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Supporting Information

Miniaturized Biosensors to Preserve and Monitor Cultural Heritage: from Medical to Conservation Diagnosis

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1. Experimental Procedures

1.1 Reagents

The polyclonal anti-collagen type I rabbit antibody and the unconjugated polyclonal anti-rabbit goat antibody have been purchased from AbCam (Cambridge, UK). Polyclonal anti-ovalbumin rabbit antibody, horseradish peroxidase (HRP)-labelled polyclonal anti-rabbit goat antibody, polyclonal goat anti-HRP antibody, blocking agent bovine serum albumin, ovalbumin, porcine gelatin (type A, from porcine skin), and gold (III) chloride trihydrate (ACS reagent) have obtained from Sigma-Aldrich Co (St. Louis, MO). The chemiluminescence (CL) detection reagent Supersignal ELISA Femto has been purchased from Thermo Scientific Inc. (Rockford, IL). The other reagents were of analytical grade and were employed as received. Phosphate buffered saline (PBS) was prepared as follows: 10 mmol L^{-1} Na_2HPO_4, 2 mmol L^{-1} KH_2PO_4, 137 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, pH 7.4

Rabbit skin glue for arts, Gypsum ($CaSO_4 \cdot 2H_2O$) and inorganic pigments: blue smalt (potassium glass containing cobalt oxide), red ochre (Fe_2O_3) and Minium (Pb_3O_4) have been purchased from Zecchi (Florence, Italy).

1.2 LFIA strips

Strips have been prepared from nitrocellulose membranes (Hi-flow plus 180 membrane cards from Millipore, Billerica, MA) employing a XYZ3050 platform (Biodot, Irvine, CA) as previously described. [1] Briefly, from bottom to top of the strip, collagen from porcine skin and ovalbumin have been dispended to form the two test lines (T-lines). Different amounts of the proteins have been used for the colorimetric and the CL devices. In particular, 0.6 g L^{-1} of collagen and 0.015 g L^{-1} of ovalbumin have been found to be optimal for CL detection while 0.1 g L^{-1} and 0.4 g L^{-1} of collagen and ovalbumin, respectively have been selected for the fabrication of the colorimetric device. The control line (C-line) has been formed by depositing the polyclonal anti-rabbit goat antibody (0.25 g L^{-1}) or by the goat anti-HRP antibody (diluted 1:1000 v/v) for the colorimetric and the CL devices, respectively. Reagents have been deposited at the density of 1 µL cm^{-1}, keeping a distance of 4 mm among the lines, followed by drying at 37°C for 60 minutes under vacuum. For reducing background signal in the CL device, the nitrocellulose membrane was saturated with 1% BSA (w/v) in PBS buffer (5 min at room temperature), washed with PBS added of 0.05% of Tween 20 and finally dried at 37°C for 60 min under vacuum.[2] Red-coloured gold nanoparticles (AuNPs) were prepared by tetrachloauric acid reduction as described in [1]. AuNPs have been conjugated to anti-collagen and anti-ovalbumin antibodies by passive absorption.[1] In details, 10 µL of the antibody solution have been mixed with 1 mL of AuNPs and 0.1 mL of borate buffer (sodium borate-boric acid 20 mM pH 8.0). After a 30-min incubation at 37° C, the free AuNP surface was saturated by adding 0.1 mL of 1% BSA (w/v) in borate buffer. AuNP-labelled antibodies have been obtained by centrifugation (15 min at 10000 x g) and washed twice with borate buffer added of 0.1% BSA. The two conjugates (AuNP-anti-collagen antibody and AuNP-anti-ovalbumin antibody) have been mixed and diluted with borate buffer supplemented with 1% BSA, 0.25% Tween 20, 2% sucrose, and 0.02% sodium azide (conjugate buffer) to reach a final optical density of 1 and 2 for labelled anti-ovalbumin and labelled anti-collagen antibodies, respectively. The mix of AuNP-labelled antibodies has been dispensed onto a glass fibre conjugate pad at a flow rate of 8 µL cm^{-1} and dried at room temperature for at least 2 h. The conjugate pad was treated with the conjugate buffer and dried at 60° C for 60 min before application of the AuNP-labelled antibodies.

