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Nucleic Acid Extraction using a Rapid, Chemical Free, Ultrasonic Technique for Point-of-Care Diagnostics

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Mycobacterium Tuberculosis (MTB)

Facts:

- Currently infects one-third world's population
- Multi-drug resistant (MDR) strains at epidemic proportions in developing countries
- Extensively drug resistant (XDR) strains have emerged (i.e. Fluoroquinolone resistant)

Impact of Technology:

- Rapid extraction of DNA for PCR and sequencing means antibiotic treatments are effective

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Cellular Disruption – Lysis Methods

Types:

- Physical, Chemical, Thermal, Enzymatic, and Ultrasonic
  - Chemical: Interferes with PCR and delay sample analysis
  - Thermal: causes sample loss and complicates sample extraction in microfluidic applications.
- Ultrasonic
  - Integrated with nano/microfluidic devices
  - Chemical-free
  - Cavitation
  - Shearing
  - Ablation

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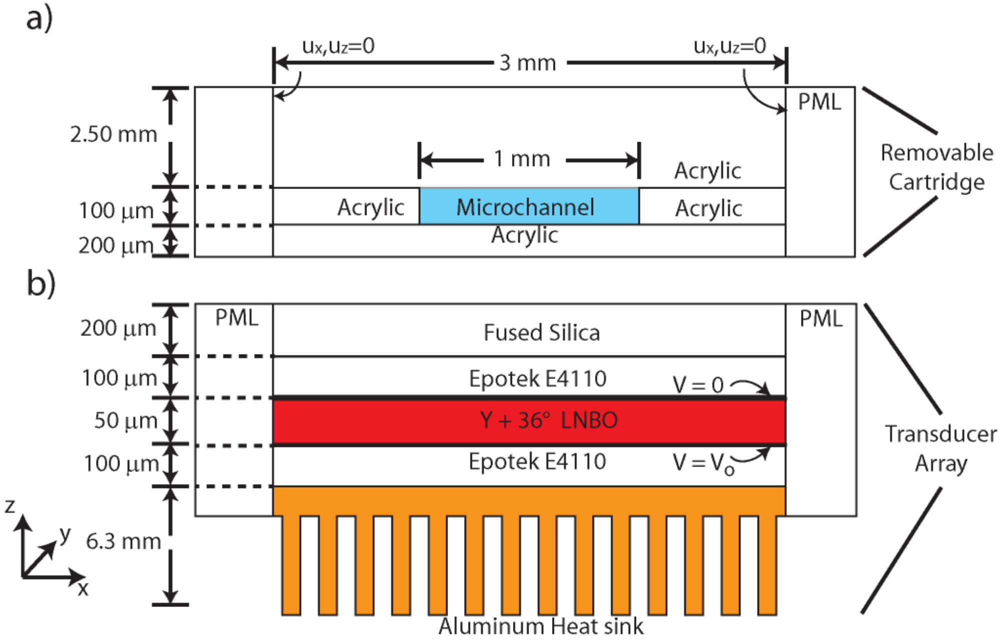
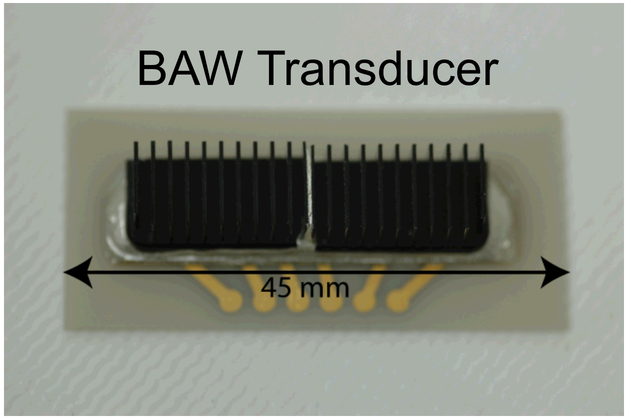
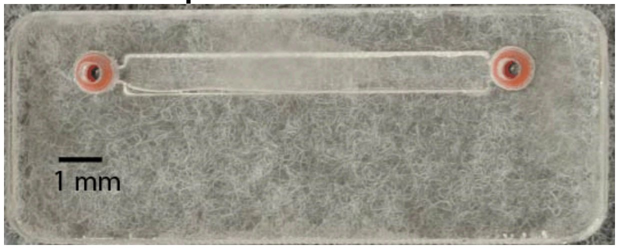
Bulk Acoustic Wave Transducer

