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Integrated micro-optical fluorescence detection system for microfluidic electrochromatography

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ABSTRACT

We describe the design and microfabrication of an extremely compact optical system as a key element in an integrated capillary-channel electrochromatograph with laser induced fluorescence detection. The optical design uses substrate-mode propagation within the fused silica substrate. The optical system includes a vertical cavity surface-emitting laser (VCSEL) array, two high performance microlenses and a commercial photodetector. The microlenses are multilevel diffractive optics patterned by electron beam lithography and etched by reactive ion etching in fused silica. Two generations of optical subsystems are described. The first generation design is integrated directly onto the capillary channel-containing substrate with a 6 mm separation between the VCSEL and photodetector. The second generation design separates the optical system onto its own module and the source to detector length is further compressed to 3.5 mm. The systems are designed for indirect fluorescence detection using infrared dyes. The first generation design has been tested with a 750 nm VCSEL exciting a 10^{-5} M solution of CY-7 dye. The observed signal-to-noise ratio of better than 100:1 demonstrates that the background signal from scattered pump light is low despite the compact size of the optical system and meets the system sensitivity requirements.

Keywords: micro-optics, fluorescence, VCSEL, electrochromatography, diffractive optics, substrate-mode, microfluidics, chemical sensing

1. INTRODUCTION

A miniaturized, highly integrated free space optical system has been developed to detect laser induced fluorescence as part of a liquid phase chemical analysis system for portable field use. This system is an excellent example of a microsystem. Microsystems is an emerging technology in which electrical, optical, and mechanical functions are combined at the chip level into compact, lightweight, and [ultimately] low cost modules with performance equal to or even exceeding those of conventional macroscopic systems. Just as the invention of the integrated circuit revolutionized the electronics industry, the development of integrated microsystems is expected to revolutionize an even broader range of fields, extending beyond electro/optomechanical systems to the fields of biology, chemistry and medicine. And just as the integrated circuit combines formerly discrete devices (transistors, resistors, capacitors) onto a single chip with increasing functionality at decreasing cost, the integrated microsystem combines formerly separate subsystems (electronics, photonics, optics, fluidics, mechanics) onto a single multichip module. This results in significant size reduction and, hopefully, as the technology matures, to increased function with reduced cost.

One of the primary areas of activity for the microsystems work ongoing at Sandia National Laboratories (and around the world) is chemical detection and analysis for medical, industrial, and forensic applications.[1] Applications in such fields as hazardous waste remediation, anti-terrorism,

nonproliferation, and biotechnology are driving the development of miniaturized chemical analysis systems. Desirable attributes of such systems include small size, lightweight, low cost, and high sensitivity. These attributes lead to a system which can be handheld, deployed in the field in large numbers, and which will provide fast, accurate analysis previously requiring large, laboratory-based systems.

A class of analytical techniques suitable for microsystem integration are electrokinetic capillary separations in conjunction with optical fluorescence detection. These processes, referred to as chemical electrochromatography , capillary electrophoresis and other variations, use narrow capillary channels, typically tens or hundreds of micrometers wide, to perform chromatographic separations of fluids.[2] In these techniques the analyte fluid is driven electrokinetically by applying a voltage and making use of electroosmotic flow to move the fluid, with both charged and neutral molecules, through the channels. Electrokinetically driven separations can be used to resolve different species in a mixture based on their differential physical properties. For example, in capillary electrophoresis species in a in a liquid placed under an electric field (typically a few hundred volts/cm) have different velocities based primarily on their charge-to-mass ratio. Therefore, arrival time at a fixed detector can be used to identify a given compound. These capillary separation systems can be miniaturized as etched channels in planar, wafer-like substrates. As the separated components pass through the channel, identification by fluorescence under optical excitation provides an extremely sensitive and versatile detection method. In many cases, direct fluorescence from the chemical sample may be observed. In other cases, fluorescence is observed by tagging the chemical sample with an appropriate dye. Indirect fluorescence is an alternate detection method in which dye fluorescence is quenched by the presence of other chemicals. This two step process of separation of a complex solution and then detection of the separated components provides a chemical analysis technique with both selectivity and sensitivity. Until now, the optical detection systems available for combination with capillary channel separation were too large and delicate for portable applications. This work describes the design, fabrication and performance of micro-optical fluorescence detection systems for integration with capillary-channel separation systems on a common transparent substrate.

