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Multi-modal particle manipulator to enhance bead-based bioassays

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Abstract

By sequentially pushing micro-beads towards and away from a sensing surface, we show that ultrasonic radiation forces can be used to enhance the interaction between a functionalized glass surface and polystyrene micro-beads, and distinguish those that bind to the surface, ultimately by using an integrated optical waveguide implanted in the reflector to facilitate optical detection. The movement towards and immobilization of streptavidin coated beads onto a biotin functionalized waveguide surface is achieved by using a quarter-wavelength mode pushing beads onto the surface, while the removal of non-specifically bound beads uses a second quarter-wavelength mode which exhibits a kinetic energy maxima at the boundary between the carrier layer and fluid, drawing beads towards this surface. This has been achieved using a multi-modal acoustic device which exhibits both these quarter-wavelength resonances. Both 1-D acoustic modeling and finite element analysis has been used to design this device and investigate the spatial uniformity of the field.

We demonstrate experimentally that 90% of specifically bound beads remain attached after applying ultrasound, with 80% of non-specifically bound control beads being successfully removed acoustically. This approach overcomes problems associated with lengthy sedimentation processes used for bead-based bioassays and surface (electrostatic) forces, which delay or prevent immobilization. We explain the potential of this technique in the development of DNA and protein assays in terms of detection speed and multiplexing.

Keywords: acoustic radiation forces; bio-sensor; frequency switching; micro-beads; optical waveguide

1. Introduction

Bead-based assays are becoming increasingly important in microfluidic sensor systems with the advantage that the bead surface forms a disposable sensing element and high bead surface area coupled with active mixing. These assays may be realized using magnetic beads held in devices using magnetic forces [1], however acoustic radiation

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forces can be used to manipulate a wider range of beads, including magnetic and non-magnetic beads, as they rely on acoustic properties only, as well as enabling finer control over bead position. Despite the advantages that acoustically enhanced bioassays could bring, it remains a challenge to move beads consistently to the detection area and in a uniform pattern. Thus a thorough understanding of the 2-D nature of the acoustic field in such a system is needed to ensure that beads can be suitably controlled. In this paper we investigate the axial acoustic field distribution using a 1-D acoustic impedance transfer model [2], but also the lateral variation of the field is modeled in detail using finite element analysis. Thus the uniformity and suitability of the 2-d field may be judged. To aid this assessment a simulation of force potential, $\langle \phi(r) \rangle$, is used to provide a means of predicting bead migration.

2. Device Design

2.1. General

In order to enhance bead-based assays, it is proposed that acoustic radiation forces are used to a) push beads to the detection surface and b) discriminate between beads that bind to the surface and those that don't by subsequently repelling unbound beads. Fig.1 illustrates two acoustic modes which provide these two functions. Firstly, Fig.1(a) shows a "to-reflector" quarter-wave mode can be used to move beads to the detection surface and secondly Fig.1(b) shows a "to-carrier" quarter-wave mode to force beads off the surface. In both cases, there must be sufficient radiation force at the wall to enhance binding and overcome surface forces, respectively, which dictates where pressure nodes and antinodes are positioned in relation to the surface (noting that radiation force is typically zero at these nodal points). Fig.1 therefore also indicates how these nodal points have been positioned some way into the reflector surface to ensure that beads are forced onto surface.

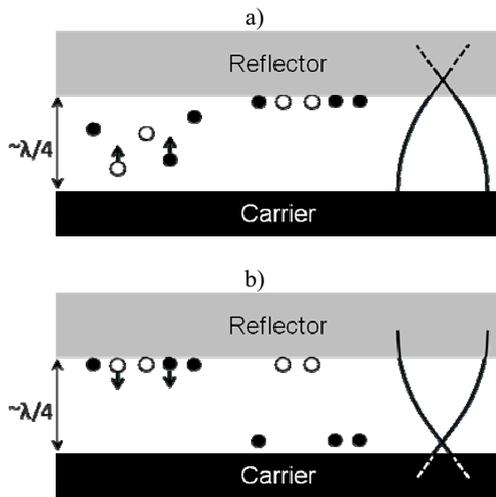


Fig.1 Multi-modal function of device showing force on beads, final location and pressure field for a) "to-reflector" mode; beads and immobilized bio-molecules move up to waveguide surface whereupon functionalized beads (O) will attach to the reflector surface, and b) "to-carrier" mode; unbound beads (●) are pulled away and towards the carrier layer surface.

