Perra, Emanuele; Hayward, Nick; Pritzker, Kenneth P.H.; Nieminen, Heikki J.

**An ultrasonically actuated needle promotes the transport of nanoparticles and fluids**

*Published in:*
Journal of the Acoustical Society of America

**DOI:**
10.1121/10.0012190

Published: 01/07/2022

**Document Version**
Publisher's PDF, also known as Version of record

**Published under the following license:**
CC BY

*Please cite the original version:*

This material is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.
An ultrasonically actuated needle promotes the transport of nanoparticles and fluids
Emanuele Perra, Nick Hayward, Kenneth P. H. Pritzker, et al.

Citation: The Journal of the Acoustical Society of America 152, 251 (2022); doi: 10.1121/10.0012190
View online: https://doi.org/10.1121/10.0012190
View Table of Contents: https://asa.scitation.org/toc/jas/152/1
Published by the Acoustical Society of America

ARTICLES YOU MAY BE INTERESTED IN

Automatic segmentation and classification of mice ultrasonic vocalizations
The Journal of the Acoustical Society of America 152, 266 (2022); https://doi.org/10.1121/10.0012350

Four decades of near-field acoustic holography
The Journal of the Acoustical Society of America 152, R1 (2022); https://doi.org/10.1121/10.0011806

Dependence of binaural gain for infrasound on interaural phase difference
The Journal of the Acoustical Society of America 152, 163 (2022); https://doi.org/10.1121/10.0012220

Decadal community structure shifts with cold pool variability in the eastern Bering Sea shelf
The Journal of the Acoustical Society of America 152, 201 (2022); https://doi.org/10.1121/10.0012193

Effects of spatialized water-sound sequences for traffic noise masking on brain activities
The Journal of the Acoustical Society of America 152, 172 (2022); https://doi.org/10.1121/10.0012222

A survey of sound source localization with deep learning methods
The Journal of the Acoustical Society of America 152, 107 (2022); https://doi.org/10.1121/10.0011809
An ultrasonically actuated needle promotes the transport of nanoparticles and fluids

Emanuele Perra,1 Nick Hayward,1 Kenneth P. H. Pritzker,2,a) and Heikki J. Nieminen1,b)

1Medical Ultrasonics Laboratory (MEDUSA), Department of Neuroscience and Biomedical Engineering, Aalto University, Espoo, 02150, Finland
2Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, M5S 1A8, Canada

Abstract:
Non-invasive therapeutic ultrasound (US) methods, such as high-intensity focused ultrasound (HIFU), have limited access to tissue targets shadowed by bones or presence of gas. This study demonstrates that an ultrasonically actuated medical needle can be used to translate nanoparticles and fluids under the action of nonlinear phenomena, potentially overcoming some limitations of HIFU. A simulation study was first conducted to study the delivery of a tracer with an ultrasonically actuated needle (33 kHz) inside a porous medium acting as a model for soft tissue. The model was then validated experimentally in different concentrations of agarose gel showing a close match with the experimental results, when diluted soot nanoparticles (diameter < 150 nm) were employed as delivered entity. An additional simulation study demonstrated a threefold increase in the volume covered by the delivered agent in liver under a constant injection rate, when compared to without US. This method, if developed to its full potential, could serve as a cost effective way to improve safety and efficacy of drug therapies by maximizing the concentration of delivered entities within, e.g., a small lesion, while minimizing exposure outside the lesion.

© 2022 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1121/10.0012190
(Received 31 May 2021; revised 3 June 2022; accepted 13 June 2022; published online 7 July 2022)
[Editor: Bradley E. Treeby]

I. INTRODUCTION

During recent decades, high-intensity ultrasound (HIU) methods for actuation have been broadly investigated. In fact, the capability of delivering a considerable amount of acoustic energy into small targets and the ability to actuate matter from distance is of interest to a number of medical applications. HIU waves carry non-ionizing radiation, therefore mitigating safety concerns and, thus, allowing a variety of treatment strategies.1

High-intensity focused ultrasound (HIFU), an application of HIU, permits deposition of thermal energy to a focal volume leading to, e.g., ablation,2,3 hyperthermia,4,5 or drug release from thermally sensitive drug vehicles such as thermo-sensitive liposomes (TSL).6,7 Primarily non-thermal applications of HIFU include sonoporation,8,9 sonophoresis,10,11 and drug transportation.12 Other approaches are based on the use of exogenous microbubbles (MB) which, upon undergoing inertial cavitation, further enhance the drug delivery effect by means of microjet formation that arises from collapsing bubbles.13 In addition, it has recently been demonstrated that when the MBs are loaded with drug carriers, such as nanoparticles, the molecular uptake into tumor cells was increased, resulting in an overall improvement of the therapeutic effect.14,15 However, the limitations of these approaches include unwanted biological effects potentially induced on the tissue, e.g., thermal effects caused by the ultrasound (US) absorption of biological tissues, thermal and mechanical effects induced by cavitation,16 and the difficulty to generate a proper focus at certain anatomical regions due to the acoustic shadowing of bones and presence of gas in the respiratory system.17 For example, tumors located at the hepatic dome are difficult to treat, because the right lower lobe of the lung, containing air preventing sound propagation, partially covers this area, hence limiting targeted delivery of acoustic energy. In order to overcome this issue, the artificial pleural effusion medical procedure has been widely used to facilitate the HIFU treatment of tumors in the hepatic dome. However, this procedure is highly invasive, risky, and can lead to complications, thus it requires an experienced operator.18 Also, the reflected acoustic energy may cause skin burns if uncontrolled,19 for which reason the invasive procedure of partial rib resection has even been recently proposed and evaluated as a way to create an acoustic pathway for HIFU treatments.18 Moreover, the translation of organs during breathing cycles can impact the precision, safety, and efficiency of the HIFU treatment methods.20

Given these limitations, percutaneous injections via hypodermic needles still provide an alternative and cost-effective technique to administer drugs, large molecules, nano-, and microparticles for the treatment of pathologies within organs that are hardly accessible with US. However,
considering the importance and extensive use of hypodermic needles, limited research has been conducted to explore how the combination of medical needles and nonlinear ultrasonics (NLU) could add value to medical applications such as enhanced drug delivery. Conventional approaches such as localized injection techniques rely on spreading the therapeutic agent uniformly and in a great volume within the target. However, most of the percutaneous injection techniques have limitations such as the low absorption rate of the substance and the restricted amount of drug that has to be administered, often resulting in limiting the therapeutic effect;\textsuperscript{21} thus the improvement of the current needle designs and functionality must be considered.\textsuperscript{22} New ways for delivery may be beneficial for the treatment of soft tissue pathologies with localized and targeted strategies\textsuperscript{23} in the liver as well as other anatomical locations.

