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Analysis and Correction of the Additive Phase Effect Generated by Power Change in a Mach–Zehnder Interferometer Integrated to an Optical Trap

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Abstract: Immersion microscope objectives stand out for their large numerical aperture, which improves the optical resolution of imaging systems such as those used in microscopic interferometry. These objectives increase the gradient forces of a beam focused through them, forming an Optical Trap (OT). However, many studies on microscopic interferometry neglect the contributions of different optical materials in the system that are also exposed to laser radiation, perhaps simply assuming transparency. In this work, a Mach–Zehnder interferometer and an OT, which share several components (including the same oil immersion objective), were coupled. Here, the response of the interferometer to a progressive increase in the OT laser power, while the interferometer laser power remains constant, is reported. Changes in laser power affect the oil temperature, altering its refractive index and volume, which in turn causes a phase shifting on the transmitted wavefront. Optical phase analysis is applied in the three-dimensional measurement of the damage produced by the OT on a paint film. This study suggests that the refractive index variations in the immersion oil affect interferograms because they will then exhibit an additive phase term that must be considered in that final measurement. Additionally, the OT geometry changes with the power increase.

Keywords: optical metrology; microscopy; interferometry; optical trap; immersion oil



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1. Introduction

Interferometric techniques take advantage of light interference, which is a phenomenon that occurs when two or more coherent waves, i.e., with the same wavelength, amplitude, and linearly polarized vibration planes, overlap in space; interference (constructive–destructive) can be observed in the form of bright and dark fringes when projecting the interfering beams onto a screen. If two waves in a vacuum interfere, the phase difference ($\Delta\phi$) with which they arrive at the point of interference is due to the difference in the length of the optical paths (OPD) traveled by each wave [1]. Depending on the interference pattern morphology and the experimental conditions, the most suitable method to calculate the complete phase difference map is selected, since each method has its own advantages and disadvantages. For example, when analyzing a dynamic sample whose interference generates open fringes, the Fourier method is commonly chosen because it requires only one interferogram that is captured in a fraction of a second [2]; on the other hand, if the sample is static or quasi-static, and the interference fringes are closed, a phase-shifting method is usually applied, which requires capturing several interferograms [2]. The optical setup designed to generate the light interference is called an optical interferometer. Through the fringe pattern generated by light interference, it is possible to measure physical quantities of objects such as Young’s modulus, topography, mechanical stress, temperature, refractive index, etc. Over the years, interferometric techniques have been combined with various optical techniques, such as in tomographic phase microscopy in flow cytometry [3], optical

tweezers to carry out biological characterizations [4], holographic optical tweezers [5–7], or optical trap spectroscopy [8]. Although the most common applications of optical tweezers are in biology [9,10] and in micromanipulation of other types of particles [11], applications are not limited to these functions.

On the other hand, in 1970, Arthur Ashkin reported particle trapping using the radiation pressure force of a continuous laser [12]. Radiation pressure (P_{rad}), at normal incidence, is determined with the expression $P_{rad} = I(R + 1)/c$, where I is the irradiance, R represents the surface reflection coefficient, and c is the speed of light [13]. When the beam emitted by a laser is focused via a lens, the pressure at the beam waist will be higher than in the rest of its optical path and will be directed toward the beam center. This makes it possible to keep a micrometer- or sub-micrometer-scale particle trapped in the beam waist, which gives it the name optical trap. The use of different laser power levels for optical traps has been reported in the literature, ranging from a few mW to a Watt or more in the specimen plane, depending on details of the laser and setup, objective transmittance, and the desired stiffness [14].

Generating an efficient optical trap requires that the beam focused via the lens comes from a large Numerical Aperture (NA); therefore, it is necessary to use an immersion microscope objective (100X and $NA \approx 1.3$). However, many radiation pressure studies neglect the optical contributions of immersion media used for their implementation [15–17], often due to their transparent nature, or simply because such media are a constant in every optical trap. Common immersion media, such as water or oil, have effects of the same type but on a different scale; thus, their contributions are a factor to consider in works that use immersion where trap geometry is of interest, or where interferometric quantifications are performed through phase recovery [18]. Phase is a particular characteristic of waveform cycle, usually expressed in terms of angular units. Phase changes are relevant in the context of light interference effects, and this will be further discussed in Section 2. Although oil is commonly used as an immersion medium, there are also experiments using water immersion objectives [19]; experimental conditions determine whether one or the other is chosen [20].

Regarding the influence of an immersion medium in an optical trap, it has previously been reported how the optical excitation of oil can influence the change in the trap focal length [21], and the same effect has been proven by testing immersion oils of different refractive indexes [22]. It has also been reported that by combining a confocal microscope and an optical trap, when using independent laser sources that share the same immersion objective, the change in the optical trap laser power changes the confocal focus position [23,24]. The variation in the beam convergence geometry has been considered as an effect on fluid pressure [25], as well as a change in the quantum state of the matter and radiation [13], and even the thermal effects of this convergence that are induced by modulation in radiation power have been characterized [26]. However, to the knowledge of the authors of this work, no reports have been found in the literature that suggest a change in the laser beam power of an optical trap generates any change in the phase of the wavefronts that are transmitted by the optical elements of the same trap.

In this work, a conventional optical trap is combined with a Mach–Zehnder interferometer, where independent laser sources that share several optical components are used. Among the shared elements is the oil immersion objective, which generates the optical trap; in Section 2, the systems are described separately, followed by their coupled implementation. In preliminary experiments, it was detected that upon changing the power of either one of the two laser sources, the interferograms showed an additive phase shift; the cause and mechanism of this effect were unknown at the time. This manuscript presents a methodology to measure and correct the contribution of such additive phase, due to some component from the optical tweezers or the interferometer, discarding the possibility that it could be due to a direct contribution of the sample under study.

In Section 3 of this work, we first describe the method implemented to identify the contribution that each experimental arrangement component in common could have on the

phase shifting observed; our experimental results have allowed us to determine that the phase shifting is caused by the oil immersion microscope objective. Consequently, a study of the optical, thermal, and morphological behavior of the oil implemented on a macroscopic scale outside the trap–interferometer setup and exciting it with a higher power laser is shown below. The foregoing is also due to the fact that within the trap–interferometer setup it is not possible to individually analyze the oil characteristics. Although this analysis allows an understanding of the general behavior of immersion oil, the particular calibration on the optical trap must be implemented in situ, as indicated below.

Finally, in Section 4, we present the phase calibration to be performed on interferometer measurements when a change in laser source power is involved. In this case, the optical trap is used to generate deformations on a black paint film at different powers, while interferometry is used to measure the deformation due to thermal stress applied by the optical trap. Since the change in trap laser power adds a phase shifting to the interferograms that measure the deformation, it is necessary to calibrate this additive phase term in the trap–interferometer system, to subsequently remove it from measurements.

2. Experimental Setup and Theoretical Analysis

2.1. Optical Trap Setup

The optical trap system used (see scheme in Figure 1) consists of a THORLABS™ (Newton, NJ, USA) butterfly laser diode type infrared (IR) laser source (model BL976-SAG300, with a wavelength (λ_{IR}) of 976 nm, and a maximum power of 350 mW) and an oil immersion microscope objective (Nikon brand, model MRP71900, with 100X magnification, NA = 1.25, and a working distance (WD) of 0.23 mm). The immersion oil (Non-Drying Immersion Oil for Microscopy, Cargille Type LDF [27]) has a refractive index (n) of 1.5181. The substrate shown in Figure 1 is a PEARL coverslip (used as a slide) of $24 \times 50 \times 0.13$ mm in dimensions and with a refractive index of 1.52. Therefore, the maximum distance available for particle trapping is approximately 100 μm , which means that to keep the optical trap above the slide, the immersion oil must not exceed approximately 100 μm in thickness; this is controlled by moving the slide along the trap optical axis. The sample under study is illuminated using a white LED light source through a condenser lens in the opposite direction to the IR laser.

