

Development of a Temperature Controlled Cell for Surface Enhanced Raman Spectroscopy for in situ Detection of Gases

Master's Thesis Project in Engineering Physics 30hp

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Abstract (Sammanfattning)

[English]

This work describes a master's thesis in engineering physics at Umeå University carried out during the spring semester of 2014. In the thesis the student has constructed and tested a temperature controlled cell for cooling/heating of surface-enhanced-Raman-spectroscopy (SERS) substrates for rapid detection of volatile substances. The thesis was carried out at the Swedish Defence Research Agency (FOI) in Umeå, Sweden. A Linkam Scientific Instruments TS1500 cell was equipped with a Peltier element for cooling/heating and a thermistor temperature sensor. A control system was constructed, based on an Arduino Uno microcontroller board and a pulse-width-modulated (PWM) H-bridge motor driver to control the Peltier element using a proportional-integral (PI) control algorithm. The temperature controlled cell was able to regulate the temperature of a SERS substrate within -15 to +110 °C and maintain the temperature over prolonged periods at ± 0.22 °C of the set point temperature. Gas phase of 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane (isoflurane) was flowed through the cell and SERS spectra were collected at different temperatures and concentrations. This test showed that the signal is increased when the substrate is cooled and reversibly decreased when the substrate was heated.

Keywords: temperature control, Raman scattering, surface enhanced Raman spectroscopy SERS, SERS substrate, volatile substances, Peltier module, thermistor, PWM, H-bridge, PI(D) control.

[Svenska]

Detta dokument beskriver ett examensarbete för civilingenjörsexamen i teknisk fysik vid Umeå Universitet som utförts under vårterminen 2014. I examensarbetet har en kyl-/värmecell för temperaturkontroll av substratytor för ytförstärkt ramanspektroskopi (SERS) för snabb detektion av farliga flyktiga ämnen konstruerats och testats. Arbetet utfördes vid Totalförsvarets forskningsinstitut (FOI) i Umeå, Sverige. Utgångspunkten var ett Linkam Scientific Instruments TS1500 mikroskopsteg, vilket utrustades med ett Peltierelement för kylning/värmning och en termistor för temperaturövervakning. Ett styrsystem baserat på ett Arduino Uno mikrostyrenhetskort konstruerades med ett motordrivkort (H-brygga) vilket använder pulsbreddsmodulering (PWM) för att reglera spänningen till Peltierelementet utifrån en PI-regulator. Den färdiga cellen klarade att reglera temperaturen på ett SERS-substrat i ett temperaturspann på ungefär -15 till +110 °C med en temperaturstabilitet på ± 0.22 °C av måltemperaturen. Cellen testades sedan på flyktiga ämnen för att visa dess funktion. Difluorometyl-2,2,2-trifluoro-1-kloroetyler (isofluran) i gasfas, med instrumentluft som bärargas, flödades genom cellen och SERS-spektra erhöles vid olika koncentrationer och temperaturer. Vid samtliga koncentrationer visades att lägre temperatur ger ökad signalstyrka. När ytan sedan värmdes upp sjönk signalen reversibelt tillbaka till ursprungsvärdet.

Nyckelord: temperaturkontroll, ytförstärkt ramanspektroskopi, SERS, flyktiga ämnen, Peltierelement, termistor, PWM, H-brygga, PI(D)-regulator.

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1 Introduction

The aim of this master's thesis project in engineering physics is to investigate and modify a Linkam TS1500 temperature control microscopy stage [1] for in situ studies of gas adsorption on gold substrates aimed for surface enhanced Raman spectroscopy (SERS). Molecular vibrational spectra of volatile substances in a controlled environment are probed with a Raman microspectroscopy system. If the SERS substrate is cooled, gas surrounding the substrate can be condensed onto the SERS surface. The cooling step thus increases the number of analyte molecules in close contact to the substrate surface, leading to increased signal strength i.e., the gases will be detected more easily. Conversely, if the temperature of the substrate surface is increased volatile analyte molecules will desorb from the surface and evaporate, leading to regeneration of the SERS substrate.

Thus, the stage must have means to cool, and thereby condense, gases of interest onto a substrate surface, as SERS requires close proximity between the analyte substance and the substrate surface. In order to release the condensed analyte gas, the stage must also be able to heat, and thereby regenerate, the substrate surface. Accordingly the contractor wishes that the temperature of the SERS substrate to be contained within the stage must be monitored and regulated without interfering with the detection method. It was also of interest to measure the temperature of the gas surrounding the SERS substrate in the cell for comparison with the substrate temperature. The stage must accept commercially available SERS substrates, named Klarite[®], as well as other substrates, so flexibility in that respect is of key concern. The temperature of the SERS substrate should be possible to regulate within a temperature span of desirably -20 to 200 °C, or as close to this as possible, primarily to allow condensation and evaporation of analytes of interest. A temperature stability of 0.5 °C is desired. The regulation and monitoring of the temperature in the cell is preferred to be managed via computer, instead of using separate controller units, in order to simplify operation. The temperature controlled cell should also be able to accept vibration and gas flow sensing devices, preferably also controllable by the computer interface.

To introduce the student in Raman spectroscopy and particularly to SERS an experiment was conducted in which Klarite[®] substrates were submerged in BPE/ethanol solutions and spectra were collected as functions of concentration and time. Another introductory SERS experiment was conducted using the resulting modified stage in which gas-phase acetone was flowed through the temperature controlled cell and spectra were collected as a function of temperature and time. These experiments are summarized in Appendix B and C of this document. The temperature controlled cell is then used with a SERS/Raman instrument setup for gas phase detection of volatile substances in order to demonstrate that it works properly.

2 Background

2.1 Project Background

Society today has an increasing demand for fast and accurate methods for detection and identification of harmful substances, such as chemical, biological, radiological, nuclear and explosive (CBRNE) substances, for defense and homeland security applications as well as in industry, medical applications and other civilian applications [2]. It is often demanded that the detection method can be used in the field and that devices are compact, portable, and easy to use and that small amounts of substances can be detected [3].

Surface Enhanced Raman Spectroscopy (SERS) is a versatile non-destructive detection method capable of fulfilling these needs, as it offers rapid and accurate means to identify trace amount of harmful substances, with little or no need for sample preparation. High reproducibility SERS substrates are today commonly fabricated, and few are commercially available, e.g. Klarite® substrates (Renishaw Diagnostics, Ltd) and many of these are showing good sensitivity together with high reproducibility. Due to its attributes, SERS has potential to be used in homeland security applications [3].

The Swedish Defense Research Agency (abbreviated FOI after the Swedish name Totalförsvarets forskningsinstitut) is an assignment-funded governmental research institute under the Ministry of Defense (Försvarsdepartementet), which focuses on research, analysis and development of technology. Examples of research areas include detection of, and protection against, harmful substances such as CBRNE substances. Main clients are the Swedish Armed Forces (Försvarsmakten) and the Swedish Defense Material Administration (Försvarets Materielverk) but FOI also carries out research for civilian clients [4].

Prior to this master's thesis, a pilot study was carried out at FOI where semi volatile substances in gas phase were detected using SERS. All tested substances were easier to detect after cooling the SERS substrate, which indicates that temperature control is desired for gas phase detection with SERS. Another result was that the SERS substrate could often be regenerated by increasing the temperature to a certain level. The resulting modified stage can be used in future research for a variety of applications of gas-phase detection with a range of SERS substrates and analyte substances, as well as other applications where temperature control is needed. This makes the project relevant as a master's thesis.

2.2 Temperature Measurement

To measure the temperature of the substrate surface in the TS1500 stage, a suitable technique must be chosen. Contact thermometry, in which the temperature sensor is in contact with the surface, seems to be an adequate choice, instead of non-contact thermometry. There are several types of temperature sensors, and examples of widely used sensors are thermocouples and resistance thermometers such as thermistors and platinum resistance temperature detectors.

2.2.1 Thermocouple

A thermocouple consists of two different conducting metal wires, with atoms at different Fermi levels, which are joined together in one end, the so called "hot" /measurement junction, and to copper wires in the other end, the so called "cold"/reference junction(s). When the measurement junction is at another temperature than the reference end of the thermocouple a voltage difference

will arise due to the Seebeck effect. The voltage difference is related to the temperature difference between the two junctions and tables are used for translating the voltage into temperature [5, p. 34].

Thermocouples have rapid response and can operate over large temperature ranges. Type K thermocouples, i.e., Chromel-Alumel thermocouples, are the most common and have an operating temperature span of -200 to 1 350 °C and an accuracy of about ± 1.5 °C. The thermocouple is a differential measurement sensor; it measures the temperature at the measurement junction compared to the temperature at the reference junction. This means that a thermocouple measurement setup will require a stable reference temperature, i.e., *cold junction compensation*, in order to give accurate temperature readings [6].

2.2.2 Resistance Thermometers: Platinum Resistance Thermometers & Thermistors

A resistance thermometer is a sensor in which the resistance varies with temperature. The temperature of the object can thus be related to the resistance of the sensor filament. The most common types of resistance thermometers are described below.

Platinum Resistance Thermometers

A common type of resistance thermometer is the platinum resistance temperature detector (RTD), which is also known as platinum resistance thermometer (PRT). Platinum has the characteristic that its resistance increases with increasing temperature, so called *positive temperature coefficient* (PTC). Features of the RTD include high stability, approximate linearity, interchangeability and accuracy.

The relationship between temperature and resistance in the platinum filament is given by the Callendar-Van Dusen equation, equation 1 below:

$$R(T) = R(0) \cdot [1 + A \cdot T + B \cdot T^2 + (T - 100)C \cdot T^3] \quad (1)$$

T is the temperature in °C, R(0) is the nominal resistance at 0 °C. The A, B and C are constants that are derived from experimentally determined parameters.

An example of an RTD is the Pt-100 sensor. The platinum filament in the Pt-100 sensor has a resistance of 100 Ohms at 0 °C. RTDs, such as the Pt-100, have fairly linear resistance dependence to temperature [7]. Response times for RTD sensors are usually longer than for thermocouples or thermistors, e.g., 10 s versus 0.5 s, respectively [8].

To measure temperature with a RTD one must supply it with a constant measuring current. This current will have the effect that the sensor will heat up. Therefore measuring currents must be kept low in order to avoid substantial errors due to self-heating of the sensor. See the *Thermistors* section below for further information about self-heating.

As resistance thermometers relates the filament resistance to temperature, and the resistance of a platinum filament is relatively close to the resistance of the lead wires, resistance in the lead wires will affect the temperature measurement. This problem is usually circumvented by using three- or four-wire measurement connections, so called Kelvin measurements [9].

Thermistors

Another kind of resistance thermometer is the thermistor. A thermistor is basically a resistor whose resistance varies with temperature, and usually consists of metal oxides. As opposed to platinum resistance thermometers, a thermistor's resistance usually decreases with increasing temperature.

This is called *negative temperature coefficient* (NTC). Note, though, that there are also PTC thermistors [5, p. 56].

