

## Article

# Laser Scatterometric Device for Inline Measurement of Fat Percentage and the Concentration Level of Large-Scale Impurities in Milk

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**Abstract:** A compact laser scatterometric device for determining the fat percentage of milk filling a cylindrical tube has been designed. The device operates by detecting the angular distribution of the scattered radiation of a semiconductor laser using an axial array of photodiodes. We have experimentally found that the light-scattering indicatrix in cow milk has a monotonous dependence on milk fat content. The intensity at side- or forward-scattering angles normalized to the backscattering intensity proves to be a reliable, informative parameter. A polynomial approximation for the calibration curve of fat percentage versus normalized scattering intensity is constructed to enable fat content measurements in the fairly wide range of ~0.01–10%. Furthermore, the intensity at forward scattering angles responds to the presence of large-scale particles in milk. The device was tested in a laminar flow regime at milk flow rates up to 100 mL/s.

**Keywords:** laser sensors; milk quality control; milking systems; scatterometry



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## 1. Introduction

The efficiency of milk production is directly related to automated quality control, which is based on various sensing technologies. One of the important technological factors for the effective management of dairy farms is the monitoring of the composition of milk produced. A dairy farm is a fairly broad concept and includes both high-tech large farms with a high degree of automation, and small farms without milking robots and most automation systems. Whilst dairy farms use milking machines of different types and characteristics, all of them need a system to control the flow rate and composition of milk. Obtaining information on the quantitative content of milk components (fat, proteins, lactose, somatic cells, progesterone, amino acids, etc.) underlies the assessment of milk quality, as well as diagnosing the nutritional balance and clinical condition of cows [1,2]. In particular, the content of fat and protein is considered to be the main criterion that determines the market value of milk. The real-time control of milk composition is especially important for promptly responding to deviations in the parameters of the physiological state of animals and for the timely adjustment of diets when milk yields are reduced. Currently, there is no unified system compactly built into milk pipelines that provides a simultaneous measurement of both the flow rate and the component percentages of milk [3]. Most small farms only operate flow meters installed on the milking machines, whilst the analysis of milk quality is carried out by a stand-alone sample analyzer, or milk samples are sent for periodic testing to specialized laboratories. For high-precision analysis of the composition

of milk, such laboratories use expensive stationary instruments. Obviously, the efficiency of dairy production can be significantly increased with the use of inexpensive and compact devices for inline analysis of the milk composition during the milking process.

In accordance with the ICAR requirement to use control devices that do not lead to a significant pressure drop in the milk hose, when developing milk quality sensors for dairy farm equipment [4,5], optical diagnostic methods are primarily promising, since these methods can perform non-contact and non-destructive diagnostics with high sensitivity and speed [6–10].

The existing devices for online assessment of the content of the three main components in milk (fat, protein, and lactose) use spectral methods, for the most part, infrared (IR) spectroscopy [3]. However, the incorporation of IR transmission spectroscopy into milking systems has the disadvantage of requiring a thin layer of milk, which reduces milk pumping rates. IR reflection spectroscopy avoids this problem, but the accuracy of the analysis decreases due to the influence of diffuse scattering on the reflectance intensity. High-precision systems using Fourier transform infrared (FTIR) spectroscopy are very expensive and have larger dimensions overall. A more compact and less expensive alternative to FTIR spectrometers are commercially available near-infrared (NIR) analyzers [11], such as Afimilk analyzers [12], but these require specialized computer software. Light-scattering analyzers appear capable of further improving price–performance ratio. Some approaches to measuring the content of milk components based on light-scattering effects have been reported in [13–16]. The spatial distribution of diffuse transmission from a layer of milk which is a few millimeters thick is measured with a digital camera using three LEDs emitting at different wave lengths, and then analyzed by a regression model to determine the content of fat and protein [13,14]. The optical transmission and internal reflectance of milk, which are sensitive to the fat level due to diffuse scatter, can be measured using fiber optic technologies [15–17]. Judging by the technologies on offer on the milking equipment market, despite the availability of the principles for sensing the milk composition in the cited works, there are no commercial implementations of such sensors.

