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Technical Note

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A versatile lock and key assembly for optical measurements with microfluidic platforms and cartridges

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ABSTRACT: A novel and versatile optical reader for microfluidic platforms is presented. The reader includes a modular insertion port based on the lock and key concept for reproducible alignment with a miniaturized optical detection system comprising an interchangeable light emitting diode (LED) and a photodiode. The modular nature of the insertion port allows the use of microfluidic platforms in variable shapes and fluidic configurations. Three different analytical methodologies based on absorbance or fluorescence measurements were used to demonstrate the flexibility and reproducibility of the proposed experimental setup.

INTRODUCTION

The miniaturization of (bio)analytical systems has substantial advantages[1, 2] such as reduced sample and reagent consumption, response times and costs, in addition to increased automation, reliability, and portability. The increasing trend toward miniaturization in this field has led to the development of microfluidic platforms affording integration and simplified automation of the whole analytical process. These miniaturized systems typically use optical detection methods[3, 4] (particularly those based on absorbance or fluorescence measurements).[3, 5–7] The growing progress in microelectronic, optoelectronic and telecommunication technologies has enabled the development of improved small optical components meeting the requirements for incorporation into low-cost instruments.[3, 8] Among others, such advances have facilitated the development of compact, robust, inexpensive equipment based on miniaturized analytical systems using light emitting diodes (LEDs) or LED lasers as light sources and photodiodes as sensitive detectors.[3, 5, 8]

A number of compact, monolithic analytical systems of this type have been reported[9–13] that are more than acceptable as regards miniaturization, integration and portability, and have enabled convenient online, in situ and field measurements. In fact, these systems perform quite well and provide adequate sensitivity and good precision. However, these miniaturized detection systems are specially designed for use at a single operating wavelength in a specific platform configuration and are thus scarcely versatile. In fact, altering the associated microfluidic design usually requires complete rearrangement of the optical components. Integrating LEDs of different wavelengths can increase versatility,[14] albeit at the expense of increased complexity. Developing a customizable optical detection system capable of operating and at variable wavelength in different microfluidic manifolds would dispense with the need to redesign the whole detection system for each particular application.

This technical note reports a simple, versatile, inexpensive portable optical detection system for the readout of microfluidic platforms called "Optical Lock and Key Reader". The proposed system has several advantages such as the ability to operate under ambient light conditions, perform fluorescence and absorbance measurements, use different LEDs, interchange microfluidic platforms of variable shape and size, and precisely align the detection flow cell across the optical beam. A modular insertion port similar to a cuvette holder[15–17] or a cartridge reader[18] allows microfluidic platforms to be easily assembled and precisely aligned, thereby increasing the robustness of the ensuing measurements. Also, LEDs can be used in two different positions for absorbance and fluorescence measurements, and be easily exchanged via a "plug and play" type mechanism. The operational principle of the proposed system presented in this work was validated by using various analytical methodologies. However, the system can be used for other, more complex analytical purposes (e.g., enzymelinked fluorescence immunoassay[19]) by adapting the proposed methodology to the microfluidic system of the platform concerned.

EXPERIMENTAL SECTION

Chemicals and Materials. All solutions used were prepared in double distilled water. 4-Methylumbelliferone (4-MU), potassium chloride (KCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), diethylamine, sulfanilamide (SAM), and *N*-α-naphthylethylenediamine (NED) were all purchased from Sigma–Aldrich (Barcelona, Spain). Sodium nitrite ($NaNO_2$) and orthophosphoric acid (H_3PO_4, 85 %) were supplied by Panreac Química, S.A.U. (Barcelona, Spain). NitriVer 3 reagent was purchased from Hach Lange (Düsseldorf, Germany).

The colorimetric reagent used to determine nitrite ion in the continuous flow mode was a 1:15 NED:SAM mixture (3.9×10^{-3} M/5.8×10^{-2} M) in 1.4 M H3PO4 that remained stable for 2 weeks if stored refrigerated. Nitrite was determined in the batch mode by placing an amount of 2–5 mg of NitriVer 3

solid reagent in a special chamber of the microfluidic cartridge that was then sealed with Greiner EASYseal microplate sealer from Greiner Bio One (Wemmel, Belgium). Nitrite solutions were prepared on a daily basis by dilution in deaerated water of an appropriate volume of a stock solution containing 1000 mg NO_2^- L^{-1}. Fluorescence determinations in the continuous flow mode were done by using 4-MU standard solutions prepared in DEA buffer (100 mM diethanolamine, 50 mM KCl, and1 mM $MgCl_2$).

