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Quantitative Fluorescence Assays Using a Self-Powered Paper-Based Microfluidic Device and a Camera-Equipped Cellular Phone

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Abstract

Fluorescence assays often require specialized equipment and, therefore, are not easily implemented in resource-limited environments. Herein we describe a point-of-care assay strategy in which fluorescence in the visible region is used as a readout, while a camera-equipped cellular phone is used to capture the fluorescent response and quantify the assay. The fluorescence assay is made possible using a paper-based microfluidic device that contains an internal fluidic battery, a surface-mount LED, a 2-mm section of a clear straw as a cuvette, and an appropriately-designed small molecule reagent that transforms from weakly fluorescent to highly fluorescent when exposed to a specific enzyme biomarker. The resulting visible fluorescence is digitized by photographing the assay region using a camera-equipped cellular phone. The digital images are then quantified using image processing software to provide sensitive as well as quantitative results. In a model 30 min assay, the enzyme β -D-galactosidase was measured quantitatively down to 700 pM levels. This Communication describes the design of these types of assays in paper-based microfluidic devices and characterizes the key parameters that affect the sensitivity and reproducibility of the technique.

Introduction

Traditional colorimetric point-of-care assays are easy to conduct and require simple, straight-forward analysis. The most common colorimetric assays are those conducted on paper or plastic (e.g., dipsticks) in which the user simply adds a sample to a paper or plastic device, or dips the device in the sample.¹ Quantitative results are possible in these assays if the intensity of color is measured using a specialized reader, or if the color is digitized using a camera-equipped cellular phone at a defined time point and then measured using image processing software.² The drawback to conducting quantitative colorimetric assays this way is the limited sensitivity provided by a single-step assay. Typically, the limits of detection for these assays range from millimolar to micromolar values for stoichiometric reactions between the analyte and the substrate, extending down to micromolar to nanomolar values for catalytic assays (e.g., when the analyte is an enzyme that is capable of producing more than one molecule of color during the course of the assay).³ Extending the sensitivity of quantitative, cell phone-based, single step colorimetric assays to sub-nanomolar detection limits would enable detection and quantification of classes of analytes that are not currently accessible to these types of assays. If this level of sensitivity were made possible in a low-

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cost, easy-to-use format (e.g., on paper), then it would provide a useful step towards creating a new generation of point-of-care diagnostics.

In this Communication we describe a strategy that enables quantitative fluorescent assays with sensitivity for enzyme biomarkers in the high picomolar range, while still maintaining the ease of use and ease of interpretation expected for a single-step colorimetric paper-based assay (Figure 1). Our approach uses fluorescence as the readout,⁴ but the fluorescence is in the visible region, therefore we measure the readout using a digital camera (similar to a colorimetric assay). Our efforts are aimed towards resource-limited environments such as remote villages in the developing world,^{3c,5} therefore our assay design uses paper and a camera-equipped cellular phone to create an inexpensive excitation source and reader.

The paper is used to form a three-dimensional (3D) paper-based microfluidic device that contains an internal single-use fluidic battery that powers a LED, which is integrated into the paper device;⁶ expensive and long-lasting external batteries are not needed for these assays. The battery turns on when an aqueous fluid is added to the paper device, and the LED illuminates a sample region where the assay takes place.^{6,7} Photographing the visible fluorescence from the assay region enables quantitative measurements of the levels of a desired analyte.⁸ Thus, this work describes the concept of quantitative fluorescence assays in paper, identifies the analytical parameters that affect the sensitivity and reproducibility of the method, and demonstrates the method by quantitatively detecting a model enzyme, β -D-galactosidase.

Other methods for conducting fluorescence assays in point-of-care environments are available, but they often use specialized electronic devices (such as fluorescence microscopes, handheld fluorescence readers, stationary fluorescence scanners, or microplate readers)⁴ that are appropriate for some point-of-care settings, but that may be too expensive and require too much power for use in extremely resource-limited environments (e.g., remote villages). Alternatively, camera phones have been used as the imaging device when paired with specialized excitation sources,⁹ but alternative versions of this solution would be valuable as well.

