NANOSTRUCTURED SURFACE ENHANCED RAMAN SPECTROSCOPY SENSOR FOR MARINE POLLUTANTS

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NANOSTRUCTURED SURFACE ENHANCED
RAMAN SPECTROSCOPY SENSOR
FOR MARINE POLLUTANTS

BY
TIMO KÜSTER

A MASTER’S THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
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OF

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Excess concentrations of nitrate and phosphate in seawater can lead to harmful algae blooms that damage coastal ecosystems, pose health risks and adversely impact commercial activity. Early *in situ* detection of over-nutrification is necessary for rapid response and mitigation plans. Commercial nitrate and phosphate sensors utilize UV-Vis spectroscopy methods. Those sensors show interference with ions present in seawater and are prone to biofouling, necessitating new approaches for *in situ* monitoring. Surface enhanced Raman spectroscopy (SERS) is a technique theoretically capable of single molecule detection, and therefore may be a promising approach for nitrate and phosphate detection. However, there are clear challenges as SERS sensing is negatively affected by interference in complex media and *in situ* sensing in a solution phase reduces accuracy and resolution. It is because of these challenges, in part, why much of the data reported in the literature are taken for purified samples that are then dried on a SERS substrate. Our goal is to address the engineering challenges for a SERS *in situ* seawater nutrient concentration measurement system. Batch and flow-through devices have been designed to incorporate commercially available, nanostructured gold SERS substrates. By benchmarking against 4-nitrobenzenethiol/ethanol solutions and ultrapure water spiked with nitrate and phosphate, our results show that our SERS devices can be used as a development platform for a seawater nutrient sensor, showing a route for a commercializable product that will greatly benefit scientific operations.
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PREFACE

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CHAPTER 1

INTRODUCTION

The ocean plays an essential role for life as we know it on our planet. It is habitat for millions of species, acts as a key part in climate control by storing greenhouse gasses and functions as a heat sink. It has been utilized by humans for centuries to provide food, transportation and economic success by enabling trade and tourism (Glöckner et al. 2012; Paytan and McLaughlin 2007).

However, the ocean is a complicated ecosystem. Nitrogen and phosphorous are naturally occurring in ocean water and an essential part of the nutrient cycle. The presence of these nutrients promotes the growth of marine life (Paytan and McLaughlin 2007; Baturin 2003; Vitousek et al. 1997; Zehr and Ward 2002).

Human activity, such as the use of fertilizers, discharge from wastewater treatment facilities as well as burning of combustion fuel can change the nutrient balance and lead to elevated levels of nitrogen and phosphorous in the ocean. Eutrophication is an effect describing increased plant growth in water bodies, caused by excess concentrations of phosphorous and nitrogen in ocean water. Cultural eutrophication describes eutrophication caused by human activity. If not addressed with countermeasures, especially nutrient reduction in affected water bodies, the composition of the plant life might become dominated by algae, which not only poses risk of toxin release, but can also result in oxygen depletion. Hypoxic or anoxic conditions mitigate fish populations and alter the water composition making it undesirable for utilization by humans (Smith and Schindler 2009; Conley et al. 2009).
Because of the challenges associated with elevated nutrient concentrations in ocean water, affordable, frequent, accurate and \textit{in situ} detection of nitrogen and phosphorous is of great importance to provide data to improve computer models and enable early prediction and warning mechanisms for algae blooms, so that countermeasures can be taken and negative effects associated with eutrophication can be reduced.

Current measurement methods include detection via chemical reactions, which either necessitate the collection and transport of samples to a research laboratory, adding a lag in data availability and a potential change of the sample composition due to biological activity, or require the availability of laboratory space, bulky instruments and potentially toxic materials on marine vessels (Patey et al. 2008). UV-Vis spectroscopy is a commercially available technique used for the detection of nitrate. Unfortunately, UV-vis is negatively affected by interference of the combined signal of bromide and nitrate in seawater, necessitating skilled operators to interpret and advanced equipment to collect the data (Johnson et al. 2013).

A promising technique to overcome some of the challenges associated with the previously mentioned measurement techniques is surface enhanced Raman spectroscopy (SERS). SERS is described as a molecular fingerprinting technique and is capable of single molecular detection when ordered metallic nanostructured substrates are employed (Nie 1997). SERS can be operated with lasers in the near infrared regime, showing little interference with water (Stiles et al. 2008). Highly portable handheld instruments are commercially available and offered by a variety of vendors, potentially
allowing for on site or *in situ* measurements (Anton Paar GmbH 2019; B&W Tek. 2019; Metrohm AG 2010; Serstech AB 2019).

While SERS is a promising detection platform, there are some clear challenges associated with this technique. SERS shows the highest signal intensity for analyte molecules adsorbed to nanostructured noble metal surfaces. The signal intensity decreases proportional to \( r^{-12} \) (Stiles et al. 2008) necessitating analyte molecules to be within 4 nm or less for the SERS effect to be observable. Because of this requirement, SERS measurements are commonly taken from analytes dried from solution on a substrate. In a solution phase measurement setup, the diffusive transport of analyte molecules to the surface can be a limiting factor, lowering the overall signal strength and/or imparting reduced responsiveness of the sensor (Moskovits 2005; Stiles et al. 2008).

The goal of this work is to demonstrate *in situ* SERS detection of nitrate and phosphate in aqueous solutions using commercial and custom fabricated nanostructured gold substrates. *In situ* detection is a critical step to assessing the suitability of SERS as a platform for continuous, field-deployed *in situ* nitrate and phosphate measurements. The goal was pursued through the following specific aims.

- **Aim 1.** Identify, for in-house designs fabricate, and characterize SERS active nanostructured substrates. SERS substrates were characterized by electron microscopy and benchmarked for SERS detection using 4-nitrobenzenethiol.
- **Aim 2.** Design and fabricate measurement devices for stationary and continuous *in situ* SERS detection. Devices were 3D-printed to physically secure SERS substrates and aid in the reproducibility of SERS measurements.
• Aim 3. Evaluate the detection performance of the SERS substrates for nitrate and phosphate in batch and continuous mode.
CHAPTER 2

BACKGROUND

2.1 The importance of nitrate and phosphate in the ocean

The work presented here focuses on the detection of nitrogen and phosphorus in the form of the ions nitrate and phosphate $\text{PO}_4^{3-}$ dissolved in water. Both nutrients are naturally present in ocean water bodies and play important roles in the environment for the growth of organisms, such as algae, plankton and certain bacteria (Patey et al. 2008; Conley et al. 2009). Due to e.g. use of fertilizer, wastewater discharge in the ocean and the burning of fossil fuels the bioavailability of nitrate and phosphate in many ocean water bodies has increased drastically in the past few decades and is still on elevated levels today (Paytan and McLaughlin 2007; Conley et al. 2009).

The underlying processes of eutrophication are extremely complex and vary strongly with the investigated site and their accurate description would exceed the scope of this report. For an overview of mechanisms and influencing factors we refer the reader to the study of the literature (Conley et al. 2009; Smith and Schindler 2009). Figure 1 gives a general idea about eutrophication and potential nutrient sources. The general principle is as following: excess nutrient concentrations lead to increased growth rates of phytoplankton (“small marine plants”) and macrophyte (“large marine plants”) vegetation, which are consumed by other organisms. Fixated nutrients sink to the bottom of marine water bodies in the form of fecal matter and dead organisms. Decay processes consume oxygen, leading to hypoxic or even anoxic zones. Furthermore, metabolism byproducts of certain algae and bacteria can be toxic for higher life forms such as fish or humans (Smith and Schindler 2009), lowering the
habitability of affected zones and rendering them less attractive for human activity. Taken to the extreme, affected water bodies might become inhabitable for higher life forms, leaving behind so called dead zones (Smith and Schindler 2009). Healthy water bodies act as reservoirs for greenhouse gases, with the formation of dead zones the natural balance is disturbed and the greenhouse gases are released into the atmosphere, potentially elevating climate change and leading to self-amplification effects by raising the water temperature and increasing the growth rate of algae (Glöckner et al. 2012; Paytan and McLaughlin 2007).

![Figure 1: Schematic of eutrophication and potential nutrient sources. Adapted from (PROJECT EUTROPHICATION PVT. LTD. 2015)](image)
2.2 Currently applied detection techniques

For the reasons described in the previous chapter, monitoring of nitrate and phosphate concentrations in marine water bodies is desired. Patey et al. (Patey et al. 2008) give an overview of commonly used techniques for nanomolar detection techniques of nitrate, nitrite and phosphate in marine water.

2.2.1 Nitrate and nitrite detection methods

The most widely used method for nitrate detection in sea water is the reduction of nitrate to nitrite, followed by spectral analysis of the products of the Griess reaction: the formation of a highly colored dye through diazotization with sulfanilamide (SA) and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) in the presence of nitrite (Patey et al. 2008; Correa-Duarte et al. 2015). A schematic of this reaction is given in Figure 2.

