A microretroreflector-based diagnostic platform

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We are developing a flexible platform for the detection of small quantities of analytes (e.g., virus particles, bacteria, DNA, RNA, etc.) that is based on the use of micron-scale retroreflectors. Such retroreflectors return incident radiation back to its source, making them extremely detectable and well-suited for inspection using low-cost optics. In this proposed system (see Figure 1, upper half), linear retroreflectors with two orthogonal and mutually touching mirrored surfaces are fabricated at specific locations on a substrate. (a) The retroreflectors are at this point all bright and easy to see. (b) For virus detection (as an example), the base of a retroreflector is decorated with antibodies to the virus, and, if present, the virus particles are captured by this surface. (c) After capture, gold nanoparticles, coated with a secondary antibody, are introduced into the system, (d) attach to the virus, and drastically reduce the retroreflector brightness. If no virus is present, the reflectivity is unaffected. The change in brightness is determined by comparing the intensity of the assay retroreflector to that of reference retroreflectors fabricated in close proximity.

We have fabricated micron-sized retroreflectors (see Figure 2A-B), confirmed that they are extremely detectable using a simple microscope (see Figure 2D), have decorated the retroreflectors with HyHEL-5 (an antibody that recognizes Hen Egg Lysozyme, HEL), have deactivated the antibodies everywhere except in front of a single assay reflector surrounded by reference reflectors, and have demonstrated the selective assembly of gold nanoparticles on the assay reflectors (see Figure 2C). We observe a reduction in the brightness of the assay reflector of about 10% when gold nanoparticles are present.

The inspection tool (see Figure 1 lower half) consists of a 12x magnification, broadband illumination microscope. Surface reflections are not collected by the optics; the retroreflectors direct the signal towards the optics (back to the source of the illumination) which is imaged using a standard CCD camera. An automated routine has been written that detects the groups of four retroreflectors (tetrads), of which three are reference reflectors and one is an active assay reflector, and is used to automatically calculate the intensity of the assay signal.

Other particles can be used to modulate the reflectivity of the assay reflector, including magnetic sample-prep beads and products of enzymatic reactions. This work is sponsored by the Western Regional Center of Excellence (NIH grant number U54 AI057156).

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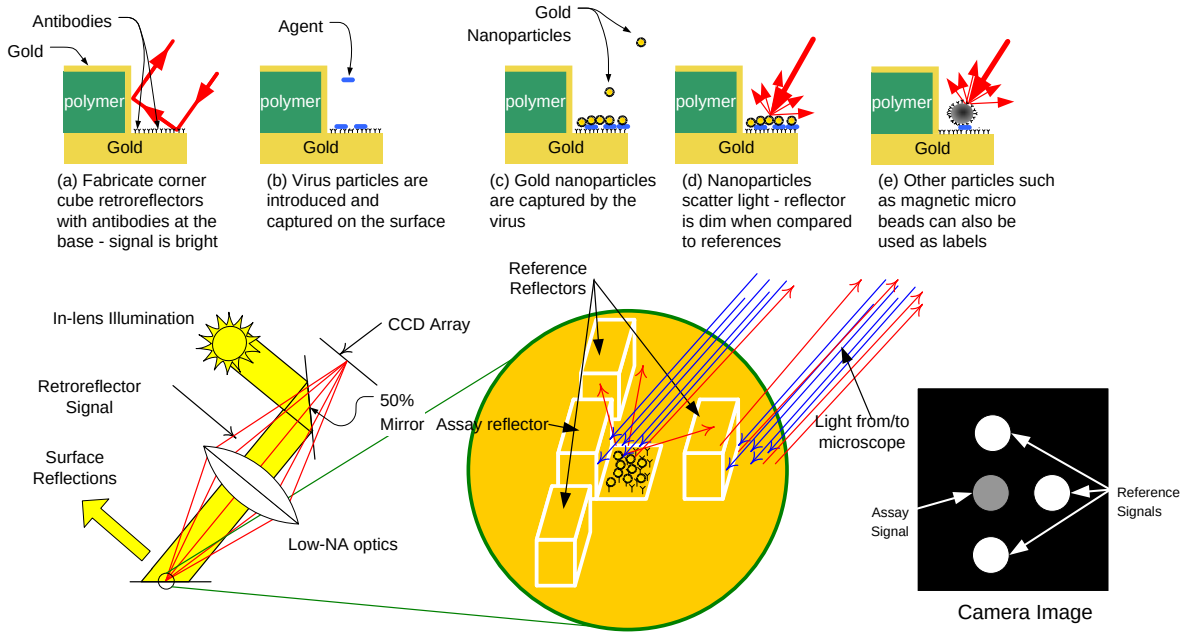

