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Wide Line Surface-Enhanced Raman Scattering Mapping

Wide line laser focusing technology for surface-enhanced Raman spectroscopy (SERS) substrate mapping is developed. The designed microscope enables collecting thousands of spectra in a few seconds without missing any “hot spots” during mapping of the SERS substrate. The SERS signal averaging across the line in the WL mode displays extremely high signal-to-noise ratios. The advantages of the WL technique for the SERS-based sensing are verified using different analyte molecules, that is, p-coumaric acid and melamine. Results show that the limit of detection can be improved by one order of magnitude compared to results obtained using a commercial Raman microscope.

Wide Line Surface-Enhanced Raman Scattering Mapping

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Surface-enhanced Raman spectroscopy (SERS)-based molecular detection at extremely low concentrations often relies on mapping of a SERS substrate. This yields a large number (>1000) of SERS spectra that can improve the limit of detection; however, the signal collection time is a major constraint. In this work, a wide line (WL) laser focusing technique aimed at fast mapping of SERS substrates is presented. The WL technique enables acquisition of thousands of SERS spectra in a few seconds without missing any of the electromagnetic “hot spots” in the illuminated area. In addition, the SERS signal averaging across the line in the WL mode displays extremely high signal-to-noise ratios. The advantages of the WL technique for SERS-based sensing are verified using different analyte molecules, that is, p-coumaric acid and melamine. Results show that the limit of detection can be improved by one order of magnitude compared to results obtained using a commercial Raman microscope.

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) is a well-established analytical tool in various biosensing applications that allows detection of analytes down to the single molecule level.^[1,2] It has been shown that the dominant part contributing to SERS is the electromagnetic (EM) enhancement mechanism,^[3,4] which is based on extreme amplification and localization of the incoming optical field by metal nanoparticles that support localized surface plasmon resonance (LSPR).^[3] Efficient LSPR coupling between nanoparticles leads to EM enhancement factors >10⁸. Sites containing highly confined optical fields in the vicinity of metallic nanostructures are often referred to as the EM “hot spots.”^[5,6]

For SERS, despite its phenomenal sensitivity, reliable quantification of target molecules has been hindered by i) non-uniform nature of the plasmonic near fields, ii) fluctuation in the SERS signal intensities due to unspecific binding,^[7] and iii) poor wafer-to-wafer reproducibility and uniformity of SERS substrates.^[8] To circumvent these issues, large area SERS

mapping is an advantageous approach for obtaining statistically reliable and reproducible results which significantly improves limit of detection (LoD).^[8–12] The method is particularly relevant for quantification of low-abundance molecules, for example, at sub-nanomolar concentrations where recording large SERS maps also greatly reduces the error of estimated molecular concentrations.^[10,13,14] Typically, if Raman active molecules display high affinities toward a SERS surface, satisfactory SERS signals can be obtained. However, a multitude of important molecules, such as p-coumaric acid^[15] and glucose^[16] do not bind to nanoplasmonic surfaces. In these cases, obtaining reliable SERS spectra already at concentrations <1 μM is

a challenging task. The SERS signal intensity is low and typically comparable to the SERS substrate background originating from, for example, surface contaminants. Here, large area SERS mapping is highly suitable. However, the Raman signal collection time is still a major constrain. For example, if we use the following conditions: exposure time $t = 0.1$ s, diffraction limited step size, 785 nm laser excitation wavelength, and a 10× magnification objective, the time required to measure the surface area of, for example, ≈20 mm² is around 7.7 days (step = (0.66*0.785 μm)/0.3 = 1.73 μm). Since this is practically impossible, the SERS map acquisition step size is usually increased to 10–100 μm, which still leads to a collection time of 32–3.2 h, respectively. Decreasing the step size might be counterproductive since at ultra-low analyte concentrations (<nM), the probability for a target molecule to be situated in the EM “hot spot” is extremely low, that is, most “hot spots” are unoccupied. This leads to a very few detectable points on a SERS surface. Even though statistical interpretation of such SERS data has been theoretically investigated,^[13] fast acquisition of SERS signals over a large surface area with a diffraction limited step size is still challenging and has not yet been addressed.