Cyclic Olefin Co-polymer (COC) sheets and films in different grades and thicknesses were purchased from TOPAS Advanced Polymers GmbH (Florence, KY, US), and poly(methyl methacrylate) (PMMA) sheets were supplied by Plásticos Ferplast (Terrassa, Spain).

Microfluidic Platforms. The platforms were fabricated as described elsewhere.[20, 21] The microfluidic systems of the different platforms or cartridges were designed by using Computer-Aided Design (CAD) software and machined onto three different layers of COC substrate on a Protomat C100/HF Computer Numerical Control (CNC) micromilling machine from LPKF Laser & Electronics (Garbsen, Germany). Substrate layers were laminated and the platform microfluidic system sealed by using the temperature diffusion bonding technique,[20, 21] which involves crosslinking polymer chains on layer surfaces under specific pressure and temperature conditions. The availability of COC in different grades[22] with also different glass transition temperatures (*Tg*) allowed platforms to be sealed with no deformation or minimal influence on the microfluidic system structure. COC grade 5013 was used for this purpose on the grounds of its good optical properties.[22] The central layer of TOPAS 5013 (*Tg*= 130 ºC) was previously laminated on both sides with TOPAS 8007 film, which acted as a sealing agent by effect of its low *Tg* value (75 ºC). The three layers were aligned and the microfluidic platform was sealed at 100 ºC under a pressure of 6 bar.

For continuous flow injection, the microfluidic platform was connected with 0.8 mm internal diameter Teflon® tubing (Scharlab, S.L., Cambridge, England) to an external peristaltic pump (Minipuls 3, Gilson, Wisconsin, US) fitted with Tygon® tubing of 1.14 mm i.d. from Ismatec (Wertheim, Germany). A six-port injection valve from Hamilton MVP (Reno, NV, US) was used to inject standard solutions.

Optical Lock and Key Reader. A miniaturized optical detection system -described elsewhere[23]- was embedded in the proposed Optical Lock and Key Reader. It consisted of an interchangeable LED and a photodetector (viz., a PIN Hamamatsu S1337-66BR large active area photodiode) mounted and integrated on a printed circuit board (PCB). The LED was modulated by a sine wave signal generated by a data acquisition card (DAQ) (NI USB-6211, National Instruments, Madrid, Spain) and which parameters (frequency and amplitude) can be changed in each measurement if necessary. The sampling frequency used to generate the modulation signal and digitalize the detected ones was adjusted to 128 times the modulation frequency to avoid problems in synchronizing signals in the DAQ. Then, the DAQ transferred the modulation signals and each detected signal from the PCB to a computer, where they were processed with several lock-in amplifiers digitally implemented in a C sharp (C#) software application. Lock-in amplification facilitated processing of raw data, increased the signal-to-noise ratio and afforded operation in ambient light without interferences.[21, 23] The whole assembly was powered by the computer.

A B5-433-B525 LED from Roithner (Vienna, Austria) with an emission peak at 525 nm was used for nitrite ion absorbance measurements because it matched the absorption maximum for the azo compound formed in the Griess reaction (540 nm). The LED was mounted in front of the photodetector (Figures 1A and B), leaving the optical flow cell of the polymeric microfluidic platforms between both components. Fluorescence measurements were made with an XSL-365-5E LED from Roithner Lasertechnik (Vienna, Austria) with an emis-

