Microfluidic sampling system for tissue analytics

A. Hokkanen,¹ I. Stuns,¹ P. Schmid,² A. Kokkonen,³ F. Gao,¹ A. Steinecker,² J. Budczies,⁴ P. Heimala,^{1,a)} and L. Hakalahti³

¹VTT Technical Research Centre of Finland, Tietotie 3, 02044 VTT, Espoo, Finland

²CSEM Swiss Center for Electronics and Microtechnology, Untere Gründlistrasse 1, CH-6055 Alpnach, Switzerland

³VTT Technical Research Centre of Finland, Kaitoväylä 1, 02044 VTT, Oulu, Finland

⁴Charité—Universitätsmedizin Berlin, Institute of Pathology, Charitéplatz 1, D-10117 Berlin, Germany

(Received 29 May 2015; accepted 3 September 2015; published online 16 September 2015)

We have developed a microfluidics based sampling system for tissue analytics. The proof-of-concept of the sampling system was demonstrated by extracting lipid samples from tissue biopsies. The sample collection system consists of a disposable silicon based multiport microneedle integrated with polymer microfluidics. The polymethyl methacrylate polymer microfluidic chip has a 10 μ l sample reservoir and actuation membranes for liquid pumping. A special automated robotic system was developed to control the positioning of the needle and the sampling procedure on preselected spots on the tissue. Real breast cancer tissue samples were used to test the feasibility of the sampling system. We successfully measured indicative cancer biomarkers from the tissue surface. Phosphatidylcholine and phosphoethanolamine were extracted from the tissue membrane with methyl tert-butyl ether solvent and detected by mass spectrometry. In the future, this tool could be used in characterization of preoperative biopsies and tumour tissues removed during surgery. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4931045>]

I. INTRODUCTION

Currently, in surgical procedures where cancerous tissue is removed, surgeons rely on preoperative imaging scans and results provided by pathologists after examination of tissue biopsies under a microscope. This approach is relatively slow, because the response time is at least 20 min. It also requires the transfer of the tissues from the operating room to the instantaneous section laboratory and preparation of frozen tissue sections. Real-time imaging tools could help oncologists to speed up the characterization of preoperative biopsies and tumour tissues that have been removed during surgery. However, in order to distinguish the cancer cells from the healthy ones by “imaging,” the molecular composition in each case needs to be known.

It has been recently shown that cancer changes lipids’ behavior and amounts in humans.^{1,2} The measurement of the oxidation levels of lipids is an indicator of cell-stress and also a biomarker for the onset of cancerous growth.³ An amount of different phospholipids has been studied as biomarkers for ovarian⁴ and breast cancers.⁵ The role of metabolites and lipids such as Phosphatidylcholines (PCs) and Phosphoethanolamines (PEs) as breast cancer biomarkers has been investigated by Eliyahu *et al.*,⁶ Hilvo *et al.*,⁷ and Butczies *et al.*⁸ Separation of breast cancer tissues from normal breast tissues was demonstrated to be feasible with high sensitivity and specificity. The same PC and PE biomarkers were also detected from urine samples by Kim *et al.*⁹ They succeeded to measure PC and PE lipids with nanoflow liquid chromatography tandem mass spectrometry.

We propose that an automated microfluidics-based sampling system could be used for extracting the lipid content from small tissue biopsies. The automated microfluidic sampling

^{a)} Author to whom correspondence should be addressed. Electronic mail: paivi.heimala@vtt.fi

system permits extraction of samples from highly defined sites of tissue samples. Homogenization, which is generally in use with tissue samples, is avoided. Our technology provides highly site-specific results instead of a crude average derived from a homogenized mixture. This lipidomics technique could be exploited as an indication for cancer after taking a biopsy or during surgery.

Intelligent surgery has been earlier investigated by other research groups. For example, Takats *et al.*¹⁰ have studied how to distinguish cancerous and noncancerous tissue during surgery by using mass spectrometer (MS). Microfluidics based approach has been applied on tissue samples by Delamarche *et al.* They have developed a microfluidic probe for performing histopathological staining of tissue samples¹¹ and built an instrument for that approach.¹² Simultaneous injection and sampling has been used by Ainla *et al.*¹³ with a multifunctional pipette. They have made single-cell electroporation using polydimethylsiloxane (PDMS) based pipette tip. Chen *et al.*¹⁴ have coupled droplets to PDMS probe for microfluidic stimulation and recording. They have used this chemistode to measure insulin secretion from a single murine islet of Langerhans.