There are three basic fast Raman mapping methods developed for spontaneous Raman scattering applications: line-focus (LF),^[17–20] line-scan (LS),^[21–23] and direct Raman or wide-field (WF) Raman.^[24–27] All three techniques require an imaging design of the Raman setup with an aberration corrected spectrograph for simultaneous acquisition of spectra from multiple points on a sample through a laser line illumination. However, the LF technique produces a static image of a laser line on a sample, and therefore, it requires a lower laser power density compared to a dynamic movement of the laser focus point in the LS technique.^[21] Since the LS method utilizes extremely high laser powers, typically ≈5–100 mW focused into

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a diffraction limited spot, the technique is generally not suitable for SERS-based sensing applications. In SERS, in order to minimize photo-induced effects and thermal processes, a laser power on the order of $\approx 10 \mu\text{W}$ at the sample is generally preferred.^[28] In the WF Raman technique, an acousto-optic tunable filter (AOTF),^[29] instead of a spectrometer, can be used to collect spectra. The technique is capable of simultaneously acquiring a 2D Raman map at a selected Raman shift, and the spectral scanning is performed via AOTF. However, there are a few limitations. First, the laser intensity should be homogeneously distributed on the sampled area, which is a significant experimental challenge. Second, the spectral resolution is usually quite low ($10\text{--}40 \text{ cm}^{-1}$) and is highly wavelength dependent. Third, the tuning range and transparency of AOTFs is limited to $\approx 100 \text{ nm}$ in the near IR range. WF Raman technique can also be realized by the use of fiber optic bundles with a so-called “round to slit” configuration whereby a wide area of a sample can be covered simultaneously.^[30] This method provides high spectral resolution. However, due to the space between fibers in the bundle and addition of optical elements in the system, this method leads to a reduced lateral resolution and decreased signal throughput. Since, the use of fiber optic bundles for Raman signal detection is challenging to combine with sample mapping, this technique is not well suited for microscopy applications.

All the aforementioned methods have been aimed at spontaneous Raman scattering measurements, and the techniques are not directly suitable/designed for SERS-based sensing applications. First of all, a SERS substrate is usually a 2D nanoplasmonic surface; hence, confocal properties of the Raman system can be omitted. Moreover, with respect to SERS, a fast Raman mapping should be able to 1) simultaneously target multiple EM “hot spots” across a large SERS substrate surface area ($\approx \text{mm}^2$); 2) perform SERS measurements at a relatively low laser power density, that is, $\approx 1\text{--}50 \mu\text{W} \mu\text{m}^{-2}$ at a sample; 3) integrate a depolarized laser illumination mode which is important for probing all randomly oriented “hot spots.”

In this work, we implemented all the aforementioned criteria and developed a new SERS substrate mapping technique termed wide line (WL) SERS. The WL-SERS method is based on the diffraction limited line (DLL) Raman mapping, where the width of a laser line is increased by at least an order of magnitude. This leads to a reduced spatial resolving power; however, a given SERS substrate area can be imaged 33 600 times faster compared to using a classical point focus SERS mapping mode. The performance of the WL-SERS method is illustrated by mapping the Raman signal of p-coumaric acid and melamine deposited on gold-coated silicon nanopillar (AuNP) SERS substrates with LSPR optimized for near-IR range,^[8] where detection limit and signal-to-noise ratio are significantly improved.

2. Results and Discussion

2.1. Wide-Line SERS Microscope

A schematic illustration of the WL-SERS experimental setup is shown in **Figure 1a**. The main parts are the following:

- (i) Laser beam delivery system where the laser point focus, DLL, and WL laser illumination modes are implemented.
- (ii) Custom-made, epi-detection-based microscope for simultaneously obtaining an optical image and a laser beam illumination profile on the surface of the SERS substrate.
- (iii) Raman beam delivery system capable of projecting an aberration-corrected laser line image onto a spectroscopic imaging sensor.

The optical paths of laser beam delivery, microscope, and Raman scattering were designed using the Zemax Optic Studio 19.4 software package. The optomechanical design of the setup was performed using Autodesk Inventor 2019. Custom-designed mechanical parts were milled from aluminum and combined with commercially available optomechanical parts.

