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Molecular Imaging with SERS-Active Nanoparticles

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Lead-in

Raman spectroscopy has been explored for various biomedical applications (e.g. cancer diagnosis) because it can provide detailed information on the chemical composition of cells and tissues. For imaging applications, several variations of Raman spectroscopy have been developed to enhance its sensitivity. To date, a wide variety of molecular targets and biological events have been investigated using surface-enhanced Raman scattering (SERS)-active nanoparticles. The superb multiplexing capability of SERS-based Raman imaging, already successfully demonstrated in live animals, can be extremely powerful in future research where different agents can be attached to different Raman tags to enable the simultaneous interrogation of multiple biological events. Over the last several years, molecular imaging with SERS-active nanoparticles has advanced significantly and many pivotal proof-of-principle experiments have been successfully carried out. It is expected that SERS-based imaging will continue to be a dynamic research field over the next decade.

Keywords

Cancer; Molecular imaging; Multiplexed imaging; Nanoparticle; Surface-enhanced Raman scattering (SERS)

1. Introduction

Because of its superb chemical specificity, Raman spectroscopy has been utilized as an important analytical tool in many research disciplines such as chemistry,^[1–2] medicine,^[3–4] physics,^[5] and material science.^[6] Raman spectroscopy has also been explored for biomedical applications, since various diseases can lead to changes in molecular composition of the affected tissues and Raman spectroscopy can provide physicians with invaluable information for real time disease diagnosis. Over the last decade, the diagnostic potential of Raman spectroscopy has been demonstrated in cancers of various organs including the esophagus,^[7] breast,^[8–9] lung,^[10] bladder,^[11] skin,^[12] among others. In addition, Raman spectroscopy is also useful for the diagnosis of certain vascular diseases such as vulnerable plaque detection in atherosclerosis.^[13]

Molecular imaging, “the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems”, has gained enormous interest over the last decade.^[14–16] Nanotechnology, an interdisciplinary research field involving physics, chemistry, engineering, biology, and medicine, has great potential for early detection, accurate diagnosis, and personalized treatment of diseases.^[17–18] With the size of many orders of magnitude smaller than human cells,

nanoparticles can offer unprecedented interactions with biomolecules both on the surface of and inside the cells, which may revolutionize disease diagnosis and treatment. To date, nanoparticles have been employed in every biomedical imaging modality, including optical imaging, computed tomography, ultrasound, magnetic resonance imaging, single-photon emission computed tomography, and positron emission tomography (PET).^[19] The most well-studied nanoparticles for molecular imaging applications include quantum dots (QDs),^[20–22] carbon nanotubes,^[23–24] nanoshells,^[25] magnetic nanoparticles,^[26] and many others.^[17, 19]

Among all molecular imaging techniques, no single modality is a perfect fit, with each having its advantages and disadvantages. The fact that Raman spectroscopy can provide molecular/chemical information of the tissue of interest makes it a promising and competitive contender in the molecular imaging arena. Aside from chemical specificity, Raman spectroscopy also possesses many other desirable properties for imaging applications, such as high spatial resolution, superb multiplexing capability,^[27] low background signal, and excellent photostability.^[28]

However, the magnitude of Raman scattering is inherently weak (1 inelastically-scattered photon in every 10^7 elastically scattered photons) which significantly hampers its biomedical applications.^[29] Over the years, several variations of Raman spectroscopy, most notably Coherent anti-Stokes Raman spectroscopy (CARS) and SERS, have been developed to enhance its sensitivity and many in vivo imaging studies with Raman spectroscopy have been successfully achieved.^[27, 29–35]

2. Surface-Enhanced Raman Scattering

Imaging with SERS has gained significant interest over the last several years.^[29] SERS is a plasmonic effect where the molecules adsorbed on a rough metal surface can result in high Raman scattering intensities.^[36] Typically, metal nanoparticles (e.g. gold) and various fluorescent dyes (such as Cy3, Cy5, and rhodamine) are used for SERS-based applications.^[37] For detailed mechanism of SERS and the design of SERS nanoparticles, interested readers are referred to several excellent recent review articles on this topic.^[29, 31] The SERS effect can increase the Raman signal intensity by up to 10^{14} – 10^{15} fold, resulting in a detection sensitivity comparable to fluorescence.^[38] To date, a variety of SERS-active nanoparticles, mostly gold- or silver-based, have been conjugated with various targeting ligands (e.g. peptides, proteins, antibodies, antibody fragments, DNA, and Affibodies) for molecular imaging applications (Figure 1). This review article will summarize the current status of molecular imaging with SERS-active nanoparticles.

