# Lorenz-Mie Light Scattering in Cellular Biology

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Abstract. The Lorenz-Mie light scattering is discussed as a tool allowing living cell characterization. The scattered light carries information about the size, shape, internal structure and refractive index of the cell. The advantages of light scattering methods consist in high speed, nondestructive, sensitive and relatively easy measurements. Light scattering methods are compatible with other methods. In light scattering in both static and flow systems. For sphere-like cells reliable size and refractive index information can be extracted. On the empirical basis, light scattering pattern can be used for the cell identification and separation purposes. The full utilization of the light scattering information is limited due to the lack of theoretical knowledge about the complex scatterer properties and efficient inversion schemes. The rapid progress in computer technique and in single-particle scattering patterns of the biological particles.

Key words: Light scattering — Living cell characterization — Size determination — Lorenz-Mie scattering

## Introduction

The interest in using Lorenz-Mie light scattering (LMLS) in cellular biology has been lasting for more than twenty years. The rapid development of the photonics (laser light sources, light detectors) and electronics (data acquisition electronics, laboratory computers) have made the light scattering (LS) techniques more available in laboratories, the biological ones included. However, besides the technical equipment the lack of information about the capabilities and limitations of LS techniques has prevented their wider utilization. The present article discusses the potentials and limitations of LMLS techniques as a source of information about biological particles, especially intact living cells.

### Theory of light scattering by small particles

The term Lorenz-Mie scattering denotes elastic light scattering by small particles. We prefer this term to that of Rayleigh scattering because of the ambiguities connected with the latter term. (We shall be using notation in which Rayleigh scattering stands for the limiting case (dipolar scattering) of the more general Lorenz-Mie scattering.) Fundamentals of the LMLS theory are in depth covered in monographs (Shifrin 1951; van de Hulst 1957; Kerker 1969; Bohren and Huffmann 1983) so only few basic information are summarized here. Elastic light scattering can be considered as arising from electron oscillations forced in the particle volume by external periodic electromagnetic field – incident light. The scattered field results as sum of elementary contributions with the account of mutual interactions and interference effects. The scattered field has the same periodicity as an incident field but it differs in the direction of propagation, intensity, phase and polarization. Scattered light parameters reflect the size and the geometry of the particle as well as its material composition. Generally, particle scattering properties can be described by the spatial distribution of its refractive index. Rather than on absolute refractive index (referring to the vacuum) light scattering pattern depends on the relative refractive index referred to the surrounding medium. Similarly, the size of the particle appears in light scattering formulae always as a dimensionless parameter relating the size to the wavelength of light.

Due to the complexity of exact solutions, only limited class of scattering problems is solved analytically (sphere: Mie 1908; ellipsoid: Asano and Yamamoto 1975; infinite cyllinder: Wait 1955; coated sphere: Aden and Kerker 1951). Best understood is the problem of the scattering by a homogeneous sphere. For the limiting cases of size and refractive index combinations some approximations can be used, which greatly facilitates the computation and interpretation of LS patterns, more complex particle models included (Table 1.).

For particle suspensions containing large numbers of particles the resulting scattering intensity is obtainable as the sum of the single particle scattering intensities. This simple rule assumes the system to be sufficiently diluted and the mutual positions of the particles to change in a random way. In the opposite case multiple scattering and interparticle interference effects must be considered. As a practical criterion for the applicability of the single-particle scattering theory may serve extinction: when the intensity of incident light falls after passing the scattering volume by less than  $\exp(-0.1)$ , then no multiple scattering correction is necessary (van de Hulst 1957).

#### Scattering geometry

The commonly accepted scattering geometry is illustrated in Fig. 1. The directly measurable quantity, the scattered light intensity  $I(r, \vartheta, \varphi)$  falls in the wave zone

Figure 1. The scattering geometry. Initial light propagates in the positive z-direction. Scattered wave is observed in the direction  $(\vartheta, \varphi)$ . For the polarized light description both initial and scattered electric field vectors are resolved into the components paralel and perpendicular to the scattering plane.  $e_{\parallel}^{i}$ ,  $e_{\perp}^{s}$ ,  $e_{\parallel}^{s}$  and  $e_{\perp}^{s}$  denote the respective unity vectors. Note that  $e_{\parallel}^{i}$  is not parallel to  $e_{\parallel}^{s}$ .



