Label-Free Somatic Cell Cytometry in Raw Milk Using Acoustophoresis

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Abstract
A microfluidic system for cell enumeration in raw milk was developed. The new method, preconditions the milk sample using acoustophoresis that removes lipid particles which are larger than a few micrometers. The acoustophoretic preprocessing eliminates the need for conventional sample preparation techniques, which include chemical solvents, cell labeling and centrifugation, and facilitates rapid cell enumeration using microscopy or coulter counter measurements. By introducing an acoustic standing wave with three pressure nodes in a microchannel at the same time as the milk sample is laminated to the channel center, lipids are acoustically driven to the closest pressure antinode at each side of the channel center and the cells in the milk sample are focused in the central pressure node. The extracted center fraction with cells becomes sufficiently clean from lipid vesicles to enable enumeration of somatic cells without any labeling step either by direct light microscopy or by coulter counting. Obtained lipid free milk fractions clearly revealed the cell fraction when analyzed by Coulter Counting. Cell counting as measured by a Coulter Counter after acoustophoretic lipid depletion aligned with the corresponding data obtained by reference measurements based on fluorescence staining and subsequent flow cytometer analysis.

Key terms
milk; acoustophoresis; ultrasound; somatic cell count; label free; microfluidic chip

CELL enumeration in biological field samples is often labor intense, requiring substantial sample pretreatment prior to analysis. As opposed to laboratory samples, there are typically a much larger number of non cellular particles in field samples that potentially interfere with cell counting instruments, regardless of the chosen technology platform. One example of such a sample is raw milk, where the number of somatic cells is an important indicator of the health status of the cow, specifically the mammary gland, which directly affects milk yield and quality (1). High somatic cell count (SCC, somatic cells/ml milk) is often a result of mastitis, an inflammation in the mammary gland. Common mastitis-causing organisms include Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli and different streptococci, typically transmitted during milking by contact to contaminated milking machines, hands, or equipment. As part of the inflammatory response the number of leukocytes increases, from ~10⁵ in an uninfected cow and ~3 × 10⁵ during subclinical mastitis to several millions during clinical mastitis, leading to an elevated SCC. Milk with an SCC of >4 × 10⁵ is deemed unfit for human consumption in the European Union while slightly higher levels are permitted in the United States (the State of Indiana has a 10⁶ SCC limit). Mastitis has adverse effects on total yield and quality of milk. Levels of casein and lactose decrease while levels of proteolytic enzymes from bacteria, lipase enzymes from the leukocytes and activated plasmin from blood all increase. More precisely, the result of the infection is lower milk and cheese yields, poor curding, bitter taste, and lower shelf life even after pasteurization. Thus it is important to rapidly identify infected animals to stop further spreading of the pathogens, treat the infected...
animals with minimal amounts of drugs, of which no residues are permitted in milk, and, in the end, maximize milk yield and quality (2,3).

Raw milk samples may be contaminated with medium sized objects such as grains of sand, parts of insects, or pieces of hair that can be easily removed with filters. However, even without these contaminants the multitude of lipid particles with sizes identical to the somatic cells (diameters 7–10 μm) will prevent the use of simple technologies like microscopy or coulter counting for cell enumeration. Instead, flow cytometry is commonly used, which alleviates the problem of erroneous cell enumeration. Flow cytometry, however, requires several steps of manual sample processing, including fluorescent staining of the cells in the sample and is commonly followed by centrifugation based wash steps prior to introduction in the cytometry instrument. Alternatively, the raw milk samples can be centrifuged and the precipitate resuspended and analyzed in a Coulter counter or by staining cells and manually counting cells in sample smears (4–10).

With the emerging possibilities of microfluidic systems, it is relevant to investigate compatible technologies that would allow integration of raw milk analysis on a single chip. Recently, it was demonstrated that lipid particles can be efficiently removed from raw milk samples using acoustophoresis to allow direct protein and lipid content analysis (11). In this report, we demonstrate how a combination of acoustophoresis and conventional Coulter Counting or phase contrast microscopy can be used to count the number of somatic cells in raw milk samples, using only an isotonic solution of NaCl as additional buffer.

