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A NEW GENERATION FIBER OPTIC PROBE : CHARACTERIZATION OF BIOLOGICAL FLUIDS, PROTEIN CRYSTALS, AND OPHTHALMIC DISEASES

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ABSTRACT

A new fiber optic probe developed for determining transport properties of sub-micron particles in fluids experiments in a microgravity environment has been applied to characterize particulate dispersions/suspensions in various challenging environments which have been hitherto impossible. The probe positioned in front of a sample delivers a low power light (few nW - 3mW) from a laser and guides the light which is back scattered by the suspended particles through a receiving optical fiber to a photo detector and to a digital correlator. The probe provides rapid determination of macromolecular diffusivities and their respective size distributions. It has been applied to characterize various biological fluids, protein crystals, and ophthalmic diseases.

INTRODUCTION

In the past few years dynamic light scattering (DLS) instrumentation has embraced several new and innovative technological advances. The immediate impact of these have resulted in the miniaturization of DLS instrumentation[1]. One of those advances is the use of fiber optics in DLS. Especially, the use of monomode optical fibers in designing and fabricating a DLS system was first initiated by Brown[2] in his 90° scattering system. Later, a lensless single fiber probe was used by Weiss and Horn[3] to study highly concentrated systems (1-40% w/v) without the adverse effect of multiple light scattering. However, since the scattering volume has to be positioned right at the tip of the fiber, the probe had to be immersed in the sample, making it no longer a non-invasive measurement. Also, the heterodyning effect caused by the internal reflection at the tip of the fiber necessitated a great deal of care in fabrication, and it could not be used for dilute samples with concentrations below 1% w/v or particles smaller than 30 nm because the reflected signal dominated the scattered signal. A lensless back-scatter fiber optic probe was developed to study concentrated particulate dispersions by Dhadwal et al.[4] Subsequently, Ansari et al[5]. and Dhadwal et al[6]. have shown the utility of their probe to the studies of cataractogenesis in excised bovine and cadaver eye lenses, and in live animals[7]. Unfortunately this probe has certain limitations and posed severe constraints in probing different parts of the eye, mainly due to a large scattering volume and a short penetration depth. Ansari and Suh[8] recently developed a new DLS probe to study nucleation and aggregation phenomena during protein crystal growth in space (microgravity) experiments. The new probe is compact, rugged, and provides accurate particle size determination of variety of colloidal dispersions in 5 seconds at extremely low laser power levels. Furthermore, it does not require any vibration isolation, index matching, or optical alignment, and it can be used with many different shapes and sizes of sample containers. These include regular spectroscopic cuvettes, capillary tubes, common laboratory utensils (beakers, graduated cylinders, conical flasks, glass/clear plastic container vessels), concentric cells containing two different solutions, and hanging fluid droplets of colloidal particles. The new probe is applied to several different applications which require characterization of various suspensions/dispersions in a wide range of particle sizes and concentrations from very dilute (water-like) dispersions to highly concentrated (milk-like) suspensions in a variety of challenging situations. These applications include monitoring protein crystallization in different hanging drop configurations suitable for microgravity experiments[9], complete eye diagnostics[10-12], zeolite crystal growth[13], study of clay-polymer interactions, particle sizing in a rotating cell to avoid sedimentation due to gravity, characterization of food colloids, microemulsion and micellar systems, and tissue and skin analysis.