Each of the components of a conventional capillary may be fabricated in microscopic form by planar, lithography-based techniques. The vertical cavity surface-emitting laser[3] (VCSEL) can provide the excitation beam. Multilevel diffractive lenses[4] fabricated in fused silica can direct the pump beam and collect the fluorescence. Etched channels in any number of substrate materials (silicon and glass,[5] quartz,[6] plastic[7]) function the same as freestanding capillaries with the added advantages of requiring lower drive voltages, less sample volume, and reduced analysis time. The goal of this work is to combine these disparate elements into a compact module, often referred to as a lab-on-a-chip. The origin of this concept is attributed to a group at the Alberta Microelectronics Centre.[8] Similar work is being driven very strongly by the Human Genome Project where high-throughput DNA analysis is needed.[9,10] Competing technologies for integrating laser induced fluorescence and capillary channels include fiber-based pump sources,[11] flow stream waveguides[12] where light propagates down the channel itself, and conventional waveguides which are fabricated on the same substrate to intersect the capillary channel.[13] The keys to the compact design of the Sandia system are the use of substrate-mode propagation for the optical path and high performance diffractive microlenses for coupling the pump beam into the substrate mode and for collecting and collimating the fluorescence from the capillary channel. Also important is the use of electronic surface-mount techniques for compact assembly of the system. In this work, we describe the design of two generations of optical subsystems, the

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microfabrication of the optical systems, and initial results from a complete integrated capillary channel electrochromatograph using direct fluorescence detection of a dye.

2. DESIGN AND FABRICATION

Two generations of optical systems have been designed and fabricated in this work. The first generation design is shown in Fig. 1. In this design, the micro-optics are fabricated onto the same fused silica substrate that contains the capillary channel. This has the advantage of requiring no alignment or assembly steps for the optical system, exclusive of the VCSEL array and photodetector. The capillary channels are formed by wet etching of the fused silica substrates prior to fabrication of the optical surfaces. Typical channel dimensions are 10 microns deep by 200 microns wide. In this design, the channels are sealed with a fused silica cover plate using photo-definable polyimide that is patterned on both substrates to form a gasket around the channels and thermally bonded under pressure. The bonding temperature is low enough for the bonding to be performed after fabrication of the optical surfaces.

VCSELs are used as the optical sources in these designs because the surface-emission and beam properties are ideal for coupling into the free-space optical system and they are readily adapted to direct surface mounting on the system. The VCSEL array is a 2 X 2 element array designed for flip-chip bonding. Only one element of the array is coupled into the optical system. The VCSEL array is flip-chip bonded onto a 2 X 4 mm fused silica submount that is in turn adhesively bonded to the cover plate of the flow module. This assembly process was described in more detail in an earlier publication.[14] The photodetector, covered by an interference filter, is mounted on the electronics subassembly that is positioned above the optics and microfluidics module. Because the fluorescence signal is shifted only 30 nm longer in wavelength than the VCSEL pump beam, the high performance interference filter is necessary to block scattered pump light. Appropriate alignment marks and a custom precision die attach system provide for accurate alignment of the various subassemblies. The two microlenses are designed for a wavelength of 750 nm and operate in reflection. The off-axis lens, with a diameter of 0.9 mm, has a deflection angle of 45° to couple the VCSEL output into the substrate mode. The on-axis lens, with a diameter of 2 mm, is an annular design that collects the fluorescence and directs it to the photodetector. This lens is designed to collimate the fluorescence so that the interference filter will efficiently transmit the fluorescence signal to the photodetector. The detector lens is very fast with a numerical aperture (NA) of 0.5. Both lenses are computer designed Fresnel zone lenses, implemented in four phase levels (two etch steps). Details of the fabrication process have been published by Wendt.[15] Additionally, two thin film metal mirrors serve to maintain the substrate-mode propagation over the 6 mm center-to-center length of the optical system. A photograph of the first generation optical system is shown in Fig. 2.

