Fast high-resolution screening method for reactive surfaces by combining atomic force microscopy and surface enhanced Raman scattering
Abstract

A fast high-resolution screening method for reactive surfaces is presented. Atomic force microscopy (AFM) and surface enhanced Raman spectroscopy (SERS) are combined in one method in order to be able to obtain both morphological and chemical information about processes at a surface. In order to accurately align the AFM and SERS images, an alignment pattern on the substrate material is exploited. Subsequent SERS scans with sub-micron resolution are recorded in 30 minutes per scan for an area of 100 x 100 µm² and are accompanied by morphological information, supplied by a fast AFM, of the same area. Hence, a complete reactivity overview is obtained within several hours with only a monolayer of reactant. To demonstrate the working principle of this method, a SERS substrate containing the alignment pattern and silver nanoparticle aggregates as catalytic sites is prepared to study the photo-catalytic reduction of p-nitrothiophenol (p-NTP).

7.1 Introduction

The characterization of reactive surfaces is complicated as there are many requirements with regards to scale, timing, environment and (chemical) specificity. Often these can not be tackled with a single analytical tool. In heterogeneous catalysis, reactivity strongly depends on the surface morphology and is subject to variations across the sample. To examine the reactivity, a surface-specific chemical analysis tool is needed, combined with knowledge about the morphological composition. Surface enhanced Raman spectroscopy (SERS) is a powerful method to gather nanoscale vibrational information about molecules close to a surface [1–3]. Applications can be found, among others, in bio-chemistry, [4, 5] forensic sciences [6, 7] and catalysis [8, 9]. Common SERS substrates are roughened silver electrodes and metallic nanoparticles [10, 11]. Alternatively, other nanostructured materials can be used, such as nanopillars, [12, 13] gold nanosponges [14] or silver islands [15, 16]. To investigate the plasmonic properties of a particular substrate, which are also important in SERS enhancement, various tools are available [17]. The SERS effect only applies to molecules very close to the surface. With as little as a monolayer of reagent a well-defined reactive volume can be obtained. The use of SERS also ensures that this limited number of molecules can still be characterized efficiently. The high SERS enhancement factors of $10^5$ - $10^{11}$, where the highest enhancement factors are observed for dyes under resonance conditions, [18] even provide single molecule sensitivity [19, 20].

Time-resolved measurements, to obtain dynamic information about chemical processes, are possible thanks to the extreme SERS sensitivity that provides a good signal-to-noise ratio, even at short integration times [8, 21–23]. In the case of heterogeneous samples, it is important to study the local reactivity of different catalytic sites [24]. SERS in itself does not provide spatial information. However, using a Raman microscope, a sample can be scanned laterally to generate a 2D SERS map of the sample with sub-micrometer resolution. The maximum achievable resolution, however, depends on the magnification of the microscope, the diffraction limit at the excitation wavelength and the time needed to obtain sufficient signal-to-noise for chemical selectivity. In addition to chemical information, structural information regarding the physical state of the reactive surface is needed to link information on reactivity to particular locations on a heterogeneous substrate. Atomic force microscopy (AFM) can be used to supply this information.

One might consider tip enhanced Raman spectroscopy (TERS), an integrated approach that yields both high-resolution structural and chemical information [25–29]. It combines the specificity of Raman spectroscopy, the sensitivity of SERS and
the high-resolution morphological information of AFM into one instrument. However, this approach may not be sufficiently robust and/or fast enough for screening purposes of areas over which heterogeneity is expected. Also, the Raman signal strongly depends on the tip shape and may suffer from tip degradation or contamination. If one aims to characterize a reactive surface over time it is extremely important that the instrument is stable throughout the experiment and does not involve actual contact with the surface once the reaction is started.

Using two separate instruments for Raman spectroscopy and AFM, benefiting from the strengths of both techniques, makes it possible to cover a larger range of dimensions and sample properties [30]. SERS allows for following a reaction without the need of actually touching the substrate, while the AFM supplies the morphological information before and/or afterwards. As the scanning of the surface does not need to be synchronous, the speed (and/or the available resolution) can be set differently for the two types of analysis.

