## **On-fiber chemiluminescence biosensing system**

Cosimo Trono<sup>a</sup>, Sara Tombelli<sup>\*a</sup>, Simone Berneschi<sup>a</sup>, Ambra Giannetti<sup>a</sup>, Francesco Baldini<sup>a</sup>, Donato Calabria<sup>b</sup>, Massimo Guardigli<sup>b</sup>, Mara Mirasoli<sup>b</sup>

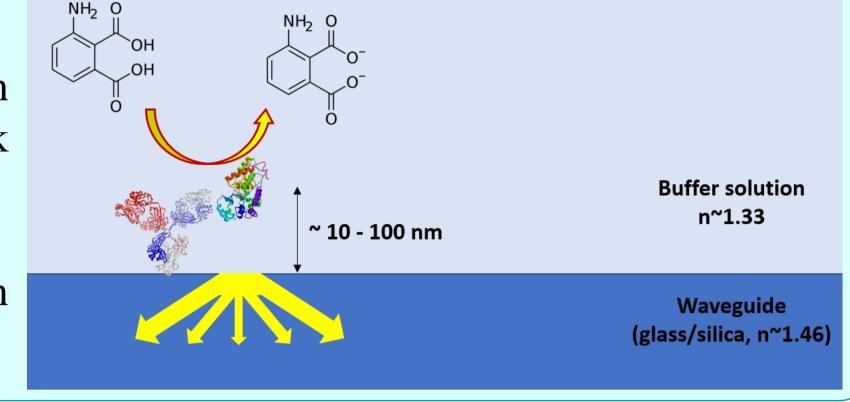
<sup>a</sup>Istituto di Fisica Applicata Nello Carrara – CNR, Sesto Fiorentino, Italy <sup>b</sup>Dipartimento di Chimica ''Giacomo Ciamician'', Università di Bologna, Bologna, Italy

AIM. The aim of the present work is the preliminary investigation of optical fiber-based platforms for the optimal implementation of chemiluminescence-based biosensing.

**THE IDEA**. The common approach in the implementation of a chemiluminescence-based detection system is to have the chemiluminescence reaction and consequent emission of photons from bulk solution inside an assay vial, and to collect the light by "looking at" the vial content.

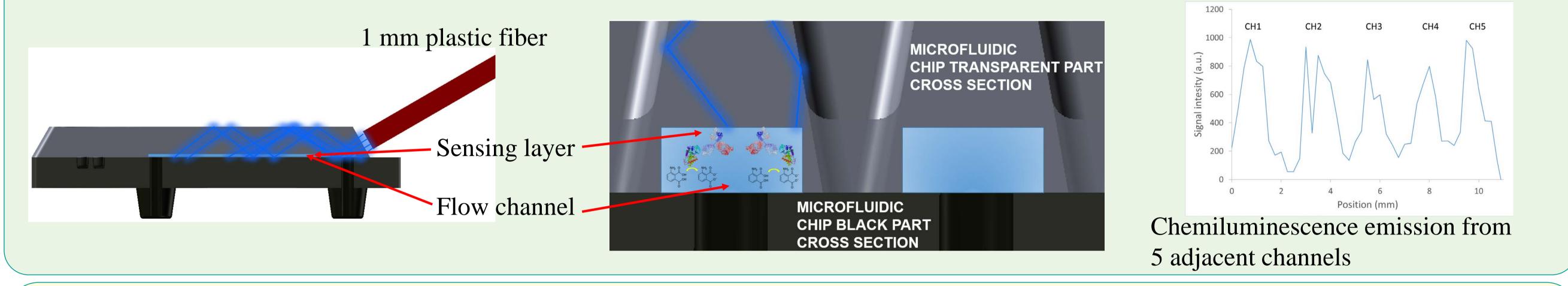
The main idea of this work is to "force" the chemiluminescence reaction in the close proximity of an optical waveguide that safely contains and guides the generated light up to the detector.

**APPROACH 1**. Optical platform, originally designed for fluorescence measurements, adapted for chemiluminescence detection. The core of the platform is a  $NH_2$  functionalized microfluidic chip in Topas with 13-microchannels (50 µm high, 600 µm width, 10 mm long). Implemented assay for preliminary measurements: immobilization of ovalbumin (100 µg/mL) onto 5 adjacent channels via carbodiimide chemistry, binding to anti-ovalbumin-HRP (1:2000), injection of chemiluminescence mix.