3. Imaging of EGFR

The epidermal growth factor (EGF) and EGF receptor (EGFR) were among the first growth factor ligand-receptor pairs discovered.^[39–40] Subsequently, EGFR was found to be a member of a receptor tyrosine kinase family, the human epidermal growth factor receptor (HER) family. The HER family consists of four closely related members: EGFR (HER1 or ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4).^[41] Together, the HER family controls a complex network of ligand-receptor interactions and cellular responses known as the HER-kinase axis.^[42–43]

EGFR is a 170 kDa protein which plays a critical role in tumor cell proliferation, differentiation, and survival.^[42, 44] EGFR overexpression has been associated with a number of solid tumor types such as head and neck cancer, breast carcinoma, lung cancer, bladder cancer, and colon carcinoma.^[43, 45] In addition, EGFR expression is often associated with more aggressive tumors, poor prognosis, and resistance to treatment with cytotoxic agents.

Therefore, EGFR is one of the most extensively studied targets in oncology and many monoclonal antibodies (mAbs) have been developed against EGFR for cancer therapy.^[46] For SERS-based imaging applications, those that target the EGFR are studied the most extensively, since anti-EGFR mAbs/ligands are widely available.

Gold nanorods can exhibit strong SERS effect, which makes them suitable for biomedical applications.^[25] In one early study, antibody-conjugated gold nanorods were applied for oral cancer detection.^[47] When conjugated with anti-EGFR antibodies, these gold nanorods were found to have strong Rayleigh (Mie) scattering. Molecules near the gold nanorods in the cancer cells exhibited Raman spectra that were greatly enhanced, sharpened, and polarized which may serve as diagnostic signatures for cancer cells. Such a strong polarization characteristics was attributed to the anisotropic alignment of the assembled gold nanorods with respect to the cell surface due to antibody-EGFR recognition, which may be utilized as important spectroscopic fingerprints in future cancer diagnostics.

In another report, colloidal gold nanoparticles were synthesized and conjugated to an anti-EGFR antibody.^[48] It was found that the SERS spectra of saliva from the closely-packed gold nanoparticle films was differentiable between those acquired from normal individuals and oral cancer patients, which may potentially serve as a screening assay for early diagnosis and phenotyping of oral cancer before any irreversible symptoms appear. Subsequently, multi-color PEGylated gold nanoparticles encapsulating Raman reporter dyes have also been conjugated to anti-EGFR antibodies for targeted detection of EGFR in cancer cells.^[49]

In one pioneering study, non-invasive SERS-based tumor detection was achieved in animal models.^[33] Small molecule Raman reporters (i.e. fluorescent dyes) on the surface of gold nanoparticles were stabilized by thiolated polyethylene glycol (PEG), which exhibited large optical enhancements. These PEGylated SERS nanoparticles, with light emission in the near-infrared window (700–900 nm), were found to be brighter than QDs (commonly used nanoparticles for fluorescence imaging).^[21–22] After surface conjugation of tumor-targeting single-chain variable fragment (ScFv) that specifically binds to EGFR, these SERS nanoparticles could home to EGFR on human cancer cells and in xenograft tumor models (Figure 2). Interestingly, although Raman spectra of the SERS nanoparticles were reported, a Raman image was not generated for the tumor-bearing mice.

Recently, biocompatible SERS nanoparticles were developed by chemisorption of Raman reporters on gold colloid, where lipoic acid was used as the linker to covalently attach a number of reporter molecules on the surface.^[50] After selecting the nanoparticles with the highest SERS signal intensity and stability, in vitro SERS screening was performed in cancer cell lines using anti-EGFR antibody-conjugated nanoparticles. Significant SERS signals were observed from EGFR-positive cells but not from the control cells. In another report, EGF peptide-conjugated SERS nanoparticles were used to directly measure circulating tumor cells in the presence of white blood cells in the peripheral blood of patients with squamous cell carcinoma of the head and neck.^[51]

Affibody molecules are based on the 58 amino acid (7 kDa) three-helix bundle scaffold of the staphylococcal protein A-derived Z domain.^[52–53] Despite the small size of Affibody molecules, their binding area is similar to an antibody-antigen recognition interface which enables high affinity binding. Using phage display techniques, Affibody molecules against a number of targets have been generated. Recently, Affibody-functionalized fluorescent SERS gold-silica nanoparticles were reported as multimodal contrast agents for molecular imaging of EGFR.^[54] The signal observed from EGFR-positive tumors was found to be significantly

higher than that from the EGFR-negative tumors, which was validated by competitive inhibition and ex vivo flow cytometry analysis.