To quickly scan a large surface for chemical changes, a Raman microscope with line scan mode is most suitable [31, 32]. In this mode the laser is projected on the sample as a line while it is raster-scanned. The 2D CCD detector area can be utilized to record multiple spectra (for all points on the line) simultaneously, which greatly increases the acquisition rate. Dynamic information is obtained by scanning the same area multiple times using a low laser power.

Aligning a sample in two different tools is, however, not straightforward. For this purpose we use a SERS-active structured substrate. A grid is applied in the evaporation step of Au on glass to prepare a substrate suitable for screening studies. The grid contains a large number of well-defined 100 x 100 µm² squares (see Fig. 7.1). This enables alignment of the AFM and SERS images with sub-micron accuracy through optical comparison and more detailed overlaying using the measured data.

To demonstrate the working principle of our newly developed method, the well studied photo-catalytic conversion of p-nitrothiophenol (p-NTP) to p,p'-dimercaptoazobisbenzene (DMAB) is used as a model system (see Fig. 7.2) [20, 33, 34]. This conversion is induced by light and catalyzed by silver [8, 35]. Hence, the reaction speed can be tuned by the illumination intensity and wavelength. The structured substrate is covered with a self-assembled monolayer (SAM) of p-NTP as the reactant and silver nanoparticles (AgNP) are spread over the surface as stable reaction centers. Under these conditions, the AgNP will aggregate and form small clusters that are relatively far apart. As a result, the reactivity of these nano-scale hotspots mimic typical heterogeneity on a reactive surface.

In this study, 633 nm excitation is used to access the plasmon resonance ranges of gold, silver and silver-gold gap mode [36]. In this way, strong SERS signals of
7.1 Introduction

Figure 7.1: Interferometry image of the TEM grid pattern on a glass substrate. The outer diameter of the circle is 3 mm. The insets show an AFM phase image of the gold surface (a), the 100 x 100 µm² squares (b), and a line scan of the height profile (c). The thickness of the gold is about 10 nm.

the whole surface can be obtained, while the silver hotspots act as the catalytic sites only when they are illuminated. It is known that thermal conversion at the gold substrate can be achieved when the plasmon resonance is excited at higher wavelengths. Using 633 nm excitation this effect was however not observed. The function of the gold substrate in the present paper is to hold the alignment pattern and to create a stable SERS background signal for reference reasons. Variations in binding, signal-enhancement and unwanted thermally-induced reactivity of the analyte can be monitored this way. Exciting the gold silver gap mode allows us to establish a controlled conversion only at the hotspot locations.

Alternatively, much higher conversion speeds could be obtained using 514 nm excitation, that may be alternated with SERS measurements at a longer excitation wavelength (matching the gold plasmon resonance). However, for this model study, controlled conversion and monitoring are done with the same excitation wavelength.

We show that this method can be used to obtain full high-resolution reactivity information and morphological data of a large area of 100 x 100 µm² containing hundreds of active sites within a few hours.
7 Fast high-resolution screening method for reactive surfaces by combining atomic force microscopy and surface enhanced Raman scattering

![Molecular structures and SERS spectra](image)

**Figure 7.2:** Molecular structures and SERS spectra of the reactant p-NTP (a) and product DMAB (b) as measured on our substrate; λ\text{ex} = 633 nm, laser power: \( = 1.5 \cdot 10^4 \text{ W/cm}^2 \), \( t_{\text{int}} = 1 \text{ sec} \).

### 7.2 Materials and methods

#### 7.2.1 Sample preparation

p-NTP (technical grade, 80%) is obtained from Sigma Aldrich Corp., St. Louis, USA. SERS substrates are prepared on standard glass coverslips (Thermo Fisher Scientific Gerhard Menzel B.V. & Co., Braunschweig, Germany). Before use, they are cleaned with anhydrous ethanol (Ethanol absolute, VWR International, Fontenay-Sous-Bois, France) in an ultrasonic bath. To create the alignment pattern a few TEM grids (Cu, 3 mm, 150 mesh, SPI, West Chester, USA) are spread over the surface of a coverslip before mounting it in the sputter machine (K550x, Quorem Emitech Ltd, East Grinstead, UK). A 10 nm layer of gold is sputtered on the glass slide to create a SERS active surface (conditions: \( I = 15 \text{ mA} \), atmosphere = \( 10^{-4} \text{ mbar} \) argon). Finally, the TEM grids are carefully removed leaving the structured gold patterns. The interaction of the gold and the glass surface causes a reorganization into small islands without the need for additional annealing (see Fig. 7.1a). All substrates contain patterns of easy-to-locate 100 x 100 µm\(^2\) regions suitable for combined AFM and SERS studies.