**Materials and Methods**

**Microfluidic System**

The principle of using acoustic standing wave forces for particle manipulation has been well researched and in the past decade a transition to a chip integrated microfluidic format has gained attention (12–15). In acoustophoresis, the primary acoustic radiation force \( F_{\text{rad}} \) [Eq. (1)] is utilized to focus particles into either nodes or antinodes according to their acoustic contrast factor \( \Phi \) [Eq. (2)], which is dependent on the density and compressibility of the particles compared to the surrounding medium. Studies have shown that ultrasonic cell manipulation in microfluidic systems are gentle to cells (16,17). Dense particles (i.e., cells) tend to have a positive contrast factor in most commonly used flow media including water, and they, consequently, focus into the acoustic pressure nodes. Less-dense particles (i.e., lipids) have a negative contrast factor and thus focus into pressure antinodes. The primary acoustic radiation force scales linearly with an increased contrast factor, \( \Phi \), and the particle volume, \( a' \), and for a polystyrene particle of diameter 10 μm the highest achievable force to date is ~100 pN corresponding to an acoustophoretic velocity of 1,000 μm s\(^{-1}\).

\[
F_{\text{rad}} = 4\pi q^2 \Phi k_y E_0 \sin(2k_y \gamma)
\]  
(1)

Primary acoustic radiation force: \( F_{\text{rad}} = 4\pi q^2 \Phi k_y E_0 \sin(2k_y \gamma) \)

**Acoustic contrast factor:** \[
\Phi = \frac{\kappa_\omega - \kappa_o}{3\kappa_o} + \frac{\rho_o - \rho_a}{2\rho_p + \rho_o}
\]  
(2)

and \( a \) (≈10 μm) is the particle radius, \( \Phi \) is the acoustic contrast factor, \( k_y = 2\pi/\lambda \) is the wave vector, \( E_0 \) (≈100 J m\(^{-3}\)) is the acoustic energy density, \( y \) is the distance from the wall, \( \kappa_\omega \) (≈2.5 × 10\(^{-10}\) Pa\(^{-1}\)) is the isothermal compressibility of the particle, \( \kappa_o \) (≈5 × 10\(^{-10}\) Pa\(^{-1}\)) is the isothermal compressibility of the fluid, \( \rho_p \) (≈1,030 kg m\(^{-3}\)) is the density of the particle (cell) and \( \rho_o \) (≈1,000 kg m\(^{-3}\)) is the density of the fluid.

To enable efficient lipid removal from raw milk samples, an acoustically resonating microfluidic chip was fabricated in silicon with an anodically bonded glass lid (11,13). The acoustophoresis channel was 25-mm long, having a width of 1,125 μm and a depth of 150 μm, as shown in Figure 1. The channel was provided with trifurcation inlets and outlets, each with a separate fluidic connection for the center flow and a common fluidic connection for the two side flows.

Four syringe pumps (WPI sp210iwz, World Precision Instruments, Sarasota, FL) were used to control the flows, sheath inlet (Q\(_{\text{sheath}}\)), sample inlet (Q\(_{\text{milk}}\)), side outlet (Q\(_{\text{side}}\)), and center outlet (Q\(_{\text{center}}\)). Samples were collected using loop valves (V-451, IDEX Health and Science LLC, Oak Harbor, WA) with a sample volume of 100 μl. During sampling a volume corresponding to three times the loop volume was allowed to flow through the loop.

Sample and buffer syringes, 1 ml at the centers and 10 ml at the sides, (BD Plastipak, Becton Dickinson, Franklin Lakes, NJ) were connected to the flow chip using Teflon tubing (TIG Teflon tubing 58697-U, Supelco, Bellefonte, PA) and PEEK luer slip connectors (P-659 and F-120X, Upchurch Scientific, Oak Harbor, WA). Silicone tubing glued around inlets and outlets at the bottom of the chip constituted fluidic docking ports into which the Teflon tubes were pushed to fit tightly. To avoid sedimentation of the somatic cells, a Teflon encapsulated magnet was placed in the sample syringe and continuously moved during the experiments by a magnet stirrer.

Acoustic actuation of the channel was performed with a piezoelectric transducer (12 × 12 × 1 mm\(^3\), PZ26, Ferroperm Piezoceramics AS, Kivistgard, Denmark). The transducer was glued to the bottom of an aluminum block, Figure 2. To ensure good ultrasound coupling, an ultrasound gel (Aquasonic Clear, Parker Laboratories, Fairfield, NJ) was applied.