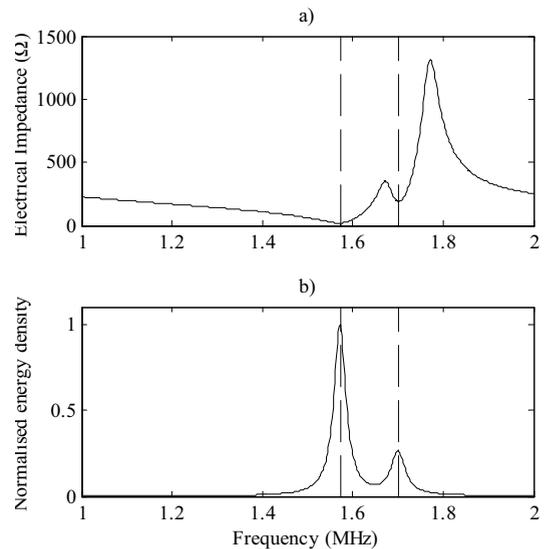


Fig.2 Matlab 1-D model results for a) electrical impedance and b) acoustic energy density within the fluid layer. Dashed lines correspond to predicted resonant modes at 1.572MHz and 1.700MHz.

2.2. Transfer Impedance 1-D Model

The final design was arrived at through successive iterations, investing the behavior as predicted by a 1-D transfer impedance model [2] implemented in Matlab. This represents the structure as a layered resonator with layers of infinite extent, thus ignoring any lateral acoustic modes.

This is sufficiently accurate, however, to arrive at a suitable multi-modal design. The transducer representation used by Hill et al. [2] was replaced by a KLM circuit model [3] extending the operating frequency of the model beyond the first resonance. For each design iteration the average acoustic energy density in the fluid layer versus frequency is inspected; this allows all the resonances supported by that configuration to be identified and examined to determine their resultant acoustic force potential. Of the modes described in Fig.1, the reflector quarter-wave mode is found to be the most sensitive to geometry, so it was easier to start the process with a design that easily supported this mode, with subsequent changes aiming to enhance the strength of the carrier layer quarter-wave mode.

These design iterations produced the final design, the geometry of which is recorded in Table 1. Troughs on the resulting modeled impedance data and peaks in the acoustic energy density clearly show two modes (Fig.2). These correspond to the carrier quarter-wave and reflector quarter-wave modes which are located at 1.57 and 1.70MHz respectively. The smaller energy density associated with the reflector quarter-wave mode is indicative of the design challenge that this mode presents.

Table 1. Geometry of device

Layer	Material	Thickness (μm)	Density (kg/m^3)	Sonic velocity (m/s)
Transducer	PZ26	1000	7700	4529
Carrier	Alumina	650	3860	10520
Fluid	Water	162	1000	1480
Reflector	BK7 glass*	1762*	2500	5872

*Thickness and properties chosen for optical detection system.

2.3. Finite Element (FE) Model

In order to gain a more detailed understanding of the behavior of the device, including lateral forces, an axisymmetric finite element model was implemented using the ANSYS package.

2.3.1. Construction of Model

The modeling of lateral fields has been described by Neild et al. [5], Manneberg et al. [6], Lilliehorn et al. [7] and Townsend et al. [8], and this work extends that of Townsend, incorporating a finite element representation of the transducer and using the results to visualize the force distribution in three-dimensions. Even in layered resonator designs which are most suited to a one-dimensional approach, significant lateral variations in acoustic radiation forces can be observed [8], which finite element analysis could be used to model.

A full verification of this Finite Element approach is beyond the scope of this paper, but Fig.3 represents some initial verification: it compares the transfer impedance model to a finite element model with boundary conditions set to mimic layers of infinite extent. The finite element model is based in ANSYS, and uses a strip of FLUID29 (2-D harmonic acoustic) elements; carrier and reflector layers are represented by 2-D structural solid elements, PLANE42; and the PZT by the piezoelectric formulation of the 2-D Coupled-Field Solid PLANE13. Varying the parameters of the transmission line model, it is found that for the parameters considered here, the air beyond the

reflector and transducers has little effect on the energy density profile, and is not included in the ANSYS model. The acoustic fluid elements are not formulated to allow any fluid damping in the fluid body, which makes predictions of acoustic amplitudes less accurate, particularly for half-wave devices where the fluid damping is important, however in the future, other element types such as FLUID79 which can include fluid damping will be explored in the future. All materials data is taken from manufacturers' data tables.

