Integrating optics and microfluidics for time-correlated single-photon counting in lab-on-a-chip devices

A. Cleary, A. Glidle, and P. J. R. Laybourn
Department of Electronics and Electrical Engineering, University of Glasgow, Oakfield Avenue, Glasgow G12 8LT, United Kingdom

S. García-Blanco
Institut National d'Optique (INO), Sainte Foy, Quebec QC G1P 4S4, Canada

S. Pellegrini, C. Helfter, and G. S. Buller
School of Engineering and Physical Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, United Kingdom

J. S. Aitchison
Department of Electrical and Computer Engineering, 10 Kings College Road, University of Toronto, M5S 3G4 Ontario, Canada

J. M. Cooper[a)]
Department of Electronics and Electrical Engineering, University of Glasgow, Oakfield Avenue, Glasgow G12 8LT, United Kingdom

(Received 20 April 2007; accepted 25 July 2007; published online 17 August 2007)

The authors describe the integration of low-loss optical waveguides with lab-on-a-chip structures to produce an integrated optical-microfluidic platform for time-correlated single-photon counting of fluorescent molecules. Waveguides were fabricated using electron beam densification of planar silica on silicon, eliminating any requirement for depositing upper cladding silica layers. Microfluidic channels were dry etched directly through the waveguides and the device was sealed using a poly(dimethylsiloxane) gasket. Time-resolved fluorescence lifetime measurements of the fluorophore nile blue were used as a model system to demonstrate the operation of the microfluidic device, with dye concentrations as low as 1.5 nM (equivalent to <6000 molecules) being measured. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2772175]

The need for sensitive compact sensors in the areas of health care and biotechnology has driven the development of integrated analytical systems, known as "lab-on-a-chip," or micrototal analysis systems (μTAS).[1] It has previously been shown that it is possible to use planar silica technology such as silica on silicon for μTAS, where functional optical structures are integrated in the same plane as the network of microfluidic channels. The end of the waveguide can be fabricated so that it forms an interface with the microfluidic channel, enabling small volumes of analyte to be probed by the light from the waveguides. Fluorescence detection within such integrated optical platforms has already been proved as an attractive analytical method due to its sensitivity,[2–4] although other techniques including surface-enhanced Raman spectroscopy have also since been developed.[5] In most cases, there has been a need to focus the collected light from the end of the waveguide onto a detector,[2–4] although recently both the sensing element and waveguides have been integrated with microfluidics to generate a truly integrated, on-chip device.[6]

Time-correlated single-photon counting (TCSPC) has many applications, including time-of-flight metrology[7] and time-resolved photoluminescence microscopy[8] of semiconductor microstructures. Within analytical biochemistry, the technique has been used in fluorescence lifetime analysis in macroscopic samples[9] and DNA analysis.[10] Recently, free space optics have been used to collect fluorescence signals from microfluidic systems using a single photon avalanche diode (SPAD).[11,12]

Time-correlated measurements enable both the intensity and the fluorescent lifetime (i.e., the decay time) of a particular molecule to be obtained. Typical decay times of such molecules are in the nanosecond range, although femtosecond and microsecond lifetimes have been reported.[13,14] Despite the potential for multiplexed single molecule detection, TCSPC has not yet been implemented in an integrated optical-microfluidic system.

Planar silica devices fabricated using flame hydrolysis deposition (FHD) of silica have long been of interest to the telecommunications industry[15] although the technology has previously been used in optical biosensing at visible wavelengths.[3,16] FHD waveguides are generally defined using standard photolithography and dry etch techniques,[15] although in such cases additional FHD cladding layers always need to be deposited after processing the waveguide core in order to allow integration of microfluidic channels. As an alternative, we have previously shown that waveguides can be written directly, using electron beam densification of the doped silica to generate an appropriate change in the refractive index and leaving a planar surface suitable for further integration, without the need for upper silica cladding layers.[17,18] Complex optical structures can be formed, and the nature of the core is such that evanescent wave applications will, in future, be possible.

