

Holographic microrefractometer

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In-line holographic microscopy of micrometer-scale colloidal spheres yields heterodyne scattering patterns that may be interpreted with Lorenz-Mie theory to obtain precise time-resolved information on the refractive index of the suspending medium. We demonstrate this approach to spatially resolved refractometry with measurements on calibrated refractive index standards, and use it to monitor chemical concentration in a microfluidic channel. Using commercially available colloidal spheres as probe particles and a standard video camera for detection yields values for the fluid's refractive index at the position of each probe particle in each holographic snapshot with a demonstrated resolution of 2×10^{-3} refractive index units (RIU) and a potential resolution surpassing 10^{-4} RIU. The combination of spatial resolution, temporal resolution, multi-point *in situ* access and technical simplicity recommend this technique for cost-effective lab-on-a-chip applications.

Optical probes at the heart of many lab-on-a-chip systems require precise time-resolved information on the refractive index of the fluid medium. Considerable effort, therefore, has been focused on developing techniques to measure the refractive index of picoliter samples. One approach is to integrate a refractometer into the microfluidic device, with examples including instruments based on beam deflection¹, photonic crystal resonators^{2,3}, Fabry-Pérot interferometers^{4,5}, and other microfabricated optical resonators⁶⁻⁸. The best of these can achieve a resolution of 10^{-7} RIU⁶. Their principal disadvantage is the need to microfabricate the refractometer and integrate it with the microfluidic system.

Chemically synthesized colloidal spheres dispersed in the fluid medium also have been used as probes of the local refractive index through spectroscopy of their whispering gallery modes⁹. When these modes are excited by broadband fluorescence of dye molecules¹⁰ or quantum dots¹¹ embedded in the probe sphere itself, the local refractive index can be measured with a resolution of 2.5×10^{-4} RIU¹¹. The necessary spectroscopic measurements, however, limit this approach to addressing one probe particle at a time. The fluid medium, moreover, must be chemically and physically compatible with

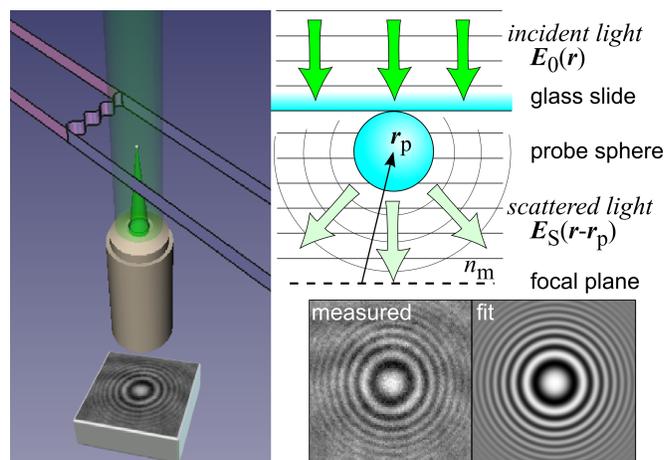


Fig. 1 Holographic microrefractometry based on holographic video microscopy. A probe particle affixed to a microfluidic channel scatters laser light into a pattern that may be analyzed to measure the refractive index of the fluid in the channel.

the specially synthesized spheres.

Here, we describe a complementary approach to microrefractometry based on holographic imaging of suspended colloidal spheres. This method yields time-resolved measurements of the refractive index at multiple points simultaneously using a standard video camera as a detector. Since virtually any type of colloidal sphere can act as the probe, this technique is compatible with a wide range of chemical conditions.

Our method is based on in-line holographic video microscopy¹²⁻¹⁴, shown schematically in Fig. 1, in which a sample is illuminated with a collimated beam of coherent light. Light scattered by a probe particle interferes with the unscattered portion of the illumination in the focal plane of an otherwise conventional optical microscope. The interference pattern is magnified and its intensity is recorded with a video camera. Each digitized image is a hologram of the probe particle in its medium that can be interpreted with predictions of the theory of light scattering to measure the particle's refractive index relative to that of the medium, with a resolution better than one part per thousand¹³⁻¹⁵. If the particle's refractive index is separately calibrated, this measurement yields the refractive index of the medium.

