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Noninvasive particle sizing using camera-based diffuse reflectance spectroscopy

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Diffuse reflectance measurements are useful for noninvasive inspection of optical properties such as reduced scattering and absorption coefficients. Spectroscopic analysis of these optical properties can be used for particle sizing. Systems based on optical fiber probes are commonly employed, but their low spatial resolution limits their validity ranges for the coefficients. To cover a wider range of coefficients, we use camera-based spectroscopic oblique incidence reflectometry. We develop a noninvasive technique for acquisition of apparent particle size distributions based on this approach. Our technique is validated using stable oil-in-water emulsions with a wide range of known particle size distributions. We also measure the apparent particle size distributions of complex dairy products. These results show that our tool, in contrast to those based on fiber probes, can deal with a range of optical properties wide enough to track apparent particle size distributions in a typical industrial process. © 2016 Optical Society of America

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1. INTRODUCTION

Particle size distribution of emulsions and other dispersions is a key parameter controlling their quality. Droplet size distribution affects properties such as stability, appearance, and viscosity. Due to its importance, it is very useful to noninvasively monitor changes in particle size distribution during processing. We propose to do this by measuring optical properties using an instrument for spectroscopic oblique incidence reflectometry. More specifically, we measure the reduced scattering coefficient \( \mu_s' \), which can also be computed from size distributions and refractive indices of particles using Lorenz-Mie theory [1, 2]. This enables us to construct an inverse method that finds information about particle size distribution from the measurements provided by a noninvasive optical instrument.

Fermentation of milk to produce yogurt is an example of a process where particle size measurements are important. The dispersion of casein micelles (colloidal protein particles) in milk is modified by the increasing acidity, caused by bacterial action. Starting from a standard milk product, such as low fat milk, a starter culture of lactic acid bacteria is added and, over time, these bacteria convert lactose into lactic acid. This acidification destabilizes the casein micelles, causing aggregation and cluster formation. We can think of it as modifying the particle size distribution of the casein micelles. Finally, the growing aggregates will form a gel—the milk has become yogurt [3]. The particle gel in yogurt scatters light more than the casein micelles in the original milk. The reduced scattering coefficient of a milk is around a factor of two larger after fermentation [4]. This means that the state of gelation (structure formation), or the apparent particle size distribution of a constituent, has an influence on the optical properties of an emulsion.

There are several ways to measure particle size distribution. However, if the sample is highly scattering, noninvasive measurement of particle size distribution is most easily accomplished using reflectance. The reduced scattering coefficient of a sample can be measured using diffuse reflectance [5]. By comparing measured and computed values, we can estimate the particle size distribution of a given sample using diffuse reflectance spectroscopy [6].

One of the challenges of using diffuse reflectance spectroscopy is that an estimate of the total incident flux is required [5, 6]. We avoid this problem by using oblique incidence reflectometry [7, 8], where the total incident flux is not required. This technique also exists in a spectroscopic version [9], which
has been used for measuring the particle size distribution of a concentrated titanium dioxide suspension [10] and skin [11]. The original technique for oblique incidence reflectometry [7] used a laser as source and a camera as sensor. To enable spectroscopic oblique incidence reflectometry, the following techniques [8–10] used optical fiber probes instead of a camera as sensor. While the fiber probes enable use of a spectrophotometer, they significantly lower the spatial resolution, which narrows the range of reduced scattering coefficients that the instrument is able to measure.

To use oblique incidence reflectometry for particle size measurement during processing, we need to measure a wider range of optical properties than the optical-fiber-based technique offers. We therefore return to using a camera, which has high spatial resolution, and obtain high spectral resolution by using a supercontinuum laser [12]. The instrument is illustrated in Figure 1, and the challenges in using a camera as the sensor were addressed in previous work [13]. The challenges were mostly in dealing with the noise and the speckle that appears with higher spatial resolution, but also in dealing with the low dynamic intensity range, lens distortion, and vignetting of the camera. With these problems solved, the instrument provides spectrally resolved measurement of the reduced scattering coefficient \( \mu' \) from 5 cm\(^{-1} \) or less to at least 70 cm\(^{-1} \). This validity range can be adjusted by adjusting the configuration, optical components, and camera resolution of the instrument. In this previous work [13], the optical properties were measured, but there was no investigation of inversion to obtain information about the apparent particle size distribution of the emulsions.