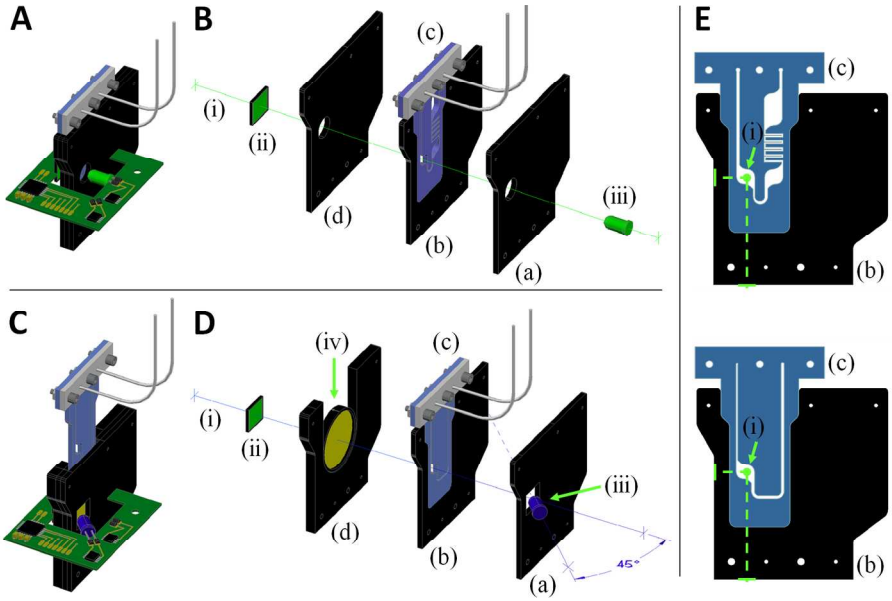

Figure 1. Schematic depiction of the lock and key concept. A) Microfluidic platform inserted into a complementary "lock" structure. B) Elements of the absorbance configuration as aligned with the (i) optical beam, (ii) photodiode, and (iii) LED. (a) Front mask, (b) lock piece of insertion port, (c) microfluidic platform, (d) back mask. C) Platform upon insertion. D) Elements of the fluorescence configuration: (ii) photodiode, (iii) LED aligned and tilted 45º with respect to (i), (iv) filter, (a) front mask, (b) lock piece, (c) microfluidic platform, (d) filter support. E) Front view of the elements of the lock and key model. The "lock" piece (b) can be fitted with multiple platforms or cartridges (c) containing various microfluidic manifolds, but the flow cell is always perfectly aligned through the optical beam (i).

sion peak at 365 nm coinciding with the excitation wavelength of 4-MU.[24] The excitation LED was tilted by 45º from the normal to the optical flow cell in order to minimize interferences in the detector (Figures 1C and D). An MF460-60 bandpass emission filter from Thorlabs (Munich, Germany) was used to prevent excitation light from reaching the detector —the maximum fluorescence emission of 4-MU peaks at 445 nm.[24] In both cases, the LED was mounted into a socket connector on the surface of the PCB that fitted the LED leg pins together.

The PCB was embedded in a customized PMMA structure (Figure 2A) that additionally accommodated the lock and key insertion port for the microfluidic platform, and the optical filter when required. The structure was designed by using CAD software and fabricated on the CNC milling machine.

RESULTS AND DISCUSSION

Design of the Optical Lock and Key Reader. The reader consists of two essential components, namely: (a) a miniaturized optical detection system including a PCB, an optical filter —required for fluorescence measurements—, and a DAQ; and (b), an insertion port for the microfluidic platforms and a support structure for embedding the PCB.

The most salient advantages of the optical detection system are its good performance and the ability to operate under ambient light conditions. Moreover, the use of a lock-in amplification via computer software and a DAQ is a low cost solution to reduce noise instead of a commercial lock-in amplifier. The versatility of the proposed system can also be increased by using exchangeable LEDs to monitor a wide range of analytical processes. Also, the LED can be positioned at two different angles with respect to the optical beam (Figure 1), namely: normal to the flow cell for absorption measurements and tilted 45º for fluorescence measurements. In the latter, the design of the insertion port allows an optical filter to be fitted between the optical detection flow cell and the detector (see Figure 1) in order to suppress reflected light from the excitation LED. The versatility of the proposed system allows it to be further modified to improve the quality of the optics and their sensitivity to some extent.[3, 5, 8] Also, collimation, focusing and the use of apertures or light baffles is possible, albeit at the expense of greater instrumental complexity.

One major advantage of the reader is that the microfluidic platform can be accurately, reproducibly positioned for correct alignment of the optical detection flow cell along the optical beam. This is a result of the insertion port having a lock and key design (Figure 1E). Thus, microfluidic platforms –the "key"- and the insertion port –the "lock"- have complementary shapes so that the former can fit exactly into the latter. An offset of 100 µm is used to make insertion port wider than the microfluidic platform in order to avoid friction and facilitate the insertion and ejection. The system affords exact alignment, and hence repeatable insertion of platforms for accurate optical readings. The results thus obtained are described in detail under *Alignment repeatability of the Optical Lock and Key Reader*.

