

# On the measurement of the effective refractive index of biological colloids

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**Abstract.** We address the problem of measuring the refractive index of biological materials. We limit our analysis to the case of colloids and consider the use of common automatic critical-angle refractometers to measure their effective refractive index. We provide rough guidelines to when these refractometers will incur in large errors and thus alternative techniques should be used.

## Introduction

The refractive index (RI) of materials can be used to obtain information on their chemical composition and microstructure. Many biological materials are optically inhomogeneous on a scale comparable to the wavelength of visible light, and thus they are optically turbid. Two questions arise when dealing with biological media: What does the effective refractive index of a turbid medium mean? and how can we measure it? To answer properly these questions we must recall that light propagation in turbid media is divided in two components: the coherent and the diffuse components. The coherent component corresponds to the average field whereas the diffuse component corresponds to the light fluctuations around its average. By average one usually means the configurational average of the system. Diffuse light gives a turbid appearance to the material. It has been shown by many researchers that the coherent wave in a turbid medium propagates with an effective wavevector. From it, one can assign an effective refractive index to the medium. This effective refractive index is in general complex and its imaginary part takes into account attenuation of the coherent wave due to absorption and scattering by the inhomogeneities.

## The effective refractive index in colloidal media

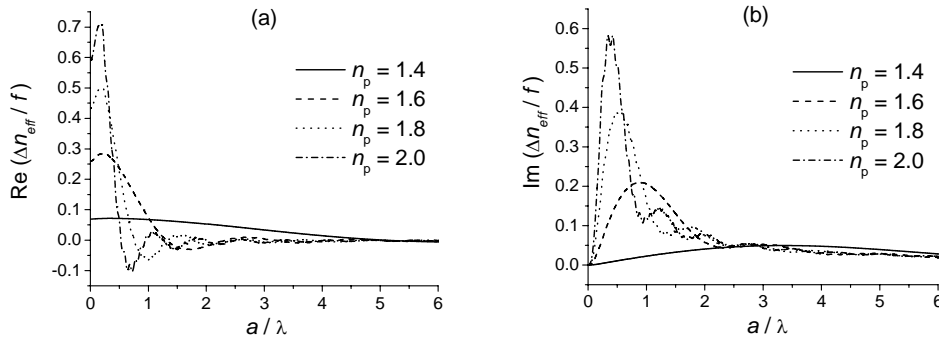
Let us now restrict our discussion to colloidal media. A colloid is defined as a suspension of particles in a homogeneous phase which we call the matrix. Many biological materials are in fact colloids. A clear example of a biological colloid is blood. But, many tissues can be modeled optically to some extent as colloidal media as well. In recent years, we have developed a Coherent-Scattering model for the coherent light propagation and reflection from dilute colloidal media and tested it experimentally [1,2]. We have found that in general we can not use without restraint the effective refractive index of a turbid colloid as we would do in the case of a homogeneous substance. For instance, when the size parameter of the colloidal particles is not small compared to one, the coherent reflection of light from a turbid colloidal medium is not properly described by the usual Fresnel reflection coefficients. This fact indicates that one may incur in sizable errors when measuring the effective refractive index of colloidal media by optical reflectance methods. More recently we have come to realize that the effective medium associated with a turbid colloidal medium has a non-local character, that is, it is spatially dispersive, and this explains why the Fresnel reflection coefficients are not readily applicable [3].

For dilute colloids, in which the volume fraction occupied by the particles ( $f$ ) is small compared to one, the effective refractive index can be well approximated by the so-called van de Hulst formula [3]. If we assume all colloidal particles have the same refractive index and are spherical, all with the same radius  $a$ , the van de Hulst formula takes the form,

$$\tilde{n}_{eff} \approx n_m \left( 1 + i \frac{3f}{2x_m^3} S(0) \right), \quad (1)$$

where  $n_m$  is the RI of the matrix medium which is assumed to be a transparent homogeneous substance,  $S(0)$  is the forward scattering amplitude of a colloidal particle of radius  $a$  immersed in the matrix and it is a function of the particles' RI ( $n_p$ ) and that of the matrix ( $n_m$ ),  $x_m$  is the so-called size parameter and is given by  $x_m = n_m k_0 a$  where  $k_0 = 2\pi/\lambda$  and  $\lambda$  is the wavelength in vacuum.

In Fig. 1 we plot the real and imaginary parts of the effective RI increment per unit volume-fraction  $f$  as a function of the relative particle size,  $a/\lambda$ , for a few examples. We assumed the particles are immersed in water ( $n_m = 1.33$ ) and considered particles with refractive index  $n_p = 1.4, 1.6, 1.8$  and  $2.0$ .



**Fig. 1.** Plot of the effective refractive index increment per unit of volume fraction  $f$ . (a) real, and (b) imaginary parts.

