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Observation of the interferences between the emitted beams in a 4Pi microscope by partial coherence interferometry

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We propose a modified 4Pi microscope setup for observing solely the interference resulting from the superposition of the beams emitted by fluorescent species placed between two microscope objectives. A scanning Michelson interferometer is coupled to the 4Pi microscope. Interferences between the beams emitted by fluorophores deposited on a cover glass are observed, thanks to partial coherence interferometry technique. © *2005 American Institute of Physics*.
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Improving the axial spatial resolution in optical microscopy is a challenging task for many applications. In conventional microscopes and for large numerical aperture (NA) lenses the lateral resolution is about four times better than the resolution obtained along the longitudinal direction. In 4Pi fluorescence microscopy, the coherent addition of the excitation light fields at the focus[1] and the fluorescence fields at the detector lead to a three- to fivefold axial resolution improvement.[2–5] This significant improvement results from the superposition of two systems of fringes: one produced by the illumination wave fronts, and the other produced by the emission wave fronts. However, due to the weak interference signal produced by the emitted wave fronts, the phenomena observed in 4Pi microscopes are usually dominated by effects produced by the pump beams. In this Letter we propose an experimental setup in which the interferences between the emission wave fronts are observed solely, thanks to partial coherence interferometry (PCI). The setup, which is derived from an arrangement proposed by Sheppard and Gong,[6] is an alternative to the classical 4Pi-C microscopes.

When a broadband source illuminates an interferometer I_1 whose optical path difference (OPD) Δ_1 is much greater than the coherence length l_c of the source, no interferences can be recorded by a photodiode placed at the output of I_1. However, in certain cases, when the output light from I_1 is sent to a second interferometer I_2, an interference signal can be observed. In particular, the signal S recorded by a photodetector placed at the output of I_2 exhibits a correlation peak when the OPD Δ_2 of I_2 is varied so that $|\Delta_2-\Delta_1|<l_c$.[7,8] When $\Delta_2=\Delta_1$, the amplitude of the correlation peak reaches its maximum value. The curve of Fig. 1 represents the signal S as a function of Δ_2, for $\Delta_1 \gg l_c$. Each variation $\delta\Delta_1$ of Δ_1 produces a lateral shift of the correlation peak in signal S. $\delta\Delta_1$ can be measured with interferometric resolution either by varying Δ_2 or by maintaining Δ_2 to a constant value.[8]

In our experimental setup (Fig. 2), a laser is used to excite a subwavelength sheet of fluorescent species (noted FL, with a coherence length l_c) located between two microscope lenses. The fluorescence is emitted through beam 1 and beam 2 that are superimposed after reflection of beam 1 on mirror M1. Lens L and mirror M1 act as an image inversion system used to preserve the spatial coherence between the two emitted wave fronts. The optical thickness of O1 and the distance between M1 and O1 are such that the OPD Δ_1 between beam 1 and beam 2 is much larger than l_c. The group (FL, O1, O2, M1, and L) acts as an interferometer I_1 whose OPD Δ_1 depends on the distance between FL and M1. After being superimposed, beam 1 and beam 2 are sent to a scanning Michelson interferometer I_2 in order to record the output signal S. The dichroic mirror DC and the notch filter NF are used to reject the laser light. The signal recorded by detector D is the result of the superposition of beam 1 and beam 2 interfering again in I_2. The displacement of FL along the optical axis modifies the value of Δ_1 which can be recovered by recording signal S.

In our experiment FL was an ~50 nm-thick layer of latex carboxylate modified FluoSpheres® (diameter of 20 nm), sandwiched between two cover glasses. The central wavelength of the fluorescence emission is $\lambda_0=525$ nm with a coherence length l_c close to 30 μm. The excitation beam at $\lambda=488$ nm was provided by an argon-ion laser. Detector D was an avalanche photodiode working in photon-counting regime. Figure 3 presents the signal S recorded as a function of Δ_2. One can see that a curve similar to that of Fig. 1 has been recorded. The correlation peak obtained for $\Delta_2 \approx 91.6$ mm is due to the interference between beam 1 and beam 2. The value of Δ_2 depends on the distance between FL and M1. The reduction of visibility of the correlation peak

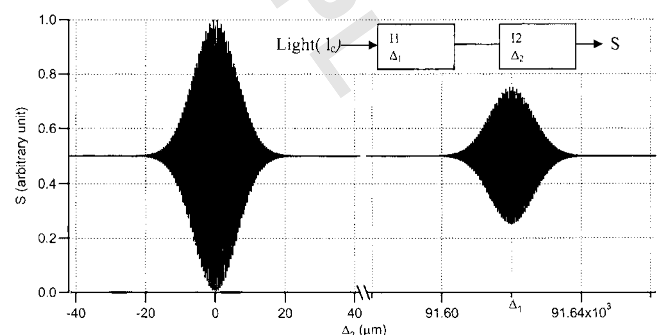

FIG. 1. I_1 and I_2 are assumed to be two-beam interferometers. The typical shape of a PCI signal recorded by the detector as a function of the OPD Δ_2 of I_2. Here the OPD of I_1 is $\Delta_1=9.162$ cm. The power spectral density function of the source is assumed to be a Gaussian function. In the calculation $\lambda_0=525$ nm and $l_c=30$ μm.

