

---

# Luminescent biosensors and optical microscopy: an innovative integrated approach for the study of materials in cultural heritage

Martina Zangheri\*<sup>†1</sup>, Giorgia Sciutto<sup>1</sup>, Massimo Guardigli<sup>1</sup>, Mara Mirasoli<sup>1</sup>, Laura Anfossi<sup>2</sup>, Fabio Di Nardo<sup>2</sup>, Silvia Prati<sup>1</sup>, Claudio Baggiani<sup>2</sup>, Rocco Mazzeo<sup>1</sup>, and Aldo Roda<sup>1</sup>

<sup>1</sup>Department of Chemistry “Giacomo Ciamician”, Alma Mater Studiorum, Università di Bologna, Bologna, Italy – Italy

<sup>2</sup>Department of Chemistry, University of Turin – Italy

## Abstract

The characterization of materials composing a painting is essential for authentication purposes, assessment of the conservation state and for establishing the appropriate restoration strategy. Among the pictorial components, protein and organic substances are particularly widespread since materials like eggs, milk and animal glues were used as binders for pigments or as protective varnishes. Immunological methods, which allow identifying the proteins and their biological origin, represent a powerful approach for the identification of these components as an alternative to the traditional analytical-instrumental methods. Immunochemical imaging techniques, widely used in the field of clinical chemistry and histology to localize antigens within tissues and cells, have been applied to pictorial samples to identify and simultaneously locate different analytes through a single analysis. Herein, we present our results employing chemiluminescence (CL) microscopy imaging, demonstrating high diagnostic potential in the analysis of pictorial samples. To enable prompt interventions, a challenging task is the development of portable and easy-to-use biosensors that can be used in situ by restorers using minimum quantities of samples. We report about the development of portable and ultrasensitive biosensors for the detection of protein binders, based on a simple pre-treatment of the sample followed by a non-competitive immunoassay which is carried out exploiting CL-based Lateral Flow Immunoassay (CL-LFIA) technique. The CL detection is performed using a thermally-cooled CCD camera. In particular, the developed method allowed the simultaneous identification of two proteins, ovalbumin and collagen. The procedure involved the use of a disposable cartridge containing the nitrocellulose membrane, the reagents necessary for the immunoassay, valves and microfluidics for flow activation. This biosensor was used for the analysis of samples from different paintings (fresco, oil painting and tempera), showing the great potentialities of the proposed integrated approach.

**Keywords:** biosensor, optical microscopy, cultural heritage, chemiluminescent imaging, immunoassay, ELISA

---

\*Speaker

<sup>†</sup>Corresponding author: [martina.zangheri2@unibo.it](mailto:martina.zangheri2@unibo.it)