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Integrated Separation and Optical Detection for Novel On-Chip Chemical Analysis

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Abstract

This report represents the completion of a two-year Laboratory-Directed Research and Development (LDRD) program to investigate miniaturized systems for chemical detection and analysis. The future of advanced chemical detection and analysis is in miniature devices that are able to characterize increasingly complex samples, a "laboratory on a chip". In this concept, chemical operations used to analyze complicated samples in a chemical laboratory--sample handling, species separation, chemical derivitization and detection--are incorporated into a miniature device. By using electrokinetic flow, this approach does not require pumps or valves, as fluids in microfabricated channels can be driven by externally applied voltages. This is ideal for sample handling in miniature devices. This project was to develop truly miniature on-chip optical systems based on vertical cavity surface-emitting lasers (VCSELs) and diffractive optics. These can be built into a complete system that also has on-chip electrokinetic fluid handling and chemical separation in a microfabricated column. The primary goal was the design and fabrication of an on-chip separation column with fluorescence sources and detectors that, using electrokinetic flow, can be used as the basis of an automated chemical analysis system. Secondary goals involved investigation of a dispersed fluorescence module that can be used to extend the versatility of the basic system and on-chip, intracavity laser absorption as a high sensitivity detection technique.

Keywords: chemical analysis, capillary electrophoresis, capillary electrochromatography, microfluidics, vertical-cavity surface-emitting lasers, fluorescence spectrometer, diffractive optics, substrate-mode optical interconnects.

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Introduction

Chemical analysis techniques based on high-performance separations in capillaries using optical detection methods provide powerful tools that are widely used to analyze complex chemical mixtures. A separation step in liquid samples is usually required since the broad spectral features in liquids often precludes analysis of its components on the basis of optical probes (such as spectroscopy) alone. Currently, SNL/CA is doing state-of-the-art work in two areas of high performance chemical separation and analysis--capillary gel electrophoresis for DNA sequencing and capillary electrochromatography (CEC) for environmental trace-contaminant identification. In both cases, high voltages are used to drive samples through free-standing capillaries (about 100 micron diameter) that are coupled with sensitive laser-based detectors.

The key to the separation of the chemical components of a complex mixture in a capillary is electrophoresis--the motion of charged species in a liquid under the influence of an external electric field--for ions and electrokinetic flow--the bulk flow of an ionic solution in a capillary due to an applied electric field--for neutral (uncharged) species. Ions separate due to different ion mobilities and through sieving effects when gel-filled capillaries are used, whereas neutral species separate chromatographically by interaction with a stationary surface, often the surface of small beads (about 3 micron diameter) packed into the capillary. As the separated components pass by a point near the end of the column, identification by fluorescence under optical excitation provides an extremely sensitive detection method. The direct fluorescence of the chemical species can be

identified in some cases. In other cases, the components can be "tagged" with a fluorophore before detection by specific reactions with dye molecules that can be easily excited and identified in the separation column.

These chemical analysis systems would benefit greatly from miniaturization. In the case of CEC for neutral species, the required driving voltages and the analysis time are significantly reduced. For DNA sequencing using capillary electrophoresis (CE), miniaturization is the first step towards the massive parallelism required for high-throughput sequencing programs. In general, miniaturization leads to devices that are field portable, versatile in that they may contain many different kind of columns and detectors, and mass producible.

These devices are well-suited to miniaturization. Electrophoresis and electrokinetic flow occur in micromachined channels as well as they do in free-standing capillaries--so separation channels can be constructed in flat substrates. Manipulation of fluids in these channels does not require pumps, valves, etc.--just applied voltages to the solutions in them.[1,2] Once the separation system is built into a flat substrate, then the key technical development at SNL/NM that enables the miniaturization of an entire chemical analysis system is the combination of vertical-cavity surface-emitting lasers (VCSELs) and diffractive optical elements with surface-mount packaging technology to assemble the elements together to form compact optical systems. With this technology we can combine the separation technology with the optical excitation and detection functions as integrated and miniaturized systems.

