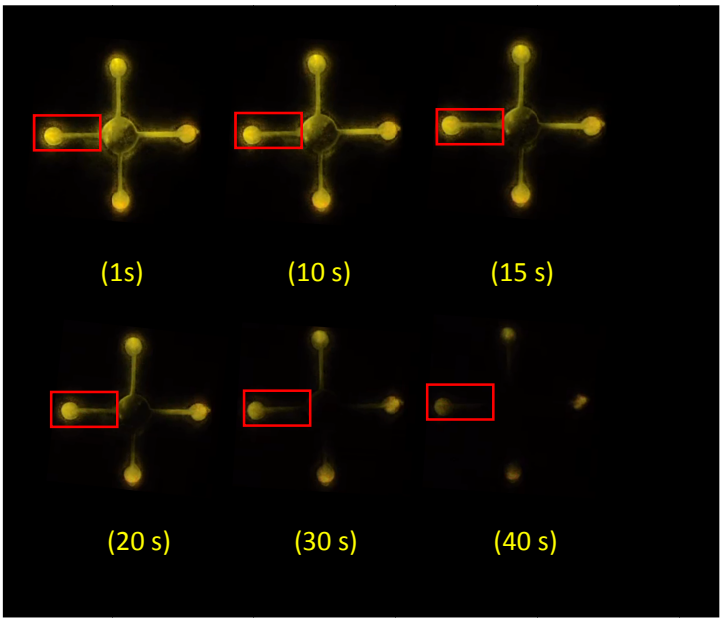
ELECTRONIC SUPPLEMENTARY INFORMATION

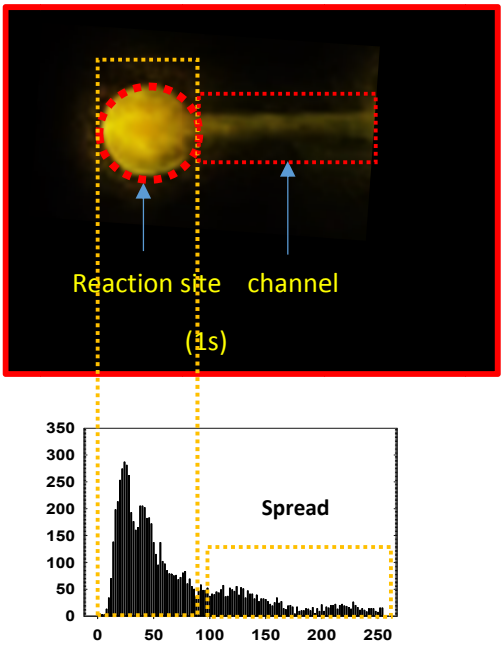
S1: All-paper microfluidic device: design and experimentation

Table S1 is a compilation of the recently developed paper microfluidics based lab on chip devices. **Table S1, Entries. 1-11** are colorimetric paper microfluidic devices that are used to detect various biomolecules. Liu *et al* made devices by wax printing chromatography papers to colorimetrically detect rabbit IgG as low as 330 ppM. Yao *et al*, using NC membranes (pore size: 0.45 µm), developed a gold nanoparticle labelled immunoassay that could detect human IgG as low as 1 ng/ml. various other biomolecules like glucose, uric acid, acetoacetate, nitrite (0.5 mM and upwards) were detected in biological fluids such as blood, urine and saliva (**Table S1: entries 9-11**) using paper based colorimetric assays. Most of the above mentioned devices might have to account for signal intensity loss due to spreading before it can adopted for credible CL detection. This loss of signal intensity can also affect the sensitivity of the chemiluminescent assay.