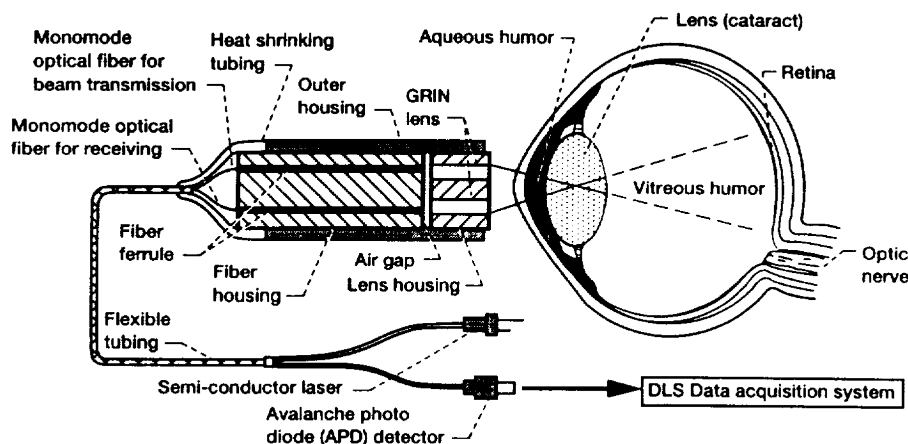
Protein crystals have been experimentally grown in 28 US Space Shuttle flights. One preferred method is to grow these crystals in hanging drops (~30μL) in a reduced gravity environment offered by the Space Shuttle orbiter or space station. In laboratory settings, DLS has been used to study protein solutions and crystal growth processes by Casay and Wilson[14], Malkin and McPherson[15], and references therein. However, in hanging droplets and in microgravity experiments, elaborate instrumentation and optical alignment problems have made in-situ and on-line applications difficult. Ansari et al[8,9]. have demonstrated that such experiments are now feasible. The new probe has

been successfully used in various earth and space-bound (microgravity) protein crystallization system configurations[9].

Cataracts remain the major cause of blindness affecting about 50 million people each year worldwide. It is estimated that over $5 billion will be spent this year in treating cataract patients in the United States alone[16]. There is no medical treatment to prevent or halt the progression of a cataract; nor is there any way to reverse a cataract once it is formed. The only known treatment is surgical. However, a medical treatment could be possible if we understand how a cataract forms and what makes it grow[16]. In order to find a medical treatment for cataracts, first, we must be able to detect a growing cataract in early stages of formation. The ability of such a detection will be useful in patient monitoring and in the development and testing of possible "anticataract" drugs. The technique of dynamic light scattering (DLS) or quasi-elastic light scattering (QELS) was first applied to the study of cataractogenesis in the pioneering work of Tanaka and Benedek[17]. An extensive review of using QELS to study cataracts has been given by Bursell et al[18]. DLS/QELS being a non-invasive and quantitative technique seems to hold promising potential in its use as a routine ophthalmic device. But its commercial scope as an ophthalmic diagnostic tool in clinical settings hitherto has not been materialized. Since the original application of QELS, some twenty years ago by Tanaka and Benedek[16], every subsequent study focused on rather easily accessible part of the eye i.e. the aqueous humor and the lens. Most recently Ansari et al[12]. in the USA and Rovati et al[19]. in Switzerland reported the first experiments directed to the studies of the vitreous humor. Ansari et al[10,11]. have also shown recently that the new probe alleviates many major concerns discussed earlier when used as an ophthalmic diagnostics device and is much superior in performance when compared with the earlier reported work[5-7]. Furthermore, the major problem of probing different parts of the eye has been solved. In this paper we report the characterization of several solutions at different particle sizes (7-800nm in diameter) and concentrations (0.0001-10.0% w/v), and the characterization of bovine lenses and the eyes of live animals (rabbits and mice).

EXPERIMENTAL PROCEDURE AND SETUP

A fiber optic probe comprising two monomode optical fibers and two GRIN micro lenses, as illustrated in Figure 1, provides a compact and remote means of studying the dynamical characteristics of the macromolecules in a sample. A 13 mm diameter fiber optic probe contains the necessary optics to perform DLS measurements at a scattering angle of 161.5°. Two monomode optical fibers, each housed in a stainless steel ferrule, are mounted into a separate stainless steel housing. An air gap (0-0.5 mm) is intentionally left between the fiber housing and the lens housing in order to produce a tightly focused spot in the scattering volume. The two optical fibers in their housings are aligned and fixed into position off-axis with the GRIN lens. The two housings are placed inside