In a recent study, we demonstrated how an ultrasonically actuated hypodermic needle is able to generate acoustic streaming when the needle is actuated in water.\textsuperscript{24} The underlying mechanism for the generation of directional fluid flow might be associated with the presence of multiple sharp edges at the bevel locations, which are known to create intense steady-state flows when oscillating at low frequencies (<20 kHz) and ultrasonic frequencies (>20 kHz).\textsuperscript{25} This phenomenon, usually referred to as sharp-edge streaming, has been widely studied and extensive literature can be found on the topic. On a microscale, oscillating sharp structures are able to induce strong fluid flows around their tip, which can be exploited in microfluidic applications for the manipulation of cells,\textsuperscript{26,27} homogeneous micromixing of fluids within microfluidic channels,\textsuperscript{28–30} and for creating micropumps with controllable flow rates.\textsuperscript{31} Ultrasonically excited hypodermic needles (f = \(58–130\) kHz) having outer diameters in the range of 0.6–0.8 mm have been also used with a similar intent, e.g., to trap and rotate small particles,\textsuperscript{32,33} to promote the generation and manipulation of small water droplets at the tip of a hypodermic needle,\textsuperscript{34} or to increase the concentration of micro- and nanomaterial at specific locations along the needle shaft by means of acoustic-streaming-induced micro-vortices.\textsuperscript{35,36} Therefore, we hypothesize that the exploitation of directional fluid flows generated by an ultrasonic needle might be beneficial for addressing some of the limitations associated with standard needle injections in soft tissues, by further enhancing the transportation of fluids and nanoparticles within the target.

In this study, we investigate the capability of an ultrasonically actuated medical needle\textsuperscript{24} to translate nanoparticles and fluids in soft tissue under the action of NLU phenomena. The hypodermic needle, when used in combination with a pressure source such as a syringe, is employed to act as a fluid conduit. This allows operators to control the deposit of high volumes of fluid into the target location. When actuated with US, the needle also serves as a waveguide, which conducts the ultrasonic power to the target.\textsuperscript{24} We, therefore, aim to demonstrate how a vibrating hypodermic needle is capable of generating acoustic streaming, which can be used to enhance the drug distribution within a target [Fig. 1(a)]. Numerical soft tissue modeling is used to simulate the acoustic and flow fields generated by the ultrasonic needle in a porous medium. The numerical results are then validated experimentally by comparing the penetration front distribution of the tracer over time in different concentrations of agarose gels with similar settings as those from the simulation environment. The potential of using US in combination with a hypodermic needle for the delivery of fluids and entities is finally studied numerically in a liver tissue model, exploring its potential in improving percutaneous ethanol injection (PEI) for the treatment of liver cancer.

II. METHODS

A. Numerical simulations

Figures 1(b)–1(d) represent the physical model adopted in the simulations. The schematics depicted in Fig. 1(b) show a cross section of the three-dimensional (3D) replicate of the device used in the experiments, comprising a Langenvin transducer, an S-shaped waveguide, and a 21 G × 80 mm hypodermic needle. The needle tip is partially placed into a 10 mm × 12 mm cylinder which is considered to be a porous medium, where the different equations are solved by using the computational software COMSOL Multiphysics v5.5.\textsuperscript{37}

The different physics involved in the finite element model (FEM) are the following: electrostatics in the piezolectric stack and its respective stress-charge constitutive relation ("Electrostatics" module); elastodynamics in all domains except for the sample domain ("Solid Mechanics" module); acoustics ("Pressure Acoustics, Frequency Domain" module), fluid dynamics ("Brinkman Equations" module) and solute transport within the sample domain ("Transport of Diluted Species in Porous Media" module). First, the equation of motion within the ultrasonic device, the acoustic pressure field, and the acoustic velocity field inside the sample domain are solved in the frequency domain by applying a potential difference of 15 V across the faces of the piezoelectric rings. Then, the stationary fluid flow within the porous domain is then evaluated by solving the Brinkman equations.\textsuperscript{38} In order to couple the acoustics domain to the equations of fluid motion, the Reynolds stress, which arises from the sound attenuation in the fluid and is responsible for generating streaming,\textsuperscript{39} is considered as the force term in such equations. Finally, the equations governing the transport of diluted species inside a porous medium are solved in the time domain. In this step, in order to account for the solute transport by convection, the solution of the Brinkman equations is considered to be the background flow velocity field.

Free tetrahedral elements were used to mesh the 3D model, considering at least 20 nodes per wavelength according to the speed of wave propagation in each material. The number of nodes per wavelength was considered appropriate to minimize the local approximation errors.\textsuperscript{40} The viscous boundary layer has been modeled with ten boundary layer elements with a stretching factor of 1.2. Since we were interested in analysing the acoustic-related phenomena at
the needle tip, the mesh resolution was increased in this region by applying the “Corner Refinement” and the “Refine” operator (regular refinement, number of refinements \(= 4\)) along the cutting edges of the needle tip. A mesh convergence study was performed to determine its optimal mesh size. It was found that an element size in the range 0.003–0.229 mm (maximum element growth rate \(= 1.05\), curvature factor \(= 0.2\)) in the sample domain was appropriate to obtain accurate and consistent results [Fig. 1(c)]. A detailed list of the model parameters is given in Table I.

In the following sections, the mathematical equations used in the numerical simulations are described.