Strips for the colorimetric device have been fabricated by layering a cellulose sample pad, a glass fibre conjugate pad, the nitrocellulose membrane and a cellulose pad as the adsorbent pad (Millipore, Billerica, MA) cut into sections (5 mm width by means of a CM4000 guillotine, Biodot, Irvine, CA) and inserted into plastic cassettes (Kinbio, China) equipped with a sample well and a reading window. Strips for the CL system have been fabricated as above, just omitting the conjugate pad and the plastic cassette.

1.3 The CL analytical cartridge and procedure of use

The fluidic element has been obtained by sandwiching a laser micro machined channel network, made of adhesive polypropylene (thickness approximately 190 µm) between two polypropylene layers (200 µm thick). The upper polypropylene layer presented embossed reservoirs for reagent pouches, LFIA strip and PDMS-filled valves, while the smooth lower layer enclosed the cartridge. As shown in Figure S1a the LFIA fluidics cartridge included the following components:

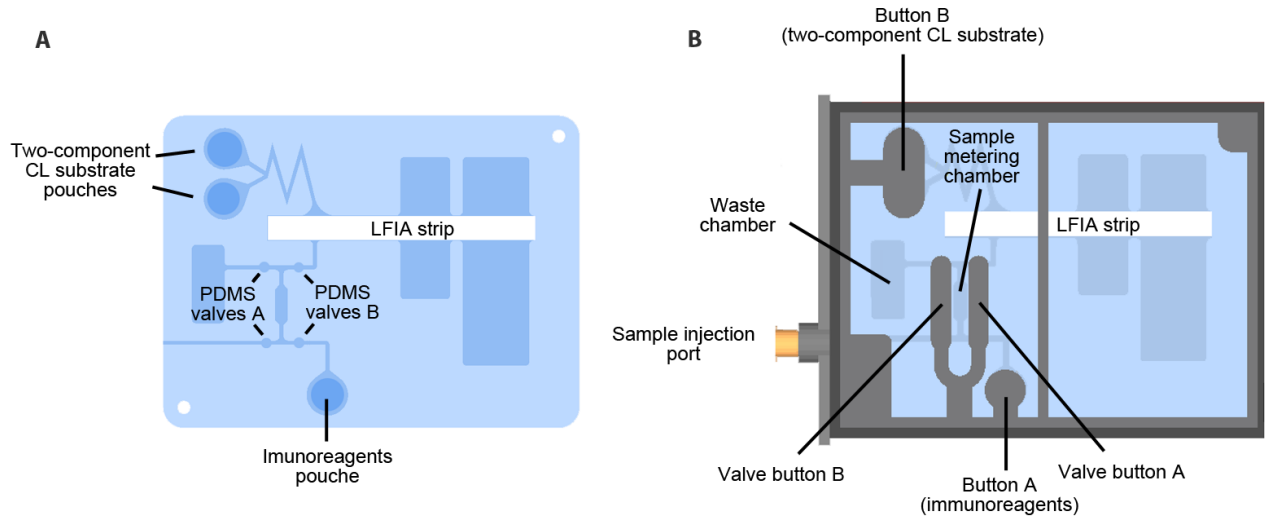
- Sample Injection Port
- Sample Metering Chamber of a defined volume (35 µL)
- Sample Waste that contains the sample overfull upon Sample Metering Chamber filling
- Cavity that perfectly fits the LFIA Strip (Strip placement)
- 4 PDMS valves for regulating the sample and immunoreagents solution flows that are actuated by direct pressure (finger pressure provides sufficient force to tightly close the valve)
- 3 Pouches that contain reagents in the liquid form:
  Immunoreagents pouches: 30-µL reservoir for the immunoreagents solution (PBS containing 3% (w/v) BSA, anti-ovalbumin antibody (diluted 1: 500, v/v), anti-collagen antibody (diluted 1:2000, v/v) and HRP-labelled anti-rabbit antibody (diluted 1:5000, v/v)
  Two-component CL substrate pouches: two 40-µL reservoirs for the two components of the SuperSignal ELISA Femto HRP CL cocktail.
- Micro channels that suitably connect the sample injection port, the sample metering chamber, the sample waste, reagents pouches and the LFIA Strip sample pad.
- A rectangular cavity that holds an adsorbent pad (additional to that present on the LFIA Strip) to further promote flow of reagents along the LFIA Strip.