2.1.1. Laser Beam Delivery

The laser beam delivery layout consists of two geometries: the first geometry is a point illumination mode (**Figure 1b**), and the second geometry is for producing the DLL and WL sample illumination modes (see **Figures 1c** and **1d**, respectively). A pair of motorized flip mirrors are used for switching between point and DLL/WL illumination modes. The DLL-focus mode is achieved when a sample is illuminated by a diffraction limited laser line, that is, $d_{\text{DLL}} = 2.8 \mu\text{m}$ in width using a 785 nm laser excitation wavelength and $10\times$ magnification lens. The WL mode is obtained by expanding the width of the laser line, that is, $d_{\text{WL}} > 2.8 \mu\text{m}$ and up to $64 \mu\text{m}$, which is achieved by adjusting the position of a cylindrical lens f_3 in the laser delivery path (see **Figure 1a**). The laser beam profile is modified using a set of cylindrical lenses $f_1\text{--}f_5$, including laser line generator lens f_6 . Detailed description of beam modification in laser beam delivery system was described in our recent work.^[31]

In this work, we utilized a multimode high-power laser (785 nm, $P_{\text{max}} \approx 500 \text{ mW}$) with a line-shaped beam output profile as an excitation source. The obtained distribution of the laser intensity in the DLL and WL modes is shown in **Figures 1c** and **1d**, respectively. Since the laser output profile consists of multiple modes, $\approx 15\text{--}20\%$ variation in the laser intensity across the entire length of the focused laser line ($L_{\text{DLL/WL}} \approx 2 \text{ mm}$) at the sample focal plane was observed. The variation was corrected by normalizing the laser intensity at each pixel on the CCD camera.

In order to image the entire $\approx 2 \text{ mm}$ long DLL/WL laser profile on a sample, the aperture for the laser beam (after a lens f_7) was designed to fit the field of view (FOV = 2.2 mm) of the $10\times$ near-infrared (NIR) microscope objective. A WL-SERS high-speed Raman mapping of $100 \mu\text{m}$ trans-1,2-bis (4-pyridyl) ethylene (BPE) in ethanol solution deposited on the AuNP substrate, displaying only minor aberrations, is shown in **Movie S1**, **Supporting Information**.

2.1.2. Microscope Construction

One of the main components in the Raman microscope is a dichroic beamsplitter (BS) that reflects the incident laser

Figure 1. WL SERS microscope: a) WL SERS microscope optical design, b) point, c) DLL, and d) WL laser illumination modes in SERS microscope. The orientation of EM vectors (blue arrows in (b)–(d)) represents depolarized laser irradiation. Laser polarization in WL mode is parallel to the surface of the SERS chip.

wavelength (785 nm) and transmits the Stokes-shifted Raman spectrum. In this case, the laser beam is reflected from BS1 (Figure 1a) and guided through a set of mirrors into the 10× microscope objective, and then focused on the SERS chip surface in either the point, DLL, or WL illumination mode (see Figure 1b–d).

The sample surface is imaged by a camera while collecting SERS spectra, which simplifies the measurement procedure. This was realized by combining the incident laser beam with visible light from a light emitting diode (LED) using BS2 (Figure 1a). To ensure that the white light source is not contributing to the recorded SERS spectra in the 785–1000 nm wavelength range, the NIR emission from the LED source was suppressed (on the order $\approx 10^6$) by an edge filter (Ed3).

An example of the WL-SERS surface scanning process is shown in Movie S1, Supporting Information. The camera projection optics is designed to enable imaging of the entire microscope objective FOV with a homogeneous sample illumination. This is usually not the case for most commercial Raman microscopes that only utilize the central part of FOV.

2.1.3. Raman Beam Delivery

The back-scattered Raman signal is collected and collimated into a parallel beam using a 10× NIR microscope objective O1

(see Figure 1a). The beam is then reflected by a mirror M4, BS2, and propagates through BS1, Ed1, and the lens f13. The optical path from sample to spectrograph slit is designed to transmit an aberration-free image of the DLL or WL laser profile. This is achieved by combining a low numerical aperture (NA) collimating lens with a high NA focusing lens in the imaging spectrograph. The low NA collimating lens f14 allows one to insert the lens f13 with equal NA. Therefore, the focal length of f13 (113 mm) is sufficiently long for delivering the DLL/WL image from the sample surface to the slit, and with correct input angles at the spectrometer. In this way, beam cutting inside the spectrograph is avoided. Alternatively, the image delivery can be obtained using two intermediate lenses between a microscope objective and a lens; however, this leads to losses in the Raman signal and increases aberrations.^[19,20]

Due to the low NA of the slit and collimating lenses, even at $d_{\text{slit}} = 100 \mu\text{m}$ slit, the pixel size limited spectral resolution is $\approx 1.8\text{--}2.5 \text{ cm}^{-1}$ throughout the Raman Stokes shift range of $350\text{--}2200 \text{ cm}^{-1}$.

