Patterning of antibody-coated surfaces using energetic helium ions

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In this work, we expose antibody-coated surfaces to a collimated helium ion beam to form biologically active patterns with high spatial resolution that are part of a retroreflector biosensor platform. The retro-reflecting structures consist of two mutually orthogonal reflective surfaces that appear very bright when imaged with a microscope because they return incident light directly back to the imaging system. The intensity of the reflected light is attenuated by the analyte-responsive biochemically driven assembly of micro- and nanoparticles or products of enzymatic reactions on the surface of the reflectors. The patterning of these structures by an ion beam ensures that only a subset of the reflectors is analyte-responsive, with the others being kept as references of constant brightness.

Figure 1 shows a top-down electron microscope image of four 4 x 8 x 5 micrometer linear retroreflectors, arranged so that three of the structures are placed in a row and the fourth is in front of the central reflector. All of the reflectors are coated with gold and decorated with antibodies to Norwalk virus particles through gold-thiol chemistry. The samples are then exposed to a 3 kV beam of helium ions at an angle such that the front retroreflector shadows the area in front of the middle retroreflector from the beam. In this way, the antibodies that are part of the central reflector are not damaged and form the biosensing element. The sample is dipped into a solution of BSA, which passivates the beam-exposed regions of the sample, leaving behind three always-bright reference reflectors.

When the sensor is placed into a solution containing Norwalk virus-like particles (NVLPs), the particles are captured on the surface by the antibodies. After rinsing, the sample is exposed to a solution containing 40nm spherical gold nanoparticles conjugated with secondary antibodies to the virus, which bind to the captured NVLPs. The SEM image shows that the regions containing the gold nanoparticles are delineated from the passivated regions with very high spatial resolution, which is due to the excellent collimation of the ion beam. A similar observation was made when using enzyme molecules (alkaline phosphatase) with secondary antibodies to NVLPs in place of the gold nanoparticles. After the enzyme was exposed to its substrate (BCIP/NBT), the reaction product accumulated on the retroreflector surface only in the regions where the antibodies were still active, as shown in Figure 2. At the conference, we will describe our ion exposure process in more detail and summarize the conditions that result in selective surface activation and passivation.

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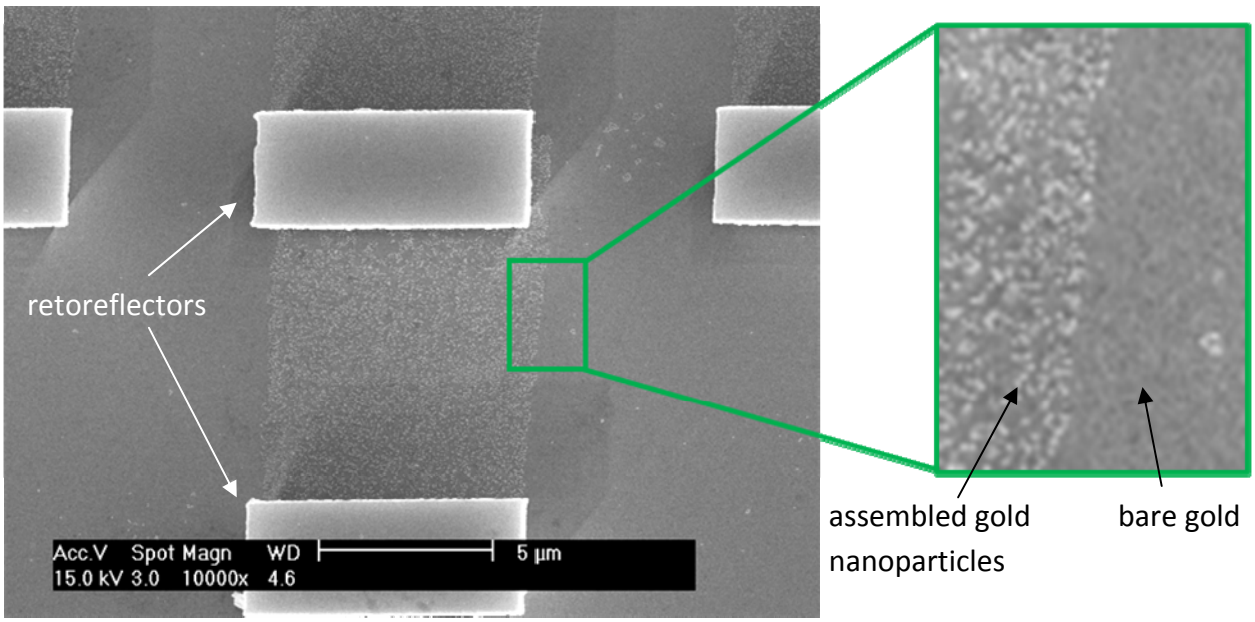

Figure 1: Scanning electron micrograph of gold nanoparticles assembling only in the regions that were shadowed by the retroreflector structures during the ion beam exposure. The zoomed-in image shows the high spatial resolution of the patterning step.

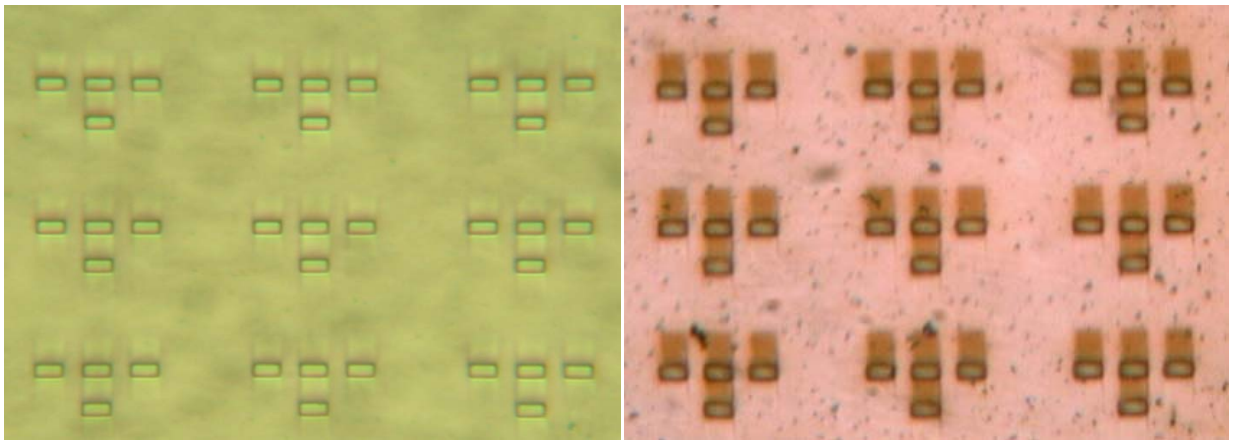

Figure 2: Optical microscope images of the retroreflectors before and after the enzymatic reaction: the enzyme product accumulates only in the shadowed regions where antibodies are not damaged by the ion beam.