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SPECIAL ISSUE - RESEARCH ARTICLE

### Laser intensity limits in surface-enhanced linear and nonlinear Raman micro-spectroscopy of organic molecule/Au-nanoparticle conjugates

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### Abstract

Laser light, illuminating surface-enhanced Raman scattering-active nanostructured CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> thin-film samples with reporter molecules/Au nanoparticle conjugates on the CeO<sub>2</sub> surface, may cause irreversible modifications of the conjugates and of the surface structure field-enhancing properties. As a result, the observed Raman signal decreases or vanishes. The limits of the laser light intensity suitable for nondestructive spectroscopic studies have been assessed using continuous and quasi-continuous wave (modelocked ps-pulse) laser radiation at different wavelengths. This radiation was used as a pump for linear and nonlinear Raman microspectroscopy of reporter molecules adsorbed on the surface of such a plasmonic metamaterial. Reducing laser power below certain levels allowed reproducible mapping of surface-enhanced Raman scattering and surface-enhanced coherent anti-Stokes Raman scattering signal strengths at the reporter molecule Raman shifts.

### KEYWORDS

Au nanoparticles, plasmonic nanostructures, SECARS, SERS, SERS-active substrates

### **1** | INTRODUCTION

Scientific research around surface-enhanced Raman scattering (SERS)-active surfaces is being conducted already for 45 years. The number of papers is constantly

growing, and the achievements, frontiers, problems, and shortages are summarized in a number of recently published books and review articles.<sup>[1-12]</sup> Presently, the investigations are devoted, among other topics, to clarification of physical mechanisms of laser light interaction



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with SERS-active surfaces<sup>[13-15]</sup></sup> and, in particular, of surface-enhanced coherent anti-Stokes Raman scattering (SECARS),<sup>[16-21]</sup> as well as to possible applications of SERS in biology, biochemistry and medicine, [4,6-8,11,22-<sup>25]</sup> chemistry,<sup>[9,11,26]</sup> and pharmacy.<sup>[27]</sup> Special interest in recent years is the development of linear and nonlinear versions of SERS excited by femtosecond and picosecond laser sources.<sup>[28-33]</sup> At the same time, it is still important to search for new structures and surfaces that not only promise high electromagnetic field gain and hence high sensitivity for reporter molecules but also provide high reproducibility of their SERS properties, good stability in the surrounding atmosphere, and high resistance to laser power. The problem of radiation resistance and reproducibility of high-gain SERS media had been already addressed.<sup>[34]</sup>

As a rule, SERS detection of minor quantities of the molecules of interest is realized either by dripping some solution containing these molecules on a SERS-active surface or by submersion of such a surface in that solution (before further drying it out). But as an alternative, there exists a somehow special group of SERS-active structures consisting of organic molecules covalently bound to noble metal (mainly Au or Ag) nanoparticles (NPs). In case this organic molecule can take part in a selective chemical reaction with some biomolecule of analytical interest, this SERS-active structure can provide detection in the few-molecule limit. As an example, Au NP-bound mercaptophenylboronic acid molecules can bind to glucose tailings.<sup>[35,36]</sup> and hence serve as sensitive sensors of glycated albumin or hemoglobin.

A specific feature of covalently bound reporter molecule/Au NP conjugates deposited on a dielectric surface is the structure's excellent long-term stability of Raman-enhancing properties and resistance to an impact of ambient atmosphere. This feature may ensure many-months-long SERS activity of this type of surfaces even at the simplest storage conditions.<sup>[37]</sup> Within such an approach, a randomly nanostructured cerium dioxide-faceted dielectric film, deposited on a thin aluminum sublayer at an aluminum oxide ceramic substrate (CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub>), has been recently reported to show surface enhancement of spontaneous Raman scattering.<sup>[37,38]</sup> The first experiments with SERS<sup>[38]</sup> and SECARS<sup>[39]</sup> by 5-thio(2-nitrobenzoic acid) (TNB)/Au NP conjugates, rarely spread over the surface of the CeO<sub>2</sub> film, have shown a feasibility to obtain signals with high spatial contrasts ("bright spots").