For the axisymmetric model shown in Fig.4, the same element types were used; the additional spacer layer was also represented by PLANE42 elements. In order to mimic the energy scattering nature of the gasket, the right-side of the fluid layer is bounded by a 50% absorbing boundary condition.

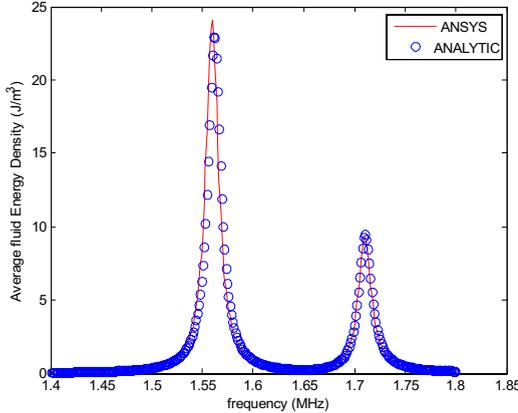


Fig.3 Predicted acoustic energy density for 1-d system and comparison between FE and transfer impedance models.

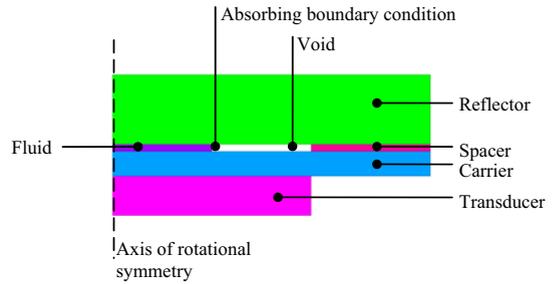


Fig.4 Construction of FE model (ANSYS).

2.3.2. Calculation of force potential

For a harmonically varying sound field, where the fluid particle velocity, u , has amplitude U and fluid pressure, p , has amplitude P , the average kinetic and potential energy densities can be calculated [9] as

$$\langle \bar{E}_{kin}(r) \rangle = \frac{1}{T} \int_0^T E_{kin}(r) dt = \frac{1}{T} \int_0^T \frac{1}{2} \rho_0 u^2 dt = \frac{1}{4} \rho_0 U^2, \tag{1}$$

and

$$\langle \bar{E}_{pot}(r) \rangle = \frac{1}{T} \int_0^T E_{pot}(r) dt = \frac{1}{T} \int_0^T \frac{1}{2} \frac{p^2}{\rho_0 c^2} dt = \frac{1}{4} \frac{P^2}{\rho_0 c^2}, \tag{2}$$

where T is the period of the harmonic wave, ρ_0 is the density of the fluid, and c the speed of sound in the fluid.

Post processing of the FLUID29 u and p output parameters allows these equations to be implemented. The following equations derived by Gor'kov [10] allow the radiation force to be derived from an arbitrary standing wave field and the acoustic potential and energy densities. The acoustic radiation force (a time averaged quantity) is given by

$$\langle F(r) \rangle = -\nabla \langle \phi(r) \rangle, \tag{3}$$

where the force potential, $\langle \phi(r) \rangle$, is given by

$$\langle \phi(r) \rangle = -V \left[\frac{3(\lambda - 1)}{2\lambda + 1} \langle \bar{E}_{kin}(r) \rangle - \left(1 - \frac{1}{\sigma^2 \lambda} \right) \langle \bar{E}_{pot}(r) \rangle \right], \tag{4}$$

and where λ is the ratio of particle density to fluid density, σ the ratio of speed of sound in the particle to that in the fluid, V the particle volume, and $\langle \bar{E}_{kin}(r) \rangle$ and $\langle \bar{E}_{pot}(r) \rangle$ the time averaged kinetic and potential energy densities of the sound wave in the fluid.

2.3.3. Simulation results

Plotting the acoustic force potential, $\langle \phi(r) \rangle$, is a convenient way of visualizing the acoustic forces. A particle will tend to move towards regions where $\langle \phi(r) \rangle$ is low (indicated blue in Fig.5) with a force proportional to the spacings of plotted contour lines.