We show here the results of an automated microfluidic sampling system. It uses robotics and microscope imaging for positioning the microneedle and sampling from real tissue biopsies. With this kind of system, the oncologist could use microscope imaging to select positions for microneedle based molecular sampling.

II. MATERIALS AND METHODS

A. Silicon microneedle

The blunt-tip silicon microneedle chips were designed. They have two out-of-plane channels inside one needle body and two in-plane connection channels to interface with the polymeric microfluidic chip. Two separate channels were needed to simultaneous solvent injection and sample aspiration during the sampling event. The structure of blunt silicon microneedle is presented in Figure 1(a) and a fabrication process is depicted in Figures 1(b)–1(j). The fabrication process started with patterning the in-plane connection channels through a hard mask (e.g., silicon dioxide) layer on a double-side polished silicon wafer (Figure 1(b)). Next, the out-of-plane channels inside the needle body were etched almost through the wafer using a thick photoresist mask (Figure 1(c)). Then, the photoresist was removed, and the in-plane connection channels were etched about 30 μm deep using the hard mask (Figure 1(d)). The needle wafer was then oxidized and fusion bonded to the cap wafer (Figure 1(e)). Next, the connection holes were patterned and etched through the cap wafer and connected with the in plane connection channels (Figure 1(f)). On the other side of the bonded wafer, a shallow well pattern was first etched through the oxide layer (Figure 1(g)). After a thick resist lithography, the needle body was sculptured by anisotropically etching away the surrounding silicon about 200 μm deep (Figure 1(h)). Then, the photoresist was removed and the shallow well was etched using the patterned oxide as mask until the out-of-plane channels were revealed (Figure 1(i)). In the end, a HF etching step was used to open the channels and a thin layer of the silicon dioxide was grown on the whole wafer to make the channel surface hydrophilic (Figure 1(j)).

B. Polymer microfluidics

The polymer chip was used both as an integration platform for a Si microneedle and the liquid storage. It was fabricated on PMMA (polymethyl methacrylate, Plexiglas 99524, Evonik Röhm, Germany). The 100 μm deep and 400 μm wide fluidic channels were hot embossed to a 375 μm thick foil. Channels were lidded by solvent bonding (ethanol-ethylacetate) with a 375 μm thick PMMA foil, which contains two drilled 5 mm wide holes. The holes were covered with a 125 μm thick elastic TPU (thermoplastic polypropylene, Sto-Nor Polymer Film AB, Sweden) membrane to enable pneumatic actuation. The silicon microneedle chip was bonded to the polymer chips by using adhesives (EpoTek 353-ND, Epoxy Technology Inc., USA).

FIG. 1. (a) Silicon microneedle structure showing the solvent injection and sample aspiration during microneedle extraction. The fabrication process was as follows: (b) mask for connection channels, (c) etch the needle channels, (d) etch the connection channels, (e) wafer bonding, (f) mask and etch the connection holes, (g) mask the shallow well and the needle body, (h) etch the needle body, (i) etch the shallow well to the head of needle tip, and (j) etch the channel opening and oxidize the channels.

C. Microneedle modelling

Flow behaviour in the microneedle was modelled with the Comsol Multiphysics software. Density and kinematic viscosity values are the most essential parameters in the model. For methyl tert-butyl ether (MTBE) solvent, these values are 740 kg/m^3 (Ref. 15) and 0.47 cSt ,¹⁶ respectively. Boundary condition used in the modelling for the outlet is 0 Pa without viscous stress. No slip was anticipated in the channel walls. The fluidic channels of the microneedle have thermally oxidized hydrophilic walls, so the channel wall was not expected to induce any flow resistance. Two different size microneedles were modelled: $150 \mu\text{m}$ outer diameter (OD) and $250 \mu\text{m}$ OD. In the smaller microneedle, the FEM model had 8055 mesh elements, while in the larger microneedle, 4921 mesh elements were used. The number of mesh elements was higher in the smaller needle to enable better simulation of the narrow channel dimensions.