Results and Discussion

Our design for a paper-based fluorescence assay requires three key elements: (i) a 3D paper-based microfluidic device that is capable of generating power to turn on an integrated surface-mount LED;^{6,10} (ii) an assay region on the paper-based device that enables the assay solution to be illuminated by the LED while simultaneously minimizing background signal arising from the LED when the intensity of the fluorescent signal is captured using a camera-equipped cellular phone; and (iii) an appropriately-designed assay reagent that responds selectively to the desired analyte and switches from non-fluorescent (or weakly fluorescent) to highly fluorescent in the presence of the analyte.¹¹ The design considerations associated with each of these three elements are described in detail in the following sections.

Design of the paper battery and the fluorescence excitation source

Figure 2 shows the design of the paper battery, the connection between the battery and the LED, as well as the arrangement between the LED and the assay region on the paper device. The paper device was assembled using layers of wax-patterned paper and patterned double-sided adhesive tape.¹⁰ This 3D layered assembly contains an internal fluidic battery^{6,7a} that is composed of several hydrophilic regions of paper that each contain the minimum quantities of metal salts and metal electrodes needed to generate multiple galvanic cells linked in series and parallel. Together these cells are capable of powering the LED for the few minutes that are required to conduct the fluorescence assay. This type of battery uses

only microgram quantities of metals and metal salts, and therefore adds only ~\$0.15 to the cost of the device; it also minimizes concerns over the disposal of hazardous waste when the device and internal fluidic battery are discarded.

Copper tape is used to connect the galvanic cells to one another in the battery (Figure 2b,c), and to connect the grouping of galvanic cells to the LED (Figure 2b). Figure 2b further shows the distribution of water from the entry point in the 3D microfluidic device to the galvanic cells. Once the metal salts in the galvanic cells dissolve, the resulting power turns on the LED, which is oriented in Figure 2b to illuminate the sample region from the horizontal direction. This arrangement allows us to capture pictures of the fluorescent readout from a direction that is perpendicular to the direction of the LED excitation source (Figure 1).

This perpendicular configuration is critical for obtaining quantitative results for the fluorescence assays. For example, in our seminal work on fluidic batteries and paper-based fluorescence assays,⁶ a UV LED was oriented in the same direction that the picture was taken, which prevented our ability to obtain quantitative results from assays (only qualitative results were possible when using a camera phone as the reader). Another critical difference between this work and our previous study is the choice of fluorophore: previously we used 7-hydroxycoumarin, whereas now we use fluorescein. This change allows us to use a LED that emits at 472 nm (rather than at 375 nm, which was necessary to excite 7-hydroxycoumarin¹²), thus substantially reducing the size and complexity of the fluidic battery required to power the LED (the fluidic battery that powers a UV LED that emits at 375 nm required 24 galvanic cells, whereas the fluidic battery that powers the LED in the current study requires only 6 galvanic cells). The λ_{ex} of fluorescein is 494 nm,¹³ which is close to the λ_{em} of the LED (472 nm) in the paper device.

Design of the region for the sample

The region on the paper device that contains the assay solution must prevent the sample from wetting the LED, as well as minimize scattering of light emitted by the LED. We addressed both of these issues by inserting a 2-mm high section of a clear straw into an appropriately sized chamber in the 3D paper-based microfluidic device (Figure 2d). The bottom of this chamber is double-sided adhesive tape, which holds the straw in place and provides a seal between the junction of the straw and the paper-based device to prevent the sample from leaking into the device. The bottom of the chamber inside the straw is covered with a disk of black electrical tape to minimize background signal. The sample region holds 75 μL of fluid.