However, the tightly focused laser light that is common for SERS and SECARS microspectroscopy can easily result in irreversible local destruction of either the active centers or the surface structure itself, which both are responsible for the appearance of "bright spots." This can cause severe systematic errors in the estimates of the obtainable reporter molecule detection limits. In case of the above-mentioned Au NP-based plasmonic complexes immobilized on a dielectric surface, the optical damage threshold can be even lower than in the case of "usual" SERS-active substrates that typically represent a nearly continuous metallic layer deposited on a solid dielectric. One of the reasons for this can be lower efficiency of heat dissipation from irradiated separate reporter molecule/Au NP complexes as compared with that from the illuminated zone of a substrate with a metallic layer. It is very important to take into account this very low level of laser damage threshold when performing sensor-type measurements in biological or medical applications.

The importance of keeping in mind laser power issue in SECARS experiments was outlined in the study of Crampton et al.,<sup>[40]</sup> whereas detailed studies of operational regimes in picosecond and femtosecond SERS experiments have been performed in the study of Pozzi et al.<sup>[41]</sup> In particular, for benzenethiol molecules on Ag film-over-nanospheres substrates at ultra-hyphen;high vacuum conditions SERS signals excited by 1-ps 795-nm laser pulses were found not to degrade up to peak intensities of  $\approx 1000 \text{ mW}/\mu\text{m}^2$ .

The present work is aimed to study the possibilities of detecting reproducible SERS and SECARS signals from TNB molecules at the Au NPs/CeO<sub>2</sub> SERS-active surface without destruction of the surface itself or the conjugates. In this respect, the laser-induced irreversible photodegradation of local SERS or SECARS signalgeneration efficiency is investigated using a set of microspectrometers. Optical damage thresholds are evaluated for different excitation wavelengths at laser intensity levels acceptable for Raman diagnostics of reporter molecules, both in continuous wave (CW) and high repetition rate picosecond-pulse modes of laser operation. The data provided by linear and nonlinear modalities of Raman spectroscopy are compared.

### 2 | EXPERIMENTAL

### 2.1 | Samples

The preparation of the investigated CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> samples, of Au NPs and Au NPs/reporter-molecule conjugates, as well as the methods of the conjugates immobilization on the CeO<sub>2</sub> film surface, has been described in detail previously.<sup>[37,38]</sup> The alleged influence of the specific properties of such a film on the additional electromagnetic field enhancement and Raman gain has been also discussed.

More extensive description of the samples, including an example of a scanning electron microscope image of their surface in Figure S1, is presented in Data S1. During the measurements, all the samples were examined in open air at ambient temperature.

### 2.2 | Laser Raman microspectrometers

In the experiments, the same samples were being investigated using three different laser Raman microspectrometers, in which CW or high repetition rate picosecond laser sources were employed.

For spontaneous Raman point spectral measurements at a He-Ne laser 633-nm excitation, a LabRAM HR Evolution-UV-VIS-NIR (HORIBA Scientific) diffraction limited spatial resolution system has been used. Laser radiation was focused onto a sample surface into a  $\approx 1.1$ -µm diameter spot by the Olympus MPlan N 100×/0.90 NA objective with the focal depth of  $\approx 0.8$  µm. A computer-controlled attenuator allowed accurate reduction of the laser power on the sample surface (13 mW maximum) up to the factor of 10<sup>3</sup>. The system spectral resolution was specified to be about 1 to 2 cm<sup>-1</sup> with a 100-µm entrance slit of a 800-mm focal length spectrograph and a 1,800 grooves/mm grating.

For both spontaneous Raman point spectral measurements and mapping, an inVia Reflex confocal Raman microspectrometer (Renishaw PLC), built around a Leica DM2700 microscope, has been used. The system was equipped with a high NA 250-mm focal length 1,200 grooves/mm grating spectrograph and two CW excitation lasers: a 532-nm diode-pumped solid-state laser (50 mW), and a 785-nm semiconductor laser (100 mW). Raman mappings have been performed with the excitation light tightly focused by spherical optics (the "StreamHR" mode) to a nearly diffraction-limited spot (diameter ~0.8 µm). Raman spectra were registered by a 2-D charge-coupled device (CCD) array while moving a high-precision two-coordinate sample holder. A computer-controlled motorized laser beam attenuator, incorporated into the system, was providing a 0.005-100% range of maximum laser power.