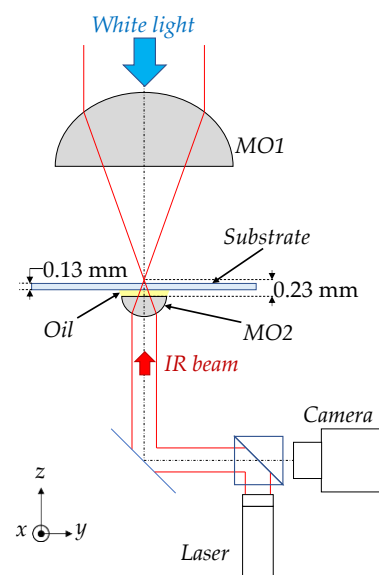


Figure 1. Representation of the usual operation of an optical trap. (MO1—Condenser Microscope Objective, MO2—Oil Immersion Microscope Objective).

2.2. Interferometer Setup

As mentioned before, a Mach–Zehnder interferometer was integrated into the optical trap system. The experimental setup corresponding to the interferometer used is shown in Figure 2. The interferometer light source is a KIMMON KOHA laser (Sukagawa, Japan) (model KBL-100-A) with a wavelength (λ_B) of 405 nm and a maximum power of 100 mW; in this laser, the coherence length decreases below 300 μm when a power lower than 1 mW is selected [28], and this makes it possible to obtain interferograms that are free from spurious interference, and also to perform non-invasive measurements (without affecting the object under study).

The laser beam passes through a beam expander system and is incident on a 50:50 beam splitter. The reflected part—the reference arm—is incident on a mirror that is attached to a piezoelectric (PZT), which is controlled by a THORLABS variable voltage source (model TDC001), in order to apply the phase-shifting technique in the interferogram demodulation; then, continuing on its path, the wavefront is redirected to a second beam splitter, also 50:50. On the other hand, the part transmitted by the beam splitter at the laser output passes through Microscope Objective 1, MO1 (Edmund brand, model 59880, of 100X, with an NA of 0.8 and a WD of 3 mm), and then, after going through the sample under study, light is captured by a second microscope objective (MO2), to be redirected via a mirror, until it crosses the second beam splitter. Here is where the beams recombine, and interference is generated.

The image-capturing video system consists of a THORLABS camera (model DCC1240c) based on a CMOS sensor with a pixel size of 5.3 μm and a resolution of 1280×1024 pixels. The field of view generated between Microscope Objective 2 and the camera is $61.24 \times 48.99 \mu\text{m}$.

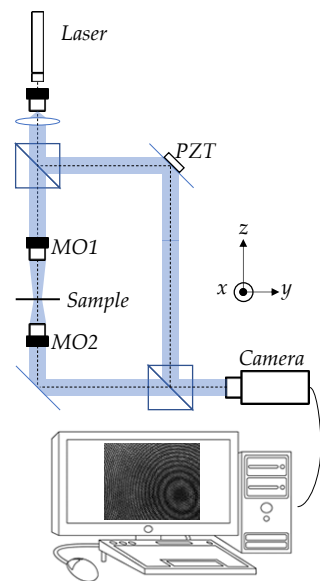


Figure 2. Scheme of a Mach–Zehnder interferometer. (MO1—Long Working Distance Microscope Objective, MO2—Oil Immersion Microscope Objective).

With the system in question, it is possible to obtain interference patterns such as the one shown in Figure 2, whose capture was performed with the LabVIEW™ program, while image processing was carried out with the MATLAB™ programming language.

The phase difference ($\Delta\phi$) between the two interfered wavefronts, previously mentioned, is given by [1]

$$\Delta\phi(x, y) = \frac{2\pi}{\lambda} \text{OPD}(x, y), \quad (1)$$

where λ is the wavelength and OPD is the optical path difference.

An idealized model of an interference pattern with time-phase shifting modulation ($I(x, y, t)$) is described using the following:

$$I(x, y, t) = a(x, y) + b(x, y)\cos[\phi(x, y) + \omega_0 t]. \quad (2)$$

Here, $a(x, y)$ is the background signal, $b(x, y)$ is the modulation signal (or fringe contrast), ω_0 is a phase carrier, and, for simplicity, the phase difference term of interest will hereafter be referred to as ϕ . If the $I(x, y, t)$ term is known, a system of at least three equations is necessary to determine $\phi(x, y)$, due to the infinite possibilities in the other parameters; in practice, it is common to introduce a variation that can be a sequential increase of $\omega_0 t = 2\pi t/N$ (also called “phase shifting” or “phase step”), N being the total number of fringe patterns [29]. It is important to note that t only indicates the instant at which the phase shifting is applied; it is not directly related to a specific time.

For each point p in (x, y) on the screen where the interference pattern is projected (in this case, each pixel of the CMOS sensor), the difference in the trajectories traveled is different, but directly related, according to

$$h(x, y) \times \Delta n = m(x, y)\lambda = \frac{\phi(x, y)\lambda}{2\pi}, \quad (3)$$

where h is the thickness of the object under study (through which the object beam was transmitted), Δn is the difference of the refractive index with respect to the reference beam, and m refers to the fringe order, which is proportional to the phase difference.

In this work, the four-step +1 algorithm was used, since it is robust to detuning errors. The wrapped phase (ϕ_W) is obtained by [30]

$$\phi_W = \tan^{-1} \left(- \left[\frac{I_2 - I_4}{\frac{1}{2}I_1 - I_3 + \frac{1}{2}I_5} \right] \right), \quad (4)$$

being the interference pattern subscripts (1–5) the corresponding time step numbers, as related to Equation (2).

Expression (4) gives a phase value wrapped in the range from $-\pi$ to π (hence the origin of its name), so that an unwrapping process is necessary to eliminate discontinuities not present in the actual measurement, and thus estimate a continuous phase.

2.3. Integration of Interferometer and Optical Trap

Figure 3 corresponds to the setup resulting from the integration of the optical trap with the Mach–Zehnder interferometer, previously explained. Based on the optical trap setup, the blue laser replaces the white light source, while the condenser objective is replaced by the long working distance objective. Before the blue laser enters this objective, the expander and the first beam splitter are placed, and finally, the reference beam is redirected to the splitter in front of the camera; it should be noted that the low numerical aperture of the long working distance objective, together with the low power of the blue laser, does not allow the generation of a significant gradient force that would disturb the optical trap. The rest of the optical trap system remains unchanged; so, both configurations share the large numerical aperture microscope objective (MO2) and, consequently, the same immersion oil.

In this way, the interaction of the optical trap with the structures under study can be quantitatively analyzed using the integrated Mach–Zehnder interferometer. Yet, as mentioned above, if the power of either laser source changes, the interferometric measurements will have an additive phase effect that must be compensated for.

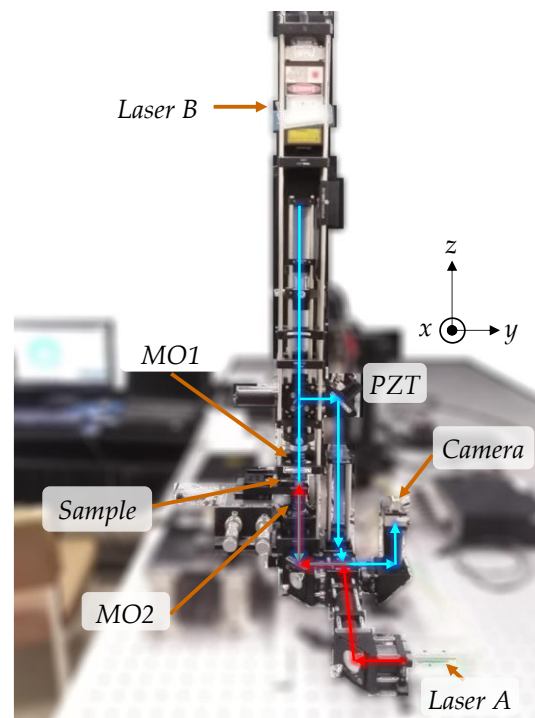


Figure 3. Optical Trap—Mach-Zehnder interferometer experimental setup. (MO1—Long Working Distance Microscope Objective, MO2—Oil Immersion Microscope Objective).

