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# Microfluidic integration on detector arrays for absorption and fluorescence micro-spectrometers

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**Abstract**

We describe a new approach for miniaturizing spectrometers by combining replica molded elastomeric micro-channels with filtered silicon detector arrays. Elastomers are excellent transparent materials, which provide hermetic seals to silicon dioxide and allow sensitive absorption and fluorescent spectroscopy in the visible and near-UV wavelength range. When integrated on dense detector arrays, such spectroscopy can be conducted on picoliter sample volumes. Elastomeric fluidic systems also permit easy integration of spectroscopic measurements with control functions for reaction monitoring and biological drug delivery and analysis systems. Here we present some results from our first experiments in which we explore the sensitivity of spectroscopic measurements within microfluidic channels. We show that multi-channel spectrometers defined on complementary metal oxide semiconductor (CMOS) silicon detector arrays can be used to obtain absorption signatures even for dilute dye solutions.


*Keywords:* Microfluidics; Spectrometers; Hyper-spectral imaging; Polydimethylsiloxane

## 1. Introduction

Over the past few years, the use of replication molding for the definition of microfluidic systems in elastomers has allowed the rapid development of compact analysis systems useful for chemical sensing and biological diagnostics. For example, fluorescently activated cell sorters [1] based on pumps, valves and channels defined in RTV silicone elastomers have demonstrated excellent throughput and sorting accuracy. These have been fabricated inexpensively into very small and robust microfluidic devices. Chemical surface pretreatment of specific areas within a flow channel has led to the possibility of developing very compact disease diagnostic chips, and even single molecule sizing systems can be built from elastomeric flow channels [2]. In all of these applications, the overall size of the analysis system is typically limited by the dimensions of the optical excitation and detection components, and miniaturization of the read-out optics is therefore very desirable. However, miniaturization of grating-based spectrometer geometries ultimately is limited by a reduction of the spectral resolution, which can be predicted from the optical path lengths between the grating and the detection slit. For example, multi-wavelength 4 mm × 12 mm spectrometers operating at 1500 nm typically yield a measured spectral resolution of approximately 1 nm. This compromise between resolution, insertion losses and size has in the past limited the minimum size of such optical analysis systems. Much better spectral performance can be obtained by using dielectric filters, which can be directly deposited onto detector arrays to form multi-wavelength detector arrays. Such filtering has in the past been used for monolithic hyper-spectral imaging applications. Filtered detector arrays offer an inherent opportunity for the miniaturization of spectroscopic instruments in microfluidic applications, with the additional opportunity of obtaining low-resolution "lens less" images of the contents in the flow channel. CMOS imagers were chosen for their ease of use and commercial availability. Imager elements based on CMOS technology also offer compatibility with other CMOS processes such as VLSI for integrating onboard signal processing.

One of the most important advantages of using elastomeric flow channels is the inherent transparency of the elastomer material in the visible wavelength range. Many semiconductor based microfluidic structures previously proposed have suffered from the inability to perform optical analysis of the device's contents in the visible and near-UV

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spectral ranges. Due to the absorption edge of silicon, for example, optical measurements in flow channels defined by this material are typically limited to the infrared range and visible/UV spectroscopy is virtually impossible to perform without using very elaborate geometries. For applications such as biochemistry, this poses a severe limitation since many absorption and fluorescence experiments are based on visible/UV fluorescent dyes. Silicone elastomers circumvent this problem since they are optically transparent and have similar UV absorption characteristics to those of glass. This property enables the easy integration of elastomer microfluidic devices with standard optoelectronic sources and detectors. Moreover, silicone elastomers are simple to integrate on top of already fully fabricated detector arrays, forming a hermetic seal to the passivation layer of the detector arrays.

Miniaturization of absorption spectrometers will advance rapidly over the next few years, due to development of short wavelength LEDs and faster computer interconnects, as well as the development of inexpensive and high-quality CMOS imaging arrays. In this paper, we describe the performance of a compact and inexpensive spectrometer geometry with spectrally filtered detector arrays underneath elastomeric microfluidic channels.