Spectra and microimages of SECARS and SERS radiation were recorded, with a high spatial resolution, using a Confotec CARS confocal CARS Raman microspectrometer (SOL Instruments Ltd., Belarus), described in detail in the study of Fabelinsky et al.<sup>[39]</sup> The CARS part of the spectrometer was based on a diode-pumped passively mode-locked Nd<sup>3+</sup>:YVO<sub>4</sub> laser (1064 nm, 7 ps, 85 MHz, 5 W, ~5–7 cm<sup>-1</sup> linewidth) and an optical parametric oscillator (OPO) synchronously pumped by the frequency-doubled

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1,064-nm radiation (2 W). The signal beam of the OPO (690–990 nm, 6 ps, 150–350 mW,) and a part of the 1,064-nm beam, with parallel polarizations, were collinearly overlapped and employed, respectively, as pump ( $\lambda_p$ ) and Stokes ( $\lambda_s$ ) radiation in the CARS process to generate narrow lines of the coherent anti-Stokes signals at  $\lambda_{aS} = \lambda_p/(2 - \lambda_p/\lambda_s)$ . The signals could be detected by the system within the Raman shift range of 990–3,580 cm<sup>-1</sup>. In its present configuration, the microspectrometer provides the possibility to record a frequency-shift dependence of CARS efficiency (a CARS–spectrum) only by manual, point-by-point tuning of the pump laser wavelength near a Raman resonance. The readings of the OPO control unit are used as the wavelength reference.

Two additional CW lasers integrated into the system — a 22-mW single-frequency 532-nm diodepumped solid-state laser and a 10-mW 633-nm He-Ne laser — provided the possibility to record Raman and SERS spectra. The OPO output around 785 nm could also be used as a Raman and SERS excitation source.

All the beams were collinearly overlapped and focused on the sample using an inverted microscope with a 40×/0.6 NA objective (the focal depth of ~1.6  $\mu$ m). The beams were adjusted to have the same focal spot diameters  $\approx 1 \ \mu$ m. Thus, all the signals were obtained from the same point of the sample surface with an accuracy of  $\approx 0.1-0.2 \ \mu$ m

At epi-detection, either CARS or incoherent Raman radiation, propagating in the backward direction and collected by the same objective, was spectrally filtered within an appropriate range and directed to the entrance slit of a grating spectrograph (520-mm focal length, 600 grooves/mm). CARS signals could be detected by either a cooled photomultiplier tube module or a cooled 2,048  $\times$  122 elements CCD array photodetector working in the "full vertical binning" mode, both with a 16-bit analog-to-digital converter. Raman spectra were detected by the same CCD detector.

Signal mapping was performed during the laser beam focal spot 2D scanning by galvo-driven mirrors and recording; at each spatial point, the spectrum of Raman scattered radiation with both coherent and incoherent contributions. The galvo-scanner moves the focal spot across the selected sample surface area by the steps of about 0.5–1  $\mu$ m (or more). In each spatial point, the spectrum is accumulated by the CCD array during the preset exposure time, typically  $\approx$ 0.5–1 s. Then, the spatial distribution of the power scattered into a preset spectral interval can be calculated. This matrix is presented as a microimage, or a map, of a signal strength. In case of the resonant signals, the signal strength is derived as the corresponding line amplitude

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above the level of the nonresonant background radiation.

### **3** | **RESULTS AND DISCUSSION**

## 3.1 | SERS excited by 633-nm CW laser radiation

Primarily, the investigations of irreversible optical damages of the Au NPs/CeO<sub>2</sub> SERS-active surface by laser radiation were carried out with the help of the HORIBA Raman microspectrometer. The optical microscope imaging camera allowed the faceted structure of a sample surface to be distinguished, with the larger islands of Au NPs represented as darker spots. The quality of the images was sufficient for targeting the laser beam spot, observed on the surface, to a sample area of interest and for distinguishing bigger surface damages after laser irradiation, if any.