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We illuminate the sample with a collimated beam from an array of fiber-coupled diode lasers (iFlex Viper). Scattered light is collected with a 100× numerical aperture 1.4 oil immersion objective (Nikon, Plan Apo VC) and is relayed to a gray-scale video camera (NEC TI-324A II), which records holograms at 30 frames/s. The intensity measured at position \mathbf{r} in the focal plane results from the superposition of the incident field $\mathbf{E}_0(\mathbf{r})$, which we model as a plane wave linearly polarized in the \hat{x} direction, and the field $\mathbf{E}_s(\mathbf{r} - \mathbf{r}_p)$ scattered by a particle at position \mathbf{r}_p relative to the center of the focal plane:

$$I(\mathbf{r}) = |\mathbf{E}_0(\mathbf{r}) + \mathbf{E}_s(\mathbf{r} - \mathbf{r}_p)|^2. \quad (1)$$

For spherical probe particles, $\mathbf{E}_s(\mathbf{r})$ is described by the Lorenz-Mie theory of light scattering¹⁶ and is parametrized by the radius a_p of the particle and by its refractive index $n_p(\omega)$ relative to that of the medium $n_m(\omega)$. This latter dependence enables us to use holograms of colloidal spheres to monitor the refractive index of the medium with spatial resolution set by the scale of the measured scattering pattern and temporal resolution set by the camera's frame rate.

Measured holograms of colloidal spheres are normalized by measured background images¹³ and fit to the predictions of Lorenz-Mie theory for each particle's position \mathbf{r}_p , radius a_p , and relative refractive index $n_p(\omega)/n_m(\omega)$. The fitting procedure has been described in detail elsewhere^{13,14}.

We validate the technique by analyzing holograms of 1 μm diameter colloidal silica spheres (Thermo Scientific, Catalog Number 8100) dispersed in a set of ten refractive index standards (Cargille Laboratories, series AAA) with refractive indexes ranging from 1.30 to 1.39 RIU. Probe spheres are attached to the upper glass surface of a microfluidic channel as shown in Fig. 1. The glass surface is initially cleaned by washing in deionized water, sonicating in acetone, and oxygen plasma etching. An aqueous dispersion of spheres in deionized water then is spread over the clean dry surface and allowed to dry for 2 hours in a 10-100 Torr dessicator. The decorated surface is then exposed for 20 hours to a room-temperature vapor of hexamethyldisilazane (HMDS 99.9%, Aldrich) in a closed glass container. The resulting coating permanently affixes the spheres to the glass and also minimizes fluid imbibition that otherwise might have changed the spheres' effective refractive indexes¹⁵. The other wall of the channel is formed by a clean number 1 glass cover slip.

The prepared channel is mounted on the stage of the holographic microscope for measurements. A typical $80 \times 70 \mu\text{m}^2$ field of view includes 5 spheres whose holographic images are sufficiently widely separated to be analyzed independently. Each sphere's refractive index is calibrated by filling the channel with a refractive index standard (nominally $n_m = 1.3300$ at 589.3 nm, corrected to $n_m = 1.3291$ at 640 nm) and fitting its measured hologram as previously described¹³. The spheres' holograms are not appreciably affected by contact

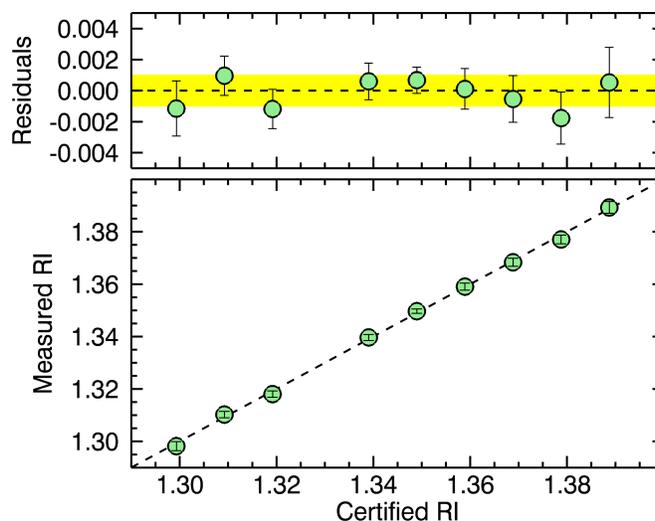


Fig. 2 Comparison of measured and certified values of the refractive index of nine refractive index standards, based on calibration of probe spheres with a tenth standard. The shaded region in the plot of residuals corresponds to the estimated 10^{-3} RIU uncertainty in the fit values for the refractive index.

with the upper wall, except that additional light is projected onto the few pixels closest to the forward-scattering direction. Because these pixels' influence on the fits is smaller than the overall measurement uncertainty, no effort is made to correct for them. Thus calibrated, the same spheres then are used as probes of the refractive index of other fluids flowing down the channel.