We can see from Fig. 1 that the RI increment due to the particles is at most of order  $f$ , but it is generally smaller. This means that in dilute colloids ( $f \ll 1$ ) the contribution of the particles to the RI is small. Nevertheless, relevant information of many materials is commonly found in small variations of the RI. Note that as the particle radius increases, both, the real and imaginary parts of the effective RI increment reach a maximum value and then decreases to zero. Note also that the real part decreases faster than the imaginary part. This means that for sufficiently large particles the effective refractive index becomes equal to the RI of the matrix, that is  $n_{eff} \rightarrow n_m$  even if it looks turbid.

### Measurement of the effective refractive index of colloidal media

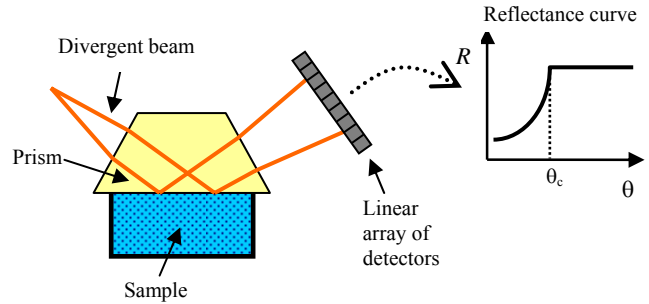
In some cases, we may be interested in measuring the absolute value of the effective refractive index. In some other cases, we may be interested in measuring accurately the refractive index increment due to the particles, that is  $\Delta n_{eff} = n_{eff} - n_m$ .

**Automatic critical-angle refractometers.** The most common way to measure the refractive index of liquid media is to use a critical-angle refractometer (C-AR) of the Abbe type. Modern automatic refractometers are based on measuring the reflectance ( $R$ ) versus angle-of-incidence ( $\theta_i$ ) curve around the critical angle ( $\theta_c$ ) in an internal reflection configuration. In fact, critical-angle measurements are being applied to biological tissue [4,5,6]. In a critical angle refractometer, the sample is placed in contact with the base of a prism of higher refractive index,  $n$ . The critical angle is defined by  $\theta_c = \sin^{-1}(n_2/n_1)$  where  $n_2$  and  $n_1$  are the refractive indices of the sample and of the prism respectively and are supposed to be real quantities. At the critical angle the reflectance curve has a sharp  $90^\circ$  bend to total-internal-reflection and its angle-derivative  $\partial R/\partial \theta_i$  is discontinuous. Modern automatic critical-angle refractometers reflect a divergent monochromatic beam from the base of a prism in contact with the sample and project the reflected light onto a linear array of photodetectors as

depicted in Fig. 2. The signals from the photodetector array generate directly a reflectance versus angle of incidence curve and the critical angle is located.

When the sample has a complex refractive index, that is, when  $n_2$  is not real, there is no longer a critical angle and the discontinuity on the derivative of the reflectance curve disappears. Nevertheless, when the imaginary part of  $n_2$  is not too large, a continuous but sharp bend of the reflectance curve is found and there is a clear maximum of the angle-derivative at the inflexion point of the reflectance curve ( $\theta_{\max}$ ). An automatic critical angle refractometer would locate the inflexion point as it would be a critical-angle for a transparent sample and would give the refractive index of the sample as  $\text{Re}(n_2) = n_1 \sin \theta_{\max}$ . This, would actually be a good approximation in the case of absorbing homogeneous substances as long as  $\text{Im}(n_2)$  is not too large [7].

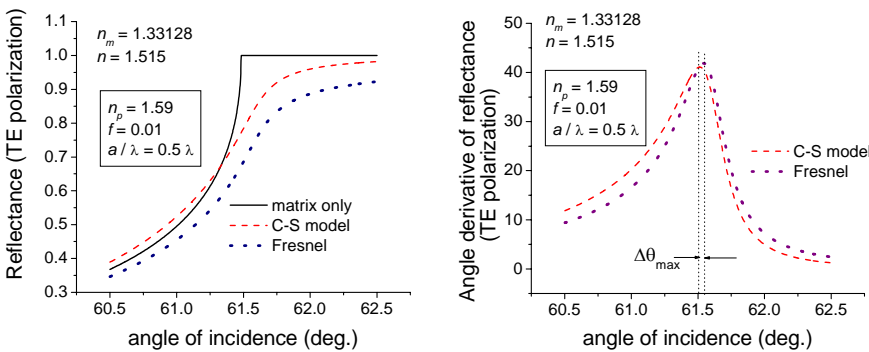
However, if a C-AR is used with turbid colloidal media errors will occur even for small values of the imaginary part of  $n_{\text{eff}}$ . To estimate these errors and give some guidelines to when, and when we can not use a C-AR with colloidal media, we have calculated the reflectance curves in an internal-reflection configuration, using the Coherent-Scattering (C-S) model developed in references [1,2] and compared them with the curves predicted by the Fresnel reflection coefficients.