![Figure 2: Schematic of Griess reaction. With SA representing sulfanilamide and NED representing N-(1-naphthyl)-ethylenediamine dihydrochloride. Adapted from (Correa-Duarte et al. 2015).](image)

This method yields the total amount of nitrate and nitrite. Because the Griess reaction and its variations are specific to nitrite, the nitrate concentration can only be assessed through conducting the analysis twice: before and after the nitrate reduction step (Patey et al. 2008). Many variations of the Griess reaction have been developed
over the years, with reported limits of detection \( LOD = 3 \cdot \sigma_{\text{blank}} \), \( \sigma_{\text{blank}} \) being the standard deviation of the blank) as low as 1.5 nM for nitrate through a combination of the Griess reaction with segmented continuous flow analysis (SCFA) and a liquid waveguide capillary cell (LWCC) system (Patey et al. 2008). UV absorbance spectroscopy, as well as fluorescence spectroscopy and fluorescence quenching are additional methods used for nitrate detection. The reported limits of detection are between 6.9 (fluorescence spectroscopy) and 40 nM nitrate (UV absorbance spectroscopy) (Patey et al. 2008). UV absorbance spectroscopy has been successfully tested with 27 spatial profiling float systems deployed over 3 years at several ocean locations ranging from the subtropical ocean, over the Southern Ocean to the Arctic Ocean (Johnson et al. 2013). Johnson et al. reported a limit of detection of 0.4 µM for their system. Sensor drift and initial correction of the data was necessary to account for sensor variation (Johnson et al. 2013).

A potentiometric method with an ion-selective membrane permeable for nitrate was tested for monitoring of a river over two months. A limit of detection of 0.007 mg nitrate reported as nitrogen (~0.5 µM nitrate) was reported. The stability of the electrode was reported to be 5 months under laboratory conditions (Le Goff et al. 2003).

2.2.2 Phosphate detection methods

Nanomolar detection of phosphate is possible through the molybdenum blue method developed by Murphy and Riley (Murphy and Riley 1962): Phosphate reacts with ammonium molybdate under acidic conditions to 12-molybdophosphate. This complex is reduced by ascorbic acid or stannous chloride to a phosphor-MB complex that can be detected using spectroscopic methods (Patey et al. 2008). Many variations
of the Molybdenum blue reaction have been developed over the years, with reported limits of detection ($LOD = 3 \cdot \sigma_{blank}$, $\sigma_{blank}$ being the standard deviation of the blank) as low as 0.8 nM for phosphate through a combination of the Molybdenum blue reaction with segmented continuous flow analysis (SCFA) and a liquid waveguide capillary cell (LWCC) system (Patey et al. 2008).

An extension to the molybdenum blue method is the nowadays widely used magnesium induced coprecipitation “MAGIC” method, developed by Karl and Tien (Karl and Tien 1992). Phosphate dissolved in the sample is pre-concentrated by sodium hydroxide induced precipitation of brucite (Mg(OH)$_2$) from the solution and adsorption of the phosphate to the precipitate. The precipitate can be removed from the solution through centrifugation and is dissolved in acid and tested with the regular molybdenum blue method. Limits of detection as low as 0.2 nM can be achieved with this method (Patey et al. 2008). The nature of MAGIC requires large sample volumes of up to 250 mL and consists of several steps, possibly introducing contamination and making it challenging to automate (Patey et al. 2008).

Other methods utilize chemiluminescence – luminol (3-aminophtalhydrazide) emits blue light when oxidized. The method yields a strong signal and limits of detection comparable to the MAGIC method. It is not specific to phosphate and requires a pre-concentration step (Patey et al. 2008).

2.2.3 Summary of findings

Our review of the available measurement methods shows that the detection of nitrate and phosphate at nanomolar concentrations is possible. Many of the described methods require either manual handling, extensive know-how for the data analysis,
availability of reagents or large volumes of sample. Automatization of these methods is often difficult and requires additional instruments that might exceed the confined space on a marine vessel (Patey et al. 2008).

It is desirable to overcome these challenges to enable scientists to analyze water samples on research vessels without the need to store hazardous reagents that pose a risk of harming the environment. The availability of an easy to use, highly specific and cost efficient measurement method would enable researchers worldwide to collect more accurate data and would possibly allow for the early detection of excess nutrient concentrations to enable counter measures and to prevent or mitigate harm to humans and the environment. It is also obvious that the currently available measurement techniques are not sufficient to fulfill the requirements of such a sensor. A promising technique to overcome some of the most pressing challenges is Surface Enhanced Raman spectroscopy (SERS), which will be discussed in the following section.

2.3 Surface Enhanced Raman Spectroscopy

Raman scattering is an effect described by C.V. Raman in 1928 (Raman and Krishnan 1928). It is based on the inelastic scattering of incident light on molecules and results in a spectrum unique to each molecule and is therefore considered a molecular fingerprinting technique (Kneipp et al. 2002).

Figure 3 shows a schematic of the Raman scattering process. Incident light photons with a frequency $h\nu_L$ are scattered by a molecule. Scattering can be elastic or inelastic. Elastic "Rayleigh" scattering describes a scattering process in which the photon energy and therefore the frequency $\nu_L$ is not changed. If energy is exchanged between the incident photon and the scattering molecule the scattering is considered
inelastic. Inelastic scattering leads to a change of the vibrational energy of the molecule $h\nu_M$ and the energy of the scattered photon $h\nu_L$. Two types of inelastic scattering are possible – increase and decrease of the incident photon energy $h\nu_L$ through the scattering event. Transfer of energy from the photon to the molecule leads to a decrease in the photon energy and to a decrease of the photon frequency. According to the relation $\lambda = c/\nu$ (with $c$ being the speed of light) an increase of the photon wavelength $\lambda$ occurs in this case. This process is called Stokes scattering. If energy is transferred from the molecule to the photon the photon frequency increases, leading to a decrease of the wavenumber. This process is called anti-Stokes scattering and requires the scattering molecule to be in an excited energy level through e.g. a previous electromagnetic interaction, or an elevated temperature. The occurring wavelength shifts can be measured and used to obtain information about the structure of the investigated molecules (Kneipp et al. 2002).
Figure 3: Schematic representation of Raman scattering. Incident photons $h\nu_L$ are inelastically scattered from molecules. The energy of the characteristic molecular vibrations $h\nu_M$, results in scattered photons of lower frequency $h\nu_S$ (Stokes scattering) or higher frequency $h\nu_{aS}$ (anti-Stokes scattering). Image adapted from (Kneipp et al. 2002).

Compared to effects like fluorescence, Raman scattering is a very weak effect with Raman cross sections being 12-14 orders of magnitude lower than fluorescence cross sections (Kneipp et al. 2002). Partly because of this it was neglected as a scientific tool for several decades (Li et al. 2015). An advantage of Raman spectroscopy compared to fluorescence spectroscopy is the higher resolution of the Raman peaks compared to the broad adsorption/emission bands observed in fluorescence spectroscopy (Mosier-Boss 2017).

It was the discovery of Surface Enhanced Raman Spectroscopy (SERS) in the 1970s with reported Raman signal enhancements of up to $10^6$ (Fleischmann et al. 1974; Jeanmaire and van Duyne 1977; Albrecht and Creighton 1977) that made Raman spectroscopy more appealing to the scientific community. Nowadays signal
enhancements as high as $10^{15}$ can be achieved, allowing for single molecular detection (Nie 1997; Stiles et al. 2008).

With the discovery of SERS, utilization of Raman spectroscopy was investigated for a wide range of applications, such as in vivo detection of glucose levels in animal models (Stuart et al. 2006), as well as for explosive (Dasary et al. 2009) and drug abuse screening methods (Andreou et al. 2013). Monitoring of single molecular electrochemical processes was investigated (Cortés et al. 2010). SERS can be used for the monitoring of chemical reactions (Kundu et al. 2004) as well as for process and quality control in the food and pharmaceutical industry (Zheng and He 2014; McNay et al. 2011) and the detection of pesticides used in agriculture (Pang et al. 2016). Product developments such as fiber and handheld analyzers allow for easy on-site application of the previously mentioned techniques (Lucotti and Zerbi 2007; Zheng et al. 2014). SERS is considered a non-destructive technique (Du et al. 2013).

The surface enhancement effect is most likely to occur in the presence of nanostructured noble metal surfaces ranging from 10 to 100 nm in size (Moskovits 2005). SERS is a near-field effect that is strongest for analyte molecules adsorbed to the metal surface and scales with a factor of $r^{-12}$, with $r$ being the distance between analyte molecule and surface (Stiles et al. 2008). The distance dependency was shown experimentally by measuring SER spectra of pyridine adsorbed on silver film over nanosphere substrates covered with aluminum oxide ($\text{Al}_2\text{O}_3$) multilayers of varying thickness. A decrease of the SERS signal intensity by a factor of ten for an increase of the distance $r$ by 2.8 nm was observed in these experiments (Stiles et al. 2008).
Even though controversially discussed in the past (Moskovits 2005), two different mechanisms are believed to be responsible for the $10^6$ signal enhancement that was historically observed for SERS - electromagnetic enhancement with a contribution of $\sim 10^4$ and chemical enhancement with a contribution of $\sim 10^2$ to the total enhancement (Stiles et al. 2008; Moskovits 2005).

