by Shane Woody and Jason Tucker

# A Research Tool for Sample Preparation and Microfluidics

Precision ultrasonic microvortexing is an underexplored area in biophysics research, a pathway to automation for portable lab-on-a-chip (LOC) systems, biodetection, assays, and screening. A critical component in LOC is the need for novel approaches to sample acquisition and preparation. For sample acquisition, it is highly desirable to have rapid collection, concentration, and transport of low concentrations of sample to targeted areas.

In diagnostic studies, researchers are making great strides in enhancing the specificity and sensitivity of detection technologies. However, one of the challenges is improving throughput and sensitivity. One way to enhance the sensitivity and speed of the detector is to use third-party technology that can rapidly concentrate biomaterial to the detector site in seconds to speed up the detection rate. An application example is forensic science, which requires the collection of low quantities of target DNA at a site quickly and with high yield.

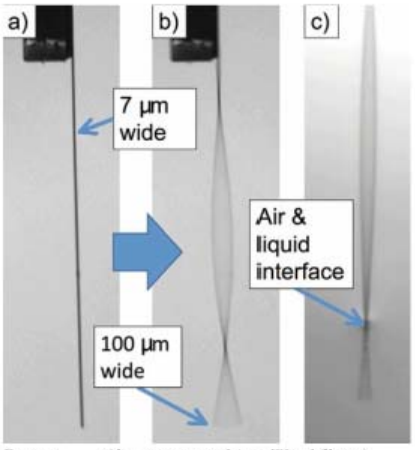


*Figure 1 Closeup images of AccuWand fiber a) turned off in air, b) turned on in air, and c) turned on in phosphate-buffered saline (PBS) liquid.*

## Sample preparation tool

The AccuWand research tool (**InSituTec**, Charlotte, NC) uses a novel approach to rapidly collect cells and nucleic acids in seconds in low concentrations without lysing or degrading the sample. This article discusses the device, highlights case studies, and discusses applications.

### Principle of operation

AccuWand is a patented technology that generates very pronounced mechanical waves on small-scale structures (*Figure 1a* and *b*). As shown in the photomicrographs, the device uses a microscale fiber that is 7 µm wide (about 1/30 the size of a human hair) and configured in lengths from 1.5 to 3 mm. Once the fiber is energized or turned on, the fiber modulates and flexes significantly back and forth at 32,000 cycles/sec to form a very elastic wave. The wave's mechanical amplitude can be easily increased or decreased from a front panel on a supplied electronic unit. Adjusting this wave's amplitude up or down will cause the AccuWand's maximum modulation velocity per cycle to also increase or decrease. For example, the tip's maximum modulating width shown in the figure is 100 µm. A cyclical frequency of 32,000 cycles/sec combined with a 50-µm amplitude will yield a maximum tip velocity up to 10 m/sec in air, which is strikingly high for a microscale structure. The pronounced modulating tip can modulate continuously for weeks without fatigue or failure to the structure, which corresponds to 115 million cycles/hr.

A unique phenomenon is observed when the modulating fibers are immersed into microfluidic samples (Figure 1c). Initially, it was thought that the modulating fibers would completely clamp or attenuate in liquid due to the surface tension of the liquid. However, the modulation is observed to attenuate by only 30–40% in liquid, and the tip velocity can be programmed up to 3 m/sec. This velocity is extremely high for a modulating structure in the presence of liquid. Figure 1c demonstrates a portion of the fiber in the liquid.

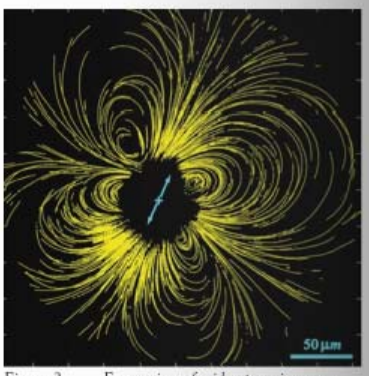


*Figure 2 Formation of wide-spanning quadrupole expanding a distance 300× from the AccuWand tip (courtesy of Brown University).*

