Nanoplex™ biotags: Near-IR Excited, Highly Multiplexed Nanoparticulate Optical Detection Tags for Diagnostic Assays

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ABSTRACT

Oxonica has developed a novel optical detection tag for use in point of care diagnostics, cellular imaging assays, and life science assays. Nanoplex™ biotags are silica coated Au nanoparticles that, by virtue of surface enhanced Raman scattering (SERS) active molecules at the glass-metal interface, act as detectable tags. Many unique biotags can be generated by varying the Raman active molecule adsorbed on the nanoparticle, thus allowing multiplexed detection to be performed. Importantly, because the biotags are excited in the far red or near-IR, and can be detected with low-cost instrumentation, they are ideal for performing assays in both tissue and whole blood. We will present data showing the versatility of this novel nanotechnology, and the applications to diagnostics.

Keywords: SERS, diagnostics, Raman, nanoparticles,

1 NANOPLEX™ BIOTAGS

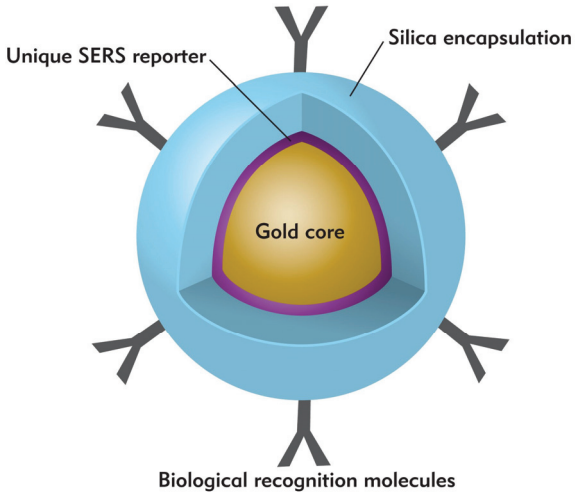


Figure 1: Cartoon depiction of a Nanoplex™ biotag

Nanoplex™ biotags comprise a 50-nm diameter Au nanoparticle core, an adsorbed layer of reporter molecules, and a 20-nm thick silica (glass) shell. The Au nanoparticle is SERS-active at 785 nm, an excitation wavelength where background interference from either absorbance or fluorescence in biological systems is minimal. The SERS spectrum is defined by the reporter molecule adsorbed to the Au surface. The silica coating locks in the reporter molecule and provides a surface on which biomolecules can be easily conjugated. A cartoon depiction of the biotag can be seen in Figure 1. By changing the reporter molecule adsorbed to the surface, many "flavors" of biotags can be synthesized. This allows multiplexed assays to be performed. We have developed over two dozen distinct biotags [1, 2].

2 ADVANTAGES OF NANOPLEX™ BIOTAGS

The biotags have a number of advantages for biological assays:

- The distinguishable spectra of the biotags allow for high levels of multiplexing, due to the narrow spectral peaks (1/50th of fluorescence) permitting the efficient use of bandwidth. In addition, all biotags are simultaneously excited with a single, variable wavelength.

- The near-IR excitation is compatible with whole blood detection, and other biological matrices. In fact, Nanoplex™ biotags can easily be detected in whole blood using 785 nm excitation, as shown in Figure 2.

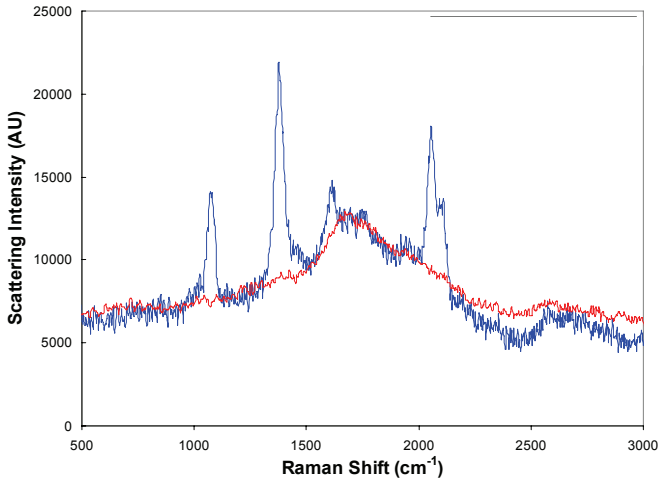


Figure 2: Red line shows Raman background of whole blood. Blue line shows Raman spectra of biotags in background of whole blood.

