SERS Nanotags for Point of Care Diagnostics

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ABSTRACT

An unmet need in point-of-care diagnostics is to develop tests that perform on par with the central laboratory. Oxonica has developed a series of nanoscale optical detection tags based on surface enhanced Raman scattering (SERS) that can potentially meet these criteria. These glass-coated, reporter-loaded gold nanoparticles are excited in the near-infrared, allowing detection in complex matrices that exhibit significant background, and multiple types can simultaneously be quantified using a portable reader. This paper describes the design strategy, construction and characterization of these novel nanomaterials. Data are presented that highlight the advantages of SERS nanotags as optical labels, and use of these materials in biological applications is presented.

Keywords: SERS, nanoparticles, point-of-care, bioassays, diagnostics

1. BACKGROUND

Raman scattering is a laser-based optical spectroscopy that, for molecules, generates a fingerprint-like vibrational spectrum that is much narrower than fluorescence. Raman scattering can be excited using monochromatic far-red or near-IR light, photon energies too low to excite the inherent background fluorescence in biological samples. Since Raman spectra typically cover vibrational energies from 300-3500 cm^{-1}, one could envisage measuring a numerous tags simultaneously, all with a single light source.

However, Raman is weak, and to increase the signal, one typically resorts to surface enhanced Raman scattering (SERS [1]). In SERS, molecules in very close proximity to nanoscale roughness features on noble metal surfaces (Au, Ag, Cu) give rise to million- to trillion-fold increases [known as enhancement factors] in scattering efficiency. First recognized in 1977, the phenomenon has been studied intensely for the past three decades from both experimental and theoretical perspectives.

SERS preserves all the essential features of normal Raman scattering, and can be observed from molecules on a variety of roughened noble metal substrates. More importantly, it can also be used to detect molecules adsorbed to individual metal nanoparticles, and has been used to demonstrate detection of single molecules [2]. Accurate models of SERS from single particles have been developed and recently refined [3].

We have developed a series of novel, nanoparticle-based optical detection labels for life science applications. Called Nanoplex™ biotags (described henceforth as SERS nanotags), these particles offer significant advantages for development of robust, multiplexed, ultrasensitive biological assays.

SERS nanotags (Figure 1) comprise (i) monodisperse 10-150 nm diameter SERS-active metal nanoparticles (e.g. 30-nm diameter Au, 20-nm diameter Ag), (ii) a submonolayer, monolayer, or multilayer of molecules or polymers adsorbed to or otherwise located at the nanoparticule surface (the "reporter"), and a 5- to 50-nm diameter encapsulant (typically silica or a polymer). A typical configuration would be 50-nm Au, a submonolayer of an organic molecule, and 20-nm thick silica shell, for an overall particle diameter of 90 nm. Because of the extreme sensitivity of SERS

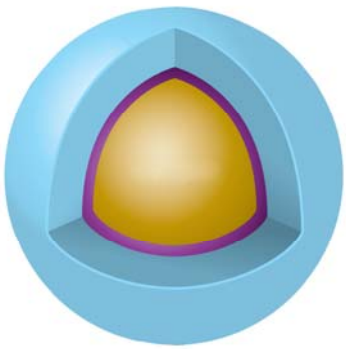

Figure 1. Cutaway of a SERS nanotag: Gold = particle, purple = reporter, pale blue = encapsulant.

toward species in very close proximity to the SERS-active surface, the spectrum of this particle consists solely of the SERS spectrum of the reporter. Becasuse Raman spectra are made up of narrow, fingerprint-like bands, this allows the generation of a series of different tags, each of which with the same core particle and

same encapsulant, but with different reporters.

This approach to optical detection tags leverages the strengths of Raman as a high-resolution molecular spectroscopy and the enhancements associated with multiplexed SERS, while bypassing the shortcomings of SERS substrate reproducibility and lack of selectivity. The encapsulant prevents unwanted species from reaching the particle surface, so the SERS spectra of the many molecules typically present in a biological matrix are not observed; it also provides a well-established surface to which biomolecules can be conjugated for bioassay development.