### Case study 1: Fluid flow studies and wide-ranging vortices

The tip's modulating frequency and pronounced amplitudes produce wide-ranging vortices in liquid samples, resulting in advanced micromixing, cell motility, and capture of targeted agents in samples. Preliminary experiments conducted by Dr. Kenny Breuer's nano and microfluidics group at Brown University (Providence, RI) used Lagrangian particle-tracking velocimetry to study the flow field generated by the AccuWand. Images were captured using a high-speed camera combined with a two-stage image intensifier. In this configuration, 512 × 512 pixel images were taken at a frame rate of 3000 frames per second (fps) and an exposure time of 100 µsec, which allows the particles to move several pixels in the image without streaking. The results reveal a complex flow field with the general appearance of a quadrupole (*Figure 2*). The maximum velocities generated are more than 1 cm/sec in local-

ized regions of about 100 µm. Mathematical streamline functions developed by **InSituTec** and Brown University have correlated experimental and theoretical 2-D flow fields. Based on this work, the center "eye" (shown in black) should exhibit fluid velocities exceeding 1 m/sec near the surface of the fiber.

The effects of these vortices enable several applications such as sample preparation, mixing, and diagnostics. First, the vortices have been measured to span more than 300 times the distance of the width of the fiber. This implies that the flow field expands beyond the area surrounding the AccuWand tip. Second, the modulating tip causes a type of quadrupole effect with four independent rotating vortices. The vortices rotate and cause biomaterial to collect and concentrate inside the vortex. The AccuWand can be displaced in the fluid sample to a desired targeted region, and the particles remain inside the vortex and thus track with the tip of the AccuWand.

## Case study 2: Collect, trap, and spin suspended polymer beads

To demonstrate particle collection and concentration, 45-µm-diam suspended polymer beads were placed in a 20-µL deionized water sample. Once the AccuWand tip was turned on and immersed in the fluid sample, polymer beads were immediately pulled into the vortex. Moreover, the rotational velocity and vortex field changes by increasing or decreasing the fiber wave amplitude. As the AccuWand was moved over a longer distance in the liquid, the beads followed the tip. This is likely due to a trapping effect caused by the vortex fields. Finally, if the AccuWand is turned off, the beads will suddenly stop in the field of view.

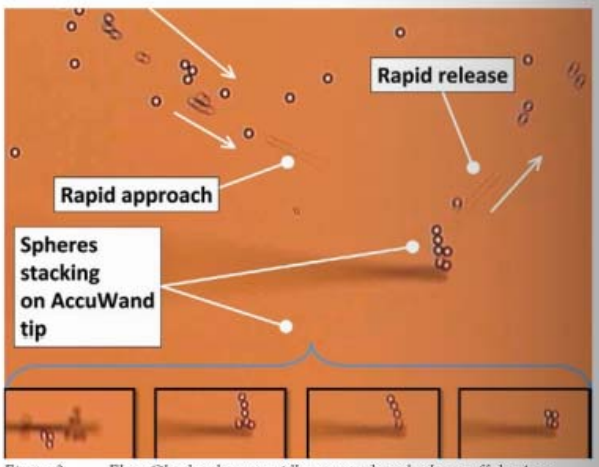


*Figure 3 Flow Check spheres rapidly approach and release off the AccuWand tip with a 40× magnification (note the overlaid images at the bottom where random patterns are formed in several seconds).*

## Case study 3: Polystyrene spheres

Flow Check® 6.0-µm polystyrene spheres (**Polysciences Inc.**, Warrington, PA) were studied at a stock concentration of $10^7$ spheres/mL. A 50-µL volume was placed onto a microscope slide, and the device was positioned into the sample and held stationary about 1 mm above the surface. The AccuWand was activated for 48 sec and observed with a fluorescent inverted microscope. The spheres pulled toward the tip of the AccuWand in seconds. A similar experiment reveals that the spheres rapidly approached toward the tip; spheres would stack atop one another and then release from the stack (*Figure 3*). This would form random patterns 60–80 times every minute. Overlaid images show some of the random patterns formed on the tip.