The data in Fig. 2 were obtained by filling the channel with samples of certified refractive index standards and fitting for the refractive index of the fluid. The fit values then are compared with the wavelength-corrected certified values. Between measurements, the channel and its probes are thoroughly cleaned by washing three times with DI water, acetone, and methanol, and then are dried with a stream of clean dry nitrogen. Each measurement is performed by analyzing 30 holograms of four probe spheres recorded over 1 s. The individual fits' precision is typically 10^{-4} RIU. The error bars in Fig. 2 reflect the 2×10^{-3} RIU range of values for n_m obtained from the four spheres. We ascribe this loss of accuracy relative to the measurements' precision to optical imperfections introduced while affixing the probe spheres to the surface.

The same data sets are reanalyzed using the certified values of the fluids' refractive indexes as inputs, this time to confirm that the spheres' measured refractive indexes do not vary with that of the medium. The absence of correlation between n_p and n_m in this analysis confirms that the treated spheres are impervious.

The data in Fig. 3 demonstrate the use of holographic microrefractometry for monitoring changes in the fluid within

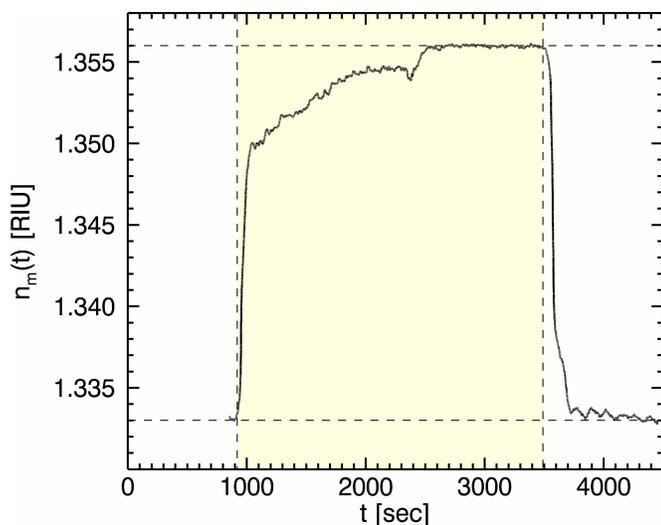


Fig. 3 Time-resolved measurement of the refractive index of an aqueous sucrose solution in a microfluidic channel. The shaded region indicates the period during which sucrose solution is flowing into the channel.

a microfluidic channel. A stream of deionized water flowing at $0.5 \mu\text{L/s}$ is abruptly replaced with a stream of sucrose solution at the same flow rate, and then equally abruptly returned to pure water. During this process, the fluid's measured refractive index increases from $n_m = 1.333 \pm 0.002$, to $n_m = 1.356 \pm 0.002$ as sugar solution suffuses the channel, and returns to its baseline value once the sucrose is washed out. The 2×10^{-3} RIU accuracy for refractive index in this measurement corresponds to a 30 mM resolution for sucrose concentration.

A naive estimate suggests that the entire volume of fluid in the channel is replaced every 2 s, and that sugar solution should entirely fill the channel in a matter of seconds. The actual half-time for replacing the water with sucrose extends to several minutes, presumably because reservoirs of fluid form in the macroscopic tubing leading to the microfluidic channel.

In addition to using randomly deposited spheres as refractive index probes, we also have used holographic optical traps¹⁷ to attach probe spheres to selected locations within microfluidic channels. In this case, probe spheres are dispersed in a 200 mM solution of Na_2SO_4 . Pressing a trapped sphere against a glass surface for roughly 10 s suffices to fix it in place. This provides a simple means to position probe spheres at specific locations.

These measurements demonstrate that colloidal spheres can be used as effective probes of the local refractive index of a fluid medium. Requiring only optical access for imaging, holographic refractometry is easily integrated with existing lab-on-a-chip systems.

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