D. Automated robotic system

Two systems for microneedle based lipid extraction were built: (i) a manual version for initial testing and (ii) an automated robotic system for user-friendly operation (Figure 2). Both systems had the same pneumatic interface in common. This allows to apply pressure changes on the membranes that cover the channels of the microneedle chip. Changing the pressure on the membrane enables the aspiration or the dispensing of liquids with the microneedle. Marsh Bellofarm T3510 controllers were used for closed-loop pressure generation. Maximum and minimum pressures were $\pm 650 \text{ mbar}$, but a pressure difference in the tip of the needle was kept at 0 mbar for a leak free sealing. The first manual version used micromanipulators for microneedle positioning, and this was supported by a vision system. This system allowed an easy assessment of critical process protocols such as tissue dissolving and extraction parameters. The automated robotic microneedle system was developed for precise and repeatable tissue extraction. It offers

FIG. 2. An automated robotic system that is used for controlling the sampling event.

the same pneumatic interface between manipulator and microneedle chip as the manual system. Additionally, a vision system with top and side cameras was part of the robotic system. An operator can simply select extraction spots from a picture and start automated tissue sampling. The positioning system consisted of two independent robots for complementary tasks: one piezo system for the high precision positioning with short travel distances of the sample and the other for coarse movement and long travel distances for logistics of the microneedle chip.

E. Mass spectrometer

Waters Triple Quadrupole mass spectrometer was used. The extracted lipid sample was first transferred from the microneedle to a steel needle (5118–15-B, 18GP, 0.033×1.5 , Efd Inc., USA) and then to a glass vial. Microneedle, steel needle, and glass vial were all disposables in order to prevent the cross-contamination of the samples. The standard sample injection interface from glass vials to MS needed $20 \mu\text{l}$ sample volume. As the sample volume after microneedle based sampling was $10 \mu\text{l}$ (without evaporation), an extra $10 \mu\text{l}$ of MTBE (306975 Sigma-Aldrich) was added to all vials to get sufficient liquid amount for MS analysis. With this, the sample dilution became 1:1. MS results were analysed with MZmine 2 software¹⁷ to get relative numerical values for measured lipids. MS analysis of the breast cancer samples was based on relative numerical values of PC and PE lipids. Signal levels of few tens of a.u. for a single lipid were noise. The lipid identification at data processing was based on an internal spectral library and tandem mass spectrometry.

F. Sample preparation

Proof-of-principle of the microneedle sampling system was demonstrated with solution of several standard lipids. This standard lipid mixture contained among others one PC and one PE lipid: 17:0 PC and 17:0 PE (850360P and 830756P, respectively (Avanti Polar Lipids Inc., USA). Both PC and PE lipid concentrations were $10 \text{ ng}/\mu\text{l}$ (10 ppm) in the sample mix.

Standard lipid droplets (1–4 droplets, each of volume $2\ \mu\text{l}$) were pipetted and dried on glass slides. Dried sample spots contain 20–80 ng PC and PE lipids.

For the proof-of-concept of tissue sampling, real patient samples were used. The project had the ethics board approval of the Charité Hospital (Reference number EA1/139/05, amendment 2008) for this purpose. Breast tissue samples were cut during surgery at Charité Hospital and snap frozen. For histopathological quality control, a hematoxylin and eosin stained (H&E) section of each tissue sample was examined by a pathologist. Samples were classified in tumour tissue (containing more than 40% tumours cells) and normal tissue (being tumour-free). Samples were cut from bulk tissue samples to Eppendorf-tubes and frozen at $-80\ ^\circ\text{C}$. Other tissue fixation methods were not considered here because the final use would be with fresh tissue samples. Before the experiments, the samples were put on glass slide and thawed from $-80\ ^\circ\text{C}$. Condensed water was let to evaporate before the sampling and measurements. During the sampling and measurements, the samples were in room temperature at $+20\ ^\circ\text{C}$.