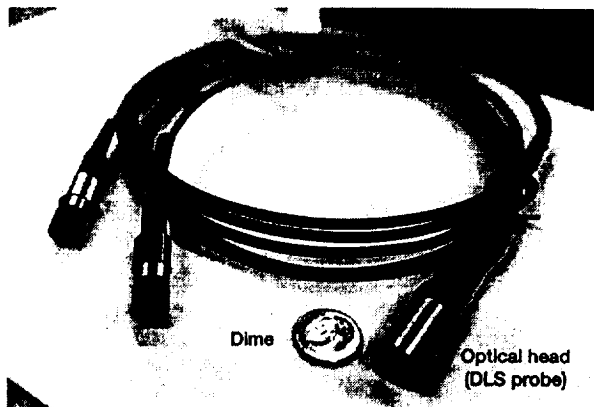


Figure 1. A new fiber optic DLS probe.

a third (outer) housing made of stainless steel, and the back end of the housing is covered with a heat- shrink tubing. The two free ends of the optical fibers were terminated with FC/PC-type male connectors for easy mating with the laser and an avalanche photodiode detector (APD). The DLS data was analyzed using the commercial software provided by the Brookhaven Instruments Company of New York.

RESULTS

First, we report a series of DLS experiments on polystyrene solutions at several different concentrations (0.0001 - 10.0% w/v) and particle sizes (32 - 800nm in diameter), and bovine serum albumin (BSA) solutions at five different concentrations (2 - 10 %), all with 30 second experiment duration. The resulting average particle sizes are shown in figure 2. The new probe accurately and reliably measures the particle size from 7nm to 800nm in diameter with concentrations ranging from 0.0001 to 10.0% w/v. Figure 3 shows DLS experiments at two different experiment durations (5 seconds and 30 seconds) using 1% BSA solution. The resulting time correlation functions (TCF) and particle size distributions (see inset) display the excellent quality of data collected even with experiment duration as short as 5 seconds.

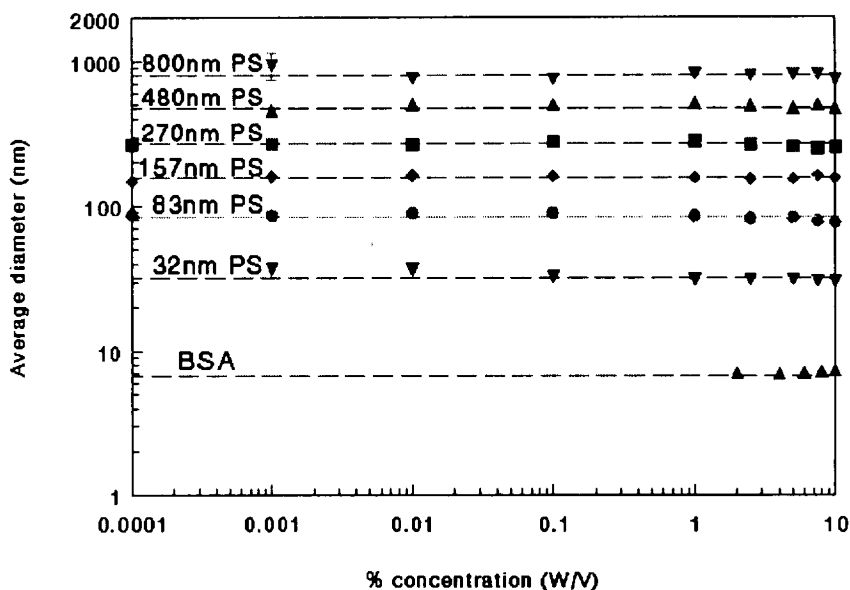


Figure 2. Calibration result with suspensions/dispersions of polystyrene and bovine serum albumin (BSA).

Figure 4 shows a 3-D DLS scan of an intact bovine eye lens, which shows detailed structure of the lens in terms of α crystalline size and its distribution in the lens tissue. In a normal eye lens, α, β, and γ crystallines (proteins) concentration is ~35 wt.%. Since α crystallines are the largest (molecular weight $\sim 1\times10^6$ daltons) in size they scatter most of the incident laser light. In the resulting average particle sizes, we find a specific trend as we move inside the lens and away from the anterior cortex. We see gradual increase in the particle size as we move from the anterior cortex to the nucleus of the lens. The same trend can also be observed as we move from the peripheral region to the nucleus. The size gradually decreases as we move out of the nucleus region and into the posterior cortex.