- The transducer is based on Y + 36° cut of lithium niobate
- Electrodes were patterned onto fused silica substrates for electrical connection
- A cartridge containing a microfluidic channel is placed onto the transducer.





Simple Microchannel



Simulation Methods

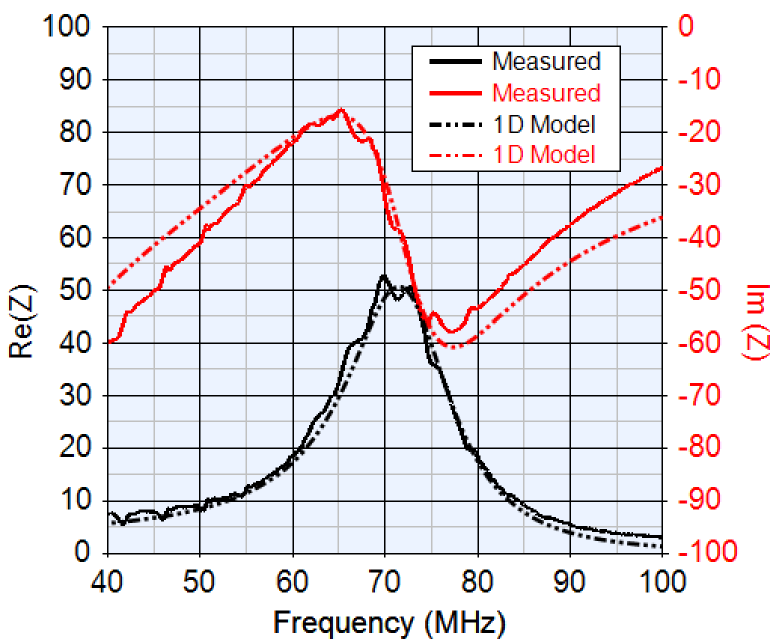
- 1D Transmission line model: Initial design
- 2D FEM: Fluid Pressure and Force on small particles
- 3D FEM: Thermal Model: Impact of heat on cell lysis

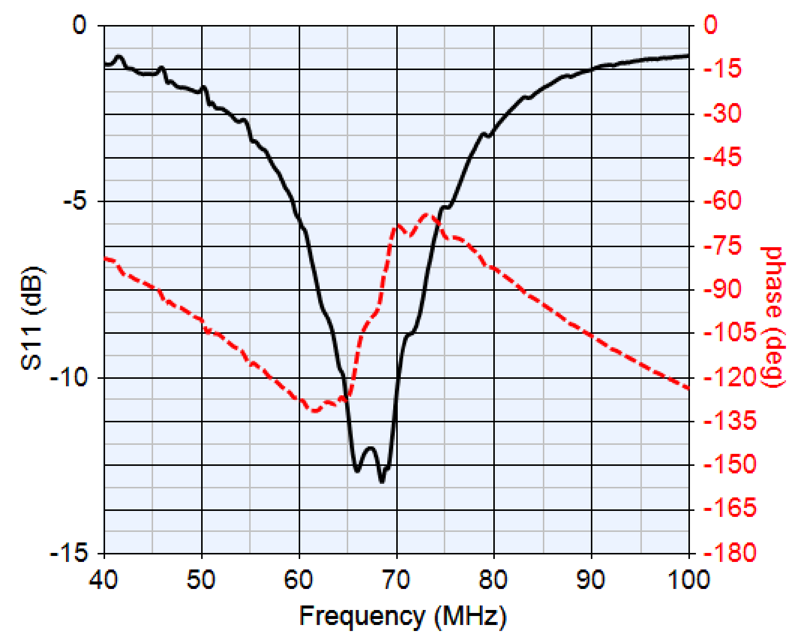
Simulations Methods

- 1D Transmission line model
  - Purpose: Fast design technique and used to determine the internal mechanical losses which leads to heat generation

