Improving diagnostic assessment by concentrating a *M. tuberculosis* sputum smear on a microscope slide using high-gradient magnetic enrichment and droplet-induced Marangoni flow

Stephanie I. Pearlman, Eric M. Tang, Yuankai K. Tao, Frederick R. Haselton
Department of Biomedical Engineering, Vanderbilt University, Nashville, TN

Background: In the developing world, microscopic examination of sputum smears for tuberculosis (TB) diagnosis remains the most common diagnostic method. However, assessment requires a time intensive 1000X inspection across the entire 2 cm^2 smear area, and this is thought to contribute to its poor diagnostic sensitivity of ~50%. A simple method to spatially concentrate the TB bacteria is one potential approach to increase sensitivity. We hypothesized that a combination of our recently published magnetic concentration method[1] and droplet-induced Marangoni flow would provide a simple method for achieving this spatial concentration.

Methods: Variables known to impact flow patterns, slide substrate, solvent thermal conductivities and solvent surface tension, were varied and droplet contact angle, droplet internal flow and final particle deposition were measured. Using goniometry, the three-phase contact angle of water on polysiloxane, polytetrafluoroethylene, indium tin oxide, and glass substrates were measured to assess droplet surface tension. To ensure maximal central deposition, the final evaporative deposition pattern of 1µm fluorescent polystyrene particles in 2µL droplets, in varying aqueous solvents, was inspected using microscopy. Optical coherence tomography was used to observe the internal droplet flow and confirm predicted Marangoni flow patterns for the selected substrate and solvent. Using the identified optimized components, standard Ziehl-Neelsen staining was compared to the Marangoni-based spatial concentration system. This was then paired with magnetic enrichment using immunospecific iron oxide nanoparticles and high-gradient magnetic separation, isolating *M. bovis* BCG (BCG) from mock patient samples containing 0 to 10^6 bacteria/mL.

Results: Spatial distribution on microscope slides was achieved with preferential deposition in the center of a droplet by induction of a Marangoni flow. The best design was found to be water repellent Rain-X, a polysiloxane glass coating and a PBS-Tween droplet solvent. Optical coherence tomography confirmed the predicted outward-oriented Marangoni flow pattern. The use of smaller, 200nm magnetic nanoparticles enabled both fluorescence and bright field imaging of BCG after sample processing. Compared to standard sputum smear sample preparation paired analysis demonstrated a volumetric enrichment of 20x and a spatial enrichment of ~28x, which together achieved a total sample concentration factor of ~560x.

Conclusions: Combining both volumetric and spatial concentration of *M. tuberculosis* from sputum improves microscopic visualization. With further refinement, this combined magnetic/Marangoni flow concentration approach is expected to improve whole-pathogen microscope-based diagnosis of infectious disease.

References:

1. Pearlman, S. I.; Leelawong, M.; Richardson, K. A.; Adams, N. M.; Russ, P. K.; Pask, M. E.; Wolfe, A. E.; Wessely, C.; Haselton, F. R., Low Resource Nucleic Acid Extraction Method Enabled by High-Gradient Magnetic Separation. *ACS Appl Mater Interfaces* **2020**.