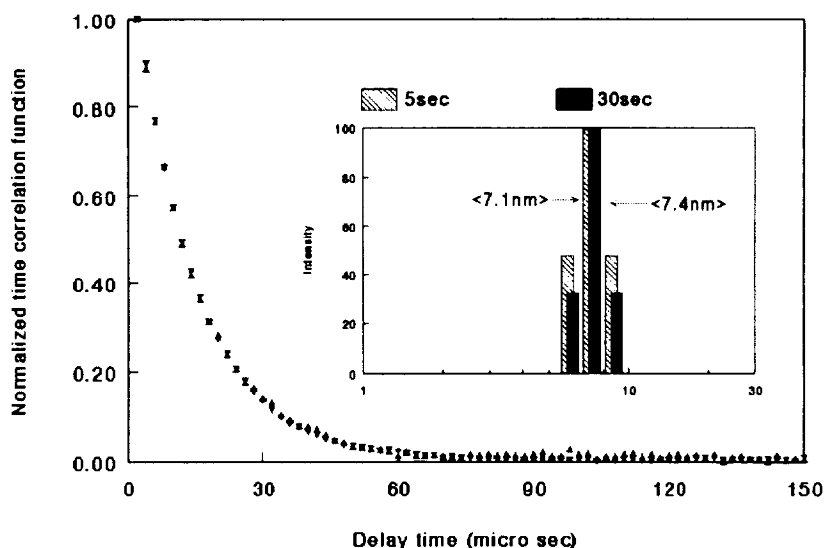


Figure 3. Normalized time correlation function (TCF) and particle size distribution for a 2% BSA solution using a laser power of 1.0mW at 5 and 30 second experiment duration.

