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On-Chip Biological Sample Preparation System using Acoustic Lysis

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Introduction

- At present biological sensors and systems lack automated front-end handling to prepare samples prior to detection.
- Consequently, samples must be manually prepared requiring additional time, effort, and equipment, often reducing the effectiveness of microsystem solution.
- The lack of efficient front-end sample handling technology impacts a broad range of biosensor systems, ultimately limiting their portability, reducing sample throughput, and causing detection variability.

Approach

- Acoustic methods are a powerful approach to manipulate biological samples in high throughput applications.
- Using Bulk Acoustic Waves (BAWs) and fluid coupled Surface Acoustic Waves (SAWs), cells can be lysed to release their intracellular components such as specific proteins and DNA.
- Two microlysing methods were compared with a commercial instrument (Misonix) on their efficacy to lyse *E. coli* (K-12) cells.
- Microlysing methods were developed as flow-through assays for subsequent analysis using a microchannel to extract DNA.

Theory

BAW: 1-D Transmission Line Model:

$$Z_e = \frac{1}{j\omega C_o} + \frac{h^2}{\omega^2 A}\cdot\frac{2(\cosh(\gamma a)-1)Z_o + (Z_r+Z_l)\sinh(\gamma a)}{(Z_r Z_l + Z_o^2)\sinh(\gamma a) + Z_o(Z_r+Z_l)\cosh(\gamma a)}$$

$$h = \frac{e_{33}}{\varepsilon_{33}} \qquad \alpha = \alpha_o\left(\frac{f}{f_o}\right)^n$$

$$\gamma = \alpha_o\left(\frac{f}{f_o}\right)^n + \frac{j\omega}{v_f} \qquad Z(\omega) = \frac{j\omega\rho}{\gamma}$$

Z_e: Electrical Impedance of Transducer
h: stress piezoelectric constant
Z_o: Film Impedance $Z_o(\omega,\rho,\alpha,v_f)$
Z_l: Impedance seen to the left
Z_r: Impedance seen to the right
C_o: clamped electrical capacitance
α: attenuation constant (Nepers/m)
v_f: Propagation velocity
γ: propagation function $\gamma(\omega,\alpha, v_f) = \alpha + j\beta$
β: ω/v_f
a: film thickness

Additional Layers (Z_l, Z_r):

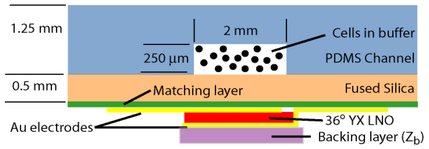
load intermediate

$$Z^+ = Z_o\frac{Z_L + Z_o\tanh(\gamma a)}{Z_o + Z_L\tanh(\gamma a)}$$

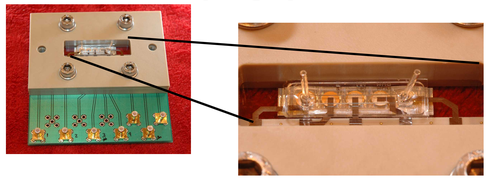
Theory

54 MHz Lithium Niobate (LNO) BAW Transducer:

d = 3 mm => A = 7.1 ·10^{-6} m^2
C_o= 46 pF
e_{33} = 5.3 F/m
h_{33} = 1.57·10^{10} N/C
k_t^2 = 0.33
V_f = 7340 m/s
Z_o= 34·10^7 Rayls
ρ_f = 4650 kg / m^3
α_o= 1.3 Np/m at f_o= 1.5 MHz
t = 51 µm
ε = 38ε$_o$
n = 1.5

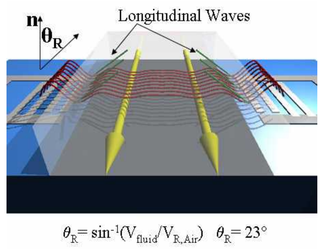


BAW Lysing System



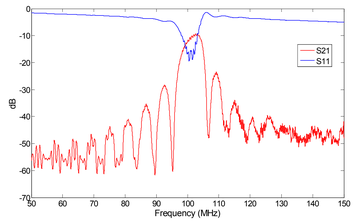
98 MHz LNO SAW Transducer:

128°YX LNO SAW



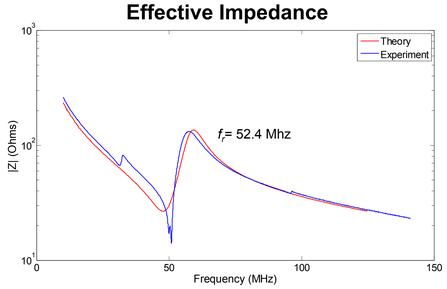
$\theta_R = \sin^{-1}(V_{fluid}/V_{R,Air})$ $\theta_R = 23°$