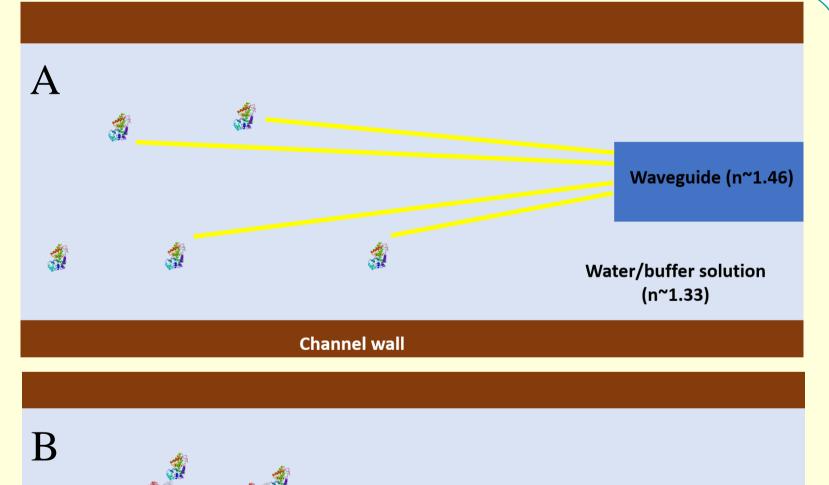








**APPROACH 2.** The challenging idea is to make the reaction occur directly on (very close to) the external lateral surface of the fiber core. The radiation emission of a dipole in the proximity of a dielectric interface (water environment – fiber silica/glass/plastic core) happens mainly in the material with higher refractive index (RI), the fiber core, and with a slope that allows the guiding by total internal reflection. The optical fiber cannot be used as it is but must be strongly modified to avoid the loss of optical signal: the core must be exposed in the reaction region, the numerical aperture of the fiber must be the higher as possible, possibly the cladding and coating must be removed in the whole length of the fiber. The aim of these preliminary measurements is the evaluation of the possibility of realizing an optical fiber chemiluminescence biosensing system in which the reaction occurs very close to the external lateral surface of an optical fiber core. Preliminary data were obtained by a modified multimode fiber inserted in a capillary used to monitor a chemiluminescence signal generated in solution (A) or after an ovalbumin immunoassay onto the fiber surface (B). Implemented assay for preliminary measurements: immobilization of ovalbumin (100 µg/mL) onto a COOH-polymer coated fiber via carbodiimide chemistry, binding to anti-ovalbumin-HRP (1:2000), injection of chemiluminescence mix.

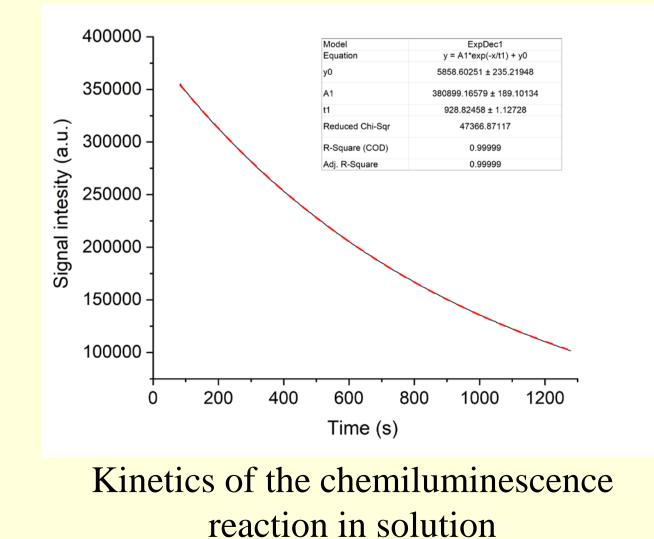


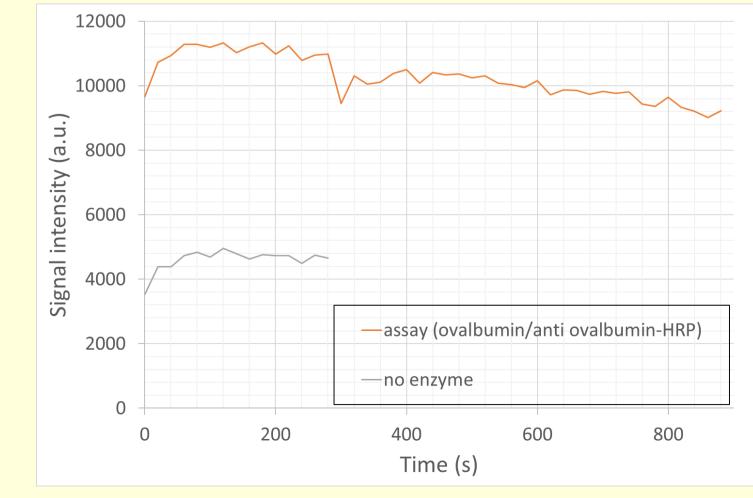


**Channel wall** 

12000 11000 10000 9000 8000 7000 600 180 300 420 540 660 780 900 1020 1140 Time (s)

Chemiluminescence mix + HRP in solution





Ovalbumin assay onto the fiber surface





Acknowledgments. This work was supported by the Italian Ministry for University and Research in the framework of "Bando PRIN 2017", project number 2017YER72K