The resistance of the thermistor is often several orders of magnitude larger than that of copper lead wires, and therefore the thermistor does normally not require three- or four-terminal measurement as the platinum RTDs do (if high accuracy is required). As with the RTDs, thermistors measure absolute temperature. However, they generally have a more narrow working range compared to RTD or thermocouple sensors [10]. Most thermistors have a maximum working temperature range of -55 to +114 °C or thereabout and work best in within approximately 50 °C of the ambient temperature, however there are thermistors capable of handling slightly larger temperature ranges [11].

The temperature is for NTC thermistors calculated approximately from the resistance of the thermistor through the Steinhart-Hart equation, equation 2 below [5, p. 57].

$$\frac{1}{T(K)} = a + b \ln R + c[\ln R]^3 \quad (2)$$

T(K) is the temperature in Kelvin, R is the resistance of the thermistor and a, b and c are constants specific to the thermistor (found experimentally and often given in the datasheet of the sensor).

A thermistor is a passive device and therefore, as in the case with RTDs, it requires a constant excitation current for temperature measurements to be made. One can use a current source to generate the excitation current, I, and measure either the resistance of the thermistor directly with an Ohm meter, or alternatively measure the voltage across the thermistor, see the circuit diagram in figure 1 below, and calculate the resistance from that voltage.

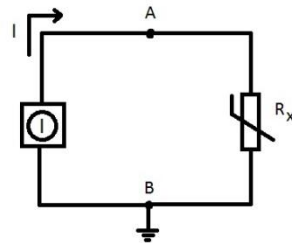


Figure 1. Circuit diagram for a simple thermistor measurement setup using a current source to generate measurement current.

If the voltage between node A and B, called U_{AB} , is measured, then the resistance of the thermistor, R_x in equation 3, is then calculated using Ohm's law.

$$R_x = \frac{U_{AB}}{I} \quad (3)$$

Then the temperature in °C is calculated approximately by subtracting the absolute zero (approx. - 273.15 °C) in the Celsius scale from the Steinhart-Hart equation which uses Kelvin, resulting in equation 4:

$$T(^{\circ}\text{C}) = \frac{1}{a+b \ln R_x+c[\ln R_x]^3} - 273.15 \quad (4)$$

Instead of using a current source to provide the excitation current, one can alternatively use a voltage source and a fix reference resistance, R_0 , connected in series with the thermistor as a voltage divider circuit. Such a circuit is illustrated in the circuit diagram in figure 2 below.

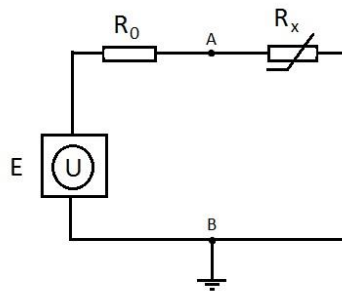


Figure 2. Circuit diagram for thermistor measurement using a voltage divider setup.

If the voltage between terminals A and B, U_{AB} , is measured, the thermistor resistance can be calculated using the following method:

U_{AB} is given by the standard voltage divider relation, equation 5 below, which is due to that R_0 and R_x are connected in series and therefore the current through $(R_0 + R_x)$ is the same as the current through R_x .

$$U_{AB} = \frac{R_x}{R_x + R_0} E \quad (5)$$

This can be rewritten as equation 6:

$$(R_x + R_0)U_{AB} = R_x E \quad (6)$$

Gathering the terms containing R_x results in equation 7 below:

$$R_x(E - U_{AB}) = R_0 E \quad (7)$$

Solving for R_x yields equation 8:

$$R_x = \frac{U_{AB}}{E - U_{AB}} R_0 \quad (8)$$

The thermistor resistance R_x can then be inserted into the Steinhart-Hart equation, i.e., equation 2, as in the previous case to obtain the temperature.

Self-Heating of the Thermistor

The excitation current passing through the thermistor element will cause Joule heating, i.e. resistive heating, in the thermistor. The dissipated power follows Joule's first law, i.e., equation 9:

$$Q = I^2 \cdot R \quad (9)$$

Where Q is power, I is current and R is resistance. For example, if an excitation current of $10 \mu\text{A}$ is applied to a thermistor with a nominal resistance of $2\,252 \Omega$ at $25 \text{ }^\circ\text{C}$, the power dissipated is as follows according to equation 9:

$$(10 \cdot 10^{-6} \text{A})^2 \cdot 2\,252 \Omega \approx 2.3 \cdot 10^{-7} \text{W} = 2.3 \cdot 10^{-4} \text{mW}$$

The power, in mW, that will raise the temperature of the thermistor by 1 °C is usually referred to as the “dissipation constant” of the thermistor in datasheets. If it has a dissipation constant of, for example, 4 mW/°C, then the dissipated power will heat the thermistor by

$$\frac{2.3 \cdot 10^{-4} \text{mW}}{4 \text{mW}/^{\circ}\text{C}} = 0.575 \cdot 10^{-4} \text{^{\circ}\text{C}}$$

Such a small error is often well inside the sensor tolerance and is thus negligible. However, if the excitation current is larger, say 1 mA; then the same thermistor will be heated by 0.575 °C. This means care has to be taken not to feed the thermistor a too large excitation current to keep the measurement as accurate as possible.

2.3 Thermoelectric Coolers (Peltier modules)

A device capable of both cooling and heating is the thermoelectric cooler (TEC), which is also known as Peltier cooler (or module). The Peltier cooler consists of series of pairs of two different semiconductor materials, with different Fermi levels, that form p-n junctions. The p- and n semiconductors are placed thermally in parallel and are connected by a conducting material in one end. When a voltage is applied to the free ends of the semiconductors, heat will be transported from one side of the Peltier module to the other due to the Peltier effect (which is closely related to the Seebeck effect). The flow of heat due to the Peltier effect, Q_{PE} , is given by equation 10 [12].

$$Q_{PE} = (\Pi_A - \Pi_B) \cdot I \quad (10)$$

Π_A and Π_B are the Peltier coefficients of material A and B respectively, I is the current. By reversing the current, the cold and hot sides are switched turning the cooler into a heater. See figure 3 below.

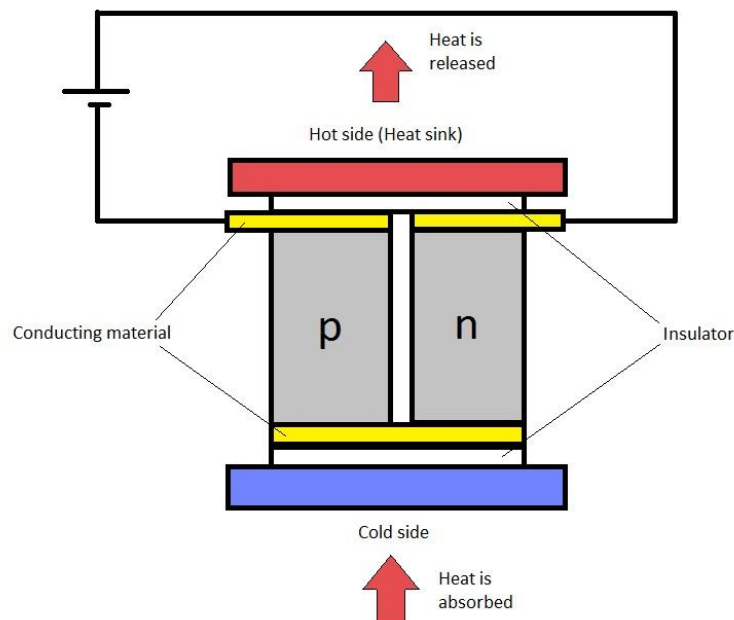


Figure 3. Illustration of a Peltier cooler.

Note that the heat released will not be due to the Peltier effect alone, there will also be Joule heating, which is independent of current direction, due to the electrical resistance of the conducting materials and lead wires. Although the cooling side and heating side can be switched by reversing the

current, the side of the Peltier module on which the lead wires are attached is often referred to as the “hot side”. This is because the Joule heating of the copper wires will heat the side that they are attached to, making it less effective for cooling than the other side. Therefore if the Peltier module is to be used for cooling, then the “hot side” should be connected to the heat sink [13].

Peltier coolers can only maintain a limited temperature difference (usually less than 70 °C) between the cool side and the hot side and as the Peltier module moves heat from one side to the other, the heat released at the hot side must be properly dissipated in order for the cooling to work and the other way around when used as a heater [14]. Therefore a heat sink should be connected to the hot side or else the Peltier cooler can overheat resulting in melting of the solders in the module.

Since a Peltier cooler has no moving parts, is being capable of both cooling and heating, and it can be made small, it is suitable for controlling the temperature of the cell.

2.4 Pulse Width Modulation (PWM)

PWM is a method of generating a “makeshift” analog signal by digital means. If a device is not capable of generating true analog signals one can simulate an analog signal by using short digital pulses. If the pulses are short enough, and the frequency is high enough, the controlled device will experience the pulses as a continuous signal [15]. The percentage of time that the voltage is on is called the “duty cycle” and determines the energy flow through the device. If the duty cycle is 70 %, i.e., the voltage is on 70 % of the time, the device experiences 70 % of full energy flow [16].

The principle is illustrated in figure 4 below, where the duty cycle is varying with time.

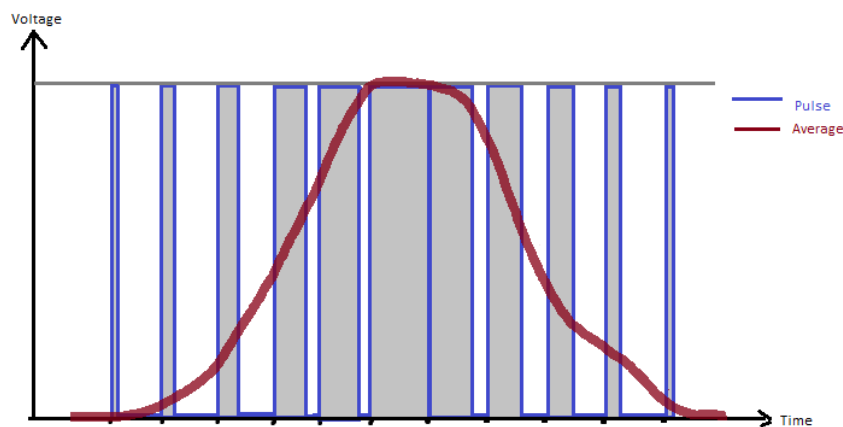


Figure 4. Illustration of pulse width modulation (PWM) showing the pulses (blue, with the area below marked in grey) and the resulting average voltage (red).

Using PWM with a Peltier cooler keeps power dissipation in the controller to a lower level than it would if a true analog/continuous signal was applied [17]. PWM will however cause rapid thermal cycling, i.e., rapid thermal contraction and expansion, in the Peltier cooler which potentially can cause degradation to the device over time. Low PWM frequencies will introduce larger temperature variations, and higher PWM frequencies instead cause more rapid thermal cycling so there is no obvious ideal frequency for the PWM signal. A study [18] shows that the level of degradation is rather constant between different frequencies and is overall small and thus not a serious reliability concern. A PWM frequency preferably in the low kHz range should be used to avoid substantial temperature variations [17].