III. RESULTS

A. Silicon microneedle

In situ liquid-liquid extraction (LLE) of lipids in tissue samples was performed with a multiport silicon microneedle. Two different size blunt microneedle types with $150\ \mu\text{m}$ and $250\ \mu\text{m}$ OD were developed. The scanning electron microscope (SEM) image of the $250\ \mu\text{m}$ needle is shown in Figure 3. The height of the needles is about $200\ \mu\text{m}$. Both needles have two separate fluidic channels that open into a shallow $30\ \mu\text{m}$ deep well on top of the needle tip. The shallow well forms a small flow cell when the needle is pressed tightly on top of the tissue sample. Fluidic channels have $30/60\ \mu\text{m}$ inner diameter (ID) and $40/80\ \mu\text{m}$ mutual spacing for 150 and $250\ \mu\text{m}$ microneedles, respectively. One of these channels was used to inject the MTBE solvent to the top of the tissue sample in order to extract the cell membrane lipids, while the other channel was used to simultaneously aspirate the extracted lipids into the needle (Figure 1(a)).

B. Polymer microfluidics

The silicon microneedle chip (Figure 4(a)) is small, only 2 by $2\ \text{mm}^2$ in size and only $\sim 5\ \text{nl}$ in volume. Therefore, it was integrated with a polymer microfluidic chip (Figure 4(b)),

FIG. 3. A SEM image of the multiport silicon microneedle. The outer diameter of the needle is $250\ \mu\text{m}$ and the height is about $200\ \mu\text{m}$. The needle has two separate fluidic channels with $60\ \mu\text{m}$ inner diameter and they are $80\ \mu\text{m}$ apart from each other.

FIG. 4. The silicon microneedle chip (a) is integrated to the polymer chip (b). A polymer microfluidic chip forms the liquid connection to the microneedle and is used as an interface between the microneedle and the robotics.

which enables injection and collection of larger liquid volumes. The dimensions of polymer chip are 35 by 42 mm², and it has two microfluidic channels, both with total volume of >10 μ l. One channel was used for solvent storage and the other for the extracted sample storage. The polymer chip also acts as an interface between the microneedle and the micromanipulator and robotics. Together with the pneumatic actuator system, the disposable polymer chip enables pressure controlled sampling (injection/aspiration) without the risk of contamination.

C. Microneedle modelling

Modelling work was focused on the liquid flows and back pressures of both blunt microneedles. The aim was to model only the silicon microneedle itself without the tissue sample and polymer microfluidics part. This was made to gain knowledge of the fluid flow behaviour in the needle tip. The sampling operation of the microneedle is presented in Figure 1(a) and cross-sections of the microneedle tips are illustrated in Figure 5. Inner diameters of flow cell structures in the microneedle tip are 100 and 200 μ m for 150 and 250 μ m microneedles, respectively.

Input fluid flow velocities in the modelling were 100 mm/s for the 150 μ m needle and 25 mm/s for the 250 μ m needle. The above modelled liquid flow velocities correspond to the volumetric flow of 0.19 μ l/s. In the extraction experiments, the volumetric flow was varied between 0.02 and 0.17 μ l/s, and 0.17 μ l/s flow gave the best results. In the real microneedle, the actuation of MTBE solvent was more difficult to control. MTBE modified the PMMA polymer surface, so that the constant flow velocity was not easily achieved. In addition, the elasticity of the TPU membrane decreased in repeated pneumatic actuation, which also may change the flow velocity of the solvent. The difference between the maximum and the minimum velocities was observed to be about ten-fold. Average values of the solvent flow velocities were, however, at

FIG. 5. Schematic structure (not in scale) of blunt microneedles showing the two fluidic channels and the liquid cavity at the tip of the needle.

FIG. 6. Modelling result showing the liquid flow distribution in blunt microneedle with outer/inner diameter of (a) 150/100 μm and (b) 250/200 μm , respectively. The inner diameter indicates the size of the flow cell on top of the needle.

the same range than the modelled MTBE flow velocity. The disposable microneedle will be used only once for sampling so that the PMMA surface modification and the TPU elasticity degradation are not that critical. By developing a flow measurement method for solvent, a feed-back loop from flow measurement to pressure controllers can be realized. This can then be used to stabilize the solvent flow.

The microneedle model used laminar flow solver for velocity and pressure calculations. Modelled fluid flow distributions are presented in Figure 6. Model focused on 200 μm long microneedle tip. Fluid inputs are at the left side channels in Figures 6(a) and 6(b). Input flow has a constant flow field, but in the microneedle channel flow distribution transforms to parabolic distribution like in the right side output channels. This parabolic flow distribution is already seen in the very beginning of the input channels.