Table 1. Lorenz-Mie light scattering theory approximations. Symbols used:  $\lambda$ , wavelenght; a, characteristic linear dimension of the particle; m, particle refractive index; R, curvature radius of the particle surface.

Size Refractive index		Approximation		
small $(a < \lambda/20)$	$2\pi ma/\lambda \ll 1$	Rayleigh scattering		
medium	$(m-1) \rightarrow 0$ Rayleigh-Gans-Debye $2\pi a(m-1)/\lambda \ll 1$ approximation			
arbitrary	(m-1)  ightarrow 0	anomalous diffraction		
large $(a > 10\lambda)$	arbitrary	Fraunhoffer diffraction		
large $(R \gg \lambda)$	arbitrary approximation	physical optics		
very large	opaque	ray optics		

(where all measurements are performed) with the distance r from the origin as  $1/r^2$ . Since the scattered wave has in general other polarization than the incident

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one, polarization properties of the light must be considered in full description. The polarization state of both the incident (i) and the scattered (s) wave can be described by the Stokes parameters I, Q, U, V, which are related by linear transformation. The transformation matrix  $S_{ij}$  is called the scattering matrix. The scattering matrix represents the full description of the scattering of generally polarized light wave by a particle. Each element of the scattering matrix is a complex function of the angles  $\vartheta$  and  $\varphi$ . The elements of the scattering matrix are directly measurable by using proper combination of optical elements (linear and circular polarizers) (Bickel and Bailey 1985).

Integral characteristics of light scattering can be described by means of extinction, scattering and absorption efficiencies  $Q_{\text{ext}}$ ,  $Q_{\text{sca}}$  and  $Q_{\text{abs}}$ . These dimensionless parameters represent the energy  $\mathbf{b}$ sses of the primary beam due to extinction, scattering and absorption, divided by the energy of incident beam passing the area equivalent to the geometrical cross-section of the particle. The term extinction comprises both scattering and absorption so that  $Q_{\text{ext}} = Q_{\text{sca}} + Q_{\text{abs}}$  The extinction, scattering and absorption cross-sections  $C_{\text{ext}}$ ,  $C_{\text{sca}}$  and  $C_{\text{abs}}$  can be obtained by multiplying the corresponding efficiencies by the geometrical cross-section.

#### **Biological particles as light scatterers**

Living cell suspensions constitute large-scale turbid polydispersions. Each of the dispersion particle itself represents a colloidal microsystem composed of the organic compounds diluted in the salt solution with the optical properties close to that encountered in outer medium.

Table	2.	Typical	cell	sizes.	After	Swanson	and	Webster	(1977)	)
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Cell type	Size		
 Bacteriophage	80 nm		
Pneumococcus	$100 \times 200 \text{ nm}$		
Mycoplasma	200–300 nm		
Bacillus influenzae	$500 \times 200 \text{ nm}$		
Salmonella	$2400 \times 500 \text{ nm}$		
Erythrocyte	7000 nm		

The size of living cells varies from several hundreds of nm to tens or even hundreds of a micrometer for some eukaryotic cells (Table 2.). In suspension of identical cell type, the cells exhibit natural size variations (Fig. 2) with the size changing during the life cycle (cell growth) and under various external conditions Figure 2. Typical size variations in a suspension of yeast cell (Saccharomyces cerevisiae strain K). The size is measured from microphotographs assuming spherical shape.



(e.g. osmotic pressure). Size variation influence on the single cell scattering pattern can be expected to be similar to that modelled in Fig. 3.



Figure 3. The effect of size variation on the light scattering pattern: angular dependence. The size and refractive index are similar to those measured for living cells in water environment, when illuminated by HeNe laser. The intensity (in arbitrary units) corresponds to the component polarized perpendicular to the scattering plane.

Refractive index	Min	Max	
Viruses	1.5	1.57	
Bacterial spore	1.46	1.54	
Walls of plant cells	1.5	1.53	
Bacterial cells	1.365	1.415	
Animal cells	1.350	1.380	

Table 3. Typical absolute refractive indexes of some biological particles. Data taken from Fichman (1967)

Since most components of the cytoplasm have similar refractive index concentration dependence, the value of the refractive index for living cells is roughly proportional to their dry weight concentration. "Effective" refractive index measured by the immersion method under physiological conditions exhibits nearly Gaussian distribution. The mean refractive index is a characteristic of each cell type, mean square deviation is of order less than 0.005 (Fichman 1967) (Table 3). The relatively narrow refractive index distribution does not significantly affect the shape (angular dependence) of the scattering pattern in forward scattering, but can lead to measurable differences in absolute levels of scattered intensities.