1. Acoustic wave propagation

The tissue is assumed to be a viscous fluid. The acoustic propagation in a viscous fluid is described by the viscoelastic wave equation,\(^4\)

\[
\nabla^2 p - \frac{1}{c^2} \frac{\partial^2 p}{\partial t^2} + \frac{\delta}{\rho c^2} \frac{\partial}{\partial t} \nabla^2 p = 0,
\]

(1)

where \(p\) is the acoustic pressure, \(c\) is the speed of sound \((\text{m s}^{-1})\), \(\rho\) is the density of the fluid, and \(\delta\) is the sound diffusivity \((\text{m}^2 \text{s}^{-1})\). The first two terms of Eq. (1) describe the linear lossless propagation of sound in a medium, while the third term is associated with viscous losses. The time-harmonic representation of Eq. (1), known as the Helmholtz equation, can be written as

\[
\nabla^2 p + k_{eq}^2 p = 0,
\]

\[
k_{eq}^2 = \left( \frac{\omega}{c_e} \right)^2,
\]

\[
c_e = c \left( 1 + \frac{i \omega \delta}{c} \right)^{-0.5},
\]

(2)

### Table I. A table of the general parameters of the numerical model at the ambient temperature of 20 °C. The speed of sound in the agarose domain was assumed to be the same as in water.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Unit</th>
<th>Water</th>
<th>Agarose (0.5%)</th>
<th>Agarose (1%)</th>
<th>Agarose (2%)</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of sound, (c)</td>
<td>m s(^{-1})</td>
<td>1482 (Ref. 84)</td>
<td>1482 (Ref. 84)</td>
<td>1482 (Ref. 84)</td>
<td>1482 (Ref. 84)</td>
<td>1575 (Ref. 85)</td>
</tr>
<tr>
<td>Attenuation coefficient, (\alpha)</td>
<td>dB m(^{-1})</td>
<td>2.178 \times 10^{-4} (Ref. 86)</td>
<td>0.165 (Ref. 87)</td>
<td>0.330 (Ref. 87)</td>
<td>0.495 (Ref. 87)</td>
<td>1.39 (Ref. 85)</td>
</tr>
<tr>
<td>Density, (\rho)</td>
<td>kg m(^{-3})</td>
<td>998.2 (Ref. 88)</td>
<td>1003 (Ref. 58)</td>
<td>1006 (Ref. 58)</td>
<td>1012 (Ref. 58)</td>
<td>1060 (Ref. 88)</td>
</tr>
<tr>
<td>Porosity, (\epsilon)</td>
<td>–</td>
<td>–</td>
<td>0.995 (Ref. 56)</td>
<td>0.990 (Ref. 56)</td>
<td>0.980 (Ref. 56)</td>
<td>0.476 (Ref. 69)</td>
</tr>
<tr>
<td>Permeability, (\kappa)</td>
<td>m(^2)</td>
<td>–</td>
<td>2.27 \times 10^{-15} (Ref. 60)</td>
<td>1.31 \times 10^{-15} (Ref. 60)</td>
<td>1.9 \times 10^{-16} (Ref. 60)</td>
<td>1.26 \times 10^{-12} (Ref. 63)</td>
</tr>
</tbody>
</table>
where \( k_{eq} \) is the equivalent wave vector, \( c_r \) is the complex speed of sound, and \( \omega \) is the angular frequency. The sound diffusivity \( \delta \), which accounts for viscous losses in a viscous fluid, is modeled as\(^{12,43} \)

\[
\delta = \frac{2c^2 \omega}{\omega^2},
\]

(3)

where \( \omega \) denotes the sound attenuation coefficient in a specific medium. Equation (2) is solved within the sample domain and has been implemented in COMSOL by adopting the “General Dissipation” model available under the “Pressure Acoustics, Frequency Domain” module.

In order to account for the viscous losses taking place within the boundary layer generated near the vibrating needle, and particularly close to its sharp edges, where the streaming is expected to be highly localized,\(^{25,44–46} \) the vibration velocity \( \mathbf{v} \) of the acoustic field, is expressed as\(^{44} \)

\[
iv = -\frac{1}{\omega \rho} \nabla p + \delta_v \nabla^2 \mathbf{v},
\]

(4)

where \( \delta_v = \sqrt{2\nu/\omega} \) is the viscous boundary layer, being \( \nu \) the kinematic viscosity of water and \( \omega \) the angular frequency. Equation (4) has been solved in COMSOL using the “Weak Form PDE” interface within the tissue domain, where the vibration of the needle has been accounted for by imposing the acoustic velocity of the fluid at the needle boundary to be equal to the structural velocity of the needle outer boundary. The “Sound Soft Boundary” condition \((p = 0)\) was instead applied on the outer boundaries of the sample domain.

2. Fluid flow in porous medium

The fluid movement inside an agarose gel or a soft tissue is modeled as an incompressible and steady flow in a porous medium fully saturated with water. The equation of motion and continuity of the fluid based on the Brinkman model are\(^{38} \)

\[
\nabla p_s - \frac{\mu}{\varepsilon_p} \nabla^2 \mathbf{u} + \frac{\mu}{k} \mathbf{u} = \mathbf{F},
\]

\[
\nabla \cdot \mathbf{u} = 0,
\]

(5)

where \( p_s \) denotes the steady state pressure, \( \mu \) the dynamic viscosity, \( \varepsilon_p \) the porosity, \( \mathbf{u} \) the steady state velocity, \( k \) the permeability, and \( \mathbf{F} \) the force term. The acoustic streaming\(^{47} \) is assumed to be the main cause of fluid motion in the tissue interstitial spaces. Therefore, the force term \( \mathbf{F} \) accounting for the momentum transfer from the acoustic wave to the fluid is given by the following time-averaged volume force,\(^{48} \)

\[
\mathbf{F} = -\frac{\rho}{2} \text{Re}[(\mathbf{v} \cdot \nabla)\mathbf{v}],
\]

(6)

where \( \mathbf{v} \) is the acoustic velocity calculated according to Eq. (4). As the time average of a quadratic periodic variable is non-zero, the magnitude of the driving force \( \mathbf{F} \) responsible for the acoustic streaming generation will be always non-zero, when an acoustic wave is travelling in a fluid. In order to account for the first-order acoustic oscillation of the needle boundaries, when computing the acoustic streaming, the Stokes drift contribution\(^{49–51} \) has been applied as a boundary condition on the moving needle walls,

\[
\mathbf{u}_2 = -\left(\frac{\mathbf{u}_1}{i\omega} \cdot \nabla\right) \mathbf{u}_1,
\]

(7)

where \( \mathbf{u}_1 \) is the first-order acoustic oscillation at the moving boundary, and the angled brackets denote time-averaged quantity. In order to verify that the acoustic streaming forcing term described in Eq. (6) is the main nonlinear acoustic phenomenon contributing to the delivery of tracer, the contribution of the acoustic radiation force (ARF) acting on the particles alone is also evaluated by considering the negative gradient of the Gor’kov potential \( U \) as follows:\(^{52,53} \)

\[
\mathbf{F}_p = -\nabla U,
\]