The microfluidic platforms can accommodate microfluidic patterns of variable complexity; however, the detection flow cell is always placed in the same relative position (Figure 1E). If needed, the size and geometric shape of the microfluidic platform can be modified for fitting to more complex microfluidic manifold configurations. In this situation, the modular design of the insertion port facilitates replacement of the "lock" structure or piece (see Figure 2). The essential requirement to be met in designing the microfluidic platform and the complementarily shaped insertion port is that the optical detection flow cell should be placed at the center of the optical beam (*X* and *Y* in Figure 2B) —dimensions *X′* and *Y′* can be altered to fit different platform configurations. If necessary, an additional dimension *Z′* can be introduced by increasing the thickness of the lock piece. This flexibility makes the Optical Lock and Key Reader a versatile tool for optical measurements on a wide variety of microfluidic platforms specially designed for specific analytical methodologies. Also, measurements can be performed in the continuous flow or batch mode, which increases the potential of the Optical Lock and Key Reader even further.

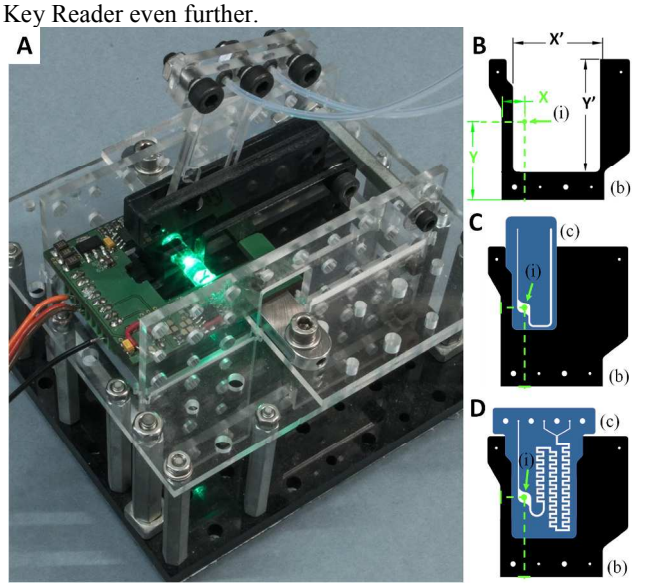

Figure 2. Photograph of A) the Optical Lock and Key Reader during the reading process showing the microfluidic platform inserted in the insertion port. B) Schematic depiction of the insertion port. The "lock" piece (b) can be designed, when required, to hold variably sized and shaped microfluidic platforms or cartridges. In some configurations, the dimensions X′ and Y′ can be modified. X and Y dimensions remain constant with respect to the center of the optical beam (i). C) and D) Schematic depiction of "key" microfluidic platforms (c) and "lock" pieces (b) with a complementary shape of the insertion port.

Analytical Validation of the Optical Lock and Key Reader. The performance of the proposed reader was validated by using it to implement various analytical methodologies based on absorbance or fluorescence measurements. We used 4-methylumbelliferone (4-MU) as the analyte for fluorescence measurements and nitrite ion (NO_2^-) as the analyte for absorbance measurements. Three different microfluidic systems were constructed for use in the batch or continuous flow mode. Batch absorbance measurements were made by loading the platform at the reader insertion port after the end of the indicator reaction. However, the continuous flow mode was preferred for optimal monitoring of the dynamic process by making repeated optical measurements of absorbance or fluorescence intensity. In addition, using the continuous flow injection technique afforded peak height measurements and hence obtaining the desired analytical information while monitoring baseline stability in a simple, expeditious manner.

Fluorescence tests

The performance of the Optical Lock and Key Reader in fluorimetric measurements was assessed with 4-methylumbelliferone (4-MU), which is a widely used standard for fluorimetric determination of enzyme activities.[24] The reagent was used in DEA buffer (pH 7.0–10.5) in order to operate under the conditions of maximum quantum yield and emission intensity.[24]

Fluorimetric measurements in the continuous flow mode. A simple microfluidic platform consisting of an inlet, a microfluidic channel, an optical detection flow cell, and an outlet (Figure 3B) was constructed for this purpose. The platform was fabricated as described above and met the specific requirements for fitting into the lock insertion port. The DEA carrier solution –where the 4-MU standard solutions were injected- was pumped into the microfluidic device through the inlet. The injected volume was 100 µL and the overall flow rate 800 µL min^{-1}. A fluorescence signal was continuously recorded by keeping the microfluidic platform inserted in the reader.