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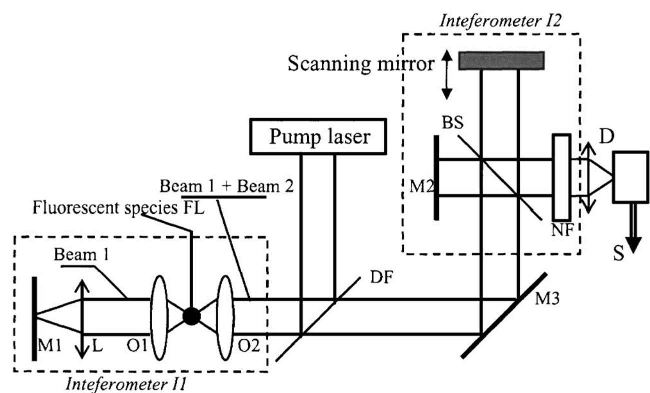

FIG. 2. Experimental setup. M1, M2, and M3 are fixed mirrors, DC is a dichroic mirror, NF is a notch filter, BS is a beamsplitter, and L is a convergent lens. M1 is in the focal plane of L. The fluorescent layer FL is sandwiched between two cover glasses (not represented). O1 and O2 are microscope objectives with a numerical aperture NA=0.3. O1 and O2 are assumed to be identical.

with respect to the theoretical value is due to the axial distribution of fluorophores on the sample. Indeed, each fluorescent molecule in FL indexed by i generates an OPD noted Δ_{1i}. Thus the correlation signal around $\Delta_2=91.618$ mm is an average of the correlation peaks obtained for all the values of Δ_{1i} which tend to blur each other if the FL layer is too thick. Chromatic dispersion, which is not compensated here, can also be a cause of the reduction of the amplitude of the correlation peak. In order to confirm the origin of the peak of Fig. 3, the pump laser was replaced by a light-emitting diode (LED) whose emission spectrum is similar to the emission spectrum of FL. We placed at the focal plane of O1 and O2, and perpendicular to the optical axis, two attached cover glasses free of fluorophores. In this case beam 1 is the beam transmitted by the cover glass and then reflected by mirror M1 and beam 2 is the beam reflected on the interface between the two cover glasses. Figure 4 shows the signal S recorded as a function of Δ_2. As expected the maximum

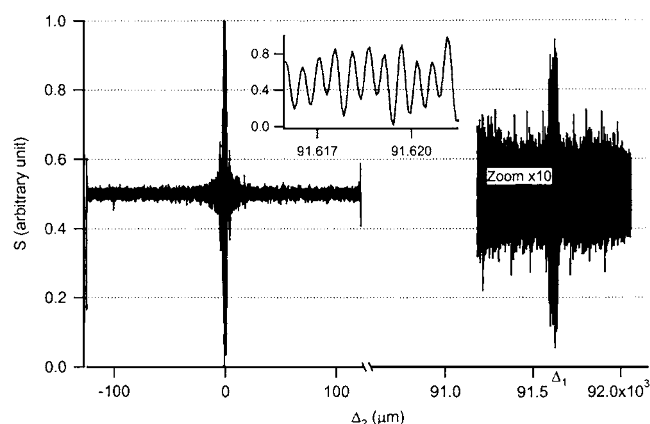

FIG. 3. Experimental results. Signal S obtained by varying the OPD Δ_2 of I_2. Around $\Delta_2=0$ the peak is the interferogram of the source. The correlation peak (zoom $\times10$), centerd on $\Delta_2=\Delta_1$, permits one to localize the fluorescent molecules with subwavelength accuracy (see the fringes of period λ_0 in the inset).

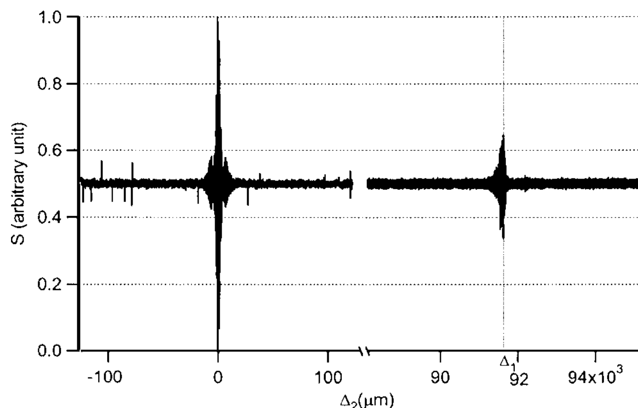

FIG. 4. Experimental results. Signal S obtained by varying the OPD Δ_2 of I_2. Same conditions as in Fig. 3 but here the sample does not contain any fluorescent molecule. The excitation laser has been replaced by a LED emitting at $\lambda_0=525$ nm with a coherence length l_c around 30 μm.

amplitude of the correlation peaks of Figs. 3 and 4 are obtained for the same values of Δ_2. When the same experiment is made with the pump laser in place of the LED and without the notch filter, the coherence length of the source is greater than Δ_1 and fringes are visible everywhere in signal S in the variation range of Δ_2. These results emphasize the fact that the correlation peak of Fig. 3 is due to the interferences between the wave fronts emitted by the luminescent sample.

The displacement of FL along the optical axis induces not only a variation of Δ_1, but also modifies the shape of the correlation peak.[9] This effect can be used to determine the direction of the axial displacement of fluorescent species.[10] In the presented setup, the two interfering beams travel almost the same paths. For this reason the microscope described in this Letter is probably less sensitive to mechanical or thermal drifts than conventional 4Pi-type C microscopes. However, for obtaining the highest amplitude of the interference signal, the chromatic dispersion has to be compensated. To do this one can add, in one arm of the Michelson interferometer, a microscope objective identical to O1 (O1 and O2 are assumed to be identical) and working as in a Linnik microscope.

As a conclusion, interferences produced by the beams emitted by fluorophores have been observed with a setup which can be an alternative to the classical arrangement of the 4Pi microscope.

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