The shape of living cells ranges from the nearly perfect sphere through ellipsoidal and rod-like forms to complex irregular forms (animal tissue cells). Nonspherical model studies indicate significant differences between spherical and nonspherical shapes (Latimer et al. 1978; Asano 1978). The scattering intensity maximum can occur at a polar angle larger than  $0^{\circ}$ , otherwise conserving the general tendency to scatter light preferentially into the small angle region. Depending on the particle orientation, extinction efficiencies can be one order higher than those of the spherical particles of equivalent volume (Fig. 4). The aspherical shape is expressed in the azimuthal angle dependence, also the light scattering pattern is dependent on the polarization of the incident wave. The scattering pattern strongly depends on the particle orientation (Fig. 5).

For cell suspension, the process of averaging over all possible particle orientations, summing over all possible shapes, and integrating over particle sizes yields a very smooth angular intensity dependence with only few basic differences between spherical and nonspherical particles. Scattering in the forward peak by nonspherical particles is very similar to that obtained from spheres (Zerull et al. 1977). However, scattering at larger angles, and in particular backscattering, can be significantly different. The extinction cross-section of a randomly oriented nonspherical particle is larger than the extinction crosssection of a spherical particle of equal volume (for sizes  $\gg$  wavelength), which leads to oversizing (Uličný 1985).



Figure 4. Extinction efficiency: asphericity effects. The arrow shows the incident light direction. Top: prolate spheroids; bottom: oblate spheroids. (Reproduced from Asano (1979)).

Biological particles themselves are inhomogeneous. The cytoplasm of living cells usually contains large numbers of discrete microscopic or submicroscopic structures. Generally, these structures differ from the rest of the cell in refractive indices



Figure 5. Aspherical particles. Orientation effects on the angular scattering pattern. Intensity vs.  $\vartheta$  dependence. The arrows show the incident light direction. The rotational axis of the prolate ellipsoid lies in the scattering plane at angle 0° (solid line) and 45° (dashed line) to the incident light direction. The particle is illuminated by unpolarized light. (Reproduced from Asano (1979)).

and as such contribute to the total scattering. Although not easily accountable by light scattering theories, such internal structures can lead to measurable effects especially at larger angles. From experimental work it has been known that the internal structures contributions are detectable in the light scattering pattern (Brunstig and Mullaney 1974; Cram and Brunstig 1973).

With the exception of some special cases of cells containing pigment grains, chlorophyll, haemoglobin or other absorbing substances, living cells absorb visible light in negligible amount, so that the imaginary part of the refractive index can be put zero in most cases. Except for the absorption bands the refractive index varies with the wavelength very slowly (Cauchy formula).

It is not surprising that the light scattering patterns obtained in real experiments exhibit an angular dependence different from those based on very simple models, especially at larger angles (Bateman 1968) Generally speaking, each of the cell type/illumination combinations can lead to an experimentally distinguishable pattern (Table 4).

It is clear that even for a monoculture cell suspension relatively wide distributions of sizes, shapes and refractive indexes occur, so that the scattering pattern observed very seldom exhibits angular structure. The random orientation of cells

Single particle parameter
 General size (volume)
Refractive index
Shape
Orientation to the incident beam
Polarization of incident light
Wavelength
Internal structure

Table 4. Possible variables forming light scattering properties.

in the suspension effectively washes out the structure even with nearly identical (aspherical) particles (Kreid et al. 1973). Information obtained can be treated as "effective" size, refractive index, shape, etc. only for narrow distributions. However, even highly averaged light scattering pattern reflects the minute changes in the state of a cell suspension (Latimer 1979). In general, quantitative interpretation of such changes is possible only by adopting some simplifications about the mechanism of the changes.