Figure 3A shows the calibration curve obtained, which fitted the equation F = 0.0354 [4-MU] + 0.0034 with a coefficient of determination r^2 = 0.9998. The curve was linear over the 4-MU concentration range 0.8–18 mg L^{-1} and the limit of detection (LOD) obtained was 0.2 mg L^{-1}. Repeatability was assessed from repeated injections (n=9) of a 9 mg 4-MU L^{-1} solution. A relative standard deviation (RSD) of 1.62 % was obtained.

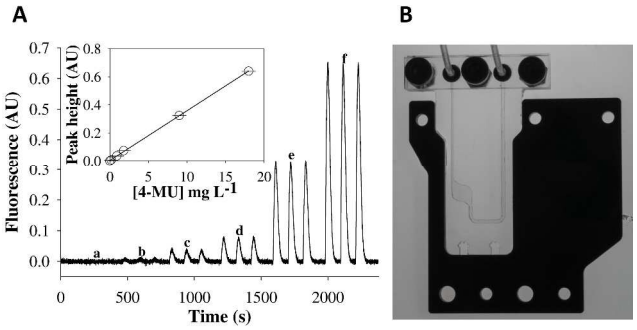

Figure 3. A) Signal recording obtained from the fluorescence measurements made with the Optical Lock and Key Reader. All solutions [(a) 0 mg L^{-1}, (b) 0.2 mg L^{-1}, (c) 0.9 mg L^{-1}, (d) 1.8 mg L^{-1}, (e) 9 mg L^{-1} and (f) 18 mg L^{-1}] were injected in triplicate. The inset shows the linear response of fluorescence intensity to the 4-MU concentration. B) Image of the microfluidic platform for fluorescence measurements and the lock piece.

Absorbance tests

Nitrite ion (NO_2^-) was determined colorimetrically using the Griess reaction. This reducing agent is widely used by the food production industry to prevent bacterial growth.[25] The Griess reaction involves the formation of a diazonium salt between sulfanilamide (SAM) and nitrite ion that subsequently forms a red azo compound with the azo dye NED in an aqueous acid medium.[25]

Colorimetric measurements in the continuous flow mode. The continuous flow determination of nitrite ion was performed in a microfluidic system consisting of two inlets, a two-dimensional meander micromixer, an optical flow cell and one outlet (Figure 4B). As before, the platform was constructed in accordance with the above-described specifications. The two inlets were used to deliver nitrite ion standards of variable concentration —or water as a carrier— and the Griess reagent. The two solutions were mixed at a merging point and the micromixer before reaching the optical flow cell for continuous recording of the absorbance. The injected volume was 100 µL and the overall flow rate 500 µL min^{-1}.

Figure 4A shows the calibration curve obtained, which fitted the equation A = 0.0042 [NO2–] + 0.0001 with r^2 = 0.9996. The linear range was 0.5–10 mg L^{-1} and the LOD 0.1 mg L^{-1}. An RSD less than 1 % was calculated from repeated injections (n = 5) of a 5 NO_2^- mg L^{-1} solution.

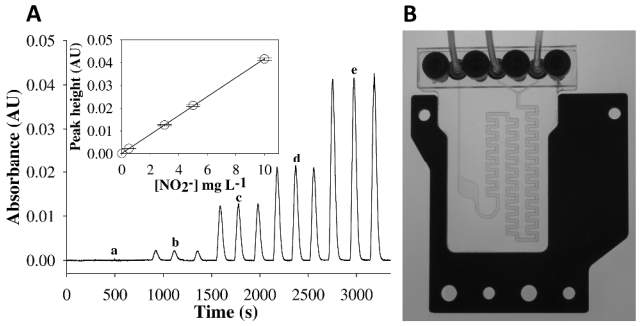

Figure 4. Signal recording obtained from nitrite ion measurements in the continuous flow mode. All solutions [(a) 0 mg L^{-1}, (b) 0.5 mg L^{-1}, (c) 3 mg L^{-1}, (d) 5 mg L^{-1}, (e) 10 mg L^{-1})] were injected in triplicate. The inset shows the linear calibration curve obtained. B) Image of the microfluidic platform and the lock piece with a complementary shape of the insertion port.