Typical transformation of a Raman spectrum obtained from a large TNB-modified Au NPs conglomerate located on the 1,900-nm thick  $CeO_2$  film surface while increasing the laser power is presented in Figure 1a. The Raman spectra were recorded at 10-s exposure time with three accumulations (i.e., 30 s/spectrum).

The laser power at the surface was being increased from 0.13 to 1.3 mW and then to 13 mW. After illumination at 13 mW ( $\approx$ 14 mW/ $\mu$ m<sup>2</sup>), a control spectrum at 1.3 mW was recorded again. It can be seen from Figure 1a that when the laser power changes from

0.13 to 1.3 mW, TNB SERS line strengths rapidly grow, whereas when the power changes from 1.3 to 13 mW, they remain nearly constant. However, the TNB lines are still distinguishable. Moreover, the  $456 \text{-cm}^{-1}$  Raman line of bulk CeO<sub>2</sub> becomes clearly visible only at about 13 mW and remains relatively strong even when the laser power is reduced back to 1.3 mW. Meanwhile, the SERS TNB line strengths decrease about tenfold compared with those of the previously recorded 1.3mW excitation spectrum. The "saturation" of the 1,338 cm<sup>-1</sup> TNB line strengths with the excitation power is demonstrated in Figure 1b for two points within the two SERS-active areas. These areas are obtained using different methods of the TNB/Au NP conjugate immobilization, the red circles referring to the spectra in Figure 1a. We assume that the described behavior can be explained by the optical destruction of the TNB/Au NP conjugates at the surface. This is confirmed by observation of the 456-cm<sup>-1</sup> CeO<sub>2</sub> line even at 1.3mW excitation after the focal area was irradiated by 13-mW laser power. The optical image of the sample surface shows that after this irradiation there appears a black spot of a physically damaged zone of the CeO<sub>2</sub> film surface.

At the same time, the strength of the  $456 \text{-cm}^{-1}$  Raman line of bulk CeO<sub>2</sub>, originating from the CeO<sub>2</sub>/Al-coated glass samples without Au NPs, linearly and reproducibly increases in significantly broader power range (see data in Figure 1b for the 2,300-nm CeO<sub>2</sub> film sample) without any noticeable surface damage. This behavior does not depend on the scale of the faceted structure and hence the film uniformity, defined by



**FIGURE 1** (a) Raman spectra from the surface of a TNB/Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> sample at different CW 633-nm excitation laser power, and (b) surface-enhanced Raman scattering (1,338 cm<sup>-1</sup> TNB line obtained at two different spatial points of the sample —  $\blacksquare$  and  $\bullet$ ) and Raman (456 cm<sup>-1</sup> CeO<sub>2</sub> line,  $\blacktriangle$ ) signal strength variations with the power of the excitation laser for the TNB/Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> and a CeO<sub>2</sub>/Al/glass sample, respectively. The spectra in (a) are to scale but shifted vertically to facilitate perception. The solid lines in (b) are the guides for the eye

the  $CeO_2$  film thickness, that has been checked for the samples with the 300- and 2,300-nm  $CeO_2$  films.

Hence, at CW 633-nm excitation, SERS signals from TNB/Au NP conjugates on the CeO<sub>2</sub> surface can be reliably investigated at laser intensities not exceeding  $\sim$ 1.4 mW/µm<sup>2</sup> ( $\sim$ 140 kW/cm<sup>2</sup>).

# 3.2 | SERS excited by 532-nm CW laser radiation

A weaker SERS-active surface damage at elevated excitation laser power of 532-nm CW radiation is illustrated, for the same sample as above, by Figure S2, where optical images of one and the same surface area before and after mapping at ~7 mW/ $\mu$ m<sup>2</sup> are presented.

