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AES Newsletter

Inside this issue:

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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. Their support is greatly appreciated.

We also thank NIH for travel grant support for 2005.

in Just-spring, when the world is puddle-wonderful... (ee cummings), AES members' thoughts turn to the San Francisco meeting in November 2006.

The annual meeting at the San Francisco Hilton from Nov. 12-17 is shaping up to be the best ever! Sixty-eight abstracts were received via the online submission process and are currently being considered by the chairs for inclusion in their sessions. The contributions cover a number of exciting topics ranging from innovations in proteomics with mass spectrometry and 2D gel characterizations to novel electrokinetic tools and microfluidic devices that analyze everything from biological samples to non-Newtonian fluids.

We are particularly excited about our plenary speakers: Dr. H. Kumar Wickramasinghe, IBM Fellow and CTO Science & Technology, will talk about "UltraFast electrophoresis at the nanoscale using Atomic Force Microscopy" (see feature article in this newsletter). Dr. Robin H. Liu (co-author Sho Fuji) from CombiMatrix Corp will talk about "Integrated Microfluidic and Electrasense Microarray Biochips for DNA Analysis." Dr. Paul Bohn from the University of Notre Dame will talk on "Nanofluidics and Mass-Limited Chemical Analysis: Nanocapillary Array Membranes as Switchable Fluidic Elements for Multidimensional Analyses." Additional plenary speakers will be featured at the beginning of various sessions.

We look forward to seeing you in San Francisco. As always, please email either of us with questions or suggestions about the meeting.

Adrienne Minerick

Victor Ugaz

Meeting Co-organizers

Systems Biology and Computational Pathways Technologies: A New Approach For Bioinformatics

BY RAMON M. FELCIANO
Ingenuity Systems

Biological understanding is a unifying theme of the pharmaceutical R&D process, and a deep knowledge of biological systems fundamentally improves our ability to comprehend gene regulation, develop disease models and therapies, predict drug toxicities, and develop predictive biomarkers. High throughput technologies afford us the opportunity to assay 10,000s of genes, proteins and metabolites at a time, providing a unique, quantitative view into the cellular landscape of a living organism. However, a primary obstacle to leveraging the power of these technologies is developing a complete framework of how these genes, proteins and metabolites interact in a coordinated fashion to trigger biological and disease processes. Systems biology and computational pathways analysis deliver new informatics approaches to overcome this obstacle.

Over the past decade, bioinformatics has become an important component of modern biological research and drug discovery. Informatics databases and tools were critical in the development and maturation of the human genome project, and a broad range of commercial and open source tools are presently available to the research community. Most bioinformatics tools, however, are designed to support a specialized analysis or algorithmic data processing task (e.g. signal processing for 2-D gel electrophoresis analysis, or statistical significance assessment for gene microarray datasets). While these tools serve a critical need in experimental data analysis, scientists are typically left to their own devices in integrating various data points to form a "big picture" of what is going on in a particular system or disease being studied. More sophisticated tools to support knowledge integration at the level of biological understanding are clearly needed [1].

Ingenuity Systems has approached this problem by developing a technology to systematically integrate, analyze, and interpret a broad range of biological data types at the level of biological pathways. Our methodology is based on the observation that pathways have long been used by biologists and chemists to document and communicate the function of biological systems, as evidenced by their long standing presence in the scientific literature and textbooks. We therefore hypothesize that biochemical pathways and systems models of biological function are the natural conceptual layer at which to integrate this information in a fashion that lends itself to actionable interpretation. The focus on "actionable interpretation" reflects our foundational belief that bioinformatics should seek to develop computational solutions that can enable the broad community of scientists to streamline their research productivity and decision making. While initial forays into a new disease area may be purely investigational in nature, the desired result of most drug discovery and development is reaching sufficient insight to enable the researcher to move forward in the research process. A good solution will therefore enable a biologist not only to understand the data, but to use this insight to decide upon the next best course of action in pursuit of their research goals.

Our platform models how molecular pathways involving genes, proteins, chemicals and other molecules affect biological function at the level of cells, tissues, organs and the whole living organism. This approach makes extensive use of biomedical ontologies, expert curated knowledge, and semantic data integration technologies that we have developed to technical maturity and scalability required for application across a broad range of disease and therapeutic research areas. By integrating knowledge-based models of biochemical pathways with novel molecular profiling datasets, we can deliver system-level understanding and modeling in support of discovery and validation of novel drug targets, diagnostic and therapeutic biomarkers, disease models, and mechanism of action for drug therapies.