The substrates are then placed in a 12 mM ethanolic p-NTP solution for approximately 24 h to create a SAM. To wash off the excess p-NTP, the samples are stored in anhydrous ethanol for 12-24 h. After removing the samples from the ethanol bath they are washed using demineralized water. All handling is done with limited exposure
Silver colloid solution is synthesized according to the method described by Lee and Meisel [1] and a droplet of 10% (v/v) solution is drop-cast on the surface. After drying, the substrate is washed with water, p-NTP solution and finally ethanol.

### 7.2.2 Raman and AFM measurements

One square of the alignment grid is selected using the integrated microscope and an AFM image of an area of 90 x 90 µm$^2$ is recorded with a resolution of 2048 x 2048 points and a speed of 0.35 Hz (Tip: NSC15, micromesh, Estonia) using a fast AFM (Dimension FastScan, Bruker Corp., Billerica, USA). This system also allows faster scanning for smaller areas after the reactivity studies, but this option is not used for the current paper. The sample is then transfered to an Invia Raman microscope (Renishaw plc., Gloucestershire, UK) and the same square can be selected quickly using the optical localization system. SERS maps are recorded using a HeNe-laser for excitation (633 nm) through a 100X objective (NA: 0.8, Olympus, Tokyo, Japan). An area of 100 x 100 µm$^2$ is mapped using the line mode of the instrument with a step size of 0.6 µm and an integration time of 1 second per point. The magnification of the objective and projection on the detector determines the resolution in the second dimension and in the current configuration is also 0.6 µm per pixel. The first SERS map is recorded with a low laser power of about $1.5 \times 10^3$ W/cm$^2$ to locate the SERS active hotspots without inducing notable conversion. Consecutive maps are recorded with a higher laser power of about $1.5 \times 10^4$ W/cm$^2$. Finally, one or more measurements at an even higher laser power (e.g. $3 \times 10^4$ W/cm$^2$) are done to confirm that conversion is completed. The order of AFM and SERS measurements can be adapted to the requirements of the application.

### 7.2.3 Data analysis

Spectral analysis is done using Matlab 2015b. Raw Raman spectra are baseline corrected and smoothened using a 2$^{nd}$ order (9 pixel) Savitzky-Golay filter. Subsequently, reactant and Raman maps are generated using the peak areas of a reactant peak (1270 - 1360 cm$^{-1}$) and product peak (1390 - 1440 cm$^{-1}$) that are calculated using trapezoidal peak integration. These ranges are selected based on the SERS spectra in Fig. 7.2. Principal component analysis can be applied to obtain a slightly better signal-to-noise ratio, but this was not a limiting factor here and the use of peak areas avoids potential issues linked to slight peak shifts that can occasionally be observed in SERS data. Hotspot identification and numbering is done by thresholding of the SERS intensities. A peak area of three times the standard deviation of
the SERS signal of the substrate is considered a hotspot. The spectra appearing at a hotspot location are normalized to the total Raman intensity between 600 and 1700 cm\(^{-1}\). The normalized peak areas of reactant and product peaks in the time-resolved maps can then be used to monitor the conversion in time. Using an automated protocol, cross sections of the data either in the XY plane or in time for an individual hotspot can be produced.

Figure 7.3: An overview of the steps that lead to alignment of AFM and SERS images. (a) Optical image at the Raman microscope (20X objective), (b) Raman map overlay (red) on optical image (100X objective), (c) AFM image of the same area (90 \( \times \) 90 \( \mu \)m\(^2\), 2048 \( \times \) 2048 pixels), (d) Zoom-in of a 10 \( \times \) 10 \( \mu \)m\(^2\) area of AFM topography and Raman intensities (red). The circles indicate large spots that can be used to align the two images. The white squares represent the zoom-in locations.