Optomechanical design and photograph of the constructed WL-SERS system is shown in Figure S1, Supporting Information. The modification of the laser beam profile in WL mode during propagation through the laser beam delivery optical path is shown in Figure S2, Supporting Information.

2.2. Beam Polarization Parameters

The LSPR excitations that generate the EM “hot spots” in the AuNP structures depend on the polarization of the incident field.^[32] Since AuNP clusters display random orientations,^[8] which is often the case for SERS substrates,^[33] illuminating a SERS substrate by a depolarized laser beam leads to more efficient excitation of the EM “hot spots,” that is, achieving higher surface-averaged EM field enhancement factors (EFs), leading to stronger SERS signals since $I_{\text{SERS}} \propto |E|^4$.^[32] The influence of a depolarized laser excitation on different nanoparticle clusters was investigated using COMSOL software package (see Figure 2a,b). The electric field distribution around different nanoparticle clusters shown in Figure 2b indicates that more “hot spots” are generated using two incident polarizations. Furthermore, the calculated ratios of surface averaged electrical field $R_{\text{EEF}} = \frac{E_{\text{avg}}}{E_{\text{avg}}^{\text{linear}}}$ and absorption R_{Abs} between orthogonal and linear polarizations for different nanoparticle configurations, shown in Figure 2a, indicate that depolarized excitation is beneficial for obtaining higher SERS signals without introducing additional heat which could cause desorption and decomposition of analytes.

To verify this experimentally, we decided to implement depolarized or circular irradiation in our WL microscope. However, the control of the laser polarization state becomes a challenging task when the angle of linear polarization differs from 0° and 90° with respect to the coordinate system shown in Figure 1a.^[34] In Figure S3, Supporting Information, we present polarization simulation results obtained in Zemax Optics Studio for the laser polarization propagation after beam reflection from mirrors. Simulation was performed at three different input polarization angles: 0°, 90°, and 45° for linear polarization (Figure S3b, Supporting Information). It is clear that polarization states 0° and 90° orientation propagate without linear phase (LA) anisotropy effects on dielectric mirrors and dichroic filters. However, if the

angle of polarization differs from 0° or 90°, LP anisotropy takes place, which is shown in Figure S3b, Supporting Information. As a result, linear polarization becomes elliptical. It is important to notice that the type of dielectric or interference coating also has an effect on the LP anisotropy.

Polarization analysis was also performed for circular input polarization (see bottom images in Figure S3b, Supporting Information). Here, we see that LP anisotropy leads to the transformation of circular polarization into elliptical on the sample focal plane. Therefore, the summarized polarization state will have a preferred orientation which will affect our depolarization experiment.

Figure 2c illustrates laser beam path in WL mode obtained in Zemax Optics Studio. Figure 2d represents polarization analysis in wide line mode from four differently oriented input polarizations (0°, 45°, 90°, and 135°). Such combination of polarization orientations makes it possible to simulate a quasi-depolarized laser beam, after propagation through a liquid crystal polymer depolarizer from Thorlabs (cat. number DPP25-B) named Depol 1 in Figure 1a. LP anisotropy becomes significant for input polarization components, which differ from 0° and 90°.^[34] Polarization simulation analysis also shows that this effect takes place at the edges of the line. However, polarization artifacts are at the level of a few degrees and can thus be neglected.

In order to check system polarization artifacts generated in the Raman beam delivery path, we performed additional polarization analysis in Zemax Optics Studio (see Figure S3c,d, Supporting Information). Our signal collection setup was designed in a way so that it has no LP anisotropy for any input linear polarization. An example, with 45° orientation of polarization, is shown in Figure S3d, Supporting Information. Due to the presence of a dichroic filter in the beam path, the signal is influenced by linear amplitude (LA) anisotropy, where S and P components of EM wave transmit with different efficiency

Figure 2. Depolarized laser excitation impact on SERS efficiency. a) Calculated ratios of surface averaged electrical field R_{EEF} and absorption R_{Abs} between orthogonal and linear polarizations for different nanoparticle configurations under same irradiance; b) simulated electric field distribution around nanoparticle clusters under orthogonal and linear incident polarizations with same irradiance; c) illustration of the laser beam path in WL mode obtained in Zemax Optics Studio; d) polarization analysis in WL mode performed in Zemax Optics Studio (depolarization effect from depolarizer was simulated as a mixture of four differently oriented EM waves); e) the enhancement of BPE SERS signal obtained on NP substrate by changing from linearly polarized (red line) to depolarized (black line) laser excitation condition. The insert graph demonstrates optical system independency to the orientation of laser polarization on the sample of liquid toluene.

through the filter. This effect leads to a tilt of polarization with a maximum angle of 2° in the entire spectral range of the Raman Stokes shift.

