Label Free Intrinsic Imaging for Capillary Electrophoresis

‘免标记内成像技術’（LFII®）带领毛细管电泳走向分析新时代

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Introduction

Label Free Intrinsic Imaging (LFII®) is a methodology of analysis which removes a whole class of systematic errors associated with labels and stains, using the absorbance characteristics of the molecule as defined by Beers Law. In proteomics, typically the peptide bond between each amino acid is detected at 214nm. LFII® has also been applied in the analysis of Peptides and Amino Acids, of small molecules such as racemic drug compounds and in Baculovirus Expression system titres. It can also analyse nucleic acids. Furthermore its unique migration time based analysis also allows it to be used for protein conformational studies.

The technology has its roots in particle physics analytical tools from the 1980’s and 1990s: The basis of this technique is Vertexing, a method adapted directly from high energy particle physics to determine the structure of matter. Vertexing uses multiple detection to reconstruct the decay point of a sub atomic particle resulting from a collision. This technique is used in the hunt for sub-atomic particles at places like CERN.

It is our ability to track an entity as it passes through multiple detectors, in the form of a Photo diode Array and correlate the space/time data that gives LFII® its power.

deltaDOT 公司專利的‘免标记内成像’技術（LFII®）与现有的直接监测无标记生物分子的CE技术相比，具有更高质量的分离功能，从而降低了每次分析的成本和时间。 deltaDOT将分子生物学和粒子物理学技术应用于生物分子分离，包括蛋白质、DNA和RNA分析。公司在仪器仪表、分子生物学、微流体、自动化、计算机信息处理和分析领域具有牢固的专利地位和丰富的专业知识，这将有助于改善贯穿药物发现和一般生命科学研究的专业知识、工作效益和处理时间。

Instrument

Capillary Electrophoresis (CE) is an efficient separation method that is widely used in biochemical and pharmaceutical research. Its application in these fields stems from its speed, high sample throughput, ease of automation, high separation efficiency, high precision and low sample volume. The first recorded demonstration of this technique was in 1967 when Hjerten demonstrated a free solution electrophoretic separation using a capillary of 3 mm internal diameter. Capillary electrophoresis has evolved into a family of techniques including capillary gel electrophoresis (CGE) and capillary zone electrophoresis (CZE) which were both translated from traditional formats. Other modes of this family include capillary iso-electric focusing (cIEF), micellar electrokinetic chromatography (MEKC) and non-aqueous CE (NACE).

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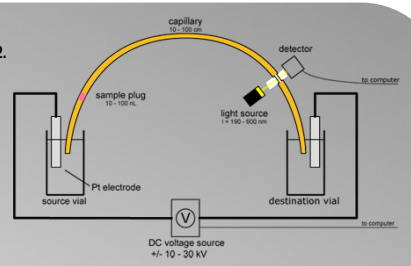


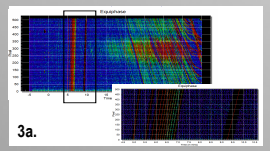
The **PEREGRINE I** instrument is an automated single capillary system incorporating a photodiode array detector. Samples and reagents are delivered using two, twenty four position, carousels on either side of the main cover. The capillary is mounted within a Peltier temperature controlled aluminium block housed under the main cover.

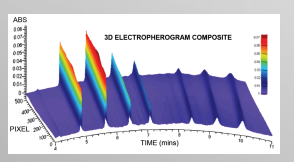
A schematic of a CE system is shown in Figure 2b highlighting its basic components. A narrow bore glass capillary, typically between 20 and 200 µm internal diameter, is used to bridge two buffer reservoirs. Prior to separation the capillary is filled with sieving matrix following which the sample is injected into the capillary using a pressure differential or by applying a voltage across the capillary. The capillary end is then replaced in a buffer reservoir and a field applied across it. This induces migration towards the outlet and detector. The sample migrates through the capillary and separates into its constituent components. On traversing the detection window each component generates a signal. This signal is converted to a trace called an electropherogram.

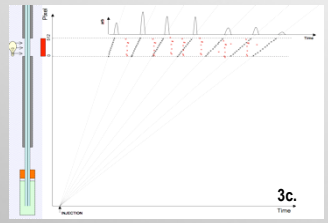
Vertexing

An Equiphase map (Figure 3a) is a three dimensional view of the separation, where the axes are distance, time and absorption. The Equiphase Vertexing algorithm searches for and calculates the size and position of all analyte peaks. The 3D image generated allows a macromolecule to be tracked as it migrates past the detector.

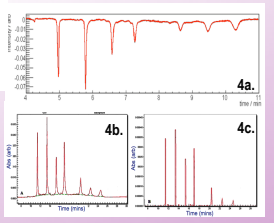
The slope of the track generated is used to calculate the velocity of the analyte. A schematic of an Equiphase map is shown in Figure 3c with the associated single pixel electropherogram that may be derived from any pixel in the detector. An analyte traversing the detection window will appear in consecutive pixels of the PDA depending on its velocity. The velocity of an analyte is dependent on its intrinsic characteristics (e.g. molecular weight, pI and overall surface charge) and the applied field.







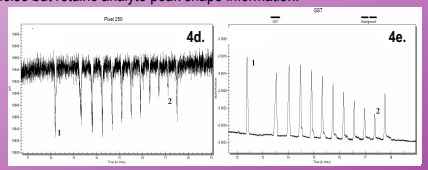
Extrapolation of analyte bands back to their injection point using their migration across a multi-pixel detector is described in Figures 3b and c. Any noise in the system, such as lamp fluctuations or bubbles will not hit the vertex (or injection time) and be ignored. The height or intensity of the peak directly correlates to the amount of analyte in the band (Figure 3b), allowing very accurate quantification. Moreover, the sample tracks can also be extrapolated forward to predict when any selected peak can be collected at the outlet end of the capillary after separation.



Signal Processing

Signal processing applied to the data obtained from the PDA can generate several data outputs. Traces from three of these have been used in herein: single pixel electropherograms, Generalised Separation Transform (GST) electropherograms and Equiphase Vertexing Algorithm (Figure 4a, b and c respectively). These traces form part of the P3EVA analysis software provided with the PEREGRINE I instrument. A single pixel electropherogram is the trace obtained from a single detector, Figure 4a shows the trace obtained from pixel 250 which is the default setting of the P3EVA analysis software. The GST algorithm is a method of signal averaging while it maximizes the signal-to-noise but retains analyte peak shape information.

The improvement in signal to noise values between single pixel and signal averaging over multiple pixels was demonstrated using two peaks from a 25 bp dsDNA ladder, Figure 4d and e. Peaks labelled 1 and 2 in Figure 4d and e were chosen as they show the highest and lowest signal in the electropherogram presented and because of the different polymer lengths (25 bp and 275 bp respectively).



The S/N values for peaks 1 and 2 at pixel 250 were 12.5 and 5.6, whereas S/N values for the same peaks using GST processed data were 115 and 36.8. This indicates an order of magnitude increase in S/N between the detection approaches.