## 2. Experimental

### 2.1. Replication molding

Replication molding, sometimes also referred to as soft lithography, dates to prehistoric times, and has been used to make phonographic records, blazed grating optics [3], and stamps for chemical patterning [4]. Through the use of replication molding techniques, we have in the past demonstrated many microfluidic devices such as peristaltic pumps, pneumatic valves, circular mixers, and fluorescently activated cell sorters [1,2,5]. Replication molding involves curing an elastomer, typically silicone based, on a microscopic mold. The mold can be generated through bulk or surface micromachining, but is typically generated via photolithography. The simplest method for creating a mold suitable for a microfluidic system consists of exposing thick UV sensitive photoresist using high-resolution contact photolithography. The resulting mold pattern is then developed and treated with a delamination agent to prevent adhesion between the mold and the cured elastomer. The elastomer can be cast on top of the mold by either pouring a thick layer, or spinning a thin layer onto the patterned surface. To define simple pneumatic valves with picoliter dead volumes, multilayer replication techniques are used, in which a pneumatic layer is aligned onto the fluidic layer. The elastomer between the two layers is usually thinner than 10 μm, and can easily be deflected with low pressures introduced from the pneumatic control source. Three of these valves can be integrated to define a peristaltic pump, to move solution over the filtered detectors, which can provide spectroscopic information about the analyte flowing in the channel. Furthermore, valves and pumps can be combined to define fluidic cell sorters with feedback in which the cell examination time can be dynamically controlled during the sorting process. Since the silicone elastomer is natively hydrophobic, the sample can be placed in 0.001 M HCl solution at 80 °C for several minutes to make it hydrophilic, if the natural hydrophobic state is an issue.

### 2.2. CMOS absorption spectroscopy

The external optical excitation and read-out equipment setup used in the experiments described here include a 588 nm light emitting diode and a CMOS camera chip. One key difference between our measurement system and more conventional cell sorting systems is that no lenses

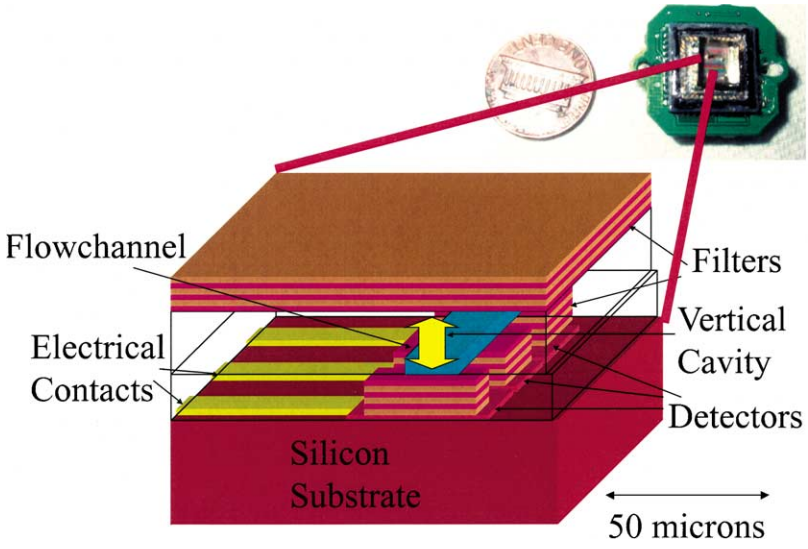


Fig. 1. Miniaturization of a spectroscopic measurement system by use of a commercial CMOS imager.

were used for imaging. We find that a very simple approach to reducing the size of a spectroscopic imaging system is to eliminate the need for focusing optics by placing the microfluidic devices directly onto the imaging detector array. This detector can consist of silicon based avalanche photodiodes (APDs), charge coupled devices (CCDs), or CMOS integrated p–n diode sensors. All of these devices are commercially available at reasonable costs. CCD arrays, although in general more sensitive, suffer from the need to read-out the entire image information in order to determine intensity information from the pixels underneath the flow channels. APDs typically require larger areas, and thus significantly reduce the resolution of the imaging system. CMOS imagers, on the other hand, offer direct control over individual pixels, and, since most of the area of the image array is typically not used, can provide much faster response times and long integration times. Although the lateral resolution of these imagers cannot match that of an optical microscope, it is suitable for most visible spectroscopy experiments on larger objects. The highest resolution of such a proximity imaging system is determined by the pixel size on the imaging array, and can be less than 10 μm. The sensitivity of the imaging system is in turn dependent on the active area of the pixel, as well as leakage currents in the pixels. Other factors that determine the performance of an imaging detector array in a spectrometer application are its sensitivity and dynamic range. The sensitivity becomes extremely important when examining picoliter volumes with a correspondingly small optical interaction length. Here we have chosen a CMOS imaging array as the sensor of our spectroscopic measurement