4. Imaging of HER2

Since no high-affinity ligand has been identified for HER2, ligand-induced activation of HER2 depends on the formation of heterodimers with ligand-activated forms of either EGFR or HER3.^[55–56] HER2 is involved in cell survival, differentiation, proliferation, metastasis, and angiogenesis.^[42–43, 57] Overexpression of HER2 has been found in a wide variety of human cancers and very high levels of HER2 expression may also cause ligand-independent HER2:HER2 homodimer formation and autoactivation.^[56]

Antibody-conjugated hollow gold nanospheres (HGNs) have been synthesized for SERS imaging of HER2 in cell culture.^[58] In comparison to silver nanoparticles, SERS mapping revealed that these HGNs exhibited significantly better homogeneous scattering properties (Figure 3). Upon antibody conjugation, these HGNs were successfully demonstrated as multimodal agents for both dark-field and SERS detection, which may be used as highly sensitive and homogeneous sensing probes for the detection of cancer markers in cells. Similar antibody-functionalized, SERS-active HGNs were also employed for SERS-based immunoassay, where detection limit of pg/mL concentrations was achieved for various proteins.^[59] Not only is this method several orders of magnitude more sensitive than conventional ELISA, the detectable dynamic range for SERS-based immunoassay is also much wider.

Subsequently, the same group also investigated antibody-conjugated gold nanorods for imaging of HER2 in cancer cells.^[60] Gold nanorods with various aspect ratios were fabricated, and their SERS enhancement effects were evaluated. For HER2 detection, anti-rabbit IgG was immobilized onto the surface of the gold nanorods while HER2-overexpressing cells were treated with anti-HER2 antibodies. Similar to the abovementioned investigation using HGNs,^[58] this study also demonstrated the potential feasibility of antibody-conjugated gold nanorods for highly sensitive targeting and imaging of cancer cell surface markers. In another report, gold/silver core-shell nanoparticles conjugated with mAbs were also developed and tested for SERS imaging of other cancer biomarkers in live cells.^[61]

In one of the abovementioned studies where biocompatible SERS nanoparticles were developed by chemisorption of Raman reporters on gold colloid,^[50] HER2 was also investigated as an imaging target. After selecting the nanoparticles with the highest SERS signal intensity and stability, in vitro SERS screening was performed in cancer cell lines using anti-HER2 antibody-conjugated nanoparticles. Significant SERS signals were observed from HER2-positive cells but not from the control cells. In addition, in vivo SERS measurement in a mouse model was also successfully achieved to detect subcutaneously injected, nanoparticle-labeled cancer cells.

5. Imaging of PSA

The prostate-specific antigen (PSA) is a 34 kDa protein produced by the cells of the prostate gland. A blood test to measure PSA is considered the most effective test currently available for the early detection of prostate cancer and rising levels of PSA over time are associated with both localized and metastatic prostate cancer.^[62–63] In 2006, the first example of SERS microscopy for selective localization of a target protein in tissue specimens with SERS nanoparticle-labeled antibodies was reported, where PSA was used as the tumor marker.^[64] Immuno-Raman microspectroscopy, which combines the high specificity of antigen-

antibody interactions with the high sensitivity of SERS, was successfully demonstrated as a novel methodology for immunohistochemistry.

Several years later, self-assembled monolayers (SAMs) of Raman reporter molecules adsorbed onto the surface of metal nanoparticles were reported for SERS detection of PSA.^[65] In this study, hydrophilic SERS labels were synthesized, where the colloidal particles were stabilized by two different PEG chains attached to the SAM, which ensures water solubility independent of the Raman reporter molecules and their properties at the SAM/solvent interface. Controlled conjugation of anti-PSA antibodies to the dual-SAM SERS labels enabled immuno-SERS microscopy for imaging of PSA in prostate cancer tissues. SERS nanoparticles functionalized with thiolated DNA have also been demonstrated to exhibit exquisite specificity, which could recognize single-base mismatches or mutations.^[66] In another report, DNA-tethered SERS-active gold/silver core-shell nano-dumbbells were reported to be capable of single molecule detection.^[67]