Modelling results confirm that liquid flow is slightly increased in the flow cell (liquid cavity on top of the needle): $\sim 1.4\times$ with 150 μm needle and $\sim 2.4\times$ with 250 μm needle (Figures 6(a) and 6(b)). According to the modelling results, the flow distribution is quite smooth over the whole area of the flow cell. Parabolic flow distribution is still possible to see in the edge of the well, but the lipid extraction is expected to be feasible over the whole flow cell area. Pressure distributions were also modelled to evaluate the operation and requirements of the pneumatic actuation system. Calculated back pressure is just a few mbars inside the microneedle channels (Figures 7(a) and 7(b)). This is not expected to cause any problem for the microneedle actuation system as the tuning accuracy of pressure controllers is in the same range.

D. Liquid-liquid extraction tests with manual microneedle system

To show the proof-of-principle of the phospholipid sampling by the liquid-liquid extraction method, we used the developed manual microneedle system for sampling standard lipids. The

FIG. 7. Modelling result showing the calculated back pressures in the liquid channel of a blunt microneedle with outer/inner diameter of (a) 150/100 μm and (b) 250/200 μm , respectively. The inner diameter indicates the size of the flow cell.

FIG. 8. (a) Standard lipid samples pipetted and dried on microscope glass slides. (b) The surface of the dried standard phospholipid sample after the microneedle sampling. The extracted sample spot is clearly visible.

extraction system is presented in Figure 1(a). The standard lipid samples on glass slides are shown in Figure 8(a). Microneedle with the OD of $150\ \mu\text{m}$ and MTBE solvent were used to extract the samples from the glass slides. As seen in Figure 8(b), all visible material from the spot beneath the microneedle tip was extracted during the sampling. The extraction time was one minute. MTBE volume in the extraction was $10\ \mu\text{l}$.

The MS results are presented in Figure 9. The first “Standard lipids” sample represents the signal of the standard lipid solution used as such in the MS analysis. The second “MTBE” sample is the extraction buffer signal as measured with the MS. The third “Sample 3” and the fourth “Sample 4” represent the microneedle extracted standard lipid samples from the glass slides. Both samples were extracted with the same microneedle. Standard lipid and MTBE references were injected directly to the MS without the use of the microneedle. The standard lipid solution components are shown in the legends: ceramide (Cer), lysophosphatidylcholine (lysoPC), PC, PE, and triglyceride (TG) lipids. The signals in the microneedle samples 3 and 4 should be compared with the “MTBE” solvent. As seen from Figure 9, the samples 3 and 4 show increased lipid values for standard lipid solutions. These values for the microneedle samples are lower than the direct undiluted standard lipid values of the first sample. The signal variation between the samples 3 and 4 for PC and PE lipids is mainly due to the clod formation and uneven spatial distribution of the dried lipid samples as can easily be observed in Figures 8(a) and 8(b).

FIG. 9. The result of the MS analysis for the lipids extracted from glass slides.

FIG. 10. (a) Tissue samples on glass slides. (b) A footprint of the 250 μm blunt microneedle on the site of extraction in the tumour tissue sample.

For the proof-of-concept demonstration, we tested first a lipid extraction from tissue with the manual microneedle system. Microneedles with the OD of 250 μm and MTBE solvent were used as presented in Figure 1(a). Larger microneedle (OD 250 μm) was used, because the objective was to measure the signal variation of the PC and PE lipids and higher signal levels are expected when the area of the sampled tissue is larger. Two different extraction solvent volumes were tested: 1 and 10 μl . It was observed that 1 μl was practically too small volume of highly volatile extraction buffer. Due to vaporization, there was almost no liquid left after 5 s extraction time. Thus, the extraction solvent volume was increased to 10 μl and this made sampling successful. Different extraction times from 1 min to 8 min were also tested. The best extraction results from the tissue were obtained with the 1 min extraction time, but optimization is still required.

Figure 10(a) shows the bulk tissue samples placed on glass slides. On the left hand slide, there is a normal sample (26N) and on the right hand slide a tumour sample (26T). In Figure 10(b), a microneedle footprint is shown on the tumour tissue 26T after extraction. It shows that the tissue morphology remains visually unmodified when the lipids are extracted from the tissue surface. Microneedle has not cut mechanically a part of the tissue. In addition, the MTBE flow has not detached any tissue material. This is the optimum case as no other tissue material as cell membrane lipids are preferred to be mixed with the extraction buffer.