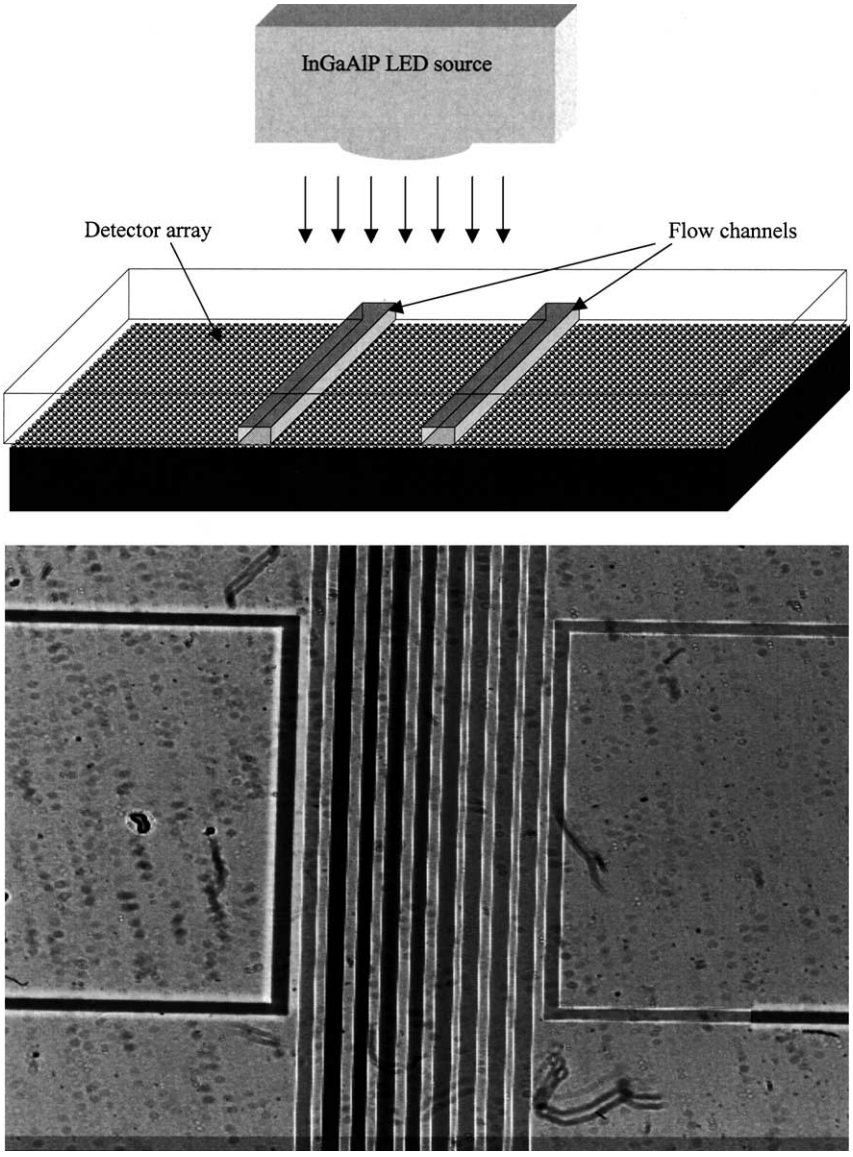


Fig. 2. Top: a schematic illustration of how the absorption spectroscopy is performed. Bottom: a PDMS microfluidic chip imaged by the APS CMOS imager. The light source is a 588 nm $\lambda_{max}$ AlInGaP LED and the channels contain 7.5 mM to 30 μM bromophenol blue solutions. The far left channel is empty and the far right channel contains water for reference. This is an 8 bit image which is considerably less than the 12 bit resolution of the imager.

system. This choice was based on the ease of directly addressing individual pixels in the array, and the opportunity for changing the integration time per pixel for more sensitive analysis. Figs. 1 and 2 show the geometry used for our spectroscopic measurements. The light source was a 588 AlInGaP light emitting diode, placed above the flow channels, which in turn were directly placed on the image array.