Design of a case for the cell phone camera

To ensure that the fluorescence assays are digitized reproducibly, we created a modified case for an iPhone 4S (Figure 3) (presumably the concepts described herein will apply to other phones and cases as well), and we included an alignment marker on the paper-based microfluidic device (i.e., the large semi-circular region in Figures 2a,d and 3c). This semi-circular region matches the diameter of a polyethylene tube (2 in. $\times$ 0.5 in. inner diameter $\times$ 0.6 in. outer diameter) that we glued around the camera hole of a standard commercial protective case for the iPhone 4S (Figure 3b). Alignment of the polyethylene tube with the semicircle on the paper-based device ensures a proper focal length for the camera (the images are always in focus), blocks external light from interfering with the color of the fluorescence assay, and ensures that the picture is acquired from the same region on every device. In the absence of these measures, the acquired images varied in intensity drastically, with ambient light particularly impacting the measurement of light emitted from the fluorescence assay (Figure S4).

We also included a circular piece (0.45–0.50 in. in diameter) of Kodak Wratten 2 No. 12 filter on the end of the poly(ethylene) tube (Figures 3b and S2). This filter removes light below 510 nm to further minimize background signal from the LED. Fluorescein emits at 514 nm,¹³ so the fluorescent signal provided by the assay is outside the wavelength cutoff of the filter used in the cell phone case (Figure S3).

Design of the assay reagent

The assay reagent used in these fluorescence assays must be non-fluorescent (or weakly fluorescent) and then become highly fluorescent when exposed to the target analyte. Several configurations of reagents are possible, but we chose to demonstrate the concept using an activity-based detection reagent that detects the presence of β -D-galactosidase (a model enzyme) through its enzymatic activity (Figure 4).¹⁴

Reagent **1** functions as follows: if β -D-galactosidase is in a sample, it hydrolyzes the β -glycosidic bond of the galactose ring in **1**. This reaction liberates the linker (the linker spaces fluorescein away from the substrate to enhance the ability of the enzyme to process the substrate¹⁵), after which the linker releases highly fluorescent fluorescein via formation of quinone methide. In principle, the design of this reagent could be generalized to detect a variety of enzyme biomarkers by incorporating their respective substrates in place of the galactose ring on **1**. One advantage of this type of small molecule detection reagent over more common biomolecule reagents is the long-term thermal stability of the small molecule¹⁶—this long-term thermal stability is a feature that will be particularly useful in resource-limited environments such as remote villages.

Synthesis of reagent 1

We prepared reagent **1** by coupling **3**¹⁷ with **4**¹⁸ through an S_N2 reaction (Scheme 1). The basic conditions of this reaction also removed the acetate protecting groups from the galactose substrate. A final deprotection of the methyl ester provided **1**. Detailed synthetic procedures are described in the Supporting Information.

Characterization of the fluidic battery

Before reagent **1** could be used in an assay in the paper-based devices, we first needed to characterize the properties of the battery in the absence of the LED to ensure that it performed reproducibly and was able to power the LED. The values for short circuit current and open circuit voltage were found to be reproducible over several different devices, providing 0.6 mA and 2.5 V (Figure 5a), respectively, which are values that are compatible with the specifications for powering the LED. We then included the LED in the paper-based device and used a variable photoresistor to measure the intensity of light emitted by the LED (Figure 5b); the intensity of light in this measurement is inversely proportional to resistance. The LED emitted light approximately 5.5 min after the addition of water to the paper microfluidic device, but required approximately 15 min to reach a consistently strong emission intensity. Measurements from five replicate devices revealed that the most accurate operating window for the fluidic battery and LED is between 15–22 min after addition of water to the battery, as denoted by the arrows in Figure 5b.