Decreasing the excitation laser power down to ~1 mW (~1 mW/ $\mu$ m<sup>2</sup>) allows one to obtain reproducible SERS and Raman maps. The examples are shown in Figure 2 for the identical surface area of the same sample as above. Here, the optical microscope camera image presented in Figure 2a well correlates with the maps in Figure 2b-d obtained at 532-nm excitation for the 456 cm<sup>-1</sup> CeO<sub>2</sub> line, as well as for the 1,338 and 1,550 cm<sup>-1</sup> TNB lines. The inVia reflex Raman microspectrometer running in the high spatial resolution "StreamHR" mode was employed,

TROSCOPY and the amplitudes of the lines above the background were taken to build the maps. In Figure 2a, the locations of the Au NP conglomerates are clearly visible against a background of the 1,900-nm thick CeO<sub>2</sub> film facets. Most likely, these conglomerates absorb and reflect a significant fraction of pump laser light that leads to a dramatic drop in the 456 cm<sup>-1</sup> Raman line strength of the underlying CeO<sub>2</sub> film in the areas located strictly below these conglomerates (seen as dark spots in Figure 2b). It can be noticed that the TNB SERS line strength distributions over the surface in Figure 2c,d are nonuniform and exhibit small "bright spots" of strong Raman signals, with a few micrometer spacing between them and the line strength span of about 1:10. Figure 2c,d illustrates a good spatial correlation of the bright spots of the 1,338 and  $1,550 \text{ cm}^{-1}$  TNB lines. Moreover, the positions of these bright spots well correspond to the locations of the Au NP conglomerates in Figure 2a.

Thus, excitation of the sample surface by both 633nm and 532-nm CW laser radiation may result in the damage of the surface itself and/or in photodegradation of the TNB/Au-NPs conjugates. The destruction threshold at 532 nm appears to be comparable with that at 633 nm, and the samples can be investigated at laser intensities below 2 mW/ $\mu$ m<sup>2</sup> (~200 kW/cm<sup>2</sup>). This value is larger than the one given in the study of Sackmann



**FIGURE 2** (a) Camera image and maps of line strengths for the (b)  $456 \text{-cm}^{-1} \text{CeO}_2$  Raman line and (c) 1,338 cm<sup>-1</sup> and (d) 1,550 cm<sup>-1</sup> TNB surface-enhanced Raman scattering lines at 532-nm CW laser excitation for the same  $23 \,\mu\text{m} \times 20 \,\mu\text{m} (0.8 \,\mu\text{m} \times 0.8 \,\mu\text{m} \text{ spatial resolution})$  surface area of the TNB/Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> sample; colors from dark blue to red correspond to the range of the line strengths ~1:10



**FIGURE 3** (a) The 1,338-cm<sup>-1</sup> TNB line surface-enhanced Raman scattering map of a  $12-\mu m \times 12-\mu m$  (1  $\mu m$  spatial resolution, 1 s exposure time) surface area of a 2,400-nm thick CeO<sub>2</sub> film TNB/Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> sample at 0.085 mW average power of 785-nm excitation and (b) SERS signal strength variation versus average excitation power in three different pixels of the map: X = 2, Y = 10 (), X = 7, Y = 10 (•), and X = 9, Y = 12 (•). The symbols on the map show the correspondence between the selected pixels and the data graphs in Figure 3b; the solid lines are the guides for the eye

et al.  $^{[34]}$  (~ 0.1 mW/ $\mu m^2$ ) for CW 515-nm irradiation of gold nanostructures prepared on a silicon wafer.

## 3.3 | SERS excited by 785-nm 85-MHz repetition rate 6-ps laser pulses

The results of SERS experiments with the picosecondpulse excitation source, also related to the sample damage threshold estimates, are presented in Figure 3. Here, one of the 1,338 cm<sup>-1</sup> TNB line SERS maps, recorded using the Confotec CARS microspectrometer at different and gradually increasing average laser powers of the OPO pump radiation at 785-nm wavelength, is presented (Figure 3a). This 12  $\mu$ m × 12  $\mu$ m map was obtained at the lowest, 0.085 mW average power at the surface within the laser spot diameter of about ~1  $\mu$ m. The 1,338 cm<sup>-1</sup> TNB line strength span in Figure 3a is from ~70 (black) to ~1,100 (white) CCD analog-to-digital converter counts, and the background signal is subtracted.