2.5 Proportional-Integral-Derivative (PID) Control

PID control is a common way of controlling a process to reach and maintain a set point, for example maintaining temperature in a closed environment. The PID algorithm is based on a control equation, see equation 11, which governs the output, $u(t)$, to the process based on the error, $e(t)$, between a process variable and a set point:

$$u(t) = P + I + D = K_p e(t) + K_i \int_0^t e(\tau) d\tau + K_d \frac{d}{dt} e(t) \quad (11)$$

K_p , K_i and K_d are gain constants that determine the contribution from each part of the equation, $e(t)$ is the error function, i.e., the difference between the current measured value of the process variable and the desired set-point. The proportional term, P , of the equation takes the current error into account. The integral term, I , takes all errors up to the present into account. The derivative term, D , estimates the future errors by the rate of change of the error function [19].

One can use all three terms of equation 11, i.e., PID control, or use parts of it, e.g., PI or PD control, depending on the needs of the specific process. For simple setups it might even be enough to use only the proportional term, P . When the error, $e(t)$, gets to zero the output of the P controller is zero. This means that there will be a steady state error (known as “droop”) between the process variable and the desired set-point. By additionally using the integral term, i.e., PI control, it will take care of the steady state error and help the process variable reach the set-point. The derivative term can be added, i.e., PID control, to decrease settling time and thus increasing stability [20].

The process of determining the gain constants of the controller is called “tuning”. There are several methods of PID tuning, and some PID controllers even have built in automatic tuning to determine the gain constants. An example of a tuning algorithm is the Ziegler-Nichols Closed Loop Method. In the Ziegler-Nichols method the controller is first set to use only the proportional term, i.e., K_i and K_d are set to zero, and K_p is increased until constant amplitude oscillations of the process variable are obtained. That gain is called the ultimate gain, K_u , which together with the period of the oscillations, T_u , are used to determine the gain constants K_p , K_i and K_d according to table 1 below [21] [22].

Table 1. Gain constants for Ziegler-Nichols tuning [21].

Type of Control	K_p	K_i	K_d
P	$0.5K_u$	-	-
PI	$0.45K_u$	$K_p/T_u/1.2$	-
PD	$0.8K_u$	-	$K_p T_u/8$
PID	$0.60K_u$	$2K_p/T_u$	$K_p T_u/8$

Other examples of tuning methods include the Cohen-Coon Open Loop Method, and of course, manually adjusting the gain constants to give an appropriate controller output.

Generally, if the system has a too low proportional gain it will be over-damped, slow to respond and may even not reach the desired set-point, i.e., a steady state error/droop. If it has a too high proportional gain, it will start to oscillate periodically around the set-point. A too small integral gain will not be able to compensate for any steady state error/droop, but a too large integral gain can cause instability. The derivative gain is rarely used in real world applications as it can cause instability if the signal has noise or if the set-point or process variable is changed suddenly [23] [24].

The integral term takes all errors up until the present into account, and therefore, if the system starts off far from the set point, the initial error values will be large and the integral term will subsequently grow quickly. This can cause excess overshoot until the opposite direction errors have decreased the integral term. This phenomenon is called “integral windup” and can be overcome by for example not using the integral term until the process variable is within a certain range of the set point.

2.6 Raman scattering process – a brief overview

Raman scattering is inelastic scattering of light by molecules. The incident photon can excite the molecule into a virtual state from which the electron can either fall back into the same vibrational state that it was excited from, i.e., elastic/Rayleigh scattering, or it can fall into another vibrational state, in which the emitted photon has either lost energy to the molecule, which is called Stokes scattering, or gained energy from the molecule, anti-Stokes scattering [25].

The so called “fingerprint” region (approx. 600 - 1800 cm^{-1}) of the Raman spectra provides information specific to the molecule, and chemical groups. This enables identification of species by matching spectral data against libraries of spectra from known molecules.

The Raman cross-section is usually small, much less sensitive than the related IR absorption of vibration spectroscopy, and about 10^8 incident photons may be required for a single photon to be Raman scattered [26].

Fluorescence is a competing photon emission process. First the molecule is excited to a higher electronic state by absorbing light where after it relaxes back into the ground state by emitting a photon. Typically this appears as broad background signals blurring the, in this case, sought Raman spectra. Luckily, for trace analysis, scientists discovered already in 1974 that Raman scattering from molecules adsorbed on nanostructured silver electrodes enhanced the Raman signal [27]. In 1997 one was even able to perform single molecule detection by SERS [28].

2.7 Surface Enhanced Raman Spectroscopy (SERS)

If the molecules of interest are brought in close contact to a metal nanostructured surface with certain properties, a large amplification, often million fold, of the Raman signal can be achieved. The enhancement factor can be as high as $10^{10} - 10^{11}$ for certain substrates [29] [30, p. 113].

The enhancement effect is generally attributed to two mechanisms: the electromagnetic enhancement and the chemical enhancement. The electromagnetic mechanism is considered to be the main contributor to the enhancement factor [31] [30, p. 116].

The electromagnetic enhancement comes from the laser excitation of local surface plasmon resonances (LSPRs), i.e., confined collective electron oscillations of the free electron cloud, in the nanostructured surface. The adsorbed analyte molecules experience an intense electric field which is created by the LSPR [30, pp. 117-118]. Only oscillations that are perpendicular to the surface contributes to scattering, so SERS substrate surfaces are often roughened to provide nanoscale features on the surface, as oscillations in flat surfaces occur in the surface plane [30, p. 117]. Small gaps in the nanostructure where the resulting electromagnetic field is very strong are called “hotspots” and have been shown sensitive enough to enable single molecule detection [32, p. 90]. This means that SERS substrates must be able to support LSPRs and consist of plasmonic material such as gold (Au) or silver (Ag) which are the most commonly used substrate materials since they

have plasmon frequencies in the visible region of the optical spectrum that is overlapping well with commonly used laser excitation wavelengths for Raman spectroscopy [29]. For some applications colloidal metal nanoparticles may be better suited than solid surfaces [31].

The chemical enhancement comes from the formation of charge-transfer (CT) complexes which are formed when the analyzed molecules chemisorbs onto the metal surface. The CT complexes can lead to change in polarizability of the molecules and thus increasing the Raman cross-section [30, p. 120].

3 Method and Equipment

3.1 Linkam TS1500 Stage

The Linkam TS1500 is a microscopy stage intended for controlling the temperature of samples from ambient up to 1 500 °C, at heating rates up to 200 °C/min. The stage body has connections for water cooling and gas connections to the interior of the cell. The stage is equipped with a heating cup with a heating element and a type S thermocouple for temperature sensing. There are two contact leads for the heating filament and thermocouple connector leads for the thermocouple. There are quartz windows both in the lid and below the heating cup [33]. Figure 5 below shows the TS1500 stage with the lid attached.

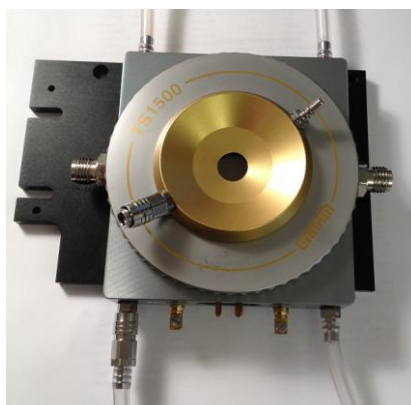


Figure 5. TS1500 stage with lid attached.

The depth between the bottom of the stage interior and the lower surface of the 1 mm thick quartz window in the lid is approx. 15.1 mm with the heating cup removed. The cooler/heater setup, SERS substrate and temperature sensor all need to fit in that space and still leave enough room to allow gas to flow over the substrate surface.

3.2 Choosing Temperature Sensor: Comparison between Thermocouples, platinum RTDs and Thermistors

Regarding the temperature sensor, the key concerns are:

- Temperature range (approximately -20 to +200 °C)
- Response time (as short as possible)
- Accuracy (± 0.5 °C)
- Two-wire sensor connection (using the existing thermocouple connector leads)
- Sensor size (as small as possible)

The platinum RTD devices such as Pt-100 sensors generally have better accuracy/tolerance than thermocouples or thermistors, and measure absolute temperature as opposed to the differential temperature measured by thermocouples.

As the cooling/heating device in the stage is being controlled by computer software relying on the temperature measured by the sensor, the response time of the sensor must be kept to a minimum so the lag in the control system is as small as possible. Otherwise the control system would set the cooling/heating according to obsolete temperature values and the system would be ineffective. The platinum RTD sensors usually have longer response times than thermocouples or thermistors, which could prove to be a problem.

As mentioned earlier a thermocouple measures a temperature difference between the measurement end and the reference end, which requires cold junction compensation. This is a potential problem with thermocouples for temperature measurement in the stage because the reference temperature has to be known and preferably constant in order to get accurate temperature readings from the thermocouple. This would require an ice bath to keep the reference end at a stable temperature of 0 °C and the ice bath require maintenance lest the ice will melt. The thermocouple connector in the stage is an S-type connector (0-1600 °C) and thus not suitable for the -20 to +200 °C temperature range, and in addition to the thermocouple's low nominal accuracy this could lead to an inaccurate temperature measurement [10].

The stage, as it was supplied, is designed to be connected to a Linkam controller unit with a contact plug connecting the controller unit to the two heater leads and the two leads of the thermocouple connector in the stage. If more leads or wires than the stage is prepared for are to be used, then one has to physically modify the stage body connection setup, adding more leads, and such changes could potentially be irreversible. As the stage is expensive it was decided that this kind of modification should be avoided as far as possible, leaving two leads available for the cooler/heater (positive and negative terminal) and the thermocouple connector for the temperature sensor.

This had the consequence that four-terminal temperature measurement was not an option. As another consequence, measuring the temperature of both the substrate surface and the surrounding gas inside the stage at the same time would be impossible without modifying the stage body with more contact leads. As the temperature of the SERS substrate is prioritized, measurement of the gas temperature would thus have to be made outside the stage in the feed.

The platinum RTD probably would not be the best choice of sensor in this case because of four-terminal requirement for accuracy and slow response time. Due to the resistance of the thermistor usually being several orders of magnitude higher than the resistance of copper lead wires, the thermistor does not need four-terminal measurements as platinum sensors do. The resistance of the thermistor is also significantly higher than the resistance of the thermocouple connector in the stage. This meant that the thermistor could be connected using the thermocouple connector without affecting the temperature measurements to a large extent.⁽¹⁾

As only two-wire connection was possible, accuracy and response time is important, and differential measurements were unpractical in this case, a thermistor seemed to be the most appropriate choice

⁽¹⁾ Assuming a 6 feet long 18 AWG S-type thermocouple connector wire, the added resistance would be approximately 0.7 Ω [46].

for the application. However this was still a compromise, because by using a common thermistor the maximum working temperature of the sensor would have to be lower than the desired 200 °C.

The following conclusions could then be made:

- Thermistor was the most appropriate sensors under the given circumstances.
- Temperature measurement on both the substrate surface and the surrounding gas *in the stage* would not be possible simultaneously.
- Thermistors usually cannot handle 200 °C which meant that maximum temperature of the SERS substrate will have to be less than maximum working temperature of the thermistor to avoid damage to the temperature sensor.

The model 44004 nonlinear thermistor element (Omega Engineering Ltd) has a working temperature span of -80 to +150 °C, a tolerance of 0.2 °C and reasonably fast response time, relatively low cost, relative small size, and can be used with two-terminal connection. This made it a suitable sensor for this application under the given circumstances.