Results

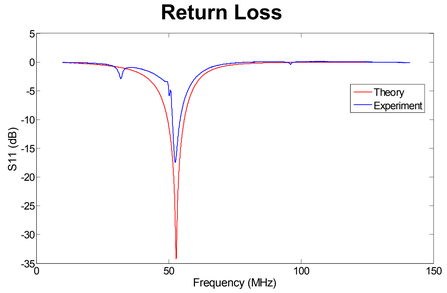


2) 54 MHz LNO BAW Transducer:

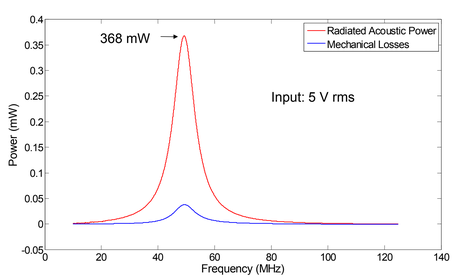
Effective Impedance



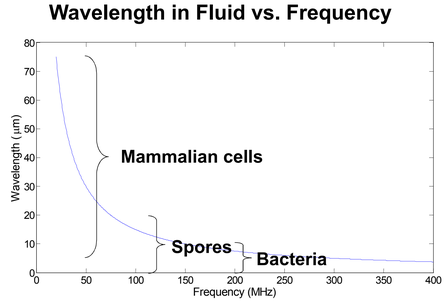
Return Loss



Theoretical Power Based on Model



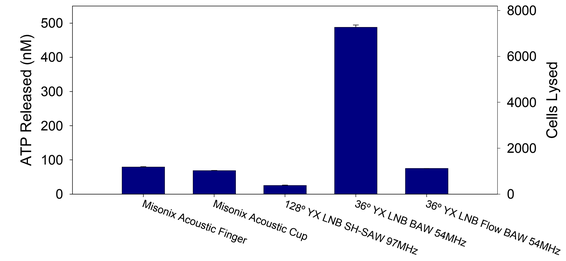
Wavelength in Fluid vs. Frequency

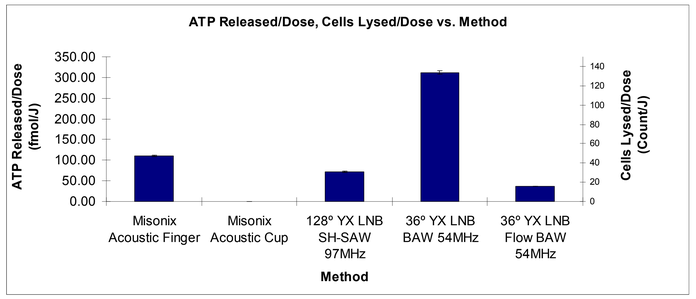


Comparison of Cell Lysing Methods: Benchtop vs. Microlysing System

- Lysing efficacy was assessed by ATP release from treated suspensions of *E. coli (K-12).*
- ATP release was measured by fitting experimental data to a standard curve generated with known concentrations of ATP using luciferase assay (Molecular Probes).
- Cells were counted and ATP was measured before and after lysing for each method.

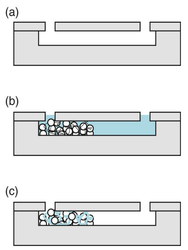
ATP Released, Cells Lysed vs. Method

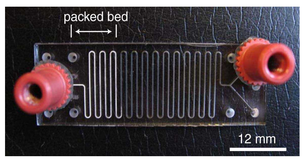


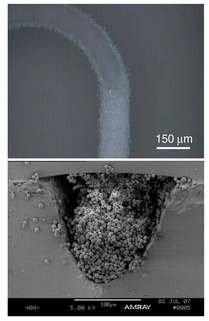


DNA Extraction:

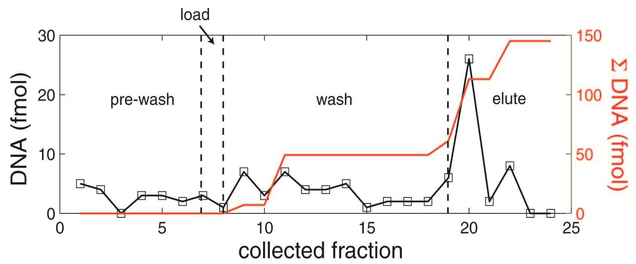
(a) Fabricate encapsulated glass microfluidic device
(b) Inject sol-gel-silica bead slurry
(c) Dry sol-gel with heat (100 °C for 3 hours)







End of the packed bed section of sol-gel immobilized silica beads (5 µm) in a channel (top). SEM of the cross-section of a packed bed section of a channel (bottom).



Extraction profile (black) and cumulative extraction (red) of DNA from a packed bed device as a function of collected fraction. Total DNA injected = 250 fmol, yielding an extraction efficiency of 100/250 = 40% (the 50 fmol eluted during the wash is not included in the efficiency calculation). Minimum detected concentration ~10 fmol.

Future Work

- Determine lysing efficacy for additional flow rates and conditions.
- Optimize DNA microchannel for handling a wide range of sample types.
- Determine lysing conditions for other biological samples.
- Integrate lysing with the DNA extraction microchannel.

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