Particle sizing requires comparison to predicted optical properties. To compute optical properties from refractive indices and a particle size distribution, we use the approach described by Frisvad et al. [14]. Our fitting of the particle size distribution is based on an assumption of a low-parameter continuous distribution with an analytical expression (lognormal distribution, for example). We use the Nelder-Mead simplex search method [15] to fit the distribution parameters.

Our main contribution is to substantiate that the above-mentioned instrument and analysis technique are useful for noninvasive particle sizing. We do this by measuring the particle size distributions of optical samples with known particle size distributions. For these measurements we get very good agreement. We also measure the particle size distributions of dairy products and compare our measurements to invasive measurements obtained using a standard instrument, based on small angle light scattering (Mastersizer 3000, Malvern Instruments, UK). Finally, we discuss the differences between measurements obtained with these two techniques.

2. METHOD

The Lorenz-Mie theory [1, 2] provides a nonlinear model for computing the reduced scattering coefficient \( \mu' \) from particle size distributions. The model assumes that the particles are spherical and scatter light independently. We would like to invert this model to go from measured spectra of reduced scattering coefficients to particle size distributions. This is achieved by modifying size distribution parameters until the difference of measured and computed coefficients reaches a minimum. To make this practical, we must limit the search space by making assumptions with respect to the size distributions.

We first specify the model, where we have the following relation between the particle size distribution \( N(r) \) and the reduced scattering coefficient \( \mu' \) of a sample [16]:

\[
\mu'_r(\lambda) = \int_0^\infty (1 - g(r, \lambda)) C_s(r, \lambda) N(r) \, dr .
\]

In this relation, \( N(r) \, dr \) is the number density of particles with radius \( r \), while \( C_s \) and \( g \) are the scattering cross section and the asymmetry parameter of a particle of radius \( r \), respectively. Given refractive indices of the particles and the host medium at a wavelength \( \lambda \), we can compute the scattering cross section and the asymmetry parameter at the same wavelength using Lorenz-Mie theory [14].

Inversion of this model to obtain \( N \) is a highly underdetermined problem as \( r \) is arbitrary and so is the refractive index of each particle in principle. We therefore make assumptions about the size distributions and use \textit{a priori} knowledge about the material. We first assume that the emulsion consists of only one or two different particle types with known refractive indices. We also consider the refractive index of the host medium to be known. Next, we consider each size distribution to be of a kind that we can describe by a simple mathematical expression. Due to the turbulent break-up of interfaces in an emulsification process, emulsions and suspensions are very likely to have a lognormal kind that we can describe by a simple mathematical expression. Lognormal distributions have been measured in milk [18, 19] and are also found in other classes of colloids, such as powders [20] and aerosols [21].

Due to our initial assumption of spherical particles, we have the following relation between the particle size distribution and the volume fraction \( v \) of a constituent:

\[
v = \frac{4\pi}{3} \int_0^\infty r^3 N(r) \, dr ,
\]

where \( r^3 N(r) \) is sometimes referred to as the particle volume frequency. The volume fraction is useful as we often have approximate \textit{a priori} knowledge about it. We often know the weight percent (wt. %) of different constituents from the production procedure, for example. Densities, however, may not be known and, in some cases, all the substance may not form colloidal particles. We can thus use wt. % as a reasonable initial guess for \( v \), but we should allow changes in \( v \).

Choosing a lognormal volume frequency distribution with mean value \( \mu \) and standard deviation \( \sigma \), we have

\[
r^3 N(r) = \frac{1}{r^3 \beta \sqrt{2\pi}} e^{-\frac{1}{2} \left( \frac{\ln r - \mu}{\sigma} \right)^2} ,
\]
We prepared six oil-in-water emulsions with different particle size distributions. They were stabilized by gum arabic and had an oil fraction of 13.1 volume percent. The refractive index of the oil was 1.50 with only slight variation. Samples with scattering properties in our range of interest, were obtained by diluting with water to give an oil content of 2–5 volume percent. Figure 2 provides the reference particle size distributions of these emulsions as measured using a Mastersizer 3000 (Malvern Instruments, UK). The sample with the smallest and the sample with the largest particles are bimodal, the other four samples are unimodal.