The LFIA Device Holder (Figure S1b) has been designed using a freely available CAD program (SketchUp Make 2014, Trimble Navigation Limited) and realized in opaque acrylonitrile butadiene styrene (ABS) polymer by Fused Deposition Modelling (FDM) 3D printing technology using a Makerbot Replicator 2X printer (Makerbot Industries).
The LFIA Device ABS Holder basically consists of two separate parts:

a) A lower part with an aperture for accommodating the upper side of the CCD faceplate, which will be in direct contact with the LFIA fluidic device during the CL measurement and a cavity that fits and holds the Unidirectional Valve
b) An upper part that fits with the lower one and that houses buttons (Button A and B located above conjugate pouches and CL substrate pouches, respectively) and Valve buttons (Valve button A in correspondence of two elastomeric valves on the right and Valve button B in correspondence of two elastomeric valves on the left).

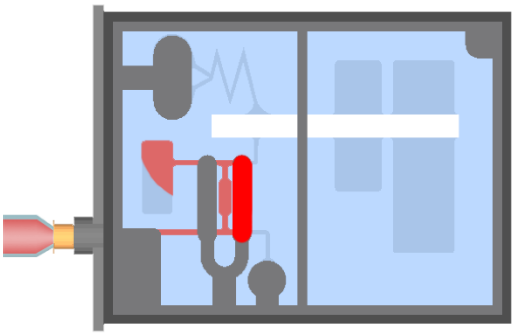
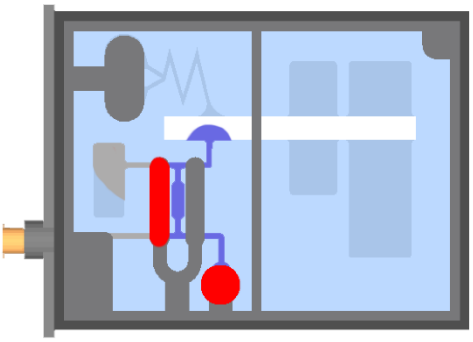
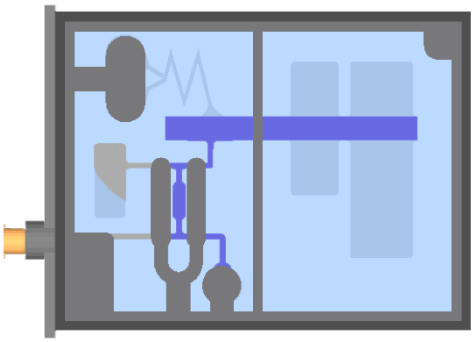
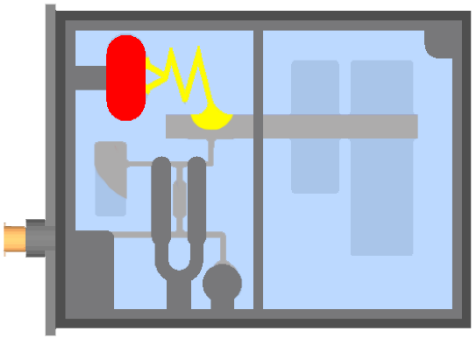
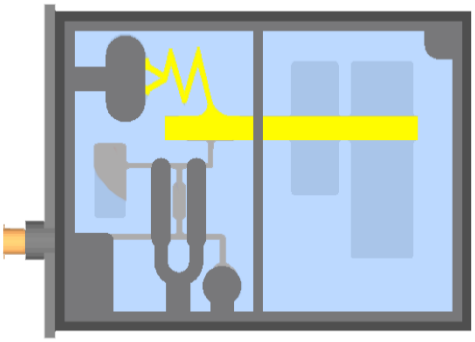
For assembling the LFIA Cartridge, the LFIA fluidics cartridge is sandwiched between the Upper and Lower part of the LFIA ABS Holder, which are then glued together.
Details on the procedure of use are described in Scheme S1.

