53d Enhanced Molecule Detection in Capillary Electrophoresis Using Label Free Intrinsic Imaging

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Label Free Intrinsic Imaging (LFII) combines UV absorption, photodiode array detection, automated fluidic handling and digital signal processing to map the space-time trajectories of bioanalytes and chemicals in capillaries. This direct absorption technique eliminates labels and dyes by using signal-to-noise enhancing image processing algorithms, and has been successfully applied to protein sizing, DNA fragment analysis, chemical detection and DNA sequencing. Imaging the molecule itself, rather than the label chemically attached to it, significantly improves resolution while controlling migration time. There are also advantages in the speed of analysis, health and safety issues, reagent costs and reduced sample size. By working with unlabeled molecules, LFII also allows real-time fraction collection in the system. This gives us the ability to actively manipulate analytes, for example by switching an identified protein for subsequent analysis and the potential to identify using advanced data-mining tools. This paper will describe the key elements of LFII. This technology utilizes dynamic multi-pixel detection and advanced signal processing algorithms to enhance the signal to noise ratio in unlabelled systems allowing increased resolution, reproducibility and sensitivity. Experiments using small molecules, nucleic acids and proteins have been performed to demonstrate the capability of the system resulting in significantly superior performance over conventional capillary electrophoresis. The analysis of this very large data set is achieved by data acquisition from each molecule as it traverses each pixel at a specific time point. This allows a space-time correlation called vertexing to be achieved. Vertexing enables a vastly increased signal to noise ratio to be acquired allowing for molecular imaging that exceeds traditional single-detector absorption imaging techniques. Here we will show how these advances allow us to image label-free single-stranded DNA template to one base resolution, to analyse complex cell lysates and to differentiate between post-translational modification forms of proteins. Label Free Intrinsic Imaging has largely come of age over the last 5 years, from fairly crude gel scanning to image unlabelled restriction enzyme fragments, to contemporary capillary and chip based systems. The LFII instruments are single platform, multi-use technologies. DNA, RNA and proteins can be analysed on the same machine. Changes in the analysis software, the chip or capillary configuration and the sieving gel matrix are the only significant changes. DNA. DNA sequencing requires a nucleotide resolution of 1 base. deltaDOT has achieved DNA sequencing on standard kit-based cycle sequencing reaction product out to read lengths of 600 bases, but are concentrating on producing fully analyzed and annotated read lengths of 200 bases. We have developed techniques whereby we can sequence in a single channel without labels. We have also used LFII in RNA analysis. Protein. Using LFII we have also successfully imaged proteins in complex mixtures. Starting with standard protein markers, we successfully imaged a range of proteins from 14.5 kDa to 205 kDa in less than 10 minutes. E.coli cell lysates have also been separated at similar rates. We achieve ‘virtual' resolutions which allow unprecedented separation and analysis of individual proteins and groups of proteins in complex mixtures. We have also begun development of an ultrafast protein folding analysis system and have preliminary results to show how LFII can be combined with other technologies to gain multiplicative analysis power. Chemicals. As part of technical due diligence for a major corporate client we have analyzed a range of chemicals both in pure form and as part of a complex beverage mixture. This set of experiments allowed us demonstrate the superb reproducibility of our systems. Root Square Deviations (RSD) of 0.18% RSD in peak area, and 0.076% in migration time have been achieved over a range of sample concentrations. Bacteria and Viruses. As part of an expansion of the application range of deltaDOT's LFII technology we have detected ‘live' (fresh from culture) samples of bacteria and established differences in mobilities between species. We are embarking on similar studies with viruses. We have identified and in part developed a range of applications based on these technology components which suggest LFII can make a powerful contribution in many areas of drug discovery, development and other biotechnology and general analysis areas.