(8)

where \( \mathbf{F}_p \) denotes the acoustic radiation force acting on the particles. The Gor’kov potential \( U \) is valid when considering particles whose radius is much smaller than the acoustic wavelength, and it is expressed as

\[
U = \frac{2}{3} \rho^3 \left( \frac{\langle p^2 \rangle}{\rho^2 c^2} - \frac{\langle v^2 \rangle}{2} \right),
\]

(9)

where \( \rho \) and \( c \) are the density and the speed of sound of the fluid, respectively, \( r_p \) the radius of the particles, \( \langle p^2 \rangle \) is the time average of the square of the acoustic pressure, and \( \langle v^2 \rangle \) is time average of the square of the acoustic velocity. The factor \( f_1 \) is given by

\[
f_1 = 1 - \frac{\rho c^2}{K},
\]

(10)

where \( K \) is the bulk modulus of the particles. The factor \( f_2 \) is given by

\[
f_2 = \frac{2(\rho_p - \rho)}{2\rho_p + \rho}.
\]

(11)

Since diluted soot particles have been used as a tracer during the experiments, the following mechanical properties of carbon black were assigned to the particles:\(^{54,55} \) \( r_p = 50 \text{ nm}, \rho_p = 1600 \text{ kg/m}^3, K = 2.3 \text{ GPa} \).

Equation (5) has been implemented in COMSOL by considering the “Brinkman Equations” interface within the sample domain and by performing a stationary study. The fluid phase was assumed to have properties of water, while the porous matrix was considered to be a hydrogel with properties reported in Table I. A “no slip” condition was applied to all boundaries of the sample domain and a pressure constraint \((p_s = 0)\) had been set to a point located on the outer wall of the sample domain.
3. Permeability and porosity

The porosity of the agarose gel was calculated as
\[ \epsilon_p = 1 - \phi, \] (12)
where \( \phi \) is the volume fraction of the agarose fiber expressed by
\[ \phi = \frac{c_{agar}}{\rho_{agar} \omega_{agar}}. \] (13)

In Eq. (13) \( c_{agar} \) is the agarose concentration (w/v) in the gel, \( \rho_{agar} \) is the density of dry agarose \((1.64 \text{ g mL}^{-1})\) and \( \omega_{agar} \) is the agarose mass fraction in a fiber \((0.625)\), determined experimentally by Johnson et al.\(^{59} \) The Carman-Kozeny equation was used to determine the hydrogel permeability, \( \kappa \), as follows:\(^{60} \)
\[ \kappa = \frac{\epsilon_p t_h^2}{k}. \] (14)

The hydraulic radius was assumed to be similar to the interfiber spacing, \( r_f \approx \frac{a}{2} \), and the average gel pore size, \( \bar{a} \), was determined experimentally by Narayanan et al.\(^{61} \) as a function of the agarose concentration. The Kozeny factor, \( k \), takes into account the direction of the gel interstitia considered as cylindrical pores randomly oriented in the 3D space. For hydrogels with high void volumes \( (\epsilon > 0.9) \) it is defined as\(^{62} \)
\[ k = \frac{(2k_+ + k_1)/3}{2\epsilon^3} \]
\[ k_+ = \frac{1 - \epsilon}{1 - \epsilon} \cdot \left[ \ln \left( \frac{1}{1 - \epsilon} \right) - 1 - (1 - \epsilon)^2 \right], \]
\[ k_1 = \frac{(1 - \epsilon)}{1 - \epsilon} \cdot \left[ 2\ln \left( \frac{1}{1 - \epsilon} \right) - 3 + 4 \cdot (1 - \epsilon) - (1 - \epsilon)^2 \right]. \] (15)

where \( k_+ \) and \( k_1 \) account for cylindrical inter-connected pores perpendicular and parallel to the fluid flow, respectively. Tissue permeability in the liver can be estimated by considering the tissue as comprised of spherical objects organized into a grid-like structure. Each sphere has a similar diameter to one hepatocyte \((24 \mu\text{m})\). By using the Carman-Kozeny model for flow around a spherical object,\(^{63} \) the permeability is calculated as follows:
\[ \kappa = \frac{d^2 (1 - \epsilon)^3}{180 \epsilon^2}, \] (16)

where \( d \) is the diameter of the spherical object, and \( \epsilon \) is the porosity of the tissue. The porosity can be easily obtained by calculating the void ratio in a cell containing a sphere with radius \( R \) encapsulated in a cube with length \( 2R \), which represents the repeating cell unit in the grid-like structure. This gives
\[ \epsilon = 1 - \frac{V_{sphere}}{V_{cube}} = 1 - \frac{4\pi(0.5)^3}{3} = 0.476. \] (17)

4. Solute transport in porous medium

The equation governing the diffusion of solute species in an isotropic porous medium is given by\(^{64} \)
\[ \epsilon_p \frac{\partial c_{tr}}{\partial t} + \mathbf{u} \cdot \nabla c_{tr} - D_{eff} \nabla^2 c_{tr} = 0, \] (18)

where \( c_{tr} \) is the concentration of the tracer \((\text{mole/m}^3)\), \( \mathbf{u} \) the velocity field derived from Eq. (5), and \( D_{eff} \) is the effective diffusion coefficient of the tracer in a porous medium. This is defined as
\[ D_{eff} = \frac{D_0 \epsilon_p}{\tau}, \] (19)

where \( D_0 \) is the diffusion coefficient of ink in water \((2.5 \times 10^{-10} \text{ m}^2 \text{ s}^{-1} \text{ at } 20^\circ \text{C})\), \( \epsilon_p \) is the porosity and \( \tau \) is the Millington-Quirk tortuosity coefficient,\(^{66} \) which is expressed as a function of the porosity as follows: \( \tau = \epsilon_p^{-1/3} \). Equation (18) has been solved by running a time dependent simulation (total simulated time = 600 s; time step = 0.1 s) with the “Transport of Diluted Species in Porous Media” interface.

