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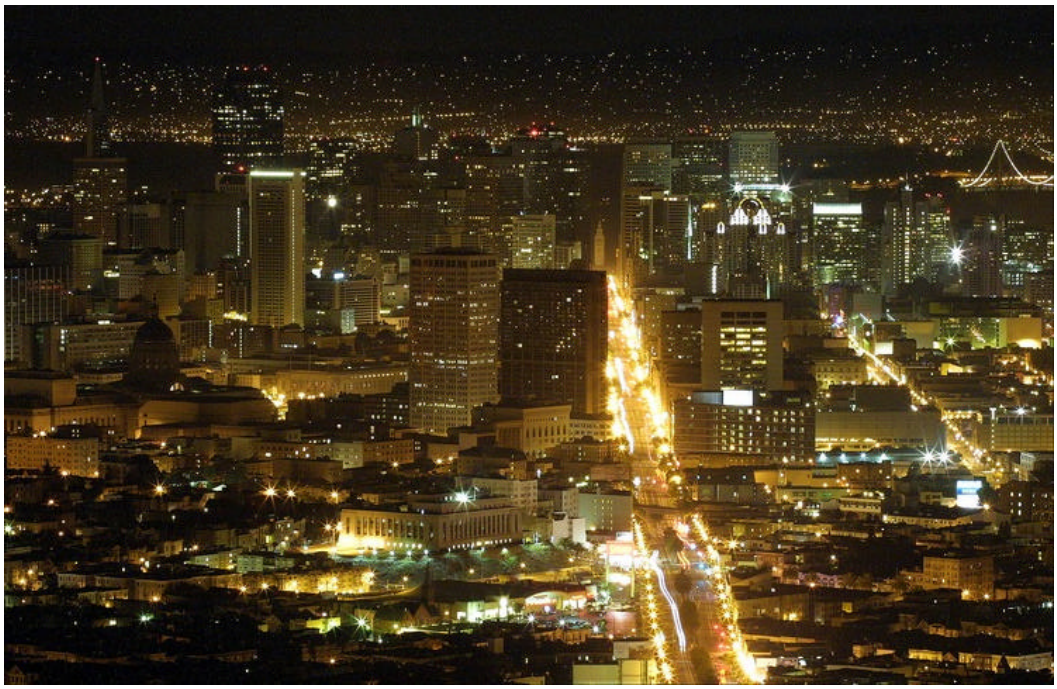
# AES Newsletter



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**Many thanks to our Sponsors for contributions funding the 2006 meeting.**

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**Our traditionally strong meetings, with sessions chaired by invited plenary speakers discussing state-of-the-art topics, would simply not be possible without funding from sponsors. These donations are greatly appreciated.**

**We also thank NIH for travel grant support.**

## San Francisco, how we love thee.... for our annual meeting on November 13-17, 2006

### News from our Meeting Organizers

The countdown is well underway, with less than one month to go until the American Electrophoresis Society's 2006 symposia, held in conjunction with the American Institute of Chemical Engineers annual meeting Nov. 13-17th at the San Francisco Hilton. This year's program is particularly strong and diverse, including sessions in the areas of cellular, genomic, and proteomic analysis, in addition to fundamental research in electrophoresis and electrokinetic flows. The AES meeting is unique in the community because it compliments AIChE's predominantly chemical engineering population by bringing together researchers from a variety of academic backgrounds who work in relevant fields as well as industrial contributors.

We are particularly excited about our plenary speakers who represent a cross-section of the current state-of-the-art in the field. Contributions in the bio-MEMS area include talks from Dr. H. Kumar Wickramasinghe from the IBM Almaden Research Center, Dr. Robin Liu from CombiMatrix Corp, Dr. Paul Bohn from the University of Notre Dame, and Dr. Lawrence Grossman from Wayne State University. Additional keynote speakers will be featured at many other sessions.

A handy summary of the AES sessions and events is included with this issue. More information can be found on the AES website www.aesociety.org The complete meeting technical program is on the AIChE website http://aiche.confex.com/aiche/2006/techprogram/D1109.HTM

We look forward to seeing you in San Francisco!

Adrienne

Victor

## A Decade of Progress in Microscale Electrical Field-Flow Fractionation

**by Bruce K. Gale, *University of Utah***

Microscale field flow fractionation (FFF) has shown significant progress since it was first reported in early 1997 [1]. The first electrical FFF systems were lucky to function for more than a few days and generated only minimal levels of retention and separation, while current systems now easily function for years and can generate multicomponent separations [2]. A variety of microscale FFF systems have now been reported, including multiple versions of normal electrical FFF (ElFFF) , cyclical electrical FFF (using oscillating fields), dielectrophoretic FFF, thermal FFF, acoustic FFF, a combined thermal-electric FFF channel, and flow FFF. The first nanoscale electrical FFF systems have recently been reported. Microscale ElFFF systems have been used to analyze and separate nanoparticles, DNA, proteins, cells, viruses, liposomes, large polymers, and other materials. ElFFF clearly improves upon system miniaturization due to the reduction in sample and carrier volumes, analysis times and more notably an increase in the separation resolution with a reduction in analysis times. Other advantages of miniaturized FFF include: parallel processing with multiple separation channels, batch fabrication with reduced costs, high quality manufacturing, and potentially disposable systems. Additionally, the possibility of on-chip sample injection, detection and signal processing favors the microfabrication of FFF systems.