In our work, we propose a prototype sensor for in-line determination of the fat content in milk, which is configured in a cylindrical geometry for compatibility with milk lines. The sensor is based on measuring the angular distribution of the light scattered from milk flowing inside an optically transparent cylindrical glass tube using a laser diode operating at a visible wavelength together with an axial photodiode array. Apart from measuring the content of main milk components, the detection of large-scale impurities with a size > 10 microns, such as somatic cells, is also an urgent task. The proposed technique has the advantage that the same device indicates the presence of abnormality caused by some large-scale impurity particles simultaneously with the measurement of fat content. Our experiments show that starting from a certain concentration level, the presence of large impurities in milk produces a relative change in the intensity of forward scattering compared to pure milk. Thus, such a type of scatterometric sensor is also able to indicate the excess concentration of large impurities above a certain level. Therefore, other conditions being equal, our sensor can be calibrated to estimate the level of somatic cell count (SCC). This sensor is not intended to replace common methods of detecting abnormalities such as conductivity measurement, through the inspection of individual parts of the udder, but provides an additional probabilistic parameter for online monitoring of milk quality with an integrated farm management system. Once the sensor detects an abnormal level of SCC, other, more accurate methods, such as microscopy, should be used to analyze the number of somatic cells more reliably.

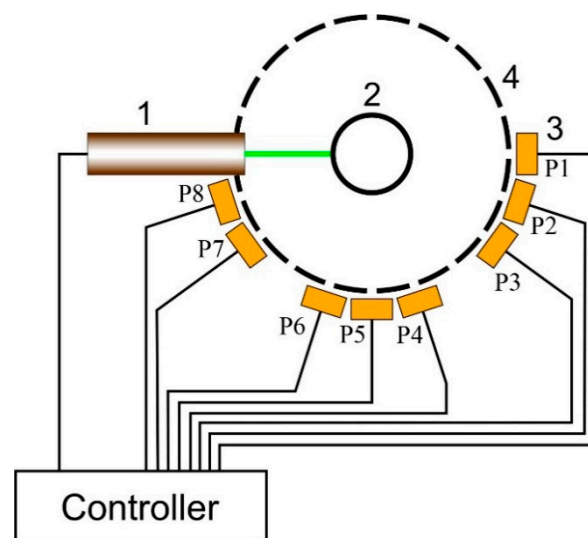
Our sensing technique has a fairly new methodological feature: in contrast to the common approach in turbidimetry, when the integral light attenuation by a turbid medium is measured, we compare the shapes of the angular distributions of the light scattered by milk with different fat percentages using dimensionless parameters, such as the ratio of the intensity at the side- or forward-scattering angles to the backscattering intensity.

Note that various scatterometric schemes have been previously developed for the characterization and control of dispersed media [18–22]. For example, the sensor designed in [18] for aerosol applications employs a laser emitting at 532 nm and axial photo-receiving geometry, as in our case. In our earlier work [23], we proposed a technique for the simultaneous determination of the percentage of fat and protein using Mueller matrix scatterometry, which requires a milk sampling procedure; the corresponding instrument layout is given in [22]. The novelty of the present work is that, firstly, our design of the scatterometric sensor is proposed specifically for a milk line, although the measurements of milk constituents are limited to fat; secondly, its implementation is simple and cheap.

