Nanoscale reaction kinetics of heterogeneous surfaces using Raman and AFM

Research thesis
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As the final part of the master Analytical science at the University of Amsterdam a master project is required. I chose to go to the LaserLaB at the VU University in Amsterdam to meet this requirement. The project I worked on was part of the PhD research of Camiel van Hoorn. This project was a collaboration between Philips lighting and the VU university. The work we did also got published in Applied Spectroscopy under the title: ‘Fast High-Resolution Screening Method for Reactive Surfaces by Combining Atomic Force Microscopy and Surface-Enhanced Raman Scattering’.
Summary

A fast high-resolution screening method for heterogeneous reactive surfaces has been developed. This method combines surface-enhanced Raman spectroscopy (SERS) and atomic force microscopy (AFM) to be able to obtain both chemical and morphological information about a reactive surface. A pattern on the substrate material makes it possible to accurately align the SERS and AFM images. The reaction on the surface can be monitored by scanning the surface at specific time intervals in linescan mode. The morphological information of the same area will be supplied by fast AFM. 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) to p,p'-dimercaptoazobisbenzene (DMAB). The developed method provides a relatively inexpensive solution for fast imaging of a heterogeneous reactive surface. With high resolution morphological data and reactive data of a few hundred individual reaction sites.
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1. Introduction

The aim of this study was to develop a tool that can be used to characterize a heterogeneous catalytic surface. The characterization of such a surface cannot be tackled with a single analytical tool because the reactivity of these kinds of surfaces strongly depends on surface morphology and will have variations across the surface. A combination of a surface-specific chemical analysis tool and knowledge about the morphology is needed to examine the reactivity. Surface-enhanced Raman spectroscopy (SERS) is a possible method to gather vibrational information about the first few nanometers of the sample surface [1–3] Common SERS substrates are roughened silver electrodes and metallic nanoparticles [4,5].

In heterogeneous samples, it is important to study the local reactivity of different catalytic sites. SERS in itself does not provide spatial information. However, using a Raman microscope, a sample can be scanned in the xy-plane to generate a 2D SERS map of the sample with sub-micrometre resolution. 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 and morphologies on a heterogeneous substrate. Atomic force microscopy (AFM) can be used to supply this information.
2. Theory

2.1 Spectroscopy

Light and matter can interact in many ways, some of these processes can give information on the physicochemical properties of matter and their surroundings. The study of matter in this way is called ‘Spectroscopy’. A very well-known form of spectroscopy is fluorescence spectroscopy. In fluorescence a photon is absorbed by a molecule and the molecule is excited to a higher energy state. By release of a fluorescence photon the molecule goes back to a vibration of its ground state (Figure 1). Fluorescence is only possible if the energy of the photon is equal or greater than the difference in energy between the ground state ($S_0$) and the first exited state ($S_1$) of the molecule.

There is another possible interaction between a photon and matter, this interaction is scattering. If a photon has the right energy to be absorbed it is very unlikely to be scattered. This likelihood of scattering increases relatively with photons of lower energy than the $S_0$-$S_1$ gap. Most of these scattered photons are scattered elastically (Rayleigh scattering). In this interaction the direction of travel will change but the photon energy will remain the same. Rayleigh scattering will therefore not contain any information about the physicochemical properties of the sample.

In 1928 the Indian physicists C.V. Raman and K.S. Krishnan discovered a far more useful form of scattering, Raman scattering. This form of scattering is called inelastic scattering, this means the photon energy of the initial photon is different from the scattered photon [6].

*Figure 1: Jablonski diagram of scattering and fluorescence processes.*
The difference in photon energy is caused by an exchange of energy between the incident photons and the molecules of the measured sample. There are two possible exchanges of energy: (I) the incident photon can lose energy to the molecule, which is known as Stokes Raman, or (II) the incident photon can gain energy from the molecule, which is called anti-Stokes Raman. The difference in photon energy between incident and scattered photons corresponds to the rotational or vibrational energy levels of the molecule [7].

The technique of Raman spectroscopy has a very low efficiency, most of the scattering of photons happens elastically (Rayleigh scattering) but this gives no information about the molecule. To increase the number of photons scattered by the Raman process a technique called Surface Enhanced Raman Spectroscopy (SERS) can be used [6]. SERS works by having the sample adsorbed to a roughened metal surface. It has been shown that the Raman efficiency can be enhanced by a factor of up to $10^{11}$ using this technique [8].