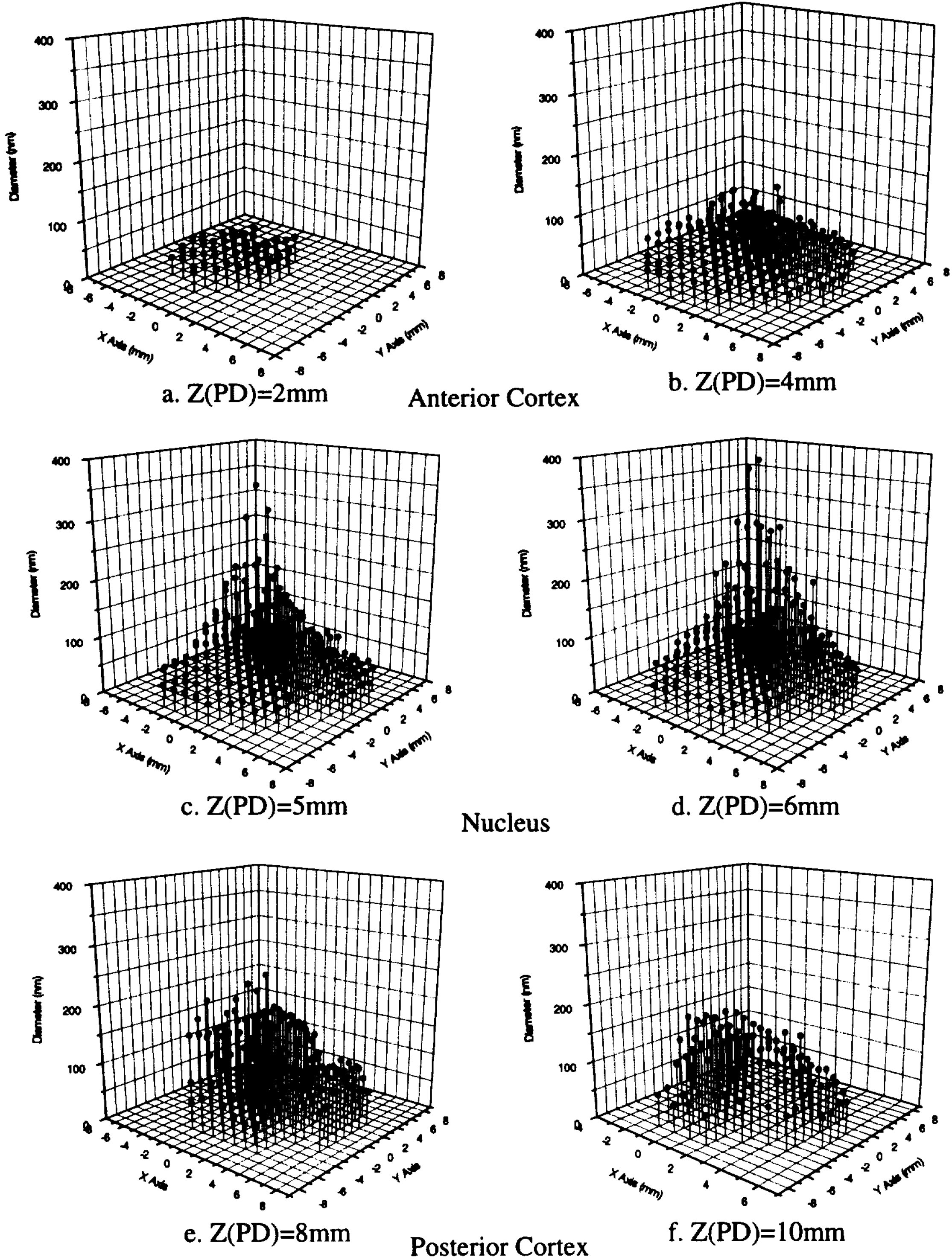


Figure 4. 3-D scanning of a bovine eye lens. The Z(PD)-axis represents the distance (penetration depth) from the front surface of the lens to the measurement point inside the lens.

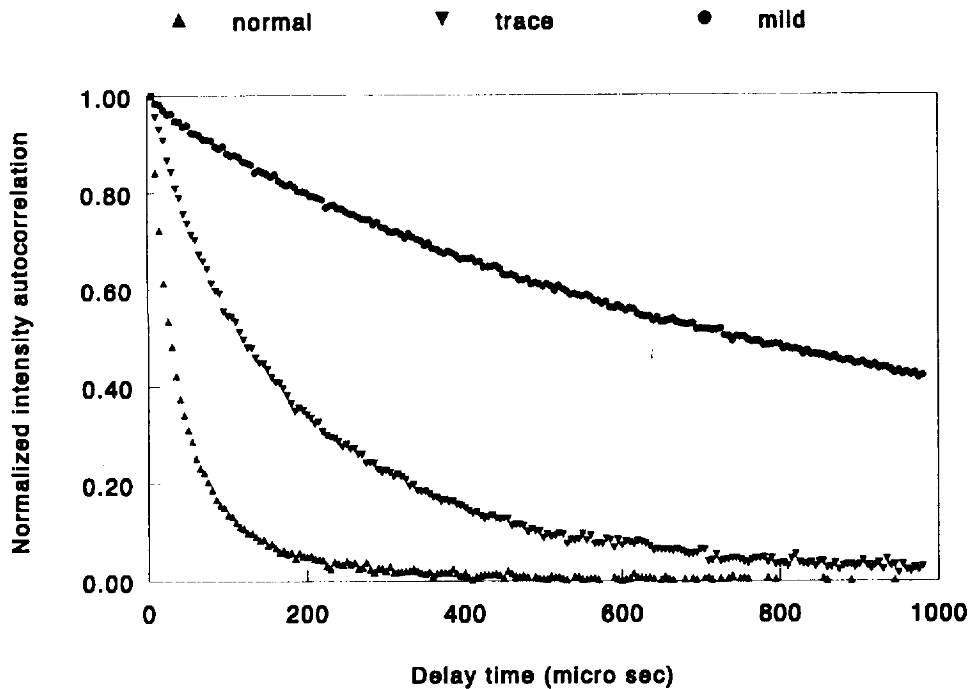


Figure 5. In-vivo cataractogenesis (DLS) measurements in Philly mice.