3.3 Installing Peltier module and Thermistor in the stage

The thermoelectric cooling approach was chosen since it is capable of both cooling and heating, that Peltier elements can be made small and only require two lead wires for both cooling and heating. A suitable Peltier module was the VT-31-1.0-1.3 Peltier Module (TE Technology Inc.) with an operating temperature range of -40 to +200 °C and a reasonable maximum power of 8.4 W. The maximum input voltage is 3.8 V and the maximum current draw is 3.6 A. The maximum temperature difference between hot side and cold side is 69 °C⁽²⁾.

The TS1500 stage body is water cooled (the water is at room temperature), and the heat from the hot side of the Peltier cooler must thus be transported from the hot side of the cooler to the stage body for dissipation. Since there is a window located in the bottom of the stage, indicated in blue in figure 6, some sort of plate of a thermally conducting material, preferably of copper or another thermally conducting material, would thus have to be connecting the hot side of the Peltier cooler to the actively “cooled” stage body. The plate could not be made too thick as this would decrease the free space above the cooler and thus decrease the space above the substrate in which the gas must be able to flow. The stage has a 0.5 mm deep circular recess of 40mm diameter in the bottom so it is 0.5 mm deeper in the center than at the edge as shown in figure 6. A 0.5 mm thick aluminum plate of 40 mm diameter and a 1.0 mm thick aluminum plate of 64mm diameter (with a cutout for the connection leads) put together as shown in grey in figure 6 were used in this case. Aluminum does not have as high thermal conduction as copper (205 vs 401 Wm⁻¹K⁻¹ [34]) but it is still a decent thermal conductor. These plates only take up 1.5mm of the overall depth of the cell while still having a large contact area to the stage body.

The Peltier cooler was then mounted on the thermally conducting plate according to figure 6 using thermally conducting epoxy adhesive. This kind of adhesive forms a strong bond, with high thermal conductivity, between the cooler and the metal plate which replaces mechanical mounting (e.g., screw mounting which would be impractical in this case). A thin layer of thermally conducting

⁽²⁾ Stated values are valid for a “hot side” temperature of 27 °C.

grease/compound was then added between the plates and the stage body to fill out any unevenness and increase thermal conduction.

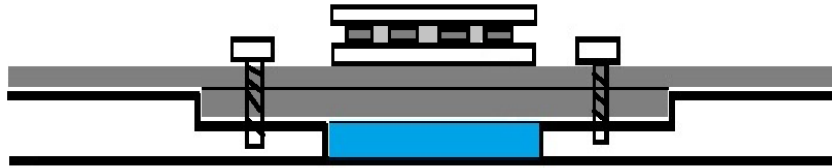


Figure 6. Peltier module mounted on thermal conducting plate (profile view).

The temperature sensor was then installed into the cell and held in place on the Peltier module or substrate chip by a metal bracket (clad in electrical insulator) holding the thermistor lead wires down, see figure 7 below, and a small portion of thermally conducting grease was applied to the sensor to increase thermal conduction between the substrate and the sensor.

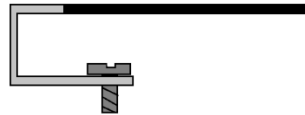


Figure 7. A metal bracket (profile view) to hold the thermistor down on the substrate surface or Peltier module surface. The black portion is covered by insulator.

3.4 Data Acquisition & Instrument Control

As a computer was to be used to monitor and control the temperature in the stage, the signal from the temperature sensor had to be connected to computer software through some form of hardware. Preferably the same software and hardware would be able to display the temperature and control the Peltier device in the stage. There are various ways of accomplishing this, however, the method presented below was chosen in this project.

3.4.1 Data Acquisition and Instrument Control Platform: Arduino

The Arduino platform is an open source electronics prototyping platform with both hardware and software available. The Arduino platform is capable of acquiring data from sensors and controlling instruments. The microcontroller board is programmed in the Arduino programming language which is based on Wiring. The software, Arduino Integrated Development Environment (IDE), is available for Windows, Mac OS and Linux and is free of charge. It is used for uploading programs to the microcontroller board, and also has a serial monitor in which one can view the data being sent to the serial port and one can also send commands to the serial port. The most common Arduino microcontroller board is the Arduino Uno R3 and it was chosen for this project [35].

3.4.2 Data Acquisition

The Arduino Uno R3 has six analog inputs which measure voltage with 10 bit precision. There is no Ohm meter on the Arduino Uno board and the voltage divider setup described in section 2.2.2 was used since there is no current source on the Arduino Uno board. To minimize the effects of noise on the temperature measurement the 3.3 V pin was used as analog reference voltage instead of the standard 5 V that comes directly from the computer USB. Additionally a series of several measurements are taken with a short delay and the average value is then calculated to further reduce effects of noise on the temperature measurement. The setup is shown in figure 8 below.

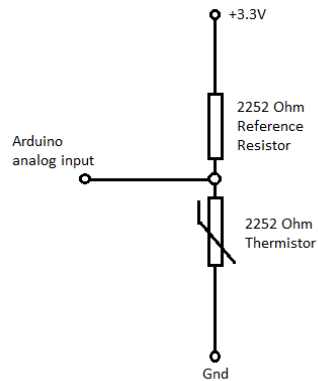


Figure 8. Voltage divider thermistor setup with Arduino.

The temperature sensor setup was connected to an analog input on the microcontroller board and by using the command *analogRead()* an integer value ⁽³⁾ is returned which represents the voltage ⁽⁴⁾ on the analog input pin. The returned value is related to the corresponding voltage as given below;

$$\text{Voltage} = \text{Value} \cdot \frac{3.3}{1023} \quad (12)$$

The temperature measured by the thermistor is calculated by the method described in section 2.2.2 of this document. The temperature, together with the time passed since the program started, can then be regularly sent to the serial port. The serial monitor in the Arduino IDE cannot save the data so an external program will have to be used if the data is to be saved; it is possible to manually copy/paste the data from the Arduino IDE serial monitor into a text file. The text file can then be opened with Microsoft Excel or similar software for data processing.

3.4.3 Instrument Control

To control the DC Peltier cooler using the Arduino platform, the Peltier cooler module had to be interfaced to the Arduino board. The Arduino Uno has 14 digital I/O pins. Six of the Arduino Uno board's digital pins ⁽⁵⁾ are marked "PWM". These can simulate analog DC signals using so called pulse width modulation (PWM) using the command *analogWrite()*. By choosing duty cycle of the PWM from 0 - 100 %, represented in 8bit precision as an integer value of 0 - 255, the average voltage can be controlled between 0-5 V. This would have been a simple and convenient way of controlling the Peltier cooler but the Peltier device, however, requires more power than the Arduino board is capable of supplying. The Arduino Uno board can only deliver currents up to the milliamp region. This means that the energy to the cooler must be generated by an external source. The Arduino board is then used to control the energy flow from the external power source through the cooler by using an external controller circuit, e.g., a DC motor driver circuit as the same principle can be applied to Peltier coolers.

⁽³⁾ 0 - 1023 (10bit precision)

⁽⁴⁾ The voltage range is 0 - 3.3 V since 3.3 V is used as analog reference voltage in this setup, note that the analog reference must be set to external in the program code using the *analogReference(EXTERNAL)* command when an external reference voltage is used or else the Arduino board can get damaged.

⁽⁵⁾ Pin 3, 5, 6, 9, 10 and 11.

External Power Supply and Voltage Regulator

An external AC to DC adapter is used to power the Peltier cooler. The adaptor (Nordic Power, model 04151A-050300) is supplying 5 V and 3 A which means that the voltage must be regulated down to a proper level for the Peltier cooler since the VT-31-1.0-1.3 has a maximum input voltage of 3.8 V. This was done by using a voltage regulator, in this case a fixed 3.3 V output LM1084 (National Semiconductor). The regulator was connected similarly to as shown in figure 9.

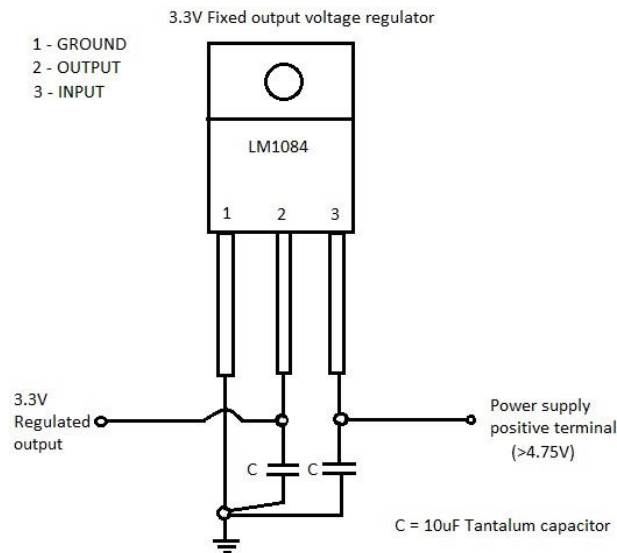


Figure 9. LM1084 3.3V fixed output voltage regulator.

The decoupling capacitors are used for stability reasons; in this case 10 μF tantalum electrolyte capacitors are selected. The 3.3 V, 3 A output from the voltage regulator is in the neighborhood of 85% of the maximum rating of the Peltier module which seemed appropriate as the Peltier module would never be overloaded in voltage or current.

Note that the voltage regulator becomes hot when high currents are passed through it and therefore a heat sink was mounted on the TO-220 casing to help heat dissipation.

H-Bridge Controller

A Low-Voltage Dual Serial Motor Controller (SMC05A), Pololu Corporation, was used to control the power output to the Peltier module. The SMC05A is meant to control the output of up to two DC motors by 600 Hz PWM in 6bit precision, i.e., 128 steps or 0 - 127, in forward or reverse direction by using an H-bridge. It can handle a 0 – 7 V operating voltage and be controlled by a 3 - 5.5 V logic voltage. Output currents of up to 5 A can be handled if one of the two outputs are used, or 10 A if the outputs are paralleled. Note that one has to reconfigure the motor controller to use the parallel mode and in this case the standard mode was used [36]. Figure 10 below is showing the SMC05A controller.

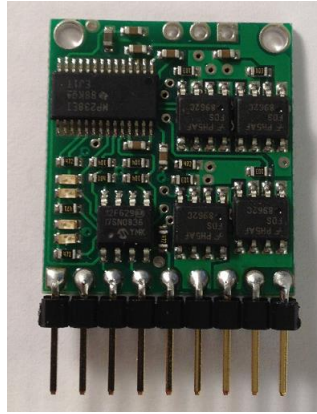


Figure 10. Pololu SMC05A Low-Voltage Dual Serial Motor Controller.

The SMC05A board is communicating via serial communication, so a software serial port is set up between the Arduino board and the motor controller board to avoid interference between the motor controller’s communication with the Arduino board and the Arduino board’s communication with the computer on the hardware serial port (UART). The SMC05A automatically detects baud rate (rates of 1200 - 19200 baud can be used).

The SMC05A uses a protocol in the following format, table 2 below [37].

Table 2. Pololu SMC05A command protocol format.

