Leaky Waveguide Devices for use in Fourier Transform Analysis of Electrophoretic Separations.

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Abstract

Presented here is a novel application of Leaky Waveguide Devices to electrophoretic separations with Fourier Transform detection. The Leaky Waveguide Device couples light into and out of a microflow channel, providing an increase in the detection pathlength relative to traditional fixed-point illumination methods. This increased path length results in an increase in the analytical sensitivity, and could also allow real-time kinetic analysis of electrophoretic analytical separations. The Leaky Waveguide Device is constructed from injection moulded polystyrene, which not only reduces the production costs per single unit, but also allows application in areas where contamination must be avoided and one-shot devices are desirable.

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1. Introduction

1.1 Micro Total Analysis Systems

The Micro Total Analysis Systems (μ-TAS) were proposed by Manz et al. in 1990 [1]. The current generation of systems has been expanded to accommodate a greater number of applications, including synthesis reactions [2], Polymerase Chain Reaction (PCR) [3] and analytical Separations [4]. The term μ-TAS is currently being succeeded by the phrase Lab-on-a-Chip [5]. Such systems are designed around a theme of miniaturising current generation analytical laboratory instruments to improve analysis times, minimise reagent and analyte volumes and reduce waste materials. It has also been shown that miniaturised reaction systems are capable of combining reagents to yield products which can not safely be prepared under the same conditions in larger scale equipment [2].

The benefits of these miniaturised analytical devices can be limited by the information one is able to obtain from them. There is very little value in using miniaturised devices to perform reactions and separations if the detection apparatus used is of laboratory scale. A far more convenient approach is to integrate the detector system to a greater or lesser extent into the μ-TAS system. This has been conducted using electrochemical [6] and optical [7,8] detection methods especially. The advantage of electrochemical detection is that electrodes operate in small volumes in much the same way as in large volumes. Optical detection on the other hand requires a pathlength over which light can interact with the analyte molecules. Traditional fixed-point detection systems, where the light enters the detection cell across the flow of analyte, result in a very small residence time for analyte molecules. Subsequently a lower than optimal limit of detection (LOD) is obtained. A method of increasing this LOD is to increase the path length of light through the channel, thus increasing the residence time for an analyte molecule in the path of the incident light. Miniaturised systems are not ideal for end-firing of light into the channel, due to the small dimensions of said channels. Other methods of allowing light to enter a channel include using grating or prism coupling. It is the latter of these methods which is used in the work presented here.

1.2 The Leaky Waveguide Device

The Leaky Waveguide Devices (LWD) [9] utilises Fresnel reflections at boundaries between materials to guide light incident to a surface along the micro channel. Since Fresnel reflections are not total internal reflections,

light is able to escape at each reflection. Hence the leaky nature of the device. (Fig. 1)

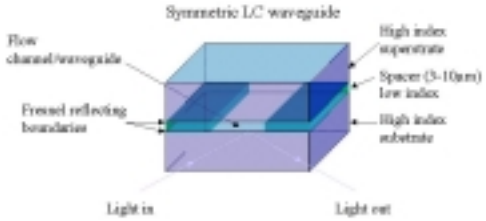

Figure 1: Leaky Waveguide Device with integrated micro-flow channel

Whilst this limits the propagation distance available to incident light, the guiding feature is more than sufficient to allow full coverage of the length of the channel (~40 mm). A prism is used to couple light into the channel, where propagation occurs. As light passes along the system, it excites fluorescence in the analyte molecules present, which have been selected to respond to the wavelength of light used. This allows simultaneous monitoring of components in the entire length of the channel.

1.3 Capillary Electrophoresis

Electrophoretic separations result from the differing mobilities of analyte molecules, under an applied electric field. The mobility of a analyte is proportional to its charge, inversely proportional to any retardation forces, such as viscous drag as defined by the physical dimensions of the solute, and to the magnitude of the applied field [10].

The migration velocity is given by the equation: -

$$V_{ep} = \mu_{ep} E \qquad (1)$$

Where; μ is the electrophoretic mobility and E is the electric field strength.

Capillary electrophoresis offers many advantages over the traditional gel electrophoresis, with speed of analysis being a major improvement. The small cross-sectional area of a capillary results in a higher resistance, such that the power dissipation and thus Joule heating is minimised. As a consequence of this, larger applied fields can be used during the separations, which in turn result in faster separations. The greater dissipation of heat from a capillary also leads to a minimising of band broadening, which is brought about by increased diffusion in a gel system. This gives rise to greater reproducibility of separations, with peak widths often approaching the theoretical limits defined by longitudinal diffusion coefficients and column efficiencies of several hundred thousand plates have been observed [11].

1.4 Image Capture and Analysis

Real-time imaging of the separation channel with off-line processing was achieved using a Baxall CD9311 CCD camera with Motion Picture video capture software and hardware (ATM Ltd., UK) (Fig. 2). This allows capture of Windows Audio Visual Image (AVI) movies at 600 x 400 pixel resolution. Sequential single frames were extracted from the AVI movies using a Labview (National Instruments, USA) based program, where they could then be processed using Scion Image (Scion Corp. USA). This is a Windows port of the Macintosh NIH-Image program, which is designed to analyse digitally captured images, specifically electrophoretic gel lanes.