Instead of applying an electrical field parallel to the flow of carrier as in electrophoresis, FFF applies the electrical field perpendicular to the flow and employs differential electrophoretic mobilities and diffusion rates to separate particles, as shown in Fig.1.

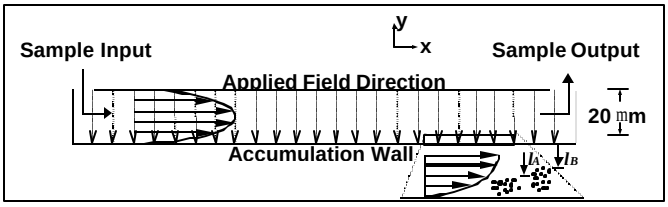


*Figure 1. Schematic diagram of FFF. The particle clouds depicted by closed circles and open circles in inset figure are sample A with average thickness $l_A$ and sample B with average thickness $l_B$. B will move faster through the channel than A since $l_B > l_A$.*

However, separation in FFF is achieved at voltages three orders of magnitude lower than those used in electrophoresis, a major advantage. Retention in ElFFF is determined by the ratio between diffusion and electrophoretic transport (D/μE). Since ElFFF is an elution method, all eluted samples can be collected for later analysis. Thus, EFFF is ideal for sample preparation in lab-on-a-chip devices, for use in nanoparticle characterization, and for use with an orthogonal analysis system.

Although electrical FFF is a powerful method, it is limited by the polarization layer formed at the electrodes when a DC field is applied. This polarization or double layer reduces the electric field in the channel to less than 3% of the applied field value and the separation efficiency suffers accordingly. However, this problem can be countered by applying an alternating or cyclical electrical field, which does not allow the build up of a double layer. Thus, cyclical electrical FFF (CyElFFF) was developed to take advantage of these miniaturized systems [3].

CyElFFF has shown significant promise using electrical fields to separate nanoparticles and colloids. In CyElFFF, the direction of the applied field is rapidly switched (~1-20 Hz) and particles oscillate near one wall or travel between the two walls of the channel. CyElFFF retains particles based on electrophoretic mobility alone and is not particle size dependent. Voltages as low as 0.25 V can retain particles easily. CyElFFF can also be tuned to maximize resolution by adjusting voltage and frequency of the applied field. Nanoparticles, viruses, and liposomes have all been characterized using CyElFFF.

Pioneering work on microscale dielectrophoretic FFF (DEP-FFF) was done by Gascoyne's group with applications in cancer research and cell separations [4,5]. These systems are technically DEP-Gravitational FFF systems in which dielectrophoretic (DEP) fields are used to drive particles away from the wall and gravitational forces drive the same particles towards the wall. Thus a balance between these competing fields determines the average particle location in the channel and the elution time.

Researchers have recently demonstrated nanoscale ElFFF using channels that "apply" the electric field naturally. In other words, the native charge on the typical surface of a nanoscale capillary drives particles to or away from the wall, just as in normal FFF. Both pressure driven flow and electroosmotic flow can be used, since at the nanoscale, electoosmotic flow profiles begin to approach those for pressure driven flow. Separations of particles in 40 nm channels based on charge have been demonstrated [6] and the theory and models derived [7].

Even with work in this area progressing well, there is still significant work remaining to make ElFFF a standard process in scientific and research circles. More information on microscale FFF can be found at www.mems.utah.edu/Project_Pages/FFF.htm

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## Issues in Achieving In-depth Proteomic Profiling

by Katheryn A. Resing, *University of Colorado at Boulder*

Recently, there has been increased need for improved protein and peptide analysis of complex samples using mass spectrometry (MS). A typical approach utilizes collision induced dissociation of peptides to generate fragment ions. The information in the resulting "MSMS" spectra is used to identify the parent peptide sequence by searching protein databases for a sequence with best fit to the MSMS spectrum. When analyzing peptide digests of purified proteins on a reverse phase column interfaced with MS (LC/MSMS), sequence assignments for >75% of the high quality MSMS are typically achieved, yielding >80% protein sequence coverage. When applied to complex samples, the MS scan rate becomes limiting, requiring fractionation of the sample. A popular approach is the MuDPIT approach (MultiDimensional Protein Identification Technology), where peptides are fractionated by strong cation exchange before LC/MSMS; very complex samples require prefractionation of the proteins. The quandary is that in-depth profiling of complex samples by MuDPIT is very difficult to achieve, because protein sequence coverage is low (1-35%), and usually no more than 30% of the high quality MSMS spectra can be assigned to peptides with high confidence.

Most work in this area has focused on improving search program scoring and validation (reviewed in 1). When the number of MSMS is small, manual analysis can be used to evaluate the chemical plausibility of the observed fragmentation, but that method is prohibitive for complex samples. Instead, investigators often rely on search program-generated scores, using an acceptance threshold equal to the highest score obtained by chance alone. The latter can be determined by searching databases where protein sequences are randomized or inverted, and all assignments are false positive.

