

Beyond iSCAT Sensitivity: Mechanistic Control of Photodamage in Label-Free Biosensing

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Summary:

This work investigates photodamage in optical biosensors by examining antibody–antigen binding kinetics in label-free digital immunoassays. Blue light induces a rapid loss of bound analyte, while orange light provides a more stable response, highlighting strong wavelength dependence. Mechanistic studies identify immobilized antibodies as the most sensitive component, undergoing irreversible, ROS-mediated degradation under blue illumination. These results show that biosensor performance must be evaluated beyond sensitivity, and that shifting to longer wavelengths, such as orange, can mitigate photodamage while maintaining functionality.

Keywords: Digital immunoassays, interferometric scattering microscope (iSCAT), photodamage

Introduction

Ultrasensitive optical imaging based on interference provides a powerful route to probe nanoscale biological systems by converting small phase variations of an electromagnetic field into measurable intensity changes [1]. Interferometric scattering (iSCAT) microscopy has emerged as a particularly robust form of image-plane based inline holography that detects the interference between light scattered by a nanoparticle and a well-defined reference field, typically the back-reflection from a planar interface such as a glass/solution. This configuration enables detection of weak scattering signals, allowing quantitative and sensitive measurements down to single viruses or proteins. [2], [3].

Blue light has become a commonly used illumination source for label-free imaging due to better resolution, higher scattering crosssections, smaller depth of fields which lead to optimal signal-to-noise measurement regimes. Nevertheless, beyond sensitivity, photodamage induced by short-wavelength illumination poses a challenge, as it is a well-established concern in biological imaging, particularly in the context of live cells and organisms[4]. Yet its impact on surface-based biosensing platforms has remained largely unexplored. For instance, photo-induced chemical reactions and degradation of biological components, may affect the stability and functionality of sensing interfaces [5]. In response to these limitations, there has been a gradual shift in optical methods toward the use of longer wavelengths, such as red or near-infrared light,

in optical detection techniques including fluorescent labeled and interferometric scattering microscopy approaches without describing the underlying mechanisms of photodamage [6], [7].

In this context, one possible mechanism underlying such destructive effects is the generation of reactive oxygen species (ROS) under short-wavelength illumination [4], [5]. In confined environments such as closed microfluidic channels, locally produced ROS can accumulate and interact with both the sensing interface and the analyte. These species are known to induce oxidative damage to biological components, including lipid membranes, proteins, and capture probes such as antibodies, potentially altering their structure and binding capability.

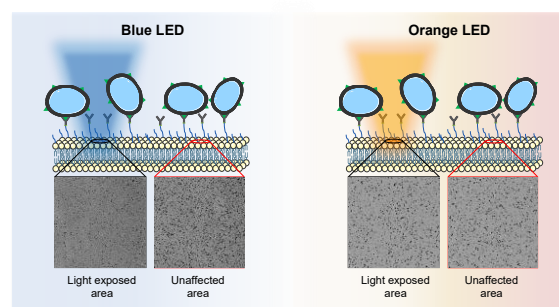


Fig. 1. Schematic representation and corresponding iSCAT images illustrating analyte detection in regions exposed to blue and orange illumination, compared to non-illuminated areas.

Within this study, the influence of blue LED illumination on the integrity of the surface chemistry

and sensing performance was systematically investigated. In parallel, alternative illumination conditions based on longer wavelengths, such as orange LED light, were explored to reduce photodamage while maintaining sufficient detection sensitivity.

Materials and Methods

Binding kinetics of biotin-functionalized liposomes, were investigated on a cell-mimicking supported lipid bilayer (SLB) to model an immunoassay-type sensing interface (Fig. 1). Two illumination sources were used to monitor analyte binding within the illuminated region. Specifically, short-wavelength blue LED light (455 nm) and longer-wavelength orange LED light (615 nm) were sequentially applied to the sensing area, while real-time measurements were performed using iSCAT microscopy.

To investigate the mechanism behind the reduced binding observed under blue light (Fig. 1), the stability of the main components of the sensing system was evaluated: (i) the SLB, (ii) the immobilized biorecognition element (anti-biotin antibodies), and (iii) the analyte (biotinylated liposomes). In addition, binding kinetics were measured at different light intensities to determine the operational range and safe monitoring time for both blue and orange LED illumination to identify dose-dependent effects and characteristic time scales of degradation. SLB integrity was assessed by evaluating surface passivation after light exposure. Since intact SLBs suppress non-specific adsorption, any light-induced disruption would be reflected as an increase in background binding. Antibody stability and functionality were probed by comparing analyte capture efficiency on antibody-functionalized regions that were either pre-exposed to light or kept as non-illuminated controls. Analyte stability was examined using biotinylated gold nanoparticles (AuNP) as a structurally robust sample. Additionally, AuNPs were introduced as local ROS scavengers to probe the contribution of ROS to the observed reduction [8].