2. CHARACTERIZATION

Figure 2 shows typical data obtained using quinolinethiol (QSH) as a reporter. The left panel shows the SERS spectrum from particles, and the right panel shows TEM data inidicating uniform glass coating; the overall particle size is roughly 65 nm.

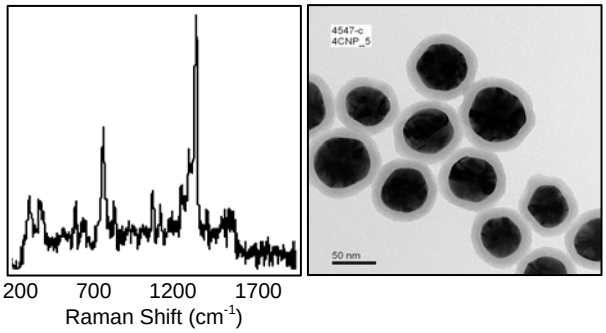

Figure 2. (Left) Spectrum of a SERS nanotag made using QSH as a reporter; (Right) TEM image.

It is important to show that the signal results solely from *SERS* of the tags, as opposed to *Raman* of reporter molecules in solution. Data in Figure 3 show

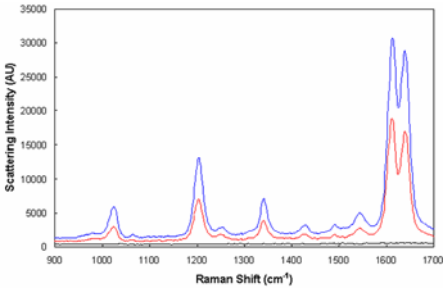

Figure 3. Raman spectrum of 0.1 µM BPE (black), SERS spectrum of BPE tags (red), and SERS spectrum of BPE added to aggregated Au colloid.

this point via a comparison of the signal intensity of bis(pyridyl)ethylene (BPE)–based nanotags to the Raman signal intensity of the same concentration of reporter used to fabricate the tags. These data show unequivocally that the signal results solely from the tags, and that the spectrum is identical to that obtained for adsorption of BPE to aggregated Au.

SERS spectra can be acquired from these tags at wavelengths where the core metal is active for SERS. For example, Figure 4 shows data obtained at 633 nm excitation and 785 nm excitation of BPE tags. The latter has become a wavelength of choice for biological experiments, as there is virtually no signal due to background fluorescence. It is also important to note that using 785 nm excitation, SERS nanotags can be seen easily both in whole blood and tissue.

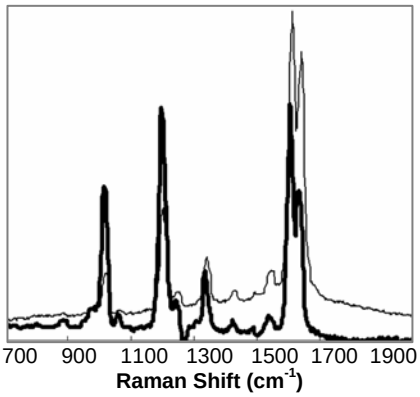

Figure 4. Raman spectrum of BPE SERS nanotags excited at 633 nm and 785 nm.

One of the key advtanges to SERS nanotags is the ability to multiplex using multiple "flavors" of tags. In other words, it is possible to create numerous labels, each with one or more unique Raman features, that allows multiple species to quantified simultaneously. While this is very straightforward using organic or particle-based fluorophores in the uv or visible, it is very hard do so in the near infrared. In contrast, this is extremely simple to do with SERS nanotags. Figure 5 shows the spectra of 5 different nanotags. Note that just over half the spectrum is shown. Nevertheless, one

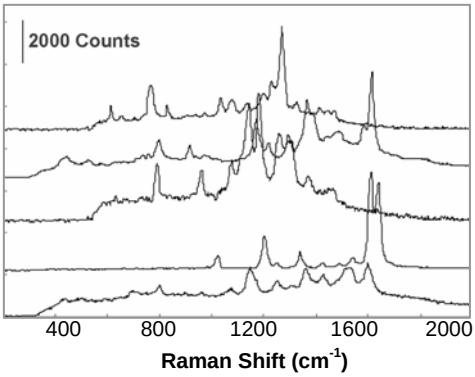

Figure 5. Comparison of tags made with five different reporters. From top to bottom: QSH, malachite green isothiocyanate, mercaptobenzamidazole ,BPE, and Bodipy.

