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An examination of specific cellular organelle-targeting nanotags using combined 3D Raman and SERS imaging

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Demonstrating a novel capability, beneficial to research on cellular delivery of NPs — thermal therapy of cancer, drug delivery into cells and intracellular bio sensing

Surface enhanced Raman spectroscopy (SERS)

- Metal nanoparticles (NPs), e.g. Ag and Au, greatly enhance the Raman signals of the adsorbed molecules through the NPs' surface plasmon and electric field effects
- Enables high sensitivity cellular imaging when internalised by cells

Organelle targeting

- Cellular organelles can be specifically targeted by using NPs functionalised with peptides

High sensitivity intracellular SERS imaging

- NPs can be tagged with reporter molecules to create nanotags
- SERS bands are sharp and suitable for multiplexing
- Nanotags can be recognised by their SERS signatures, especially in a multiplex[i]
- Imaging of nanotags enables their locations and distribution to be visualised in a non-destructive manner

Reference: i. McAughtrie *et al* (2013). Chem Sci. 4:3566-3572

Materials

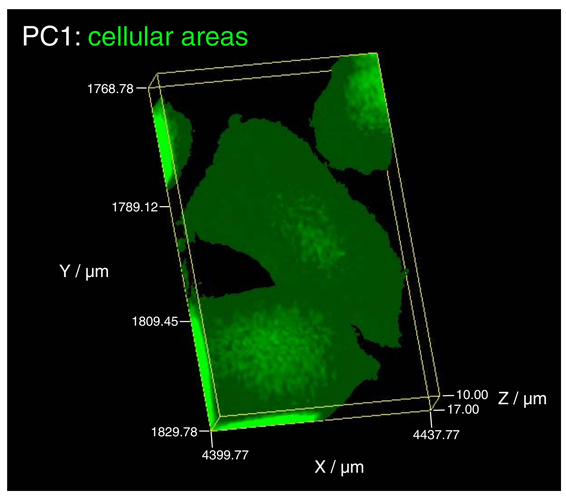
- Chinese hamster ovarian (CHO) cells seeded on to calcium fluoride windows
- Trans-Golgi network (TGN)-targeting NPs: tagged with SDYQRL peptide and 4-mercaptobenzoic acid (MBA) reporter molecule

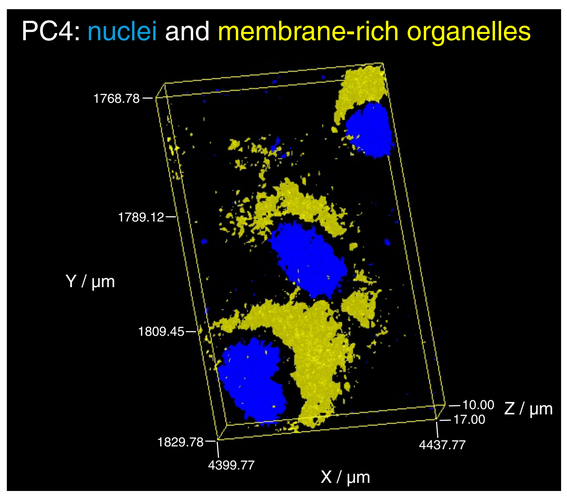
Method

- inVia confocal Raman microscope (Renishaw plc)
- Excitation: 532 nm for cell signals; 633 nm for SERS signals of nanotags
- StreamLineHR™ volume imaging, run concurrently
- Step sizes: (x,y) 0.5 µm, (z) 1.0 µm
- 60× water immersion objective (Olympus)
- Spectral analysis and image generation (WiRE software):
  - Cell image: principal component analysis (PCA)
  - Nanotags: direct classical least squares (DCLS)