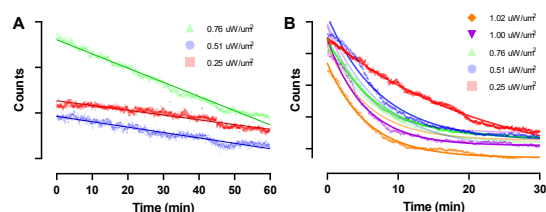


Fig. 2. Real time monitoring of liposome binding kinetics under (A) orange LED for 60 minutes and (B) blue LED for 30 minutes were depicted.

Results

A clear dose-dependent response to illumination intensity was observed for both blue and orange

light sources (Fig. 2). Under orange illumination, the assay maintained >80% binding efficiency after one hour, indicating stable sensing conditions. In contrast, blue illumination led to a rapid and pronounced decrease in particle counts. Even at low power densities, an initial loss of bound analyte was observed, followed by a plateau phase where a fraction of particles remained on the surface. Importantly, this effect occurred at power densities significantly lower than those typically used and reported in iSCAT and related interferometric techniques, suggesting that blue light can perturb the sensing system under relatively mild conditions. This limits its suitability for long-term kinetic measurements and indicates it may be more appropriate for single measurement endpoint-type assays.

Mechanistic studies indicated that the immobilized antibodies were the most affected component, as reduced binding was observed for both liposome and gold nanoparticle analytes on pre-exposed areas. The damage to antibodies was irreversible; however, the presence of local ROS scavengers reduced the extent of degradation when binding occurred prior to light exposure, supporting the role of ROS in this process.

References

- [1] R. W. Taylor and V. Sandoghkar, "Interferometric Scattering Microscopy: Seeing Single Nanoparticles and Molecules via Rayleigh Scattering," *Nano Lett.*, vol. 19, no. 8, pp. 4827–4835, Aug. 2019, doi: 10.1021/acs.nanolett.9b01822.
- [2] P. Kukura, H. Ewers, C. Müller, A. Renn, A. Helenius, and V. Sandoghkar, "High-speed nanoscopic tracking of the position and orientation of a single virus," *Nat. Methods*, vol. 6, no. 12, pp. 923–927, Dec. 2009, doi: 10.1038/nmeth.1395.
- [3] J. Ortega Arroyo *et al.*, "Label-Free, All-Optical Detection, Imaging, and Tracking of a Single Protein," *Nano Lett.*, vol. 14, no. 4, pp. 2065–2070, Apr. 2014, doi: 10.1021/nl500234t.
- [4] Y. Nakashima, S. Ohta, and A. M. Wolf, "Blue light-induced oxidative stress in live skin," *Free Radic. Biol. Med.*, vol. 108, pp. 300–310, Jul. 2017, doi: 10.1016/j.freeradbiomed.2017.03.010.
- [5] E. Kim, S. Kim, M. Kim, and D. Min, "Photooxidative molecular damage under blue light," *Exp. Mol. Med.*, pp. 1–18, Jan. 2026, doi: 10.1038/s12276-025-01609-8.
- [6] A. L. Antaris *et al.*, "A small-molecule dye for NIR-II imaging," *Nat. Mater.*, vol. 15, no. 2, pp. 235–242, Feb. 2016, doi: 10.1038/nmat4476.
- [7] D. Loewenthal *et al.*, "Continuous mass photometry by single molecule trapping," Feb. 03, 2026, *bioRxiv*. doi: 10.64898/2026.02.02.703204.
- [8] J. Kim, H. Park, G. Saravanakumar, and W. J. Kim, "Polymer/Aptamer-Integrated Gold Nanoconstruct Suppresses the Inflammatory Process by Scavenging ROS and Capturing Pro-inflammatory Cytokine TNF- α ," *ACS Appl. Mater. Interfaces*, vol. 13, no. 8, pp. 9390–9401, Mar. 2021, doi: 10.1021/acsami.0c15727.