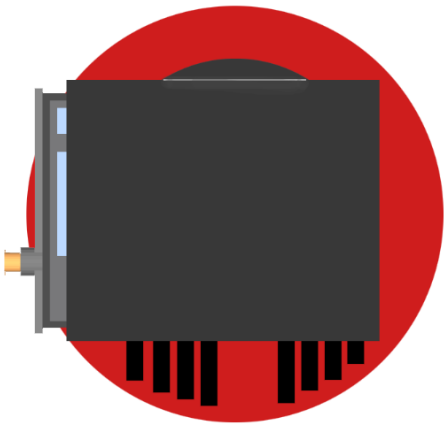
Figure S1. Scheme of the CL analytical cartridge: a) The fluidic element; b) The device holder.



SUPPORTING INFORMATION

Scheme S1. Scheme of the procedure of use of the analytical cartridge.

	2a) Press valve button A 2b) Inject sample using a syringe until the metering chamber is filled 2c) Release valve button A
	3a) Press valve button B 3b) Push button A to transfer sample and conjugate to the LFIA strip 3c) Release valve button B
	4) Incubation (30 min)
	5) Push button B to transfer the two-component CL substrate to the LFIA strip
	6) Incubation (15min)

	7) Insert cartridge into the CL reader
	8) Acquire image
	9) Using ImageJ software v.1.46 (National Institutes of Health, Bethesda, MD) measure the mean photon emission in the areas corresponding to C-line and T-lines and in the areas below and above each line (background signal).
	10) Insert the data on the spreadsheet to obtain the concentration of the target analytes.

1.4 Instrumentation

Chemiluminescent measurements were performed using an ultrasensitive charge coupled device (CCD) camera (MZ-2PRO from MagZero, Pordenone, Italy, equipped with a Sony ICX285 sensor), coupled with a round fiber optic taper (25/11 mm size, Edmund Optics, Barrington, NJ) placed in contact with the CCD sensor.

Calibration curves for ovalbumin and collagen have been obtained employing standard solutions prepared by adding ovalbumin and collagen to the desired concentration, comprised between 0 (blank) and 100 µg mL^{-1}. To obtain quantitative information, the mean photon emission was measured in the areas corresponding to C-line and T-lines of the LFIA strip and each was subtracted of the mean background signal measured in two adjacent areas below and above the line. The T-line/C-line ratio was calculated for each analyte and then converted into B/B_0 ratio by dividing it for the T-line/C-line ratio measured in the absence of the target analyte (B_0, i.e., maximum T-line/C-line value). Calibration curves were obtained by plotting B/B_0 values against the log of analyte concentration and obtaining a sigmoidal curve fitted with a four-parameter logistic equation. To obtain the analyte concentration in real samples, B/B_0 value has been calculated as described above and interpolated on a stored calibration curve, employing a suitable spreadsheet.

1.5 Standard paint reconstructions and historical samples

Standard samples have been prepared according ancient painting treatises.[3] Briefly, the preparation layer was made with gypsum (190 g) and rabbit glue (10 g dissolved in 150 mL of hot water). Pigment layers, obtained by mixing inorganic pigments and rabbit glue or egg, have been applied over the gypsum ground. Whole egg-tempera has been prepared by mixing egg white, yolk, and water in a 1:1:1 (v/v) ratio.

The ratio between pigment and binder has been varied according to the nature of the materials, to obtain a mixture suitable for the application as layer. Historical paint samples, submitted to the analysis, have been collected from easel and mural paintings. The first case of study was a painted wood panel by Baldassare Carrari (1460–1516), an Italian painter of the Renaissance period, representing *the Virgin with the Holy baby and Saints* and exhibited in the city museum of Ravenna, Italy. In addition, a wall painting attributed to Giuseppe Milani (1719–1798) and located in the dome of the Abbey of S. Maria del Monte, Cesena (Italy), dated to 1774 has been also investigated.

Samples have been submitted to the extraction procedure by suspending about 0.5 mg of powder in 200 µL of PBS and sonicating the suspension for 30 min. The suspension has been let to settle down and 60 µL of supernatant were collected for the analysis.

2. References

[1] F. Di Nardo, C. Baggiani, C. Giovannoli, G. Spano, L. Anfossi *Microchim. Acta* **2017**, 184:1295–1304

[2] M. Zangheri, F. Di Nardo, L. Anfossi, C. Giovannoli, C. Baggiani, A. Roda, M. Mirasoli *Analyst* **2015** 140:358-65

[3] Cennini C, Frezzato F, *Il libro dell'arte*, Neri Pozza, Milano, **2004**.