Effect of the LED and the optical filter on the reproducibility of the fluorescence measurements

In initial experiments, we measured the ability of the paper-based excitation source and the camera equipped cellular phone to collectively provide reproducible and predictable measurements for various concentrations of fluorescein. These experiments used multiple paper-based devices, each containing a different LED, although all LEDs were sold as

identical components. The images from the experiments were analyzed using Adobe® Photoshop®, where the median green value for each sample was measured using the histogram function. The range of concentrations of fluorescein used for the experiment was 250 nM to 4 μ M. Concentrations of fluorescein above 4 μ M saturated the signal on the camera phone, and concentrations below 250 nM did not provide sufficient signal for accurate measurement. These tests revealed that differences in fluorescein concentration could be measured, but that the error in the measurements was substantial (Figure 6a).

We hypothesized that the primary source of error was caused either by slight variations in intensity of light emitted by the LEDs and/or by differences in the wavelength of maximum emission between otherwise identical LEDs. To test this hypothesis, we prepared a second calibration curve using multiple paper-based devices, but using only one LED for all devices. (Note: the optical filter was not included in the camera phone for these measurements). The improvement in reproducibility is noticeable (Figure 6b). Clearly before this design of a paper-based fluorescence excitation source can be generalized, the intensity of each LED must be measured and noted prior to incorporating it into a device. Alternatively, each device could be calibrated on site by measuring the intensity of a standard solution prior to performing an assay (note the section below on re-use of the paper-based excitation source).

Finally, we repeated the experiment using a single LED, but this time we used a camera equipped cellular phone that included the optical filter in the appended polyethylene tube. This filter minimized background signal from the LED and further improved the reproducibility of the measurements (Figure 6c).

Quantification of β -D-galactosidase

Based on the results from the fluorescein experiments, we next tested whether we could generate a calibration curve for quantifying the model enzyme β -D-galactosidase using reagent **1**. The duration of exposure of **1** to β -D-galactosidase was 30 min and photographs of the fluorescent signals for the samples were obtained 15 min after adding water to the paper-based excitation source. The same LED was used in all assays, although it was transferred between many paper-based devices for replicate measurements. Likewise, the calibration curve was generated using the optical filter on the camera equipped cellular phone. The results of these measurements are shown in Figure 7a, which reveals a linear dynamic range for β -D-galactosidase from approximately 0.7 nM to 12 nM. The limit-of-detection¹⁹ is 0.7 nM, as determined from the linear portion of the calibration curve (Figure 7b). This calibration curve indicates that the assay platform is capable of providing quantitative fluorescence assay results while minimizing the amount of equipment needed for the assay (i.e., only a paper-based device and a cell phone camera are needed). The detection reagent and LED can be altered as desired in order to detect and quantify a variety of targets.

Finally, it is worth noting that while the paper-based excitation source was designed for single use, we found it possible to use the same device for multiple assays while the intensity of the LED remained in the reproducible operating window (Figure 5b). Re-use is possible simply by removing the sample from the assay region (Figure 1), and then washing the region $1 \times 75 \mu$ L with water and $3 \times 75 \mu$ L with the next sample. This procedure, of course, is only applicable to samples in which excess is available for use as a wash solvent. Using this procedure, we were able to conduct 18 assays using a single paper-based excitation source.