**Figure 1.** Schematic top view of the microfluidic channel (not to scale). Trifurcations at both ends with connected sides simplify the fluidic setup. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]
between the chip and the aluminum tip. An acoustic frequency of 1.970 MHz, corresponding to three half acoustic wavelengths across the channel, with a 30 kHz modulation (FM) was used. The aluminum block acted as a heat sink. The frequency modulation prevented acoustic hot spot formation.

The piezoelectric transducer was actuated by a signal generator (HP 33220A, Hewlett-Packard, Palo Alto, CA) and an RF-amplifier (75A250A, Amplifier Research, Souder ton, PA). The total amplification from the function generator to the output of the RF-amplifier is nominally 30 dB, but due to an impedance mismatch typically half of the power is reflected from the transducer. The assembly of the flow chip and ultrasound transducer was placed under a microscope, and imaged during the separation process. The complete system can be seen in Figure 3.

Label-Free Analysis

As the aim of the project is to provide label free detection and counting of cells, thus eliminating the need for chemicals used to dissolve lipids and stain cells in conventional cell enumeration, several techniques were employed to investigate the samples. Original sample cell numbers were analyzed using a flow cytometer (Fos somatic FC, FOSS Analytical A/S, Hillerd, Denmark). Protein and lactose concentrations were measured to verify that the raw milk samples used were typical in composition. Collected samples after acoustophoresis treatment were then analyzed using microscopy as well as Coulter counting (Multisizer 3, Beckman Coulter, Brea, CA) and flow cytometry (BD FACS Canto, Becton Dickinson, Franklin Lakes, NJ). Cells were labeled by adding staining solution (Fos somatic staining, FOSS Analytical A/S, Hillerd, Denmark) 1:20 to the raw milk at room temperature, stirring the sample and letting it sit for a few minutes. The microscope inspection was carried out by alternating between phase contrast and fluorescent mode to verify that objects thought to be cells really were somatic cells and not false positives. In fluorescent mode a mercury lamp (Osram HBO, Osram AG, Munich, Germany) was used in combination with a 520-550 nm excitation, 565 nm mirror and 580 nm emission filter cube (UMWIG-2, Olympus, Hamburg, Germany). Samples from a chip with the ultrasound turned off were inspected using the same protocols to further illustrate the difficulties encountered when trying to count cells in samples with normal lipid content concentrations. Samples were all left to rest for a few minutes before observation in the microscope to allow somatic cells to sink to the bottom and lipid vesicles to float to the top of the observation chamber.

Acoustic Power and Flow Control Optimization

A well-defined flow in the channel is a prerequisite for efficient separation of lipids from the raw milk sample while losing only a minor fraction of the cells. With the present choice of flow rates and acoustic operation the lipid particles, having a lower density than both milk serum and water, were extracted from the sample stream and focused in the two antinodes immediately at either side of the channel center, Figure 4. Because the cells have a higher density and speed of sound (i.e., \( \Phi > 0 \)) than the milk serum, they were focused into the center node and remained in the milk serum, and thus the lipids were separated from the milk by balancing the flow rates at the chip outlet.

It is preferable to maximize the acoustic force that particles are exposed to in the channel since this allows even low contrast (\( \Phi \)) as well as small lipid particles to separate. With a fixed channel length the total lateral displacement of particles may be increased by lowering the flow rate, thus increasing particle exposure time in the acoustic field, or by an increase in acoustic power input. At low flow rates or high acoustic power, lipid particles, reported in our earlier work (11), tended to accumulate along the channel walls. The current three node design now addresses these problems and allows lower flow rates (or a higher acoustic power). By introducing three acoustic nodes into the system and using sheath flow to laminate the milk sample into less than one-third of the total channel width in the channel center, the two side nodes act as acoustic barriers that prevent lipids from travelling further into the sheath fluid and reaching the channel walls. However, at very low flow rates, the lipids can still be trapped in hot
spots at the trifurcation zone of the inlet and outlet channels as illustrated in Figure 5.

When lipids aggregate at the inlet trifurcation the flow and separation becomes asymmetric. Typically, large lumps of lipids are also released from the first aggregate at an irregular rate, which prevents optimal separation. Likewise, if lipid lumps build up at the outlet trifurcation the side outlets may become partially blocked, causing flow disturbances where some of the lipids flow into the center outlet and some of the cells flow into the opposite side outlet.