We also tested our method on the commercial milk and yogurt products listed in Table 1. The chosen products span the range of optical properties typically appearing in a milk fermentation process. We used a Mastersizer 3000 to measure the particle size distributions. We did this for the milk products, but not for the yogurt products as the necessary sample stirring and dilution would destroy the gel network [22]. The measured particle size distributions are in Figure 3. For each distribution, the mode in the 10 to 100 nm range is due to protein particles (casein micelles) and the mode(s) around 1 μm (and above) is due to fat particles (globules). However, it is important to keep in mind that the Mastersizer cannot allow for particles with different refractive indices. This means that we cannot think of these measurements as ground truth reference distributions. Nevertheless, it is still interesting to compare our results with the Mastersizer measurements.

The instrument we used for spectral measurement of the reduced scattering properties is as described in previous work [13] (see Figure 1). To measure apparent particle size distributions, we compare to Lorenz-Mie theory [14]. In our calculation of the spectral reduced optical properties, we assume that the oil droplets in the emulsions have a refractive index 1.50. For the

### Table 1. Fat and Protein Content of the Homogenized Dairy Products for which we Measure Apparent Particle Size Distributions

<table>
<thead>
<tr>
<th>Type</th>
<th>Product name (g/100 g)</th>
<th>Fat (g/100 g)</th>
<th>Protein (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat milk</td>
<td>Arla Lærkevang Minimælk</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Reduced fat milk</td>
<td>Arla Lærkevang Letmælk</td>
<td>1.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Whole milk</td>
<td>Arla Lærkevang Sødmælk</td>
<td>3.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Low fat plain yogurt</td>
<td>Arla A38 naturel</td>
<td>0.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Reduced fat plain yogurt</td>
<td>Arla A38 naturel</td>
<td>1.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Whole milk plain yogurt</td>
<td>Arla A38 naturel</td>
<td>3.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

3. MATERIALS

We prepared six oil-in-water emulsions with different particle size distributions. With these equations (1–4), we use piecewise linear integration to compute the spectrum of reduced scattering coefficients that corresponds to a given set of parameters \( v, \mu, \) and \( \sigma \).

Having means to measure a spectrum of reduced scattering coefficients [13], we use a fitting algorithm to choose the parameters \( v, \mu, \) and \( \sigma \) such that the mean residual between measured and computed spectra is minimized. We use the Nelder-Mead simplex search method [15] as it is a derivative-free fitting algorithm. To run this direct search algorithm, we need initial guesses for all three parameters.

If a sample is composed of several particle types or multiple modes, each particle type \( p \) or mode will have its own set of distribution parameters \( (v_p, \mu_p, \sigma_p) \). This means that a material such as milk, with two single mode particle types (fat and protein), will require the fitting algorithm to find six parameters instead of three.

Spherical particles and independent scattering are reasonable assumptions in a medium like milk, as the fat and protein particles are reasonably spherical and the volume fractions of these inclusions are usually well below 10%. However, in a particle gel like yogurt, particle-particle interactions occur. This leads to diffraction effects that change the integral (1). Thus, when applying our particle sizing method to structured materials like yogurt, we will be measuring apparent particle size distributions. We cannot use such measurements to say much with respect to the actual particle size distribution. However, we can detect structural changes during processing by detecting changes in the apparent particle size distribution, which is very useful.

![Fig. 2. Reference particle size distributions of oil-in-water emulsions (A–F) as measured using a Mastersizer 3000.](image)

![Fig. 3. Particle size distributions of the commercial milk products listed in Table 1 as measured using a Mastersizer 3000.](image)
milk and yogurt samples, we assume milk fat particles of refractive index 1.462 [23] and casein particles of refractive index 1.503 [24]. The initial guesses used for the Nelder-Mead simplex search algorithm are in Table 2.

### Table 2. Initial Guesses Used for the Nelder-Mead Simplex Search Algorithm

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mode</th>
<th>( \nu )</th>
<th>( \mu (\mu m) )</th>
<th>( \sigma (\mu m) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, C–E</td>
<td>oil</td>
<td>0.03</td>
<td>0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>B</td>
<td>oil 1</td>
<td>0.03</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>oil 2</td>
<td>0.002</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>F</td>
<td>oil 1</td>
<td>0.002</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>oil 2</td>
<td>0.03</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>dairy product</td>
<td>casein</td>
<td>0.04</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>fat</td>
<td>( b )</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*These initial guesses were based on our approximate *a priori* knowledge of the materials.

*Here we use the fat fractions listed as wt. % in Table 1.