Byte 1	Byte 2	Byte3	Byte 4 etc.
Start byte (0x80)	Device type (0x00)	Data byte 1	Data byte 2 etc.

When a start byte (128, 0x80 in hexadecimal notation,) is sent on the serial port, the serial device/devices knows that a command is to be issued and “listens” to see if the command is meant for it/them (determined by the device type byte, it should be 0, 0x00 in hexadecimal notation, for SMC05A). If the command is meant for the device in question, it reads the following data byte(s) and executes the command.

To set the output and direction a four-byte packet of the following format, refer to table 3, is sent on the software serial port to the SMC05A:

Table 3. Packet for setting output level and direction for SMC05A.

Byte 1	Byte 2	Byte 3	Byte 4
Start byte (0x80)	Device type (0x00)	Output ID and direction (0x00 or 0x01, i.e., 0 or 1)	Output level (0x00-0x7F, i.e., 0 - 127)

The output ID is specifying which of the two outputs (0 or 1) the command should be executed on, and the direction (1 for forward or 0 for backward) specifies the direction of the current through the device. Note that the SMC05A’s interface protocol is compatible with other Pololu devices and thus multiple devices can be controlled by the serial line. Note that all SMC05A devices on the serial line respond to output IDs 0 and 1. Therefore other output IDs, e.g., 2 and 3 (refer to the user’s manual for other configurations than the default configuration), must be used to control specific outputs on

specific controllers if multiple controllers are used. Since only one SMC05A board and only one output is used (Output 0) in this application this byte will be 0x00 for “backward” or 0x01 for “forward”. Sending the example packet in table 4 below on the software serial port will issue the command “forward direction at output level 78” to the SMC05A controller (hexadecimal representation of 78 is 0x4E):

Table 4. An example packet, issuing the command "Forward direction at output level 78".

Byte 1	Byte 2	Byte 3	Byte 4
0x80	0x00	0x01	0x4E

The SMC05A controller was connected according to figure 11 below. Note that “Power supply” in figure 11 refers to the regulated output of the voltage regulator. Since only one Peltier cooler is used pin 6 and 7 on the SMC05A board are not connected.

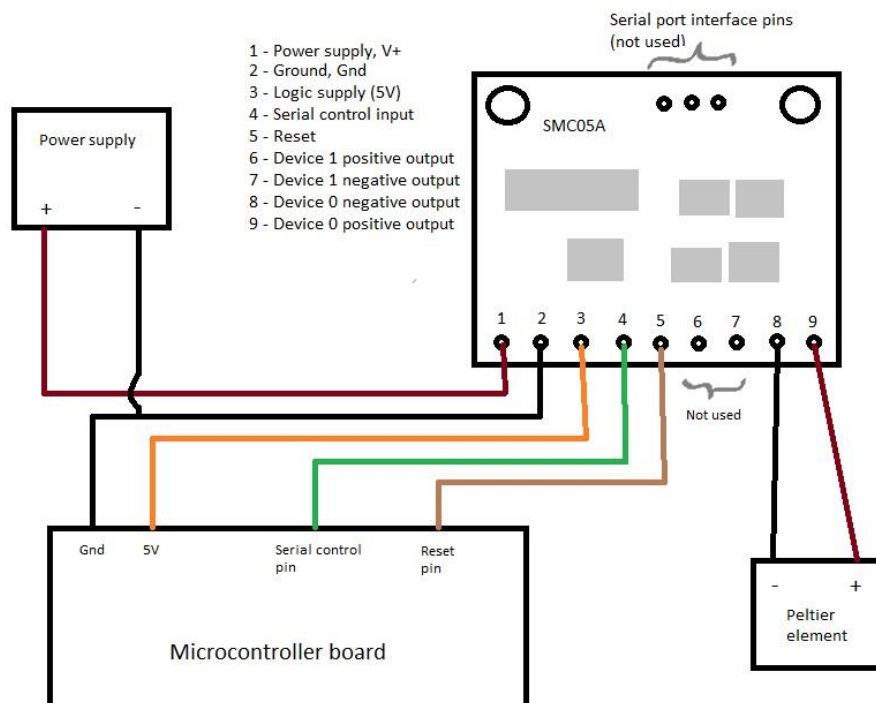


Figure 11. SMC05A setup with Arduino microcontroller.

PID Control

There is a free PID library available for the Arduino platform [38]. This PID controller is a single stage controller, either for heating or cooling. However, the direction of the controller, which decides if the output should be increased or decreased if the process variable is above or below the target set-point, can be changed as the program runs effectively making it a dual stage controller, i.e., controlling cooling or heating depending on what the set-point is. In this case the output to be controlled is the supplied voltage to the Peltier cooler, represented by a 6bit value. The process variable, also called *input*, is in this case the 10bit value (integer value 0-1023) reading of the

temperature sensor and the set-point is a 10bit value representing the desired target temperature of the substrate surface.

The controller was in this case tuned manually by a variant of the Ziegler-Nichols method described earlier in section 2.2.4 of this document. The gain constants K_p , K_i and K_d were set to zero and K_p was then increased until the temperature started to oscillate around the set point, meaning that the critical gain was achieved. Thereafter gain constants were set to the PI controller values of table 1. PI control was used since the system is relatively fast responding, and derivative control could therefore easily cause instability.

Regulation System Casing

The electronic components of the regulation system were soldered onto an Arduino prototyping shield which fits directly onto the Arduino Uno R3 microcontroller board. The Arduino microcontroller board with the shield attached was then mounted in a plastic box which was equipped with a cooling fan, to increase heat dissipation from the H-bridge controller and the voltage regulator, and connections for power supply, temperature sensor and Peltier module.

3.5 Tests of the Temperature Control Cell

3.5.1 Temperature Control System Test – Experimental Procedure

A test of the cooling of the temperature control system was made by starting at ambient temperature (~23 °C) and selecting 20 °C as target temperature. After the temperature had stabilized, the target temperature was set to 15 °C and temperature was allowed to stabilize. This procedure was repeated for 5 °C steps down to -15 °C and up again to ambient temperature.

A similar test was then made for the heating of the temperature control system (which as mentioned earlier has another set of PID gain factors). As in the previous case the test was started at ambient temperature. The target temperature was then set to 30 °C and the temperature was allowed to stabilize at the target temperature. The target temperature was then set to 50, 80, 110, 100, 80, 60, 40, 30 °C and then ambient temperature. The temperature was allowed to stabilize at each target temperature. To test the stability of the temperature regulation, the cold side of the Peltier cooler was kept at -15°C for approximately 15 minutes and the temperature was observed.

A K-type thermocouple was used as a reference thermometer and the temperature of the Peltier module surface was measured at number of temperatures and the thermocouple and thermistor values were noted.

3.5.2 Test using Isoflurane – Experimental Procedure

Isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane), see figure 12 below, is an anesthetic substance in the form of a colorless liquid with pungent odor. It is used as an inhalation anesthetic by vaporizing the liquid. As anesthetic substances can be harmful, or even fatal, if handled in the wrong way, it should only be handled by persons with adequate knowledge and training in anesthetics [39].

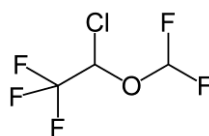


Figure 12. Isoflurane molecule [40].

The Raman instrument setup was a Horiba Jobin Yvon LabRam 800 HR confocal Raman microscope equipped with an Andor Newton back-illuminated EMC CDD and a 785 nm Sacher Lasertechnik Tiger diode laser. LabSpec 5 software was used to operate the system and for data acquisition. A 5x lens was used in backscatter geometry and the confocal hole was set to 500 μm . The grating used in the experiment was a 600 gr/mm blazed at NIR wavelengths. Due to etalon effects in the detector all spectra were corrected according to NIST Standard Reference Material 2241 using 20 s acquisitions and 20 cycles on the reference. The gas was generated using an Ohmeda Enfluratec 5 enflurane anesthetic vaporizer (note that this vaporizer is meant for use with enflurane which means that the vaporizer used in this experiment was not calibrated for isoflurane), with instrument air as carrier gas. A Brühl & Kjaer anesthetic gas monitor type 1304 was used to monitor the gas.

After warming up the 785 nm diode laser the output power was stable and the Raman microscope was calibrated against the 520.7 cm^{-1} peak of Si. Then a fresh SERS substrate (Klarite[®]) was placed in the temperature cell on top of the Peltier element, i.e., between the thermistor bead and the Peltier element. The cell was closed and connected to a water cooling system (water at ambient temperature, ca. 23.5 °C) and to the gas feeding system. The temperature control system was set to maintain the substrate at ambient temperature, 23.5 °C. An optical density filter was used to reduce the illumination power on the sample surface, and subsequently about 8 mW was focused on the SERS substrate. A spectrum was collected before any gas flow was generated. A flow of 1 L/min of instrument air, which was used as carrier gas, was applied and the gas system was checked for leakage using leakage detection spray. As no leakage was detected, a concentration of 5 % isoflurane was applied (the vaporizer was set to 5 %, but the monitor went off scale above 5 % so the actual concentration was unknown but above 5 %). A few spectra were collected to determine appropriate acquisition time and number of cycles. The temperature was then set to +3 °C and was allowed to stabilize at that temperature. An additional couple of test spectra were collected and the appropriate acquisition settings were determined to 20 s and 2 cycles, and the optical density filter was changed to yield a power delivered at the surface of 20 mW.

Three spectra were collected in succession to show that the spectra are reproducible. The vaporizer was then set to pure instrument air, i.e., 0 % isoflurane, to clean the surface and after two minutes a spectrum was collected to ensure that the isoflurane signal had disappeared. The vaporizer was then set to 3 %, the monitor showing approximately 5 %, and two minutes were allowed to pass to let the cell be completely filled with the gas before three spectra were collected in succession. The vaporizer was again set to 0 % isoflurane and after two minutes a spectrum was collected to ensure that the isoflurane signal had disappeared. The laser was then focused on another nearby spot on the substrate surface to avoid laser damage on the SERS substrate. Another spectrum was collected at this new spot. The vaporizer was then set to 1 % isoflurane, the monitor showing approximately 2.5 % (probably because the vaporizer is intended for enflurane), and after two minutes three spectra were collected in succession.

The temperature was then set to -10 °C and was allowed to stabilize before three spectra were collected. The vaporizer was then set to 3 % isoflurane, the monitor showing approximately 5 %, and after two minutes had been allowed to pass, three spectra were collected. The vaporizer was then set to 5 %, the monitor being off scale at above 5 %, and after two minutes three spectra were collected. The vaporizer was then set to 0 % isoflurane, i.e., pure instrument air.

The temperature was then set to $-13\text{ }^{\circ}\text{C}$ and was allowed to stabilize. The grating was set to 600 cm^{-1} . A time mapping was prepared, i.e. a kinetic analysis in which the signal is monitored while the analyte is binding to, or being released from, the surface. The software was set to collect spectra with 2 s acquisition every other second during a minute for a total of 31 spectra. At the same time as the mapping was started, the vaporizer was set to 5 % and was allowed to be open for 5 s before the valve was closed. After the time mapping had been completed, a gain of 160 was then applied to the sensor and a similar time mapping was performed with the new sensor setting. The total mapping time was then changed to 2 minutes (still 2 s acquisitions every other second). At the same time as the mapping was started, the vaporizer was set to 5 % isoflurane and after one minute the valve was closed. When the third time mapping was completed, the software was set to collect 4 s acquisitions every 5 s during three minutes. At the same time the mapping was started, the vaporizer was set to 5 % and the valve was kept open for one minute before it was closed.