Since the typical size of an elastomer microfluidic channel is on the order of 50–250 μm wide by 10–20 μm deep, the absorption path length is quite small compared to more conventional cuvette-based absorption spectrometers with interaction lengths 100–1000 times larger. According to the Beer–Lambert law, the absorbance $A$ is proportional to the concentration of the absorbing material $c$ and the absorption path length $l$, so that

$$A = \varepsilon c l$$

where $\varepsilon$ is the molar absorption constant or molar absorptivity. Thus, the difference in the expected detected intensity of a channel filled with reagent versus a channel filled with water is very small for dilute solutions. Therefore the higher the sensitivity of the detectors in the sensor array, the greater the concentration range that can be detected.

Our first absorption experiment was performed using a 10-bit resolution black and white CMOS imager provided by NASA's Jet Propulsion Laboratory. This imager has a typical pixel size of 12 μm, a dynamic range >65 dB, and a responsivity >1.3 μV/photon at room temperature. The active imaging area consisted of 512 × 512 pixels. First, we determined the minimum concentration of dye, which can be detected in this system. We tested the absorptivity of various concentrations of bromophenol blue (Aldrich Chemical Company, Inc., #62625-28-9) on a calibrated Shimadzu BioSpec 1601 spectrophotometer with solution filled into 1 cm cuvettes. The molar absorption constant was then calculated and a curve fit was applied to generate the control data for a 14 μm channel. Next, a polydimethylsiloxane (PDMS) microfluidic chip consisting of eleven 100 μm wide by 14 μm deep channels spaced 100 μm apart was placed directly on our CMOS imaging chip. The channels were filled with each concentration of interest and one channel was filled with water for background measurements. Fig. 2 shows a typical image of light transmission through the multi-channel silicone structure observed by the CMOS imager. The illumination source consisted of a Yellow AlInGaP LED with $\lambda_{max} = 588$ nm and $I_0 = 1500$ mcd, and was optimized for the absorption peak of bromophenol blue. Although it may be difficult for the reader to distinguish the difference in the lower concentrations, the imager is more sensitive than the human eye and can readily distinguish differences down to the sub-micromolar range. The results of the experiment are summarized in Fig. 3. A similar test was conducted on Orange G, excited with light at 470 nm. Measurements were made by averaging the values from 5 mm long sections of the flow channel. Each of these sections has an approximate volume of 7 nl. Since the

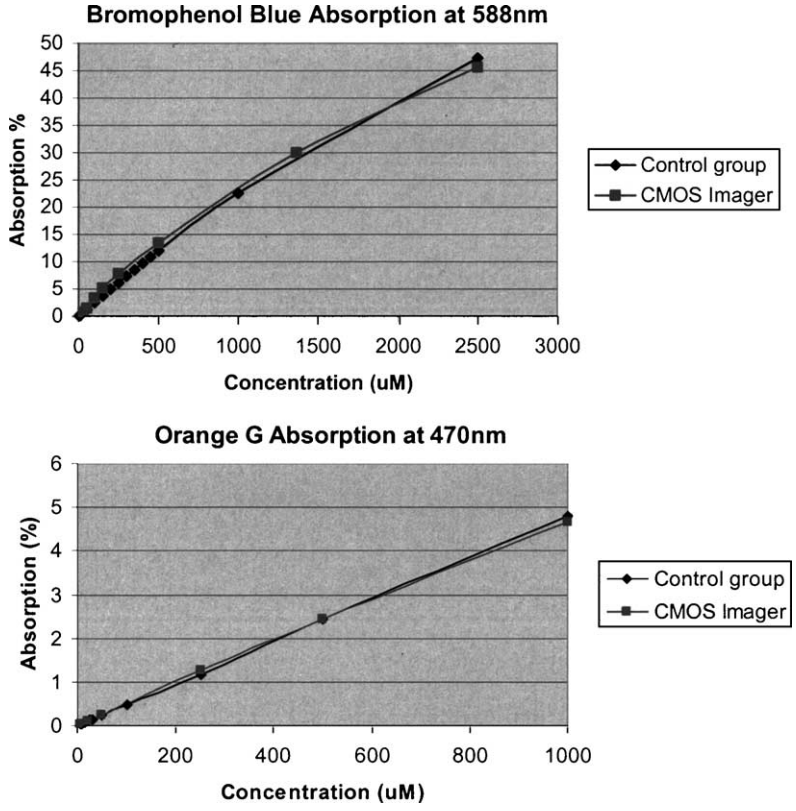


Fig. 3. Absorption spectra of various bromophenol blue and Orange G concentrations taken with a Shimadzu spectrophotometer and the APS CMOS imager.