A simplified situation occurs when single-cell scattering can be observed. Sphere-like cell scattering can be interpreted without significant problems using the Mie theory (Tycko et al.1985). For aspherical particles proper orientation must be maintained in order to obtain reproducible results. In flow systems the partial orientation can be reached by the proper nozzle design (Kachel et al. 1977). Numerical studies based on the physical optics approximation (Ravey 1988) suggest that the approximate shape of a particle can be obtained as the inverted ratio of the angular position of the intensity minima measured in the two principal planes. The size of a particle cannot be obtained in such a straightforward manner, because this parameter depends on the refractive index.

### **Experimental techniques**

In general, light scattering measurements can be divided into two basic groups, extinction (transmission) measurements and angular dependence measurements. The conceptual scheme of the apparatus for light scattering angular measurements is presented in Fig. 7. This type of apparatus is called the polar nephelometer.

#### Light sources

At present, almost exclusive light sources used in scattering experiments are lasers. The excellent collimation of the laser beam together with the monochromacity and



Figure 7. Principle of extinction measurements. Field stop S prevents the scattered light from being measured.

high intensity have opened up new experimental possibilities, i.e. measurements of the single particle scattering. Among the most often used lasers are HeNe, HeCd and ion argon lasers. In the near future infrared laser diodes may become used instead of visible lasers; they provide a worse structural resolution and geometrical characteristics of the beam, but are more compact, reliable and in perspective more available.

Using lasers as light source poses the question of the influence of inhomogeneous illumination on the particle scattering. The assumption of a homogeneous illumination is acceptable only when the particle is small compared to the beam width. For spherical particles solutions were found for illumination by a converging wave (Chew et al. 1977), and by a gaussian beam approaching  $TEM_{00}$  mode of lasers (Gouesbeth et al. 1988).

Often, linearly focused beams are used, especially for measurements combined with TOF (time of flight) and the laser scanning technique (Schrader and Eisert 1986). This is possible only for particle diameters substantially larger than the halfwidth of the beam waist.

### Sample preparation.

The principal requirement for the sample preparation is a high purity of the sample, i.e. the absence of other particles in the scattering volume, especially larger than those investigated. The sample must be prepared in a clear, dust-free environment using clean solutions, carrying media and chemicals. The usual treatment includes redistillations, centrifugation and filtration through membrane filters (Hofer et al. 1989).

Another experimental obstacle represent the occurence of particle aggregation and the presence of cellular fractions. The separation of single tissue cells can be obtained by the filtration techniques, enzymatically, and by sonification. Another problem during the sampling and treatment is the preservation of the relative frequencies of various cell types and prevention of morphological changes (Kaplow 1977).

## Detection technique

The light detection technique strongly depends on the type of measurement (static or flow), the size of the particles, the scattering angle interval and the required spatial resolution (scattered intensity level). In flow measurements it is usual to include peak sample and hold circuits for holding the light flash which typically lasts for about 1 microsecond, a time necessary for its digitization and storing in computer memory. Using lasers as light sources, the sensitivity of the detector is not as critical, even for single-particle scattering. Using photomultipliers, light scattered by a 0.05 micrometer particle can be detected (Bouland and Madelaine 1988). Also, special hardware solutions have been offered able to measure, store and treat several tens of independent light scattering parameters in real time at rates up to about 30,000 single cells per second. Such a high throughput requires the use of customized electronics with high-speed A/D converters, hardware dividers and multipliers (Kachel et al. 1981), intensively used DMA channels for communication with computer memory and "hardwired" logic for specialized treatment (Bartholdi et al. 1980). Spatial resolution which is sometimes required can be obtained by using optical fibers or densely spaced diode arrays or CCD arrays. Photomultipliers working in the analog pulse mode are suitable when the number of parameters measured are small. Short light pulses converted to current pulses are preamplified and processed by peak-sense-and-hold circuits with subsequent A/D conversion. Because of large dynamical range of possible values, it is suitable to use logarithmic amplifiers. An alternative to the photomultiplier represents PIN photodiode and/or diode arrays. Solid state detectors (photodiodes and their arrays, CCD elements, avalanche photodiodes, PIN diodes) have advantages in a more compact and reliable design, uniformity of the response, large dynamic range of linearity and they being resistant to the overloading. Their disadvantage is a reduced sensitivity compared to PMT. Special diode arrays (Recognition Systems Inc.) were developed for light scattering experiments with the entire light sensitive field being divided into two semicircular areas; semicircular segments allow to measure the radial distribution of the scattered intensity in small angle region integrated over angle  $\varphi$ ; radial segments allow to measure the  $\varphi$  dependence indicating particle asymmetry. With the growing radius of the rings their area also grows to account for the fall of the intensity scattered at larger angles.