Conclusions

Single-step quantitative fluorescence assays with picomolar detection limits using essentially paper, a straw, an appropriate detection reagent, and a cell phone offer a simple approach to achieving sensitive point-of-care assays. With the overall strategy in place, and the operating parameters defined, the assay strategy now can be refined for a variety of applications. For example, future efforts will include (i) designing signal amplification reagents that release more than one equivalent of fluorescein in response to an enzyme analyte,²⁰ (ii) establishing an approach to normalizing the assay results when using commercial LEDs that vary in intensity, and (iii) expanding the scope of the approach to include analytes other than the model enzyme β -D-galactosidase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. (a) Yetisen AK, Akram MS, Lowe CR. *Lab Chip*. 2013; 13:2210. [PubMed: 23652632] (b) Li X, Ballerini DR, Shen W. *Biomicrofluidics*. 2012; 6:011301.(c) Shah P, Zhu X, Li CZ. *Expert Rev Mol Diagn*. 2013; 13:83. [PubMed: 23256705] (d) Rolland JP, Mourey DA. *MRS Bulletin*. 2013; 38:299.(e) Phillips ST, Lewis GG. *MRS Bulletin*. 2013; 38:315.(f) Zhao W, Ali MM, Aguirre SD, Brook MA, Li Y. *Anal Chem*. 2008; 80:8431. [PubMed: 18847216] (g) Dungchai W, Chailapakul O, Henry CS. *Anal Chim Acta*. 2010; 674:227. [PubMed: 20678634] (h) Jokerst JC, Adkins JA, Bisha B, Mentele MM, Goodridge LD, Henry CS. *Anal Chem*. 2012; 84:2900. [PubMed: 22320200] (i) de Souza FR, Alves GL, Coltro WKT. *Anal Chem*. 2012; 84:9002. [PubMed: 23072590] (j) Kim S, Jung E, Kim MJ, Pyo A, Palani T, Eom MS, Han MS, Lee S. *Chem Commun*. 2012; 48:8751.(k) Wang W, Wu W-Y, Wang W, Zhu J-J. *J Chromatogr Sci*. 2010; 1217:3896.
2. (a) Martinez AW, Phillips ST, Carrilho E, Thomas SW III, Sindi H, Whitesides GM. *Anal Chem*. 2008; 80:3699. [PubMed: 18407617] (b) Shen L, Hagen JA, Papautsky I. *Lab Chip*. 2012; 12:4240. [PubMed: 22996728] (c) Lee DS, Jeon BG, Ihm C, Park J-K, Jung MY. *Lab Chip*. 2011; 11:120. [PubMed: 21109898] (d) Coskun AF, Wong J, Khodadadi D, Nagi R, Tey A, Ozcan A. *Lab Chip*. 2013; 13:636. [PubMed: 23254910] (e) Zhu H, Yaglidere O, Su T-W, Tseng D, Ozcan A. *Lab Chip*. 2011; 11:315. [PubMed: 21063582]
3. (a) Martinez AW, Phillips ST, Whitesides GM. *Anal Chem*. 2010; 82:3. [PubMed: 20000334] (b) Vella SJ, Beattie P, Cademartiri R, Laromaine A, Martinez AW, Phillips ST, Mirica KA, Whitesides GM. *Anal Chem*. 2012; 84:2883. [PubMed: 22390675] (c) Giljohann DA, Mirkin CA. *Nature*. 2009; 462:461. [PubMed: 19940916]
4. (a) Liang J, Wang Y, Liu B. *RSC Advances*. 2012; 2:3878.(b) Miyaki K, Guo Y, Shimosaka T, Nakagama T, Nakajima H, Uchiyama K. *Anal Bioanal Chem*. 2005; 382:810. [PubMed: 15883790] (c) Ryu G, Huang J, Hofmann O, Walshe CA, Sze JYY, McClean GD, Mosley A, Rattle SJ, deMello JC, deMello AJ, Bradley DC. *Lab Chip*. 2011; 11:1664. [PubMed: 21431240] (d) Pais A, Banerjee A, Klotzkin D, Papautsky I. *Lab Chip*. 2008; 8:794. [PubMed: 18432351] (e) Yoon B, Park IS, Shin H, Park HJ, Lee CW, Kim J-M. *Macromolecules*. 2013; 34:731.(f) Liu X, Zong C, Lu L. *Analyst*. 2012; 137:2406. [PubMed: 22489282] (g) Myers FB, Henrikson RH, Bone J, Lee LP. *PLOS One*. 2013; 8:e70266. [PubMed: 23936402]
5. (a) Yager P, Domingo GJ, Gerdes J. *Annu Rev Biomed Eng*. 2008; 10:107. [PubMed: 18358075] (b) Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH. *Nature*. 2006; 442:412. [PubMed: 16871209] (c) Fu E, Yager P, Floriano PN, Chrisodoulides N, McDevitt JT. *IEEE Pulse*. 2011; 2:40. [PubMed: 22147068] (d) Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P,

- Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Hay Burgess DC. *Nature*. 2006; 444:73. [PubMed: 17159896] (e) Peeling RW, Holmes KK, Mabey D, Ronald A. *Sex Transm Infect*. 2006; 82:v1. [PubMed: 17151023]
6. Thom NK, Yeung K, Pillion MB, Phillips ST. *Lab Chip*. 2012; 12:1768. [PubMed: 22450846]
7. (a) Thom NK, Lewis GG, DiTucci MJ, Phillips ST. *RSC Advances*. 2013; 3:6888.(b) Liu H, Crooks RM. *Anal Chem*. 2012; 84:2528. [PubMed: 22394093] (c) Lee KB. *J Micromech Microeng*. 2005; 15:5210.(d) Zhang L, Zhou M, Wen D, Bai L, Lou B, Dong S. *Biosens Bioelectron*. 2012; 35:155. [PubMed: 22417872]
8. (a) Yang H, Li D, He R, Guo Q, Wang K, Zhang X, Huang P, Cui D. *Nanoscale Res Lett*. 2010; 5:875. [PubMed: 20672123] (b) Ma Y, Li H, Peng S, Wang L. *Anal Chem*. 2012; 84:8415. [PubMed: 22946839] (c) Kwon H, Samain F, Kool ET. *Chem Sci*. 2012; 3:2542.
9. (a) Yuan C, Zhang K, Zhang Z, Wang S. *Anal Chem*. 2012; 84:9792. [PubMed: 23121315] (b) Zhu H, Mavandadi S, Coskun AF, Yaglidere O, Ozcan A. *Anal Chem*. 2011; 83:6641. [PubMed: 21774454]
10. (a) Martinez AW, Phillips ST, Whitesides GM. *Proc Natl Acad Sci USA*. 2008; 105:19606. [PubMed: 19064929] (b) Martinez AW, Phillips ST, Butte MJ, Whitesides GM. *Angew Chem Int Ed*. 2007; 46:1318.(c) Carrilho E, Martinez AW, Whitesides GM. *Anal Chem*. 2009; 81:7091. [PubMed: 20337388]
11. Cho D-G, Sessler JL. *Chem Soc Rev*. 2009; 38:1647. [PubMed: 19587959]
12. Atkins RA, Bliss DE. *J Org Chem*. 1978; 43:1975.
13. Martin MM, Lindqvist L. *J Lumin*. 1975; 10:381.
14. Goddard JP, Reymond JL. *Trends Biotechnol*. 2004; 22:363–370. [PubMed: 15245909]
15. de Groot FMH, Loos WJ, Koekkoek R, van Berkom LWA, Busscher GF, Seelen AE, Albrecht C, de Bruijn P, Scheeren HW. *J Org Chem*. 2001; 66:8815. [PubMed: 11749612]
16. (a) Yeung K, Schmid KM, Phillips ST. *Chem Commun*. 2013; 49:394.(b) Nuñez SA, Yeung K, Fox NS, Phillips ST. *J Org Chem*. 2011; 76:10099. [PubMed: 22092043]
17. Ghosh AK, Khan S, Farquhar D. *Chem Commun*. 1999; 24:2527.
18. Adamczyk M, Grote J, Moore JA. *Bioconjugate Chem*. 1999; 10:544.
19. Defined as $3 \times$ the standard deviation of the 0 nM sample divided by the slope of the linear region.
20. (a) Amir RJ, Shabat D. *Adv Polym Sci*. 2006; 192:59.(b) Avital-Shmilovici M, Shabat D. *Soft Matter*. 2010; 6:1073.

Fig. 1.