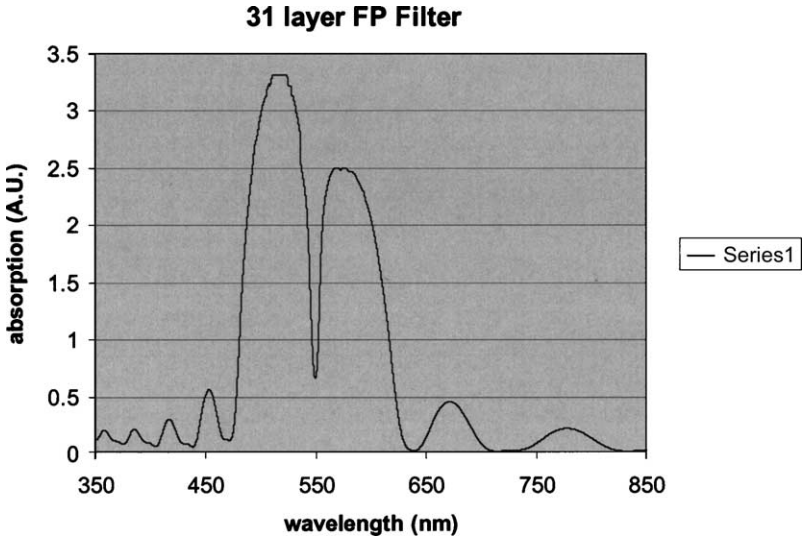


Fig. 4. Absorption spectra of a 31 layer $SiO_2/Si_3N_4$ Fabry–Perot cavity.

CMOS imager allows for individual sections to be analyzed, any area which might have imperfections such as air or droplet formations could be selectively removed. From these figures, it is seen that the monolithic CMOS device displays similar performance to the commercial Shimadzu spectrometer system over the conditions tested.

The important advantages of the imager spectrometers over more conventional absorbance spectroscopy systems include the capability to characterize spectra from picoliter volumes, and enable the observation of many channels in parallel. Individual detectors can be filtered by using $Si_3N_4/SiO_2$ multilayer Fabry–Perot cavities (Fig. 4) deposited onto the silicon detector array before definition of the fluidic structures. Filters can be deposited with a deliberate thickness variation in order to obtain a specific wavelength response for each detector in the imaging array, providing a hyper-spectral imaging array. By pumping the solution of interest over these filtered detectors and observing their response, a spectrum of the absorption or fluorescence of a very small solution volume can be obtained.

### 2.3. Fluorescence spectroscopy

During fluorescence spectroscopy, the sample under test is excited with a light source whose wavelength is close, within 40–80 nm, to the emitted fluorescent light. Typically, the pump source is much brighter than the fluorescence signal, especially for experiments involving small numbers of fluorescing dye molecules, such as when performing single cell detection. Without a very efficient filter, the pump beam saturates the imager, precluding any chance of identifying the fluorescent signal. A blocking filter, which is tuned to the pump wavelength, must be placed between the microfluidic device and the imager, and should be transparent at the fluorescent wavelength. The filter can easily be fabricated as a carefully grown dielectric thin-film mirror (Fig. 5). A typical filter, deposited by reactive sputter deposition of alternate $\lambda/4$ layers of silicon dioxide and silicon nitride, is transparent at the fluorescent wavelength, and blocks over 99% of the incident pump wavelength. We use diluted fluorescein dye to test the performance of our monolithic fluorescence system. Fig. 6 shows the absorption and emission spectra of the fluorescein dye, together with the reflectivity spectrum of the dielectric blocking mirror. Since the mirrors and flow channel are directly deposited onto the silicon CMOS detector array, we can use the lens less contact image from this array to differentiate between concentrations of fluorescein. Spectrally resolved fluorescence measurements are also possible by slowly varying the spectral position of the reflectivity edge of the dielectric blocking mirror and measuring fluorescence intensities in different sensor pixels protected with filters with different reflectivity edges. The requirement for obtaining a high-quality fluorescence image on a miniaturized chip-based spectrometer relies on very efficient blocking of the incident excitation light by the filter