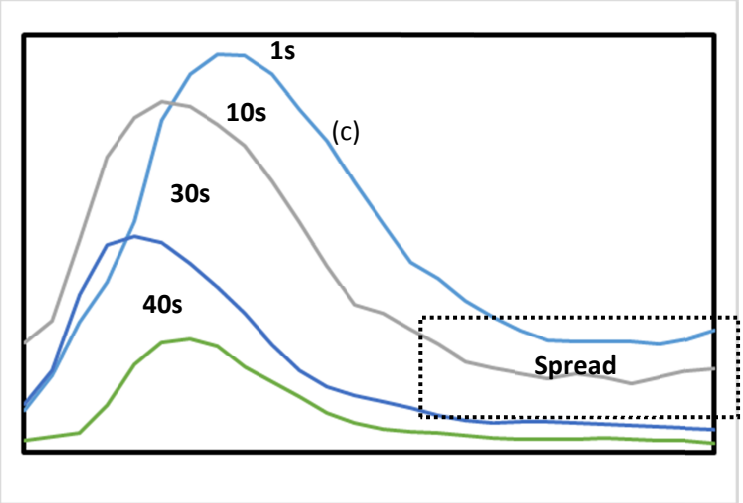
All-paper microfluidic device prototype was fabricated using paper laminated between patterned laminating sheets (3 mil). The plastic sheet was patterned to include a cross channel design which allow the reagents to flow from the center inlet towards each of the four arms. The device was prepared by using a precision craft cutter to cut the cross design. A small strip of filter paper was inserted between the two sheets and the device was thermally laminated. **Fig. S1a** and **S1b** shows the layers of the paper microfluidic device and the cross shaped cut out. **Fig. S1a** depicts the chemiluminescent intensities at various instances as observed through iPhone camera. We can notice the chemiluminescent reaction has spread across the channels from the point of reagent introduction obviously due to capillary force offered by the highly porous paper substrate. The reagents quickly diffuse away from the reagent delivery point spreading the signal along its travel. **Fig. S1b** shows the intensity profile of the reaction site and the channel during a reaction and **Fig. S1c** depicts the variation of spread at different instances of the reaction. Around 20% of net photon emission is contributed by channels at the beginning of a reaction. As reaction proceeds, the contribution of channels towards the total luminescence is reduced to less than 5% in 20 seconds.

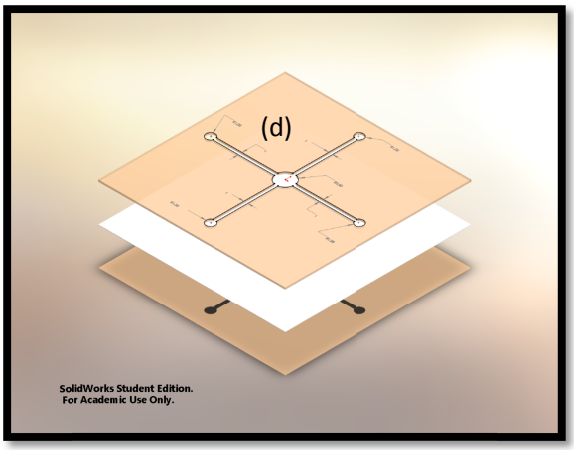


(a)



(b)