The electromagnetic enhancement is caused by localized surface plasmon resonance (LSPR) on the surface of the involved nanostructures, an effect that is visualized in Figure 4. Localized surface plasmons can be described as collective oscillations of conducting electrons of ionic metal cores (labeled “Electron cloud” and “Metal sphere” in Figure 4) caused by interaction with an electromagnetic field, in this case an incident light beam. Even though other excitations are possible, dipolar plasmon resonance is dominantly observed for small structures between 10 to 100 nm in size (Moskovits 2005). When the incident light source resonates with localized surface plasmons a local dipolar radiation field is emitted from the surface of the nano-structure, which can then excite the electromagnetic field of the analyte molecule (Moskovits 2005; Stiles et al. 2008). The magnitude of the Raman scattered field $E_R$ follows the relation

$$E_R \propto \alpha_R E_S \propto \alpha_R g E_0$$

with $\alpha_R$ being the Raman polarizability of the analyte molecule, $E_S$ describing the magnitude of the enhanced electromagnetic field at the nano-structured metal surface, $E_0$ being the magnitude of the incident light field and $g$ representing the averaged field enhancement over the surface of the nano-structure (Moskovits 2005). Additional field enhancement $g'$ can occur when the metal nano-structures scatter
Raman shifted light from the analyte. The amplitude of the SERS enhanced field $E_{\text{SERS}}$ is therefore given by $E_{\text{SERS}} \propto \alpha_R g g' E_0$. The average SERS intensity $I_{\text{SERS}}$ will be proportional to the square modulus of $E_{\text{SERS}}$ according to the following relation

$$I_{\text{SERS}} \propto |\alpha_R|^2 |g g'|^2 I_0$$

with $I_{\text{SERS}}$ and $I_0$ being the intensities of the incident and enhanced fields. At low-wavenumber bands $g$ and $g'$ become nearly identical, which allows for the simplification of above equation and yields the finding that $|E_L|^4 = |g|^4$ (Moskovits 2005).

The dipole radiation field can be generated on single structures, but is stronger in clusters of particles, with the inter-particle gaps allowing for the induction of enhanced electromagnetic fields as it is shown in Figure 5 (Moskovits 2005; Stiles et al. 2008). Areas on a SERS substrate or within a colloidal solution of SERS active particles that show enhanced Raman activity are called hot spots (Moskovits 2005).

![Figure 4: Schematic of localized surface plasmon resonance (LSPR). Adapted from (Stiles et al. 2008).](image-url)
Chemical enhancement is a much more general term and summarizes enhancement effects caused by processes such as charge transfer, that alter the electromagnetic field of the complex of nano-structured surface and analyte molecule in direct contact with each other (Moskovits 2005; Kneipp et al. 2002).

From the previously described underlying principles of SERS a series of attributes can be extracted that make a “good” SERS substrate – good meaning in this context a high signal enhancement, a high reproducibility, robustness and a long lifetime. To achieve this the surface requires the presence of homogenously distributed hot spots over the surface and must show effective analyte adsorption. The substrate must show a high resistance to photodegradation. Furthermore the availability of a standard to monitor for an eventual time dependence of the measurements is desired (Mosier-Boss 2017).

Materials commonly used for chemical detection include noble metal nanoparticles in suspension or deposited on a surface, that allow for tuning of the SERS enhancement by changing the size and shape of the particles. The SERS signal increases
with increasing particle size until the size approaches the scale of the wavelength. The signal increase can be explained by the higher number of available electrons, while the decrease in signal after reaching a critical particle size is caused by a shift of the particle excitation to non-radiative modes. The lower intensity at smaller particle sizes can be explained by a lowered conductivity of the particles as well as diminishing of the light scattering. The signal enhancement as a function of particle shape can be attributed to the increased availability of intrinsic SERS hotspots for particle shapes such as triangular or star-shaped structures (Mosier-Boss 2017).

Deposition of particles on a surface is possible through various chemical linking methods, application to filters, as well as embedding in paper matrices (Mosier-Boss 2017). It was shown that the effect is strongest for interparticle distances of 1 nm or less (Correa-Duarte et al. 2015; Moskovits 2005; Mosier-Boss 2017; Kneipp et al. 2002). Another method to immobilize nanostructures on surfaces is the fabrication on the surface itself. Fabrication of highly ordered nanostructures can be achieved through nanolithography techniques such as nanosphere lithography (NSL) and electron-beam lithography (EBL). NSL describes a technique in which nanoparticles are brought into contact with a surface and are used as a template to form metal films. EBL utilizes the solubility change of photoresist on noble metal surfaces when exposed to an electron beam. The electron beam is manipulated to draw into the photoresists to generate nanostructures on the surface that can then be used for SERS sensing (Mosier-Boss 2017).

A variety of SERS substrates is commercially available. Since the description of all of them would exceed the scope of this report we refer to the discussion by
Mosier-Boss (Mosier-Boss 2017). For this work two types of commercial substrates were tested. Gold particles incorporated in a paper matrix, distributed by Ocean Optics as “RAM-SERS-Au” (Ocean Optics) as well as gold coated silicon nanorods grown on a silicon wafer distributed as “SERStrate” by Silmeco (Silmeco ApS; Mosier-Boss 2017).

Common challenges with the application of SERS are the sensitivity of the system to inhomogeneities in the structure of the SERS substrate as well as of the inhomogeneities in the concentration of analyte over the surface. When drying after drop-casting without taking special measures, convective forces in the droplet lead to the accumulation of particles or analyte molecules on the edge of the droplet and leave behind stains after the drying process is completed. This process is described as “coffee stain effect” (Deegan et al. 1997)

Since SERS is extremely sensitive, these differences in local concentration heavily influence the signal. Several approaches exist to overcome these challenges – increasing the homogeneity of the surface as well as the distribution of the analyte above the surface and increasing the measured area by moving the laser or the substrate are common strategies to achieve this (Moskovits 2005; Mosier-Boss 2017). Another possibility is to conduct measurements in situ. Measuring in situ leads to a more homogenous distribution of molecules in the system but reduces the number of molecules in SERS active proximity to the surface. It can also add a diffusion limitation to the system. This results in a lower overall signal strength, but drastically increases the handling and automatization capabilities of SERS (White et al. 2012).
CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals being used

4-Nitrobenzenethiol (4-NBT, technical grade, 80%) was purchased from Sigma Aldrich and dissolved in 200 proof biology grade ethanol to create the benchmark solutions. Solutions were stored at a temperature of 4 °C. Nitrate solutions were prepared by mixing Sigma Aldrich sodium nitrate standards for IC of 4.4268 g/L with ultrapure water. Phosphate solutions were prepared by mixing Sigma Aldrich sodium phosphate standards for IC of 1 g/L with ultrapure water.

Ultrapure water was prepared with a MilliPore Milli Q3 UV system. Solutions were stored in vials sealed with Parafilm® at room temperature. The resistivity of the water was >18.2 MΩ.

Paper based gold nanoparticle SERS substrates marketed as “RAM-SERS-Au” were purchased from Ocean Optics and used as received. Gold sputtered silicon nanorods grown on a silicon wafer, marketed as “SERStrate Au” were purchased from SILMECO® and used as received. Whatman® Anodisc™ 13 - nano porous aluminum oxide discs with pore diameters of 20 and 200 nm and a disc diameter of 13 mm were purchased through Fisher Scientific and sputtered with gold to fabricate SERS substrates.
3.2 Equipment being used

Most Raman spectra shown in this paper were collected with one of the two following instruments. Initial Measurements were taken with a Raman Systems R3000QE spectrometer with attached fiber optic probe at a wavelength of 785 nm. The majority of the in situ studies was conducted with a Snowy Range® SIERRA 2.0 Raman spectrometer at a laser wavelength of 785 nm. The R3000QE has a laser spot diameter of 100 µm. The SIERRA 2.0 has a laser spot diameter of 40 µm. The SIERRA 2.0 instrument features an Orbital Raster Scanning mode (ORS) that moves the laser beam over a circular area with a diameter of ~2 mm, following the pattern shown in Figure 7 c). ORS was activated for all measurements with the SIERRA 2.0. If not specified otherwise a laser power of 250 mW was used for the R3000QE and a laser power of 100 mW was used for measurements with the SIERRA 2.0. Pictures of the two instruments are shown in Figure 6 a) and b). The software “PEAK”, used to communicate with the SIERRA 2.0, was provided by the manufacturer in version 1.3.68.
Figure 6: Images of Raman spectrometers being used a) Fiber optic part of Raman Systems R3000QE and b) Snowy Range Instruments SIERRA 2.0.

Scanning Electron Microscopy (SEM) images were taken with a Zeiss Sigma VP field emission scanning electron microscope, running on SmartSEM, software version 5.06.

3.3 Experimental procedures

The following subsections contain information on how the measurements for the individual experimental steps of the project were conducted. The description begins with a description of the obtaining of spectra of the measurement solutions without surface enhancement in section 3.3.1, to in situ measurements of 4-NBT, nitrate and phosphate with no flow in section 3.3.2 and under flow conditions in section 3.3.3.
The experiments were started with varying concentrations of 4-NBT in ethanol as a benchmark. 4-NBT was chosen because the thiol group binds to the gold surface and therefore a prominent SERS signal can be observed. After confirming the measurement principle measurements with nitrate and phosphate solutions were conducted.

3.3.1 Normal Raman measurements

The confirmation of literature values of observed peaks was conducted by transmittance cuvette measurements with an analyte volume of 2 mL in the SIERRA 2.0 instrument, as it is illustrated in Figure 7 a. The instrument parameters were a laser power of 100 mW with integration times Δt of 10, 20, 30, 40 and 60 s and activated Orbital Raster Scanning (ORS). The system was covered with an ambient light blocking laser protection housing. Background spectra were collected and automatically subtracted with the instrument software. Spectra for each integration time were collected in triplicate and averaged. For the 4-NBT benchmark 200 proof ethanol as well as a 10 mM 4-NBT/ethanol solution were tested. Measurements of nitrate and phosphate solutions were conducted with as received nitrate and phosphate stock solutions and repeated with 0.25 M concentrations of the two analytes.

Figure 7 c) shows the laser pattern of Orbital Raster Scanning. ORS was used to decrease the influence of surface variability by measuring a spot of ~2 mm in diameter on the surface, while maintaining the high intensity of a small laser spot of ~40 µm diameter. ORS was activated for the transmittance cuvette experiments to be in better agreement with the later conducted top to bottom measurements, illustrated by Figure 7 b) and described in section 3.3.2.
3.3.2 In situ batch measurements

All measurements in in situ batch mode were conducted in the bottom to top measurement mode. Commercially available Silmeco® “SERStrate” substrates were tested and used as received.