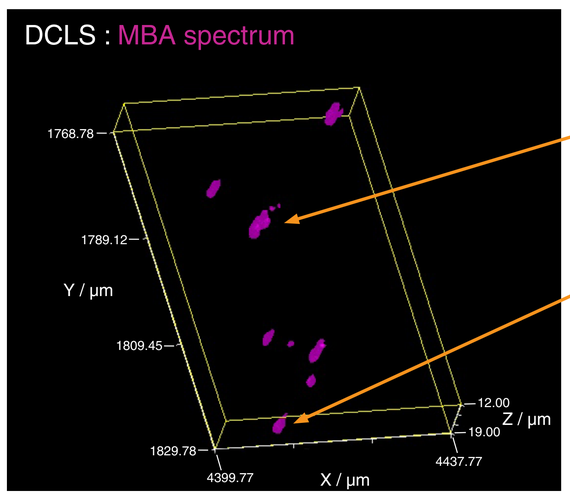
Results and discussion

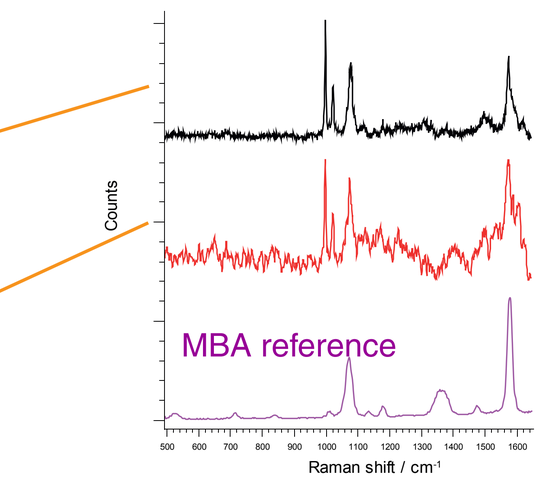
Volume Raman images of CHO cells generated by PCA



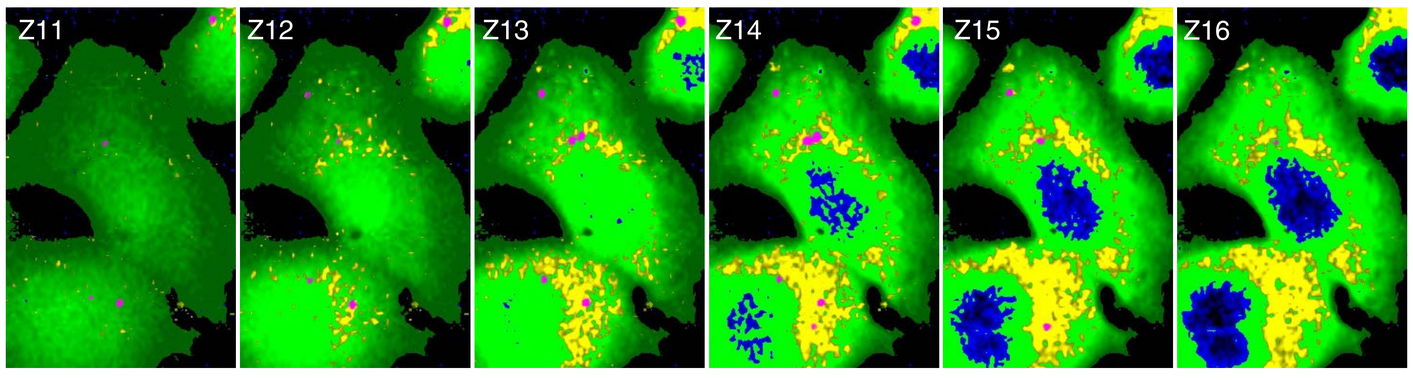


Volume SERS image of nanotags generated by DCLS





Composite PC images of cells and DCLS images of nanotags overlaid on individual x,y planes (left to right = top to bottom)



- The SERS signals of MBA enabled the identification and visualisation of nanotags
- Nanotags were located within the cells, confirming the intracellular uptake of the NPs
- The nanotags co-localised with the membrane-bound organelles; none found in the nuclei
- The results suggest successful TGN targeting
- The results can be verified using fluorescence staining

Conclusions

- Volume Raman images of CHO cells and volume SERS images of the nanotags in the same cells were successfully generated using inVia
- SERS imaging offers high sensitivity and is non-destructive
- This combined technique locates NPs, and is a powerful tool for examining the uptake, and functionalization
- Combined 3D Raman and SERS imaging is useful for research on cell targeting and on the cellular delivery of substances