The graphs illustrating SERS signal strength variation with the average excitation power in some particular pixels of the map in Figure 3a are presented in Figure 3b. For comparison, the pixels with the smallest, intermediate, and largest SERS signal levels at the lowest power have been selected. It is clearly seen that the slopes of the signal growth in these pixels also differ from each other within an order of magnitude. Most probably this is due to different Raman signal enhancement provided by various Au NP structures present within the laser spot. It is observed that the signal growth starts to slow down or even decrease (depending on the initial signal level) at the average excitation power as low as  $\approx 0.5$  mW. The similarity with the two graphs in Figure 1b can be noted. Such a behavior may result, presumably, first from TNB/Au NP conjugates optical damage and then Au NP clusters and islands destruction, or vice versa, or both.

Thus, SERS signal strength dependences presented in Figure 3b show that the average excitation power should be kept well below 0.5 mW (that corresponds to the intensities 0.5 mW/ $\mu$ m<sup>2</sup>, or 50 kW/cm<sup>2</sup> — in average, and 1,000 mW/ $\mu$ m<sup>2</sup> — in a peak). This peak laser intensity value is in a good correspondence with the data from the study of Pozzi et al.<sup>[41]</sup> obtained using a 1-ps 795-nm laser to probe quite a different SERS-active structure.

To summarize, the presented SERS experimental data suggest that more or less "safe" pump laser power density for our samples is estimated to be below 2 mW/ $\mu$ m<sup>2</sup> at 532 nm and ~0.4 mW/ $\mu$ m<sup>2</sup> at 633 nm for CW radiation, and less than ~0.5 mW/ $\mu$ m<sup>2</sup> in average for the high-repetition rate 6-ps source at 785 nm.

## 3.4 | SECARS excited by 85-MHz repetition rate 6-ps laser pulses

Sample surface optical damage in picosecond-pump SECARS experiments was investigated using the 1,338 cm<sup>-1</sup> TNB Raman line resonant pump laser beams at  $\lambda_p = 931$  nm and  $\lambda_S = 1,064$  nm provided by the Confotec CARS system. Our results show that after



**FIGURE 4** The 1,338 cm<sup>-1</sup> TNB line surface-enhanced coherent anti-Stokes Raman scattering maps of the same 24  $\mu$ m × 24  $\mu$ m surface area of a TNB/Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> sample (1  $\mu$ m spatial resolution, 1 s exposure time) at two different levels of laser powers: (a)  $P_p = 70$   $\mu$ W,  $P_S = 125 \,\mu$ W and (b)  $P_p = 300 \,\mu$ W,  $P_S = 534 \,\mu$ W;  $\lambda_p = 931 \,\text{nm}$ ,  $\lambda_S = 1,064 \,\text{nm}$ , and  $\lambda_{aS} = 828 \,\text{nm}$ . The pixel coordinate axes are the same as in Figure 3a. The symbols in Figure 4a indicate the locations, in which the values for the power dependences presented in Figure 6 were taken

one mapping scan even at moderate pump laser intensities the Au NPs/CeO<sub>2</sub> active surface can be severely destroyed in the entire scan region, as it is demonstrated in Figure S3.

At significantly lower (nondestructive) pump laser intensities, the maps of SECARS signal at  $\lambda_{aS} = 828$  nm, similar to those presented as an example in Figure 4 for a 2,400-nm thick CeO<sub>2</sub> film sample, can be recorded and investigated as a function of the increasing laser intensities. The SECARS signal strength is defined by the product  $P_p^2 \times P_S$ , where  $P_p$  and  $P_S$  are the pump and the Stokes beam power, respectively. (Note that the



**FIGURE 5** Surface-enhanced coherent anti-Stokes Raman scattering signal strengths in the row #9 of the map in Figure 4a (indicated by the arrow) recorded in two sequential scans ( $\blacksquare$ , the first and  $\bullet$ , the second) at the same lowest levels of laser powers,  $P_p = 70 \ \mu\text{W}$  and  $P_s = 125 \ \mu\text{W}$ . The solid lines are the guides for the eye

same colors in Figure 4a,b correspond to different values of SECARS signal amplitudes).