7.3 Results and discussion

Fast screening of reactive substrates requires detailed chemical information as well as high-resolution morphological data. This method, that combines AFM and SERS, provides detailed reactivity information about a large sample area using a single AFM image and a series of SERS images of the same area. Accurate alignment of the data is crucial for meaningful reactivity data. Fig. 7.3 shows the steps that lead to the overlay of SERS maps and AFM images. Using the optical microscope of both the
Raman instrument and AFM, a specific square in the alignment pattern is selected (Fig. 7.3a). Note that in these maps the SERS background is subtracted and thus only the hotspots remain (red). Larger clusters, when they are present, (white circles in Fig. 7.3b and Fig. 7.3c) can be used to align the AFM and SERS images accurately. For samples containing only small hotspots, spatial size selection in the AFM image can be used to simplify the correlation procedure, i.e. selecting clusters that cover a particular area rather than those that have a specific height. More information about specific reaction sites can be obtained by zooming in on a region of interest (Fig. 7.3d). Clearly, not all particles in the selected area induce significant signal enhancement, therefore reactivity is also not expected at all locations.

The combined data is useful for two reasons: (i) statistical analysis of the whole sample can be made based on, for example, the correlation between physical dimensions of reactive sites and reaction speed and (ii) relevant individual hotspots can be localized and studied in detail.

In our model study, SERS scans with an illumination time of 1 second per pixel provide a good trade-off between signal-to-noise ratio, reaction control and acquisition time. A series of scans shows the development of the reaction at the various sites. In Fig. 7.4a all hotspots are highlighted in red. Fig. 7.4b and 7.4c show hotspots that have reached 50% conversion after one and six seconds, respectively. After every subsequent measurement more clusters light up, indicating differences in reaction speed rather than distribution of reactive versus non-reactive sites. Typical spectral changes for a single reactive hotspot are shown in Fig. 7.4d.

On many reactive surfaces it is interesting to study the correlation between certain sample properties and the local reactivity. In our model system, for example, the data can be divided into groups based on signal enhancement or cluster size. In Fig. 7.5a the red dashed curve represents the average conversion pattern of clusters with a low signal enhancement (up to a factor 1.2 compared to the analyte signal at the gold substrate) and in green all other clusters. This enhancement factor appears limited because the signal is generated in a small area in the illumination spot. However, if for example a particle of 50 nm is responsible for the enhancement factor of 1.2, compared to the SERS background, in a 500 x 500 nm² illuminated area (100 times larger) the real enhancement of this particle is a factor of 20. Fig. 7.5b represents an alternative approach to asses the data. It shows the curves of the same data for clusters smaller than five particles (red dashed) compared to larger clusters (green) based on the AFM data. In Fig. 7.5a there is a slight difference between the average reactivity of clusters with a different signal enhancement. The size of the cluster (Fig. 7.5b) seems to be less important for the reactivity. Note that for smaller or less intense hotspots on average, the reaction starts one second later than for larger
Fast high-resolution screening method for reactive surfaces by combining atomic force microscopy and surface enhanced Raman scattering

Figure 7.4: SERS maps of reactant and product intensities measured in line mode using 633 nm excitation at $1.5 \cdot 10^4$ W/cm$^2$. (a) shows the location of potential reactive sites based on an increased reactant signal (peak area at 1325 cm$^{-1}$). (b) and (c) show the formation of product (peak area at 1420 cm$^{-1}$) when the cluster reaches 50% conversion after illumination for one and six seconds, respectively. (d) shows the spectral changes during the reaction from 0 to 14 s at one specific reaction site.

or more intense hotspots. A more detailed analysis of this difference is outside the scope of this paper.

To obtain a better understanding about the activity at individual hotspots, reaction data about these locations can be extracted. To indicate the difference between various reaction sites, Fig. 7.6 shows the topography and SERS overlay of an area containing several reactive sites. For three hotspots the individual reaction curves are shown. Fig. 7.6b shows the reactivity of a larger cluster that reaches its maximum conversion in about seven seconds. Fig. 7.6c shows the reaction of another particle that proceeds much faster as indicated by the steeper slopes. Also the peak intensities after one second are closer together, indicating that already during the first (high-power) SERS scan part of the $p$-NTP is converted. Fig. 7.6d shows data for a third, non-reactive hotspot.