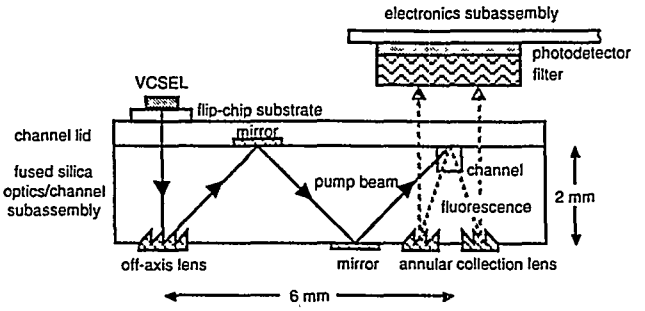

Figure 1. Schematic cross section along the optical path of the first generation chemical analysis system. The off-axis lens couples the VCSEL pump beam into the substrate mode and two mirrors direct the beam to the capillary channel. The annular collection lens collimates the fluorescence to the detector. Both diffractive optics are coated with Au and operate in reflection.

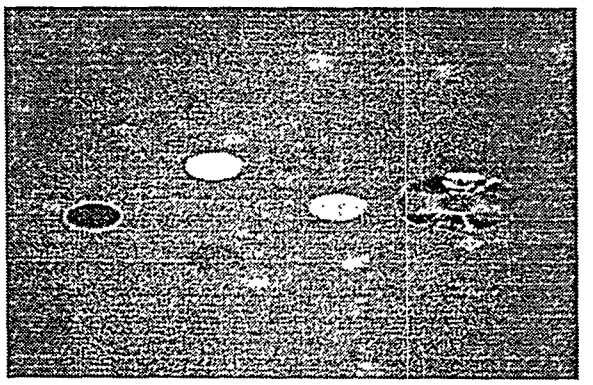

Figure 2. Photograph of the first generation diffractive optical system, viewed from an oblique angle. Visible from left to right are the off-axis lens (dark oval), the two metal mirrors (bright ovals), and the collection lens (annulus). The capillary channel is not shown.

The second generation design is shown in Fig. 3. In this design, the micro-optics are fabricated on a separate fused silica substrate from the capillary channel. This modular design allows for more efficient fabrication of the optical subassembly, in parallel with fabrication of the capillary channels, and can incorporate disposable fluidics modules. The design allows for use of opaque flow channel substrates with transparent covers. In this design the flow channels can be sealed by high temperature processes prior to assembling the optical module onto the substrate, which the first generation design did not allow. The VCSEL array is again flip-chip bonded to the 2 X 4 mm submount. The submount is then adhesively bonded to a ceramic frame that provides the spacing to the first diffractive optical surface and also provides the solder pads for wiring to the VCSEL array. The length of the optical system has been reduced by almost a factor of two from the first generation design. Alignment of the various subassemblies is accomplished similarly to that for the first generation. The two microlenses are also designed for a wavelength of 750 nm but now operate in transmission. The off-axis lens, with a diameter of 0.5 mm, has a deflection angle of 26.5° to couple the VCSEL output into the substrate mode. The on-axis lens, with a diameter of 2.8 mm, collects and collimates the fluorescence to the photodetector. The detector lens is again an aggressive design with a high NA of 0.78. The detector

lens was implemented in two different versions. One is a conventional Fresnel element and the other is an optimized design that iteratively varied the diffractive features while staying within the fabrication constraints of our process. The optimized design resulted in a significant improvement in diffraction efficiency.[16] As above, both lenses are computer designed Fresnel zone lenses, implemented in four phase levels. The fabrication process is very similar to that used for the first generation design. Also as above, two thin film metal mirrors serve to maintain the substrate-mode propagation over the shortened 3.5 mm center-to-center length of the optical system. A photograph of the second generation optical system, without any active components, is shown in Fig. 4.