Observations show a strong correlation between lipid aggregation and the loss of cells during separation. It is also believed that the lipid lumps tend to trap some of the cells, which cause even worse separation when these cells are transported with the lumps into the waste outlet. A similar phenomenon is observed when milk is centrifuged—although the cells are heavier than the milk serum, a significant fraction of the cells are trapped in the lipid layer at the top.

To avoid lipid aggregation while maintaining good separation and throughput, the system was investigated at varying transducer actuation voltages and flow rate settings. With the transducer actuated at 30 V, corresponding to a maximum force of \( \sim 100 \) pN acting on a 9 \( \mu \)m particle, lipids started to aggregate even at flow speeds of 500 \( \mu \)L min\(^{-1}\). This might be because the no-slip conditions at the channel top and bottom will cause lipids to aggregate there at relatively high acoustic settings. At 20–25 V actuation, corresponding to \( \sim 50–75 \) pN force, separation was stable and no lipid aggregation occurred.

Taking these inherent system properties into consideration, transducer actuation was set to 22 V and microfluidic conditions set to \( Q_{\text{sheath}} = 420 \) \( \mu \)L min\(^{-1}\), \( Q_{\text{sample}} = 30 \) \( \mu \)L min\(^{-1}\), \( Q_{\text{cell}} = 60 \) \( \mu \)L min\(^{-1}\), and \( Q_{\text{waste}} = 390 \) \( \mu \)L min\(^{-1}\). These settings facilitate symmetric and stable separation of the lipid particles into bands that flow into the side outlets, as shown in Figure 6. Cell-sized particles will be subject to a maximum acoustic force of \( \sim 25–50 \) pN at this acoustic setting. A center outlet flow of 60 \( \mu \)L min\(^{-1}\), result in samples that consist of 50% milk and 50% sheath liquid when no ultrasound is applied (later visible in measurements, Figure 10b). An isotonic solution of NaCl in deionized water (9 g NaCl added to 1 L Milli-Q water) was used for the sheath liquid, such that the mixing of sample and sheath liquid does not change the liquid osmolality.

**RESULTS AND DISCUSSION**

**FTIR Analysis**

To quantify the lipid depletion efficiency of the system, an FTIR spectrum was obtained from a center outlet sample, with ultrasound active as well as inactive. The result of a representative measurement is shown in Figure 7, where the ester...
linkage peak at 1,750 cm$^{-1}$ (dashed circle) indicates a factor 6 in lipid depletion. This aligns with our previously reported experiments, which had longer acoustic exposure times, thus resulting in higher depletion rates (11). Most important, though, is that the removed lipid vesicles composed the larger size fraction as measured by Coulter counting and multiangle light scattering. The data suggest that sufficient amounts of lipid vesicles in the size range of cells have been removed to enable label free somatic cell detection.

**Phase Contrast Microscopy**

Center outlet samples with ultrasound active and inactive were collected, and phase contrast as well as fluorescence microscopy were used to investigate the feasibility of label free somatic cell detection in acoustophoretically preprocessed samples. The top of the observation chamber was imaged in phase contrast mode and the bottom in both phase contrast and fluorescent mode. Figure 8 shows the schematic set-up for imaging the two samples, where a thick layer of lipid vesicles were observed floating at the top of the observation chamber for the untreated sample, Figure 8a, whereas the acoustophoretically "polished" milk sample displayed a significant reduction of interfering lipid vesicles, Figure 8b. The corresponding microscope images are seen in Figures 9a and 9d, respectively.

When focusing at the chamber bottom in the treated sample, it was possible to discern somatic cells, Figure 9e. This was not possible in the untreated sample due to lipid vesicles obscuring the cells. It can also be noted that the untreated sample displayed some larger lipid vesicles that can be mistaken for a cell, marked as "false cell," Figure 9b. When switching to fluorescent mode, it was possible to observe the cells in both samples, Figures 9c and 9f. Comparisons between phase contrast and fluorescent images revealed the "false cells."

Figure 6. The figure shows the flow at the outlet side of the channel during acoustophoretic lipid separation from milk. The larger lipid particles are separated symmetrically into two bands that flow into the side outlets, while the milk constituent flow into the center outlet. The broader central band of light scattering casein micelles and small lipid particles visualizes the lipid depleted milk stream. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

Figure 7. FTIR spectrum of the sample collected from the center outlet, with and without the ultrasound applied. The peak at 1,750 cm$^{-1}$ (dashed circle) originates from the C–O–C ester linkage bond, and is a measure of the lipid content in the milk. The measurement show a factor of 6 in lipid depletion of the treated sample (blue) compared to the untreated sample (red). [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]
This finding opens the route to the development of automated image analysis as a detection mode for somatic cell counting in raw milk without elaborate chemical preprocessing protocols.