## 2. Experimental Setup and Materials

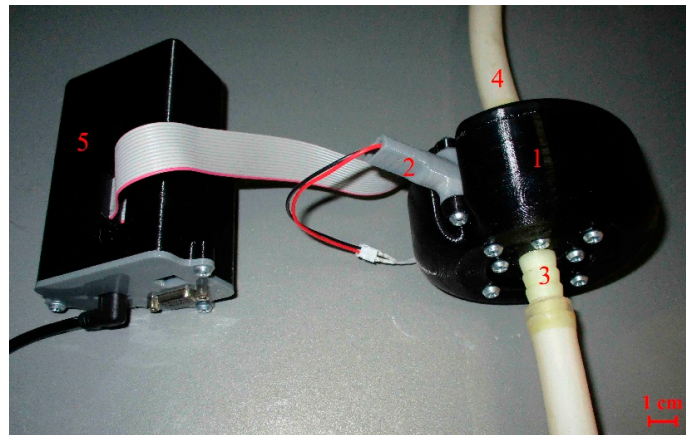
### 2.1. Device Design

We assembled an experimental setup in accordance with the optical scheme shown in Figure 1, which is a laser scatterometer sensing the fat content of milk. The radiation of a laser diode with a wavelength of 532 nm and a power of 5 mW undergoes diffuse scattering in milk flowing through a transparent cylindrical quartz tube (outer diameter 15 mm, wall thickness 1 mm). The ends of the tube are terminated with fittings for connecting milk hoses. The angular distribution of the scattered light intensity is recorded by a set of eight photodiodes arranged around a circle with an angular step of  $18^\circ$ . The photodiodes have a silicon photosensitive element of  $3 \times 3 \text{ mm}^2$  area with a plastic molded lens. The radius of this circle is chosen to correspond to the focus of an imaginary cylindrical lens, into which the tube would turn when filled with water. The photodiodes P1–P8 are divided by gaps into three groups covering the angular ranges of forward scatter ( $0^\circ, 18^\circ, 36^\circ$ ), side scatter ( $72^\circ, 90^\circ, 108^\circ$ ), and backscatter ( $144^\circ, 162^\circ$ ). Before reaching the photodiodes, the scattered light field is limited by rectangular diaphragms 1 mm wide, made in a plastic ring. The photodiodes operate in the photoconductive mode. Being amplified, the photodiode signals are digitized by an ADC ADS 1256 connected to an Arduino Leonardo microcontroller. The microcontroller switches on the laser module in the square-wave mode.



**Figure 1.** Optical scheme of the laser scatterometric sensor: (1) laser diode with a wavelength of 532 nm; (2) cylindrical tube of 15 mm diameter filled with flowing milk; (3) axial photodiode array P1–P8; (4) ring with rectangular diaphragms.

Figure 2 shows a photograph of a prototype device that implements the scheme in Figure 1.



**Figure 2.** Photograph of the prototype sensor shown schematically in Figure 1: (1) sensor enclosure; (2) laser diode holder; (3) herringbone fitting for 14 mm hose; (4) silicone tube connected to the pumping system; (5) controller unit.

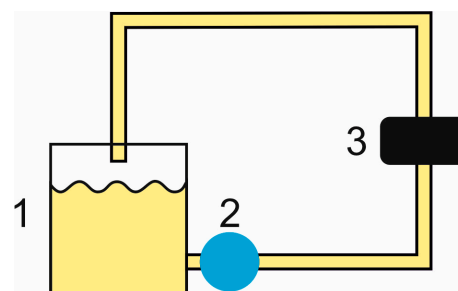
The plastic parts of the device were made using FDM 3D-printing technology.

## 2.2. Operation Algorithm

The laser diode illuminates in the mode of rectangular pulses with a duration of 5 sec. Sets of photocurrent samples from all 8 photodiode channels are repeated at a frequency of 1 sec<sup>-1</sup> and subjected to median filtering. Then, the median of dark current is subtracted from the median of the photocurrent during laser illumination. This photocurrent median difference is taken as the intensity value at the corresponding scattering angle. We have established a sensitive parameter—the ratio of the intensity at a certain angle of side scattering (72–108°) or forward scattering (0–36°) to the intensity at one of the backscattering angles (144°, 162°), which is functionally related to the fat content. The controller calculates the decimal logarithm of this ratio and then, using the calibration curve, outputs the corresponding value of fat content.

## 2.3. Pumping System

The pumping system that was used in the experiments with the laser scatterometric sensor in flow mode is shown in Figure 3.



**Figure 3.** Block diagram of the pumping system used for testing the sensor: (1) milk container; (2) centrifugal pump; (3) laser scatterometric sensor.

Milk samples were poured into a 2 L container. Centrifugal pump circulates milk in the system at a pumping speed of up to 100 mL/s pipe.