The System Concept

The basic building block of the instrument is a transparent substrate of fused silica with etched channels. The channels can be fabricated by conventional photolithography and wet etching processes with dimensions of 50-100 microns. To fit longer separation channels into small areas, channels may be etched in a serpentine pattern as shown in figure 1. Initially, simple electrophoretic separation techniques in open channels have been employed, but more advanced versions can incorporate beads or gels in the capillaries. The channels can be sealed by a bonding

of a fused silica plate to the etched side by a number of different processes. The assembly then has two optically-flat surfaces on which the optical functions of the system can be implemented. VCSELs can be used as excitation sources, with either direct illumination of the channels by surface-mounting them over the channel or the VCSEL beam can be directed to the desired location by diffractive optical elements that can provide focusing and direction. Figure 2 shows an example of such a beam path using substrate-mode propagation of the beam by launching the beam at an angle that allows total internal reflection in the substrate. Detection of the fluorescence signal is by commercial detectors in open packages bonded to the substrate. For simple fluorescence systems, the detector can have interference coatings applied to its surface for spectral selectivity.

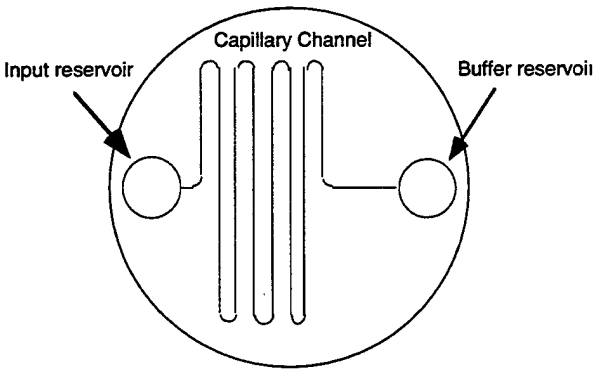

Figure 1. Drawing of capillary channel in a silica substrate. 10-30 kV between the reservoirs will induce electrokinetic flow along the capillary.

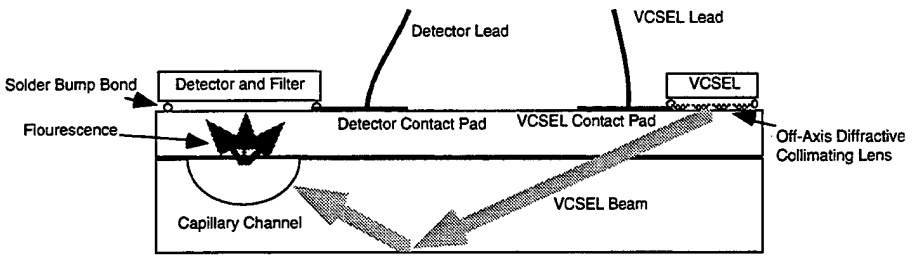

Figure 2. Cross section of a portion of the capillary channel showing one approach to providing excitation from a VCSEL and detection by a solid state detector.

SNL has the capability to fabricate VCSELs over a range of wavelengths, including the visible, so that a variety of fluorescent tag dyes are available. If wavelengths not presently available from VCSELs are needed for specific applications, it is possible to use external fiber coupled sources and still maintain a highly integrated and small system. The system described in the previous paragraph is a basic instrument that can prove the validity of the concept and provide useful analysis capabilities. We also considered two advanced analysis methods that would provide added versatility and sensitivity to the system. The first advanced approach was to include dispersion of the fluorescence signal as part of the optical system. Fluorescence signals that may include a number of wavelengths can be dispersed by diffractive optical elements fabricated on the chip to form a low resolution spectrometer. For many chemical analysis situations, tag dyes with separations of 5-10 nm are available and in this way, multiple species can be detected with a single detector and a single pump laser. This approach was not pursued during this project, but is worth considering for a follow-up program. A second approach used the recently developed SNL capability to use compact, external-cavity VCSEL structures for intracavity absorption spectroscopy. In this approach, a VCSEL is fabricated in which there is a gap between one of the mirrors and the gain region of the VCSEL. If a substance in the gap region either absorbs the stimulated emission light in the cavity or, by variations in index of refraction, locally changes the optical length of the cavity, the light output of the laser is strongly perturbed. This is a technique that has been explored in this project and is reported later in this document.