When the time mappings had been completed, the acquisition settings were returned to 20 s acquisitions and 2 cycles. The vaporizer was then set to 5 % isoflurane, the monitor being off scale above 5 %. The temperature was set to $+3\text{ }^{\circ}\text{C}$ and was allowed to stabilize. A spectrum was collected after two minutes. The temperature was then set to $25\text{ }^{\circ}\text{C}$ and was allowed to stabilize before a spectrum was collected. Then the temperature was set to $+50\text{ }^{\circ}\text{C}$ and a spectrum was collected after the temperature had stabilized.

To ensure that the right substance is detected, a “normal” Raman spectrum of liquid phase isoflurane was collected for comparison using a handheld Raman spectroscopy instrument First Defender RMX®, Thermo Fischer Scientific, measuring directly in the storage bottle.

4 Results & Discussion

4.1 System Description

4.1.1 The Modified Stage

The modified stage can be seen in figure 13 below.

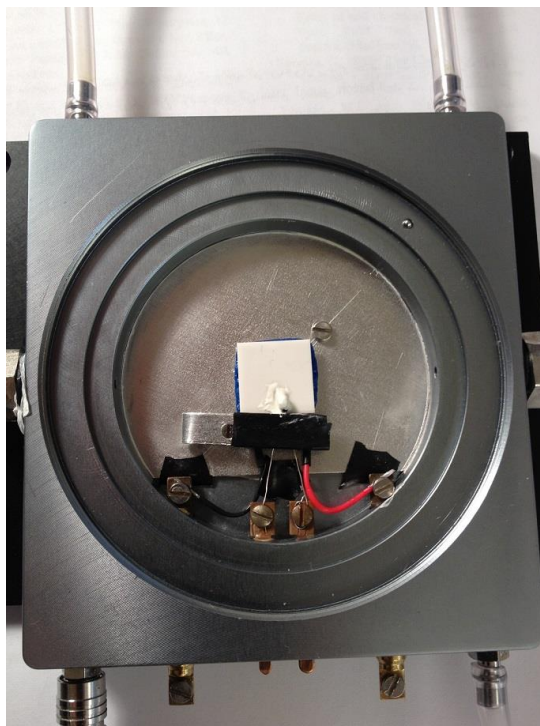


Figure 13. Top down view of the interior of the modified stage.

Note that the black thermistor bead is submerged in a drop of white thermal compound. The SERS substrate is inserted between the thermistor bead and the Peltier module surface when SERS measurements are to be made.

4.1.2 PID Control

The target temperature should be reached in a reasonable amount of time; however, it should not overshoot the target temperature since the analyte could for example be unintentionally released from the substrate surface. This meant that a compromise between fast response, low or no overshoot and high stability was desired. Values of $K_p = 1.5$, $K_i = 0.5$ for cooling, i.e., ambient temperature and below, and $K_p = 1$, $K_i = 0.3$ for heating, i.e. above ambient, worked in this case.

The set-point can be changed by the user when the program is running. This is done by sending the desired target temperature (e.g., 45 or -5.50 for 45 °C or -5.50 °C respectively) on the Arduino hardware serial port by using for example the Arduino IDE's built in serial monitor. The program senses when a message is sent on the serial port and converts the incoming temperature to a 10bit value and stores it in the set-point variable of the PID controller. The output of the PID controller is by default a 8bit value (integer value 0 - 255) to be compatible with the PWM pins of the Arduino board and thus needs to be rescaled to fit the output range of the SMC05A, i.e., 0 - 127, and the corresponding voltage is applied to the Peltier cooler using the method described under the *H-bridge Controller* header.

Note that the set point should be ramped in an appropriate way, e.g., 5 °C or 10 °C steps, in order not to damage the Peltier cooler, or other equipment, by thermal shock as the system has rapid response and can cover large temperature differences in short time period.

4.1.3 System Overview

An overview of the temperature regulation system can be seen in figure 14 – 16.

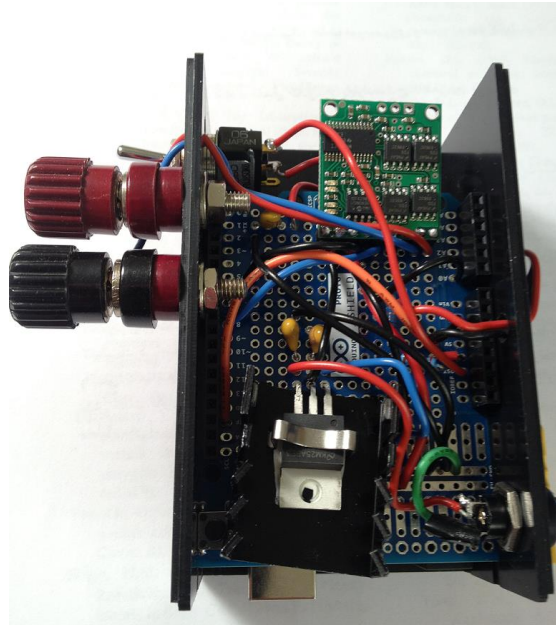


Figure 14. Top down view of the regulation system.

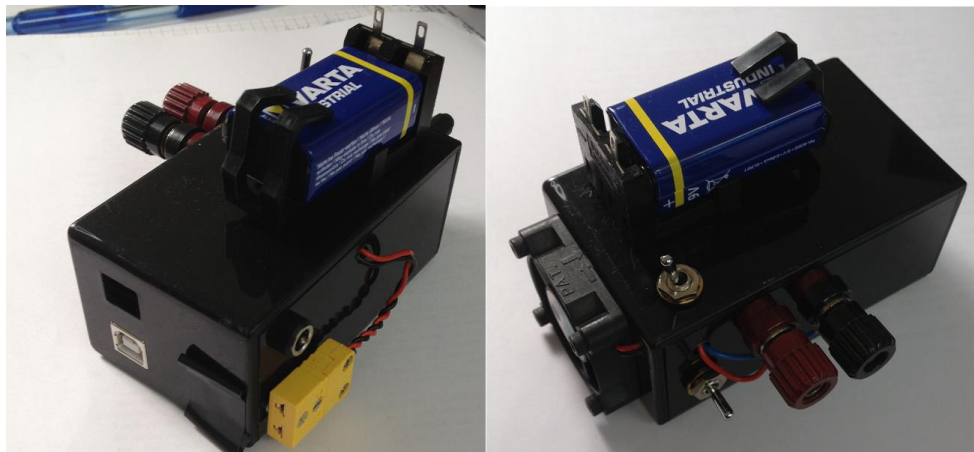


Figure 15. The regulation system box with fan-cooled lid attached.

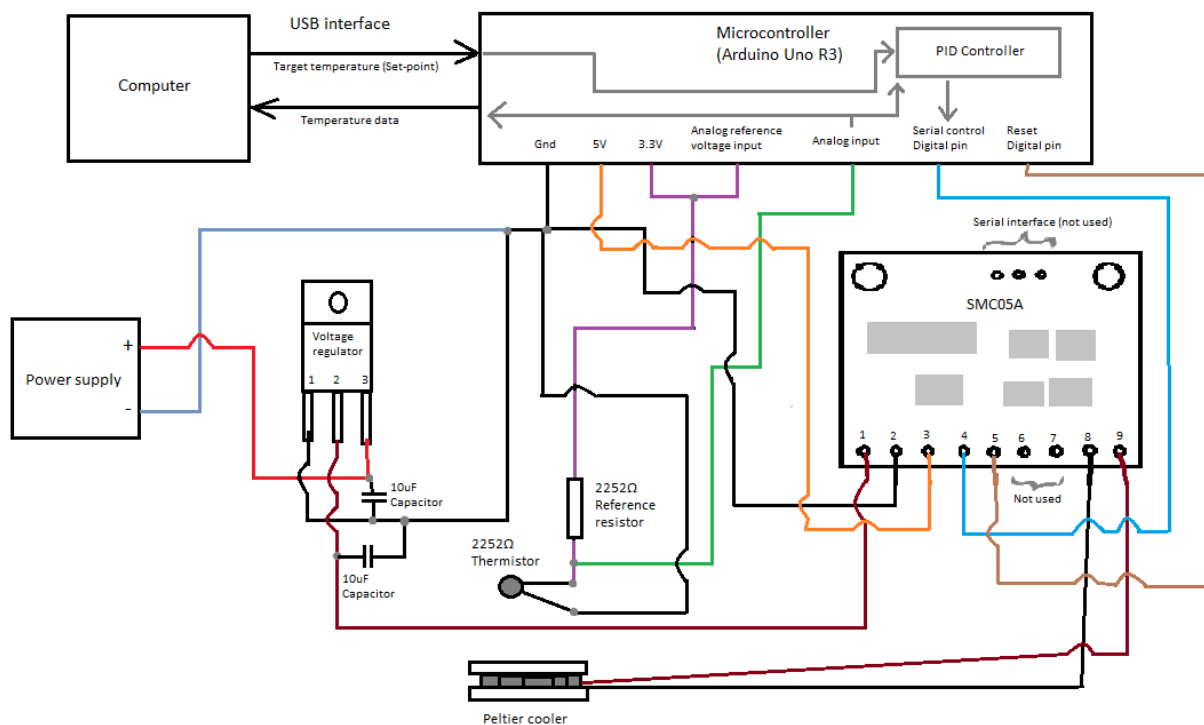


Figure 16. Overview of the data acquisition and instrument control system.

The user is selecting a target temperature (set-point) by sending the desired temperature on the hardware serial port to the microcontroller; the default set-point is 25 °C. The program running on the microcontroller is recognizing when a temperature value is sent on the serial port and stores the value into a variable. The temperature is then converted into a 10bit precision value, 0 - 1023, which is corresponding to the value that would be read on the analog input pin when the temperature sensor is at that temperature. This value is then used as set-point for the PID controller. The program then measures the temperature by the method described in the temperature measurement section of the report. This temperature is thereafter sent via the serial port to the computer so the user can read the temperature and the elapsed time since the program started. The program then decides if the PID controller should be in direct or reverse mode (cooling or heating) depending if the set-point temperature is above or below the ambient temperature (room temperature, defined as 25 °C in the program). The PID controller then computes the appropriate output, and the program sends the corresponding command on a software serial port to the H-bridge controller which in turn sets the voltage supplied to the Peltier cooler at the corresponding level and direction. The loop then starts again and the set-point is kept at the last set value until a new set-point is sent on the serial port.

The source code for the Arduino sketch, *kylcell.ino*, can be found in Appendix C.

4.2 Test Results

4.2.1 Results of Temperature Control System Test

Figure 17 shows the result from a test run of the cooling of the temperature control cell. The system is almost exactly critically damped with virtually no overshoot and the temperature response is rapid.