**Coulter Counting**

The quantitative agreement between the SCCs obtained by the manual procedure using cell staining, centrifugation and flow cytometry, and the new proposed method (acoustophoresis in combination with Coulter counting) was investigated. Samples from three cows suffering from mastitis were analyzed using both techniques. The resulting SCCs for each sample are shown in Table 1 together with Coulter counter data for untreated samples.

Treated samples were also compared to centrifuged samples as well as untreated raw samples using Coulter counting, Figure 10. In the raw samples (solid green line), it was not possible to distinguish cells from lipid vesicles due to the high amounts of lipid vesicles in the sample. (b) When using acoustophoresis to deplete the sample from lipids, cells can be detected without any need for labeling. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

![Figure 8](image-url)

**Figure 8.** Schematic cross section of the observation chamber. Chamber top and bottom illustrate the focal position in Figure 9 (top illustrates Figs. 9a and 9d and bottom Figs. 9b, 9c, 9e, and 9f). (a) It is not possible to detect unlabeled cells in an untreated sample due to the high amounts of lipid vesicles in the sample. (b) When using acoustophoresis to deplete the sample from lipids, cells can be detected without any need for labeling. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

![Figure 9](image-url)

**Figure 9.** a—c, show microscope images of an untreated sample taken at different focal depths as outlined in Figure 8a. (d—f), show the corresponding images of an acoustophoretically pretreated sample, as outlined in Figure 8b. (a) When focusing on the top of the observation chamber in an untreated sample, a thick layer of lipid vesicles was seen. By focusing at the bottom of the chamber and then alternating between phase contrast and fluorescent imaging modes, we observed that the lipid vesicles obscured the somatic cells. Also, lipid vesicles form “false cell” aggregates (dashed insert square), b and c. When focusing on the top of a treated sample, and comparing to an untreated sample, the efficiency of the lipid removal becomes apparent (a vs. d). In a treated sample the cells can be clearly seen at the chamber bottom in phase contrast mode (e). When switching to fluorescent mode the visually observed cells in (e) were also confirmed (f). [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]
overlapping size distribution. However, in the acoustophoretically treated samples, a cell peak (solid black line) centered at \( \sim 8.6 \mu m \) diameter emerged once the lipids were removed. Comparisons with centrifuged samples and standard fluorescent flow cytometry confirmed that the peak consisted of somatic cells. The acoustically treated sample has a slightly higher particle count as compared to the centrifuged sample (solid yellow line), indicating a non complete removal of all lipid vesicles in the cell size regime, Figure 10a. Samples passing through the microchip with the ultrasound turned off, as well as samples left in the inlet syringe, were unaffected, except for a 1:2 dilution in the chip sample which corresponds to the sheath liquid/milk ratio in the center outlet, cf. solid green line vs. solid yellow and blue lines Figure 10b.

**Table 1. Raw milk measurements**

<table>
<thead>
<tr>
<th>RAW MILK SAMPLE NO.</th>
<th>TRADITIONAL FLOW CYTOMETRY SCC (CELLS/ML RAW MILK)</th>
<th>TREATED SAMPLE CC SCC (NO. OF 7.2–10.5 ( \mu m ) PARTICLES/ML)</th>
<th>UNTREATED SAMPLE CC SCC (NO. OF 7.2–10.5 ( \mu m ) PARTICLES/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.03 ( \times 10^6 )</td>
<td>3.93 ( \times 10^6 ) (2% error)</td>
<td>12.06 ( \times 10^6 ) (199% error)</td>
</tr>
<tr>
<td>2</td>
<td>0.97 ( \times 10^6 )</td>
<td>1.22 ( \times 10^6 ) (26% error)</td>
<td>3.25 ( \times 10^6 ) (235% error)</td>
</tr>
<tr>
<td>3</td>
<td>5.76 ( \times 10^6 )</td>
<td>5.73 ( \times 10^6 ) (1% error)</td>
<td>8.27 ( \times 10^6 ) (46% error)</td>
</tr>
</tbody>
</table>