In a CRADA with Beckman Instruments, Inc., SNL/CA has built a prototype multi-capillary instrument for DNA sequencing based on capillary gel electrophoresis and diode laser-based fluorescence detection. The application of VCSEL based microoptical systems is particularly well suited for DNA analysis. The match is based on the availability of extremely effective dyes (CY-5, CY-7, TOTO-3, TOPRO-3, and other derivatives) used to label the DNA that have strong absorption at 650 and 750 nm. Fluorescence based detection in this wavelength region is currently being used in a new DNA sequencing instrument to be released this summer by Beckman

Instruments and is also being using in a Sandia DNA sizing project for the intelligence community. These dyes by themselves can be used for evaluating various fluorescent detection schemes. They can be used to evaluate the separation efficiency of the devices as well as the detection sensitivity. Plugs of dye can also be used to visualize sample movement through the channels under the influence of the applied voltages.

These performance tests can then evolve into more practical demonstrations as more mature devices are developed. These and similar dyes can be used to tag actual chemical species of interest, including biological molecules or metal ions. An approach that opens up the use of the devices to almost any species is the "indirect fluorescence" approach in which a fluorescent dye is added to the buffer solution that fills the separation channel. In this scheme a constant fluorescent background is observed until a non-fluorescent target species passes the detector and a dip in the fluorescent signal is observed.

Microfluidics results:

The primary goal of this project was to develop the key components of the on-chip chemical analysis system. These components consist of the capillary flow channels fabricated in a fused silica substrate, the VCSELs for fluorescence excitation and microoptics to be fabricated on the substrate for direction and control of the VCSEL output. Other important parts of a final system are the detector and the mounting of the components on the substrate. The flow channels, VCSELs and microoptics were fabricated in the CSRL at SNL/NM and testing of the parts performed at SNL/CA. Special external cavity VCSELs for intracavity absorption detection were also fabricated and integrated with similar capillary flow channels. These were evaluated independently at SNL/NM and can be integrated into a system with the capillary electrophoresis separation system.

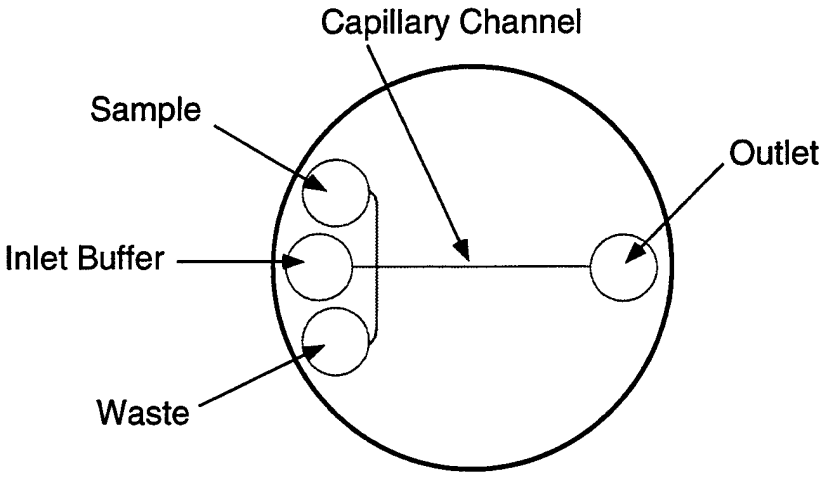

Figure 3. Schematic drawing of flow channel layout for control of electroosmotic flow in channels by switching voltage between the various reservoirs.