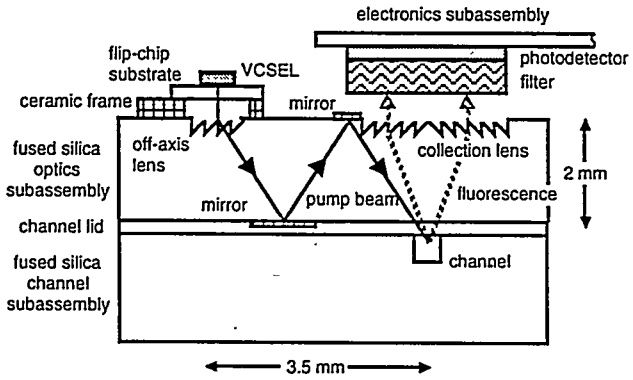

Figure 3. Schematic cross section along the optical path of the second generation chemical analysis system. The optical subassembly is now independent of the capillary channel, and the diffractive lenses operate in transmission.

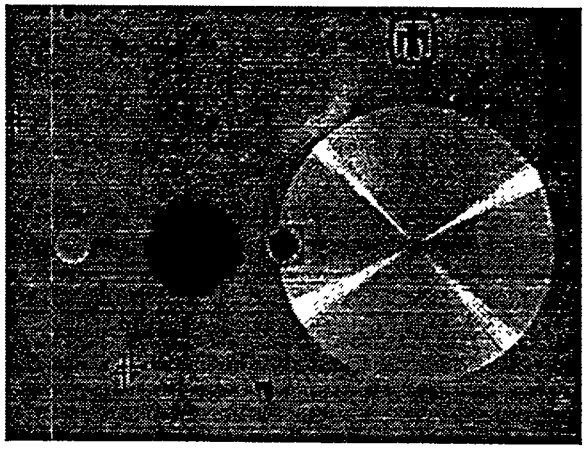

Figure 4. Photograph of the second generation diffractive optical system, viewed normal to the surface. Visible from left to right are the off-axis lens (small light circle), the two metal mirrors (black circles), and the collection lens (large notched circle).

In both designs, fabrication of the optical surfaces begins with conventional optical lithography and standard semiconductor processing to define three sets of Cr/Au alignment marks on the optically flat, fused silica substrate. The different sets of alignment marks are used for electron beam lithography, dual-side optical lithography, and for assembly of the subassemblies.

The electron beam lithography is performed on a JEOL JBX-5FE thermal field emission system operating at 50 kV. Lithographic challenges of the microlenses include submicron features (0.15 μm minimum lines and spaces), relatively large areas (1-3 mm^2), and an insulating substrate (fused silica). These challenges are met by utilizing optimized electron beam lithography processes.[17] While this is not a low-cost technique, it is the most efficient and flexible during the development phase. Once a design of the optical subassembly is finalized, it should be possible to substitute a fabrication technique for the lenses or for the entire optical subassembly that is more suitable for low cost, mass production. Possible techniques include plastic injection molding[18] or embossing.[19]

3. EXPERIMENTAL RESULTS

Characterization of the first generation optical subassembly began by testing the module independently of the other subassemblies to confirm that the optical design performed as expected. First, the output characteristics of a flip-chip mounted VCSEL was measured. Then, that same VCSEL was mounted onto the fused silica substrate containing the optical system. By coupling the laser excitation beam from the substrate surface (where it would ordinarily be incident on the channel) with an index-matched 45° prism, the pump beam power after transmission through the optical system was measured. The overall efficiency of the excitation part of the optical system is measured to be at least 37%. In the first generation device, direct measurement of the diffraction efficiency of the reflective collection optic was not performed. While the maximum possible first order efficiency of a [low NA] four-level diffractive optic is predicted by scalar diffraction theory to be 81%, it is expected that the very fast detector lens used in this work will have a lesser maximum efficiency, even before accounting for imperfections in fabrication.[20] The transmissive collection optic of the second generation design was characterized for diffraction efficiency. The conventional Fresnel element has a diffraction efficiency of 46% and the optimized design has 58% efficiency. The efficiency of the laser excitation optics of the second generation system has not been measured yet.