An asymmetric Y-structure[3] was chosen for the sensor chip to allow for continuous monitoring of the waveguide

[a)]Electronic mail: jmcooper@elec.gla.ac.uk

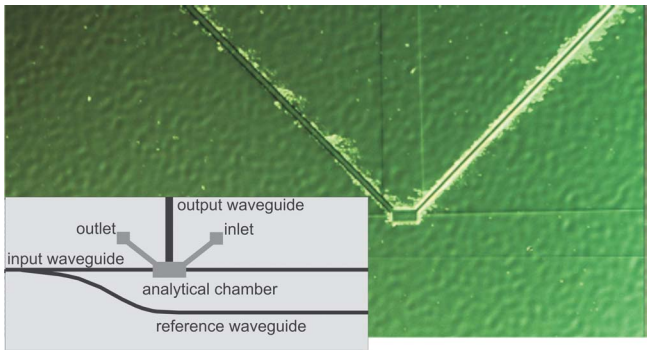

FIG. 1. (Color online) Optical micrograph of the area around the analytical chamber of the chip used for TCSPC fluorescence measurements at 5× magnification, where the analytical chamber intersects the input waveguide. The inset shows a schematic of the complete chip (not to scale).

input, while providing a reference branch. FHD silica was deposited in house, as described previously[3] with the resulting glass layer having a thickness of 2 μm and a core/undercladding index difference of 0.75%. The wafers were then mechanically diced into 1.0×1.5 cm^2 devices using a fine diamond saw. A thin layer (30 nm) of NiCr was deposited onto the substrate to prevent charging of the substrate during the electron-beam irradiation process described below.

OPTIWAVE BPM program (Optiwave, Canada) was used to predict the asymmetric Y-branch splitting ratio, using 2 μm wide single mode waveguides. A ratio of 80:20 was chosen to allow the majority of the excitation light to be directed into the analytical chamber. The simulated waveguide layouts were transferred directly to a Leica electron-beam writer to define the waveguide patterns into the silica layer. The refractive index change produced in the irradiated area was measured as 5×10^{-3} for an electron dose of 1 C cm^{-2} and electron energy of 50 keV.

Channels, 9×80 μm, and an analytical chamber, $100\times50\times9$ μm^3 (45 pl), were both defined photolithographically and were produced by dry etching through the electron beam densified waveguide core and undercladding using CHF_3 chemistry within an Oxford PlasmaTechnology BP80 instrument (Fig. 1). The single mode waveguide was 2 μm deep giving an effective analytical volume of collection of approximately 10 pl (notwithstanding scattering and angles of collection).

A thin layer of spun polydimethylsiloxane (PDMS) was used as a gasket to seal the channels and to provide an upper cladding for the waveguides. Microfluidic inlets were mechanically formed in the PDMS to create a microfluidic interconnect, and a fluorescent dye was introduced under pressure driven flow. Nile blue dye in methanol (Aldrich, UK), with excitation and emission maxima at 636 and 686 nm, respectively, was used as a model fluor.

Prior to measurement, the device was placed in an ultrasonic bath of acetone for 5 min, followed by methanol for 5 min. It was then washed in Piranha solution for 10 min, and finally washed exhaustively in reverse osmosis water. Fluorescence excitation was provided by a PicoQuant 630 nm pulsed diode laser, operated at 20 MHz, with a pulse duration of 100 ps. A 20× objective was used to launch the laser beam into the input waveguide. The optical input was optimized by monitoring the emission intensity from the reference waveguide. The dye emission was collected using a