Flow channels were fabricated in two and three inch diameter fused silica substrates using photolithography and wet etching. The channels have cross sections of 10 X 100 microns and are configured with reservoirs and intersections to allow control of sample sizes and flow by applying voltages to induce electroosmotic flow. Cover plates were fabricated from fused silica plates by abrasive machining to form additional reservoir volume with access holes for electrodes to be inserted into the reservoirs.

Sealing of the cover plates to the flow channels was a much more difficult task than originally thought. A number of techniques described were tried. Some of these include direct thermal diffusion bonding of the fused silica plates[3], use of photodefinable polyimide as a sealing material and the use of various adhesives. Thermal bonding of fused silica was abandoned as it required such high temperatures that, even if it worked well, we would be severely limited in the number of processes, such as metallization, that could be performed on the substrates prior to bonding. The polyimide sealing is still being developed, but has so far been unsuccessful. The great attraction of the polyimide bonding is that it is several microns thick and can accommodate steps on the substrate surface, such as thin-film metal electrodes. Two adhesive bonding techniques were used in order to demonstrate the microfluidic system. UV (ultraviolet)-light curing optical cements could be wicked by capillary action into the space between the top and

bottom wafers and fixed by UV light before the fluid contaminated or blocked the flow channels. This technique worked well for simple straight channels, but not for serpentine channel patterns where the adhesive could not be uniformly spread in the spaces between the channels. For the more complex channel designs, silicone adhesive could be spin-coated on the cover plate and then would form a bond across the whole wafer when they were pressed together. The only disadvantage of this procedure is that the channel surface covered with silicone adhesive had different electrical properties from the fused silica, which lead to flow dispersion when doing electrochromatography.

A sealed system with open microchannels and integrated buffer reservoirs was obtained using the UV-setting adhesive. This system was wired with platinum electrodes and successfully tested for flow and electrical continuity. A schematic of the channel layout is shown in Figure 3. Fluorescent dye was placed in the channel and voltage-driven electroosmotic flow was demonstrated as a first step towards electrokinetic separation in the channel. Under voltage control, solutions were flowed between reservoirs. The result of this can be seen in Figure 4, where two of the channels have been filled with dye using electroosmotic flow. Through appropriate switching, a plug of dye was sent down one channel to simulate voltage-controlled sample introduction. Flow between reservoirs and sample introduction are the two operations required for chemical analysis in a planar format. Such a chemical analysis was demonstrated on this device by separating a mixture of laser dyes. The separated components were detected using VCSELs which were mounted separatedly from the planar flow channels.

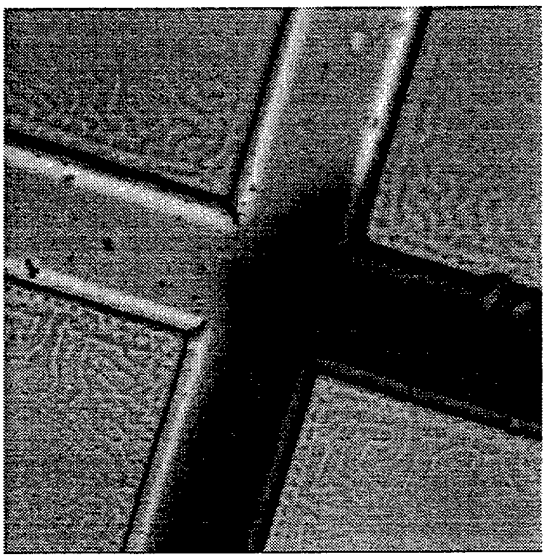

Figure 4. Photomicrograph of dye flowing through intersection of flow channels under applied voltage. The flow is "turning the corner" in the intersection as the ionic solution flows from one reservoir to another. Such intersections can operate as valves by switching voltage between reservoirs.