**Figure 10.** Relative cell counts from Coulter measurements of the three raw milk samples (1.0, 4.0, and 5.8 M SCC mL\(^{-1}\)). Graph (a) compares acoustophoretically treated, untreated and centrifuged samples of sample 1 \((n = 4)\). Graph (b) shows that cells even in the lowest SCC sample 2 \((n = 5)\) can be seen (cell peak emerges). It also shows that samples are unaffected, except for a 1:2 dilution, when run in the system with the ultrasound turned off (blue and dark green lines vs. light green line). Graph (c) shows both center and side outlets for sample 3 \((n = 5)\) to illustrate more stable cell counts compared to lipid vesicle counts (smaller error bars for cell sized particles). Graph (d) shows how the Coulter measurements of treated samples scales according to the flow cytometry data with the 970 SC \( \mu L \) \(^{-1}\) (1.0 M, black line) sample having a peak that is \( \sim 1/6 \) of the 6,758 SC \( \mu L \) \(^{-1}\) (5.8 M, purple line) sample. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]
The different milk samples that were treated acoustophoretically, suggest a good correlation between the Coulter counter data in these samples. The number of cells also corresponded well with the values measured with traditional flow cytometry using a Fossmatic FC+ instrument (a sample of ~2.5 mL raw milk was mixed with an Ethidium bromide based staining agent, surrounded by a sheath liquid and then passed through a flow cell in which fluorescent flow cytometry was performed by detecting emitted light from stained cells using a laser and a photomultiplier tube). For example, in sample 2 the flow cytometer measurement indicated 0.97 × 10^6 SC mL⁻¹ whereas the corresponding mean number of particles in the cell-peak region 7.2–10.5 µm, as measured by Coulter counting was 1.27 × 10^6 SC mL⁻¹ Figure 10b. It can be noted that without the acoustic pretreatment of the sample the corresponding Coulter counter data indicated 3.25 × 10^6 SC mL⁻¹. A number totally dominated by the lipid vesicles. The respective data for sample 1 and 3 are also shown in Table 1. The relative deviation in flow cytometry cell count and Coulter counter data averaged 10% (2, 26, and 1%, respectively). A reason for the deviation between flow cytometry data and particle numbers obtained by Coulter counting is that the high amount of lipid vesicles compared to cells makes the system sensitive to fluidic imbalance, i.e., a minor flow disturbance may cause leakage of lipid vesicles into the center outlet and thus generate an over estimation of the somatic cell count. The cases where the cell counts actually was lower by means of Coulter counting versus flow cytometry, may be explained by the fact that samples with a significant number of cells having lipid vesicles adhering to the cell membrane may be deflected into the lipid fraction. Although these values suggest that the current setup might not be suited for exact SCC counts, it can still be used as a fast indicator of healthy or diseased live stock, not requiring elaborate chemical sample processing.

CONCLUSION

Acoustic focusing of cells in flow cytometry has already entered the market in the recently launched Attune® flow cytometer (Life Technologies, Carlsbad, CA) and have demonstrated valuable improvements in sample throughput and simplified sample processing. Presumably these benefits could also be harvested in the application of somatic cell counting in milk. The work presented herein, however, aims at taking, acoustic sample handling one step further in an effort to move away from flow cytometry as the analytical platform and rather pave the way to imaging cytometry at microchip level offering a less complex route forward to the next generation somatic cell counting systems in the dairy industry.

In this perspective the reported phase contrast microscopy images of raw milk samples after lipid depletion using acoustophoresis, demonstrates a simple strategy to the development of a system for somatic cell counting in raw milk, using automated image analysis without elaborate lipid solvation and cell staining preprocessing protocols. The ability to discern the “cell peak” in all samples including the one with 0.97 × 10^6 SC mL⁻¹, Figure 10b, indicates that it may be possible to detect early stages of mastitis (current thresholds advocate cut-offs between healthy and sick live stock at between 1 and 5 × 10^6 SC mL⁻¹ with cows typically reaching 0.97 × 10^6 SC mL⁻¹ after 2–3 days of inflammation). Comparisons with conventional methods as well as the correct ratio between the three samples that were analyzed using flow cytometry demonstrates that the method is able to quantify somatic cell counts in raw milk within an approximate accuracy of 10%. This opens the route to a possible integration of a Coulter counter unit on chip. Coulter counters integrated with microfluidic systems have been reported previously (18–21) and on-going work in our group now targets the development of a microfluidic system with integrated acoustophoretic and Coulter counter functionality.

LITERATURE CITED