	Author	Materials	Method of preparation	Detection	Spread/Smear	Analysis
1	Andres W. Martinez et al. (2008)	Whatman chromatography paper 1	paper patterned with photolithography and baked	glucose [0.5 mM] and protein [4uM] in artificial urine	YES	colorimetry
2	Wei Wang et al. (2010)	Whatman chromatography paper 3mm	cut into tree-shaped sheets soaked in citric buffer solution	protein - bovine serum albumin (BSA) in artificial urine [0.08 mg/mL]	YES	colorimetry
3	Yao Lu et al. (2009)	Three different brands of NC membrane (0.45 µm pore size)	polystyrene patterning by rapid prototyping technique	gold nanoparticle-labeled immunoassay of anti-human IgG and human IgG (hIgG) [1ng/mL-10 ug/mL]	YES	colorimetry and fluorescence
4	Sarah J. Vella et al. (2012)	Whatman chromatography paper 1	wax ink covered with plastic sheath (lamination sheets)	alkaline phosphatase, ALP [15 U/L]; aspartate aminotransferase, AST [44 U/L], in blood; Protein [7 g/L]	NO	colorimetry
5	XY Liu et al. (2011)	3D-uPADs with choromatography paper; allows for strip that can be slid for access to other channels	wax printing for channels; test strip patterened with photolithography	rabbit IgG [330 pM]	NO	colorimetry
6	W Dungchai et al. (2010)	Whatman chromatography paper 1	photolithography	glucose [0.5 mM], lactate [1 mM], and uric acid [0.1 mM]detection	YES	colorimetry
7	GG Lewis et al. (2012)	digital assay made by stacking wax patterned paper and tape	wax patterning	hydrogen peroxide [10 mM]	-	colorimetry
8	Xu Li et. al. (2010)	Grade 4 Whatman filter paper	ink jet printing	NO2- [156-2500 uM] and uric acid [0-1600 uM]	YES	colorimetry
9	Fenton et al. (2008)	Whatman chromatography paper 1, nitrocellulose membranes, vinyl cover	computer controlled knife cutter	glucose [75 mM] and albumin [1.5 uM]	YES	colorimetry
10	Scott A. Klasner et al. (2010)	Fisherbrand qualitative filter paper	wax printing	glucose [3-50 mM], acetoacetate in artificial urine, nitrite in artificial saliva [25-250 uM]	YES	colorimetry
11	AK Ellerbee et al. (2009)	Whatman chromatography paper 1, sensitivity vary with thicknesses	photolithography	BSA [0-40uM] in artificial urine	YES	colorimetry
12	Jacqui L. Delaney et al. (2011)	Whatman chromatography paper 4; screen-printed electrode laminated on	inkjet printing using Alkenyl ketene dimer (AKD)-heptane solution (2% v/v); baked	2-(dibutylamino)-ethanol (DBAE) [0.9uM] & nicotinamide adenine dinucleotide (NADH) [72uM]	YES	ECL
13	Jacqui L. Delaney et al. (2013)	Grade 4 Whatman filter paper	lamination	2-(dibutylamino)ethanol (DBAE) [100 uM]; proline [100uM - 10mM]	NO	ECL
14	Z Nie et al. (2010)	Whatman chromatography paper 1 with Ag/AgCl ink electrodes	photolithography or wax printing	glucose [0.22 mM] in artificial urine	-	ECL
15	Kei Ge et al. (2012)	Whatman chromatography paper 1 with screen printed electrodes	wax printing	human AFP [0.15 ng/mL], CA125 [0.6 U/mL], CA199 [0.17 U/mL]and CEA [0.5 ng/mL]	YES	ECL
16	J. Yu et al. (2011)	Whatman chromatography paper 1 channels, covered with tape	XY knife plotter	glucose [2mM] and uric acid [47 mM]	YES	CL
17	KS Lok et al. (2012)	Continuous flow PMMA substrate	CO_2 laser system for design transfer	1 pg/mL to 0.1 mg/mL of cobalt (II) in vitamin B_{12}	NO	CL
18	X. Chen et al. (2013)	PDMS	lithography	1.0×10^{-10} to 1.0×10^{-3} mol/L of cobalt (II) ions	NO	CL
19	Wen-Bin Lee et al. (2011)	PDMS	PMMA mold	C-reactive protein [10, 2.5, 0.625, 0.125, 0.0625, 0.0125 mg/L]	NO	CL
20	S. Casolari et al. (2013)	Mylar	Precision cutter	Blood ALP activity	NO	CL

S2. Optimization experiments

The protocol optimization experiments were conducted in confined paper reaction sites as in **Fig. S2**. The dimensions of the smartphone accessory was designed after a series of preliminary experiments obtained using a light sealed box and smartphone.