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or more features within this spectral window are clearly unique to each reporter.

In a multiplexed assay, a single spectrum will be acquired, and it will be necessary to deconvolve that spectrum into its components, in a quantitative fashion. In principle, this is a simple task, insofar as the component spectra are known in advance, and commercial least-squares software packages can be used. However, in practice, the variable background caused by trace fluorescence, as well as the variable Raman background of the biological sample itself and the support (e.g. glass vs. plastic), each contributes a small amount to the baseline, making accurate deconvolution more challenging. Accordingly, we have developed software that is more efficient at deconvolution; an illustration with two tags is shown in Figure 14, and demonstrates both excellent linearity and quantitation capability.

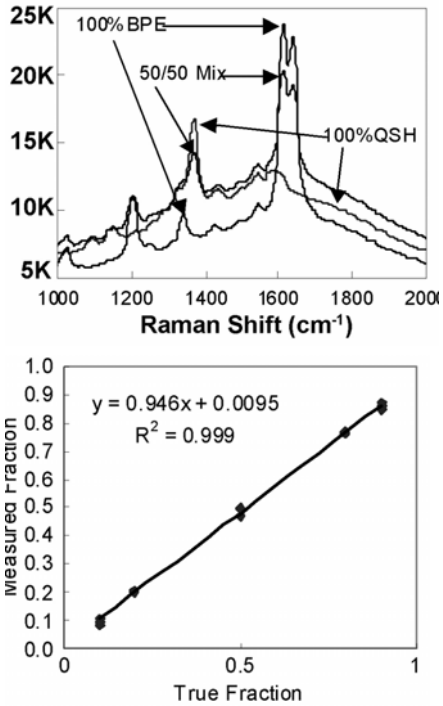

Figure 6. Analysis of mixtures of bis(pyridyl)ethylene (BPE) and quinolinethiol (QSH) Tags. Left: Spectra of solutions containing 0%, 50%, and 100% BPE tags (remainder QSH). Right: Plot of determined amount of QSH (by least squares fit) vs. true amount from solutions containing 10%, 20%, 50%, 80%, and 90% QSH.

3. BIOASSAYS

We have also spent considerable effort to show that the particles can be used for a wide variety of bioassays, a process that involves four steps. First, we showed a variety of chemical functional groups can be introduced to the outer surface of the glass to allow bioconjugation. We have been able to introduce aldehydes, epoxides, alcohols, amines and thiols successfully. Currently, the latter (-SH) is our preferred functional group, as it can be reacted with the heterobifunctional reagent sulfo-SMCC (Pierce) to crosslink the thiol group to amines on proteins. Second, we showed that biotinylated Abs could be attached to the streptavidin- or neutravidin-coated particles, with no loss of activity, and with minimal non-specific binding. Third, we demonstrated direct conjugation of Abs to the particle surface, again with retention of biological function and specificity. Finally, and most importantly, we have demonstrated the utility of the biofunctionalized particles in two different Ab-based assay formats: on protein microarrays, and in a lateral flow device.

Figure 7 shows a one-plex chip-based sandwich immunoassay for IL7, using commercially spotted capture Ab on glass. Without optimization, we achieved a limit of detection between 10-100 pg/ml, which is comparable to many commercial enzyme immunoassays. We then demonstrated the potential for multiplex assays, by probing three different analytes (ovalbumin, bacillus globigii, and C-reactive protein) on adjacent spots of a protein chip, using detection antibodies conjugated to distinct SERS tags. The data show a clean signal at each spot, with no cross reactivity (Figure 8). With these results, we proceeded to demonstrate that the SERS nanotags could be used in no-wash assays. In particular, we carried out a lateral flow immunoassay for Bot tox, using Tetracore's lateral flow cartridge. In this experiment, we opened the device, removed the pad containing their colorimetric detection reagent (which comprises colloidal Au conjugated to a secondary Ab), and replaced it with a conjugate comprising SERS nanotags conjugated to the same Ab (kindly provided by Tetracore). The data (Figure 9) show minimal non-specific binding, and successful capture of the conjugate in the presence of antigen, with a non-optimized LoD equal to their commercial product.