3. Separate Analysis per Individual Components

In previous experiments, using the interferometer-trap configuration, it was noted how, as the optical trap trapping force increased or decreased, the interferograms exhibited a phase shifting directly proportional to the change in laser output power. The same effect, although with a different ratio, was observed when keeping the trapping force constant and now changing the interferometer laser power. Since, in both cases, the phase shifting was directly proportional to the change of power, and since the direction of propagation of both lasers is opposite to each other, a mechanical effect caused by the radiation pressure could be discarded as the cause of the phase addition; otherwise, the phase shifting for some of the sources should be inversely proportional to the change in power. Thus, the cause of the above effect must be in one or some of the optical elements on the object arm that the optical trap and the interferometer share. To identify these elements and their contribution to the phase addition effect, the following experiments are proposed; they include independent external analyses for the elements located around the optical trap.

3.1. Identification of Optical Elements Contributing to Phase Shifting

Considering the study published in [24], in which a variation in the confocal microscope optical geometry as a function of IR laser radiation is reported, the first experiment of the manuscript presented here aimed to study the optical trap position with respect to the optical element in which it is formed (no trapped particle), i.e., the relative trap position was changed, moving the substrate from the sample immersion medium (located above the slide) down to the immersion oil, going through the slide. At each height interval, the IR laser power was changed, evaluating the power required for the phase addition to correspond to a value of 2π radians. Since power is directly proportional to intensity ($\text{Power} = \text{Intensity} \times \text{Area}$), it follows that the intensity-phase ratio is also linear. Experimentally, the laser power can be manipulated, so this variable is used in this work.

If the power-phase ratio were to remain constant in the different media, then no medium responsible for generating the additive phase could be identified. However, the results presented in the graph in Figure 4 provide evidence that by placing the trap in the

immersion oil, the phase addition is more sensitive to the change in power when compared to the slide glass and the medium in which a sample would be immersed over the slide, which in this case was air. It is observed how less power is required to cause a phase shifting of 2π when the trap is placed in the oil (between -130 and $-240\ \mu\text{m}$).

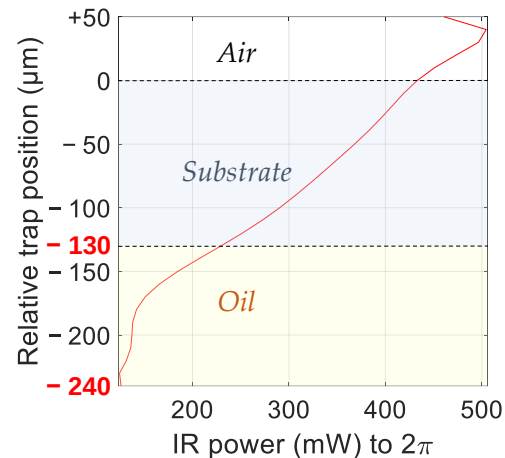


Figure 4. IR power required to cause a 2π shift depending on trap position.

To corroborate the major contribution of oil to the detected phase shifting, independent tests of oil, water, air, and glass were conducted on a macroscopic scale outside the optical trap. Since in the trap–interferometer setup it is not possible to measure variables separately, three systems were designed: in order to study the refractive index, another Mach–Zehnder interferometer was mounted, and the element was contained; to measure the temperature, a thermocouple was used; to characterize the topography, and then calculate the volume, a Michelson interferometer was used. In the subsequent sections, the optical, thermal, and morphological analysis experiments of these elements, particularly oil, are described. Thus, by knowing the contributions of each material, it will be possible to calibrate and correct measurements.

3.1.1. Refractive Index Test

The experiment proposed above makes it possible to identify that there is a different phase contribution from each element, but it does not make it possible to determine the contribution of each of these or whether only one of them contributes. Therefore, an experiment (Figure 5) was performed by assembling a Mach–Zehnder interferometer outside the optical trap system to test each element separately on a macro scale (air, glass, distilled water, and oil), while they were excited with a second laser beam (different from the interferometer). The interferometer had a He–Ne laser source with a λ of 633 nm (λ_{Pr}) and a power of 12 mW. The beam was spatially expanded and filtered. In addition, it had two non-polarized 50:50 beam splitters. On the observation plane was a PIXELINK (Ottawa, ON, Canada) camera (model PL-B741F) with a CMOS sensor, 6.7 μm pixel size (1.3 Mpx), and 1280 $\times$ 1024 pixel resolution. The camera sensor was exposed without having a lens attached.

The laser used to excite the elements of interest was a SPECTRA-PHYSICS (Milpitas, CA, USA) solid-state laser (model Excelsior-532-100) with a λ_{Ex} of 532 nm and a fixed power of 100 mW; the beam diameter that is obliquely incident (45°) on the sample was 0.7 mm. This angle of incidence was determined due to the setup geometry, which is not critical in the results. Although it would have been possible to excite on-axis with the excitation laser, in order to prevent the exposed beam from hitting the camera sensor and saturating or damaging it, it was necessary to illuminate obliquely, resulting in that angle.

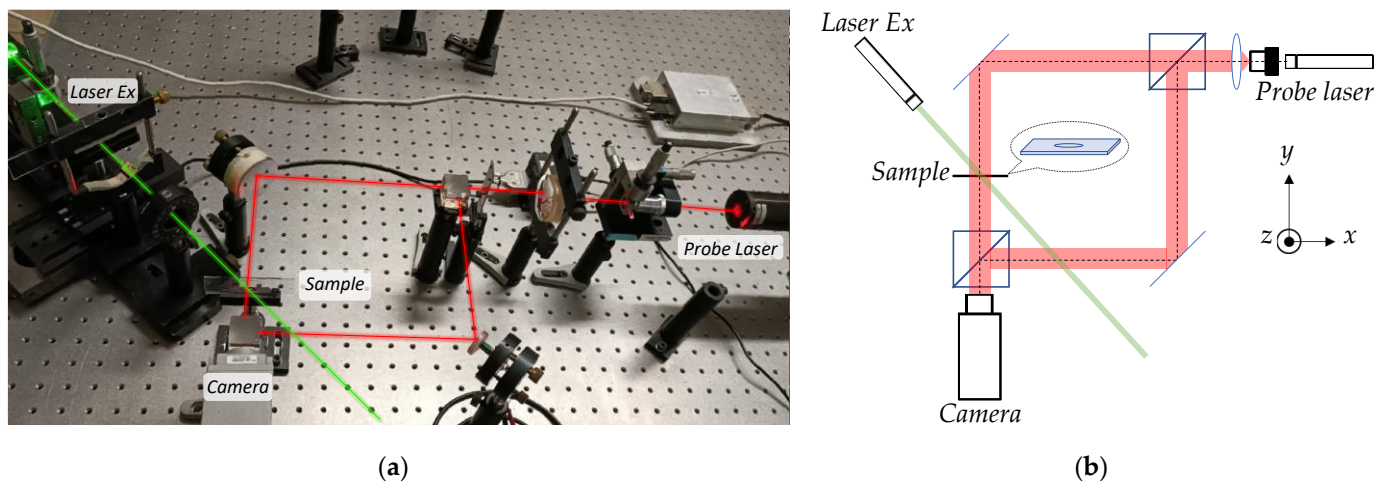


Figure 5. Mach–Zehnder interferometer used to study refractive indexes: (a) experimental setup photo; (b) Scheme.

To study glass, water, and oil, an optical container was fabricated consisting of a 1.1 mm thick glass slide with a central perforation of 10 mm in diameter. A coverslip was placed on each side of the slide; one was fixed with optical adhesive and the other was attached only through atomic interaction with each element in question.

To characterize air, the container was completely removed. For the glass analysis, a regular slide was placed, without perforation. For distilled water, the container filled with this liquid was positioned in the area of interest, and the experiment was carried out in a similar fashion with the immersion oil.