The MS results of the microneedle extracted samples 26N (normal) and 26T (tumour) are shown in Figure 11. Both samples are from different positions of the same patient and the same tissue sample. Normal and tumour samples were classified by a pathologist. A new clean microneedle was used in sampling for each sample. Extraction time was again 1 min. Just a relative change is shown in the results and signal levels of few tens of a.u. are noise. Increased values of the PC and PE lipids present in the cancerous tissue are found in the tumour samples after microneedle sampling and MS analysis. Figure 11 presents only PC and PE lipids that indicate biomarkers for breast cancer.^{7,8}

The normal and tumour samples have also been tested with a standard extraction method with a homogenized mixture. This has been done in a previous work with the same samples.^{7,8} The PC and PE signal levels were $0\text{--}n \times 10$ a.u. and 1000–100 000 for the normal sample and the tumour sample, respectively. Concentrations of standard lipids in Figure 9 were on the same level as PC and PE lipids in homogenized tumour samples with standard sampling⁷ without microneedle. Standard curve for the determination of lipid concentrations is normally done by adding standard lipids to a homogenized normal tissue sample.

E. Tissue sampling with the robotic system

With the robotic system, the needle can be automatically located on the site of interest and the lipid sample can be extracted on the spot. In the experimental tests, the samples were taken randomly on the tissue, because the normal and tumour tissue samples were separate.

FIG. 11. MS result from the samples extracted with the manual microneedle system. Sample 26N is normal tissue and sample 26T is cancerous tissue.

Microneedles with the OD of $250\ \mu\text{m}$ were used and extraction time was the same 1 minute. The MS analysis results of the extracted samples 14N (normal) and 14T (tumour) are shown in Figure 12. Both samples are from the same patient and from the same tissue sample, but from different positions. A new clean microneedle was used both for normal and tumour samples. The tumour sample 14T was extracted five times 1–5 with the same microneedle. The normal tissue samples were almost like oil after thawing, because the fat content was so high in these samples. This complicated the analysis, because the microneedle channels got easily clogged. Thus, the repetition of the normal samples was difficult. Figure 12 presents only PC and PE lipids that were supposed to be as biomarkers for breast cancer.^{7,8} Clearly increased values of those lipids were found from the tumour sample. The PC and PE signal variation that is seen

FIG. 12. MS results from the samples extracted with the robotic microneedle system. Measured sample 0 is normal 14N and samples 1–5 are tumour 14T.

between the tumour samples 14/1–5 becomes most probably from the different spatial extraction positions, because microneedle extraction is highly site-specific.

The signal level of the normal sample 14N is below the noise level as it was also with the normal sample 26N in Figure 11. The PC and PE lipid values of the tumour samples in Figures 11 and 12 are clearly above those of the normal samples, but below the signal level of the homogenized tumour sample. The sample dilution in the MS analysis is one potential reason for the lower signal levels. It is possible to improve the extraction efficiency by optimizing the extraction time. However, the flow velocity measurement for the MTBE solvent flow needs to be developed first.

IV. CONCLUSION

We have shown the proof-of-concept of extracting both the PC and PE lipids from the fresh frozen and thawed breast cancer tissue samples with the automated robotic microneedle sampling system. In future, this system could be suitable for use during surgical operations. It can help surgeons in making decisions about the extension of the operation needed for fully removal of the cancerous tissue without sacrificing too much of the healthy tissue.

It is possible to increase the microneedle diameter at least tenfold ($10\times$) from the size used in these experiments. This means hundredfold ($100\times$) larger area beneath the microneedle tip and also potentially $100\times$ higher amount of lipids in the extracted sample. In the same time, sacrifices are made in focusing of the sample spot to larger areas. With smaller needles, a more precise sampling can be done and the dilution of the cancerous sample with the healthy tissue during analysis can be minimized. The microneedle design should be investigated further to prevent the occasional clogging of the needle.