Experiments in “batch mode” were carried out using a 3D-printed beaker capable of forcing the hydrophobic SERStrate substrates to submerge and stay in position. No air layer was visible after submission in water. The beaker volume is 0.75 mL. Parts were printed using polylactic acid (PLA) filament using a MakerBot Replicator+. A schematic of the beaker with the projected path of the laser beam and the position of the substrate is shown in Figure 8. The upper grey part fits tightly in the beaker and forces the substrate to stay in position, when filled with analyte solution.
Figure 8: Exploded view of 3D-printed beaker, with SERS substrate (golden), 3D-printed beaker cap, to hold the substrate in place and LASER beam.

Measurements were taken with the Raman Systems R3000QE as well as the Snowy Range SIERRA 2.0. The R3000QE instrument was run with a laser power of 250 mW and with an integration time of 30 s for each data point. The SIERRA 2.0 was run at the maximum laser power instrument setting of 15, which equals to a laser power of 100 mW and an integration time of 5 s. SIERRA 2.0 measurements were conducted with enabled ORS. Before each concentration series, the 3D-printed beakers were washed with ultrapure water and dry blown with compressed nitrogen. The systems were carefully assembled and placed under the laser, using the grey 3D printed spacing system shown in Figure 9 to ensure that the same spot on the substrate was hit for every measurement.
Figure 9: 3D-printed positioning piece for sample holders for use with Snowy Range SIERRA 2.0.

After assembly the laser was focused on the substrate and the background of the dry substrate was measured in triplicate and averaged. The system was covered with an ambient light blocking laser protection housing. Background spectra were collected and automatically subtracted with the instrument software for every spectrum. Spectra for each integration time were collected in triplicate and averaged. The system was then filled with 0.75 mL of pure solvent (ethanol or ultrapure water) and the laser was refocused to account for the focal change caused by the introduced media. Measurements were started as soon as the laser was focused. Each concentration was measured either every two or 5 minutes for up to one hour. Due to evaporation of ethanol the 4-NBT measurements had to be stopped after approximately 20 minutes. Concentrations were changed by pouring the solution out of the beaker and rinsing it three times with 0.75 mL of solvent. The beaker was then filled a fourth time with solvent and spectra of the cleaned substrate in solution were taken in triplicate to investigate the cleaning capabilities. After measuring, the fourth solution was poured out, and the beaker was re-filled with 0.75 mL of solution of the next higher
concentration. The measurement procedure was repeated until the highest concentrated solution was reached. After each experiment the substrates and substrate holders were dried, moved to a petri dish and sealed with parafilm. The so prepared systems were stored at room temperature in the dark for future analysis.

3.3.3 In situ continuous flow measurements

A flow channel was designed and manufactured using 3D-printing technology. The system is shown in Figure 10 and consists of a 525 µL flow chamber with a fit for the laser lens of the Raman spectrometer. Silmecos® SERStrate substrates can be mounted inside the channel. Lens and fluid are separated through a microscope cover glass slip. The device has connectors for 1/16” tubing.

A picture of the assembled system without the Raman spectrometer is shown in Figure 10. The opening that can be seen on top of the system fits the lens of the Snowy Range SIERRA 2.0 instrument and ensures that the distance between lens and substrate is kept constant as well as that ambient light is blocked.

Triplicates of dry spectra were collected before each run as before with the batch system. To test the measurement capabilities under continuous flow, liquid was pumped through the system at a rate of 0.18 mL/min, using a peristaltic pump, resulting in a residence time of 2.9 min. The Reynolds number in the measurement chamber for water and ethanol at 20 °C was calculated to be $Re_{\text{water}} = 0.54$ and $Re_{\text{ethanol}} = 0.36$, indicating laminar flow conditions for both cases. With laminar flow, diffusion through the boundary layer over the SERS substrate is going to be the dominant mechanism to force analyte molecules into the SERS active distance of the substrate. The Reynolds number calculations are shown in appendix A-2. Solutions with increasing analyte
concentrations were tested. Measurements were taken every 71 s with an integration time $\Delta t$ of 10 s, automated background subtraction and activated ORS for 30 minutes for each concentration.

Each measurement series was started by filling the device with solvent (ethanol or ultrapure water). The concentration was increased by switching the flow channel inlet to the next higher concentration. After testing the highest concentration, solvent was pumped through the device for 60 minutes, to clean the system. Spectra were continuously collected with the same parameters as before while flushing the device. After the flushing step the devices were emptied, dried and the inlets and outlets were sealed with parafilm and stored at room temperature for future analysis.

![Prototype of 3D printed flow channel attached to peristaltic pump before contact with Snowy Range SIERRA 2.0.](image)

*Figure 10: Prototype of 3D printed flow channel attached to peristaltic pump before contact with Snowy Range SIERRA 2.0.*
3.4 Evaluation methods

3.4.1 Baselining

Raman spectra were baselined using the TBB Baseline method, a polynomial fit method implemented in the “PEAK” software distributed with the Snowy Range instrument. The sensitivity of the method was left at the standard parameter of 115 out of 1000. Baselined spectra were exported to Microsoft® Excel for further analysis. Figure 11 shows the comparison of a raw spectrum of 1 mM 4-NBT collected in the *in situ* batch measurement setup. The baselined data was manually shifted by a value of 50,000 a.u. to allow for a better comparison of the spectral features. The figure shows the same signature peaks for both data sets, but the background is straightened, meaning that the method produced reliable baselining results. Throughout our data analysis this finding was consistent.

![Figure 11: Comparison of TBB baselined data with raw data for 1 mM 4-NBT spectrum measured in the in situ batch measurement setup with an integration time $\Delta t = 5$ s. The baselined data was manually shifted by 50,000 a.u. to allow for the easier comparison of spectra features.](image-url)
3.4.2 *Standard normal variate*

Baselined data was normalized as necessary by standard normal variate method, described in (Gautam et al. 2015), according to the following equation:

\[ Y_{snv}(x) = \frac{Y(x) - \bar{Y}}{\sigma} \]

Where \( Y_{snv}(x) \) describes the standard normal variate modified peak intensity at a given wavenumber \( x \). \( \bar{Y} \) represents the average intensity of the entire spectrum and \( \sigma \) stands for the standard deviation. The method can produce negative values for the baselined spectra. In case of the presence of negative normalized intensities, the entire spectrum was manually shifted to zero, to allow for the comparison of normalized intensities by a common starting point.

3.4.3 *Time dependency*

The individual spectra of a concentration series were investigated for the presence of the characteristic peaks of the analyte of interest. If characteristic peaks were present the peak intensity was plotted as a function of time. For data sets showing no time dependency the individual spectra were averaged over the entire time series, resulting in a single spectrum of averaged intensities. For data sets showing a time dependency only intensities after reaching a steady state were used for the averaging procedure. Peak intensities for the characteristic peak of interest were extracted and plotted as a function of concentration. Correlation functions were applied to allow for the quantification of analyte concentrations in unknown solutions. This was done for spectra before and after normalization.
3.4.4 Limit of detection calculation

The limit of detection LOD for the different substrates was calculated by applying a linear fit of the form

\[ y = m \cdot x + b \]

to the plot of characteristic peak intensity over analyte concentration. Only the linear portion of the plot was considered. The following equation, described in (Shrivastava and Gupta 2011; Armbruster and Pry 2008) was used to calculate the limit of detection from the slope \( m \) and the standard deviation \( \sigma_b \) of the y-intercept \( b \):

\[ LOD = \frac{3 \cdot \sigma_b}{m} \]
CHAPTER 4

RESULTS AND DISCUSSION

Various SERS substrates, commercially available as well as in-house designs have been tested in a preliminary study that is shown in Appendix A-1. Initial benchmark measurements with 4-NBT dried out on the substrates showed superior performance of the SILMECO® SERStrate substrates compared to the other tested substrates. Therefore, the work presented here focuses on the measurement capabilities of SILMECO SERStrate substrates for the detection of nitrate and phosphate in aqueous solutions. The findings in this chapter will be discussed by analyte, beginning with 4-NBT measurements in section 4.1 followed by nitrate and phosphate measurements in section 4.2.
4.1 Discussion of 4-NBT measurements

The following discussion of 4-NBT measurement results follows the individual experiments of the experimental procedure and will guide the reader through the various process steps of the method evaluation. The discussion will begin in section 4.1.1 with transmittance cuvette Raman measurements of 4-NBT to confirm the characteristic peaks shown in Table 1, followed by the discussion of testing of in situ batch measurements in a 3D printed beaker in section 4.1.2. Section 4.1.3 will focus on the evaluation of measurements in a 3D-printed continuous flow channel before section 4.1.4 goes into detail about the obtained levels of detection for 4-NBT in ethanol solution.