The fast screening method presented in this work is set up such that the number of
7.3 Results and discussion

Figure 7.5: Graphs showing the correlation between the reactivity and cluster properties. (a) Raman enhancement (compared to the analyte signal at the gold substrate) versus reactivity, (b) cluster size versus reactivity. Excitation power: $1.5 \times 10^4$ W/cm$^2$. The first three seconds are illuminated with low intensity ($1.5 \times 10^3$ W/cm$^2$) to mark the starting point of the reaction. The last two seconds, the maximum laser power of $3 \times 10^4$ W/cm$^2$ is used. Graphs are normalized to full conversion.

Data points is weighted against speed. To gain access to reliable reaction constants, e.g., as has been calculated by van Schrojenstein Lantman et al., [8] much more data points may be required. Our screening method does provide reaction data about individual hotspots rather than an average value over a larger area. It is specifically meant for heterogeneous surfaces on which differences between the reactive sites occur. These differences can be quickly observed and studied in depth in further experiments.

When sub-nanometer resolution is needed to resolve the morphological differences that may be relevant in explaining a variation in reactivity, regions of interest can be selected and high resolution images can be acquired at a later state.

On the basis of the screening method, interesting locations could be selected without inducing a reaction on the active sites as the signal-to-noise ratio is sufficiently high to obtain SERS maps with a very low laser power density. Such sites can then be studied with point mapping with slightly higher spatial resolution rather than line scanning as the relevant area is significantly smaller. An example in Fig. 7.7a shows a high-resolution (10 x 10 µm$^2$, 2048 x 2048 pixels) AFM image with larger and smaller particle clusters. The Raman enhancement of these hotspots is indicated in red in Fig. 7.7b and the product formation in green. Not all hotspots show additional Raman enhancement and only a few clusters show conversion. Larger clusters can act as multiple hotspots and only parts of them show relevant reactivity. Point mapping is of course much slower than line mapping, and the actual gain in spatial resolution is visible, but not very large as even with the line scan approach the imaging resolution approaches the diffraction limit.
7 Fast high-resolution screening method for reactive surfaces by combining atomic force microscopy and surface enhanced Raman scattering

Figure 7.6: AFM topography image with SERS map overlay a) and reaction curves for specific reaction sites ((b), (c), (d)). The colored areas indicate selected sites, that show different reactivity. (b) Orange: slow reaction; (c) Green: fast reaction; (d) Red: no reaction. Gray: other SERS active sites - here used for alignment only (data not shown). The AFM gray scale in (a) is 200 nm.

7.4 Conclusion

We have developed a fast screening method for reactive surfaces using a combination of AFM and SERS. With this method, the optimum settings of both techniques can be utilized without the need for a complex integrated system, which makes this methodology relatively inexpensive and enables high-resolution examination of large inhomogeneous sample areas. The AFM provides important high-resolution morphological information, while SERS is used to obtain sub-micron chemical reactivity data. The non-synchronous nature of our approach ensures that the resolution and speed for the individual techniques can be set individually. During a reactivity study no contact with the surface is needed, while SERS provides sub-micron chemical information with an extremely low detection limit that makes it possible to work with a monolayer of reactant on a surface.

Separate SERS scans can be recorded in 30 minutes for a 100 x 100 µm² area with a pixel size of 0.6 x 0.6 µm². Hence, complete reaction curves of hundreds of individual reaction sites can be collected within a few hours. The accurate alignment of the data obtained from both techniques is ensured through the use of an alignment pattern in the substrate material. This makes it possible to link morphological features to reactivity and/or determine other potentially valuable information about reactive surfaces. Although the presented method is meant for fast screening of larger surface areas, it is shown that the alignment approach also works well when selected locations
**Figure 7.7:** AFM image (a) and SERS map (b) of the same 10 x 10 µm² area. Red color in the SERS map indicates the reactant intensity, green color indicates product formation. AFM resolution: 2048 x 2048 pixels, SERS resolution: point map mode with a stepsize of 0.2 µm, λ_ex = 633 nm.

need to be further investigated using high-resolution AFM imaging and/or diffraction-limited Raman mapping.
7 Fast high-resolution screening method for reactive surfaces by combining atomic force microscopy and surface enhanced Raman scattering

Bibliography


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