For example, biomarker researchers have leveraged this technology to develop gene and protein signatures enriched for biological pathways relevant to the target phenotype, as well as gain confidence in biomarker candidates associated with functions relevant to patient outcomes and assayable as a candidate serum biomarker. Computational pathways analyses can identify a set of transcriptionally regulated genes that are significantly correlated with signaling pathways and disease processes, and whose protein targets are secreted and detectable in blood, resulting in actionable biomarker hypotheses that can be validated through follow up proteomics assays [2-4]. Other use cases for computational pathways analyses include identifying biomarkers that are mechanistically based and causally associated with the disease, adverse effect, or clinical endpoint, and prioritization of biomarker candidates based on biological relevance to the disease model or patient population being studied (see http://www.ingenuity.com/solutions/ for other examples). We believe this technology platform enables new levels of biological understanding and insight that can transformationally enable biomedical research at an unprecedented rate.

1. Giallourakis, C., et al., Annu Rev Genomics Hum Genet, 2005. 6: p 381-406.

2. O'Toole, M., et al., Arch Neurol, 2005. 62(10): p 1531-6.

3. Pass, H.I., et al., N Engl J Med, 2005. 353(15): p 1564-73.

4. Wu, X., et al., Chem Biol, 2004. 11(9): p 1229-38.

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UltraFast Electrophoresis at the Nanoscale using Atomic Force Microscopy

BY KEREM UNAL, JANE FROMMER, H. KUMAR WICKRAMASINGHE
IBM Research Division, Almaden Research Center

Since its birth, atomic force microscopy (AFM) has evolved from a microscopy technique to a family of tools to alter and characterize surfaces at the nanoscale. We tailored an AFM to perform electrophoresis in milliseconds and with sample quantities smaller than zeptomoles (10^{-22} moles)[1]. The electrophoretic medium comprises a few water layers on the AFM tip surface while the driving force for differentiation is a large electric field applied over the length of the tip. Enhanced differential mobilities are observed stemming from the partial screening of charges in this peculiar experimental configuration.

Several alterations to a regular AFM probe are required to move molecules along its surface. The back of an AFM cantilever is coated with a metal to establish an electric field with the substrate surface along the length of the tip. A trench (150nm wide and 0.7-0.8mm deep) is fabricated with a focused ion beam at the base of the AFM tip to confine molecules and serve as a reservoir for the molecules to be separated. Finally, the surface of the probe must be modified for adsorption compatibility with the solute without irreversible binding, in our experiments with low molecular weight poly(ethyleneglycol) silanes.

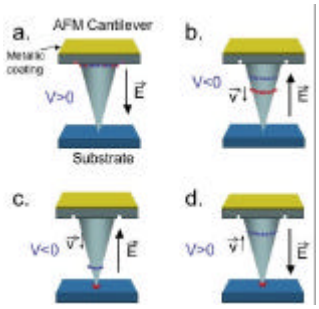

Figure 1. Scheme of operation for mobility measurements using a modified AFM probe

Electrophoretic experiments are performed within the 2-3nm condensed water layer formed on the surface of the AFM probe at the relative humidity conditions (35-50%). Molecules are loaded onto the tip by bringing the tip into brief contact with a droplet of aqueous solution without buffers to avoid additional ions in the electrophoresis medium. The tip is then moved to a clean spot on the substrate. To establish a common starting point for migration, the molecules are driven with a voltage (0.1-1.0V) away from the substrate, toward the trench at the wide base of the AFM probe, and maintained there with an electric field between the backside of the AFM cantilever and the substrate (Fig. 1a). The release of molecules is initiated by applying a voltage pulse (-0.1 to -10V) of inverse polarity to the confinement polarity, driving the molecules toward the substrate (Fig. 1b). Only the molecules that have diffused from the reservoir to the high field region near the base of the tip take part in the electrophoretic process during the release cycle. If the pulse width t is larger than the time t_0 required for the molecules to travel the tip length, the molecules are released from the tip and deposited on the substrate (Fig. 1c). Those molecules with lower mobilities that remain on the tip are driven back to the top of the AFM tip by once again reversing polarity (Fig. 1d). Close control over the pulsing protocol allows one to deliver discrete, detectable populations on the substrate. For detection, it is critical that the deposited molecules are immobilized on the substrate surface to avoid surface diffusion. We utilized an amino terminated surface (3-aminopropyl-tri-ethoxysilane, APTES) to take advantage of electrostatic interactions with solute DNA fragments. The transfer of molecules on the substrate surface is detected by scanning the substrate surface with the same AFM tip working in lateral force microscopy mode, a technique sensitive to changes in the friction of the tip with the surface.

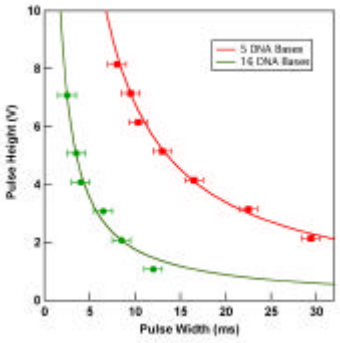

Figure 2. Threshold pulse height as a function of the pulse width for 5-and16-base long DNA fragments.