B. Experiments

1. Hydrogel preparation

Hydrogels with agarose concentrations of 0.5, 1, and 2\% (w/v agarose powder/EDTA) were prepared by adding, respectively, 0.25, 0.5, and 1 g of agarose (catalogue number: 10377033 Agarose Low-Melting, Nucleic Acid Recovery/Molecular Biology Grade, Thermo Fisher Scientific, Waltham, MA) to 50 ml of 50 mM EDTA buffer (catalogue number: 11836714 Thermo Scientific EDTA, Thermo Scientific, Waltham, MA). The solution was heated up to 96°C, while being mixed with a magnetic stirrer (Heater and magnetic stirrer C-MAG HS series C-MAG HS 4 model, IKA, Staufen, Germany), and allowed to cool down at room temperature \((22^\circ \text{C})\). When the temperature of the solution had reached 42°C, 3 ml of agarose gel was poured into different polystyrene cuvettes \((\text{external dimensions} = L \times W \times H = 12 \text{ mm} \times 12 \text{ mm} \times 45 \text{ mm}, \text{ wall thickness} = 1 \text{ mm})\) and left to solidify for 2 h.

2. Sonication in agarose

A custom-made ultrasonic device\(^{24} \) was employed to enable flexural waves in a hypodermic needle and mediate the transport of the tracer in different agarose concentrations. The design consisted of a Langevin transducer coupled to a hypodermic needle \((21 \text{ G}, \text{ length} = 80 \text{ mm})\) (model: 4665465100 STERICAN, B Braun, Melsungen, Germany) via an S-shaped 3D printed aluminum waveguide (3D Step Oy, Ylöjärvi, Finland). The needle tip was first moistened with 40\% (v/v ink/de-ionized water) diluted ink.
boundaries are clearly visible. The first frame of the video footage in which all needle lenses were applied to the needle for a duration of 10 min by using an RF amplifier (model: AG 1012LF, Amplifier/Generator, T&C Power Conversion, Inc., Rochester, NY) in combination with a function generator (model: Analog Discovery 2, Digilent, Inc., Henley Court Pullman, WA). The total acoustic power (TP) employed in the experiments was 20 mW, which, according to our simulation, corresponded to a needle oscillation displacement of 16 \mu m and a maximum pressure amplitude of \sim 250 kPa. The needle action was imaged throughout the duration of the experiment with a high-speed camera (model: Phantom V1612, Vision Research, Wayne, NJ) in conjunction with a macro lens (model: Canon MP-E 65 mm f/2.8 1–5x Macro Photo, Canon Inc., Ōta, Tokyo, Japan) using the following settings: sample rate = 100 fps, exposure = 9900 \mu s, resolution = 768 pixels \times 768 pixels, lens aperture = f/16. Eventually, the projected area of the tracer distribution over time was quantified and analyzed in MATLAB (R2020b)\textsuperscript{67} from the recorded images as follows:

\begin{equation}
A_{\text{tracer}}(x, y) = \int I_{\text{bw},0}(x, y) - I_{\text{bw},1}(x, y) \, dx \, dy,
\end{equation}

where \( I_{\text{bw},0}(x, y) \) are binary frames generated by thresholding the frame-set \( I(x, y) \) with the Otsu method\textsuperscript{68} and \( I_{\text{bw},1}(x, y) \) is the first frame of the video footage in which all needle boundaries are clearly visible.

III. RESULTS

Figure 1(b) represents the geometry of the 3D model employed in the simulations. In this design, the waveguide converts the longitudinal motion provided by the transducer to a flexural movement of the needle. This creates flexural standing waves in the needle with greatest displacement at its tip. By this means, the needle tip is made to oscillate sideways within the \( xz \)-plane, acting as a dipole-like sound source. The emitted sound is anticipated to exert an acoustic radiation force on the liquid, promoting the convection of fluid through the interstitia of the porous medium. The model was validated by comparing the tracer delivery dynamics calculated numerically, i.e., cross-sectional area of tracer distribution, expansion velocity of the penetration front, and tracer concentration profiles, to those measured experimentally, i.e., projected area of tracer distribution, expansion velocity of the penetration front and tracer absorbance profiles.

A. Characterization of delivery in different agarose gel concentrations

Hydrgels with agarose concentrations of 0.5, 1, and 2\% (w/v, agarose/EDTA) were subjected to 10 min of sonication applied with the ultrasonic needle, while the needle tip was moistened with 40\% (v/v, ink/de-ionized water) and inserted 5 mm deep into the specimen. The projected area of the spatial distribution of the tracer was imaged throughout the experiment and quantified during the post processing. Figure 2(a) represents different time frames of the ultrasonic mediated delivery of nanoparticles in hydrogels acquired at 0, 100, 400, and 600 s from the beginning of the sonication. The projected area of nanoparticle distribution increased over time, covering an area of 1.85 mm\textsuperscript{2} on average after 10 min in 0.5\% agarose gel [Fig. 2(b)]. As the gel concentration increases, the area of distribution decreases, measured to be 1.35 and 0.35 mm\textsuperscript{2} in 1 and 2\% agarose gel, respectively. These results were in line with the numerical simulations, where the cross section of the 3D model exhibited pronounced distribution of nanoparticles around the needle tip, which grew over time [Fig. 2(c)]. Similar to the experimental observations, the delivery of nanoparticles (diameter < 150 nm) was confined to a smaller region when higher concentrations of agarose gel were used [Fig. 2(d)]. Both the experimental and numerical results did not exhibit any significant passive diffusion of the tracer in the agarose gels across a 600 s time window, when no acoustic power was applied to the needle.

Figure 3(a) depicts the absorbance map of a 0.5\% agarose specimen after the sonication was applied. The color intensity represents the light absorbance calculated as \( A = \log_{10}(I_0/I) \), where \( I_0 \) is the intensity of the incident light, considered as the background pixel intensity, and \( I \) is the intensity of the light after it passed through the sample. A black polygon showing the needle contour has been overlapped on the absorbance map since the absorbance values in that region are not representative of the tracer concentration. After 10 min of sonication, the penetration front of the tracer extended to 0.6 mm on both sides of the needle tip along the \( x \)-axis and 0.6 mm from the tip along the positive \( z \)-direction. Considering the simulated concentration map assessed on a cross section passing through the needle centerline and parallel to the \( xz \)-plane, the tracer expanded to 0.7 mm radially from the tip along the \( x \)-axis and to 0.2 mm along the positive \( z \)-direction [Fig. 3(b)]. The spreading velocity of the penetration front, evaluated on a line parallel to the \( x \)-axis with an offset of \(-0.5\) mm, was on average 2.2 \mu m s\textsuperscript{-1}, calculated across a time window of 100 s, while after 200 s was found to spread at a constant velocity of 0.6 \mu m s\textsuperscript{-1} [Fig. 3(c)]. Numerically, these velocities were calculated to be 2.6 and 0.5 \mu m s\textsuperscript{-1} across the same time windows [Fig. 3(d)]. Figures 3(e) and 3(f) show a 3D reconstruction of the simulated volume diffused by nanoparticles after 10 min sonication, revealing that the delivery is localized at the needle tip. The side and front view suggest that the delivery action is predominantly within the \( xz \)-plane [Fig. 3(e)] and marginal on the \( yz \)-plane. This has to do with the flexural mode induced in the needle, which is made to oscillate sideways within the \( xz \)-plane, with the greatest displacements at the tip. By this means, the tip acts as a dipole-like sound source, generating an acoustic intensity vector.
field with the highest magnitudes located at the needle tip and with the same direction of needle motion.