# **Applications of LMLS in biology**

The main advantages of the LMLS methods are the speed and the relative simplicity of measurements, the nondestructive character of the interaction, and the remarkable sensitivity to various changes in the optical properties of the scatterer. On the other hand, inverting experimental data to quantities desired often is a non-trivial task. Extracting the data often requires extensive computations, which in general are only plausible for close-to-model particles . For biological particles with the known variability this means that the scattering patterns may be interpretable only in regions insensitive to small changes in parameters which are out of interest. The other possibility is to study numerous populations and to interpret only statistical parameters.

LMLS methods are suitable for *in situ* measurements. Non-destructive measurements allow the reuse of the investigated particles, or to simultaneously apply other investigation methods (the main advantage with biological particles). Light scattering measurements do not require any fixation or staining, so that biological function is not affected. Light scattering methods are especially suitable where high speeds or large statistics of events are required. With detailed knowledge of light scattering by individual particles, very detailed high precision data can in principle be obtained (Chylek et al. 1983). Unfortunately, but a very limitted class of biological objects fit these criteria (cell ghosts, protoplasts, sphered red blood cells).

Even with no theoretical information, empirical light scattering parameters (e.g. angular dependence of some elements of the scattering matrix) can serve as sensitive indicators of various cell states (Salzman et al. 1975; Loken et al. 1976; Eisert 1979).

## Particle sizing and counting

Particle sizing belongs to the most widespread use of LMLS. For not very large particles extinction methods are appropriate, for larger particles angular intensity methods are suitable.

Inversion problems for wide size distributions have lead to the development of methods able to measure single cell scattering.

Detailed description of the particle sizing methods can be found in chapter 7 of the Kerker's monograph (Kerker 1969), for more recent works see Gouesbeth and Gréhan (1988).



Figure 8. Scattering efficiency vs. size for homogeneous spherical particles. Size parameter means the circumference of the sphere in wavelenght units. Refractive index m = 1.05 corresponds to that measured for living cell suspended in water.

#### Extinction methods

Within a limited size range (the upper limit depending on the refractive index and the shape) particle extinction changes monotonically with the particle size. Thus, various methods for extracting particle size distributions from the extinction vs. wavelength dependence can be obtained. The optical scheme for the apparatus for extinction measurements is presented in Fig. 8. Note that as much light scattered under small angles as possible has to be excluded from the viewing angle of the light detector. Often omitted, this fact can substantially alter the extinction measurements, especially when large particles are present. For a sufficiently diluted sample, light intensity decreases as  $I/I_0 = \exp(-NC_{ext}l)$ , where N is the particle concentration,  $C_{ext}$  is the extinction cross-section, and l is the length of the scattering volume. Fig. 8 illustrates a typical  $Q_{ext}$  vs. size dependence for spherical homogeneous particles with a refractive index close to that for living cells. Inverting is sensitive to experimental noise, as well as to the uncertainty of the refractive index. Extinction crosssection is very sensitive to the shape of the particle, always resulting in larger apparent size estimated from extinction measurements for nonspherical particles. The extinction efficiency for non-spherical particles can differ from that for spherical ones by one order of magnitude. Assuming random orientation of non-spherical particles, even ideal monodisperse populations of identical particles will show finite width size distributions. This seeming multimodality must be considered when particles depart significantly from the spherical shape. Correction factors for the asphericity and orientation effects can most easily be obtained from of various approximations (RGD, anomalous diffraction, physical optics approximation). In general, inversion of the experimental data requires some apriori assumptions about the nature of the particles under investigation, otherwise no unique solution is possible or the computations take much more time than necessary.

#### Angular intensity methods

#### Low angle scattering methods

Most popular methods of particle characterization by light scattering are based on low angle scattering. The low angle light scattering is practically insensitive to variations of refractive index, reflecting only the size and the shape of the particle (Hodkinson 1966). In this region diffraction on the particle edges strongly predominates over the other mechanisms. Practically all scattered energy is concentrated in the narrow cone at low angles. Thus, forward scattering is considerably easier to measure and to interpret than scattering to larger angles.