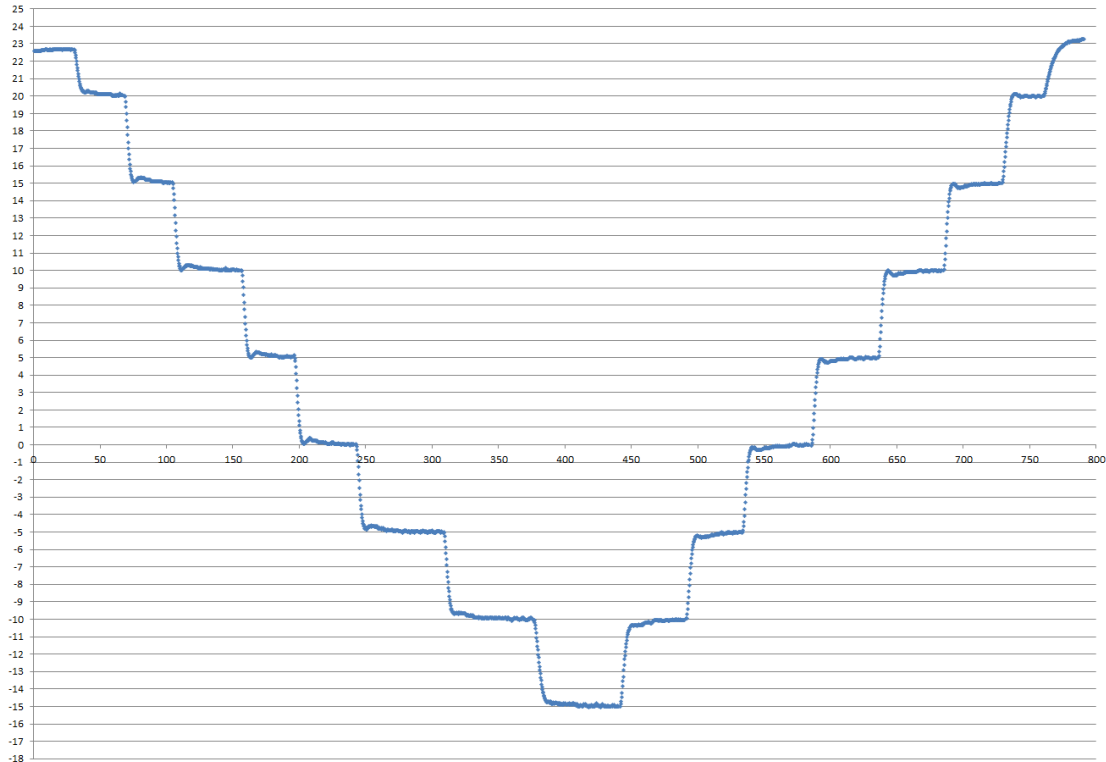


Figure 17. Temperature [°C] vs. time [s] plot from a test run of the cooling of the temperature control cell.

The results from the corresponding test of the heating of the temperature control cell are found in figure 18 below. Note that at 80 °C the target temperature was changed before the temperature had stabilized. Also note that the system is under-damped up to about 60 °C and is over-damped above that temperature. This means that above room temperature and up to about 60 °C there is some overshoot and above 60 °C there is little or no overshoot. This is a compromise between being able to reach high temperatures and keeping the overshoot as low as possible.

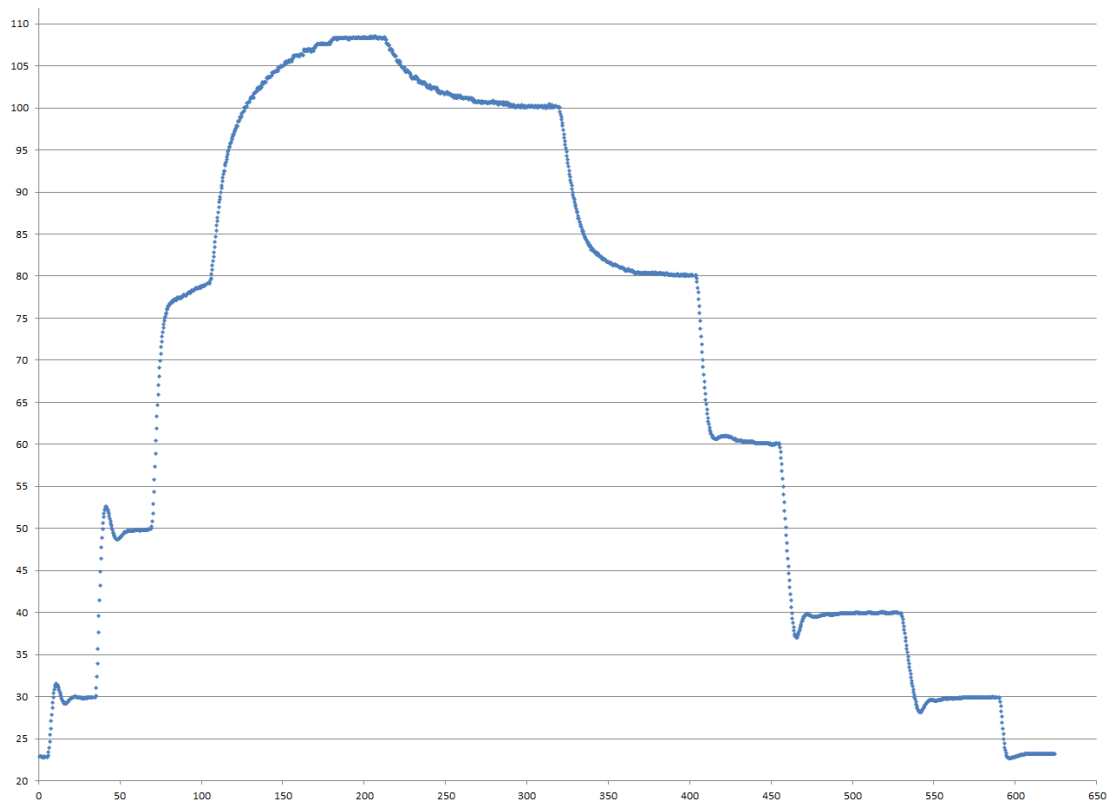


Figure 18. Temperature [°C] vs. time [s] plot from a test run of the heating of the temperature control cell.

The stability test showed that the temperature is kept within ± 0.22 °C of the set point during the test, with a standard deviation of 0.04, which is well within the 0.5 °C stability requirement. The temperature is not showing any signs of drift from the set point over time.

Table 5 below shows temperature readings from the thermistor and from a K-type thermocouple placed on the surface of the Peltier module just next to the thermistor. The temperature readings agree fairly well. One has to consider that the K-type thermocouple has considerably less accuracy than the 44004 thermistor element which is used in the cell (± 0.5 % of reading ± 1 °C] for the thermocouple compared to ± 0.2 °C for the thermistor) and it would have been better to use a platinum RTD as reference but as no platinum RTD was available at the time the thermocouple would have to suffice. The size of the thermistor sensor is larger than the size of the measurement junction of the thermocouple. The round geometry (bead shape) of the thermistor requires that thermal compound is used to increase the thermal contact between the thermistor and the SERS substrate or Peltier module surface. Both of these reasons probably contributes to that the thermistor reads less extreme values than the thermocouple.

Table 5. Thermistor temperature readings and K-type thermocouple temperature readings for verification.

Thermistor temperature [°C]	Thermocouple temperature [°C]
-10.0	-12.0
0.0	-0.8
10.0	9.4
22.7	22.4
30.0	30.2
50.0	51.4
70.0	72.3

4.2.2 Results of Test Using Isoflurane

In this section the results of the SERS experiment using the temperature control cell to detect isoflurane are presented and discussed.

A “normal” Raman spectrum of isoflurane in liquid phase, and a SERS spectrum of isoflurane (5 % concentration) collected at -10 °C, is shown in figure 19 with wavenumbers of significant peaks noted. The liquid phase spectrum was collected using a handheld Raman instrument measuring directly in the isoflurane storage bottle.

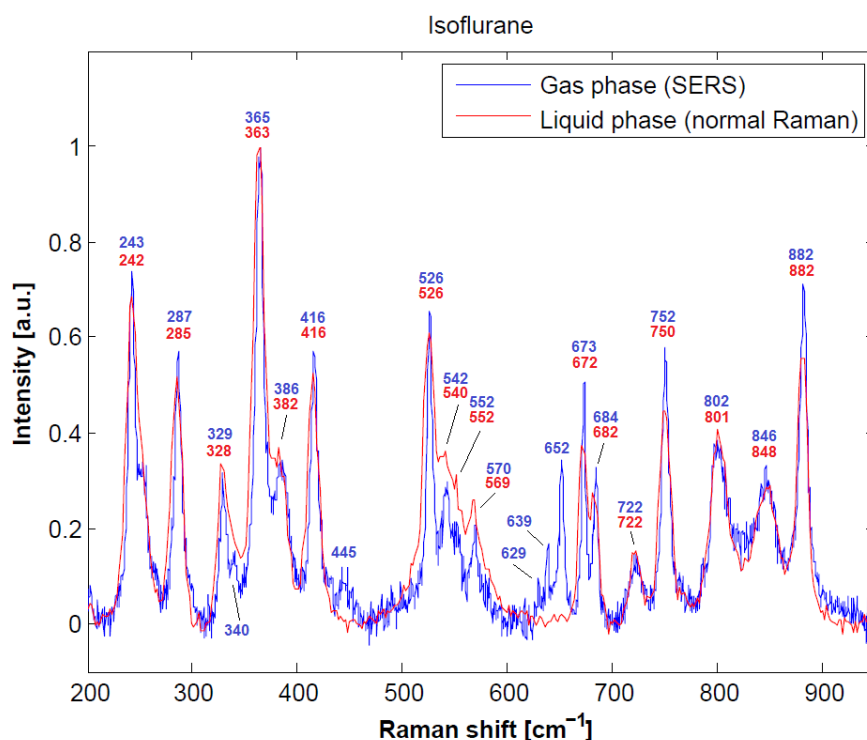


Figure 19. Comparison between Raman spectrum of isoflurane in liquid phase and SERS spectrum of isoflurane in gas phase. Note that the spectra are normalized.

The similarity between the spectra in figure 19 is striking; it is indeed isoflurane that is detected. Although additional peaks, e.g., at 629, 639 and 652 cm^{-1} , are showing up, all significant peaks are seen in the SERS measurement, and it is conclusive that isoflurane vapor is in-situ monitored by SERS.

The origins of the extra peaks are not further investigated, but one should bear in mind that a SERS spectrum does not necessarily overlap a spectrum originating from “normal” Raman scattered photons.

The background spectrum, collected at ambient temperature before any gas flow was applied, is presented in figure 20.