The enhancement factor has been studied and the prevailing theory says that the enhancement is predominately caused by the plasmonic effect. This theory states that the enhancement is caused by the excitation of localized surface plasmons of a metal substrate. Surface plasmons are a collective delocalized electron oscillations at the surface between a dielectric and a conductor, for example air and a metal. Surface plasmons can be excited when the energy of the plasmon oscillation is matched by the wavelength of the incident light. By using a metal nano particle the surface plasmons will become trapped and thus will not be delocalized. These localized surface plasmons induce a local strong electromagnetic field when excited. This electromagnetic field cause the enhancement of the Raman. This is due to the Raman intensity relation to the electric field $E$ and wavelength $\lambda$ as shown below [9]

$$I \propto \frac{E^2}{\lambda^4}$$

The strong local electromagnetic field close to a scattering particle will cause a large SERS enhancement. The SERS effect is strongest at the surface of the sample. Therefore, only molecules close to the surface benefit from the large enhancement factors, which explains why often monolayers of molecules are used as sample. The special resolution of Raman or SERS by the deflection limited spot size. This makes a special resolution lower than the wavelength of the laser almost impossible in practice.

The peaks in a (surface enhanced) Raman spectrum are very specific for the analyte. This makes Raman a good technique for identification, also any changes in the analyte can be monitored by consecutive measurements of the same sample. This makes SERS and Raman in general very applicable for reaction monitoring either in situ or in the lab.
2.2 Atomic force microscopy

Atomic force microscopy (AFM) is a technique to image the topology of a surface at the nanometer scale. One of the advantages of AFM is that it works on almost any material and that it does not require special sample preparation [10].

In atomic force microscopy, a sharp needle is raster-scanned over the sample surface in order to obtain a topography image with special resolution of a few nanometers. The measurement of the vertical displacement is done indirectly, the tip is most commonly attached to a micro-cantilever made of a flexible material commonly silicon. Interaction forces between the tip and the sample make the cantilever bend. The cantilever deflection is monitored by a laser, focused on the cantilever. The reflected beam is then detected by a position sensitive photo diode (Figure 2) to measure this bending [11].

Figure 2: Simplified representation of an AFM system [10].
There are three main measurement modes, contact mode, tapping mode and non-contact mode. In contact mode the cantilever is kept in contact with the surface while it is scanned across the sample. In this mode the lateral force exerted on the sample can be high which might hamper the applicability for fragile or soft samples. The force may also cause damage or change the sample in this way. These drawbacks led to the invention of tapping mode. In tapping mode the cantilever is oscillating at a frequency close to its resonance frequency and touches the sample only at the end of every oscillation. The amplitude of oscillation changes by the height of the sample. Non-contact mode is especially suitable for well-defined flat surfaces. In this mode the cantilever set at a few nanometers above the sample. The tip is attracted to surface which changes the frequency of the oscillation of the cantilever [10].

A feedback mechanism applies a voltage to the z-piezo in order to keep the cantilever at constant height of the sample throughout the measurement. The z position of the piezo is used to build the topography image. The image of the surface is made up points where the height is measured, and is displayed by applying a colour gradient to the height. The measured height data can also provide peak-to-valley distances, standard deviations of the height, and the slope of gradients. This data can be useful for the functionality of a biological or technological samples or in our case the monitoring of chemical reactions.
2.3 Samples

2.3.1 p-nitrothiophenol

For these experiments the dimerisation reaction from p-nitrothiophenol (p-NTP) to \(p'-\)dimercaptoazobisbenzene (DMAB) is used as a model system [12]. The reaction described above is induced by light and catalysed by silver. Strong changes in the Raman spectrum indicate product formation. The reaction can be monitored in time by following the increase of the aromatic N=N stretch vibration at 1420 cm\(^{-1}\) [13] (Figure 3 b), and the decrease of the symmetrical vibration of C-NO\(_2\) at 1330 cm\(^{-1}\) (Figure 3 a). During conversion of p-NTP to DMAB the peak at 1330 cm\(^{-1}\) will not fully disappear due to an overlap with the bending vibration of the C-H on the aromatic ring [14].

The reaction described above is induced by light.

