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AES Newsletter

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Don't forget, our next meeting in Cincinnati is just around the corner….

Report from Adrienne Minerick and Victor Ugaz, our meeting organizers:

Dear Electrophoresis Enthusiasts,

The 2005 Annual AES meeting has shaped up nicely. On Monday, our program kicks off with a plenary session featuring three outstanding researchers. Mark Molloy will discuss "Drilling Deeper into the Proteome," Anup Singh will awe us with his talk on "Intergated Microfluidic System for Oral Diagnostics," followed by Brenda Korte who will give an enlightening talk on "Funding Opportunities in Technology Development at the NIH." The remainder of Monday will feature two sessions on Advances in Proteomic Analysis with a focus on electrophoresis - based approaches. Tuesday morning the Biomedical Applications and Bioinformatics sessions will take place. On Tuesday afternoon, we'll transition to BioMEMS and Microfluidics with a focus on Cell & Biomolecule Analysis. Wednesday's talks will cover Biomedical/Bioanalytical CE and Microdevice Technology. Thursday will include sessions on Electrokinetic and Electrophoretic Applications. A special AES poster session will run from Monday through Thursday; the poster reception and awards will be Tuesday evening. Please join us for these stimulating and exciting contributions to the broad field of electrophoresis.

Many thanks to all the session chairs and co-chairs for their hard work!

Adrienne

Victor

Many thanks to our Sponsors for contributions funding the 2005 meeting.

Bio-Rad Laboratories

CBS Scientific

Danisco Genencor

GE HealthCare

Nonlinear Dyanmics

Proctor & Gamble

Proteome Systems

Scimagix

Syngene

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.

For authors and talk abstracts (TOPICAL A), go to: http://aiche.confex.com/aiche/2005/preliminaryprogram/index.html/

Dielectrophoresis: Tools to Manipulate Cells in Microdevices

Lab-on-a-chip and micro-Total Analysis Systems (mTAS) have generated much excitement in recent years due to their potential to provide high resolution, low cost, and rapid analysis for a wide range of biological and chemical applications. The pursuit of these fully integrated analytical devices has spawned the development of micron scaled technologies to 1) isolate specific cells of interest, 2) amplify the information if necessary, 3) accurately measure an indicator for the cell, and 4) deliver or measure a quantitative result. Electrokinetic tools are attractive and utilized with many of the 1 through 4 stages described due to selectivity and ease of interfacing within microfabricated devices.

This brief discussion focuses on one relatively new electrokinetic technique with far-reaching and versatile appeal: dielectrophoresis (DEP) is the application of an alternating current in a nonuniform geometry. At radio frequencies, cells are selectively controlled due to their inherent intramembrane polarizability characteristics, which cause them to respond uniquely from other cells at specific AC frequencies. Genetically or geometrically similar cells have more similar, but still distinct, responses at given frequencies. The dielectrophoretic force acting on the polarized cell causes it to move either up or down the electric field gradient created by the non-uniform electrode geometry.

Dielectrophoresis can be considered a competitive alternative to the more conventional methods of cell concentration and separation, such as centrifugation, filtration, fluorescence activated cell sorting, or optical tweezers. Because DEP can operate directly on native, unlabeled cells, it eliminates the expense, labor and time of labeling and tagging, as well as the development and validation of such labels and tags. The same basic DEP method has the (probably unique) capability of isolating and analyzing a wide range of particle types (cells, bacteria, viruses, DNA and proteins) using one basic procedure.

After their selective isolation and recovery by DEP, cells remain viable for further analysis, processing or cell therapy. Multiple parameters on individual live cells can be determined and, if desired, specific cell types can be collected. Because it utilizes electronic signals, the technology is capable of extensive automation, is inexpensive and is portable. DEP can also operate under sterile conditions.

Although the ability to selectively isolate cells without harming them is important for many biomedical applications, there are also advantages to be gained by being able to destroy selected target cells. DEP appears capable of achieving this objective. Just as there are limitations in the use of electrofusion and electroporation (mainly associated with the fact that not all cell types can be treated with the same ease, and with irreproducibility between different laboratories), we can expect that efficient and reproducible protocols for selective cell destruction by DEP will not be so easily achieved as the DEP isolation of viable target cells. But it is a worthwhile challenge.

This then prompts the discussion of two possible DEP applications. The first is a pure DEP analysis of an intact cell possibly able to provide enough information that the traditional subcellular efforts to accomplish DNA isolation and polymerase chain reaction (PCR) amplification for genetic detection would be obsolete. The second is to contribute to the first two stages of a mTAS system – to nonchemically lyse cells such that the DNA-PCR-detection sequence could continue. We expect to see exciting developments in each of these areas.