6. Imaging of Folate Receptor (FR)

Folic acid is a vitamin (i.e. B₉) that is essential for the proliferation and maintenance of all cells. Because folate is often a limiting nutrient in human serum, up-regulation of FR on cancer cells may enable malignant cells to compete more aggressively for this vitamin.^[68] Many cancers express high levels of FR, include cancers of the ovary, lung, kidney, breast, brain, colon, as well as myeloid cells of the hematopoietic lineage.^[69]

Recently, a dual-modal imaging probe, based on mesoporous silica-coated gold nanorods, was reported for cancer cell targeting.^[70] Fluorescence and SERS signals, generated independently by using different excitation wavelengths, enabled the investigation of the folic acid-conjugated probe in HeLa cells which overexpress FR. Both fluorescence imaging and SERS mapping verified cellular uptake of the probe by endocytosis. When compared with gold nanorods without the silica shell, this probe also has remarkably reduced cytotoxicity.

7. Imaging of β 2-Adrenergic Receptor

Adrenergic signaling is initiated by ligand binding to the adrenergic receptors contained in nanoscale multiprotein complexes at the cellular membrane.^[71–72] The β 1- and β 2-adrenergic receptors on the surface of cardiomyocytes mediate distinct effects on cardiac function and the development of heart failure by regulating production of the second messenger cyclic adenosine monophosphate.^[73] In one study, SERS of antibody-functionalized silver nanoparticles was employed to study the β 2-adrenergic receptor aggregation state on mouse cardiac myocyte cells.^[74] After rational design of the nanoparticles, which took into account the proximity and orientation of the Raman reporter to the nanoparticle surface for optimal SERS imaging of membrane protein aggregation states, scanning electron microscopy analysis revealed distinct SERS-active clusters of between 10 and 70 nanoparticles per signaling domain of β 2-adrenergic receptor clusters on the cellular membrane. It was suggested that this technique may be generally applicable in studying other cell surface receptors and explore their distribution on cell surfaces.

8. Nuclear-Targeting Probes

SERS imaging with a nuclear-targeting, peptide-functionalized gold nanoparticles was demonstrated in cell culture.^[75] Conjugation with SV-40 large T nuclear localization signal (NLS) peptide successfully directed the gold nanoparticles to the cell nucleus. As a real-time detection technique, SERS-based imaging provided structural information for both nucleic acids and proteins without notable disturbance caused by the functionalization (Figure 4). It

was suggested that this class of nanoprobes, non-toxic and biocompatible, could potentially be used for drug delivery and cancer therapy.

Recently, another report described a co-functionalized nanoprobe and biodelivery platform for both tracking and imaging.^[76] The nuclear targeting peptide employed in this study was an HIV-1 protein-derived TAT sequence, a well-studied peptide that can transport various cargos through the cell membrane, as well as to localize small cargos to the cell nucleus.^[77] Two-dimensional SERS mapping was used to track the spatial and temporal progress of the nanoparticle uptake in cancer cells at various time points. Silver nanoparticles co-functionalized with the TAT peptide showed significantly enhanced cellular uptake over the control nanoparticles without the targeting moiety. Superior to other tracking techniques such as fluorescence/light microscopy, this strategy may have potential clinical applications in early detection and selective treatment of disease in affected cells, as well as in basic research for understanding the inner workings of living cells and how they respond to certain exogenous stimuli.

9. Imaging of Apoptosis

Apoptosis, programmed cell death, plays a critical role in the maintenance of cells by providing controlled cell deletion to balance cell proliferation.^[78] At the molecular level, apoptosis represents a collection of intricate pathways which involves many proteins. The bcl-2 proteins are a family of proteins involved in apoptosis.^[79–80] Some of these proteins (e.g. bcl-2 and bcl-XL) are anti-apoptotic, while certain others (e.g. Bad, Bax, or Bid) are pro-apoptotic. Phosphatidylserine (PS) is typically located in the cytosolic leaflet of the plasma membrane lipid bilayer. However, PS redistribution from the inner to the outer leaflet is an early and widespread event during apoptosis.^[81] Annexin V, a 36 kDa protein, can bind to PS in the presence of Ca^{2+} with high affinity.^[82]

Fluorescent SERS nanoparticles (F-SERS dots), composed of silver nanoparticle-embedded silica spheres with fluorescent organic dye and specific Raman labels, have been used for imaging of apoptosis in cells and tumor tissues.^[83] Annexin V was conjugated to these F-SERS dots for apoptosis imaging. In addition, anti-Bad or anti-Bax antibodies were also conjugated to F-SERS dots for multi-parametric analysis of apoptosis. It was found that the F-SERS dots, with no significant toxicity in several cell lines, could be used to monitor apoptosis effectively and simultaneously in both cells and tissues through fluorescent as well as Raman signals.^[83]