Figure 3: SERS spectra and structures of (a) p-NTP and (b) DMAB measured on a gold substrate; Excitation wavelength of 633 nm, laser power of \(1.5 \times 10^4\) W/cm\(^2\) and integration time of 1 s [15].
2.3.2 Self-assembled monolayers

Metal surfaces have the tendency to adsorb organic materials, this is due to the lowering of the free energy of the metal and the environment. These adsorbates can change the stability of nano-structures in the metal and change the interfacial properties. Most surfaces coated with adsorbates are not well defined and because of this ‘random’ coating there are usually no specific chemical functionalities or reproducible physical properties on the surface of the coating [16].

A self-assembled monolayer (SAM) is a system where the organic adsorbate is organized in a crystalline (or semi-crystalline) structure. This crystallinity is acquired by the adsorption of molecular constituents from solution or the gas phase onto a solid surface. The constituents organize spontaneously into a crystalline (or semi-crystalline) structure. The spontaneous organization is caused by a head group with higher affinity for the metal surface. Thiols have a high affinity for noble metals, making it possible to generate a crystalline organic surface with a highly alterable functionality [16].

SAMs are very useful for reaction monitoring. This is thanks to the molecules all being immobilised on the surface, which creates a small, very stable reaction volume. In the case of monitoring using Raman, all molecules being in the same orientation give a more clear spectrum. This is because all the molecules are ‘exited’ in the same direction and the sample is more homogeneous.

Figure 4: SAM of (right) pNTP and (left) DMAB on gold coated glass.

2.3.3 Silver nano particles

To complete the model system silver nano particles (AgNP) will be drop cast on the SAM. The AgNP will aggregate and form small clusters on the surface that will be relatively far apart. These clusters act as a catalyst in the dimerization of p-NTP. This makes many small reaction hotspots on the surface of the sample, this mimics typical heterogeneity on a reactive surface.
3. Experimental

3.1 Sputtering

SERS substrates of p-NTP (technical grade, 80%, Sigma Aldrich Corp., St. Louis, USA) are prepared on glass coverslips (Thermo Fisher Scientific Gerhard Menzel B.V. & Co., Braunschweig, Germany). The coverslips are cleaned with ethanol (ethanol absolute, VWR International, Fontenay Sous-Bois, France) in an ultrasonic bath for 5 min. Transmission electron microscopy (TEM) grids (Cu, 3 mm, 150 mesh, SPI, West Chester, USA) are placed on the coverslip before sputtering 10 nm of gold (K550x, Quorem Emitech Ltd, East Grinstead, UK) to create a SERS active surface. After sputtering the TEM grids are removed leaving a pattern of 100 µm x 100 µm squares used for alignment between the AFM and Raman measurement.

To test what thickness of the gold substrate would give the best SERS signals we tested different conditions:

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<th>Table 1: Sputtering 5 times at 15 mA.</th>
<th>Table 2: Sputtering 2 times at 30 mA.</th>
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For the SERS measurements a SAM of p-NTP is created by placing the coverslip in a 12 mM ethanolic p-NTP solution for approximately 24 h. The excess of p-NTP is washed off by a ½-1 day bath in ethanol absolute. The ethanol is washed of using demi water. From the moment p-NTP is used the light exposure of the samples is limited to prevent the p-NTP from reacting. A silver colloid solution is synthesized using the method described by Lee and Meisel [1]. A 10% (v/v) solution is drop-cast and dried on the SAM. The sample is then rinsed with p-NTP solution and demi water.
3.2 Developed method

3.2.1 Sample preparation

The sample preparation was done as described in chapter 3.1, using the 5 times 15 mA current. These samples gave the clearest peaks in the p-NTP spectrum.

![Sample preparation image](image)

*Figure 5: Interferometry image of the TEM grid pattern on a glass substrate. The circle has an outer diameter of 3 mm. a) an AFM phase image of the gold surface, b) the 100x100 mm squares, c) and a line scan of the height profile [15].*

3.2.2 Atomic force microscopy

Atomic force microscopy is used to localize and characterize the clusters of silver nano particles. This is done by selecting one square in the grid pattern using the optical microscope integrated in the AFM (Dimension FastScan, Bruker). An area of 90 μm x 90 μm is scanned with a resolution of 2048 x 2048 points and a speed of 0.2 Hz (Tip: NSC15, Micromesh, Estonia).
3.2.3 Surface enhanced Raman spectroscopy