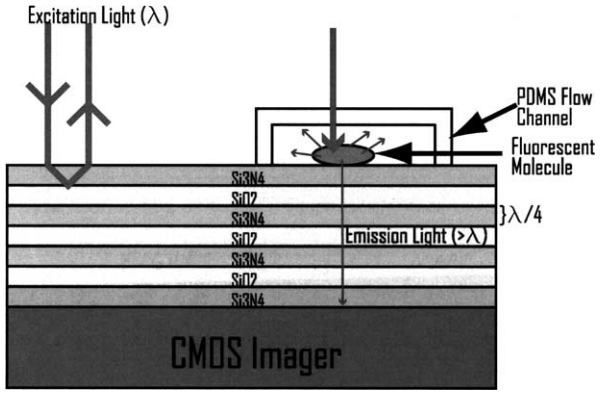


Fig. 5. A quarter wavelength dielectric thin-film filter grown on the CMOS imager to block the excitation light, but pass, with minimal loss, the emission light.

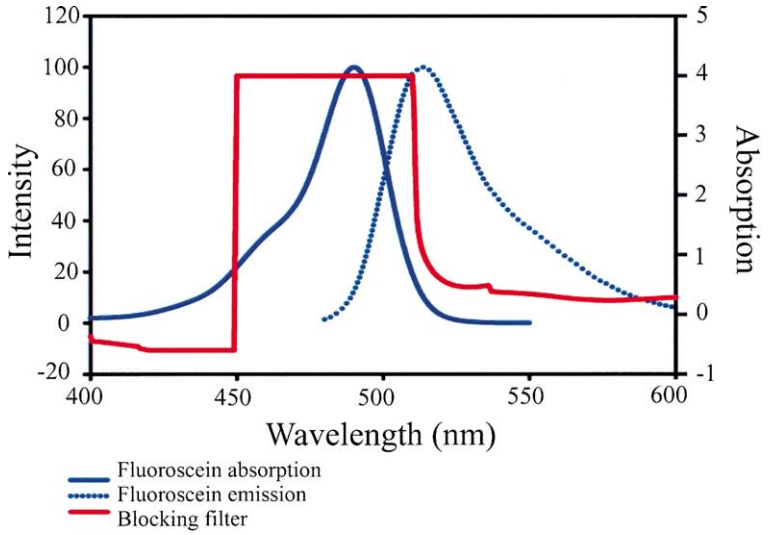


Fig. 6. Fluorescent absorption and emission spectra for fluorescein. Blocking filter absorption spectrum shown in relation to second *y*-axis. The emission peak corresponds to a region just outside the block band where the transmission is roughly 50%. This can easily be improved by constructing a narrower band-blocking filter.

whose absorption spectrum is shown in Fig. 6. Otherwise the excitation light would overwhelm the fluorescing signal. Fig. 7 depicts a sample image acquired with our system. In the figure, two channels were filled with different concentrations of fluorescein and illuminated with laser light. The channels were 100 μm wide and spaced 100 μm apart. The channel that resides in between the two test channels was filled with water for reference purposes. The figure illustrates that virtually all