*Table 1: Characteristic 4-NBT peaks and their assigned modes as well as the molecular structure of 4-NBT. Printed bold is the main peak. Spectral data and mode assignments adapted from (Kim et al. 2003).*

| Wavenumber (cm⁻¹) | Mode assignment     | C-C stretching mode | Symmetric stretching of NO₂ | C-H bending mode | C-H bending mode |
|-------------------|---------------------|---------------------|-----------------------------|------------------|------------------|
| 1573              |                     | 1346                | 1110                        | 1082             |                  |
| Molecular structure of 4-NBT |                     |                     |                             |                  |                  |

![Molecular structure images for C-C stretching mode, Symmetric stretching of NO₂, C-H bending mode, C-H bending mode]
4.1.1 Normal Raman measurements

The confirmation of literature values was conducted by transmittance cuvette measurements as described in section 3.3.1, p. 22. The results are shown in Figure 12. The expected peaks of 4-NBT are highlighted with arrows. The characteristic 4-NBT peaks are in good agreement with the literature (Kim et al. 2003), but very small in comparison to the ethanol peaks. This means that 4-NBT can be detected with our instrument and is therefore a suitable benchmarking molecule for our purposes. However, the circled peak at 879 cm\(^{-1}\) is the main ethanol peak and is cut off due to the limitation of the photodetector to 60,000 counts, meaning that the detectability in the absence of a SERS substrate is limited with our instrument. The dominance of the ethanol signal limits the use of longer integration times in solution, because of overcompensation of the 4-NBT peaks. As the thiol group of 4-NBT binds to gold the detectability is suspected to be higher using SERS. This will be shown in the following section 4.1.2.
Figure 12: Comparison of Raman spectra of 200 proof ethanol and 10 mM 4-NBT in ethanol solution measured in transmittance cuvette configuration with an integration time of $\Delta t = 10$ s.

4.1.2 In situ batch measurements

After confirming that the detection of 4-NBT using Raman spectroscopy is possible, testing of in situ batch measurements was conducted with the 3D printed device, discussed earlier and shown in Figure 8.

Figure 13 shows a comparison of the averaged spectra of ethanol as well as 1 mM 4-NBT in ethanol solution. The laser power was 100 mW and the integration time was set to 5 s. As expected, the 4-NBT spectra are clearly visible and show a much higher intensity than the normal Raman measurements discussed in section 4.1.1 and shown in Figure 12. It must be noted that the symmetric NO$_2$ stretching mode was shifted from 1346 cm$^{-1}$ to 1331 cm$^{-1}$ for 4-NBT in the presence of a SERS substrate and is most likely related to the covalent binding of the molecule to the surface. A shift of the wavenumber to lower values as present here means that the scattered light has a shorter wavelength and therefore higher energy. Since the scattered light energy
depends on the incident beam energy and the scattering event, less energy must have been transferred during the scattering process in presence of a SERS substrate. By covalent binding of the 4-NBT molecule to the gold surface the intermolecular composition changes, which as a result causes the observed peak shift.

The higher signal intensity is due to the covalent binding of the thiol group to the gold surface and the surface enhancement effect. This also means that the local 4-NBT concentration was increased at the surface of the SERS substrate compared to the solution. 4-NBT solutions of increasing concentrations were tested. The results presented in Figure 14 are normalized by the standard normal variate method to account for signal variations due to changes of the liquid level in the system, and were obtained from the Raman Systems R3000QE instrument. They show a strong correlation between the 4-NBT concentration and the intensity of the most dominant peak at a wavenumber of 1331 cm\(^{-1}\). Similar results were achieved with the SIERRA 2.0. The obtained calibration curves are compared in Table 2.

| Table 2: Comparison of linear calibration functions obtained with Snowy Range SIERRA 2.0 and Raman Systems R3000QE. |
|---------------------------------------------------------------|
| **Instrument** | **m** | **b** | **R\(^2\)** |
| Sierra 2.0 | 0.0017 | 0.05 | 0.98 |
| R3000QE | 0.0002 | 0.0014 | 0.99 |

These findings indicate that the designed measurement setup, consisting of the 3D-printed beaker and a SILMECO SERStrate, is capable of \textit{in situ} detection of 4-NBT in ethanol solution.
Figure 13: Averaged spectra resulting from in situ measurements of ethanol and 1 mM 4-NBT/ethanol solutions in 3D printed substrate holder with SILMECO SERStrate. The ethanol spectrum was shifted manually by 40,000 a.u. to allow for easier comparison of spectral features.

Figure 14: Peak intensity at 1331 cm\(^{-1}\) for increasing concentrations of 4-NBT in ethanol solutions using the Raman Systems R3000QE.
4.1.3 *In situ continuous flow measurements*

We believe the use of flow channels can support the diffusion of analyte molecules to the laminar boundary layer on the surface of a SERS substrate, leading to a higher quality signal. To test the influence of flow on the measurement system the experiments discussed here were conducted. The general functionality of the system was assessed with 4-NBT in a similar way as before for the *in situ* batch system. The 4-NBT concentration in the inlet was kept constant and only changed after the chosen measurement time for a given concentration. Due to binding of 4-NBT molecules to the surface the actual 4-NBT concentration that was measured was different from the concentration in the inlet stream. As the substrate was not changed in-between concentration measurements, 4-NBT from the previous experiment was already attached to the substrate surface, further altering the measured concentration. Nonetheless an increase of inlet concentration was expected to show an increase in signal strength.

The flow channel was filled with pure ethanol, while SERS spectra were collected. An overview of representative averaged spectra at various 4-NBT concentrations is given in Figure 15. With increasing concentration, the intensity of the four main 4-NBT peaks at 1079, 1109, 1331 and 1573 cm\(^{-1}\) increases. This finding already shows that 4-NBT can be detected using our flow channel design and a SERStrate substrate.
Figure 15: Representative spectra collected from 4-NBT/Ethanol solutions in a 3D-printed flow channel with an integration time of 10 s and activated ORS. Three different magnifications were chosen to show the various spectral features.

To gain an understanding of the system response to increasing concentrations, an event that will be observed in an on-site application such as the deployment of a measurement device on a buoy, a plot of the intensity of the main peak at 1331 cm\(^{-1}\) over the entire experimental time is shown in Figure 16. The ideal residence time of 2.9 minutes of the device is represented by the labeled bar on the right side of the graph. The real residence time of the device is much higher, as backflow can occur in the chamber inlet due to the presence of edges and small quantities of liquid can get trapped in rough surface features on the wall of the device resulting from the additive
manufacturing process. An indicator of the real residence time of the system can be obtained from observation of the response to the final flushing step with ethanol. The signal obtained during the flushing step, labeled as “Ethanol (flush)” plateaus after ~11 minutes of running time, which is an indicator for the overall response time of this system. This also shows that the system deviates from the previously calculated ideal residence time by a factor of \( \frac{11\,\text{min}}{2.9\,\text{min}} = 3.79 \). From this finding follows that the inlet concentration of the flow channel remains at the previous lower 4-NBT concentration for ~11 minutes after increasing the concentration.

The system shows a clear response to increasing concentrations. The curves for inlet concentrations of 0.01 to 1 \( \mu \text{M} \) are still raising when the next higher concentration is introduced, meaning that the system had not reached an equilibrium state for these concentrations. The Reynolds number \( Re_{\text{ethanol}} \) had a value of 0.36 under the tested flow conditions, meaning that the system was in the laminar flow regime. Characteristic for laminar flow through a channel or tube is the presence of a stationary boundary layer at the edges of the system, in this case the walls of the flow channel and the SERS substrate at the bottom of the device. We suggest that the transport of analyte molecules from the stream into the boundary layer and ultimately to the surface of the SERS substrate was diffusion limited. According to Fick’s law diffusion rates depend on the concentration gradient and the diffusion coefficient. The diffusion coefficient depends on the type of molecule, the medium as well as the temperature. As all these parameters are close to constant in our system the diffusion coefficient must be close to constant, too. As the concentration gradient is increased in the system by increasing the concentration at the system inlet, the diffusion rate from the stream into the boundary
layer increases too. The concentration versus time curve for 100 µM 4-NBT in ethanol shows a decrease of the growth rate with increasing measurement time, indicating that the system was approaching an equilibrium state for the given parameters. This observation indicates that the concentration gradient at an inlet concentration of 100 µM 4-NBT was high enough to force diffusion of 4-NBT molecules in measurable concentrations through the boundary layer. Increasing the concentration further leads to a signal increase over time. The sudden decrease in signal that can be observed around 180 minutes is related to air bubbles that had been introduced to the system while the concentrations were changed. By removing the air bubble, the signal could be regained. Switching the inlet to pure ethanol to flush the system at the end of the measurement series leads to a lowered signal increase speed.

Plotting the normalized averaged peak intensity after 11 minutes of the most dominant 4-NBT peak at 1331 cm\(^{-1}\) against the concentration at the inlet, leads to the plot shown in Figure 17. The standard deviation for all concentration series is low and is overlapped by the plot markers. The data shows a power law correlation spanning the concentration range from 10 nM to 1 mM, with an \(R^2\)-value of 0.98. As the system was not in equilibrium the power law correlation most likely results from the diffusion and chemisorption processes between incoming solution and the SERS substrate. A limit of detection calculation is not reasonable, as the system was not in equilibrium.
Figure 16: Plot of characteristic 4-NBT peak intensity at 1331 cm\(^{-1}\) as a function of time for increasing concentrations ranging from 10 nM to 1 mM.

Figure 17: Averaged normalized peak intensity at a wavenumber of 1331 cm\(^{-1}\) as a function of 4-NBT concentration at the inlet in a 3D-printed flow channel with SILMECO SERStrate substrate. The first 11 minutes of each inlet concentration measurement were ignored to account for the system response time.

Because the laser of the Raman spectrometer was focused on the surface of the SERS substrate, the obtained signal intensity is a measure of the 4-NBT concentration.
at the substrate surface. Figure 17 only shows the concentration at the inlet of the system. To gain an understanding of the concentrations at the surface the SIERRA 2.0 calibration curve shown in Table 2, p. 35 and the averaged normalized intensity data shown in Figure 17 were used to calculate the surface concentrations in the flow channel. The results of these calculations are presented in Figure 18 and show the relation between the inlet concentration and the concentration at the surface of the substrate. The dotted 45° line represents the idealized case that the inlet concentration equals the surface concentration. Examining Figure 18 shows that for low 4-NBT concentrations of 10 and 100 nM the surface concentration is close to the ideal case. As expected, the surface concentration increases with increasing inlet concentration. It can also be seen that the surface concentration increases much slower than the inlet concentration, which indicates that a transport limitation of 4-NBT molecules to the surface must be present.