$$Z_e = \frac{1}{j\omega C_o} + \frac{k_t^2 v_t^2 \rho_t^2}{C_o t_t \omega^2}\left[\frac{2\left[\cosh(\gamma t_t)-1\right]Z_t + (Z_L+Z_R)\sinh(\gamma t_t)}{(Z_L Z_R + Z_t^2)\sinh(\gamma t_t) + Z_t(Z_L+Z_R)\cosh(\gamma t_t)}\right]$$

$$P_m = \frac{1}{2}\mathrm{Re}(Z_e)\left|\frac{V_{in}}{Z_e}\right|^2 - \frac{1}{2}R_p\left|\frac{V_{in}}{Z_e}\right|^2 - P_a$$





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2D FEM Fluid Pressure Model

Piezoelectric – Acoustic Pressure Model

Acoustic fluid interaction

$$\nabla\cdot\left(-\frac{1}{\rho_f}(\nabla p - q)\right)-\left(\frac{\omega^2}{\rho_f c_f^2}\right)p = Q$$

$$\rho_f = \frac{Z_f k_f}{\omega} \qquad c_f = \frac{\omega}{k_f} \qquad k_f = \frac{\omega}{c_s} - i\alpha \qquad Z_f = \rho_o c_s$$

Piezoelectric transduction

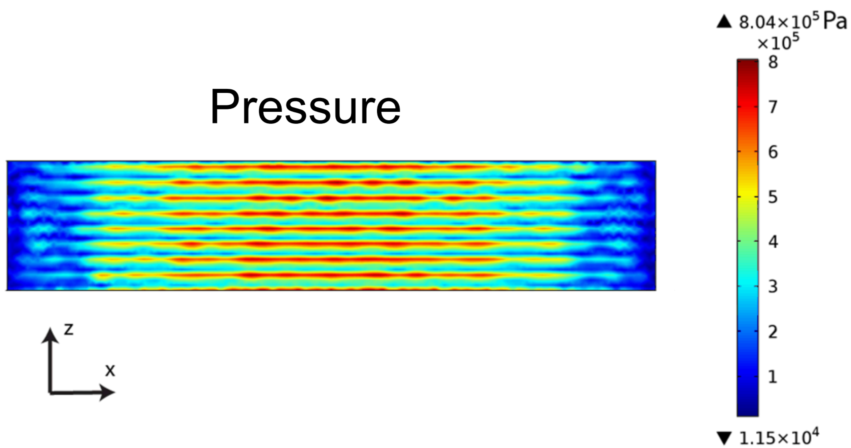
$$\rho\frac{\partial^2 u}{\partial t^2} = \nabla\cdot c^E : \nabla_s u - \nabla\cdot(e\cdot E)$$

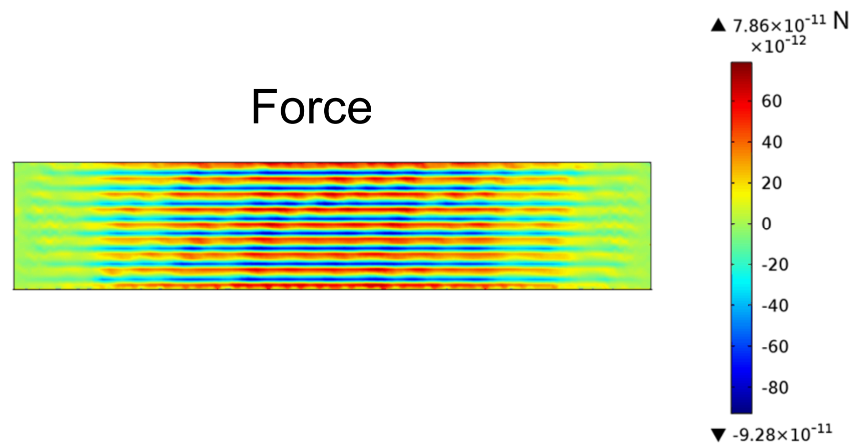
$$\nabla\cdot(e:\nabla_s u) - \nabla\cdot(\varepsilon^s\cdot\nabla\phi) = 0$$

→ Pressure and velocity in the fluid!