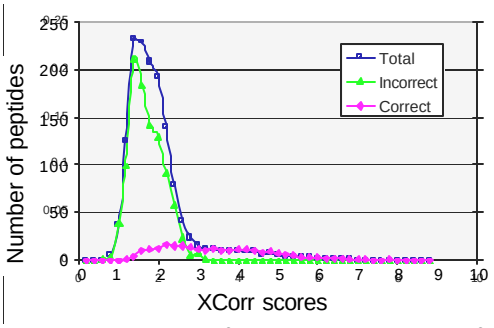


*Figure 1. Distribution of Sequest XCorr scores for MSMS spectra of a complex sample. Comparison of scores for a full MuDPIT dataset (blue), correctly identified, manually validated "hits" (pink) and the remaining MSMS spectra (including alternative charge assignments).*

Fig. 1 shows the histogram of scores for MH2+2 ions in a small MuDPIT dataset of human proteins, where manual analysis was used to validate the assignments made by the Sequest search program. The inverted database search shows that a false discovery rate (FDR) ~0.5% can be achieved using an acceptance threshold of XCorr=3.3 (high confidence threshold = HCT). However, this occurs at the expense of false negative rate ~45%, thus rejecting many correct assignments. In order to capture more data, researchers often lower acceptance thresholds, then try to filter false positive cases. A method commonly used requires a minimal difference in scores between the top two assignments, which validates 10-20% additional MSMS than those accepted by the HCT.

An alternative validation method uses independent inputs to increase statistical discrimination. For example, peptide physicochemical properties can be used to filter false positives, by using exact mass measurements of the parent peptide, or by comparing observed vs. calculated elution times from RP chromatography. We extended the use of independent inputs by evaluating consensus between three MSMS scoring algorithms and applying several physicochemical filters, and found that 77% of the manually validated data could be accepted, yielding 35% greater data capture than the HCT (2).

Another approach simulates the intensities and m/z of fragment ions, then scores for similarity between resulting theoretical MSMS and observed spectra. Early studies used statistical analyses of large datasets to predict ion intensities, but the best results have been obtained by simulating gas phase fragmentation chemistries. The feasibility of the latter approach was shown by Z. Zhang (3), who modeled rate constants for peptide fragmentation based on proton affinity at the peptide bond, availability of protons, intrinsic cleavage kinetics determined by the adjacent amino acids, and other reactions such as C-terminal rearrangement and loss of H2O, NH3 or CO. We have found that similarity scoring between observed and theoretical spectra is able to confirm >95% of the manually validated sequence assignments in a MuDPIT dataset (4).

Despite these advances, when applied to a wide range of datasets, 30-70% of the high quality MSMS still cannot be assigned. Many can be identified as MSMS of "source" fragment ions, generated inside the MS as ions are accelerated through a regions containing gas (e.g. at the source, or as ions enter, cycle in, or exit from the helium-filled ion trap). Others are revealed as "chimera spectra", when two or more parent ions are trapped together in the isolation step, and a composite MSMS spectrum is produced. The Aebersold lab has estimated that 28% of MSMS from SCX fractionated peptides of a yeast extract are chimeras (5), and our own studies show as many as 45% MSMS are chimeras in mammalian datasets (4). Newer MS instruments may not solve the chimera problem, because even with high resolution instruments, 2-3 Da width in the isolation window is required. Generation of chimera spectra with high intensity source fragment ions from abundant proteins is probably an important reason why lower abundance proteins and low intensity fragment ions are so difficult to detect; this would account for the difficulty in achieving in-depth profiling and high sequence coverage.

Improving peak capacity during peptide fractionation is a possible solution, and may be viable as ultra high pressure HPLC on 75 micron ID columns becomes robust enough for 24/7 data collection. Another possible solution is to fractionate ions in the gas phase, for example by ion mobility spectrometry (IMS), where ions are resolved by through a gas-filled drift tube. Recent reports indicate that sensitivity problems that compromise

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Electrophoresis past, present and future

the usefulness of IMS are being solved (6), although the generation of source fragment ions generated in the drift tube has not been addressed. In the meantime, utilizing judicious protein and peptide chromatography protocols or focusing on specific subcellular fractions may provide the best means of side-stepping the complications of instrument generated fragment ions and chimera spectra.

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# Results of AES 2006 Councilor Elections

*Three councilor seats were open for election and all have been filled. One standing councilor, Dr. Bob Stevenson, volunteered to run again, while two new candidates, Dr. Sharon Sauer and Dr. Joe Biernacki, volunteered as well. We are once again lucky that our applicants, tapped by the standing council, are of the highest caliber. Their photos are below; biosketches will be in the January newsletter. The candidates will be formally approved at the San Francisco meeting in November.*

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Our meeting co-organizer, Adrienne Minerick, has been productive in many ways. The AES proudly welcomes her and husband Rob's new baby into the world: Madison Dagny Minerick, HS class of 2024.