10. Imaging of pH

Using SERS of 4-mercaptobenzoic acid (pMBA) on gold nanoaggregates, spatially resolved probing and imaging of pH in live cells was demonstrated (Figure 5).^[84] Measuring intracellular pH with sub-cellular resolution is critical to the understanding of various physiological processes such as trafficking and recruitment of intra- and extracellular molecules.^[85] In this study, SERS-based pH nanosensors, measuring the relative intensity of pairs of spectrally narrow Raman lines in the same spectrum, allowed for quantitative measurement of pH without any correction regarding cellular background absorption/emission signals.^[84] SERS with pMBA was capable of imaging the pH values in sub-cellular structures in the range from pH 6.8 to pH 5.4. With two-photon excitation using surface-enhanced hyper-Raman scattering (SEHRS) of pMBA on nanoaggregates, SEHRS spectra of pMBA were shown to exhibit a spectral signature suitable for measurement and differentiation of pH values between 8 and 2. Such extended response range of SEHRS-based pH sensor may enable the probing of a variety of sub-cellular compartments including those of extreme pH (e.g. very acidic lysosomes).

11. Multiplexed Imaging

The key advantages of SERS-based Raman imaging over fluorescence imaging (the most widely used optical imaging technique) is the superb multiplexing capability, good quantitation, and lack of confounding background signal from autofluorescence.^[29, 32] In particular, multiplexed imaging can be extremely powerful in future research where different agents can be attached to different Raman tags to enable the interrogation of multiple biological events simultaneously in vitro or in vivo. The abovementioned F-SERS dots, composed of silver nanoparticle-embedded silica nanospheres, organic Raman tagging materials, and fluorescent dyes, have been employed to detect various biomolecules.^[86] These F-SERS dots demonstrated highly sensitive, selective, and multifunctional characteristics for multiplexed targeting, tracking, and imaging of cellular and molecular events in living organisms. Expression levels of three proteins (CD34, Sca-1, and SP-C), simultaneously expressed in bronchioalveolar stem cells in the murine lung, were quantitatively compared in the tissue using SERS-active nanoprobe. It was suggested that such immunoassays may become next-generation labeling technologies for simultaneous analysis of multiple biomolecules.

In another study, SERS nanoparticles composed of a gold core, a Raman-active molecular layer, and a silica coating were used for Raman imaging in vivo.^[27] A minimum detection sensitivity of 8 picomolar of SERS nanoparticles was observed in living mice. As a proof-of-principle, in vivo multiplexed imaging of four different SERS nanoparticles was demonstrated. In a follow-up study, the superb multiplexing capability of Raman spectroscopy was further explored.^[35] Spectral fingerprints of 10 different SERS nanoparticles were successfully separated after subcutaneous injection into a living mouse (Figure 6). The five most intense and spectrally unique SERS nanoparticles were selected and intravenously injected to image their natural accumulation in the liver (most nanoparticles will accumulate in the liver when intravenously injected due to the reticuloendothelial system uptake),^[19, 87] which were also all identified and spectrally separated by non-invasive Raman imaging. More importantly, linear correlation of the Raman signal with SERS nanoparticle concentration was observed for both subcutaneous and intravenous injection. Although no molecular targeting was involved in these in vivo multiplexing studies, the superb multiplexing capability of Raman over other imaging techniques was clearly demonstrated, which sets the foundation for future quantitative, targeted, and multiplexed Raman imaging in vivo.

12. Summary and Outlook

A wide variety of SERS-active nanoparticles have been conjugated with various targeting ligands and investigated for molecular imaging applications (Figure 1). Since many of these studies used different SERS-active nanoparticles, their in vivo biodistribution characteristics will be very different and should be examined carefully. Development of novel SERS-active nanoparticles for molecular imaging applications needs to proceed in tandem with the assessment of any toxicological side effects. Robust chemistry for modification of SERS-active nanoparticles is the key to success in future biomedical and clinical applications. For better performance in vivo, future generations of smaller SERS nanoparticles will be needed since the currently used SERS nanoparticles are typically around 100 nm in diameter which can lead to significant uptake in the reticuloendothelial system (e.g. the liver and spleen).