## Case study 4: Concentrating cells

Cells are pulled into the vortex and spin at extremely high acceleration and velocity. The rate of spin can be precisely controlled by adjusting the amplitude wave of the AccuWand. Preliminary studies have shown that crystal violet-stained *Staphylococcus aureus* G+cocci (strain UAMS-1) were rapidly captured in this vortexing flow field (*Figure 4*). These images were captured at 40× 10–20 sec after introduction into the liquid. The crystal violet color visible below the tip shows a high cell concentration. Similar cells of the same strain underwent vortexing using the AccuWand for 20 min at full power. Standard spread plate methods revealed that the cells remained viable and free of damage.


*Figure 4 Rapid sample collection. Vortexing violet-stained S. aureus (strain UAMS-1), attracted in 30 sec at a concentration of $10^4$ per mL.*

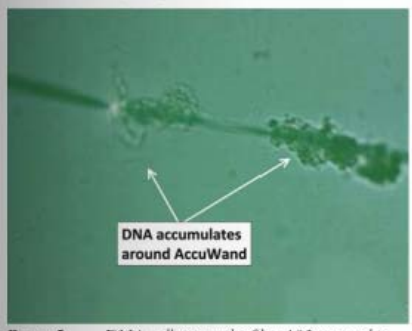


*Figure 5 DNA collects on the fiber 180 sec total in liquid and DNA concentrates more.*

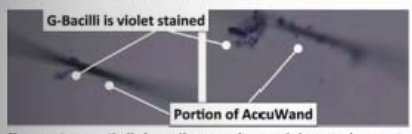


*Figure 6 Cellular adhesion observed due to ultrasonic microscale vortexing.*

## Case study 5: Collecting DNA

A number of experiments were conducted to assess the applications of the AccuWand in molecular biology. Chromosomal DNA was isolated from *Escherichia coli* K12 (MG1655) with the Bactozol™ kit (**Molecular Research Center**, Cincinnati, OH). The DNA was then purified using the Wizard® *Plus* miniprep kit (**Promega**, Madison, WI). A 50-µL droplet with a concentration of 0.7 µg/mL was investigated. The AccuWand tip was immersed, and DNA immediately entangled around the fiber (*Figure 5*) in seconds. The DNA were also assessed for degradation. The AccuWand was immersed and activated for 20 min using the maximum wave. Later, the samples were diluted 1:10 and electrophoresed in a 0.8% agarose gel at 50 V. There was negligible damage from the AccuWand on the DNA in solution.

## Case study 6: Cellular adhesion and viability

*S. aureus* and *E. coli* bacteria were investigated using the AccuWand, and the bacteria of $10^4$/mL rapidly accelerated to the AccuWand tip in seconds. Large formations occurred due to cellular adhesion by ultrasonic microvortexing (*Figure 6*). Cell damage and viability were evaluated. Both *S. aureus* and *E. coli* were then collected and diluted serially 1:10, 1:100, and 1:1000 and placed onto Trypticase soy agar (TSA) as spread plates. The plates were incubated overnight and compared the following day against a control set by colony-forming unit (CFU) counts for viability. Additionally, spot tests were performed with dilutions $10^{-1}$ through $10^{-5}$ as a control for the first assay. The CFU/mL of the bacterial cultures was not affected negatively by their introduction to the AccuWand. The CFU counts on the 1:10 and 1:100 plates were too numerous to count (TNTC), while on the 1:1000 plates there was an average of 3–4 colonies per plate for the untreated controls of both species as well as the experimental solutions.

## Laboratory bench applications

The first AccuWand product line is targeted for researchers in the life sciences, specifically biophysics, microfluidics, and assay development. Each device is manufactured on a disposable cartridge that is placed into a small, portable unit that easily mounts to the microscope stage. The unit is then connected to a compact electronics box that sits next to the microscope. The user operates the standing wave from the electronics unit to concentrate targeted agents to the fiber, process specimens, etc. The company is also working on advanced assay developments, microfluidics, nanofluidics, and diagnostics to integrate this technology into more complete systems.

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