**Figure 18:** Log-log plot of concentration at inlet vs. concentration at substrate of surface calculated from calibration curve applied to normalized data after 11 minutes and theoretical response for an ideal system in which the inlet concentrations equals the surface concentration.
4.1.4 Limit of detection

The 1331 cm\(^{-1}\) peak intensity versus 4-NBT concentration plot of \textit{in situ} batch measurements presented in section 4.1.2, Figure 14, showed a clear correlation and can therefore be used to carry out a limit of detection calculation according to the procedure described in section 3.4.4. The data follows a linear trend of the form \(0.0002 \cdot x + 0.0014\) and can therefore be used for the limit of detection calculation without further modification. Using the Excel line estimation function “linest” yields the standard deviation of the y-intercept \(b\) to be \(\sigma_b = 0.0085\) and therefore the limit of detection for the \textit{in situ} batch measurement device is LOD = 111 nM 4-NBT.

The continuous \textit{in situ} measurement data presented in Figure 17 was not in equilibrium and would therefore not yield a reasonable result for a limit of detection calculation.

4.2 Detection of nitrate and phosphate

After showing in the previous section 4.1 that the developed measurement system works with 4-NBT solutions, the evaluation of aqueous nitrate and phosphate solutions using SERS will be evaluated following the same procedure as before for 4-NBT in ethanol.

Table 3 shows literature values for Raman peaks of nitrate and phosphate for comparison in the next sections.
Table 3: Selected characteristic peaks of crystalline sodium nitrate and 1 M aqueous sodium nitrate solution as well as sodium phosphate. The main peaks are printed bold. Nitrate data was adapted from (Daniel R. Lombardi et al. 1994; Rousseau et al. 1968; Waterland et al. 2001) and phosphate data was adapted from (S. K. Sharma et al. 2006; Toupy-Krauzman et al. 1979). Crystalline sodium nitrate mode assignments adapted from (Rousseau et al. 1968), 1 M sodium nitrate from (Waterland et al. 2001) and sodium phosphate assignments from (Toupy-Krauzman et al. 1979).

| Crystalline NaNO₃ | Wavenumber (cm⁻¹) | Mode assignment | Symmetric stretching |
|-------------------|------------------|-----------------|---------------------|
|                   | 1385.2           | ν₃ vibrational mode |                      |
|                   | **1067.5**       | Symmetric stretching | ν₄ vibrational mode |
|                   | 726.1            |                 |                     |

| 1 M NaNO₃ (aq.)   | Wavenumber (cm⁻¹) | Mode assignment | ν₄ vibrational mode |
|-------------------|------------------|----------------|---------------------|
|                   | **1047.7**       | Symmetric stretching |               |
|                   | 719.0            |                 |                     |

| Sodium phosphate Na₂HPO₄ | Wavenumber (cm⁻¹) | Mode assignment | ν₃ vibrational mode of tetrahedral (PO₄³⁻) | Symmetric stretching |
|--------------------------|------------------|-----------------|-------------------------------------------|---------------------|
|                          | 1290             | In plane mode of (P)O–H groups | 997 | 911 |
|                          | **997**          | ν₃ vibrational mode of tetrahedral (PO₄³⁻) | 911 | 911 |
|                          | 911              | Symmetric stretching | 911 | 911 |
4.2.1 Normal Raman measurements

In order to quantify the detectability of nitrate and phosphate with our SERS measurement setup, the peak locations of both analytes must be known. Figure 19 shows Raman spectra collected for 0.25 M nitrate and phosphate stock solutions measured with the transmittance cuvette Raman setup available in the SIERRA 2.0 instrument. A clear peak is observed for nitrate at a Raman shift of 1047 cm\(^{-1}\). The phosphate peak at 989 cm\(^{-1}\) is less dominant, but clearly visible in the spectrum. These findings are consistent with the values of the main peaks shown in Table 3.

![Raman spectra of 0.25 M aqueous solutions of a) Nitrate; b) Phosphate with sodium as the counterion measured at an integration time of 20 s and at a LASER power of 100 mW.](image)

4.2.2 In situ batch measurements

The hydrophobic nature and the low weight of the SERStrate substrates, that were used for the investigation of the in situ SERS detectability of nitrate and phosphate made it challenging to submerge them in aqueous solutions, as it is illustrated in Figure 20 a). Because of this, the previously described and in Figure 20 b) displayed 3D printed beaker was designed. The beaker is fully submerged in the picture to illustrate the
concept. The actual *in situ* measurements were taken by filling the beaker with 0.75 mL of analyte solution, and followed the procedure described in section 3.3.2.

**Figure 20:** a) SERStrate floating in beaker b) Prototype of 3D printed beaker submerging SERStrate in water; later measurements were conducted by filling the beaker with, rather than submerging it in, analyte solution.

Figure 21 shows characteristic spectra averaged over the measurement time for *in situ* nitrate measurements at increasing concentrations taken with the Snowy Range Instruments SIERRA 2.0. The observed signal intensities are very small in comparison to the more dominant features of the spectra, therefore a close-up of the data range of interest is shown. The inset shows the full spectra for comparison. A peak shift from 1047 cm\(^{-1}\) to 1079 cm\(^{-1}\) was observed. This behavior is in good agreement with the literature (Daniel R. Lombardi et al. 1994). The peak intensity at 1079 cm\(^{-1}\) increases with increasing nitrate concentration.
Figure 21: Development of shifted nitrate peak at 1079 cm\(^{-1}\) as function of concentration. Top: full spectrum, bottom: Close-up of the characteristic peak.

Figure 22 shows the characteristic plot of the normalized intensity of the previously identified characteristic nitrate peak at 1079 cm\(^{-1}\) as a function of the measurement time over the course of one hour for the water background, as well as for a concentration of 332 nM nitrate. The measurement for pure water shows a weak periodic background signal with values between 0 and 0.11. The time frame of the periodic signal lays between 4 and 5 minutes. With the assumption that the used ultrapure water, as well as the substrate and the beaker, were free from contaminants additional possible explanations of the periodic variation can be found: either a physical change of the structure of the surface or a change of the chemical composition of the adsorbed layer of molecules is possible. A change of the orientation of the gold covered
nanorods seems possible, but a change of the adsorbed layer, such as ad- and desorption of analyte molecules is more likely, as only an non-specific attraction of the nitrate ions to the surface is to be expected. The normalized intensity for a concentration of 332 nM nitrate is higher than the background and the periodic behavior is still observable. With few exceptions this same trend can be observed for all investigated spectra for the Snowy Range Instruments SIERRA 2.0.

![Graph](image)

*Figure 22: Plot of peak intensity over time for the characteristic peak intensity at 1079 cm\(^{-1}\) for water and a concentration of 332 nM nitrate collected with SIERRA 2.0.*

Figure 23 and Figure 24 show the development of the normalized nitrate peak as a function of concentration for measurements conducted with the Snowy Range Instruments SIERRA 2.0 and the Raman Systems R3000QE device. The characteristic peak on the Raman Systems R3000QE was shifted to 1072 cm\(^{-1}\) instead of 1079 cm\(^{-1}\).
Figure 23: Peak intensity of characteristic nitrate peak at 1079 cm\(^{-1}\) as a function of concentration for Snowy Range Instruments SIERRA 2.0.

Figure 24: Peak intensity of characteristic nitrate peak at 1072 cm\(^{-1}\) as a function of concentration for Raman Systems R3000QE.

Figure 23 shows the plot of the normalized intensity of the characteristic peak of nitrate collected with the SIERRA 2.0 instrument. The normalization was necessary to account for the instrument variation, as well as the potential influence of the changing...
liquid level in the measurement system and to increase the comparability between the two instruments. Two trends are observable in the data set, that are both described well with a linear equation. The drop of intensity of the 443 nM nitrate measurement can be attributed to the collection of data at two different times and a slight change of instrument focus during the process.

The trends shown in Figure 24 resulting from the in situ batch measurements data collected with the Raman Systems R3000QE differ from the results obtained with the SIERRA 2.0. The range of concentrations from 22 to 221 nM nitrate, shown in Figure 24, can be described by a linear fit. Interestingly the intensity drastically drops off at higher concentrations. This is an indicator for substrate failure at concentrations above 221 nM nitrate for the Raman Systems device. As this behavior was not observed for any other measurements it can most likely be attributed to a poor substrate.

The data recorded with the SIERRA 2.0 yielded a slope of 0.001 for the linear fit in the lowest concentration regime, while the R3000QE had a slope of 0.003 for the concentration regime below 221 nM nitrate. This indicates that the R3000QE was more responsive to the nitrate signal and can be explained by the longer integration time and higher laser power used with this instrument. The fact that both instruments yielded linear fits shows that nitrate detection was successful and will allow for the calculation of detection limits in section 4.2.4.

Figure 25 shows normalized representative spectra for increasing phosphate concentrations measured in the in situ batch setup on the SIERRA 2.0 instrument. The observed characteristic peak is shifted to 1001 cm\(^{-1}\) from the peak at 989 cm\(^{-1}\) measured...
in normal Raman mode. A raise of the peak intensity with increasing phosphate concentration can be observed.