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At the lowest powers used, the overall pattern is reasonably well reproduced when sequential mapping scans are performed at the same level of laser powers. This is clearly evidenced by Figure 5. There, the SECARS signal amplitude variation along the row #9 of the map in Figure 4a (at Y = 9, indicated by the arrow) is presented.

The reproducible SECARS signal map in Figure 4a represents an example of data obtained at the lowest levels of, presumably, Raman-resonant excitation of Au NP-bound TNB molecules. Here, the bright spots in the recorded SECARS images, with the average distances between the spots of the order of 5  $\mu$ m, are assumed to correspond to the spatial distribution of TNB/Au NP conjugates of various sizes. In this case, the Au NP clusters and islands are supposed to enhance all or some of the electromagnetic fields with  $\lambda_p$ ,  $\lambda_s$ , and  $\lambda_{as}$ .

However, as it can be found by comparing the signal strength values and distribution patterns in Figure 4a,b (the product  $P_p^2 \times P_S$  differs by ~60 times), the brightest spots become "suppressed" by the laser beams, and the relief of the pattern is "flattening" as the laser powers are growing. The flattening manifests itself both in the enhancement of the number of pixels that deliver a significant SECARS signal and in the saturation and/or degradation of the signal strength in the pixels exhibiting the highest signals at the lowest laser powers. This can be seen while proceeding from Figure 4a to b, obtained at the highest excitation level. In particular, the average relative SECARS signal strength point-to-



**FIGURE 6** Surface-enhanced coherent anti-Stokes Raman scattering signal strength variation in six spatial points of the sample surface (see Figure 4a) at increasing powers of the excitation lasers. The rightmost set of data corresponds to  $P_p = 300 \ \mu\text{W}$ ,  $P_S = 534 \ \mu\text{W}$  (Figure 4b). The solid lines are the guides for the eye. The symbols in the data graphs coincide with those plotted in Figure 4a to indicate the pixels, in which the values for the graphs were taken

point variation over the image area decreases from  $\sim$ 240% in Figure 4a to  $\sim$ 110% in Figure 4b.

The graph in Figure 6 presents SECARS signal strength dependence on the laser power product  $P_p^2 \times$  $P_{\rm S}$  in six different arbitrarily selected points of the sample surface area represented in Figure 4. Note that the laser powers were gradually increasing during the experiment. It is seen from the graph that the values measured in these points vary in a different way. Namely, the strengths in some of these points — which originally, at the lowest excitation levels, provide the bright spots generated apparently by TNB/Au NP conjugates (e.g., at [X = 17, Y = 9], [X = 22, Y = 15], and [X = 22, Y = 15], and [X = 12, Y = 15], = 8, Y = 17]) — become saturated somewhere below  $P_p^2 \times P_S \approx 6 \times 10^{-3} \text{ mW}^3$  ( $P_p = 150 \text{ }\mu\text{W}$  and  $P_S =$ 267 µW). In contrast, originally, "dark" spots (e.g., at [X = 18, Y = 9] and [X = 21, Y = 2] still demonstrate noticeable growth of SECARS signal strength, and even nearly linear dependences of the signals (like at X =10, Y = 17], but with somewhat different slopes) on the product  $P_p^2 \times P_s$ . The slope of these dependences is  $\sim (0.1-0.2) \times 10^{-3}$  counts/ $\mu$ W<sup>3</sup> and rather corresponds to the level of Raman nonresonant signals defined in the study of Fabelinsky et al.<sup>[39]</sup> as ~4  $\times$  10<sup>-3</sup> counts/ $\mu$ W<sup>3</sup>. These signals result, presumably, from two-color four-wave mixing processes near one- or two-photon absorption resonances<sup>[10,12,14,42-45]</sup> within various unmodified Au NP structures immobilized on the CeO<sub>2</sub> film surface.