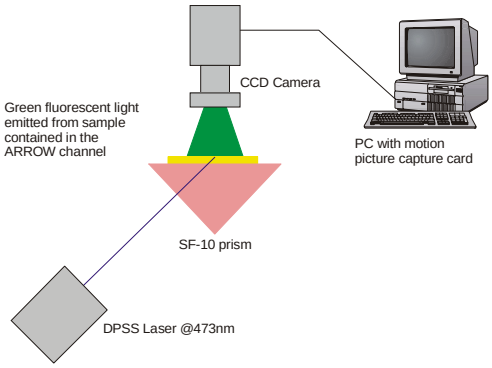

Figure 2: Equipment Arrangement

On-line processing is achieved using an Electrim ECD-2000N 10-bit CCD camera and capture board (Electrim Corp, USA). The arrangement of the apparatus is the same as in Fig. 2. Software written in-house (NJG) allows real-time pixel line scan and Fourier Transform analysis of the data. These data are then saved as a text file.

1.5 Micro Analysis Devices

The separation device is fabricated from injection moulded polystyrene. The mould is produced using EdgeCAM software (Pathtrace, UK) to create milling files for use

on a Datron CAT3DNC CNC Milling Machine (Datron Technology, UK). This machine is capable of milling to resolutions of between 2-3 microns. The moulds are then used in a Babyplast Injection Moulder (Chronoplast S.A. Spain) to allow production of the finished devices, or chips. A slight modification to the mould design allows the insertion of pre-formed copper or gold electrodes prior to injection of the polystyrene, such that the chips possess integrated electrodes. The channels are 100 µm wide, and are arranged on a 40 x 40 mm device as shown in Fig. 4, as are the injection moulded prisms. A single sided 500 µm thick adhesive coated polyester sheet (Plastic Art, UK) is used to seal the channel, and a double-sided 80 µm thick polyester tape ARCARE 8725 (Adhesives Research, USA) is used to adhere the injection moulded prisms to this structure.

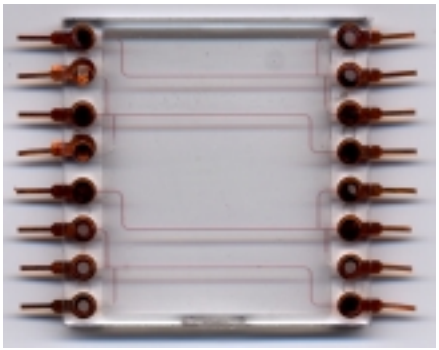

Figure 4: The Injection moulded separation device, with integrated electrodes.

The initial work was conducted using a photopolymer sandwich layer coated onto a glass substrate [8]. Access wells were either drilled into a cover slip. The photopolymer was a conventional positive working liquid photoresist (Shipley SJR5740), spincoated to produce a layer approximately 10 µm thick (Fig. 5).

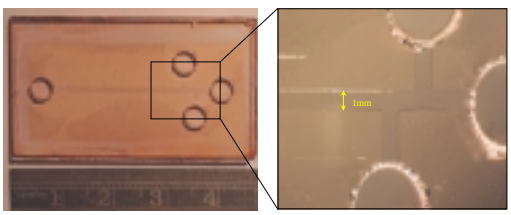

Figure 5: Liquid Photoresist chip.

2. Experimental

Fluorescein which absorbs in the blue region is excited using a 473 nm Diode Pumped Solid State Laser (Laser 2000, UK), and migrates at rates determined by the electrophoretic mobility. A Brandenburg PMT PSU was used to supply the drive voltage, which was maintained at 500 V DC. A ground switching circuit was constructed in house to allow discrete bands of dye and buffer to enter the separation channel.

A crystal-controlled clock is used to provide accurately timed repeated injections of sample into the separation channel. This provided a set of bands whose peak-to-peak separation depended on the electrophoretic mobility. The fluorescence intensity along the channel is recorded (Fig. 6 and 7), then spatially Fourier Transformed to produce the electropherogram (Fig. 8). Since the separation is continuous, successive Fourier Transforms can be averaged together to provide even better signal-to-noise performance than that provided by the multiple injections of sample.

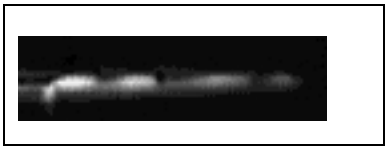

Figure 6: Single frame captured from a single component injection

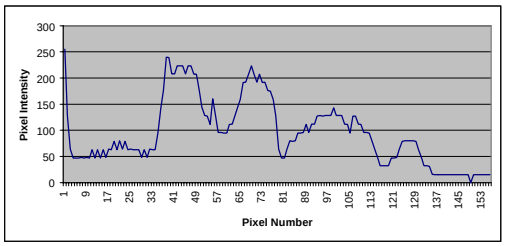

Figure 7: Pixel intensity plot of Fig. 6.

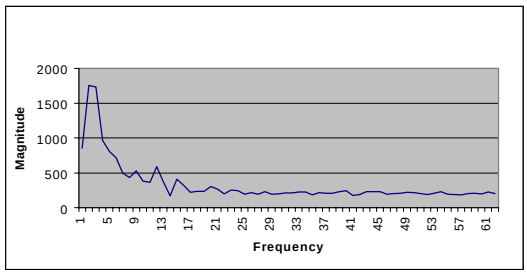

Figure 8: Fourier Transform electropherogram of Fig.7.

3. Conclusions

Preliminary data shown here indicate that the combined approach of using Leaky Waveguide Device miniaturised electrophoretic separation systems with Fourier Transform analysis is a viable technique, which is capable of increasing the signal-to-noise ratio substantially.

The combined approach of using one-shot injection moulded chips allows applications in situations where contamination must be a avoided, and the cost per unit volume is significantly reduced by using polystyrene as an alternative to glass or silicon.

4. References

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