Figure 25: Development of normalized characteristic phosphate peak as a function of phosphate concentration in the 3D printed batch system collected with SIERRA 2.0. Top: full spectra, bottom: Close-up of characteristic peak.
Figure 26: Characteristic plot of peak intensity at 1001 cm\(^{-1}\) vs time for ultrapure water and 1 µM phosphate in ultrapure water solution.

Figure 26 shows a plot of the peak intensity of the characteristic phosphate peak as a function of time for water and a phosphate concentration of 1 µM. The signal for a concentration of 1 µM phosphate is slightly higher than the signal of water. Both signals stay relatively constant over time, indicating that no time dependency of the signal is present. This behavior compares well to the observations made previously for nitrate measurements and supports the hypothesis that 4-NBT has a specific affinity for the gold surface, while nitrate and phosphate undergo nonspecific adsorption and desorption processes.

Figure 27 shows data of increasing phosphate concentrations collected with the Snowy Range SIERRA 2.0 device. A steady increase of the signal from 10 nM to 1 µM phosphate in water is observed, while maintaining low standard deviations. Further increasing the concentration to the stock solution at 10 mM phosphate results only in a
minor increase of the signal from 0.78 at 1 µM to 0.80 at 1 mM phosphate, indicating that the sensor became saturated in between these two concentrations.

We were unable to detect phosphate with the Raman Systems R3000QE, therefore no comparison can be made.

**Figure 27:** Normalized peak intensity of characteristic phosphate peak at 1001 cm⁻¹ for SIERRA 2.0 as a function of concentration.

In summary it can be stated that nitrate as well as phosphate were detected using *in situ* SERS measurements. Nitrate was detected with both instruments. Phosphate was detected with the SIERRA 2.0 but not with the R3000QE.

One concern with *in situ* SERS measurements is the overall higher distance of analyte molecules from the SERS substrate, as compared to measurements of analyte dried out on SERS substrates. 4-NBT shows a chemical affinity to gold surfaces through its thiol group, a mechanism that is not present for nitrate and phosphate, meaning that there is no specific affinity of these molecules to the surface. Figure 28 shows a comparison of normalized 4-NBT, nitrate and phosphate data, recorded with the
SIERRA 2.0. The data is shown normalized to account for slight variations in substrate composition as well as instrument focus and to increase the comparability. Comparing the three datasets, the normalized intensity of the characteristic 4-NBT peak at 1331 cm\(^{-1}\) is higher than the characteristic peak intensities of nitrate and phosphate for concentrations of 100 nM and above. The chosen standard normal variate method is influenced by the noise within the data series, with higher values meaning less noise. This means that the signal of 4-NBT is less noisy than nitrate and phosphate which can be explained by 4-NBT molecules binding to the surface, while nitrate and phosphate do not bind to the surface and therefore result in a noisier signal.

These results indicate that it is reasonable to attempt continuous \textit{in situ} measuring of aqueous nitrate and phosphate solutions in a 3D-printed flow channel.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure28.png}
\caption{Comparison of averaged normalized peak intensity of 4-NBT, Nitrate and Phosphate recorded with Snowy Range SIERRA 2.0. The characteristic peak intensities shown are 1331 cm\(^{-1}\) for 4-NBT, 1079 cm\(^{-1}\) for nitrate and 1001 cm\(^{-1}\) for phosphate.}
\end{figure}
4.2.3  *In situ continuous flow measurements*

Experiments with *in situ* SERS measurements of nitrate and phosphate in 3D-printed flow channels of the form previously shown in Figure 10, were conducted. The functionality of the system was shown before by benchmarking with 4-NBT in section 4.1.3. The same flow parameters as for the 4-NBT in ethanol solution measurements were chosen.

Figure 29 shows a close-up of the wavenumber regime of the previously identified characteristic peaks for nitrate solutions, measured in a 3D-printed flow channel with a SILMECO SERSstrate substrate. The presented spectra are averaged out of 25 individual spectra. The signal around the previously identified peak of 1079 cm$^{-1}$ stays on the order of the water signal. The same is true for the other expected peak at 1047 cm$^{-1}$, except for the highest investigated concentration of 71.4 mM nitrate and the following flushing step. The highest concentration was already detectable without the presence of a SERS substrate, as it was shown before with the stock solution measurements presented in section 4.2.1, Figure 19 a). Because of the lack of the previously observed peak shift and the high concentration required to obtain a signal it is likely that no surface enhancement occurred for these measurements with our device.

Figure 30 shows a plot of the peak intensity of the nitrate peak at 1047 cm$^{-1}$ as a function of time while the system was flushed. The signal shows scattering, with a downward trend towards the background noise. This behavior is expected as nitrate does not bind strongly to the SERS substrate and is flushed out by the incoming stream of ultrapure water. The initial signal strength of the flushing step was 268 a.u. and decreased over time. After a measurement time of 24.5 minutes the signal approaches
zero for the first time and does not exceed a value of 158 a.u. for the rest of the experiment, indicating that most of the nitrate solution was flushed out and that SERStrate substrate can be cleaned from nitrate by rinsing with water under the circumstances tested in the flow channel.

![Graph](image.png)

**Figure 29:** Close-up of significant spectra of various nitrate/water solutions measured in a 3D-printed flow channel with SILMECO® SERStrate substrates, an integration time of 10 s and activated orbital raster scanning.
Figure 30: Time dependency of peak intensity at 1047 cm$^{-1}$ for the flushing step after a concentration of 71.4 mM nitrate in a 3D-printed flow channel.

The experiment was conducted in the same way with solutions of phosphate in ultrapure water. Representative spectra of the experimental findings are shown in Figure 31. As the intensity of the characteristic peak, previously identified to be around 989 cm$^{-1}$, was very small a closeup of the area of interest is shown in the bottom of the figure. It can be observed that the water filling step, labeled as “water (fill)” shows a lower signal strength than the other presented spectra. This becomes particularly clear by examination of the peak associated with the optical filter of the system between 200 and 400 cm$^{-1}$. The spectrum collected while filling with water yielded an average filter peak intensity of ~2000, while the other spectra showed intensities of this peak much closer to ~15,500. It is possible that the flow channel still contained small amounts of air, changing the laser focus and therefore influencing the signal strength. Figure 31 also shows a small peak at a wavenumber of 997 cm$^{-1}$. This finding is in good agreement with the previous in situ batch mode phosphate measurements that showed a peak at
wavenumbers between 989 cm\(^{-1}\) and 1001 cm\(^{-1}\). The peak intensity is nearly constant and therefore independent of the concentration.

Summarizing the continuous \textit{in situ} SERS measurements it was shown that nitrate stock solution at a concentration of 71.4 mM was detected with our measurement setup. Phosphate was not detected in the continuous \textit{in situ} measurement mode.

![Graph showing representative spectra of varying phosphate concentrations in ultrapure water.](image)

\textbf{Figure 31:} 3D-printed flow channel, representative spectra of varying phosphate concentrations in ultrapure water; Top: full spectra, bottom: zoomed to characteristic peak.

\subsection*{4.2.4 Limit of detection}

Plots of the characteristic nitrate peak versus the concentration for \textit{in situ} SERS measurements in batch mode for the Snowy Range Instruments SIERRA 2.0 and the Raman Systems R3000QE instrument were shown in section 4.2.2. The data for the
3D-printed *in situ* batch system measured with the SIERRA 2.0 instrument, presented in Figure 23, followed a linear trend for concentrations from 22 to 332 nM nitrate. In order to show the entire span of concentrations, the data was presented as a semi-log plot. This means that the signal intensity for water had to be excluded from Figure 23. Figure 32 shows the linear region of this measurement series including the water signal at a wavenumber of 1079 cm\(^{-1}\). The resulting linear fit yields a slope of 0.001 and a y-axis intercept of 0.0067. Using the Excel line estimation function “linest” yields the standard deviation of the y-intercept \(b\) to be \(\sigma_b = 0.0094\) and therefore the limit of detection for the *in situ* batch measurement device is LOD = 34 nM nitrate in ultrapure water.

![Graph](image)

*Figure 32: Resulting signal for the linear concentration range spanning from 0 to 332 nM nitrate for *in situ* batch measurements, conducted with SIERRA 2.0.*

Repeating this procedure and including the water results in the plot based on data collected with the R3000QE system, previously shown in Figure 24, yields Figure 33. The resulting linear fit has a slope of 0.003 and a y-axis intercept of 0.051. Using
the Excel line estimation function “linest” yields the standard deviation of the y-intercept \( b \) to be \( \sigma_b = 0.029 \) and therefore the limit of detection for the \textit{in situ} batch measurement device is LOD = 30 nM nitrate in ultrapure water. Because of the lack of signal in the continuous \textit{in situ} SERS measurements no limit of detection for these measurements could be obtained.

The limits of detection for nitrate calculated for both instruments in the \textit{in situ} batch setup are very similar, indicating that the calculated limits of detection are independent of the tested instruments and can be attributed to the tested devices.