Colorimetric measurements in the batch mode. The microfluidic platform for determining NO_2^- in the batch mode was constructed in a disposable microfluidic cartridge configuration. The microfluidic system included an inlet, a reagent storage chamber, an optical detection flow cell, an outlet, and a simple microfluidic channel connecting all elements (Figures 5B and 5C). The analytical procedure started with deposition of NitriVer 3 in the reagent storage chamber, which was then sealed with an adhesive foil for microplates. Next, 120 µL of a nitrite ion standard solution at an appropriate concentration was loaded in the microfluidic cartridge to dissolve the preloaded solid reagent and form the colored product to be measured at the optical detection flow cell. After the reaction product was obtained, the microfluidic cartridge was inserted and ejected in triplicate in the Optical Lock and Key Reader for measurement (3 seconds per replicate).

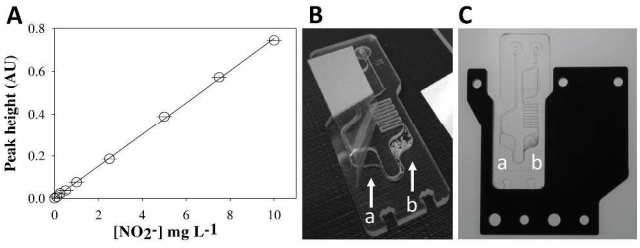

Figure 5. A) Calibration curve for the determination of nitrite ion in the batch mode. All solutions (0.1, 0.25, 0.5, 1, 2.5, 5, 7.5 and 10 mg NO_2^- L^{-1}) were measured in triplicate. B and C) Images of the microfluidic cartridge and the lock piece for batch absorbance measurements showing a) the optical flow cell and b) the reagent storage chamber.

Eight different nitrite concentrations were measured to construct the calibration curve of Figure 5 A. The above-described overall procedure was followed to measure each individual nitrite ion standard solution in triplicate. A linear response was thus obtained over the concentration range 0.1–10 mg L^{-1} that

fitted the equation A = 0.0751 [NO_2^-] + 0.0015 with r^2 = 0.9995. The calculated limit of quantitation (LOQ) and detection (LOD) for nitrite ion was 0.05 and 0.002 mg L^{-1}, respectively. Repeatability was assessed by repeating the whole analytical process 9 times with a standard solution containing 2.5 mg NO_2^- L^{-1} and calculated to be 3% as RSD (n = 9).

The results were compared between the continuous flow and batch modes. The path length of the microfluidic platform was 1 mm in both modes. The differences in LOD can be ascribed to differences in reaction time and dilution factor between the two modes. The latter depends on sample injection volume, flow rate and microfluidic manifold used. Obviously, a better LOD was obtained in the batch mode. The sample is not diluted while mixing with NitriVer 3 solid reagent and the cartridge is read when reaction time is attained. On contrast, the transient signal obtained in the continuous flow mode is affected by sample volume injected and dilution suffered by the sample in the micromixer, both chemical and hydrodynamic variables.

Alignment repeatability of the Optical Lock and Key Reader. As stated under *Design of the Optical Lock and Key Reader*, we assessed the influence on measurement reproducibility of the insertion/ejection procedure of the microfluidic platform into/from the insertion port. A cartridge was loaded with a 2.5 mg L^{-1} NO_2^- standard solution as described under *Colorimetric Measurements in the Batch Mode*. Once the resulting colored product was formed, the cartridge was successively inserted and ejected 9 times. Absorbance measurements were found to be subject to an RSD less than 1% (n = 9). Based on these results, it can be affirmed that the insertion port permits a reproducible alignment with the flow cell and the optical beam.

The overall results testify to the analytical viability and versatility of the Optical Lock and Key Reader in terms of linear ranges and detection limits. Also, the low RSD values obtained confirm the reproducibility of both the optical measurement process and the insertion/ejection procedure. Furthermore, the reader can be adapted to more complex analytical procedures when needed, as it has been demonstrated.[19]

CONCLUSIONS

The proposed Optical Lock and Key Reader is a simple, robust, versatile, inexpensive system for batch or continuous absorbance and fluorescence measurements on microfluidic devices. Alignment problems are minimized by using a modular insertion port to facilitate rearrangement of the system with multiple microfluidic platforms. In this way, the usefulness of the system is not limited to a single optical detection setup with exchangeable LEDs; rather, the reader is open to further modification and integration of supplementary optical components with a view to expanding the range of analytical procedures amenable to implementation on these microfluidic platforms.

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Author Contributions

The manuscript was written and its final version approved by all authors. Also, all authors contributed equally to the work reported herein.