Figure 6 shows the changes in the refractive index of the four analyzed media after 20 s of excitation with the green laser. These results allow us to identify oil as the element that makes the greatest contribution to the additive phase to be removed from the measurements made with the optical trap and Mach–Zehnder interferometer system. In both air and glass, only problems of interferogram detuning are observed, without any contribution from the excitation of those elements. However, the change in the refractive index of distilled water and immersion oil is easily observed; specifically, taking the difference between the maximum and minimum values for each figure, Figure 6c (5.47×10^{-6}) and Figure 6d (4.47×10^{-5}), the change in oil is 8.17 times higher than that of water.

The refractive index changes shown in Figure 6 were calculated from the phase recovered from the interferograms corresponding to each element by the following expression:

$$n_f = n_i - \left(\frac{\phi}{2\pi} \frac{\lambda_{Pr}}{h} \right), \quad (5)$$

where n_f is the medium refractive index when it responds to the excitation laser, n_i is the unexcited medium refractive index, and h is the sample thickness.

It is now possible to evaluate, to a first approximation, how much the trap position (the immersion objective working distance, WD) would change if the trap were totally immersed in the oil and the refractive index change were to change uniformly throughout the oil, using the following equation:

$$WD = \frac{d}{\tan\left(\sin^{-1}\left(\frac{NA}{n}\right)\right)}, \quad (6)$$

where d is the distance from the optical axis to the marginal beam at the output of MO2. Figure 7 plots the behavior between the change in refractive index (x-axis) and the corresponding change in working distance (y-axis), which is equivalent to the change in trap position [21] or to the focus position in a confocal microscope [24].

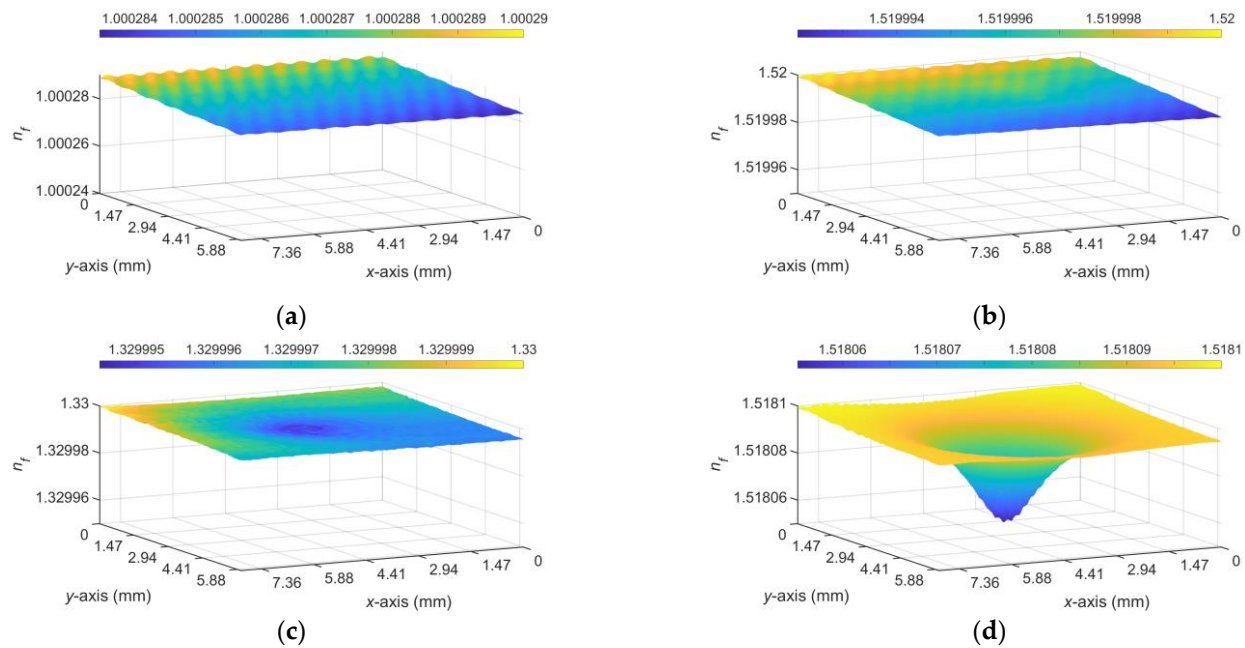


Figure 6. Change in the refractive index when laser radiation excites: (a) Air; (b) Glass; (c) Distilled water; (d) Immersion oil.

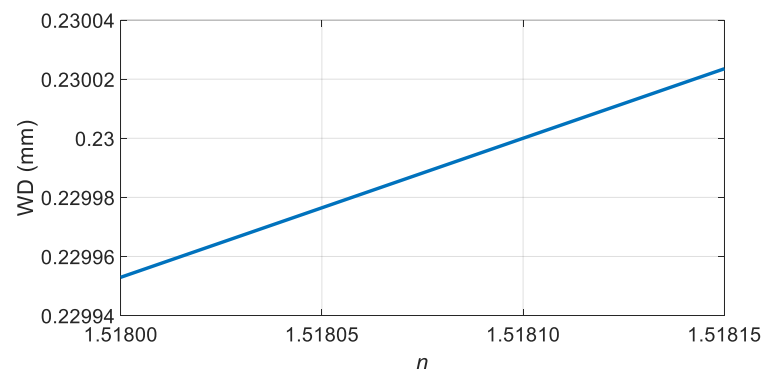


Figure 7. Behavior of the refractive index of the immersion oil and the working distance of an oil immersion objective.

In addition, Liu reports that the higher the objective NA, the less power is required for a given ΔWD . It is agreed that, as the laser power is increased, the WD decreases; furthermore, the trap gradient force is higher, the oil is excited, and the oil's n decreases [21].

3.2. Thermal and Morphological Analysis of Oil

The results from the independent analysis of the elements allow us to disregard the contribution of air, water, and glass, focusing attention only on the immersion oil, which is the one that contributes most to the phase addition. In this regard, laser excitation is changing the oil temperature, which causes a change in refractive index, and at the same time, a change in oil volume; in what follows, two experiments implemented to analyze both effects separately are proposed.

3.2.1. Temperature Tests

Thermal analysis of immersion oil was performed by exciting it with the same laser as in the previous experiment ($\lambda_{Ex} = 532 \text{ nm}$) and measuring the temperature with a low-cost type K thermocouple, whose sensor is 1.15 mm in diameter. Although this thermocouple is relatively big, our laboratory technical limitations force us to use it. Figure 8

shows the experimental configuration set up for this purpose, where the laser is observed being incident in a normal orientation in the container center. Unlike optical metrology techniques that are usually full-field and do not require direct contact with the sample, the thermocouple must be immersed in the oil to make not only a local measurement of temperature, but also that of the surrounding oil. Therefore, when the thermocouple is immersed in the oil, it can be directly excited by the laser beam, mixing its own temperature with that of the oil, which would generate incorrect measurements.

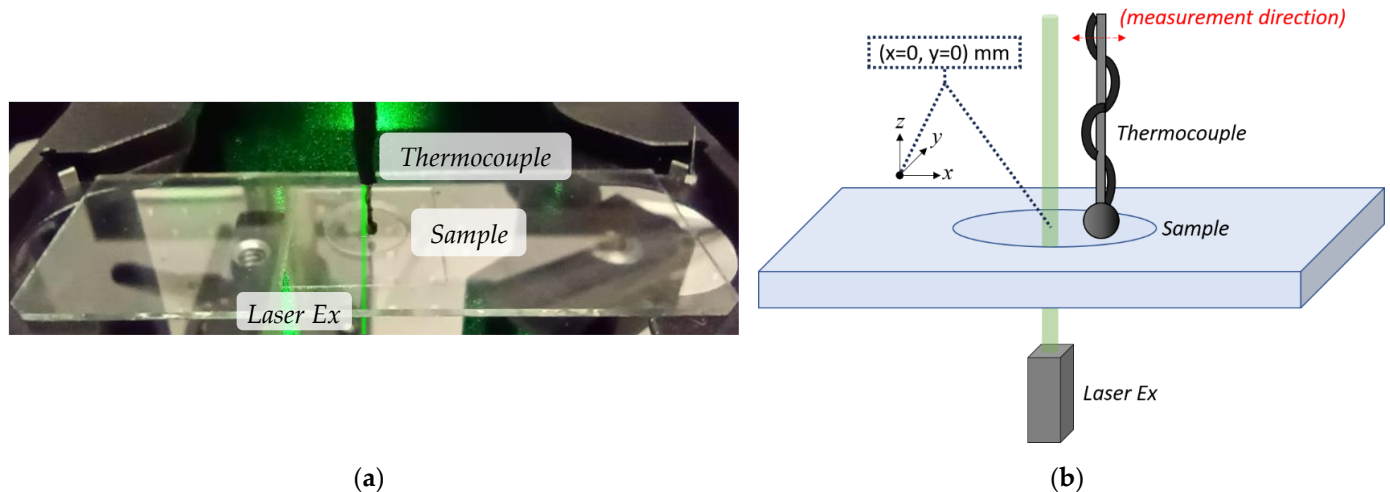


Figure 8. Experimental setup used to analyze thermal behavior: (a) photo; (b) scheme.