The initial steep slope of the  $P_{aS}$  versus  $(P_p^2 \times P_S)$  dependences in the bright spots is evaluated to be

 $\approx 0.003$  counts/ $\mu$ W<sup>3</sup>. This value correlates with the  $P_p^2 \times P_S$  product-normalized peak SECARS signal, presumably generated by TNB/Au NP conjugates, which had been estimated previously<sup>[39]</sup> to reach  $\approx 0.13$  counts/ $\mu$ W<sup>3</sup>. The ~40 times distinction between the normalized SECARS signal strengths presented in this work and those from the study of Fabelinsky et al.<sup>[39]</sup> can be ascribed to the difference in the Au NP surface densities specific for the investigated samples.

As a result, the assumption can be made that it is TNB/Au NP conjugates that are primarily photodestructed at the increase of the excitation laser intensity, whereas the TNB-unmodified Au NP clusters and islands provide the linear growth of the anti-Stokes signal strength with the increase of the product  $P_p^2 \times P_s$ .

Hence, the data presented in Figures 5 and 6 also suggest that for our particular samples and CARS Raman microspectrometer, average lasers powers  $(P_p +$  $P_{S}$ ), recommended for more or less nondestructive and reproducible measurements, should be kept below 0.4-0.5 mW (corresponding to intensities 0.4 - 0.5 $mW/\mu m^2$ ), which is consistent with the results of our SERS measurements presented previously. It should be noted that even at the largest average laser powers mentioned above no visible "physical" damages of the sample surface, similar to those in Figures S2 and S3, have been observed.

Figure 6 demonstrates that when discussing the values of laser intensity limits in SERS and SECARS spectroscopy of organic molecule/Au NP conjugates, it should be clear that for the particular type of the samples under investigation, which contain all the possible types of Au NP structures (see Figure S1), starting from a single NP and ending with a conglomerate of several hundreds of aggregated nanospheres on the CeO<sub>2</sub> film surface, as well as Au NP plasmonic complexes, a unified exact value of the sample optical damage threshold cannot be derived. The reason for this is that the value experiences significant variations from sample to sample and even from one bright spot to another within a single sample. Because of this, by a sample damage threshold, we understand here an estimate of the pump laser intensity value below which there is a good chance to obtain reproducible SERS and/or SECARS images for a number of consecutive mapping scans.

### 4 | CONCLUSION

SERS-active surface of Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> metamaterial, with plasmonic complexes of organic reporter molecules and Au NPs immobilized on the CeO<sub>2</sub> film surface, was investigated using a set of

different laser microspectrometers. The intensity limits for CW or quasi-CW ps-pulse laser beams, employed in SERS or SECARS detection of Au NP-bound TNB reporter molecules, were experimentally evaluated. For CW radiation at 532 and 633 nm, the threshold intensity was found to be about 2 and 1.4 mW/ $\mu$ m<sup>2</sup>, respectively. For 85-MHz repetition rate sequence of 6-ps laser pulses in the range of 785–1,064 nm, the average threshold intensity was evaluated to be less than 0.5 mW/ $\mu$ m<sup>2</sup>, which corresponds to a peak intensity of 1,000 mW/ $\mu$ m<sup>2</sup>.

The results of the experiments demonstrate that extreme care should be taken about the laser power employed while using SERS-active structures for analytical purposes in linear or nonlinear Raman experiments.

However, the experiments also showed that at Raman resonant two-color laser excitation of the reporter molecules strong enough SECARS signals can be generated at laser powers that do not destroy the organic-metal conjugates, and the sensitivity of SECARS measurements still remains rather high. The high chemical imaging contrast demonstrated by the recorded SECARS microimages of Au NPs/CeO<sub>2</sub>/Al/Al<sub>2</sub>O<sub>3</sub> sample surfaces is promising in terms of reaching high SECARS detectability of the probed reporter molecules, as well as investigating the mechanisms of SECARS signal generation in further experiments.

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