After the AFM measurement, a SERS measurement is carried out on an Invia Raman microscope (Renishaw plc., Gloucestershire, UK) with a HeNe laser (633 nm). The square measured in the AFM is selected using the optical system. Mappings are recorded in Streamline mode with a step size of 0.6 µm and an integration time of 1 second per point. To obtain the best possible lateral resolution, a 100x objective (NA: 0.8, Olympus, Tokyo, Japan) is used. Streamline mode makes it possible to scan large areas in short times. This is accomplished by projecting a line on the sample with the laser and scanning 50 points at the same time. The SERS maps are 100 µm x 100 µm to make sure both maps overlap. The first map is used to localize the SERS active spots and is therefore measured with lower laser power, $1.5 \times 10^3$ W/cm$^2$, to limit the conversion. Subsequent mappings are measured with a higher laser power, $1.5 \times 10^4$ W/cm$^2$ to be able to monitor the conversion of p-NTP to DMAB. The final measurement is done at extra high laser intensity, $3 \times 10^4$ W/cm$^2$, to make sure that the conversion is completed.
4. Results

4.1 Overlaying

In order to correlate morphology data to spectroscopic information, an overlay of AFM and Raman images has to be made. To do this, the patterns seen in both AFM and Raman have to match. Larger clusters are easy to locate in both AFM and Raman images and can thus be used for a rough correlation. In figure 6 the large clusters are the spots in the yellow circles. A totally empty spot in the AFM should result in no Raman signal, these empty spots can also be used for overlaying, which is also shown in figure 6. At the bottom right an empty triangle can be found in both measurements. In the Raman measurement there is a belt of high intensity in the bottom, due to this being in-between two squares of gold, giving this part of the sample a different height and roughness of the gold, resulting in a different SERS amplification.

Figure 6: a) AFM measurement of p-NTP SAM, yellow rings and triangle are used for overlaying the AFM and Raman images; b) Raman measurement of p-NTP SAM, yellow rings and triangle are used for overlaying the AFM and Raman images.
Making an overlay isn’t always this easy, some samples don’t have large clusters of silver particles or the big spots give no Raman signals (Figure 7). For these samples a Matlab script was written to filter the sizes of the particles in the Raman mappings. Filtering is done based on the size of the AFM spots not their height as done in figure 6. These new ‘maps’ consisted of fewer particle and should make it easier to align the two maps (Figure 8).

Figure 7: a) Original AFM map; b) Raman map

Figure 8: Filtered version of figure 7a, calculated for different area ranges of silver clusters: a) between 0.01-0.16 µm², b) 0.16-1.00 µm², c) 1.00-5.00 µm², d) larger than 5.00 µm².
4.2 Reaction kinetics

The amount of information obtained from a single mapping is enormous, so only a small part of the map is shown here for analysis. Figure 9 shows the procedure used to overlay the AFM and Raman data. This starts by taking a single square in the optical microscope image (Figure 9a). This square is then measured in both AFM (Figure 9c) and Raman(Figure 9b). These two data sets can then be overlaid, when zoomed in this will give reactivity data about a single clusters in the AFM (Figure 9d).

![Figure 9: Procedure of overlaying and zooming to get to small clusters.](image)

The alignment made can be used for the different SERS scans. This makes it possible to monitor individual clusters of AgNP over time. Figure 10 a shows eight clusters with SERS enhancement, not all of them analysed and not all of them cause conversion of the p-NTP. The graphs of figure 10 b-d show the reaction curves of clusters that cause conversion, the smallest clusters finish the conversion after 4 seconds (Figure 10 c), the larger clusters are slower and finish after 6-7 seconds. This small fraction of the data may indicate that the speed of conversion is inversely related to the size of the clusters (Figures 10 b-d). This might be due to the surface area of the reactive hotspot, this will be bigger with bigger spots, so more molecules to react. The small data set makes it impossible to know for sure if this is true, more data can be found in the paper [14].
Figure 10: a) Zoom in of AFM map, The coloured areas indicate reactive sites detected by the Raman measurement. Green: reaction, red: SERS enhancement, but no reaction, yellow: data not shown. 

b-f) reactivity graphs of the corresponding area in ‘a’. The red line indicates the reactant p-NTP and the green line indicates the product DMAB.
5. Discussion

The number of data points or Raman scans in the described method is weighted against speed. To gain more reliable reaction constants similar to what has been done by van Schrojenstein Lantman et al. [17] many more data points are required. This screening method does not provide an average value of a larger area but rather information about individual hotspots on the surface. The method is therefore best used for heterogeneous surfaces on which a multitude of reaction sites exist. These different sites can be quickly observed and studied in depth in further experiments.