To prevent that problem, the thermocouple was positioned as close as possible to the laser beam while avoiding direct excitation. To find this position, an oil-free container was used, and the thermocouple was placed at the x-axis negative end (with $y = 0$ mm), at a z-height of just a few microns above the container bottom. From this point, air temperature measurements were made along the x-axis, passing through the laser beam center incident on the $x = 0$ mm, $y = 0$ mm position. The experiment was then repeated with the container filled with the immersion oil. Figure 9 also shows that the thermocouple starts to behave erratically the closer it gets to the laser beam center, where the points correspond to valid measurements and the crosses are erratic measurements.

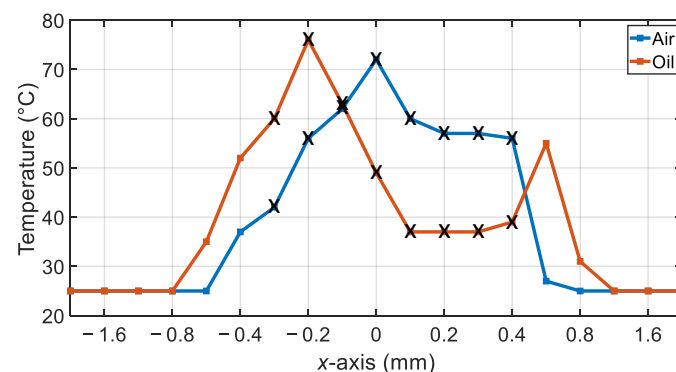


Figure 9. Air and oil temperature profiles. Crossed-out readings indicate erratic thermocouple behavior.

The results of air temperature (taken as a reference) and oil are presented in Figure 9, where it can be observed, in the profile corresponding to air, that the position closest to the laser where the thermocouple receives minimum excitation is $x = -0.5$ mm, $y = 0$ mm.

The profile for the temperature differences between air (empty container) and oil is shown in Figure 10. If above-mentioned erratic measurements, in this case between -0.3

and 0.4 mm, are discarded, it is possible to calculate—with MATLABTM fit function—an approximate temperature profile that corrects thermocouple behavior (red line in Figure 10), and which is congruent (similar in shape but reverse in direction) to the refractive index change map central profile in Figure 6d. Thus, it can be inferred that oil reaches a temperature of around 120 °C at the beam center.

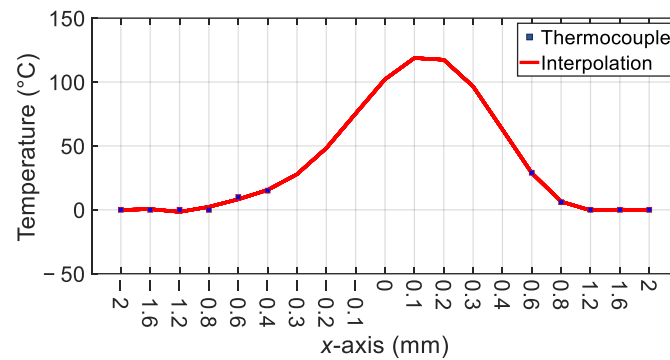


Figure 10. Oil temperature profile vs thermocouple position. Values shown as crossed out in Figure 9, were here removed and replaced by the interpolation curve.

Positioning the thermocouple at $x = -0.5$ mm, $y = 0$ mm, a temporal analysis of the oil temperature was then performed. In that experiment, measurements were taken under three different conditions: first with the laser source off for 10 s, then in a second scenario of 20 s with the laser on, and finally, a third scenario of 20 s with the laser off again; in all of them, measurements were taken continuously at 1 s intervals. The same procedure was applied in the experiment of Section 3.1.1 where the refractive index change was measured, although in that section, only the result for the 20th second of the second scenario, which corresponds to the maximum excitation time, was presented.

Figure 11 plots the thermocouple measurements as a function of time and compares them with the optical phase map maximum value corresponding to the refractive index. It is observed that in both cases, the oil gets excited and relaxes at approximately the same time intervals, whereas in this case, the thermocouple is slower to react to the change. It is important to mention that the oil returns to its initial temperature and refractive index in less than 20 s.

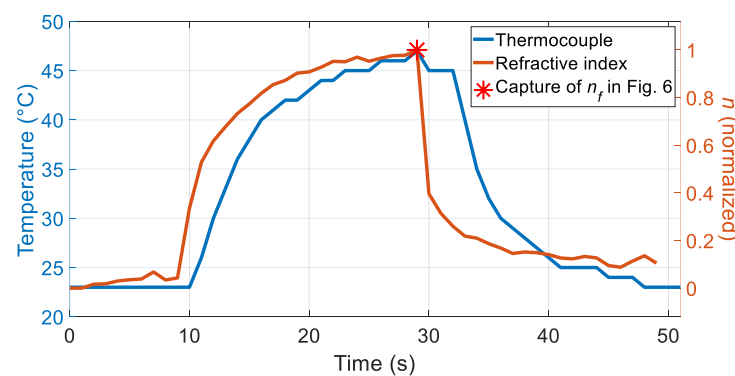


Figure 11. Comparison of thermal behavior and refractive index of oil over time. The asterisk indicates the point that was analyzed in Figure 6.

3.2.2. Morphological (Volume) Tests

To characterize the volume change caused by laser excitation, a Michelson interferometer was mounted (Figure 12), having as illumination source a THORLABS brand LED (model LED635L) that emits light with a central spectral output (λ_{LED}) of 635 nm, a half-height width (FWHM) of 15 nm, an approximate coherence depth of 25 μ m, and a half viewing angle of 7°. Since the test object is the same oil-filled container, the LED's low

coherence allows interference only with the first surface, where the oil is exposed and the volume change is concentrated; this way, spurious interference from other surfaces is avoided. For these experiments, the same λ_{Ex} excitation laser and camera that were used to analyze the refractive index were used again. In addition, the experiment sequence (without excitation, with excitation, and finally, in relaxation) was repeated. As in the temperature experiment, only the oil was analyzed.

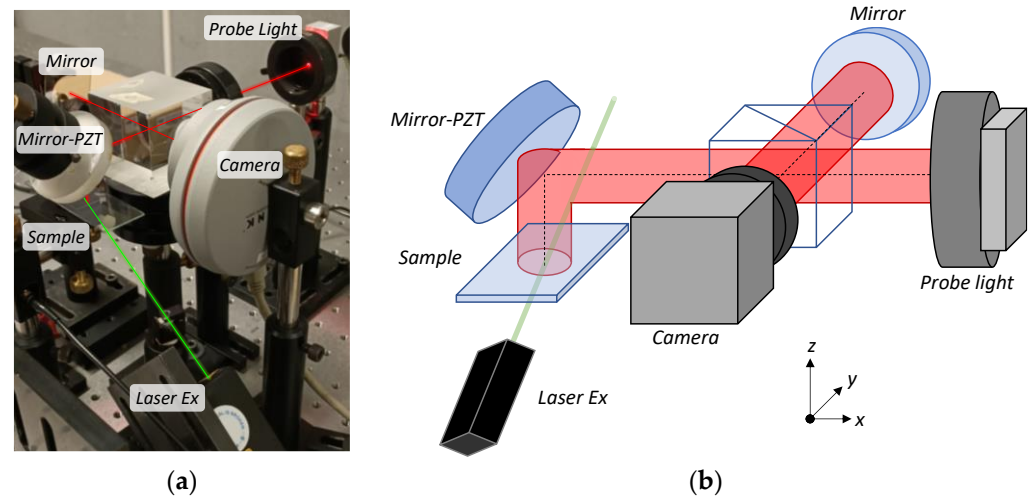


Figure 12. Michelson interferometer used to study volume changes: (a) experimental setup photo; (b) scheme.