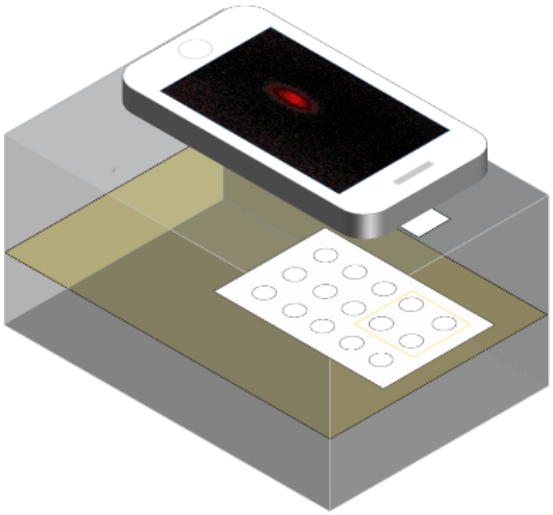
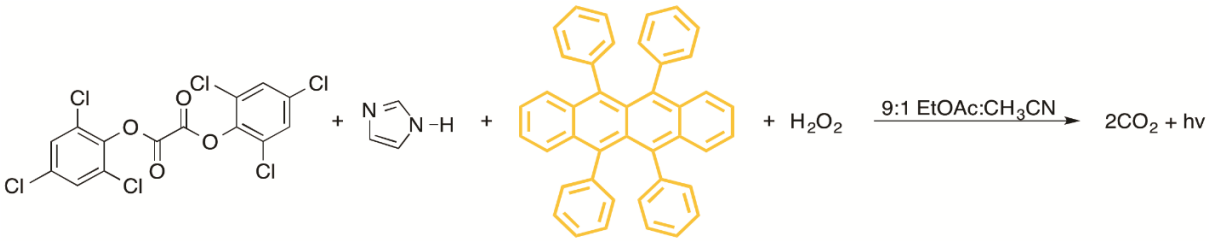


Fig. S2: Light sealed box for conducting optimization experiments in confined paper reaction sites. The reactions were recorded using a smartphone attached to the top of the box.

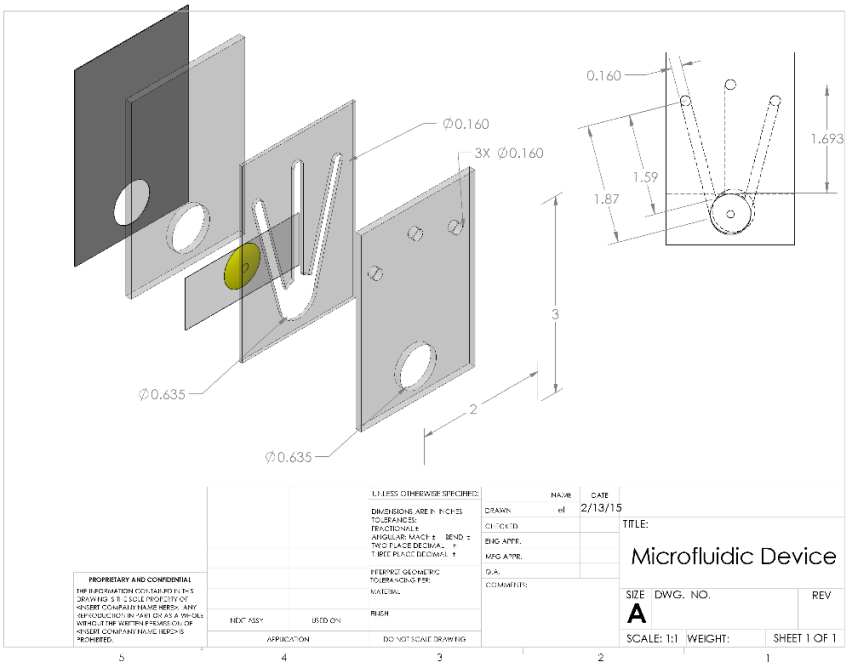
As a first step, 10 µL of 7 mM rubrene (5,6,11,12-tetraphenyltetracene) was added to a mixing chamber immediately followed by 10 µL of 60mM imidazole (1,3-Diaza-2,4-cyclopentadiene**)**. Next, 10 µL TCPO (bis(2,4,6-trichlorophenyl) oxalate), was inserted and allowed to settle for 1 minute. Each reagent is in solution with a 9:1 mixture of ethyl acetate and acetonitrile.