In conclusion, dielectrophoretic technologies are an attractive tool with wide-reaching applications in whole cell separation, manipulation, and characterization. However, much work is needed to seamlessly interface DEP modules with other microdevice components in order to attain the sought after lab-on-a-chip, systems. This will require the cooperative pursuit of research into these technologies by individuals in a variety of academic, clinical, and industrial settings. The American Electrophoresis Society currently strives to facilitate these important dialogues and invites you to become involved at the 2005 Annual Conference in Cincinnati, OH. For additional reading, we suggest the following six articles:

1, Gascoyne, P.R.C, and Vykoukal, J. *Electrophoresis*, 23, 1973-1983, 2002
2. Hughes, M.P. *Electrophoresis*, 23, 2569-2582, 2002
3. Cummings, E.B., and A.K. Singh, *Anal. Chem.*, 75(18), 4724-4731, 2003
4. Minerick, A.R., R. Zhou, P. Takhistov, and H.-C. Chang. *Electrophoresis*, 24(21): 3703-3717, 2003,n
5. Pethig, R., *Crit. Rev. Biotechnol*., 16:4, 331-348, 1996
6. Pethig, R., M.S. Talary, and R.S. Lee, *IEEE Eng Med Bio Mag*, 22(6), 43-50, 2003.

Adrienne Minerick

Ronald Pethig

An Epos of Isoelectric Focusing in the Alpha Century of Electrokinetic Methodologies

by Pier Giorgio Righetti

I was born in 1968. The year that rocked the world according to Mark Kurlansky (Vintage books, Random House, London, 2005) and quite a few others. There has never been a year like 1968, and it is unlikely that there will ever be one again; a year in which a spontaneous combustion of rebellious spirits occurred around the world. Universities were leading the protest that started at Berkeley with Jerry Rubin, spread to NYC with the civil rights movement leader Abbie Hoffman, and continued in Paris with Cohn-Bendit and his army equipped with *sanpietrinos* (the porphyry cubes thrown at the police, named after the pavement of S. Peter's square). Quite a few world leaders were swept ashore by the big surf of 1968: L.B. Johnson did not run for re-election, Robert Kennedy and Martin Luther King were assassinated, De Gaulle was soon to retire.

More humbly, in 1968, I was doing my time (not as convict, though) at MIT (Cambridge, MA) as a post doctoral fellow at the Department of Nutrition and Food Science. As luck goes, we were visited one day by a Japanese scientist who delivered a lecture on isoelectric focusing (IEF) of ferritins. Japanese truly amaze me, they are always on the forefront: nobody had ever heard of IEF and no one could quite figure out how it worked. It was instant love, a passion that devoured my life and my energies. Upon returning to the University of Milano in 1970, I spent the next decade working out every aspect of it, synthesizing novel types of carrier ampholytes, fractionating them in narrow ranges with a continuous flow IEF device, exploring ways and means of counteracting the decay of the steady-state (the infamous cathodic drift), applying it to all sorts of clinical chemistry problems (by intensive screening of fetal haemoglobins in newborns, together with G.F. Cossu, we practically eradicated thalassemia from Sardinia; see P.G. Righetti, *Isoelectric Focusing: Theory, Methodology and Applications*, Elsevier, Amsterdam, 1983). Yes, IEF was just as big a revolution in Separation Science as the student movements of 1968 were!

By the end of the seventies, though, it was apparent that IEF in soluble carrier ampholytes was beginning to show the crippling disease of age. So, we teamed up with scientists at LKB Produkter (later to be incorporated into Pharmacia) to work out a totally new concept, immobilized pH gradients (IPGs), that seemed to iron out all the wrinkles of conventional IEF. This new work of art was unveiled in April, 1982, at the electrophoresis meeting organized by D. Stathakos in Athens and acclaimed with standing ovations. At least that is what we expected. In reality, we presented these data to an almost empty room, since most of the delegates had never been to Athens before and were enjoying the lovely spring weather on the Acropolis, Licabetto and the Plaka. Discouraging as this was, it gave us leeway over the competition and set us working for another decade on all aspects of IPG development: synthesis of all possible Immobilines (from barely six to sixteen, from vacuum to plenum chemistry, as we wrote in a review), theoretical simulations for developing extended pH gradients (in those days we thought one could use them only for generating 1-pH-unit wide gradients), preparative aspects, including the idea of multicompartment electrolyzers with Immobiline membranes, that allowed harvesting ultra-clean protein species in a liquid vein. This concept was abandoned in the nineties, but resurrected recently as a pre-fractionation tool in proteome analysis. With our work, we brought democracy into the realm of electrokinetic methodologies: when running IPGs in a pH 3-10 gradient, for analysing a total cell lysate, as a first dimension of a 2D map (another great discovery of the seventies, O'Farrell *docet*), we realized that a linear pH profile would not be "democratic," but aristocratic, since it would give plenty of extra space to fewer alkaline proteins and confine the much more abundant acidic proteins into a shantytown. So, we worked out a non-linear IPG pH 3-10, the only "democratic" gradient giving equal rights to the population at large. It was the biggest mistake of our life, since we did not think of patenting it: today 99% of the scientists engaged in proteome analysis have adopted it, but not only we do not see a penny, but are not even given credit for it (that is why I still move around on a red bicycle instead of a Ferrari Testa Rossa)! See P.G. Righetti, *Immobilized pH Gradients: Theory and Methodology*, Elsevier, Amsterdam, 1990). Yes, IPGs turned out too to be a major innovative step in Separation Science.