In Figure 13, the change in the surface topography of the exposed oil at different times is presented. Figure 13a shows the displacement map when measured at 5 s, when the laser was still off; Figure 13b shows measurement at 10 s, when the laser was turned on; Figure 13c–e represents measurements made at 15, 20, and 25 s, respectively; finally, Figure 13f corresponds to the topography measured when the laser was off.

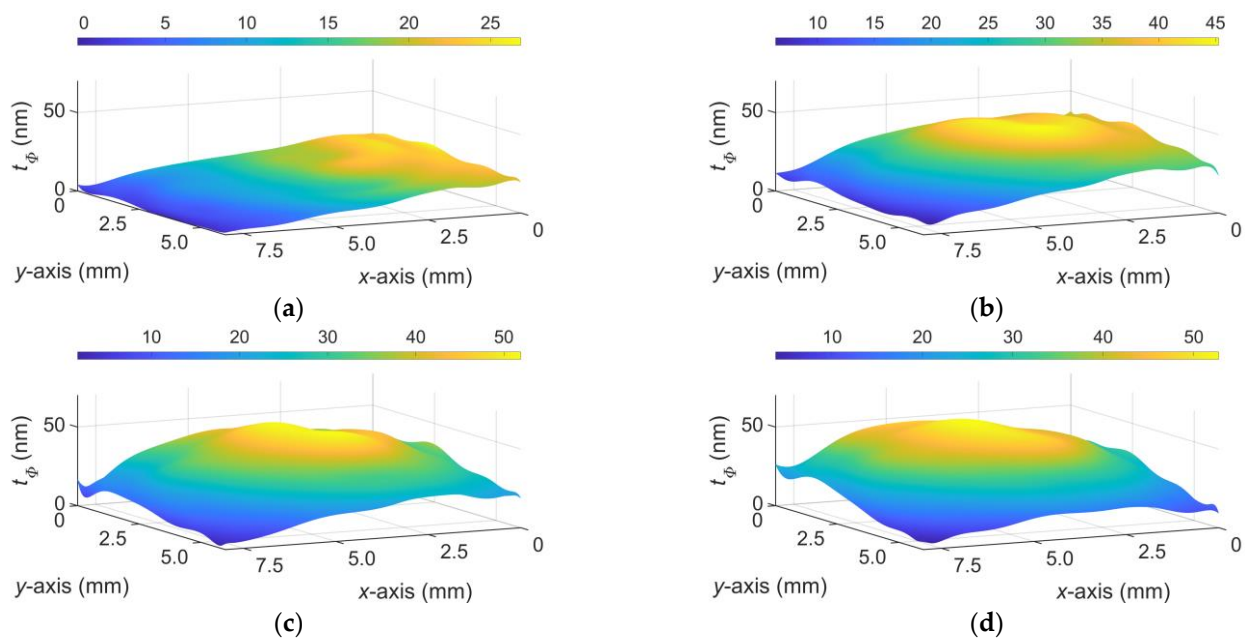


Figure 13. Cont.

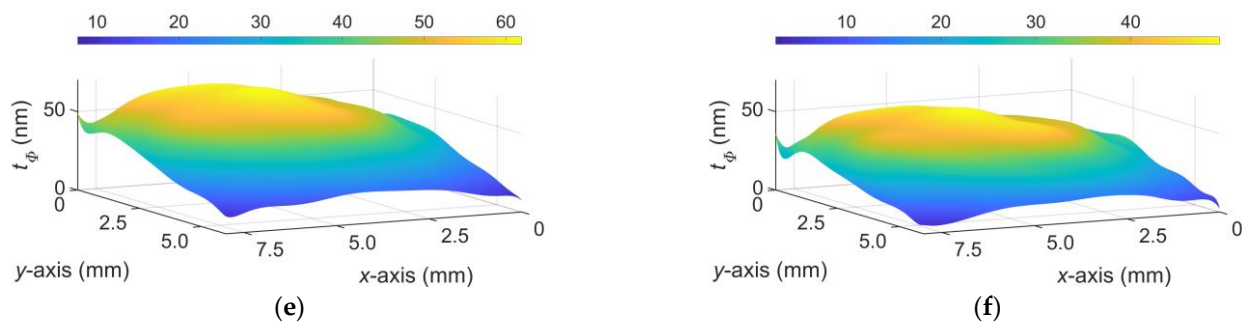


Figure 13. Topography changes in time when laser radiation excites at: (a) 5; (b) 10; (c) 15; (d) 20; (e) 25; and (f) 30 s.

For the Michelson interferometer used in these experiments, the topography values are obtained from the phase maps through the following equation:

$$t_{\phi} = \frac{\phi \lambda_{LED}}{4\pi}, \quad (7)$$

where t_{ϕ} is the change of sample topography.

By integrating the volume under the topography at each point, the total volume of each measurement can be obtained. Due to interferometer instability, the measurements present a detuning error; however, volume behavior corresponds adequately to laser excitation time, and can be related to the result of a refractive index–temperature test, since oil expands with heat. Figure 14 shows, as a function of time, the change in volume against the change in temperature and refractive index.

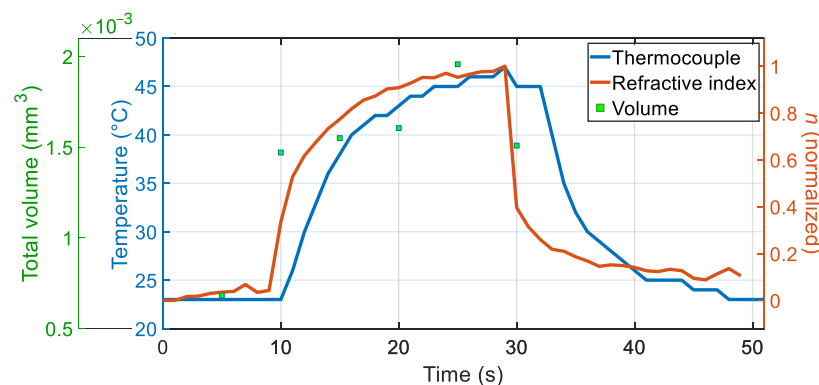


Figure 14. Comparison of temperature, refractive index and volume change of the oil over time.

As reported by Matrecitos-Avila, oil volume change does not represent a variable that affects her measurements due to equipment design, and therefore, she does not stop in its analysis [24]. However, it has been proven in this work that such change also exists and should be considered, depending on the application.

4. Calibration and Correction of the Additive Phase

Once it has been shown how immersion oil reacts when excited by electromagnetic radiation, the correction (compensation) applied to the measurements made with the Mach–Zehnder interferometer integrated to the optical trap (as mentioned in Section 2.3) is presented.

In this case, the object under test is a black paint film on which the optical trap will generate deformations and possible damage when exposed to different IR laser powers, while the interferometer measures the three-dimensional shape of the damage caused. That is, although in this case the optical trap works as an indenter, the deformation it generates

in the paint film would be of the same nature as the deformations that could be measured with the interferometer on any trapped particle.