ASSOCIATED CONTENT

Supporting Information Available

Figure S1. This information is available free of charge via the Internet at http://pubs.acs.org/.

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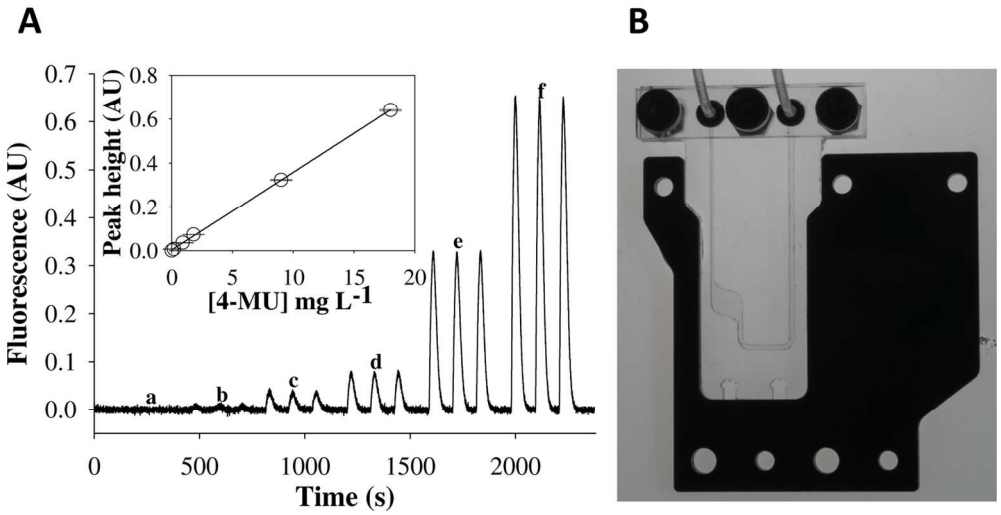

Figure 3. A) Signal recording obtained from the fluorescence measurements made with the Optical Lock and Key Reader. All solutions [(a) 0 mg L–1, (b) 0.2 mg L–1, (c) 0.9 mg L–1, (d) 1.8 mg L–1, (e) 9 mg L–1 and (f) 18 mg L–1] were injected in triplicate. The inset shows the linear response of fluorescence intensity to the 4-MU concentration. B) Image of the microfluidic platform for fluorescence measurements and the lock piece.
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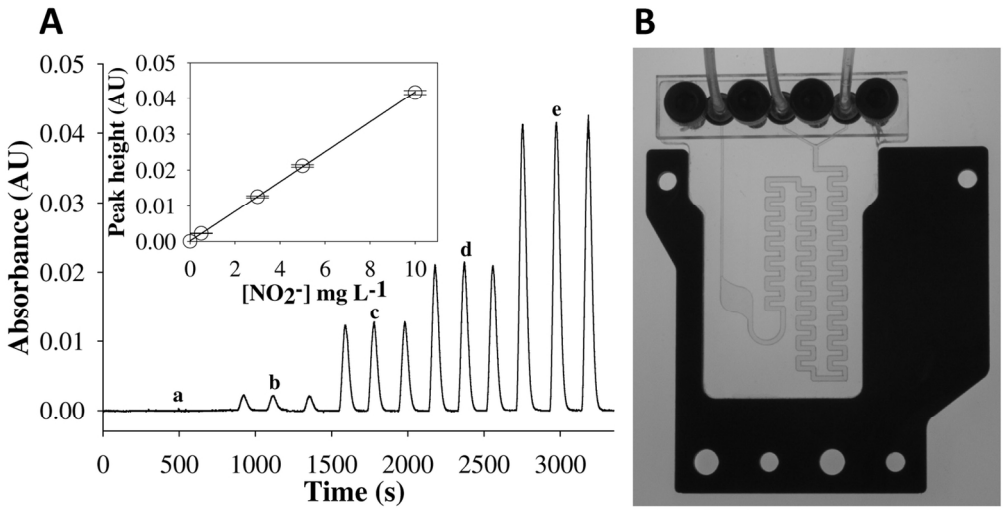


Figure 4. Signal recording obtained from nitrite ion measure-ments in the continuous flow mode. All solutions [(a) 0 mg L–1, (b) 0.5 mg L–1, (c) 3 mg L–1, (d) 5 mg L–1, (e) 10 mg L–1)] were injected in triplicate. The inset shows the linear calibration curve obtained. B) Image of the microfluidic platform and the lock piece with a complementary shape of the insertion port.
129x68mm (300 x 300 DPI)