B. Numerical simulation of delivery in a liver tissue model

In order to demonstrate that the ultrasonic actuation of a hypodermic needle can bring contribution to a clinical scenario, we simulated the delivery action in a simplified model for liver, which represents a target common in different clinical applications. The liver was modeled as a porous medium, whose permeability and porosity values were estimated using a Carman-Kozeny model, which assumes flow through a bed of spherical objects with comparable radius to one of the hepatocytes. Simulations were carried out by considering a needle injection rate of 10 L/min with delivered total TAP of 0 W and 20 mW throughout 10 s. The temporal variation of the volume diffused by the nanoparticles was investigated when a normal injection was simulated, the drug was spread only on the side of the needle opening. However, when a TAP of 20 mW was employed, the drug spread more evenly around the needle tip. The volume of nanoparticle distribution increased at an average rate of 0.31 mm^3 s^{-1}, when a flow rate of 10 L/min was applied. Yet, in conjunction with US, it was estimated to be 0.95 mm^3 s^{-1}. This resulted in a threefold increase in the total volume of delivery.

The ultrasonic actuation of the needle contributed to a fluid velocity field highly localized at the very close proximity of the needle tip. The maximum stream velocity was approximately 10 mm s^{-1} within 100 μm from the tip along the x-axis, almost five orders of magnitude greater than the average velocity within the needle shaft caused by the externally applied flow rate. The simulated acoustic...
intensity map revealed that the maximum acoustic intensity is located near the tip, emanating outwards from the needle centerline along the $x$-axis direction [Fig. 4(d)]. Since the acoustic streaming is proportional to the acoustic intensity, higher stream velocities within the same plane were also generated.

1. Characterization of acoustic streaming and Gor’kov potential

Figure 5 depicts the evaluation of some physical quantities on different cross-sections of the model parallel to the $xy$-plane located at $z = 0, 0.5, -1, -1.5 \text{ mm}$ (Fig. 5). Close to the tip of the needle ($z = -0.1 \text{ mm}$), the pressure amplitude could be as high 120 kPa, decreasing down to 70 kPa at a distance of 100 $\mu\text{m}$ along the positive $x$-direction from the needle wall [Fig. 5(a)]. At $z = -0.5 \text{ mm}$, both sides of the needle radiated acoustic pressure of similar amplitude, being 120 kPa close to the needle walls and $\sim 70 \text{ kPa}$ within 200 $\mu\text{m}$ from the needle walls. Since the needle vibrates along the $x$-direction, it can be noted how the generated acoustic field resembles the one produced by a dipole sound source, having the minimum amplitudes located on a surface perpendicular to the direction of the needle motion. At $z = -1$ and $-1.5 \text{ mm}$, the pressure amplitudes can be as high 250 kPa, with maxima located close to the concave part of the needle.

We sought to confirm whether the delivery of nanoparticles is dominated by the acoustic streaming effect or the ARF acting on the particles. To do this, the Gor’kov potential [calculated as Eq. (9)] describing the level of attraction of the nanoparticles, and the force acting on the fluid responsible for the generation of streaming, were evaluated and shown in Figs. 5(c) and 5(d), respectively. According to Fig. 5(b), the distribution of the Gor’kov potential around the needle tip would suggest that the particles tended to be repelled away from the needle outer walls. Also, the direction of the force field indicated that the liquid was always pushed outwards from the needle boundary, with greater forces localized near the cutting edges [Fig. 5(c)]. However, the force acting on the liquid [calculated as Eq. (6)] was two
orders of magnitude greater compared to the force acting on the nanoparticles alone [calculated as Eq. (8)], revealing that the latter brings a negligible contribution to the nanoparticle transport.

If one considers the acoustic streaming pattern generated at $z = 0.1$ [Fig. 5(d)], it can be noticed that high streaming velocities are highly localized at the needle cutting edges, characterized by maximum velocities of $10 \text{ mm s}^{-1}$. The streamlines indicate that a fluid flow emanating outwards along the positive $x$-direction was formed, while fluid vortices are generated near the sharp edges. Similar phenomena can be observed, when considering other cross-sections of the needle geometry located at $z = -0.5, -1, -1.5 \text{ mm}$, suggested by directional and slow movement of fluid originating from the sharp edges and flowing outwards from the needle walls [Fig. 5(d)].

A closer view of the physical quantities is given in Fig. 6, where close-up views near sharp edges are presented, identified with red squares in Fig. 6(a). Gor’kov potential and ARF exhibited high magnitudes very close to the sharp edges, with velocity vectors pointing radially away from such structures [Figs. 6(b) and 6(c)]. Correspondingly, the acoustic streaming was extremely localized at the sharp edges, exhibiting outflow of fluids emitting along the centerline of the sharp tips, and giving rise to eddies on the sides [Fig. 6(d)].