Fig.5 shows the force potential in the fluid for three modes, where the left boundary represents the centre of the chamber and right boundary the gasket wall (fluid elements shown in Fig.4). Fig.5(a) and 5(b) are both “to carrier” quarter wave modes, and show strong variation across the width of the device. 5(c) shows a “to reflector” quarter-wave, and predicts that the forces will be stronger nearer the centre of the chamber. This is observed in practice, although the pattern of force variation is more complex than the model predicts. This is probably due to poorly matched boundary conditions – both of the energy dissipation of the gasket, and the complex nature of the clamping that is not reflected in the model. The main use of the model is to provide indications of the type of variation to be expected, and give insights into the causes of these variations. In particular we note that surface and plate-wave type mode shapes have a strong influence on lateral behavior. In the future we will use the finite element models to develop designs that are less sensitive to geometric variations and material parameters to produce manipulation devices that produce more uniform action over a chamber area.

Table 2. Frequencies of operation (MHz)

Mode	1-D Model (Matlab)	FE Model (ANSYS)	Measured
“to-carrier”	1.572	1.55 & 1.61	1.508
“to-reflector”	1.700	1.72	1.712

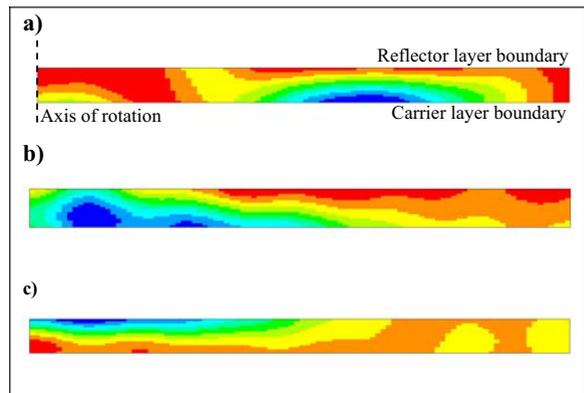


Fig.5 Plots of force potential in fluid layer elements (see Fig. 4) where blue indicates agglomeration region for a) 1.55MHz “to carrier” quarter-wave, b) 1.61MHz “to carrier” quarter-wave and c) 1.72MHz “to reflector” quarter-wave.

3. Enhancement of Microbead Assay

This section describes how the multimodal operation can be used to assist microbead based assays. In this example, a mixture of 6µm diameter streptavidin functionalized polystyrene beads (Bangs labs, CP01N) and non-functionalized control beads (Invitrogen, P24671, 6µm diameter, Cy5.5 labeled) are driven against a glass reflector which has been functionalized with a PEG-biotin coating. The “to carrier” quarter-wave is then applied to remove

the unbound control beads. In a more realistic application, the beads could be replaced by bacteria, and an antibody functionalized surface used. The finite element modeling predicts weakening axial forces at the chamber edges and that complex lateral variations exist, particularly for the “to-carrier” mode, which match observations and may be aggravated by inlet channel geometry which results in asymmetric boundary conditions. However an area of approximately 1mm^2 was found near the centre of the chamber where both the required acoustic modes were reasonably uniform. White light was coupled into the glass reflector through its end face such that an evanescent field was established close to the surface. This evanescent field illuminates any beads in direct contact with the reflector. An epi-fluorescent microscope was used to distinguish between the streptavidin beads and the fluorescent control beads.

After applying the “to reflector” quarter-wave (with 19Vpp voltage), all beads were seen to reach the surface within 1s of activation. After a 90s delay to ensure bead capture, excitation was switched to the “to carrier” mode (19Vpp). It was found that this removed 80% of the control beads, while 90% of the streptavidin beads remained attached to the surface. A short 0.5s burst of flow 2mms-1 flow through the chamber was sufficient to dislodge further Cy5.5 labeled control beads, such that 92% of them in total had been removed. This compares to the case when no ultrasound was used, where even after 270s only a small proportion of the beads were seen to be in contact with the waveguide surface, the remainder possibly repelled by electrostatic forces. Therefore, these results show that this technique could provide a new and useful type of rapid assay.

4. Conclusion

In the device presented, both quarter-wavelength modes have been demonstrated and at frequencies which tally closely with modeled results. These modes have successfully been shown to significantly enhance the immobilization of functionalized beads, with all beads brought to the surface within 1s. Future developments will look at coupling this with DNA manipulation.

A 3-D finite element model incorporating both the piezoelectric and acoustic interactions has been verified against a layered device. Applying this to an axisymmetric model of the manipulator device we obtain useful insights into the action of the device. It predicts the type of lateral force variations that are observed experimentally, but further work to improve the way boundary conditions and damping are modeled is required before we can be confident in the modeled results. The model is sufficiently accurate to be useful in the future for developing designs that are less sensitive to geometric variations and material parameters, and to produce manipulation devices that produce more uniform action over a chamber area.

Acknowledgements

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