Assume MTB: 1 μm diameter → Compute Potential Energy then Force

Microchannel





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3D FEM Thermal Model

Navier-Stokes (Fluid) – Thermal Model

fluid
$$\begin{cases} \rho(\boldsymbol{u}\cdot\nabla)\boldsymbol{u}+\nabla p-\nabla\cdot\eta\left(\nabla\boldsymbol{u}+(\nabla\boldsymbol{u})^{T}\right)=0 \\ \nabla\cdot\boldsymbol{u}=0 \end{cases}$$

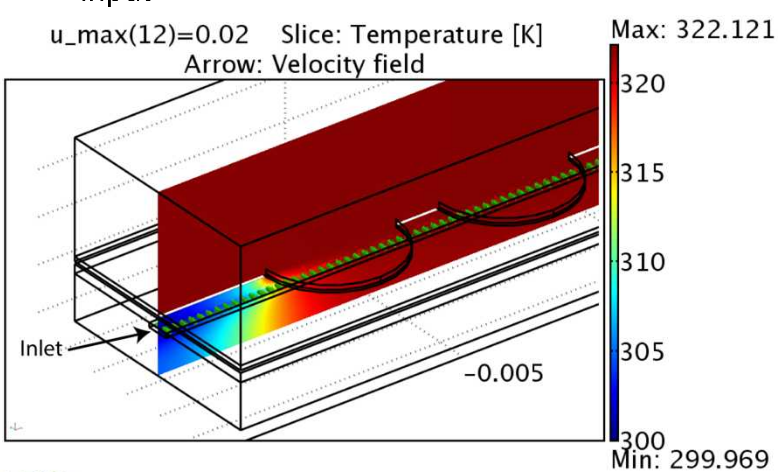
heat
$$\nabla\cdot(-k\nabla T)=Q-\rho C_p u\cdot\nabla T$$

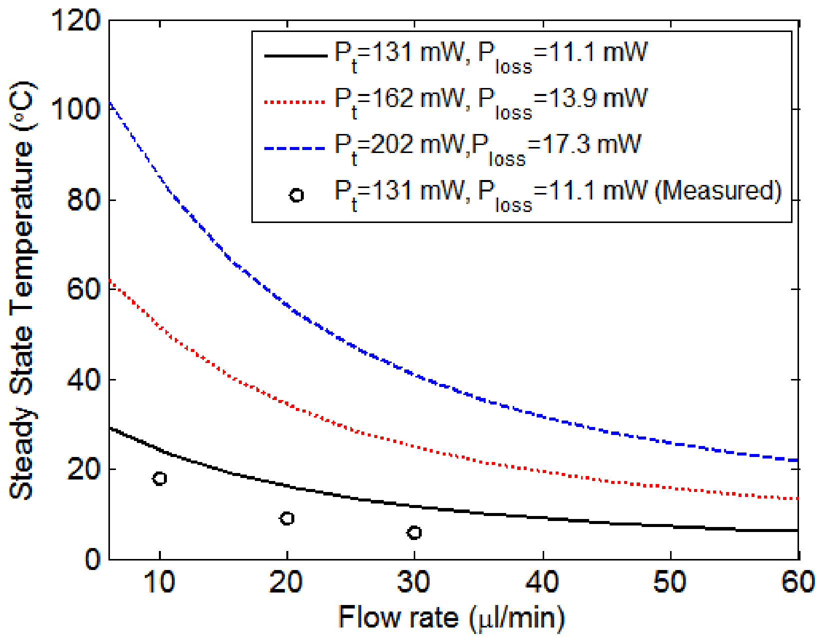
→ Temperature distribution versus flow rate!