Graphical representation of a paper-based excitation source for fluorescence assays. The excitation source is a three-dimensional (3D) paper-based microfluidic device that contains an internal fluidic battery,⁶ a surface mount LED, and a built-in cuvette for the assay solution; the cuvette is made from a section of a clear plastic straw. The bottom images in the graphic provide detailed views of key portions of the paper-based device.

Fig. 2.

Detailed view of the paper-based excitation source. (a) An expanded view of the patterned layers of paper and tape. The dimensions of the device are 2.4 cm wide $\times$ 3.6 cm long $\times$ 0.28 cm thick. (b) Cross-sectional view of the device showing the distribution of water in the hydrophilic channels in the device. The location of the cross-section is shown in layer 3 in (a). Photographs of (c) layer 4 (layers 5–12 are underneath layer 4; copper tape has been placed over the electrodes to connect the galvanic cells in this image), and (d) the top of the device (the LED is underneath the top layer of patterned paper, as indicated). The dotted white lines denote the edges of the device. An alternative and more detailed view of the device is provided in Figure S1.

Fig. 3. Photographs of the cell phone case with the appended poly(ethylene) tube and optical filter. (a) The cell phone case and black tube as purchased. (b) The tube cut to 2 in. in length and glued onto the cell phone case. The end of the tube contains a cap that further contains an optical filter ($\lambda_{\text{cutoff}} = 510 \text{ nm}$). (c) Photograph showing the alignment of the cell phone to the paper-based excitation source.

Fig. 4.

Design of the assay reagent (1). The weakly-fluorescent reagent detects the model enzyme β -D-galactosidase through an activity-based detection mechanism¹¹ to release the highly fluorescent dye fluorescein (2). Changing the substrate portion of the reagent should provide derivatives of this reagent that select for other enzyme biomarkers.

Fig. 5.

Characterization of the fluidic battery and paper-based excitation source. (a) Time-dependent short circuit current and open circuit voltage values after adding water to the entry point of the paper-based microfluidic device. (b) Time-dependent intensity of light emitted by the LED when powered by the fluidic battery. The resistance is inversely proportional to the intensity of the light. The most reproducible light is emitted between 15 min and 22 min after adding water to the input region of the paper-based excitation source. Values below the horizontal dotted line indicate when the emitted light from the LED is visible. In both (a) and (b), the data points are the averages of the measurements, and the error bars represent the standard deviations from these averages.

Fig. 6.

Characterization and optimization of the collective performance of the paper excitation source and the camera-equipped cellular phone. (a) Intensity of green for the fluorescence tests using fluorescein in 100 mM phosphate-buffered water (pH 7.6) at 20 °C. All tests in (a) were conducted using different copies of LEDs that were purchased as identical components. Likewise, the optical filter was not used in the cell phone case. (b) An identical experiment to (a), with the exception that one LED was used in all tests. (c) An identical experiment to (b), but in this case the optical filter ($\lambda_{\text{cutoff}} = 510$ nm) was included in the cell phone case. The data points in all graphs are the average of three measurements and the error bars represent the standard deviations of these averages. Representative photographs of the readouts for different concentrations of fluorescein are shown to the right of the graphs in (b) and (c).

Fig. 7.

Calibration curve for quantifying the level of β -D-galactosidase in a sample (pH 7.6, 20 °C) using a 30 min fluorescence assay. (a) Graph of the intensity of the green color for the fluorescence assays versus the concentration of β -D-galactosidase. (b) Expanded view of the dotted region in (a). The graph in (b) was used to determine the limit of detection for the assay. The data points represent the average of six measurements and the error bars represent the standard deviations from these averages.

Scheme 1.

Synthesis of reagent **1**. Reagents and conditions: (a) i. PBr_3 , CH_2Cl_2 , THF, 0 °C; ii. fluorescein methyl ester (**4**), K_2CO_3 , DMF, 50 °C (33%); (b) LiOH , $\text{MeOH}/\text{H}_2\text{O}/\text{THF}$ (49%).