APPLICATION NOTE

SURFACE ENHANCED RAMAN SCATTERING NANOPARTICLES AS AN ALTERNATIVE TO FLUORESCENT PROBES – AN EVALUATION

Summary:

Interest in using nanoparticles – specifically, Surface Enhanced Raman Scattering Nanoparticles (SERS NPs) – as an alternative to traditional fluorescent tags in biological imaging is growing. These nanoparticles can interact with living cells in various ways, potentially impairing target acquisition, localization and visualization. Maria Navas-Moreno and colleagues at the University of California Davis Centre for Biophotonics compared fluorescent probes and SERS NPs, showing that SERS NPs and fluorescent tags produced equivalent results in fixed cells, but exhibited very different patterns when interacting with live cells, due to uptake of SERS NPs by the cells. This research, published in *Scientific Reports*, demonstrates that unexpected cellular interactions with nanoparticles must be taken into account, before using SERS as an alternative to traditional fluorescent probes. This research was conducted using a Deltavision fluorescence microscope, and a home built line scanning Raman microscope, which incorporated an H117 motorized precision stage from Prior Scientific.

Introduction to imaging using SERS Nanoparticles:

Raman scattering is widely used already, especially in materials analysis. The method operates by analysing scattered light after the substrate is exposed to a monochromatic light source. Although most of this scattered light retains the wavelength of the light source (i.e., Rayleigh scattering), a small fraction of photons are shifted in wavelength. This small proportion of photons with shifted energy constitute Raman scattering, and collectively they can be used for very accurate, non-destructive chemical composition and mechanical stress analysis, among many other applications. However, a major drawback is that the Raman-scattered light is extremely faint.

Surface Enhanced Raman Scattering (SERS) is one of the methods used to enhance Raman scattering by putting the molecules of interest in the close vicinity of metallic nanostructures, resulting in signal enhancements of multiple orders of magnitude. SERS nanoparticles can be used as labels by attaching a Raman reporter (a molecule that will produce a known Raman spectrum) to a nanoparticle (typically between 1-100nm and made of a noble metal, such as gold or silver). For biological applications, an antibody can be attached to this nanoparticle to allow binding to a molecule or structure of interest, creating a SERS nanoparticle probe (SERS NP). In much the same way as a fluorescence tag allows the visualisation and localization of a target by emitting light of a particular wavelength when excited, this SERS NP allows visualisation and localization of a target by emitting a particular Raman spectrum when excited. This has a number of advantages over traditional fluorescence based probes for localizing molecules, as they are more photostable, do not suffer from photobleaching and avoid the problem of spectral overlap.

Experiment:

Maria and colleagues used both fluorescent probes and SERS to quantify the distribution of specific biomarkers found on the cell membrane – HER2 and CD44. MDA-MB-231 cells are rich in CD44 markers whilst SKBR3 cells are rich in HER2 markers. Fixed cells were stained with either SERS NPs or fluorescence tags. Afterwards, pulse chase experiments were carried out, during which cells were incubated with either SERS NPs or fluorescence tags for a period of time, before being fixed and imaged.

The SERS NPs consisted of a 50 nm gold particle encapsulated in a silica shell, with HER2 and CD44 antibodies conjugated to them, in addition to fluorescence tags. Antibodies conjugated to fluorescence tags were also included in the SERS NPs solution. Imaging was carried out using a Delta Vision deconvolution microscope for fluorescence imaging. The Raman data were acquired using a home built system, which included an H117 motorised stage, controlled via the ProScan III control system from Prior Scientific.

The Motorized Stage:

Motorized stages are widely used in microscopy and photonics, especially for higher-end applications. These stages give movement (usually in the XY). Advantages of using such motorized stages include the ability to achieve higher levels of precision when moving a sample under the objective, as well as the ability to pre-program movements, integrating the positioning of the stage within the overall imaging process, thus allowing longer, more complex and more efficient imaging. Additionally, many users find that a motorized stage greatly reduces strain on the wrist and joints.

This stage was selected for several reasons. Especially important from Maria's view was the fact that its large travel range (114 x 75 mm) ensured that a large field of view could be easily imaged. The ProScan III control system can be easily integrated with most common imaging software, thus allowing the stage to be integrated into the overall imaging process. The stage offers precision positioning, with a repeatability of just 0.2 um, and a metric accuracy of 0.059 um/mm. The H117 also benefits from Prior's patented Intelligent Scanning Technology (IST). IST takes accounts of the idiosyncrasies of each stage, and adjusts the movement accordingly, resulting in much more precise positioning.

Results:

When both fluorescent tags and SERS NPs were used to stain already fixed cells, there was excellent agreement between the two methods, as illustrated in figure one. Imaging of cells tagged with fluorescence markers, and cells tagged with SERS NPs, showed that the two methods produced very similar images, indicating that both methods accurately located the biomarkers of interest (Figure 1).