What would the next decade (the nineties) bring to us was largely determined by a 1989 meeting organized in Boston by Barry Karger: the First International Symposium on High Performance Capillary Electrophoresis (CZE). To all those who thought that this First would also be the Last, the following years would bring a great disappointment since there followed a deluge of meetings on CZE, still much in vogue today, although, all in all, CZE did not live up to the expectations, namely of surpassing HPLC: there seems to be no way for us electrophoreticists to prevail over chromatographers. And that is precisely what we found out by spending a decade working with CZE. As long as we applied the technique to DNA separations, we always got plenty of good results, but when we approached proteins… well, matters were not so smooth. No matter how hard we worked in developing good coating for the silica wall, novel acrylamide derivatives endowed with high hydrophilicity and very high resistance to hydrolysis, including some recent chemicals (quaternarized piperazines) able to spontaneously react with the silica surface and alkylate silanols, proteins had a love affair with the silica (naked or dressed up, mind you) and kept being adsorbed by this surface. Do not trust street peddlers selling you this technology for protein separations (peptides do much better, though): it hardly works and that is why, for protein analysis, we will never be able to compete with HPLC. Notwithstanding that, I did not overcome my obsession with IEF and reported plenty of IEF variants applied to a capillary format. See P.G. Righetti, *Capillary Electrophoresis in Analytical Biotechnology*, CRC Press, Boca Raton, 1996.

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Electrophoresis past, present and future

What did the third millennium bring about? Proteomics, of course, and I am now living in a lab with the walls pasted with dry, large 2D maps filled with thousands of tiny blue spots, trying to bring democracy in the field of protein analysis. You are surely aware that all proteomes are not democratic; oligarchic, at best. Take the human serum proteome, for example: the first 50 proteins represent about 99% of the total amount of protein mass but only less than 0.1% in number. They swamp and obliterate the "silent majority." So, we are presently working with "equalizer beads," a library of hexameric peptide ligands, made with the 20 natural amino acids (thus containing, in principle, some 64 million different ligands), able to capture all components of a mixture, sharply cutting the concentration of the abundant species and enriching the rare and very rare ones. Digging deep down in the mine to bring to light the "hidden proteome." Watch the current literature for the latest news.

Projections for the future? Just a major one: I want to give up and let the young generation carry the Olympic torch. It is just about time!

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Professor Righetti's article had to be shortened because of space constraints. The full text can be found on the AES website: www.aesociety.org under the Society Newsletter link.

Announcement: Councilors and Secretary needed!

Two councilors and a new secretary will be elected at the November meeting based on results of October email balloting. The 3-year terms of councilors, Pedro Arce from Tennesee Tech University and Alfred Gaertner from Danisco Genencor, as well as that of our Secretary, Jasmine Gray from GE Healthcare, will expire this year.

Duties of the AES Secretary (3-year position) are not arduous and include taking minutes at meetings, keeping a record of bylaw changes, and serving on the council. Other council members will assist with minutes whenever necessary. Similarly, the 3-year position of AES Councilor doesn't take a lot of time but nevertheless is quite important to the Society. The Council, which includes the President, Past President, Secretary and Treasurer, as well as six Councilors, meets formally in person at the annual meeting, and throughout the year by email and telephone conference. Important issues are discussed by the Council as they arise. After full consideration a vote is taken and a course of action implemented. It's also an opportunity to interact with a dynamic and intellectual group. Please notify Scott Rodkey, Vice-President (lrodkey@uth.tmc.edu) or David Garfin, President (degarfin@sonic.net) by email if you wish to nominate a member or run yourself for AES Council. Please attach a biographical sketch to the message suitable for an email ballot. Photos are welcome.

Many thanks to our three council members whose terms have expired. They've done a fine job!

Dr. Jasmine Gray
GE Healthcare
AES Secretary
2002 - 2005

Dr. Alfred Gaertner
Danisco Genencor
AES Councilor
2002 - 2005

Dr. Pedro Arce
Chemical Engineering
Tennessee Tech
AES Councilor
2002 - 2005