## 2.4. Milk Samples and Somatic Cells

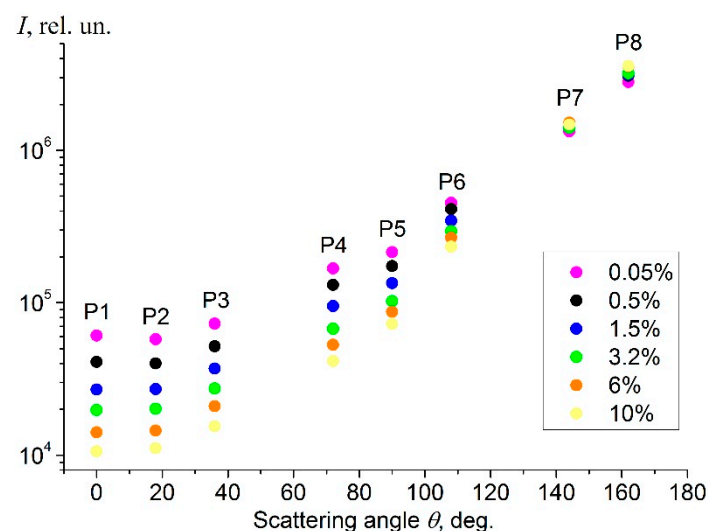
To calibrate the sensor, we carried out experiments with a series of commercially produced cow milk samples with nominal fat percentages of 0.05%, 0.5%, 1.5%, 3.2%, 6%, and 10% (milk cream) using UHT pasteurization and homogenization. The protein content was claimed to be 3.0 g per 100 g of product for all of these milk samples.

To study the sensor response to the presence of somatic cells in milk, we performed experiments with MCF-7 human breast carcinoma cells (ATCC no. HTB-22), of which the mean size is  $\sim 20 \mu\text{m}$ . These cells are a relevant model of somatic cells that appear in the milk of mammals, that of cows in particular, due to mastitis. For routine procedures with cells, the following analytical grade reagents were used: DMEM/F12 medium, fetal bovine serum (FBS), L-glutamine, penicillin–streptomycin, and 0.05% trypsin–EDTA solution (PanEco, Moscow, Russia). Cells were cultured in T-25 flasks (TPP, Trasadingen, Switzerland) at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  in an S-Bt Smart Biotherm incubator (Biosan, Riga, Latvia). The medium used was DMEM containing 10% FBS, 2 mM L-glutamine, 100 units/mL penicillin, and 100  $\mu\text{g}/\text{mL}$  streptomycin. Cells were cultured for 7 days to achieve maximum confluency ( $\sim 98\%$ ). Before the experiment, the cells were detached from the surface of the culture flask using a 0.05% trypsin–EDTA solution. Trypsin was inactivated with 10% FBS solution, cells were centrifuged at 350 g for 5 min, and then resuspended in DMEM medium without additives and counted using a counting chamber and a laboratory inverted microscope. Before being added to milk, the cell suspension was diluted so as to obtain the resulting concentration of cells in milk at a level of  $10^6 \text{ cm}^{-3}$ .

### 3. Results

#### 3.1. Scattering Indicatrix

We have recorded photodiode readings for milk samples with a nominal fat percentage of 0.05%, 0.5%, 1.5%, 3.2%, 6%, and 10% under normal conditions (temperature  $20^\circ\text{C}$ ). By calculating the photocurrent medians, we obtained the actual values of the scattered light intensity at the corresponding angles, where the photodiodes are located as shown in Figure 1. In this way, the sensor measures the angular distribution of the scattered light, which is called the scattering indicatrix. At the beginning, the scattering indicatrix for milk samples was measured in the static case, i.e., at zero flow velocity in the tube (Figure 4). Milk filling a tube with a diameter of  $\sim 1 \text{ cm}$  is a multiple scattering medium, since its optical thickness, estimated from the data on the scattering and absorption coefficients of milk in the visible range [24], exceeds 10, which means that the condition for the prevalence of multiple scattering is fulfilled [25,26]. Consequently, the scattering indicatrices shown in Figure 4 have a typical form for a multiple scattering medium; specifically, their values increase from the forward-scattering angles to the backscattering ones, which is consistent with the results of theoretical modeling for multiple scattering media [27].



**Figure 4.** Scattering indicatrix measured for milk samples with a nominal fat percentage of 0.05%, 0.5%, 1.5%, 3.2%, 6%, and 10% at zero flow velocity in the tube at a temperature of  $20^\circ\text{C}$ . Laser wavelength is 532 nm.