Therefore, the most critical part of signal collection with regard to polarization orientation sensitivity is the diffraction grating (named as grating in Figure 1a). It is important to notice that our spectrograph is based on a low polarization dependent transmitting fused silica diffraction grating from Ibsen Photonics (cat. number RAMAN NIR-1500-908). Due to low polarization dependency of the grating, we were able to carry out quantitative Raman intensity comparison between polarized and depolarized experiments as described below.

A depolarizer (Depol 1) was introduced into the laser beam delivery part of the WL-SERS setup (see Figure 1a). The depolarizer was fixed on a manual flip mount. A difference between polarized and depolarized laser excitation cases was realized by inserting Depol 1 into the laser beam path. First, we evaluated that the setup is insensitive to the polarization of the incident field by recording Raman spectra of toluene in a cuvette using the i) linearly polarized and ii) depolarized WL illumination modes. Both spectra display virtually identical Raman mode intensities (see the inset in Figure 2e). In order to be sure that the Raman beam delivery optical path is insensitive to the polarization orientation, we also verified that a Raman spectrum of toluene has virtually identical intensities at different laser polarizations. Next, SERS spectrum of $10\ \mu\text{M}$ BPE deposited on the AuNP surface was recorded in the depolarized WL mode and it shows $\approx 50\%$ increase in the SERS intensity compared to the linearly polarized WL case (see Figure 2e).

It is important to notice that simulations in Comsol (Figure 2a,b) were done under the assumption that depolarized light is formed as a mixture of two orthogonally polarized beams. However, the depolarizer produces multiple randomly polarized beams. For a correct comparison with simulation results, we repeated an experiment with two orthogonally polarized beams focused on the sample as shown in Figure S4a, Supporting Information. The orientation of the half wave plate placed before polarized beam splitter can equalize the intensity between orthogonal beams recombined on the second non-polarized beam splitter. By rotation of the half wave plate we can change polarization parameters in the sample focal plane from the linear polarized case to the case with two orthogonally polarized laser beams. In such geometry, equal total intensity is maintained automatically in both experiments. This experiment demonstrated similar increase in the SERS intensity of BPE compared to the linearly polarized case (around $\approx 50\%$) as shown in Figure 2e. For simplicity of optics, all further depolarized experiments were done with the use of depolarizer Depol 1.

2.3. Signal-to-Noise Ratio Comparison

Pixel limited spectral resolution of the WL SERS system is $\approx 1.8\text{--}2.5\ \text{cm}^{-1}$. A relationship between d_{slit} and spectral resolution of the setup was evaluated using $100\ \mu\text{M}$ of BPE deposited on the AuNP SERS substrates (see Figure 3a). The peak width of the $1602\ \text{cm}^{-1}$ BPE mode is $\approx 10\text{--}15\ \text{cm}^{-1}$. It means that the spectral resolution obtained at $d_{\text{slit}} = 100\ \mu\text{m}$ can be reduced by a factor of ≈ 4 . At $d_{\text{slit}} = 400\ \mu\text{m}$, the spectral resolution is

Figure 3. a) Spectral resolution test in WL mode. Black plot: spectral resolution dependence versus line thickness; blue plot: the linewidth of BPE peak with central position $1602\ \text{cm}^{-1}$ versus line thickness. b) Comparison of SERS microscope laser illumination modes. a) SERS spectra of BPE ($10\ \mu\text{M}$ concentration) measured on Au nanopillars substrate at point (black), DL line (red), and WL (blue) illumination modes; b) zoomed spectra in the range $1750\text{--}1950\ \text{cm}^{-1}$ for SNR demonstration. All presented spectra were acquired at equal conditions: $0.2\ \text{mW}\ \mu\text{m}^{-2}$ of laser intensity, laser wavelength $785\ \text{nm}$, exposure time $0.1\ \text{s}$, $10\times$ magnification microscope objective. SNR was measured as signal divided on RMS noise.