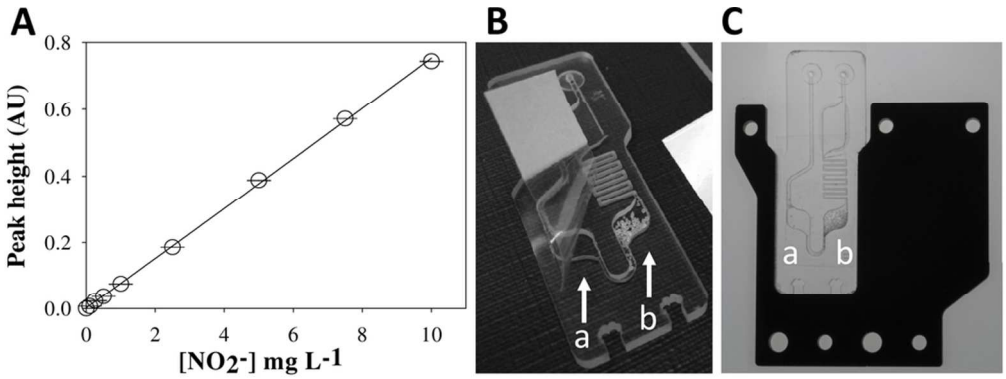
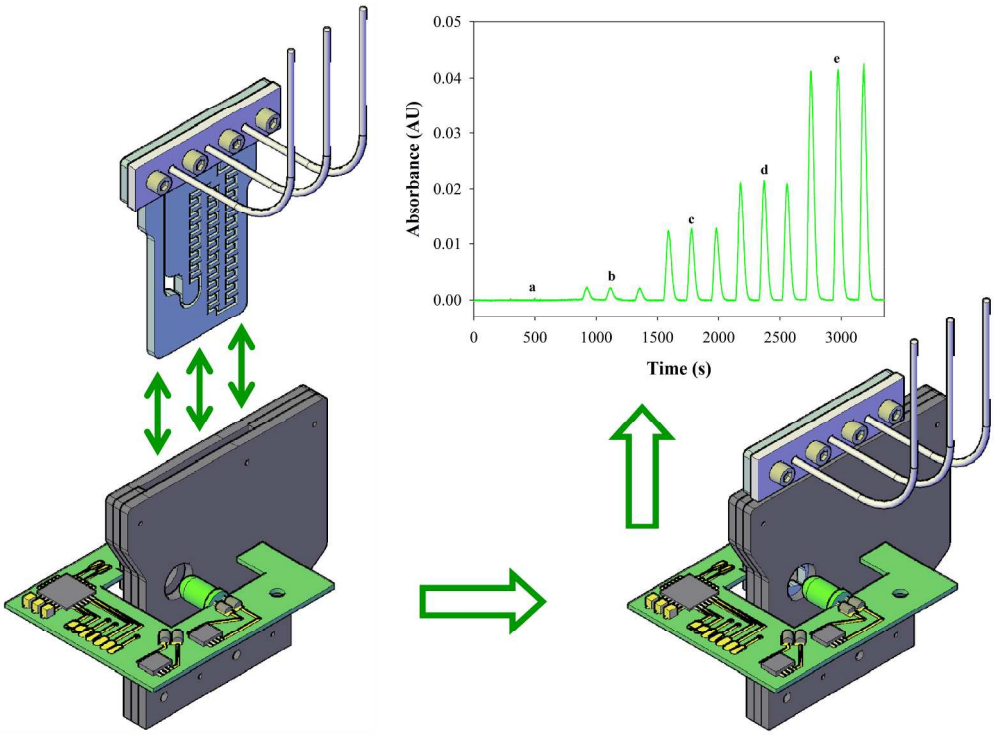


Figure 5. A) Calibration curve for the determination of nitrite ion in the batch mode. All solutions (0.1, 0.25, 0.5, 1, 2.5, 5, 7.5 and 10 mg NO2– L–1) were measured in triplicate. B and C) Images of the microfluidic cartridge and the lock piece for batch absorbance measurements showing a) the optical flow cell and b) the reagent storage chamber.
89x34mm (300 x 300 DPI)



258x189mm (300 x 300 DPI)