4. RESULTS

Using the oblique incidence reflectometry instrument (Figure 1), we acquired spectral measurements of reduced scattering coefficients, \( \mu'_r(\lambda) \) for \( \lambda \in [465 \text{ nm}, 945 \text{ nm}] \), five or six times per sample type. In rare cases, we discarded a full spectrum of measurements due to extreme noise or a large number of missing data points. We experienced no such problems when measuring the properties of the three milk types.

The quality of measurements and fits are quite similar for all the emulsions. Figure 4 shows two examples. The measured reduced scattering coefficients of the emulsions consistently exhibit oscillations. Their presence may suggest that the emulsions are less polydisperse than the Mastersizer results and our results suggest. The larger bumps in the near infrared part of the spectrum may be due to cross-talk between reduced scattering and absorption when these properties are inferred from the raw camera data.

Examples of measurements and fits for the dairy products are in Figure 5. Slight oscillations are also present in these measurements, but we observe no unexpected bumps in the near infrared. As expected (due to limitations of the instrument), noise increases with an increase in the reduced scattering coefficient. When measuring the optical properties of whole milk yogurt with reduced scattering coefficients of several hundreds per centimeter, we are reaching the limit of the instrument.

We used our inversion method to estimate the size distribution parameters (volume fraction \( \nu \), distribution mean \( \mu \), and standard deviation \( \sigma \)) for each individual fitted spectrum and then took the mean values. The resulting parameters are listed in Table 3. These results are somewhat influenced by the initial guesses in Table 2, which means that our method (in its current form) is not well suited for absolute measurements. As mentioned before, we need *a priori* knowledge of the particle composition (volume fractions, number of modes, and a rough idea of the mean and width of each mode). On the other hand, if our method is calibrated, it is very useful for monitoring changes in the apparent particle size distributions.

Figure 6 compares our emulsion results to the distributions measured by the Mastersizer 3000. We believe that these samples are not changed by either dilution or the stirring during measurement. So, in this case, we consider the Mastersizer results as reference results.

It is important to note that while the initial guesses are unchanged for several different samples, the size distribution parameters returned by the inversion method are quite different. Especially in the case of milk versus yogurt. This means that we

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**Fig. 4.** Measured reduced scattering coefficients for samples A and B (oil-in-water emulsions) and average fits. For samples with two modes, the red and blue lines show the part of the fit due to each mode.

**Fig. 5.** Measured reduced scattering coefficients for reduced fat milk and reduced fat plain yogurt and average fits. The red and blue lines show the part of the fit due to each of the two different particle types.
Fig. 6. Particle size distributions of samples A–F (oil-in-water emulsions) and Mastersizer measurements (references).

Table 3. Fitted Particle Size Distribution Parameters

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mode</th>
<th>( \nu ) (( \mu m ))</th>
<th>( \mu ) (( \mu m ))</th>
<th>( \sigma ) (( \mu m ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>oil</td>
<td>0.017</td>
<td>0.15</td>
<td>0.067</td>
</tr>
<tr>
<td>B</td>
<td>oil 1</td>
<td>0.013</td>
<td>0.14</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>oil 2</td>
<td>0.0020</td>
<td>0.93</td>
<td>0.32</td>
</tr>
<tr>
<td>C</td>
<td>oil</td>
<td>0.021</td>
<td>0.29</td>
<td>0.060</td>
</tr>
<tr>
<td>D</td>
<td>oil</td>
<td>0.014</td>
<td>0.17</td>
<td>0.073</td>
</tr>
<tr>
<td>E</td>
<td>oil 1</td>
<td>0.0025</td>
<td>0.043</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>oil 2</td>
<td>0.014</td>
<td>0.68</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>low fat milk</td>
<td>casein</td>
<td>0.026</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat</td>
<td>0.0017</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>reduced fat milk</td>
<td>casein</td>
<td>0.025</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat</td>
<td>0.012</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>whole milk</td>
<td>casein</td>
<td>0.023</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat</td>
<td>0.024</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>low fat yogurt</td>
<td>casein</td>
<td>0.049</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat</td>
<td>0.0047</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>reduced fat yogurt</td>
<td>casein</td>
<td>0.031</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat</td>
<td>0.023</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>whole milk yogurt</td>
<td>casein</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat</td>
<td>0.075</td>
<td>0.071</td>
</tr>
</tbody>
</table>

are not simply getting back what we gave as input. Considering the casein volume fractions in Table 3 for the three milk types, it is interesting to note that these values are quite close to the expected value of 0.025 (or 0.024 in the case of whole milk). One can calculate this expected value from the densities of the protein and the milk host as well as the percentage of the protein content that typically exists as casein micelles in the milk [14].