#### Intensity ratio method

This method determines the particle size by means of the two intensities at various angles  $\vartheta$ . Both angles lie in the first diffraction lobe. When properly selected, this ratio is a linear function of the particle size over a limited size range (Jovin et al. 1976). The method is insensitive to variations of refractive index, particle concentration, variations in the intensity of the light source, and for cell suspensions, also to shape variations. The obtained effective size of the particle can be interpreted in terms of the particle with average area (Latimer 1979).

#### Sloan Arrington method

The Sloan Arrington method determines the effective size of the particles from the angular position of the peak of  $I\vartheta^2$  vs.  $\vartheta$ . Various approximations imply a

constant factor for several particle types close each to other (sphere 10 4, rod 8 4, disc 9 2, all in deg  $\mu$ m), so that for a narrow distribution average size can be determined (Livesey and Billmayer 1969) The method seems to be very robust to shape variations (Uhčný 1985)

# Time of flight and particle signature function

For particles much larger than wavelength light pulses can be detected arising when the particle edge crosses the linearly focused laser beam. Since the minimal focal spot is of the wavelength size order, this limits the particle size capable of producing two peaks, the spatial distance of which (particle diameter) can be determined (Tycko et al 1987). The characteristic time dependence of the initial light intensity is called particle signature function. The particle signature function carries, besides the information about the diameter, also information about the refractive index of the particle (Chevailher et al. 1988, Sharpless et al. 1977).

# Location of maxima/minima method

When the particle shape is known, it is possible to fit the light scattering pattern measured to the theoretical one The most suitable scattering curve, namely the angular position of scattering maxima and minima, determines the particle size with a high precision (Lettieri 1988) With a spherical particle, the number of extrema equals or is less than the size parameter (van de Hulst 1957)

# Mie resonance spectroscopy

Using tunable lasers with variable wavelengths it is possible to measure light intensity scattered by a fixed particle into fixed angle interval Upon continuously changing the wavelength, the particle size parameter also changes When the particle size parameter matches the resonance condition, a very sharp intensity peak can be measured (Ashkin and Dziedzic 1981) The wavelength separation of these peaks allows to determine the particle size with a high precision (Chylek et al 1983)

# Conclusions

Practical needs require more effective solutions to the light scattering problem for more universal particles Often, real world particles cannot be treated as being spherical and homogeneous, so that more adequate models are necessary Aspherical but regular particles are solvable by using various approximation methods (RGD, anomalous approximation, diffraction theory, physical optics approximation, ray optics) or boundary condition methods Boundary condition methods are in some cases solvable in closed form (sphere, spheroids, infinite cylinder), most cases however require numerical solution (Extended Boundary Condition Method: Barber and Yeh 1975). Irregular particles must be treated by statistical methods only, with averaging over large numbers of particles. Solutions to both classes of particles can be found in (Schuermann 1980).

The searching for new experimental parameters, capable of reflecting various particle parameters, go on. Natural candidates are elements of the scattering matrix. Based on computations for elliptical particles, quantity  $1 - (S_{22}/S_{11})$  has been proposed as a measure of particle asphericity (for spherical particles, this parameter is zero) (Barber and Hill 1988). The angular dependence of the scattering matrix elements may serve as a very useful empirical identification mark, especially for complex biological particles (Bickel et al. 1976).

Single-particle experiments include various particle traps allowing particle fixation in the scattering volume by means of electrodynamic forces (Eversole et al. 1988; Bottlinger and Umhauer 1988) and/or by a combination of hydrodynamic forces and light pressure (Park and Lee 1987). Another possibility is to use microwave analog experiments (Zerull et al. 1977).

Still another technique used is optical levitation. With this technique, particle is held in equilibrium by its gravitation and vertical radiation pressure. The vertical position is determined by energy density gradient of the laser beam (Ashkin 1970).

From the practical point of view, efforts to find more effective methods for inverting experimental data are important. Most efforts concentrate to the accounting for particle asphericity. The aim of the new methods is to obtain from experimental data information about the particle shape, internal structure and/or refractive index, in addition to size information. More detailed information about the issue can be found in (Gouesbeth and Gréhan 1988).

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