The primary limitations of Raman imaging in humans are those also faced by other optical techniques. Even in the near-infrared region, light penetration beyond a few centimeters of tissue is quite difficult.^[21, 88] In clinical settings, optical imaging (including Raman spectroscopy) is only relevant for tissues close to the surface of the skin (e.g. breast

imaging), tissues accessible by endoscopy (e.g. the esophagus and colon), and intraoperative visualization (e.g. image-guided surgery). To date, most of the molecular imaging studies with SERS-active nanoparticles were carried out in cells or tissues, with very few successful examples in live animals. Much future improvement in both the imaging system and probes will be needed before SERS-based molecular imaging can become a clinical reality.

Aside from SERS-active nanoparticles, single-walled carbon nanotubes (SWNTs) have also been applied for Raman imaging applications,^[24, 27, 34, 89] taking advantage of their intrinsic G band Raman peaks produced by the strong electron-phonon coupling which can cause efficient excitation of tangential vibration upon light exposure. Since SWNTs can also be used for a variety of other applications such as drug/gene delivery, sensing, photothermal therapy, as well as multimodality imaging,^[24, 90] the combination of these applications with Raman imaging can be a fertile area of research over the next decade.

Since no single imaging modality can have all the desirable features such as high sensitivity, good spatial/temporal resolution, multiplexing capacity, low cost, and high-throughput, the combination of Raman imaging with other techniques such as fluorescence, photoacoustic,^[91] or PET imaging^[92] is a promising direction for future research. For personalized cancer patient management, ex vivo sensing and in vivo imaging are both important. Raman-based sensing (using blood samples drawn before, during, and after treatment), tissue assays, in combination with in vivo diagnostics (e.g. non-invasive imaging before, during, and after treatment) may provide a synergistic approach in predicting which patients will likely respond to a specific molecular therapy and monitor their responses to personalized therapy. Over the last several years, molecular imaging with SERS-active nanoparticles has advanced significantly and many critical proof-of-principle experiments have been successfully carried out. The next decade will most likely witness the rapid expansion of this unique imaging technique.

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Figure 1.

A variety of SERS-active nanoparticles have been modified with various targeting ligands for molecular imaging applications.

Figure 2.

In vivo SERS detection of EGFR. a) A photograph showing the experimental setup where in vivo SERS spectra were obtained from the tumor and the liver. b) SERS spectra obtained from the tumor and the liver by using EGFR-targeted (left) or non-targeted (right) SERS-active nanoparticles. Adapted from Ref. [33].

Figure 3. SERS imaging of HER2. a) A schematic illustration of anti-HER2 antibody conjugated hollow gold nanospheres (HGNs). b) Raman profiles of a HGN-labeled cell and corresponding Raman spectra at six different spots. The numbers indicate the laser spots across the cell. c) SERS mapping images of HER2-expressing cells using HGNs or silver nanoparticles. Superior homogeneous optical properties of HGNs over silver nanoparticles were observed. Adapted from Ref. [58].

Figure 4.

SERS imaging of cell nucleus. a) Merged SERS and optical transmission image of a cell after incubation with a nuclear-targeting SERS-active nanoprobe. The arrows indicate the positions where the spectra in b) were collected. Scale bar: 10 μm . The inset is a cell incubated with non-targeted nanoparticles. b) SERS spectra obtained from the nucleus of a single living cell incubated with the nuclear-targeting SERS-active nanoprobe at different locations. Adapted from Ref. [75].

Figure 5.

SERS imaging of pH in live cells. a) A photograph of a cell after incubation with the gold nanosensor. Lysosomal accumulations of gold nanoparticles are observed as black spots. b) pH map of the cell displayed as false color plot of the ratios of the SERS lines at 1423 and 1076 cm^{-1} . The values in the color scale bar determine the upper end value of each respective color. Dark blue color indicates that no detectable SERS signals exist. c) Representative SERS spectra collected in the endosomal compartments with different pH. Adapted from Ref. [84].

Figure 6.

Multiplexed Raman imaging in vivo. a) A schematic illustration of a SERS nanoparticle consisting of a gold core, a Raman active layer, and silica coating. Ten different SERS nanoparticles were used. b) Raman spectra of the 10 SERS nanoparticles, each assigned a different color. c) Multiplexed Raman imaging of 10 different SERS nanoparticles in vivo. The particles were injected subcutaneously into a nude mouse and the post-processing software separated all 10 SERS nanoparticles into their respective channels with minimal crosstalk. Top: S420, S466, S481, S421, and S403. Bottom: S440, S482, S470, S663, and S661. Adapted from Ref. [35].