By measuring the threshold pulse width for molecules to travel down the tip and arrive on the substrate surface, the velocity v of molecules moving along the known length of the AFM tip surface can be determined. For any given electric field E the mobility μ can be derived using the relation $v = \mu \cdot E$ (eq. 1). We determined the mobilities of two lengths of DNA fragments from the threshold pulse width data (Fig. 2) and the electric field. Electrophoretic mobility of $\mu_5 = 1.84 \times 10^{-5} \pm 0.02 \times 10^{-5}$ (cm^2/Vs) and $\mu_{16} = 7.13 \times 10^{-5} \pm 0.07 \times 10^{-5}$ (cm^2/Vs) for 5 and 16 bases, respectively.

The observed transit times for molecules are in the order of few milliseconds. AFM mediated electrophoresis could lead to separation times 10,000 to 100,000 times faster than in capillary electrophoresis systems.

In free solution, the macroscale mobility of DNA fragments with more than 10 bases does not scale with the number of bases since the charge-to-mass ratio is constant[2]. In our measurements, mobility *does* depend on chain length; a ratio of ~4 is found when mobilities are compared (μ_{16}/μ_5). This discrepancy is attributed to the influence of the tip surface charge, which is not fully screened by the nanometer-scale water layer on the tip. In free solution the screening of surface charges by water creates an electrical double layer with a Debye length that is miniscule

Continued on p 4

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Newsletter subscriptions are complimentary with AES membership. For more information or to join AES contact Matt Hoelter (see above). To submit articles contact Nancy Kendrick.

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AFM, from p 3

compared to the bulk water volume. In our experiments the electrical double layer is greater than the thickness of the water layer, a phenomenon that has been reported to have a dramatic influence on the conductivity of nano-fluidics and nano-pore systems.

One further application of this technique is in surface patterning. The relative position and the number of molecules deposited are precisely controlled by repeatedly applying the described pulse protocol. This is illustrated in Fig. 3 where 5-base long DNA fragments are deposited at a bias of 10 V and a scan rate of 250 nm/s using 10 ms pulses with a 30 ms period.

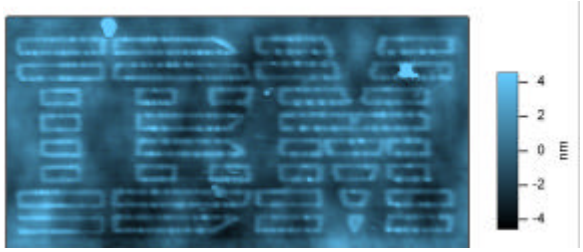

Figure 3. Surface patterning with 5 bases long DNA fragments the logo measures 3.3 by 8.8 microns.

Our recent results suggest that DNA fragments could be separated for lengths up to 100 bases. In other experiments, we measured mobilities of different molecules such as dyes, proteins and viruses showing the broad applications of this method. We therefore envision a significant impact in biology and medicine, for example by reducing DNA sample size and accelerating throughput in a sequencing procedure. The method is easily scaled in a massively parallel operation in tip arrays, such as the Millipede currently under development at IBM research.

[1] K. Unal, J. Frommer, H. K. Wickramasinghe, Appl. Phys. Lett. 88, 183105 (2006).

[2] Q. Dong, E. Stellwagen, J. M. Dagle, N. C. Stellwagen, Electrophoresis 24, 3323 (2003).

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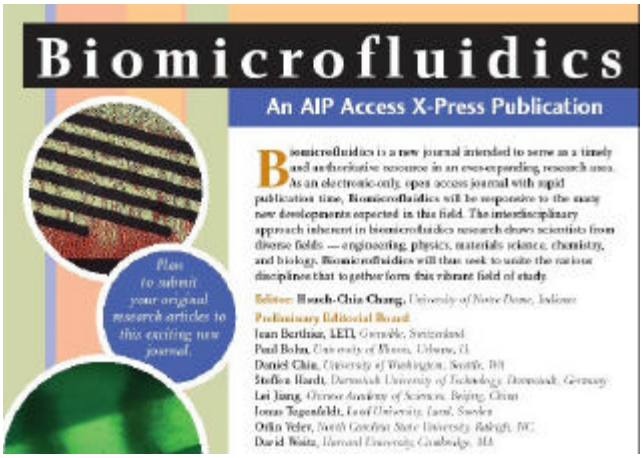
See the recent review of Victor Ugaz's "DNA Lab on a Chip" in Texas A&M Engineering News!

http://engineeringnews.tamu.edu/news/1320

The news item is based on a recent description[1] by Ugaz and Ph.D. student Faisal Shaikh of a system for isolating and concentrating small amounts of DNA that would otherwise be difficult to analyze.

[1]*Proceedings of the National Academy of Sciences of the United States of America* **103**, 4825-4830 (2006).

Like last year, a proposal has been submitted to NIH requesting support for graduate student / postdoc travel and registration expenses. If successful, the timeline on this will once again be in early October.



Submit an article from work presented at the meeting to a special edition of the new journal *Biomicrofluidics*. Contact our meeting organizers (see front page) for a full-page flier and more information.