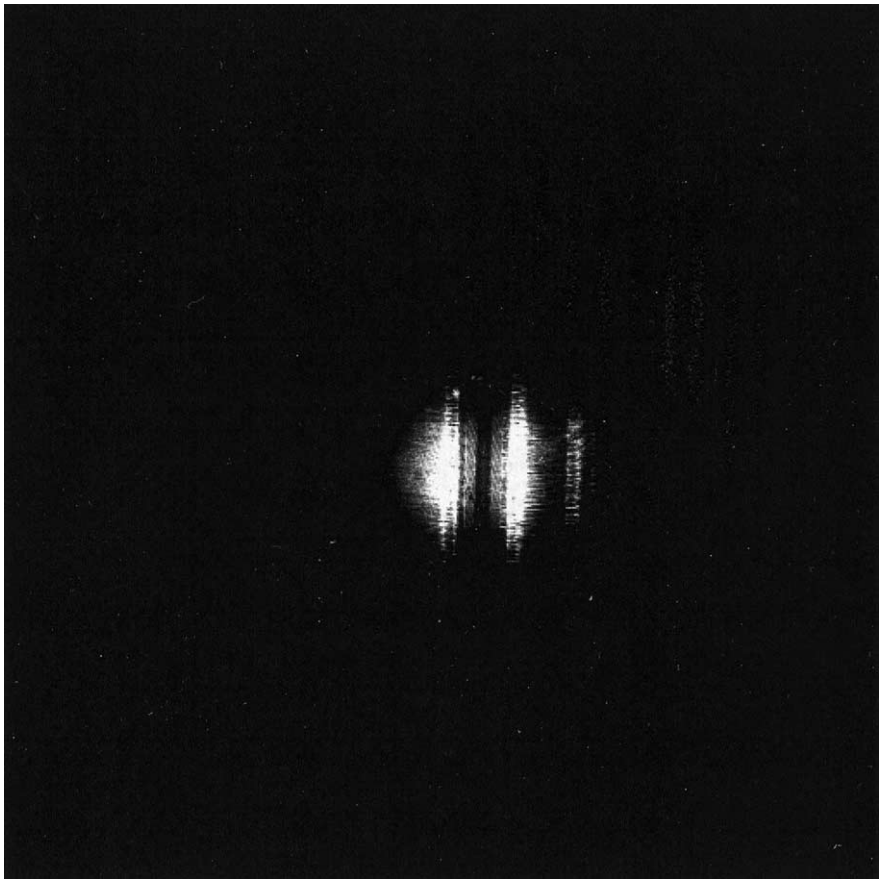

Fig. 7. The 170 and 85 μM fluorescein in 100 μm wide × 14 μm flow channels on 460 nm blocking filter illuminated by an Ar ion laser at 488 nm with nos. 2 and 5 neutral density filters in the beam path to decrease the excitation light intensity so that the imager is not saturated.

of the excitation light was blocked, and only fluorescence light was acquired by the imager. As stated previously, the reader may find it difficult to distinguish a difference between the two channels due to the loss of resolution in conversion of the imager data to picture format; however, the imager is capable of making the distinction. In fact, the concentrations of fluorescein shown in Fig. 7 locally saturate the imager. We are able to detect much lower concentrations of fluorescein and are at this time working to find the minimum concentration detectable.

### 2.4. Emission sources

We have demonstrated the monolithic integration of the microfluidic device directly on an image sensor array. The next component of the system, which must be miniaturized, is the light source. In a typical visible spectrophotometer the light source is generally a tungsten or tungsten–iodine filament lamp with some models also including light emitting diodes. In a miniature spectrometer, the appropriate light source depends heavily upon the application and heat dissipation problems. The most convenient alternatives include solid-state light emitting diodes, laser diodes, white light sources, and perhaps even the sun. For infrared analysis, the source might also be a tungsten filament lamp with a specific color filter placed directly over the microfluidic device. Although for many applications, an array of vertical cavity surface emitting lasers (VCSELs) could be desirable, such laser sources are very difficult to construct in the most interesting UV/visible wavelength range. Instead of using lasers, high finesse optical cavity filters can be defined on top of LED arrays to obtain filtered light sources, which can be directly placed on top of the microfluidic channel, which in turn is placed on top of a detector array, to create a fully functional on-chip visible spectrometer.

## 3. Conclusion

An ideal microfluidic analysis system would consist of inexpensive and disposable fluidic components, and a very compact, robust and monolithic optical excitation and measurement system. The optical read-out system could remain reusable for many experiments, but should be very sensitive and provide diagnostic imaging information on the condition of the flow channels. With the appropriate information processing, such an integrated system could yield rapid, accurate results in a very short time. This coupled with the flexibility of soft lithography shows much promise toward the definition of a spectroscopic laboratory on a chip in which either absorption or fluorescence can be measured. Here, we have described one such geometry, which satisfies some of the requirements of an integrated measurement system, in which intelligent analysis of microscopic fluid volumes can be undertaken. We believe that this approach, when optimized sensor arrays are used, can offer similar performance to commercially available spectrophotometers with the opportunity of optically monitoring many fluidic channels at the same time.

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## Biographies

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