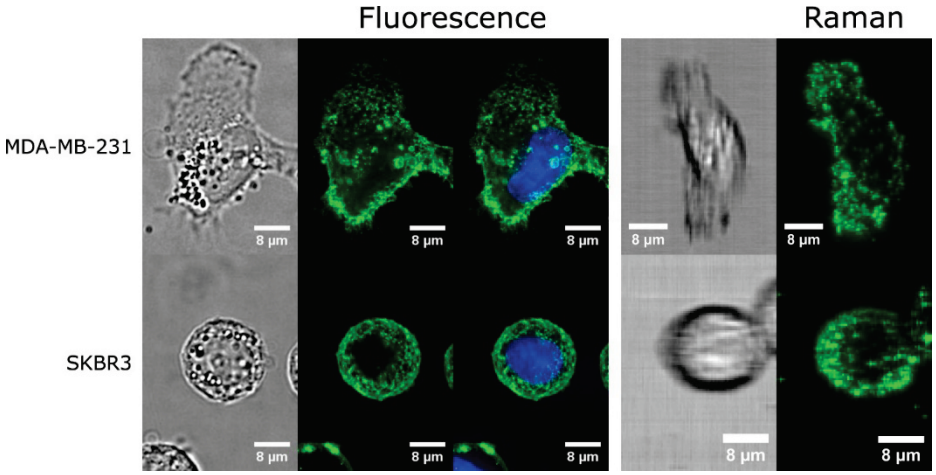

Figure 1. Side by side comparison of SERS and fluorescence images, showing strong agreement between fluorescence conjugate and SERS-conjugate labelling in fixed cells . Top: Fluorescence and Raman images of MDA-MB-231 cells stained with CD44 fluorescence and SERS conjugates, respectively. Bottom: Fluorescence and Raman images of SKBR3 cells stained with HER2 fluorescence and SERS conjugates, respectively. All images are accompanied by brightfield images for reference. Blue indicates nuclei of cells. Scale bars are 8 µm.

However, the pulse chase experiments revealed that the fluorescent tags and the SERS NPs behaved very differently. For the fluorescence tags, images taken throughout the process resembled each other closely. This observation demonstrates not only that the fluorescent tags display a very high affinity to their targets, but also that the antibody-fluorophore construct is able to accurately show the known dynamics of HER2 and CD44. In cells, CD44 and HER2 are mostly found on the surface, not internally, as observed with the fluorescent tags. However, the SERS NPs showed high levels of internalization, indicating that they had been taken up by the cells and localized within cell components, thus indicating that SERS NPs result in images that do not represent the known dynamics of these 2 proteins (figure 2).

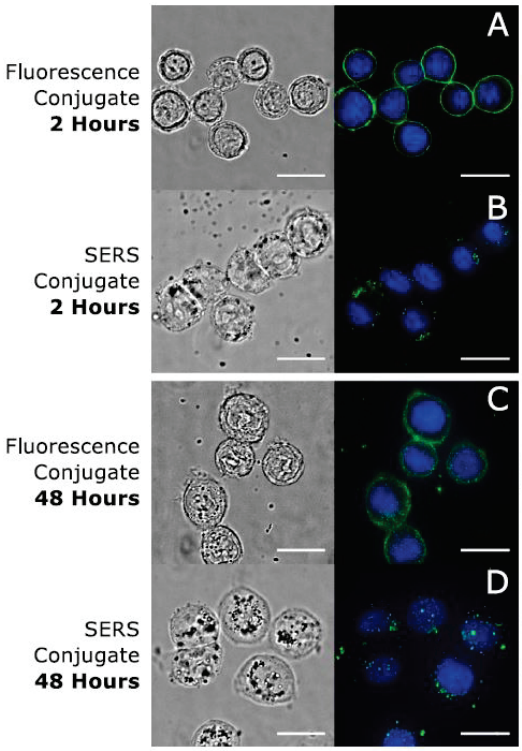

Figure 2: Nanoparticle labels are internalized and result in a different distribution than their fluorescent counterparts in live cells. (A) SKBR3 cells incubated for 2hours with HER2 and CD44 fluorescent conjugates, respectively. (B) SKBR3 cells incubated for 2hours with HER2 and CD44 SERS conjugates, respectively. (C) SKBR3 cells incubated for 2hours with HER2 and CD44 fluorescent conjugates, respectively, and allowed to incubate for additional 46hours in fresh media. (D) SKBR3cells incubated for 2hours with HER2 and CD44 SERS conjugates, respectively, and allowed to incubate for an additional 46hours in fresh media. Blue indicates nuclei of cells. Scale bars are 20µm.

Interestingly, subsequent flow cytometry indicated that non-functional – i.e., non-tagged SERS NPs – were not taken up by cells; whilst functional – i.e. tagged ones – were. Further imaging revealed further details on this process. Both CD44 and HER2 targeting SERS NPs showed uptake into cells, particularly into organelles such as lysosomes and endosomes. Furthermore, it was also shown that the antibody-nanoparticle conjugate disassociates over time.

Conclusions:

This study illustrated the fact that SEP NPs cannot be thought of simply as an alternative to fluorescence tags, but rather have their own drawbacks and benefits. When used on fixed cells, SEPS NPs conjugates generated Raman images with the same characteristics as their fluorescent counterparts, e.g., excellent specificity, but have the advantages of photostability and multiplexing. However, when used with live cells, SEPS NPs were taken up by cells, resulting in a label distribution that did not match the known dynamics of the proteins studied, unlike that obtained using fluorescent tags. Additionally, these SERS NPs began to disassociate when taken up by cells. Thus, researchers must consider very carefully whether SEP NPs are a suitable tool, especially if live cells are to be imaged.

As with all advanced imaging techniques, high quality imaging requires high quality, precision equipment. As noted by the researchers, the H117 motorized precision stage from Prior Scientific proved to be an excellent tool. Easily integrated into the overall imaging system, the stage provided an excellent combination of a large travel range and high levels of precision. Such high quality imaging equipment is absolutely vital for such advanced imaging work, and equipment from Prior Scientific can be found in laboratories all over the world. Prior's range of equipment encompasses motorized stages and focussing devices, illumination solutions, shutters, filters and robotic sample loaders. Prior's range can be used to both enhance the capabilities of a wide range of leading research microscopes, or to construct a homebuilt system for a specific application.

All images in this paper are courtesy Navas-Moreno, M. et al. (2017) *Nanoparticles for live cell microscopy: A surface-enhanced Raman scattering perspective*. Scientific Reports (7) 4471, and we thank the lead author for her assistance.

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