be applied directly to the incoherent light emitted by fluorescence spontaneously. Recently several holographic techniques have been presented that address this limitation. Scanning fluorescence holography (SFH) has the ability to produce a hologram using fluorescence emission. In SFH a Fresnel Zone Plate (FZP) from a coherent fluorescence excitation source scans the object where at each scanning position the light intensity is integrated by the detector [17,18]. Further the Fresnel incoherent correlation holography (FINCH) has been introduced to record multicolor digital holograms from objects emitting spontaneous fluorescent light [19,20]. In scanning holography mechanical scanning is needed to get 3D imaging which is a slow process and is sensitive to vibration. FINCH is a non-scanning technique that requires a filter wheel and somewhat complicated data analysis. The fundamental principle with both SFH and FINCH is to make the propagation path between object light and reference light small enough to fit within the coherence volume of the wavelength component of interest, thereby making it possible to use fluorescent light emitted spontaneously. The same principle of FINCH has also recently been utilized by Bon et al. to generate complex amplitude images in white light, and potentially fluorescent light, using a

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quadriwave lateral shearing wavefront sensor [21,22]. But in contrast to FINCH that utilizes the interference between axially shifted fields the wavefront sensor by Bon et al. relies on the interference between laterally shifted fields along the two directions of the sensor. In this study we take a different route and make use of stimulated fluorescence, which is a coherent process, and couple it to holography for imaging fluorescent species which to our knowledge has not been demonstrated previously.

When a volume of excited fluorescent molecules is probed by a certain wavelength included in its fluorescence spectrum, the stimulated LIF emission occurs. The stimulated LIF emission is a coherent process as the photons of the probe beam are locally cloned as they pass through the excited volume resulting in a gain of the probe beam, which is the fundamental process utilized in a laser. In digital holography only the light that is coherent with the source is recorded in the holograms. Therefore only the gain caused by stimulated LIF emission will be recorded and all spontaneous fluorescence and other disturbances will be filtered out. If we record two holograms without and with the pump laser beam, intensity maps that show the gain of the probe laser beam due to the stimulated LIF emission are produced. In the following the possibility of coupling pulsed digital holography with the stimulated LIF effect for time-resolved imaging is studied.

2. Experimental setup and procedure

Two experiments have been performed in this study. First we have used a spectrometer and an energy monitor to record the gain of the probe beam due to the stimulated LIF effect. Furthermore the stimulated LIF is studied using pulsed digital holography. A sketch of the experimental setup of spectroscopic detection of stimulated LIF is shown in Fig. 1(a). An injection-seeded, twin oscillator, Q-switched Nd:YAG laser system (Spectron SL804T) is used as light source. The laser system operates at 10 Hz. Stable single shot operation is not possible. Instead, fast solenoid-activated beam dump shutters allow access to a single, stable, single-frequency pulse. The frequency tripled Nd:YAG 355 nm (pulse duration 12 ns) beam is used to pump Coumarin 153 dye solved in ethanol contained in a cuvette with a width of 1 cm in the laser direction. The Coumarin 153 dye was showing strong absorbance at the pump wavelength 355 nm and its fluorescence spectrum has a peak close to 532 nm. The frequency doubled 532 nm beam from the same laser is used as a probe beam and passed through the excited volume of the dye. In the path of the probe-beam a $\lambda/2$ plate is placed in series with a polarizing beam splitter enabling control of the probe-beam energy while keeping the pump-beam energy constant. The cuvette is placed at a distance such that the angle between the pump and probe beams is almost zero, giving a maximum overlap of the two beams inside the cuvette. After exciting the dye in the cuvette, the beams are guided through a 532 nm interference filter where the pulse energy of the probe beam is measured with a computer controlled energy monitor (Ophir PE25BF). A spectrometer (AvaSpec-ULS2048) is positioned perpendicular to the propagation direction of the pump-beam, recording the fluorescence spectrum. For a certain dye concentration a series of measurements was recorded for different probe beam energies from 0.5 mJ to 5.0 mJ while the energy of the pump beam was kept constant at 1.7 mJ. For each selected probe beam energy the pump beam was initially blocked and the pulse energy of the probe beam was recorded. The pump beam was then unblocked and the recording was repeated. For each setting the energy of 200 consecutive pulses was recorded for averaging. Simultaneously the average spectrum was recorded.

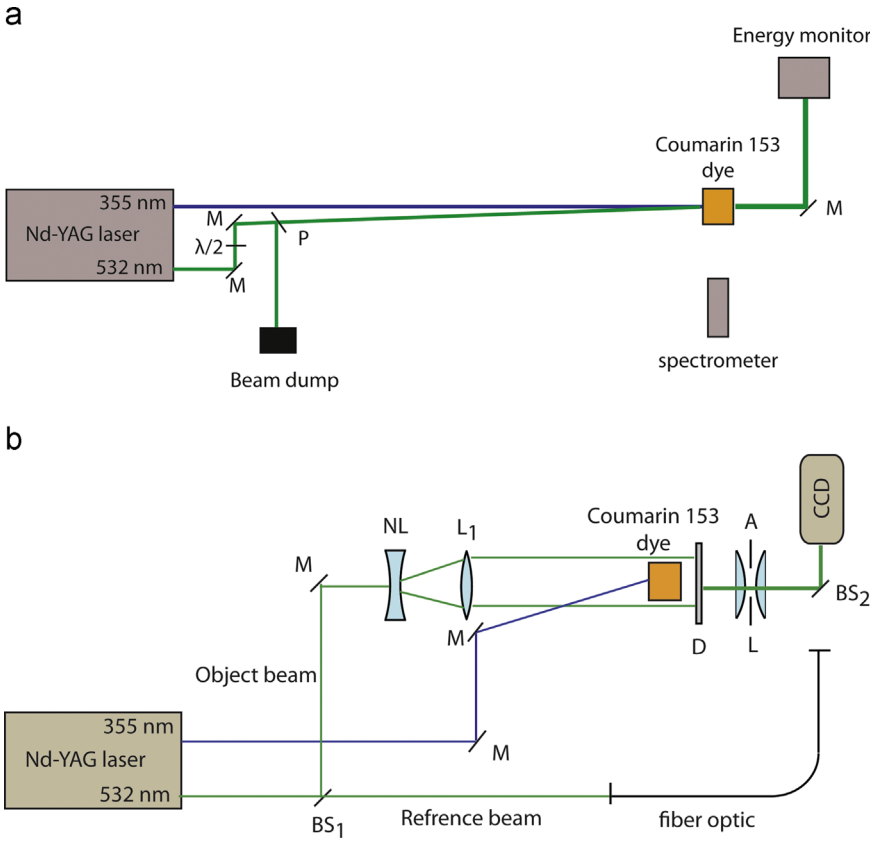

Fig. 1. Experimental setup. (a) Experimental setup of spectroscopic detection of stimulated LIF. M: mirror, $\lambda/2$: half wave plate, and P: polarizing beam splitter. (b) Experimental setup of LIF holography. M: mirror, NL: negative lens, L_1: collimation lens, L: lens system for imaging, A: aperture, D: diffuser, and BS_1 and BS_2: beam splitters. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)