4. CONCLUDING REMARKS

SERS nanotags offer numerous advantages as multiplexed optical detection labels. First, they are extremely robust: the signal comes from the *inside* the particle, and so species in solution are not seen. Moreover, the glass shell renders them insensitive to changes in ionic strength or pH, a huge boon for bioassay development. Second, all the particles are excited at 785 nm. This near-IR excitation allows assays to be carried out in complex matrices, and with minimum sample preparation, because the background optical signal (fluorescence, scattering) is typically

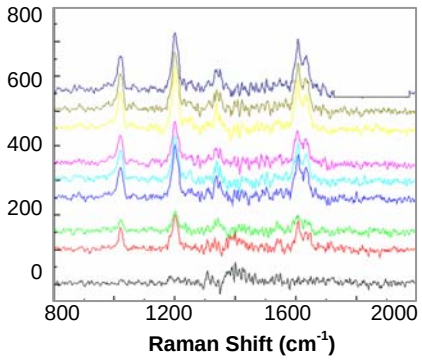

Figure 7. Titration data for IL7 sandwich immunoassay on a glass slide. IL7 concentration ranged from 10 pg/ml (bottom tracing) to 10000 pg/ml (top tracings). Y-axis: Scatter Intensity. Limit of detection was 10 - 100 pg/ml.

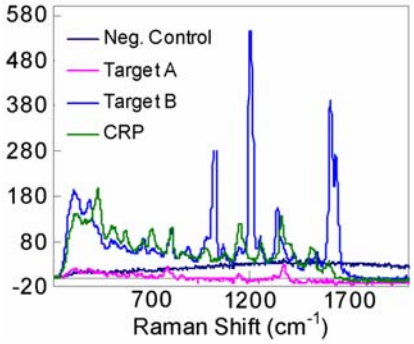

Figure 8. Immunoassay of three adjacent spots on a protein chip. Target A = Ovalbumin; Target B = Bacillus globigii; Target C = CRP.

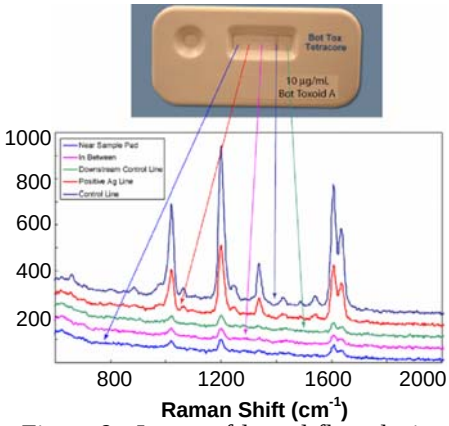

Figure 9. Image of lateral flow device for bot tox (top), and spectra obtained from each location on device (bottom), as indicated by colored lines. Y-axis: Intensity (Absorbance Units).

much more pronounced in the visible region of the spectrum. Third, the tags are highly multiplexed, meaning a large number of them can be simultaneously detected and quantified. We have made and characterized over 20 different SERS nanotags, and have used 5 at a time in biological assays. These three properties bode extremely well for the use of SERS nanotags for a wide variety of biological applications.

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5. REFERENCES

1. Cohen, L. F., Editor, "Surface Enhanced Raman Spectroscopy Faraday Discussions Vol 132", Royal Society of Chemistry, 2006.

2. Nie, S.; Emory, S. R. "Probing single molecules and single nanoparticles by surface-enhanced Raman scattering," *Science* **1997**, *275*, 1102-1106.

3. (a) Zeman, E. J.; Schatz, G. C. "An accurate electromagnetic theory study of surface enhancement factors for Ag, Au, Cu, Li, Na, Al, Ga, In, Zn, and Cd," *J. Phys. Chem.* **1987**, *91*, 634-643. (b) Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. "The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment," *J. Phys. Chem. B* **2003**, *107*, 668-677.