The Philly mouse is a good animal model of hereditary cataract[20]. Philly mice develop cataracts spontaneously between day 26 and 33 after their birth. This mouse model can be useful in the screening of anti-cataract drugs. We report a series of DLS experiments performed in Philly mice in collaboration with National Eye Institute (NEI) of National Institutes of Health (NIH). We applied our DLS probe to study the progression of cataractogenesis in this mouse model and to show the feasibility and safe use of our device in laboratory animals. Figure 5 shows DLS time correlation data on three Philly mice. It is quite challenging to perform DLS measurements in these animals because these animals have very small eyes. The lens in these animals has a diameter of less than 2 mm. The data includes a 45 day old normal mouse of the control FVB/N strain and two Philly mice roughly 26-29 days old. The eye examinations of these mice conducted with a slit-lamp apparatus concluded a normal (transparent) and two other eyes having trace and mild cataracts. These examinations were conducted within few minutes of the DLS measurements. Each measurement only took 5 seconds at a laser power of 50 μW. The changing slope of the time correlation functions is an indication of cataractogenesis. We can quantitatively monitor

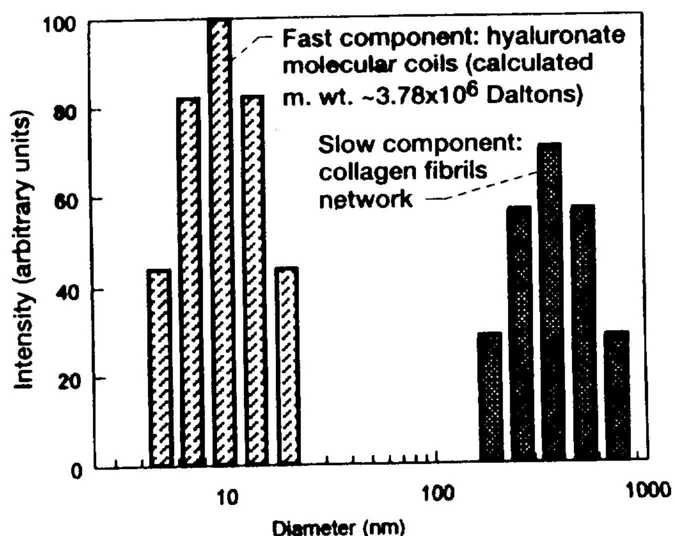


Figure 6. Size distribution for a 6 month old rabbit vitreous humor. Penetration depth from the corneal surface is 11mm.

cataractogenesis with reasonable reliability and reproducibility (5%-10%)[21].

The vitreous humor of the eye described by Sebag[22] is the least understood part of an eye. We present preliminary DLS measurements made in vivo on a six month old live rabbit. The vitreous body shows strongly two-exponential behavior, i.e. a fast and a slowly diffusing component, consistent with its gel-like properties. We see almost constant value for the fast component in this region. We ascribe this fast component due to the diffusion of hyaluronate molecular coils in water and the slow diffusion component due to the collagen-fibril network. A size distribution of the vitreous is presented in Figure 6. We calculated the molecular weight for the fast component to be 3.78×10^6 daltons. This is consistent with the range ($2\text{-}4.5 \times 10^6$) of values given by Balazs and Delinger[23]. More in-vivo experimental work is being performed at this time in our laboratory to fully understand the structure of the vitreous humor. We have recently concluded three dimensional (3-D) scans of the bovine vitreous which is reported elsewhere[12].

CONCLUSION

In this report we have briefly described a new DLS probe to characterize various biological fluids, protein crystals, and ophthalmic diseases. The new probe is compact, rugged, non-invasive, and is free of optical alignment. Furthermore, it uses very low laser power, produces data with high spatial coherence, and exhibits no adverse effect of multiple light scattering even with highly concentrated systems (up to 10% w/v with particle size of 800nm in diameter). The probe has been and is being successfully used for several different applications in various challenging environments including monitoring protein nucleation, aggregation, and crystallization in various space-bound (microgravity) hanging drop apparatus[9], eye diagnostics experiment on live animals as well as excised eyes[10-12], a zeolite crystal growth experiment[13], experiments in a rotating cell to minimize the sedimentation due to the gravitational effect, and clay-polymer interaction in a graduated cylinder.

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