![Graph showing nitrate concentration vs. normalized signal intensity at 1072 cm\(^{-1}\).](attachment:image.png)

*Figure 33: Resulting normalized signal intensity at a wavenumber of 1072 cm\(^{-1}\) for the linear concentration range spanning from 0 to 221 nM nitrate for in situ batch measurements, conducted with R3000QE.*

A calculation of the limit of \textit{in situ} detection for phosphate was conducted as before for nitrate. It was discussed earlier that only \textit{in situ} phosphate measurements conducted with the Sierra 2.0 showed a signal. Figure 34 shows the results obtained from these measurements including the water baseline value. The resulting linear fit yields a slope of 0.003 and a y-axis intercept of 0.303. Using the Excel line estimation
function “linest” yields the standard deviation of the y-intercept b to be $\sigma_b = 0.075$ and therefore the limit of detection for the *in situ* batch measurement on the SIERRA 2.0 is $\text{LOD} = 70 \text{ nM phosphate in ultrapure water.}$

*Figure 34: Resulting signal for the linear concentration range spanning from 0 to 100 nM phosphate for *in situ* batch measurements, conducted with SIERRA 2.0.*
CHAPTER 5

CONCLUSIONS AND FUTURE WORK

In this work we investigated the capabilities of SERS for the detection of nitrate and phosphate in aqueous solutions, using commercially available SERS substrates provided by nanomanufacturing company Silmeco. Silmeco “SERStrate” substrates were chosen after a preliminary trial, comparing the performance of various in-house fabricated, as well as commercially available SERS substrates by measuring against a 4-NBT benchmark.

3D-printed test devices for in situ SERS measurements in batch mode, as well as under continuous flow conditions were designed to overcome the hydrophobic nature of the SERStrate substrates and tested against a 4-NBT in ethanol solution. Both systems were shown to be working.

The developed measurement devices were then used to access the in situ SERS measurement capabilities of aqueous nitrate and phosphate solutions. In situ detection of nitrate and phosphate using commercially available SERS substrates was shown to be possible with low limits of detection in the nanomolar regime for both analytes in batch measurement mode. Continuous in situ measurements of nitrate and phosphate in water solutions showed no clear SERS activity, but a weak signal for nitrate stock solution was observed.

Building on the results presented in this report, additional work will be conducted in the future with ocean water samples to answer the question if in situ measurements can be achieved in the field. It is expected that the overall signal quality will be impaired by the complex composition of sea water. The possible presence of
other contaminants in high concentrations might overshadow the weak nitrate and phosphate signals. At the same time the presence of naturally occurring ions such as bromide will interfere with the signal too.

Microfluidic flow channels are currently investigated in our lab to reduce the influence of external factors and to improve the nitrate and phosphate signal by forcing analyte molecules on the SERS substrate surface. The use of flow channels will also allow to test for the re-usability of substrates and their long term stability. In the meantime, the substrates that are being used need optimization. The affinity for the analytes nitrate and phosphate needs to be increased and more work is needed to increase the reproducibility of the measurements. This could be achieved through the deposition of reporter molecules on the surface. One possibility that is currently investigated is the deposition of modified azo dyes on substrate surfaces.
A-1 Initial substrate evaluation

The initially conducted evaluation of substrate performance is described in the following subsections.

A-1.1 Experimental procedure

Four different types of substrates were initially tested. In house designs consisted of concentrated spiky gold nanoparticles drop casted on glass substrates and gold sputtered nanoporous aluminum oxide filters (Whatman Anodisc). Commercially available substrates tested were gold sputtered silicon oxide nanorods grown on a silicon wafer, provided by Silmeco and sold as SERStrate, as well as gold particles embedded in a paper matrix, provided by Ocean Optics as “RAM SERS Au”.

Drop deposition measurements were conducted as following with the Raman Systems R3000QE. Measurement parameters were a laser power of 250 mW for all tested substrates except the paper-based Ocean Optics substrates, which were tested at 57 mW to minimize substrate burning. The integration time for all measurements in this series was 30 s. The instrument was focused by measuring multiple times until the highest overall signal strength was achieved. After focusing, three dry measurements were taken. After that 5 µL of analyte were deposited on the substrate and measured every two minutes until dried. These experiments were conducted with increasing 4-NBT concentrations ranging from 5 nM 4-NBT to 25 µM 4-NBT. For the Ocean Optics substrates 10 µL instead of 5 µL were used to account for volume losses through soaking of the cellulose matrix.
A-1.2 Results

The graphs presented in Figure 35 to Figure 38 show a comparison of 4-NBT spectra collected on different substrates. The focus of the instrument, as well as the other instrument settings for all tested substrates were kept constant during the measurements, therefore the signal strength can be expected to be at a similar level for substrates with similar SERS activity. Spiky gold nanoparticles were concentrated and drop casted on glass substrates. They are referred to as “Drop Cast” in Figure 35. In this figure two trials are compared. Both substrates show the characteristic peaks of 4-NBT. Due to the simplicity of the fabrication process the uniformity of the substrates and therefore the reproducibility of the readings was poor, as can be seen from the signal difference between the measurements at a constant 4-NBT concentration of 7.5 µM. Increasing the concentration from 7.5 µM 4-NBT to a concentration of 25 µM 4-NBT gives the expected result that a concentration increase leads to a signal increase.

![Figure 35: SERS spectra of drop casted spiky gold nanoparticles on glass substrate with varying 4-NBT concentrations.](image-url)

"Figure 35: SERS spectra of drop casted spiky gold nanoparticles on glass substrate with varying 4-NBT concentrations."
Gold sputtered Whatman Anodisc® membrane filters (pore size 20 nm) were tested by depositing increasing concentrations of 4-NBT in ethanol solution and letting them evaporate. Figure 36 shows spectra collected on a gold sputtered Anodisc® membrane after increasing concentrations of 4-NBT in ethanol were dried on the surface. The previously determined 4-NBT peaks only appear at a concentration of 1 mM 4-NBT. The spectra collected at lower concentrations stay all on the order of the background. Comparing this sensor response with the previously observed detection capabilities of drop casted substrates leads to the conclusion that, under the given parameters, gold sputtered Anodisc® substrates are not as well suited for the detection of 4-NBT as substrates fabricated through drop casting.

Additional measurements were taken with gold nanoparticles embedded in a paper matrix, provided by Ocean Optics. The results of these measurements are shown in Figure 37. For these substrates only a weak signal intensity of 1200 was observed. Peaks can be observed at 1573 cm\(^{-1}\) and 1331 cm\(^{-1}\), but no peak is present at 1079 cm\(^{-1}\). In addition to the lack of signal the paper-based substrate got damaged through laser burning, even though the laser intensity had been reduced to account for this. These observations lead to the decision not to proceed with the investigation of Ocean Optics paper-based substrates.
Commercially available “SERStrate” substrates - gold sputtered silicon oxide nanorods grown on a silicon wafer, distributed by SILMECO were tested with increasing concentrations of 4-NBT. The collected spectra are shown in Figure 38 and show clear 4-NBT peaks, consistent with the previous spectra of drop casted nanoparticles and the highest concentration on gold sputtered Anodisc filter membranes. In comparison the SILMECO SERStrate shows the highest 4-NBT signal in this study, with an intensity as high as 27,000 counts for a 4-NBT concentration of 25 µM for the peak at a wavenumber of 1331 cm⁻¹.

The SEM image of a SERStrate shown in Figure 39 reveals a uniform distribution of surface features. The observed structure was consistent over the surface and with all investigated SERStrate substrates.

Because of these observations and the suspected higher stability for in situ applications, due to the nanorods being fixed to the surface and unlike deposited...
particles not prone to being washed off, the focus was set on the investigation of nutrient measurements with SILMECO SERStrate substrates instead of the in-house fabricated Anodisc or spiky gold nanoparticle drop cast measurements.

![Graph](image)

**Figure 37:** SERS spectra collected on Ocean Optics paper-based substrates for varying 4-NBT concentrations.

![Graph](image)

**Figure 38:** SERS spectra on SILMECO SERStrate substrates for varying 4-NBT concentrations.
Figure 39: SEM images of as-received SILMECO SERStrate.
A-2 Calculation of the Reynolds number in the 3D-printed flow channel

A schematic of the flow channel used for the continuous in situ measurements is shown in Figure 40. The area of interest for the calculation of the Reynolds number is the grey area in Figure 40 a) and b) that represents the volume above the SERS substrate. The volume can be approximated by a cube of the dimensions (LxWxH) 6.2x6.2x4.9 mm. With the definition of the Reynolds number for volume flow through a pipe

\[
Re = \frac{Q \cdot D_H}{\nu \cdot A}
\]

and the cross sectional area A (m²), the volumetric flow rate Q (m³/s), \( \nu \) being the kinematic viscosity of the fluid (m²/s) and the hydraulic diameter \( D_H \) (m) defined as

\[
D_H = \frac{2 \cdot A}{(a + b)}
\]

where a and b represent the length of the edges of the cross sectional area the Reynolds number can be calculated according to the following equation:

\[
Re = \frac{2 \cdot Q}{\nu \cdot (a + b)}
\]

The flow rate was kept constant at \( Q = 0.18 \frac{ml}{min} = 3 \cdot 10^{-9} \frac{m^3}{s} \). The values of a and b are 6.2 and 4.9 mm respectively. The kinematic viscosity of water at a temperature of 20 °C is \( \nu_{water, 20^\circ C} = 1.004 \cdot 10^{-6} \frac{m^2}{s} \) and the kinematic viscosity of ethanol at a temperature of 20 °C is \( \nu_{ethanol, 20^\circ C} = 1.5 \cdot 10^{-6} \frac{m^2}{s} \). With these values the Reynolds number for water calculates to \( Re_{water} = 0.54 \) and the Reynolds number for ethanol to \( Re_{ethanol} = 0.36 \).
Figure 40: Schematic of 3D printed flow channel a) top view with inlet and outlet indicated by arrows and measurement chamber in grey. The channel diameter is 5 mm and the dimensions of the measurement chamber are 6.2 x 6.2 x 4.9 mm b) side view, with inlet and outlet indicated by arrows, measurement chamber in grey, SERS substrate in yellow, projected laser path in red and optical window in light blue.
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