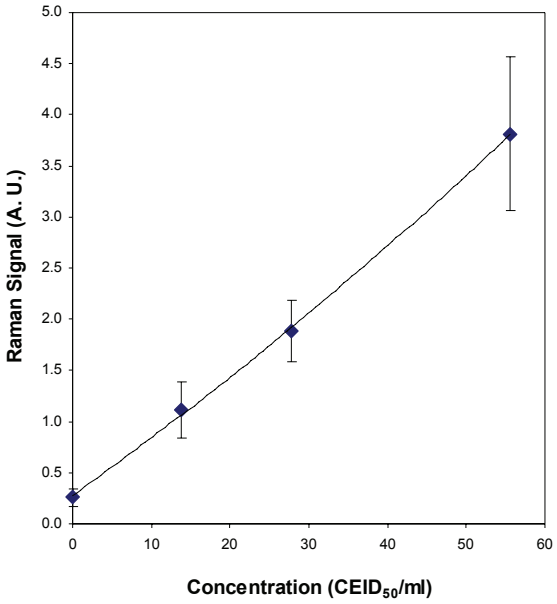
- There is no photobleaching of the biotags, even with long exposure times and high laser powers.
- The biotags are inert to matrix interference, are non-toxic, and environmentally insensitive. This allows for quantitative measurements to be made.
- A variety of Raman readers exist that can serve as detectors, including portable instrumentation. In addition, Oxonica has developed proprietary analysis software.

3. EXAMPLES OF BIOLOGICAL ASSAYS

We have investigated the use of the biotags in a number of biological assays, including

- Lateral Flow Immunoassays. Figure 3 shows titration curve results from an Influenza A lateral flow device.
- Homogeneous and heterogeneous immunoassays
- DNA analysis assays
- Immunohistochemistry and cell labeling. Figure 4 shows data from mouse tissue array using Nanoplex™ Biotags as detection tags.

Figure 3: Titration curve data for triplicate lateral flow devices for Influenza A.



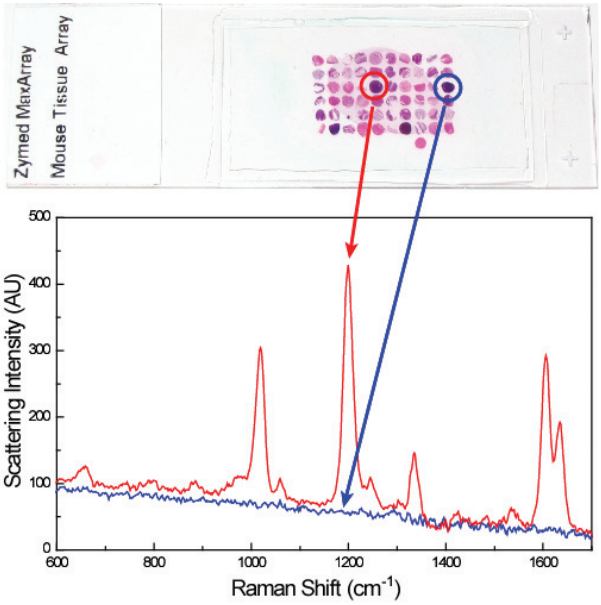


Figure 4: Blue line shows background from non-specific binding of biotags, red line shows Raman signal from specific binding to mouse array.

We will discuss results from each of these assay formats during the presentation.

REFERENCES

[1] Mulvaney, S., et al., Glass-Coated, Analyte Tagged Nanoparticles: A New Tagging System Based on Detection with Surface-Enhanced Raman Scattering, Langmuir, 19, 4784, 2003.

[2]. Freeman et al., Detection of Biomolecules using nanoparticle, surface enhanced Raman scattering tags, in Nanobiophotonics and Biomedical Applications II, edited by Alexander N. Cartwright and Marek Osinski. Proceedings of SPIE Vol. 5705 (SPIE, Bellingham, WA, 2005). Page 114 – 121.