Laminar flow at inlet:
$$u=16u_{\max}\frac{(z-z_o)(z_1-z)(y-y_o)(y_1-y)}{(z_1-z_o)^2(y_1-y_o)^2}$$

Power loss from 1D model is the heat generated :
$$\begin{cases} -n\cdot(q_1-q_2)=q_o \\ q_i=-k_i\nabla T_i \end{cases}$$

P_{input} = 202 mW, 60 μL/min





Cellular Lysis of MTB

- Used the vaccine for MTB - Bacillus Calmette Guérin (BCG)
- Compared results to a commercial sonicator
- Positive control: boiling at 95° C for 30 minutes
- Negative control: untreated sample passed though microchannel
- Assay: Measured mean cycle threshold (C_t) and standardized against the boiling method: lysis performance (*LP*):

$$LP = 2^{Ct(boil) - Ct(sample)}$$

LP < 1: worse than boiling
LP > 1: better than boiling

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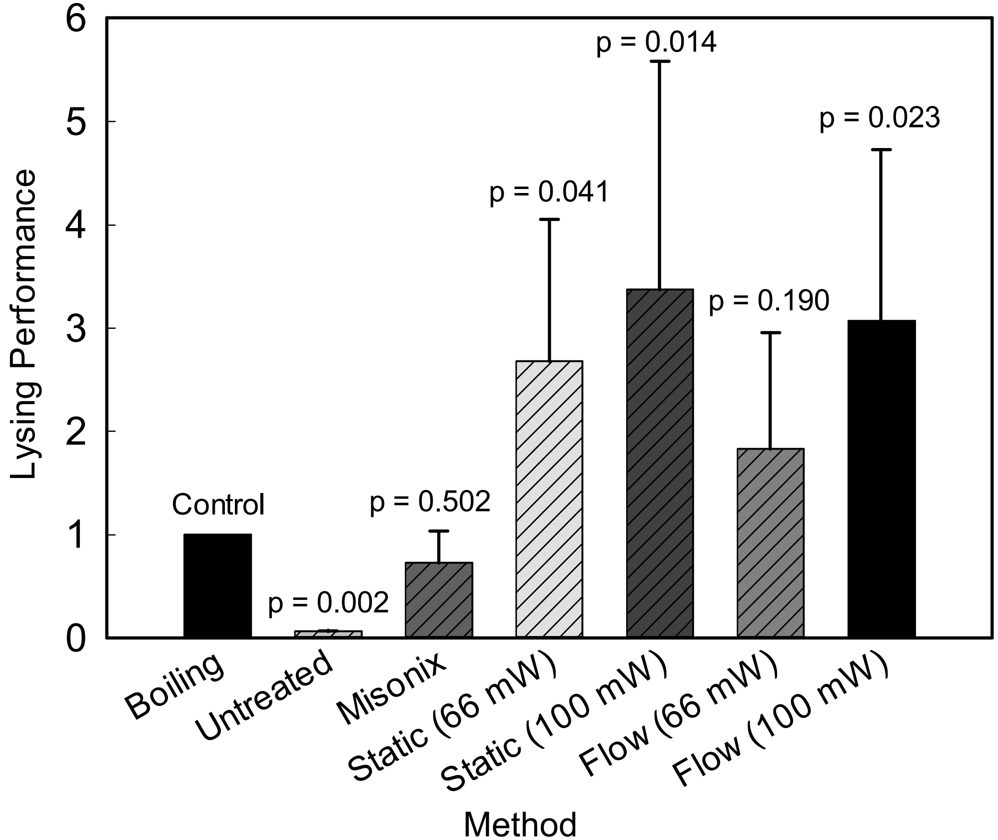
RT-PCR Analysis



t-test compared boiling to the other acoustic methods. p value is level of significance. Small p is statistically significant, large p is not statistically significant.

Thank You!

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