Figure 1: Retroreflector-based diagnostic platform.

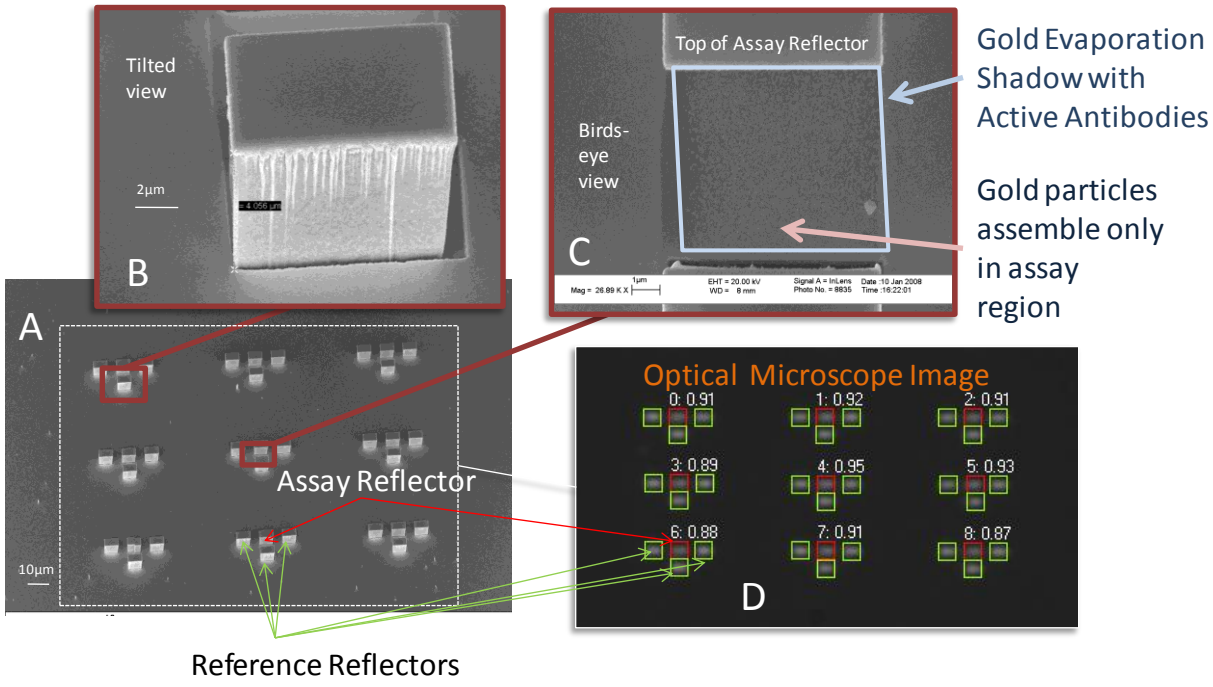

Figure 2: (A) shows an overview of the test area for one agent, where a set of nine retroreflector tetrads, consisting of three reference and one assay reflector, is imaged in one field-of-view of the CCD camera. (B) shows a close-up of a typical reflector and (C) shows a birds-eye (top down) view of the region between the front reference and the assay reflectors where gold nanoparticles have selectively assembled through HyHEL-5 binding to HEL. Only the region between the reflectors has antibodies, and assembly is not observed outside of this region. (D) shows the output from the image analysis software.