$\approx 8\ \text{cm}^{-1}$, which corresponds to $d_{\text{WL}} \approx 64\ \mu\text{m}$ ($L_{\text{WL}} \approx 2\ \text{mm}$) at the sample focal plane when using a $10\times$ microscope objective. Therefore, the maximum possible width of a laser line in the WL illumination mode is $64\ \mu\text{m}$, which is ≈ 22 times broader compared to the diffraction limited line width (d_{DLL}). It is important to mention that such line widening only becomes possible if the spectral resolution of the spectrometer is higher than the expected spectral line width of the Raman peaks.

The performance of the setup in terms of signal-to-noise ratios (SNR) has been evaluated for the point, DLL, and WL focus modes, by measuring $10\ \mu\text{M}$ of BPE deposited on the AuNP substrates in a droplet with volume $5\ \mu\text{L}$ (see Figure 3b). Results show that the WL mode yields a two orders of magnitude higher SNR compared to the point focus mode under the same measurement condition: $10\times$ magnification lens, $0.1\ \text{s}$ exposure time, $0.2\ \text{mW}\ \mu\text{m}^{-2}$ irradiance, $400\ \mu\text{m}$ slit size, $\approx 8\ \text{cm}^{-1}$ spectral resolution (see also Movie S2, Supporting Information).

This can be explained by calculating laser illumination surface areas for the three cases ($L_{\text{DLL/WL}} \approx 2 \text{ mm}$), which are $\approx 25 \mu\text{m}^2$, $\approx 6000 \mu\text{m}^2$ ($d_{\text{DLL}} = 2.8 \mu\text{m}$), and $\approx 128\,000 \mu\text{m}^2$ ($d_{\text{WL}} = 64 \mu\text{m}$) for the point, DLL, and WL illuminations, respectively. The ratios between point/DLL and point/WL illumination areas are 1:240 and 1:5120, respectively. The improvement in SNRs can be calculated as a square root of these ratios which yields 15 and 72 times higher SNRs for the two cases. Experimentally obtained SNR ratios are: point: 1:310; DLL: 1:3950; and WL: 1:21\,000 (see Figure 3b). The measured improvements in SNRs for the DLL and WL modes are 13 and 68 times, respectively, which is in good agreement with the calculated values. The difference in values can be explained by several sources of errors. First, point and WL areas can differ from the calculated values. Second, corrections for signal attenuation on the edges of the WL can be applied with wrong coefficients. It is important to notice that CCD pixels across spectral dimension were not averaged by post processing or CCD binning in both point and WL modes.

Performance of our system can be compared with existing commercially available Raman line-focus microscopes. Fundamentally, they should provide SNR at the same level as we demonstrate for DLL focus mode. Our system would have an advantage when the WL mode is applied. Based on the calculations presented above, SNR will be improved 5.2 times in WL mode compared to DLL mode.

2.4. p-Coumaric Acid Detection

p-Coumaric acid (pHCA) is a precursor of phenolic compounds which are extremely important for food^[35] and cosmetics

industries.^[36] It has been shown previously that pHCA produced by genetically engineered *Escherichia coli* cultures can be quantitatively detected using the point-focus-based SERS mapping technique with a reported detection limit (LoD) of $10 \mu\text{M}$ of pHCA in ethanol.^[15] We used an x - y step size of $100 \mu\text{m}$ to map the AuNP SERS surfaces. In order to demonstrate the performance of the WL-SERS method, different concentrations of pHCA down to $1 \mu\text{M}$ in ethanol were deposited on the AuNP substrates and detected using both 1) the point focus and 2) WL illumination modes for comparison (see results in Figure 4).

In point focus mode, 8×10 points (80 spectra, step size $263 \mu\text{m}$ in X direction, $423 \mu\text{m}$ in Y direction) for each SERS map were collected at 0.2 s per point exposure time, that is, 16 s per SERS map. A schematic illustration of the data acquisition process and averaged pHCA SERS spectra is shown in Figure 4a–c, and the process is visualized in Movie S3, Supporting Information. The estimated LoD of pHCA using the 1187 cm^{-1} and 1610 cm^{-1} vibrational mode intensities is $\approx 10 \mu\text{M}$, which is in accordance with the aforementioned study.^[15]

Next, SERS spectra from the same samples were recorded using the WL illumination mode (see Figure 4d–f). The WL field is scanned in the y -direction with a step size equal to the width of the WL profile, that is, $d_{\text{WL}} = 64 \mu\text{m}$ in this case (see Movie S4, Supporting Information). A SERS map for each concentration of pHCA is collected using 80 steps at 0.2 s exposure/step (16 s per map). Note, the total exposure time for each SERS map is the same as in the point-focus case. The obtained averaged SERS spectra for the WL mode are shown in Figure 4e. In this case, the same characteristic pHCA vibrational peaks are resolved well even at a concentration of $1 \mu\text{M}$.