To investigate the sensitivity of our inversion method, we ran a large number of trials with different initial guesses and mapped out the local minima. In this experiment, we found that the method is independent of initial guesses if we know the volume fractions in advance and constrain the standard deviation to be at least 30% of the mean, that is, if we require a coefficient of variation \( c_v = \sigma / \mu \) greater than 0.3. If the method is left as is, a 10% change in the initial guess of a volume fraction can lead to changes in the results by a factor 2 or 3, but not changes by an order of magnitude. The method is most sensitive to changes in volume fractions. Luckily, this is also the parameter that we have better knowledge of in advance.

Mean values and coefficients of variation of the lognormal size distribution of milk fat globules have been measured for homogenized milk using a spectroturbidimetric method [18]. In comparison to these measurements, our mean values are about a factor two larger, but our coefficients of variation are similar. In fact, these measurements indicate that a lower homogenization pressure was used for our low fat and reduced fat milks compared to our whole milk. This is likely as the larger fat parti-
cules are skimmed from these products before homogenization, but remain in the unhomogenized whole milk, which then may require a higher homogenization pressure.

Mean values and $\beta$ of the size distribution of casein micelles have been measured in natural cow’s milk using dynamic light scattering [25]. Our mean value for whole milk (96 nm) is in the middle of the interval of means from 77 to 115 nm that they measured in the milks of individual cows. Our $\beta$-value for whole milk (0.38 by insertion in Eq. (4)) is also within the interval from 0.27 to 0.41 that they measured.

Figure 7 shows the particle size distributions that we found for the three milk products. These can be compared directly to the measurements presented in Figure 3. In this case, we do not consider the Mastersizer results to be reference results as the Mastersizer does not allow for particles with different refractive indices. The Mastersizer finds very similar size distributions for all three types of milk. The main difference is the increase in the amount of fat which raises the mode with the larger particles while lowering the mode of the smaller particles. The latter happens as the total amount of particle content increases while the protein content stays more or less unchanged. In our results, it is interesting to note that the protein mode moves with increasing fat content toward larger apparent sizes of the casein micelles. This might be error, but could also be due to the recently discovered adsorption of the smallest casein particles onto the surfaces of the fat globules [26]. We may conjecture that a larger fat content can disguise the smaller casein particles by adsorption, whereas the stirring and dilution necessary for the Mastersizer measurements perhaps breaks this effect.

Finally, Figure 8 compares our results for the yogurt samples to our results for milk samples of the same fat content. It is interesting to note how the apparent size of the casein micelles increases due to the gel structure formation and the apparent size of the fat globules decreases. These effects are due to interference effects as the particles sit closer together in clusters. In addition, the fat globules adsorb onto the casein network, which makes it harder to distinguish them from the protein in a light scattering measurement like ours.

5. DISCUSSION AND CONCLUSION

We successfully estimated particle size distributions of oil-in-water emulsions and dairy products using a noninvasive technique. This means that we avoid sample preparation and dilution, which makes the technique suitable for inline process control and enables measurement of apparent size distributions for colloidal networks. Our approach is to use wavelength-resolved measurements of reduced scattering coefficients. We also compute these coefficients using Lorenz-Mie theory with refractive indices of host and emulsion and lognormal distributions of particles as input. Finally, we use Nelder-Mead simplex search to fit the parameters of the lognormal distributions so that predicted distributions come close to the measured references.

A key issue in the use of the Nelder-Mead simplex search method is that it deterministically finds a local minimum. This makes the method rather noise intolerant and quite sensitive to the initial guess. In this work, we rely on imperfect $a$ priori knowledge about the sample for which we are measuring the particle size distribution to set reasonable initial guesses. Another approach would be to manually (or randomly within user specified limits) pick a number of different initial guesses and choose the result with lowest local minimum out of those trials. The recently proposed stochastic Nelder-Mead simplex method [27] offers an algorithmic way of dealing with this issue.

We propose that a Mastersizer is used to obtain initial guesses. This would be the calibration of our method. Once initial guesses are in place, we find our noninvasive technique very suitable for monitoring food processes such as a milk fermentation.

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