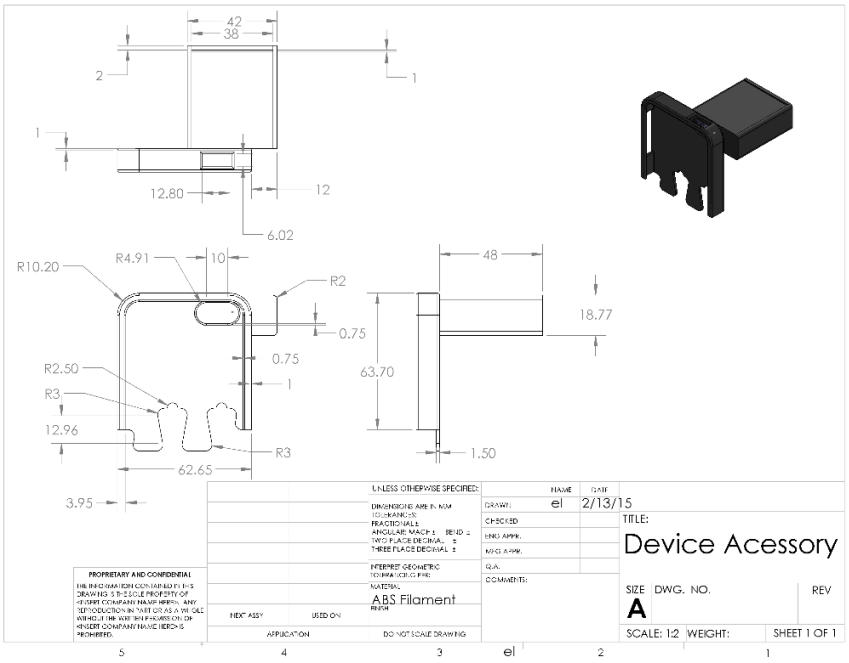
Video recording of the whole reaction is initiated just before H_2O_2 addition step. Experiments were conducted for the following H_2O_2 concentrations and 10 mM TCPO: 100 nM, 250 nM, 500 nM, 750 nM, 1 µM, 10 µM, 50 µM, 100 µM and 1 mM. Additional experiments were conducted with 15 mM TCPO with 100, 250, 500, 750 nM, and 1 µM H_2O_2 concentrations. Experiments were verified with Olympus IX73 inverted research microscope configured with RTC real time controller and Hamamatsu Orca Flash 4.0 SCMOS digital camera in a dark room environment.

S3. SMARTPHONE ACCESSORY DESIGN

The device can be made compatible with a custom-made light sealed accessory. This accessory was designed with SOLIDWORKS^{TM} and 3D printed with black ABS filament in a Stratasys uPrint SE Plus 3D printer (Stratasys Ltd., USA). The accessory is comprised of a black box (48 mm x 42 mm x 18.77 mm) attached to a custom made iPhone 5 case. The box has a light sealing slit (1 mm x 38 mm) on the top where the microfluidic device can be inserted. A hole was made in the phone case to allow the camera to view the reaction site. More details of the microfluidic device and smartphone accessory design is detailed in **Fig. S3a and Fig. S3b**, respectively**.** Digital images of the snap shots were converted to 8-bit grayscale and the mean pixel value at the reaction site of the device was analyzed and correlated with the concentration of the analyte.



(a)



(b)

Fig. S3: (a) Paper-plastic microfluidic device, (b) **S**martphone accessory design for video capture of chemiluminescent reaction

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