This system is not yet ready for detailed lipidomics studies, because further development is still needed for the control of the highly volatile extraction buffer and the optimization of the extraction time. It is possible to integrate a feedback loop to pneumatic pressure controllers from the measured flow velocity of the MTBE extraction buffer. The measurement of the extraction buffer velocity should be added to the system. Transparent polymer channel of the microneedle device makes at least optical flow detection possible. The next step is the use of unclassified tissue samples and samples from different patients to test and develop the system. The focus should be in practical questions: How to select the sampling position on an unclassified tissue sample? How does individual differences affect to the results? How many microneedle samples are needed to secure the validity of the result? How long one sample from sampling to the result will take? A thorough statistical comparison of the microneedle sampling and traditional analysis method should also be made.

ACKNOWLEDGMENTS

This work has received funding from the European Community's Seventh Framework Programme (FP7) as a task of the project ARROWS, Grant Agreement No. 257669 (Advanced interfaced microsystems Research for analysis of Real-wOrld clinical, food, environmental, and Waste samples). The authors also thank Ms. Päivi Pöhö, Mrs. Ulla Lahtinen, and Mr. Howard Rupprecht from VTT. Päivi and Ulla performed the MS analysis. Howard checked the grammar of the manuscript.

¹C. R. Santos and A. Schulze, *FEBS J.* **279**, 2610 (2012).

²F. Baenke, B. Peck, H. Miess, and A. Schulze, *Disease Models Mech.* **6**, 1353 (2013).

³P. Cejas, E. Casado, C. Belda-Iniesta, J. De Castro, E. Espinosa, A. Redondo, M. Sereno, M. A. García-Cabezas, J. A. F. Vara, A. Domínguez-Cáceres, R. Perona, and M. González-Barón, *Cancer Causes Control* **15**, 707 (2004).

⁴E. Iorio, A. Ricci, M. Bagnoli, M. E. Pisanu, G. Castellano, M. Di Vito, E. Venturini, K. Glunde, Z. M. Bhujwala, D. Mezzanzanica, S. Canevari, and F. Podo, *Cancer Res.* **70**, 2126 (2010).

⁵J. Baumann, C. Sevinsky, and D. S. Conklin, *Biochim. Biophys. Acta* **1831**, 1509 (2013).

⁶G. Eliyahu, T. Kreizman, and H. Degani, *Int. J. Cancer* **120**, 1721 (2007).

⁷M. Hilvo, C. Denkert, L. Lehtinen, B. Müller, S. Brockmöller, T. Seppänen-Laakso, J. Budczies, E. Bucher, L. Yetukuri, S. Castillo, E. Berg, H. Nygren, M. Sysi-Aho, J. L. Griffin, O. Fiehn, S. Loibl, C. Richter-Ehrenstein, C. Radke, T. Hyötyläinen, O. Kallioniemi, K. Iljin, and M. Orešic, *Cancer Res.* **71**, 3236 (2011).

- ⁸J. Budczies, C. Denkert, B. M. Müller, S. F. Brockmüller, F. Klauschen, B. Györfy, M. Dietel, C. Richter-Ehrenstein, U. Marten, R. M. Salek, J. L. Griffin, M. Hilvo, M. Oresić, G. Wohlgemuth, and O. Fiehn, *BMC Genomics* **13**, 334 (2012).
- ⁹H. Kim, H. K. Min, G. Kong, and M. H. Moon, *Anal. Bioanal. Chem.* **393**, 1649 (2009).
- ¹⁰Z. Takats, J. Denes, and J. Kinross, *Future Oncol.* **8**, 113 (2012).
- ¹¹R. D. Lovchik, G. V. Kaigala, M. Georgiadis, and E. Delamarche, *Lab Chip* **12**, 1040 (2012).
- ¹²J. F. Cors, R. D. Lovchik, E. Delamarche, and G. V. Kaigala, *Rev. Sci. Instrum.* **85**, 034301 (2014).
- ¹³A. Ainla, S. Xu, N. Sanchez, G. D. M. Jeffries, and A. Jesorka, *Lab Chip* **12**, 4605 (2012).
- ¹⁴D. Chen, W. Dua, Y. Liu, W. Liu, A. Kuznetsov, F. E. Mendez, L. H. Philipson, and R. F. Ismagilov, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 16843 (2008).
- ¹⁵See www.sigmaaldrich.com for density of MTBE solvent.
- ¹⁶See www.jhaltermann.com for kinematic viscosity of MTBE solvent.
- ¹⁷See mzmine.sourceforge.net for MZmine 2 open-source software download and documentation.