4.1. Optical Trap Tests for Phase Calibration

As a first approximation, calibration of the phase added by the immersion oil is performed by keeping the laser power of the interferometer (λ_B) constant, having previously placed the optical trap on the upper face of the slide (glass-air interface), in an area that does not contain paint. Taking as a reference the interferogram phase when the laser that generates the trap is off (0 mW), the power of this IR laser is increased in 5 mW intervals, until reaching 300 mW, with a waiting time of 5 s between increments. In order to apply Equation (4), five interferograms were taken at each power, with a step of $\pi/2$, and waiting 77 ms between steps; after making a phase measurement at each interval, the reference phase is subtracted from each of these. The result of this experiment is shown in Figure 15, where a profile for the average of phase differences was plotted, observing that the power-phase ratio is linear and uniform throughout the phase map. Thus, knowing the increase in the trap power, it is possible to know the corresponding phase that should be subtracted from the measurements made with the interferometer, since the phase shifting could be considered spatially constant.

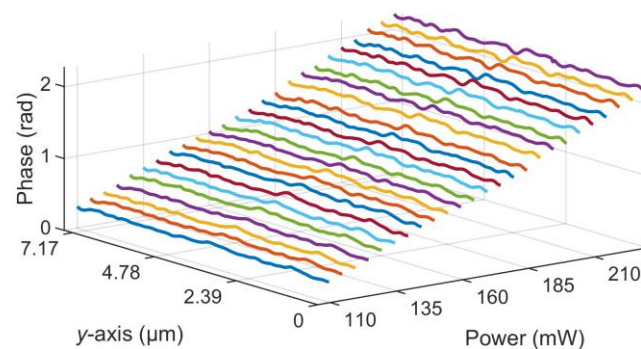


Figure 15. Phase shifting (in profiles) relative to power shifting from 110 to 230 mW.

However, as demonstrated in Section 3.1.1, phase distribution may not be constant throughout the field of observation; so, it is advisable to consider such effects when implementing the calibration. For example, Figure 16a shows the phase behavior when applying powers of 110, 140, 175, 205, and 230 mW, in a slide area where there is no sample (i.e., without paint). Likewise, in Figure 16b, the profiles of the gray plane in intersection with the phase maps are plotted, as they are indicated in Figure 16a. Since the behavior between phase and power is linear, only these powers were selected to facilitate visualization. In both figures, it is clear that the additive phase intervals are equispaced, although spatially variable.

Similarly, Figure 17 shows the phase values when the same powers are applied to an area of the slide where a paint sample has been deposited.

In both characterizations, a positive trend is perceived, which corresponds to the phase-shifting behavior in the respective interferograms. Figure 18 shows the behavior of each trend line and a sample interferogram.

4.2. Additive Phase Correction

It can be clearly observed how there is a piston in the phase maps in the characterization of a sample as the IR laser power is increased. Figure 19a–e present in phase the damage that the sample receives when the IR laser is positioned at the glass–air interface, and the sample is characterized at powers of 110, 140, 175, 205, and 230 mW.

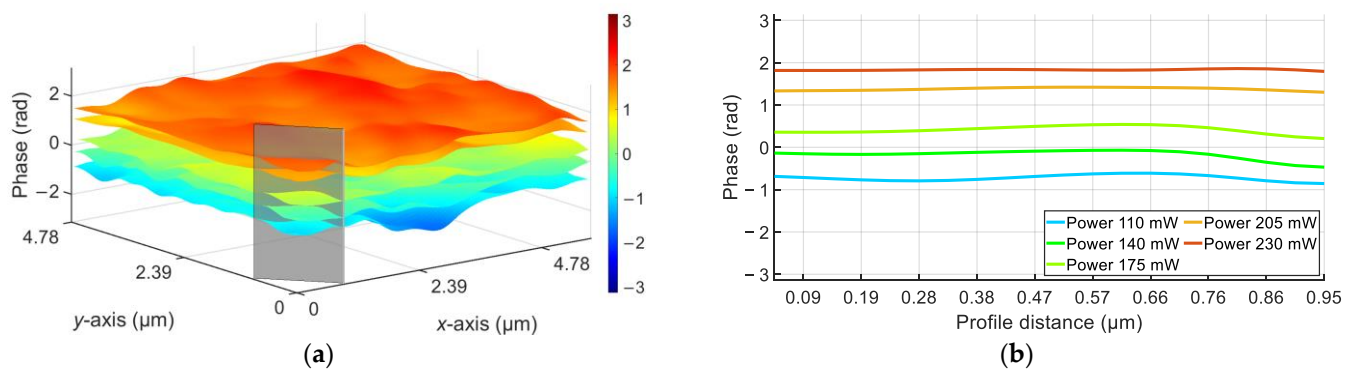


Figure 16. Phase piston behavior in glass using 0 mW power as reference: (a) phase mapping; (b) profiles.

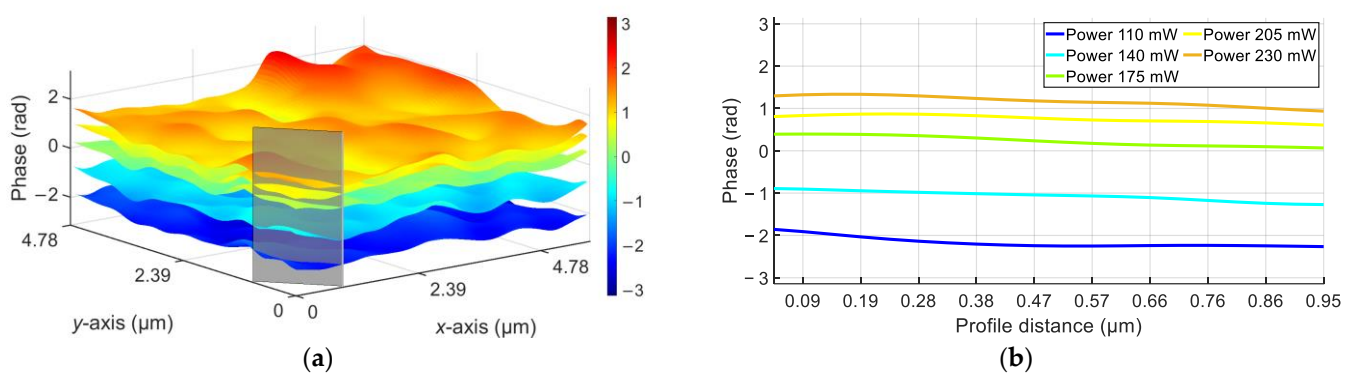


Figure 17. Phase piston behavior in paint using 0 mW power as reference: (a) phase mapping; (b) profiles.

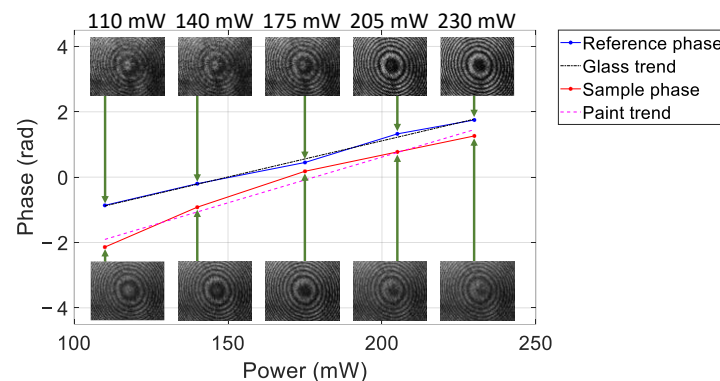


Figure 18. Phase-shifting behavioral trend shown in interferograms as well as in phase average (point) depending on the power.

Since the applied power for each damage caused to the paint is known, then it is possible to remove the corresponding additive phase from the calibration presented in the reference of Figure 18. If the additive phase map of the calibration is constant, it will be enough to also remove that constant value from the phase map of the measured three-dimensional form; in this case the damage caused by the optical trap. If the additive phase map were not constant, then a correction should be implemented by relating both phase maps, point to point. Figure 20 shows the Figure 19d phase map; from 0 to 4.78 μm corresponds to the uncorrected phase and the half from 4.78 to 10.27 μm is the corrected measurement. It can be observed, in the corrected half, how the area of the paint that has not been damaged by the optical trap remains with values close to those in Figure 19a. It is expected that the correction in each of the other phases will behave in the same way.