Fast screening of reactive substrates requires high resolution morphological data as well as detailed chemical information. This method, that combines AFM and SERS, provides detailed reactivity information about a large sample area using a series of SERS images and a single AFM image of the same area. Accurate alignment of the data is crucial for meaningful reactivity data. Using optical microscopy options in both the Raman and AFM instruments, a square in the grid pattern is selected for analysis (Figure 9). Larger clusters, when they are present, can be used to align the AFM and SERS images accurately. The difficulty of alignment of datasets showing only very small Raman hotspots has to be further investigated. The filtering on area did not result in an easier alignment of the samples that were impossible before filtering. When alignment is possible more information about specific reaction sites can be obtained by zooming in on a region of interest (Figure 8d). It is clear that not all particles in the scan area induce significant SERS signals, therefore reactivity is also not expected at all locations.

The combined data of AFM and Raman is useful for two reasons: (I) statistical analysis of the whole map 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 more 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, acquisition time and reaction control. When sub-nanometer resolution is needed to resolve the morphological differences extra AFM measurements can be conducted on the region of interest at a later stage. In regards to SERS, interesting location can be selected and scanned with very low laser power density to not induce a reaction on the reaction site. The signal-to-noise ration of SERS maps obtained in this manner is sufficiently high. These small areas can also be studied with point mapping, this gives a slightly higher spatial resolution compared to line scanning.
6. Conclusion

A fast screening method for reactive surfaces has been developed. This method relies on a combination of AFM and SERS maps obtained with different instruments, the optimum conditions of both techniques are utilized without complex integrated systems. The lack of an integrated system makes this a relatively inexpensive method, while still enabling high-resolution examination of a large inhomogeneous sample surface. The AFM provides important high-resolution morphological information, while SERS is used to obtain sub-micron chemical reactivity data. By performing the measurement in series, the resolution and speed of both techniques are unaffected by each other. No contact with the surface is needed during a reactivity study, while SERS provides submicron chemical information with an extremely low detection limit that makes it possible to work with a monolayer of reactant on a surface. Single SERS maps can be recorded in 30 minutes for a 100 x 100 µm area with a pixel size of 0.6 x 0.6 µm. Therefore, complete reaction curves of a few hundred individual reaction sites can be collected within several hours. By using an alignment pattern in the substrate an accurate alignment between the datasets can be made. Hence, morphology can be linked to reactivity and to other potentially valuable information about reactive surfaces. Although the presented method is meant for fast screening of larger surface areas, it is also possible to use the alignment approach when selected locations need to be further investigated using high-resolution AFM imaging and/or diffraction-limited Raman mapping.
7. Acknowledgements

Special thanks to Dr. Camiel van Hoorn for supervising my experimental work and helping me with the Matlab coding. Without Camiel I wouldn’t have had the chance to work on this project as it is part of his PhD.

Also very special thanks to Dr. Arjan Mank of Philips Lighting for doing most of the measurements at the High tech campus in Eindhoven. Without the equipment of Philips the project wouldn’t be such a success.

And last but not least special thanks to Dr. Freek Ariese for supervising the writing part of my thesis, it must have been hard to get all my spelling and grammatical error from my writing due to me having dyslexia.
8. References

[8] Swe Zin Oo, Design, fabrication and optimization of large area chemical sensor based on surface-enhanced raman scattering (SERS) mechanism, PhD thesis University of Southampton, 2015, 7-33
List of abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>SERS</td>
<td>Surface-enhanced Raman spectroscopy</td>
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<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
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<tr>
<td>$S_0$</td>
<td>Ground state</td>
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<tr>
<td>$S_1$</td>
<td>First excited state</td>
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<tr>
<td>p-NTP</td>
<td>$p$-nitrothiophenol</td>
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<tr>
<td>DMAB</td>
<td>$p,p'$-dimercaptoazobisbenzene</td>
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<td>SAM</td>
<td>self-assembled monolayer</td>
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<td>AgNP</td>
<td>Silver nano particles</td>
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<td>TEM</td>
<td>Transmission electron microscopy</td>
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