**IV. DISCUSSION**

These results suggest that actuating a hypodermic needle with US allows one to enhance the delivery of nanoparticles and fluids in porous media, such as soft tissues. The resemblance between the numerical and experimental results of the transport velocities and tracer distribution within the sample indicates that convection and diffusion contribute to the transport mechanisms of nanoparticles in a porous medium. The tapered double-bevelled structure of the needle provides greater needle displacements at its tip than elsewhere, therefore concentrating the acoustic intensity and amplifying the acoustic radiation pressure associated phenomena (i.e., acoustic radiation forces).
FIG. 5. Post-processing of 3D simulation results. (a) Representation of the absolute pressure field radiated from the needle boundary (oscillation direction parallel to the $x$-axis), visualized on cross-sectional planes of the needle geometry taken from locations $z = -0.1, -0.5, -1, -1.5$ mm. (b) represents the Gor’kov potential, which denotes the level of attraction of the nanoparticles within the acoustic field and (c) represents the ARF acting on the liquid, which gives rise to streaming patterns (d). (b) and (c) are normalized by their respective global maximum values identified in the 3D model. In (b), the streamlines denote the acoustic radiation force $F_p = -U U$ acting on the nanoparticles alone, being $U$ the Gor’kov potential, while the color shows the magnitude of Gor’kov potential. The resulting force acting on the nanoparticles due to the Gor’kov potential has been calculated as in Eq. (8) and compared with the ARF acting on the liquid [calculated as Eq. (6)]. The ARF acting on the liquid was measured to be two orders of magnitude greater compared to the force acting on the nanoparticles alone, revealing that the latter brings a negligible contribution to the acceleration of nanoparticles and therefore negligible effect on transport. This suggests that the main contributor to the transport is the acoustic streaming.
Evaluation of acoustic variables around the needle tip (close-up view)

FIG. 6. Acoustic variables post-processed from 3D simulation results. (a) Representation of the absolute pressure field radiated from the needle boundary (oscillation direction parallel to the x-axis), visualized on cross-sectional planes of the needle geometry taken from locations \( z = -0.1, -0.5, -1, -1.5 \) mm. (b), (c), (d) Close-up views near the sharp edges of Gor’kov potential, ARF and streaming patterns, respectively. The zoomed-in regions of the needle geometry have been identified with red squares in a. In (b), the arrows denote the acoustic radiation force \( \mathbf{F}_p = -\nabla U \) acting on the nanoparticles alone, being \( U \) the Gor’kov potential, while the color shows the magnitude of Gor’kov potential. High magnitudes of Gor’kov potential and ARF can be observed close to the sharp tips, as well as high streaming velocities concentrated at the sharp edge locations.
streaming) at this very location. Since most of the acoustic energy is confined at the proximity of the needle outlet [Fig. 4(d)], liquids or particles are allowed to be influenced by US, while being injected into the tissue, making this concept an interesting starting point for developing novel drug delivery systems combining needles and ultrasonic actuation.

A. Acoustic streaming

Since the acoustic wavelength (~45 mm) at the employed frequency of 33 kHz is considerably larger than the diameter of the nanoparticles (<150 nm), the acoustic streaming is considered to be the main nonlinear acoustic phenomenon contributing to the delivery rather than acoustic radiation force directly pushing the particles. In fact, since the ratio between the acoustic streaming induced particle velocity and the ARF induced particle velocity is proportional to the square of the particle diameter, \( \frac{d}{C_{24}} \), the ARF contribution can be neglected for particles smaller than 150 nm in diameter.51 This has also been confirmed by evaluating the ARF acting on the liquid, which gives rise to streaming, and the ARF acting on the particles alone, which is derived from the negative spatial gradient of the Gor’rekov potential. Since we have found that the latter was two orders of magnitude smaller compared to the ARF acting on the liquid, we have concluded that the force acting on the nanoparticles can be neglected and that the acoustic streaming seems to have a greater influence on the delivery effect.

B. Comparison with prior literature

Considering the acoustic streaming pattern generated by a vibrating hypodermic needle (21 G, outer diameter ~ 0.8 mm) and that generated by a simple wedge,25-27,29-31,44,45 some similarities can be noted. In such studies, the streaming seems to be localized exactly at the tip with the direction of the flow parallel to the axis of symmetry of the sharp structure. Similarly, in our study, the generated streaming was the strongest at the cutting edges [Fig. 5(b), \( z = -0.1, -0.5, -1 \) mm]. Moreover, the outflow of fluid emitting along the centerline of the sharp tips gives rise to eddies on the sides, which is typical in the sharp edge streaming. In accordance with finding from previous numerical studies,25,26,45 it can also be noted how the streaming directivity is dependent on the sharp tip angle. This can be observed for example in Fig. 5(b) at \( z = -1.5 \) mm, where the wide angle of the needle edge (~ 120°) causes the fluid flow to be mainly oriented parallel to the needle boundary, rather than emitting along the centerline of the tip [Fig. 5(b), \( z = -0.1, -0.5, -1 \) mm]. However, these strong streaming patterns enabled directional fluid flow along the positive x-axis by means of convection, which helped in translating the nanoparticles along the direction of needle vibration. Eventual disparities between our results and those found in the literature might be attributable to the complex geometry of the needle tip and the porous medium, where the streaming is assumed to take place. In fact, the geometry of a hypodermic needle is not closely comparable to that of a conical sharp edge. A hypodermic needle contains multiple sharp edges (cutting edges) which have different inclinations with respect to the needle motion. In addition, since the fluid is constrained to flow within the interstitia of the porous medium, the generated streaming is expected to be more restrictive if compared to that generated in a free fluid.24 Nevertheless, these findings are important as they raise the question of whether the shape of a hypodermic needle can be re-designed in order to generate the desired streaming patterns in specific locations of the needle tip, thus improving the delivery efficacy.

C. Cavitation

Potential cavitation activity or crack formation in the agarose specimen that might have contributed to enhancing the delivery effect were ruled out by reducing the TAP (~ 20 mW). Based on our numerical simulation, the pressure amplitudes induced in the vicinity of the needle at this power level can be as high as 250 kPa, which could be enough to enable cavitation events in water according to previous experimental results.70,71 However, given that the pressure maxima are located close to the needle wall and decay quickly with distance, potential presence of cavitation activity taking place within the needle and sample domain cannot be excluded. In addition to the discussed phenomena, microstreaming arising from cavitation activity taking place in the liquid/needle interface could be in part responsible for the observed convective transport.