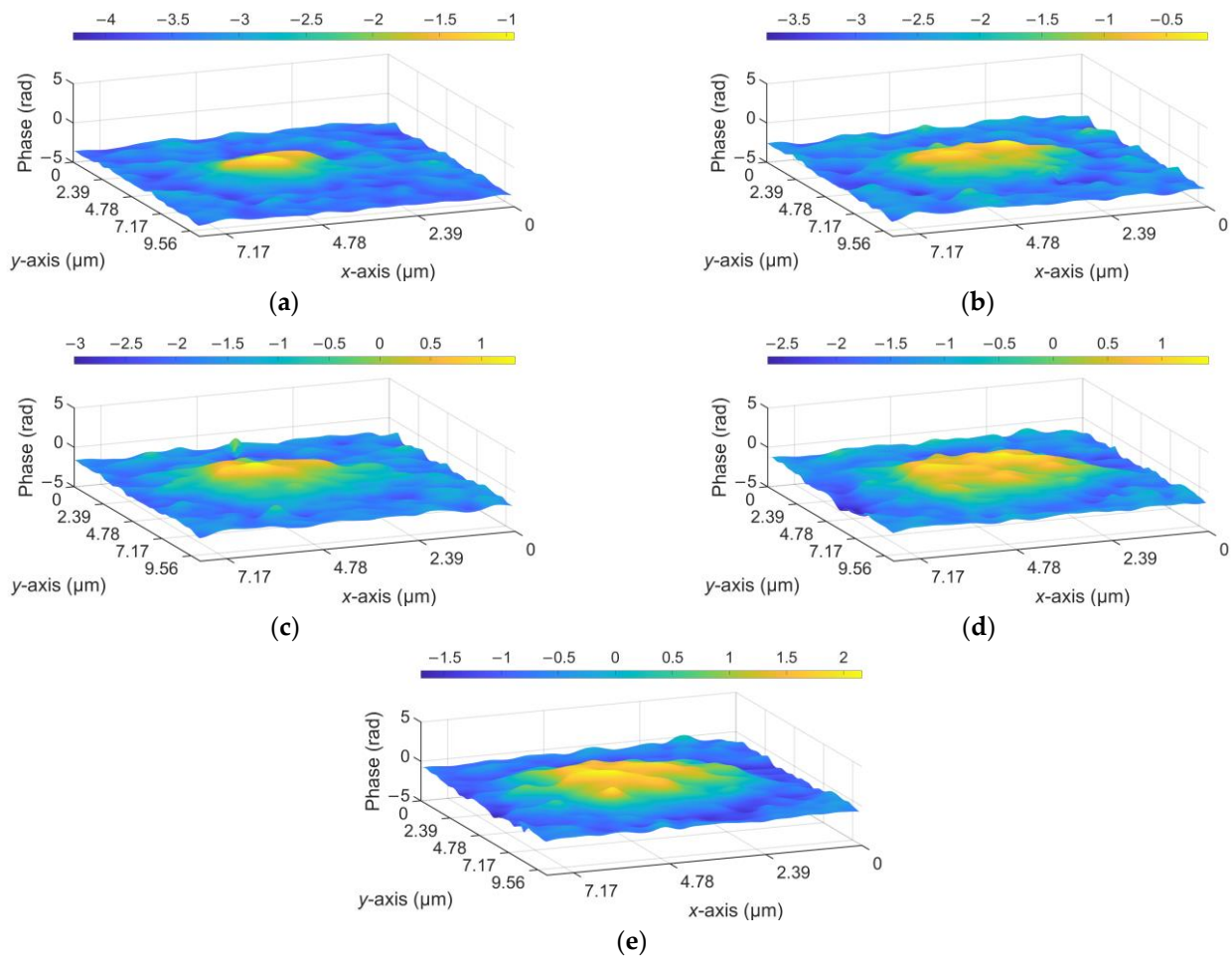


Figure 19. Paint damage (in phase terms) when the laser IR radiates at: (a) 110; (b) 140; (c) 175; (d) 205; and (e) 230 mW.

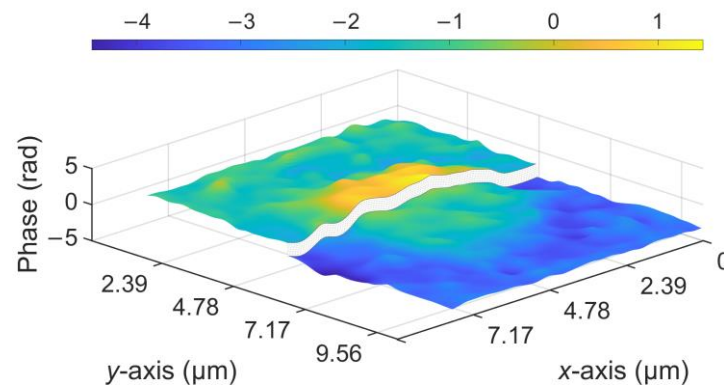


Figure 20. Measurement correction of the phase map at 205 mW of power. Left side (from 0 to 4.78 μm over the y-axis) corresponds to the uncorrected phase and the right side (from 4.78 to 10.27 μm) is the corrected measurement.

4.3. Discussion

With the data presented here, it can be established that the additive phase piston detected in the optical trap is a consequence of changes in the immersion oil. Although in Section 3.1.1 (Figure 6d), the refractive index shows a Gaussian behavior, in Section 4.1 (Figures 16 and 17), a quasi-constant phase shifting appears throughout the field of view, due to the difference of orders of magnitude of the observed areas (mm and μm, respectively). This phase piston is attributed to a thermal effect caused by laser radiation when

transmitted through immersion oil. Although the oil's morphological change (volume) does not usually affect the experiment directly, because it does not touch the sample under test, the change in temperature implies a variation in the refractive index and indicates an alteration in the beams focal point at the exit of the immersion objective used. In the experiments described in Sections 3.1.1 and 3.2, the effect of a specific wavelength was analyzed, this was only to understand the oil behavior; if other wavelengths were used, it is suggested that there would be similar results, but with different ratios. Also, the results could vary with a focused beam instead of the collimated beam used in those experiments. However, the calibration and phase correction were done with the trap's IR laser. In the case of a proper digital correction, the laser characteristics and the oil response to its exposure must be known.

5. Conclusions

When an oil immersion objective is used in an interferometric measurement system that undergoes laser power changes, an additive phase term caused by the immersion oil reaction will be present. This effect occurs when coupling an interferometer and an optical trap, where the latter is used to manipulate the object under test that the interferometer will measure. To obtain a correct interferometer measurement, that additive phase term must be removed from the measurement. In this study, it is demonstrated that the immersion oil, which is required by the microscope objective, is the component that reacts to the change in laser power, causing a phase shifting in the observed interferograms. Therefore, the immersion oil was analyzed optically, thermally, and morphologically, independent of the trap–interferometer setup. It was concluded that when there is a change in the laser source, it is necessary to calibrate the additive phase term in the trap–interferometer array, to subsequently consider it in the measurements, as presented in Section 4.1.

The direct applications of this study impact microscopes that use immersion oil and are used to measure a certain parameter. In addition, since power decreases during the useful life of a laser, it would be necessary to implement the calibration described herein on a periodic basis. Another calibration would imply characterizing the ray convergence point of an oil immersion microscope objective, depending on the application and the equipment used. For example, for techniques such as microscopic interferometry, digital holographic microscopy, or 3D flow cytometry, the phase term added due to the change in the oil refractive index would have to be considered. In the same sense, for an optical trap or a confocal microscope, there would be a change in the system's optical geometry. More robust work concerning the optical, thermal, and morphological behavior of the material discussed here is left open.

It is reiterated that the time-dependent change in volume corresponds to the change in temperature and refractive index, since oil expands with heat. In addition, as the laser power increases, the WD decreases, the trap gradient force is higher, the oil is excited, and the oil's n decreases; thus, there is a piston in the phase maps.

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