The complete first generation system, including VCSEL, channel, and photodetector, has been tested using fluorescence detection with a 750 nm VCSEL pumping a dilute solution of CY-7 dye. Collected fluorescence was measured first with only the buffer solution in the channel. Then a 10^{-4} M solution of CY-7 dye was introduced through the channel and the resulting fluorescence signal measured. The ratio of these two signal levels gives a signal-to-noise ratio of 100:1. This demonstrates that despite the compact size of the optical system, the background signal from scattered pump light is low. This analysis system is ultimately designed for indirect fluorescence detection of explosives and related degradation products. Experiments with explosive related chemicals using conventional capillary tubing have shown that a 10^{-5} M solution of CY-7 dye will be adequate. An open channel separation of CY-7 was also performed with the first generation system. A three-peak fluorescence signature corresponding to the molecular components of CY-7 is shown in Fig. 5.

Development of the capillary channel separation process has been proceeding independently of the optical system. Electrophoretic separations and indirect fluorescence detection have been performed in fused silica channels on a sample containing eleven explosives and degradation products. These separations utilized an off-chip pump and photodetector setup. Nine of the eleven chemical constituents were identified in less than one minute.[21] It remains to perform a separation and analysis of a real

sample on the fully integrated system. This will most likely be performed using the second generation optical subassembly, the assembly and test of which is in progress.

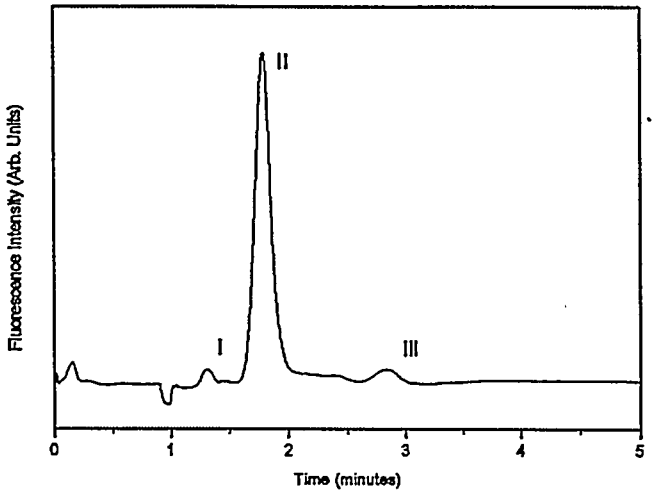

Figure 5. Open channel separation of a dilute solution of CY-7 dye showing separation into components on a timescale of a few minutes. Data was obtained with the first generation micro-optical system.

4. SUMMARY

We have described a highly integrated miniature chemical analysis system in the form of a compact capillary channel electrochromatograph with on-chip fluorescence detection. The use of high performance diffractive microlenses enables achievement of extremely compact and reasonably efficient optical detection subassemblies. The use of microfabrication techniques for the bulk of the optical system provides inherent alignment and offers the possibility for economical mass production. Alignment of the subassemblies for assembly into an integrated microsystem is performed on a custom die attach apparatus, making use of lithographic alignment marks included on each subassembly. Tests of the first generation design using fluorescence detection with a 750 nm VCSEL pumping a 10^{-4} M solution of CY-7 dye show a signal-to-noise ratio of better than 100:1, demonstrating that the background signal from scattered pump light is low despite the compact size of the optical system. An open channel separation of CY-7 dye has also been performed, further demonstrating the functionality of the overall system. The successful demonstration of two generations of optical subassemblies and the incorporation of the first generation design into a functional miniaturized chemical analysis system is a significant step forward along the path to an affordable, hand held chemical analysis system.

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