Figure 4. pHCA detection at point and WL illumination modes. a,e) Illustration of mapping algorithms at point and WL laser illumination, respectively; b,f) SERS maps of pHCA represented at peak position 1604 cm^{-1} for point and WL laser illumination modes, respectively; c,g) SERS spectra of pHCA at different concentrations on Au nanopillars substrate measured with SERS microscope at point and WL illumination modes, respectively; d,h) zoomed spectrum region of pHCA at $10 \mu\text{M}$ concentration obtained at point and WL illumination modes, respectively. Point and WL illumination SERS spectra were acquired at equal conditions: $0.2 \text{ mW } \mu\text{m}^{-2}$ of laser irradiance, laser wavelength 785 nm , exposure time 0.2 s , $10\times$ magnification microscope objective.

The results show that the WL-SERS method is highly suitable for molecular detection where both the SNR and LoD can be significantly boosted compared to the standard laser point-focus mode. In the illustrated pHCA case, the SNR and LoD are improved by a factor of ≈ 43 and 10, respectively.

2.5. Melamine Detection

Melamine is a nitrogen-rich chemical that has received much attention in recent years owing to a series of highly publicized food safety incidents.^[37] Several melamine detection methods have been developed.^[38] SERS-based detection is considered a promising technique; however, the LoD needs to be further decreased.^[11] We measured melamine using a microfluidics platform. Melamine was spiked into raw milk at concentrations 1, 20, 40, 60, 80, 100, 150, and 200 ppm. Samples with different concentrations were placed into different positions on a

SERS disk and centrifuged according to the rotation algorithm described in our previous work.^[11,39]

At the end of the centrifugation process, each SERS chip was scanned in WL mode. Raman mapping was performed on the entire SERS chip surface with 0.2 s exposure time per WL; step size in Y-direction was equal to 15 μm . Total exposure time per map was 80 s. Collected SERS maps with dimensions (X, Y, wavenumber) were averaged through the X axis.

Averaged Raman maps for different melamine concentrations are presented in Figure 5a. Graphical representation of melamine peak intensity distribution at wavenumber 674 cm^{-1} along the SERS chip length is presented in Figure 5b. Similar to the pHCA case, we were able to detect the melamine peak at 1 ppm concentration level (Figure 5c). LoD obtained on commercial research-grade point mapping Raman microscope using equal sample pretreatment procedures was at the level of 10 ppm.^[11] We consider this result an important achievement toward the development of a highly sensitive melamine detection method.

Figure 5. Melamine detection at WL illumination mode. a) Spreading distance versus Raman maps representing the distribution of melamine vibrational mode (673 cm^{-1}) through the SERS chip surface as a function of concentration (experimental parameters: 0.2 mW μm^{-2} of laser irradiance, laser wavelength 785 nm, step size 15 μm , width of line 15 μm , exposure time per WL 0.2 s, 10 $\times$ magnification microscope objective); b) graphical representation of melamine distribution through the SERS chip surface at different concentrations; c) SERS spectra of melamine at different concentrations on Au nanopillars substrate measured with SERS microscope at WL illumination mode. Characteristic peak of melamine is shown by dotted line (678 cm^{-1}).

3. Conclusion

We have developed a WL SERS microscope which provides improved sensitivity of molecular detection from SERS substrates. The system can operate in point, DL line, and WL laser focusing modes. Summarizing optical novelty of the setup, we can highlight two key benefits. First, WL illumination technique provides 8 cm^{-1} spectral resolution in the case of a sample illumination area of $2100 \times 64\text{ }\mu\text{m}$. At such conditions, the spatial resolving power of the microscope is reduced; however, the SERS substrate surface can be scanned three orders faster than the point-focus mode without losing any “hot spots.” Second, laser polarization was designed to be parallel to the sample surface and randomly polarized in the WL illumination mode. Such condition provides the most efficient way of surface plasmon excitation on the NP SERS substrate.

Based on our previous results obtained on high-end commercial Raman equipment, we can conclude that a WL SERS microscope can provide at least one order lower LoD in WL mapping mode compared to the point mapping mode at equal exposure times.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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