The graph (Figure 4) manifests a monotonic dependence of the scattering intensity,  $I$ , on the fat content at each scattering angle,  $\theta$ . It can also be seen that the scattering intensities at backscattering angles of  $144^\circ$  and  $162^\circ$  are practically the same for all fat percentages of the measured milk samples. This fact allows us to introduce a dimensionless parameter, which is a single-valued function of the fat percentage of milk and independent of the laser power, such as the ratio of the intensity at the side-scattering angles to the intensity, for example, at the backscattering angle  $144^\circ$ . Since it is more practical to handle the decimal logarithm of this ratio, the device operates with a scatterometric parameter, which we have defined as follows:

$$X_i = -\log\left(\frac{I(\theta_i)}{I(144^\circ)}\right), \theta_i = \{72^\circ, 90^\circ, 108^\circ\}. \tag{1}$$

Our experimental tests have shown that it is not advisable to use the forward-scattering angles  $\theta_i = \{0^\circ, 18^\circ, 36^\circ\}$  available in the sensor scheme (Figure 1) for measuring fat content due to the fact that the scattering intensity at these angles is more influenced by large impurity particles; this effect is further shown in Section 3.4. Thereby, fat content measurements are more stable at the side-scatter angles,  $\theta_i = \{72^\circ, 90^\circ, 108^\circ\}$ .

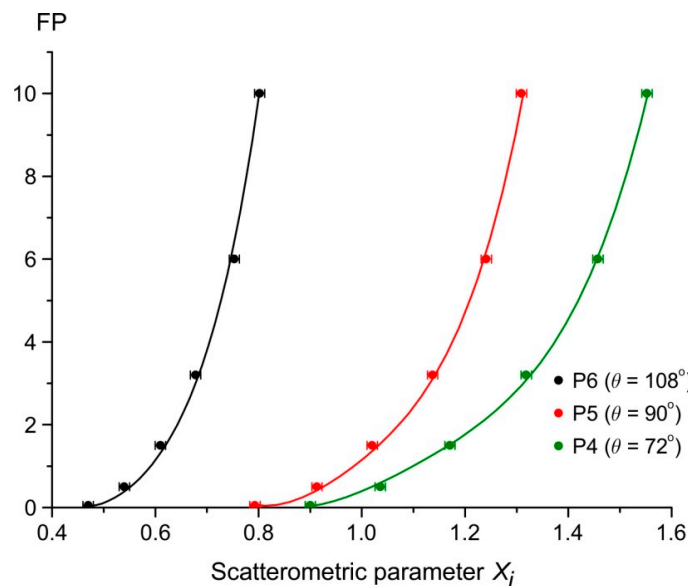
### 3.2. Calibration Curve of Fat Content

We have plotted the experimental dependence of fat content in milk,  $C$ , on the scatterometric parameter,  $X_i$ , at the side-scatter angles,  $\theta_i$  (Figure 5). The experimental points for each  $\theta_i$  are approximated by a polynomial of the fourth degree, which can serve as a calibration curve for converting photodiode signals into fat percentage:

$$C_i = A_{i0} + A_{i1}X_i + A_{i2}(X_i)^2 + A_{i3}(X_i)^3 + A_{i4}(X_i)^4, \quad i = 1 \dots N \tag{2}$$

where  $N = 3$  is the number of processed side-scatter channels that are provided by the corresponding photodiodes located at the angles  $\theta_i = \{72^\circ, 90^\circ, 108^\circ\}$ ;  $X_i$  is the scatterometric parameter of each channel. If averaging is used, then the fat percentage,  $FP$ , can be determined as:

$$FP = \sum_i C_i / N. \tag{3}$$



**Figure 5.** Dependence of fat percentage,  $FP$ , in milk on the scatterometric parameter,  $X_i$ , in the range 0.05–10% at zero flow velocity in the tube at a temperature of  $20^\circ\text{C}$ . Laser wavelength is 532 nm. The standard deviation of scatterometric parameter values is no more than 0.01.

The coefficients of  $A_{ij}$  determined from the experimental data using the least-squares method are presented in Table 1.