Microoptics results:

VCSEL arrays of 780 nm wavelength were fabricated and packaged in the CSRL for testing in the existing capillary electrophoresis testbed. The devices were designed to have multiple transverse modes for higher power output (5-7 mW) and produce focal spots sufficiently small in the optical system to efficiently pump the capillary channel region. Several fluorescent dyes, as well as fluorescently labeled DNA, have been obtained to test the applicability of VCSEL excitation to fluorescence detection. The VCSELs were used to produce fluorescence signals in separation systems composed of conventional capillary tubes, as well as the planar channels, demonstrating that the VCSELs are suitable for such detection techniques.

The optical system depicted in Figure 2 was rigorously redesigned, using commercial raytrace codes, to optimize the system performance. The major changes in the design include a collimating/collecting optic that will maximize the fluorescence signal transmitted through the filter. This design has been converted to mask data for direct write electron beam lithography. The fabrication of the optical system is continuing under the "ChemLab" LDRD program. The detector

choice has been narrowed to a silicon PIN photodiode used with a low-noise amplifier, based on testing done at SNL-CA.

We have been testing metallizations of the fused silica surfaces and VCSEL arrays that will improve adhesion for thermocompression flip-chip bonding of the devices. Test structures were bonded to the fused silica substrates. We decided to use a separate substrate for the VCSEL bonding and micro-optical fabrication that can be contacted to the flow channel substrate with index matching fluid for easy disassembly and reconfiguration of a compact experimental system.

Intracavity microlaser detection:

As a first step toward realizing an intracavity absorption detection, microoptical flow device, we assembled and tested a microcavity laser. Fused silica that had been wet etched into an array of 200 micron channels separated by 120 micron barriers was coated with a multilayer dielectric of SiO2/TiO2 to form a highly reflecting mirror of approximately 99.5% reflectivity at 850 nm in the bottom of the channels. This glass optic was assembled, in a dry condition, face-to-face with an open VCSEL structure to define a laser cavity. The cavity was photo-pumped with cw or pulsed light in the wavelength region from 641 to 752 nm. The resulting spontaneous emission spectrum was recorded with an array detector to assess the optical quality of the laser cavity. The spectrum revealed longitudinal modes that were sharp (less than 0.3nm) and widely spaced, (greater than 20 nm) to indicate a high cavity finesse. The cavity was opened and reassembled with a wet solution of 6 micron polystyrene spheres. In this case, the spheres provide lateral confinement of spontaneous light. Under these conditions, the cavity could be photo-pumped above the lasing threshold. A single, very sharp ~0.1 nm lasing line was observed in the emission spectrum. This observation suggests that the quality of the microfabricated optics is sufficiently high for microlaser cavities.[4] Work is underway to seal the VCSEL structure to the capillary channels with capillary tube inlets so that lasing experiments can be conducted with liquid flow in the cavity.[5]

Summary and Conclusions:

The progress made in this LDRD has provided the foundation for current negotiations with Beckman Instruments to develop a multichannel optical detection systems for DNA sequencing. It also provides the potential for future work in miniaturizing Sandia's DNA sizing instrument that currently uses more conventional diode laser and optics packages. The miniature chemical analysis system designed under this LDRD project is a major component of a new LDRD project to create a more general purpose "Chemlab-on-a-Chip".

References

1. Microchip Capillary Electrophoresis with an Integrated Postcolumn Reactor, S.C. Jacobson, L.B. Koutny, R. Hergenroder, A.W. Moore, Jr. and J.M. Ramsey, Anal. Chem. **66**, 3472-3476, (1994).

2. Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips, A.T. Wooley and R.A. Mathies, Proc. Natl. Acad. Sci. USA **91**, 11348-11352, (1994).

3. Fused Quartz Substrates for Microchip Electrophoresis, S.C. Jacobson, A.W. Moore and J.M. Ramsey, Anal. Chem. **67**, 2059-2063, (1995).

4. Semiconductor microlasers with intracavity microfluidics for biomedical applications, P. L. Gourley, T. French, and A. E. McDonald, SPIE vol. 2978, 186 (1997).

5. Microlaser-optical-mechanical systems for biomedicine, P. L.Gourley, Optics and Photonic News, April 1997, p. 32-36.

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