D. Safety considerations

Considering safety, the adopted frequency was relatively low compared to the MHz frequency typically employed in HIFU applications. Importantly, this limits the US-induced temperature increase. In fact, the acoustic absorption coefficient was estimated to be less than 0.5 dB m\(^{-1}\) at 33 kHz in agarose. In addition, the US exposure of the agarose specimen did not exhibit any signs of cavitation or cavitation-induced liquefaction, as confirmed optically from the high-speed video footage. This suggests that the combination of the employed TAP and sonication duration is below the threshold for deleterious cavitation events. However, these effects are minimized further away from the needle, as the acoustic intensity geometrically decays by \( 1/r^2 \), with \( r \) being the radial distance from the sound source, if one assumes spherical wave propagation of the US wave. Increased levels of TAP increase the probability of cavitation occurrences. If these phenomena were to take place in tissue, thermal and mechanical damage would occur in the regions where the acoustic intensity is greatest. Although this may represent a limitation when treating healthy tissues, conversely it might be beneficial for clinical procedures for tissue ablation, including solid organ cancers of the prostate,72 thyroid,73 pancreas,74 and many more.75

E. Potential medical applications

The idea of providing the acoustic energy locally with a conventional hypodermic needle to drive fluids and entities inside the body is first demonstrated in this study and further
improvements could be beneficial for drug delivery applications. This could be an alternative, minimally invasive, and low-cost approach to expensive ultrasonic clinical methods, such as magnetic imaging-guided HIFU, used for image-guided delivery of drugs into a target location. Specifically, this method could add value, e.g., to the common liver cancer therapy of PEI. This technique consists of injecting ethanol into the tumor in order to achieve complete ablation of hepatocellular carcinoma. The PEI procedure is usually preferred to the non-invasive HIFU approach when the tumor is located in the upper part of the liver. Since the rib cage and the lower part of the right lung partially cover this region anatomically, the formation of a proper ultrasonic focus in this area is difficult to achieve due to the poor acoustic window. The ultrasonic actuation of the vibrating needle would help in distributing ethanol locally and evenly within the tumor, by exerting a pushing force on the liquid surrounding the needle tip, thus promoting the generation of steady fluid flows. This is supported by the numerical results, which showed a threefold increase in the volume of tissue diffused by the tracer when the needle was actuated for 10 s at a TAP of 20 mW under an applied flow rate of 10 µL min⁻¹, as compared to when no US was employed. This could potentially increase uptake of fluid caused by the enhanced delivery localized near the needle tip. These benefits could potentially bring added value to this technology for PEI and similar procedures, such as precision delivery of nanoparticles as personalised anticancer drugs and radiological contrast for discrete organ imaging in preparation for surgical resection.

More generally, the local administration of drugs, combined with the ultrasonic action provided by the vibrating needle, might be advantageous compared to other commonly used routes of drug administration for cancer treatment, i.e., intravenous (IV) injection. When administered intravenously, the drug may interact with different organs, potentially giving rise to side effects. The drug may be also cleared away from the systemic circulation prematurely without reaching the tumor site, resulting in a reduced therapeutic effect. Intravenous drugs often accumulate in healthy tissues and cause toxicity. Conversely, by injecting the therapeutic agent intratumorally with an ultrasonically actuated needle, one could mitigate the aforementioned issues and also benefit from enhanced drug transport mechanisms induced by the ultrasonic action of the vibrating needle. The acoustic energy would be released directly from the needle tip, which acts as a dipole-like sound source within a target inside the body, rather than needing a sound source placed externally. This may overcome limitations related to poor beam formation and the presence of inconvenient anatomical structures, such as bones and intraluminal gas, which disrupt HIFU-based applications. Besides, considering the limited hardware needed to actuate a conventional medical needle, this solution could constitute an inexpensive and readily accessible alternative.

Based on the numerical results provided in Fig. 4(b), the ultrasonic actuation of the needle was able to increase the volumetric flow rate of the tracer up to 0.9 mm³ s⁻¹. The induced volumetric flow rate would be equivalent to a pumping pressure of 245 Pa, while it is estimated to be 95 Pa, when no US is employed. This suggests that the streaming generated by the vibrating needle tip could potentially enable a pumping behaviour through the needle conduit towards the open end of the needle. Although we have no evidence yet to support this claim, one could speculate that the dimension and the shape of the needle tip might influence the efficacy of the pumping effect, allowing one to generate tunable flows with minimal hardware requirements for a pump.

The presented technology, if optimized to its full potential, could be valuable for other medical applications. At high TAP levels, the needle vibrations are expected to induce cavitation and thermal effects. These can be of use in applications such as tumor ablation, histotripsy, or lithotripsy, where the objective is to cause mechanically pathological tissue destruction. The ability to deliver acoustic energy in a localized manner, while bypassing anatomical structures can be employed for the ablation of lung lesions, which are seldom treated with US due to the presence of air that represents an obstacle for the US wave propagation. Moreover, in our recent study, we have demonstrated that the very same technology can be used to enhance the sample yield during fine needle aspiration biopsy (FNAB), making this device clinically versatile and very promising for novel medical use. The results of the present study extend to broaden the understanding of fluid dynamics near the ultrasonically actuated medical needle, e.g., in the context of ultrasonically enhanced biopsy. Such finding could open up an avenue of research application where FNAB and delivery of substances are combined, e.g., delivery of contrast agent to enhance the needle visibility in US-guided FNAB.

V. CONCLUSION

We have demonstrated numerically that the ultrasonic actuation of a medical needle enhances delivery of nanoparticles and liquids in porous media, e.g., soft tissues with porous structure, at the proximity of the needle tip. The delivery action was also observed experimentally in different agarose concentrations, with similar results to those obtained in the computational model. Based on the assumptions made in the numerical model and the similarity between the numerical and experimental results, it appears that enhanced convection by acoustic streaming is one of the main factors of the nanoparticle transport mechanisms in porous media. A simulation study considering the enhanced delivery in a liver model was also conducted in order to demonstrate its potential for biomedical research and clinical applications. As percutaneous injections are frequently conducted under US guidance in the clinic, we must eventually evaluate our technology in combination with simultaneous US imaging to optimise compatibility with established clinical procedures. To conclude: this method, if fully developed, could provide minimally invasive and
localized drug delivery for small lesions in a safe, portable, and cost-effective manner, while minimizing unnecessary drug exposure to adjacent organs.

ACKNOWLEDGMENTS

We thank all members of the Medical Ultrasonics Laboratory (MEDUSA) at Aalto University for constructive discussions related to the topic. The Academy of Finland is acknowledged for financial support (Grant Nos. 314286, 311586, and 335799). All authors contributed to the design of the study, writing, or reviewing the manuscript, and have approved the final version of the manuscript. E.P. produced all data and conducted the data analysis. H.J.N. and K.P.H.P. have stock ownership in Swan Cytologics Inc., Toronto, ON, Canada, and are inventors within the patent application WO201800102A1. H.J.N. is also an inventor within the patent application WO2020240084A1. E.P. and N.H. do not have any competing interests in relation to this work. The datasets are available upon request. The codes are available upon request.