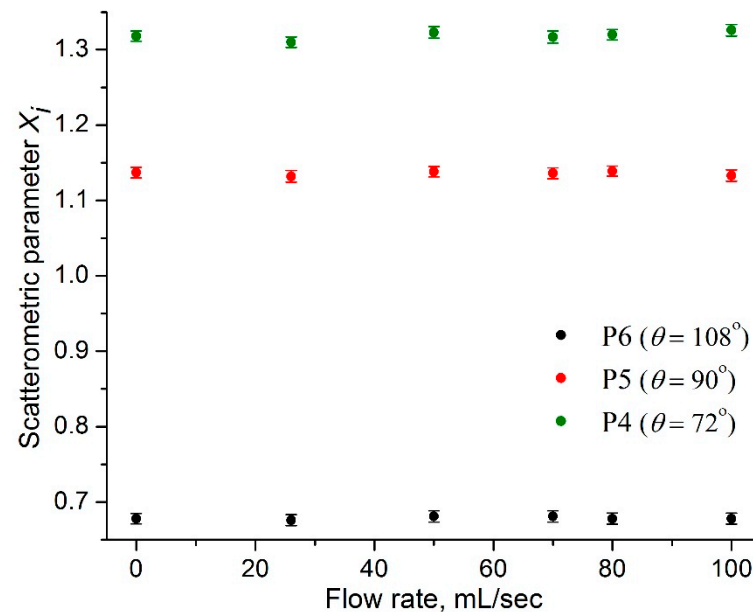
**Table 1.** Coefficients of  $A_{ij}$ .

| $(i, j)$                   | 0      | 1      | 2      | 3       | 4     |
|----------------------------|--------|--------|--------|---------|-------|
| 1 ( $\theta = 108^\circ$ ) | 57     | −397.6 | 1049.2 | −1264.5 | 602.8 |
| 2 ( $\theta = 90^\circ$ )  | 237.15 | −971.4 | 1488.2 | −1016.3 | 263.5 |
| 3 ( $\theta = 72^\circ$ )  | 137.6  | −507.6 | 695.2  | −421.6  | 96.8  |

As shown below, the calibration that was found in the static case (Figure 5) is also valid during milk flow, since the scatterometric parameter for the corresponding fat percentage remains unchanged with good accuracy at flow rates up to 100 mL/s.

### 3.3. Dependence of the Scatterometric Parameter on the Milk Flow Rate

We explored the dependence of the scatterometric parameter on the flow rate of milk in the tube in the range of 0–100 mL/s using the pumping system (Figure 3). Figure 6 shows the values of the scatterometric parameter  $X_i$  measured at three scattering angles  $\theta_i = \{72^\circ, 90^\circ, 108^\circ\}$  corresponding to photodiodes P4–P6 (Figure 1). At each flow rate, photosignals were measured for 3 min, and then the time average and standard deviation for the scatterometric parameter were calculated.

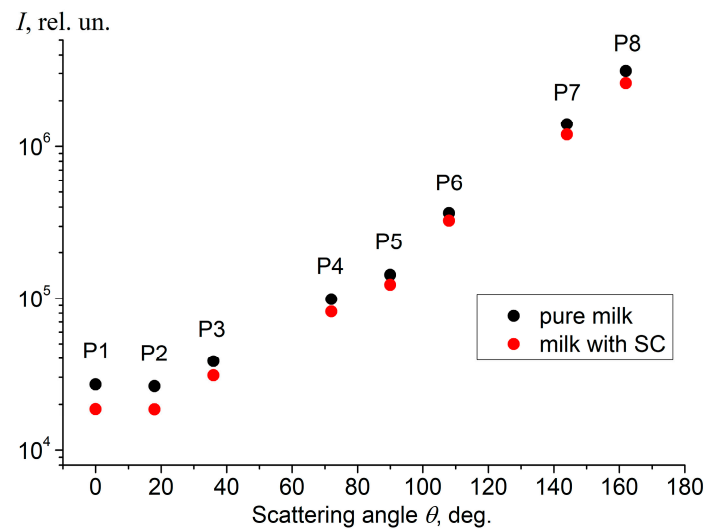


**Figure 6.** Dependence of the scatterometric parameter  $X_i$  on the milk flow rate for milk with a fat percentage 3.2% at a temperature of 20 °C.

The scatterometric parameter for other milk fat contents in the range of 0–10% is also practically constant for the milk flow rates up to 100 mL/s. This was to be expected in our case of laminar flow.

### 3.4. Influence of Large Particles on the Scattering Indicatrix of Milk

We tested the behavior of the scattering indicatrix of milk with large-scale impurities. To this end, we compared the measurements made for pure milk with a fat content of 1.5%, and the same milk where somatic cells were added with a final concentration of  $10^6 \text{ cm}^{-3}$  (Figure 7).