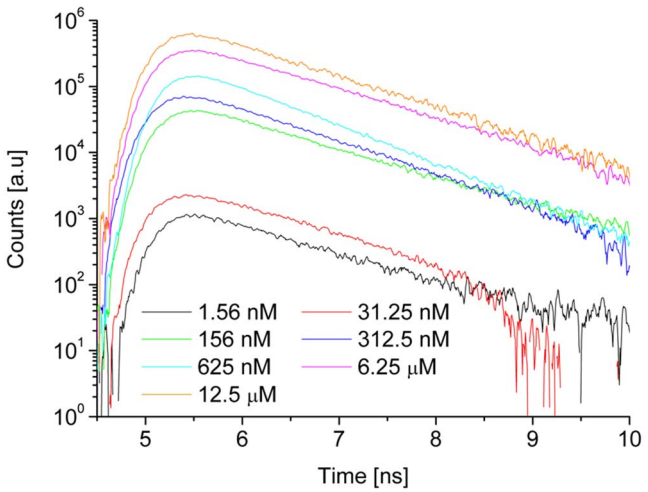

FIG. 2. (Color online) Fluorescence decays at several different dye concentrations across a range of five orders of magnitude. At higher concentrations, concentration quenching of the fluorescence decay time is observed. Note that the background auto fluorescence response from methanol (not shown here) gave a weak fluorescence response that was significantly lower in intensity than the lowest concentration of dye measured.

100 μm core fiber butt-coupled to the output waveguide, and bandpass filters were used to eliminate any stray excitation light.

Fluorescence decay times for nile blue dye in methanol, with concentrations ranging from 5 mM to 750 pM were measured. To avoid pulse pile-up in the samples, neutral density (ND) filters were used to limit the fluorescence signal reaching the detector. Once the ND filters as well as instrumental and solvent responses had been accounted for, the measured intensity response was seen to increase with concentration (Fig. 2). Measurements at each concentration were repeated five times and the mean response was used in all further analysis.

By using the SPAD detector, we were able to measure both the fluorescence intensity (Fig. 2) and the decay time

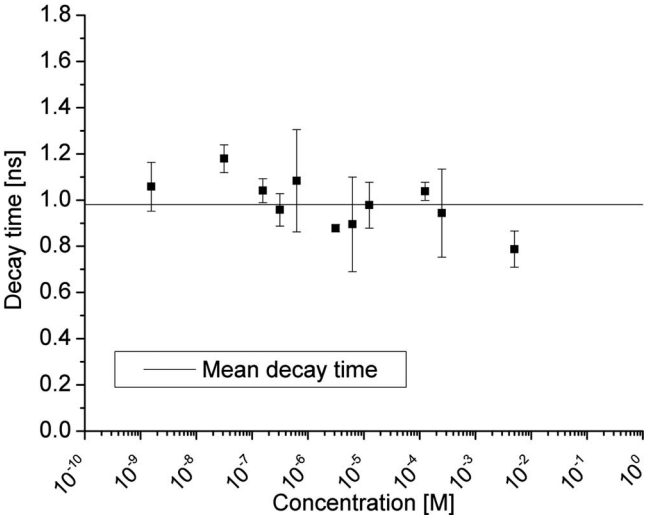

FIG. 3. Fitted exponential decay time constants from measurements of the full range of dye concentrations that were used in these experiments, with error bars (standard deviation of the mean) calculated from the mean of the five measurements taken at each concentration. The smallest measured dye concentration was 1.5 nM. The solid line represents the mean value of the fitted decay times of 0.93 ns.

(Fig. 3) of the dye. For each measurement taken, the decay time constant of the dye was found using an iterative reconvolution (where the model exponential function was convoluted with the instrumental response and compared with the measured data for the best fit).

As shown in Fig. 3, the measured decay time is constant across a change in the dye concentration of seven orders of magnitude [with a mean of 0.93 ns and a standard deviation of 0.14 ns (±15%)], providing us with a robust method for identifying the presence of a specific dye across a wide dynamic range of concentrations. For comparison, the decay time of nile blue in methanol in bulk solution was measured as approximately 1.05 ns.