**Fig. 2** Schematic illustration of a modern critical angle refractometer

In Fig. 3 we show an example considering a monodispersed dilute colloidal-medium. We can appreciate in Fig. 3a that the curves calculated with the C-S model and with the Fresnel reflection coefficients are similar in shape but are clearly separated from each other. By looking only to the shape of the curve we may actually think we are dealing with a homogeneous medium with a complex refractive index, and this would lead us to sizable errors. In Fig. 3b we plot the corresponding derivative curves ( $\partial R / \partial \theta_i$  vs  $\theta_i$ ). We can appreciate again the shape is similar but the location of the maximum,  $\theta_{\max}$ , is different in both curves.

**Errors with C-ARs.** We may define the relative error incurred by an automatic C-AR in obtaining the real part of the refractive index increment as,  $E = [1 - (\theta_{\max}^{\text{CS}} - \theta_c^{\text{matrix}}) / (\theta_{\max}^{\text{F}} - \theta_c^{\text{matrix}})]$ , where  $\theta_c^{\text{matrix}}$  is the critical angle with the matrix alone (that is, without the colloidal particles),  $\theta_{\max}^{\text{CS}}$  is the location of the inflexion point for the colloidal medium predicted by the Coherent-Scattering model, and  $\theta_{\max}^{\text{F}}$  is the location of the inflexion point predicted by the Fresnel reflection coefficients.

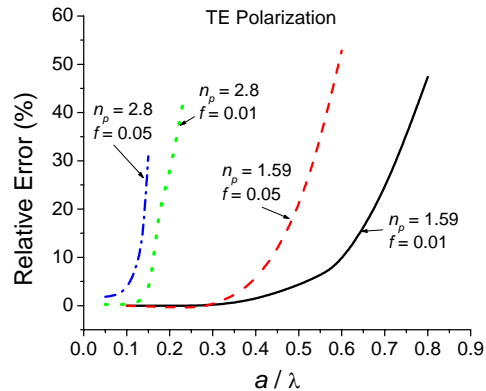


**Fig. 3.** (a) Reflectance and (b) derivative curves for TE polarization around the inflexion point in an internal reflection configuration with a colloid. The values of  $n_p$ ,  $n_m$ ,  $n$ ,  $a/\lambda$ , and  $f$  are shown in the inset.

In Fig. 4 we plot the relative errors as a function the relative size,  $a/\lambda$ , for colloidal-particles dispersed in water for two values of their RI ( $n_p = 1.59$  and  $2.8$ ) and for two values of the volume density ( $f = 1\%$  and  $5\%$ ). We can see from the graphs that for  $n_p = 1.59$  the relative error is below  $1\%$  for  $a/\lambda < 0.3$  up to  $f = 5\%$ , and for  $n_p = 2.8$  the relative error is below  $1\%$  only for  $a/\lambda < 0.1$  up to  $f = 1\%$ . The limits just quoted give us some rough guidelines to when it is safe to use a C-AR. Thus, as

the RI contrast in the colloid and the volume density increases, the size of the colloidal particles for which we could use an automatic C-AR to estimate  $\Delta n_{eff}$  and obtain correct results with reasonable accuracy decreases. Finally we may point out, that if particles are sufficiently large a C-AR would make a huge error in determining  $\Delta n_{eff}$ , but since this is very small for very large particles (see Fig. 1), the measurement will be a good approximation to  $n_m$ .

**Alternatives to measuring  $n_{eff}$ .** We could basically use a C-AR and interpret the reflectance curves with the Coherent-Scattering model. From a best fit of the model to the experimental curve we could obtain microscopic information on the colloidal particles, and then calculate the effective refractive index [2]. But there are two other options that avoid light reflection. One option consists of measuring the phase delay of the coherent wave upon propagation through a known distance through the colloidal medium. This can be done by standard interferometric techniques [8]. The second option is to use light refraction at the interface between a colloid and a transparent homogeneous medium. If proper care is taken, the RI increment due to the particles can be measured accurately and obtain physical information from it [9].



**Fig. 4.** Relative error versus  $a/\lambda$  for two values of  $n_p$  and  $f$ , and for  $n_m=1.33$  and  $n=1.515$ .

## Conclusions

The refractive index of a turbid material corresponds to the effective refractive index seen by the coherent wave. The imaginary part takes into account attenuation of the coherent wave due to scattering. This means that it can be non-zero even in the absence of optical absorption. The coherent reflection of light from a flat interface with a colloidal medium with particles of size parameter comparable to one or larger, do not follow the Fresnel reflection coefficient. This causes automatic critical-angle refractometers to measure erroneously the effective refractive index in many cases. In this case other methods based on light refraction or phase delay may be used. Finally when the size parameter of the colloidal particles is sufficiently small or sufficiently large, a C-AR will measure correctly the refractive index of the matrix medium. How small or how large should the particle be in this case will depend on their refractive index contrast.

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