**Figure 7.** Scattering indicatrix measured for milk samples having a nominal fat percentage of 1.5%: without large-scale impurities (black circles), with the addition of somatic cells (SC) at a concentration level of  $10^6 \text{ cm}^{-3}$  (red circles). Milk flow velocity in the tube is zero. The temperature is  $20^\circ \text{C}$ . Laser wavelength is 532 nm.

Note that the addition of somatic cells has little effect on the intensity ratio of side and back scatter, that is, on the scatterometric parameter at the angles ( $72^\circ$ ,  $90^\circ$ ,  $108^\circ$ ). This is an essential result, which means that fat content measurements at these angles are not disturbed by the presence of somatic cells.

Intensity ratios analogous to (1) at forward-scattering angles of  $\theta_i = \{0^\circ, 18^\circ, 36^\circ\}$  can serve as indicators of the presence of large particles in milk. It makes sense to introduce the index of large particles,  $LPI$ , as a deviation of the values of these ratios from the normal case when there are no large inclusions in milk (pure milk):

$$LPI_i = \frac{X_i^{(pure)} - X_i}{X_i^{(pure)}}, \quad \theta_i = \{0^\circ, 18^\circ, 36^\circ\}. \quad (4)$$

It follows from the graph in Figure 7 that the addition of somatic cells to milk at a level of  $10^6 \text{ cm}^{-3}$  leads to an increase in  $LPI$  from zero to  $\sim 0.1$ .

#### 4. Discussion

We propose a simple laser scatterometer having a cylindrical photo-receiving geometry, which can serve as a prototype for a compact and inexpensive sensor of fat content and concentration levels of large particles in milk. Our experiments showed that the light-scattering indicatrix of a cow-milk-filled cylindrical tube has a monotonic dependency on the fat percentage of milk. The scatterometric parameter that was defined as the logarithm of the decrease in scattering intensity from backscatter to side scatter is highly sensitive to fat content of milk in the range 0–10%. In the studied range of milk flow rates 0–100 mL/sec, the scatterometric parameter can be considered constant with good accuracy, which ensures the stability of fat content measurements. We have studied the characteristic behavior of the scattering indicatrix of milk upon the addition of somatic cells. It turned out that the scatterometric parameter at the side-scatter angles is not perturbed, and thus the fat percentage is measured correctly even in the presence of somatic cells in milk. At the same time, the scatterometric parameter at the angles of direct scattering increases due to the presence of somatic cells, reaching a relative change of about 0.1 at a cell concentration of  $10^6 \text{ cm}^{-3}$ . However, it should be taken into account that the presence of large bubbles in the milk will also affect the forward-scatter intensity and, accordingly, will prevent the correct measurement of the level of somatic cells in milk.



It is more advantageous to use a CCD camera instead of the photodiode P1 located at  $0^\circ$  to provide greater angular resolution at small angles in forward scattering, and thus increase the sensitivity to the presence of large particles so that the level of their concentration can be more accurately determined.

An important design feature of the sensor is a ring holder that allows one to change the number and position of laser modules and photodiodes around the milk tube. In particular, a UV emitter can be installed in the sensor to excite the fluorescence of casein, which makes it possible to additionally determine the protein content.

## 5. Conclusions

Our experimental results have shown that using light-scattering measurements in cylindrical geometry, it is possible to develop a compact and fast sensor of the milk fat percentage and concentration level of large-scale impurities. The proposed sensor prototype based on laser diode emitting at 532 nm and silicon photodiodes has a relatively small size and weight, which allows it to be integrated into milking systems. Such a sensor is intended for milking parlors and AMS with milking robots. We expect this type of sensor to be an optimal solution for the measurement of fat content and the concentration of large-scale impurities in milk.

**Author Contributions:** Conceptualization, A.V.S.; methodology, A.V.S. and M.E.A.; software, M.E.A.; validation, N.V.S.; formal analysis, A.V.S.; investigation, A.V.S., D.N.I., N.V.S. and M.E.A.; resources, S.V.G.; data curation, N.V.S. and M.V.V.; writing—original draft preparation, A.V.S.; writing—review and editing, N.V.S., M.E.A., M.V.V. and S.V.G.; visualization, D.N.I.; supervision, A.V.S.; project administration, S.V.G.; funding acquisition, M.V.V. All authors have read and agreed to the published version of the manuscript.

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