A limit of detection of 1.5 nM was measured, corresponding to detection of 60 zmoles (60×10^{-21} moles) dye in the microfluidic chamber of volume of 45 pl. It was noted that this figure of merit would be substantially improved if the collection volume at the end of the waveguide, as opposed to the geometric volume of the chamber, was used to calculate the numbers of molecules being detected. This measured detection limit, expressed in terms of the number of molecules in the detection volume is favorable when compared with that seen by Ruano *et al.*,[3] particularly when issues of the estimation of the measured volume are considered.

In conclusion, a FHD sensor chip, fabricated using electron beam densified Y-branched integrated optics and etched microfluidics, was interfaced to a SPAD detector and TCSPC was used to measure the fluorescence decay time of nile blue. Using these methods, a limit of detection of 1.5 nM, equivalent to <6000 molecules was measured.

[1] A. Manz, N. Graber, and H. M. Widmer, Sens. Actuators B **1**, 244 (1990).
[2] T. E. Plowman, J. D. Durstchi, H. K. Wang, D. A. Christensen, J. N. Herron, and W. M. Reichert, Anal. Chem. **71**, 4344 (1999).
[3] J. M. Ruano, V. Benoit, J. S. Aitchison, and J. M. Cooper, Anal. Chem. **72**, 1093 (2000).
[4] K. B. Mogensen, Y. C. Kwok, J. C. T. Eijkel, N. J. Petersen, A. Manz, and J. P. Kutter, Anal. Chem. **75**, 4931 (2003).
[5] F. T. Docherty, P. B. Monaghan, R. Keir, D. Graham, W. E. Smith, and J. M. Cooper, Chem. Commun. (Cambridge) **2004**, 118.
[6] S. Balslev, A. M. Jorgensen, B. Bilenberg, K. B. Mogensen, K. B. D. Snakenborg, O. Geschke, J. P. Kutter, and A. Kristensen, Lab Chip **1**, 213 (2006).
[7] S. Pellegrini, G. S. Buller, J. M. Smith, A. M. Wallace, and S. Cova, Meas. Sci. Technol. **11**, 712 (2000).
[8] G. S. Buller, J. S. Massa, and A. C. Walker, Rev. Sci. Instrum. **63**, 2994 (1992).
[9] D. J. S. Birch, A. Sanderson, A. S. Holmes, D. McLoskey, and R. E. Imhof, Meas. Sci. Technol. **4**, 797 (1993).
[10] M. Neumann, D.-P. Herten, A. Dietrich, J. Wolfrum, and M. Sauer, J. Chromatogr. A **871**, 299 (2000).
[11] I. Rech, A. Restelli, S. Cova, M. Ghioni, M. Chiari, and M. Cretich, Sens. Actuators B **100**, 158 (2004).
[12] R. K. P. Benninger, Y. Koc, O. Hofmann, J. Requejo-Isidro, M. A. A. Neil, P. M. W. French, and A. J. deMello, Anal. Chem. **78**, 2272 (2006).
[13] A. Grofcsik, M. Kubinyi, and W. J. Jones, J. Mol. Struct. **348**, 197 (1995).
[14] Y. Li, M. Baba, and M. Matsuoka, Phys. Rev. A **55**, 3177 (1997).
[15] M. Kawachi, Opt. Quantum Electron. **22**, 391 (1990).
[16] J. M. Ruano, A. Glidle, A. Cleary, A. Walmsley, J. S. Aitchison, and J. M. Cooper, Biosens. Bioelectron. **18**, 175 (2003).
[17] S. García-Blanco, A. Glidle, J. H. Davies, J. M. Cooper, and J. S. Aitchison, Appl. Phys. Lett. **79**, 2889 (2001).
[18] S. García-Blanco, C. Hnatovsky, R. S. Taylor, and J. S. Aitchison, Appl. Phys. Lett. **85**, 1314 (2004).