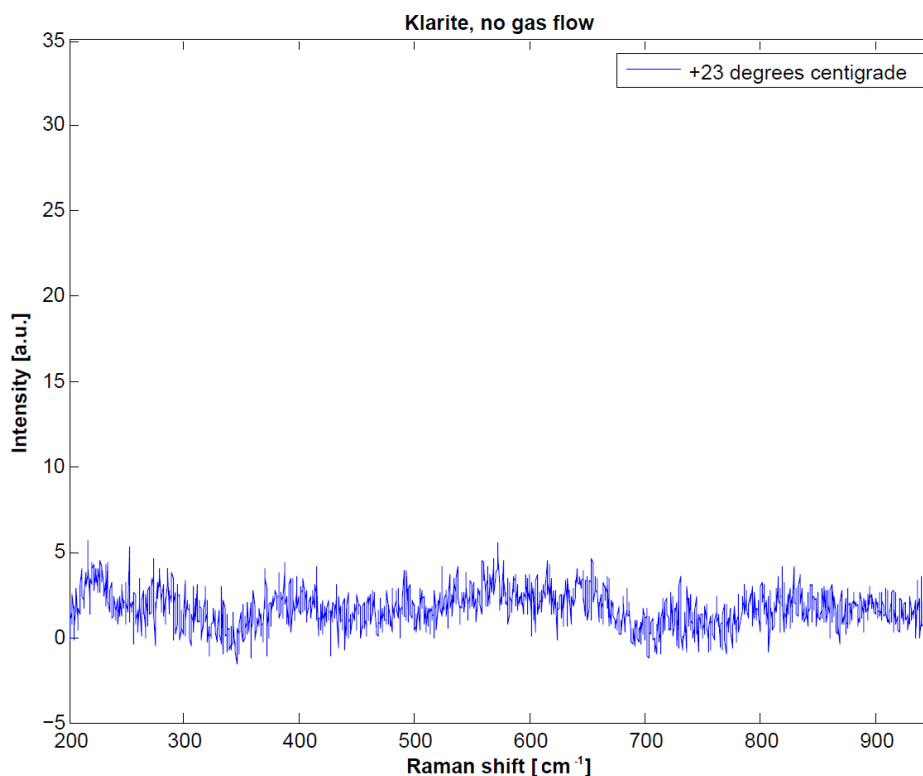


Figure 20. Background spectrum of the Klarite® substrate at ambient temperature without any gas applied.

Temperature Study and Regeneration of the Substrate

No peaks were detected after the vaporizer had been set to 5 % at 23.5 °C. The spectra collected when the temperature was set to +3 °C and the vaporizer set to 5 % clearly display isoflurane peaks.

Figure 21 below shows the three spectra collected when the temperature was set to -10 °C and the vaporizer set to 5 % isoflurane. The signal had increased significantly compared to the spectra collected at +3 °C. Apparently by lowering the temperature the amount of analyte molecules in close proximity to the substrate surface is increasing whereupon a signal enhancement is seen. Note that the three spectra are practically identical which supports reproducibility.

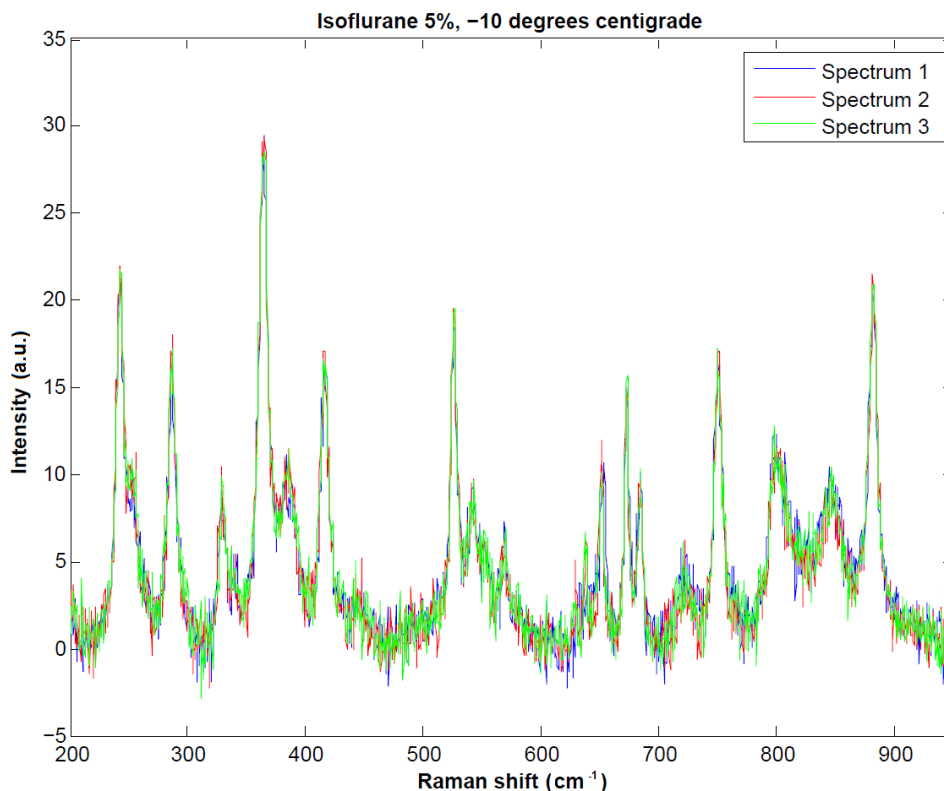


Figure 21. Three spectra, temperature set to -10 °C and vaporizer set to 5 %.

At the highest concentration, with the vaporizer set to 5 %, the temperature started to climb from -10 up to -5 °C for the first spectrum and -2.5 °C for the second and third spectra. The temperature returned to the preset temperature quickly after the isoflurane valve was closed. The condensation of the isoflurane thus seems to affect the cooling, at least when the vaporizer was set to 5 % since this phenomenon was not observed for the lower concentrations or at +3 °C, ambient temperature (23.5 °C – 25 °C) or at 50 °C. However, the temperature rise does not seem to have affected the signal to any significant extent since the three spectra are practically identical.

Spectra (mean) for the three concentrations at +3 and -10 °C are found in figures 22 and 23 respectively. Only two of the three collected spectra of 3 % isoflurane at +3 °C were used since the third contained a broad peak (between ca 680 – 780 cm^{-1} , centered at about 720 cm^{-1}) which was not visible in the first two spectra. This peak could for example be due to stray light (e.g. lighting entering the cell) or dust. Note that with the temperature set to -10 °C, figure 23, the signal is practically identical (with the exception of the three peaks at 629, 639 and 652 cm^{-1}) with the vaporizer set to 3 and 5 %, which indicates that the SERS substrate is saturated at 3 % at this temperature.

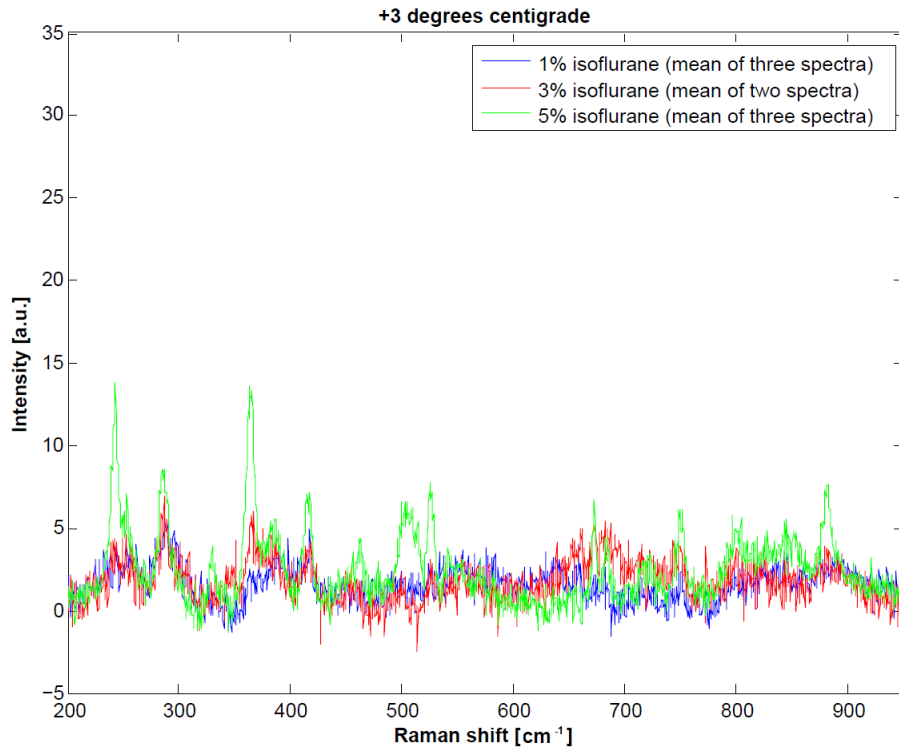


Figure 22. Spectra (mean) of the three concentrations with the temperature set to +3 °C.

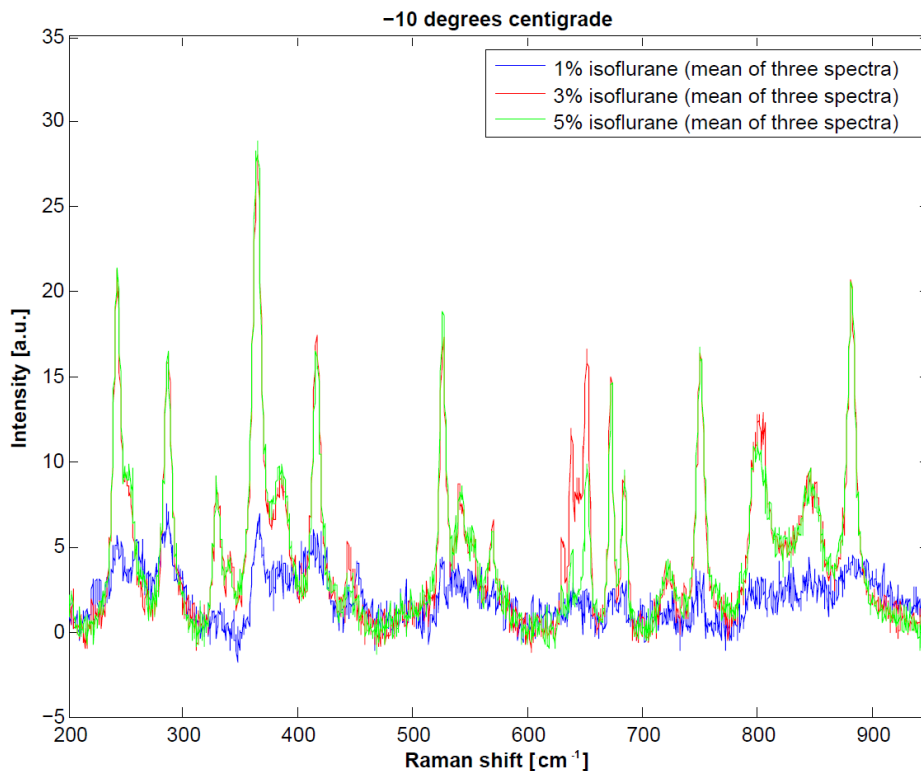


Figure 23. Spectra (mean) of the three concentrations with the temperature set to -10 °C.

When the substrate was heated, the gain of the detector was, by mistake, not reset to the previous value used before the time mapping acquisitions. This means that the signal and noise are amplified in the three spectra collected at +3, +25 and +50 °C when the substrate was heated. However, when

a damping factor of 0.05 (i.e., damped 20 times) is used on the spectra collected after the gain had been changed and plotted together with the +3 °C spectrum collected before the gain was changed, see figure 24, the spectra look rather similar.

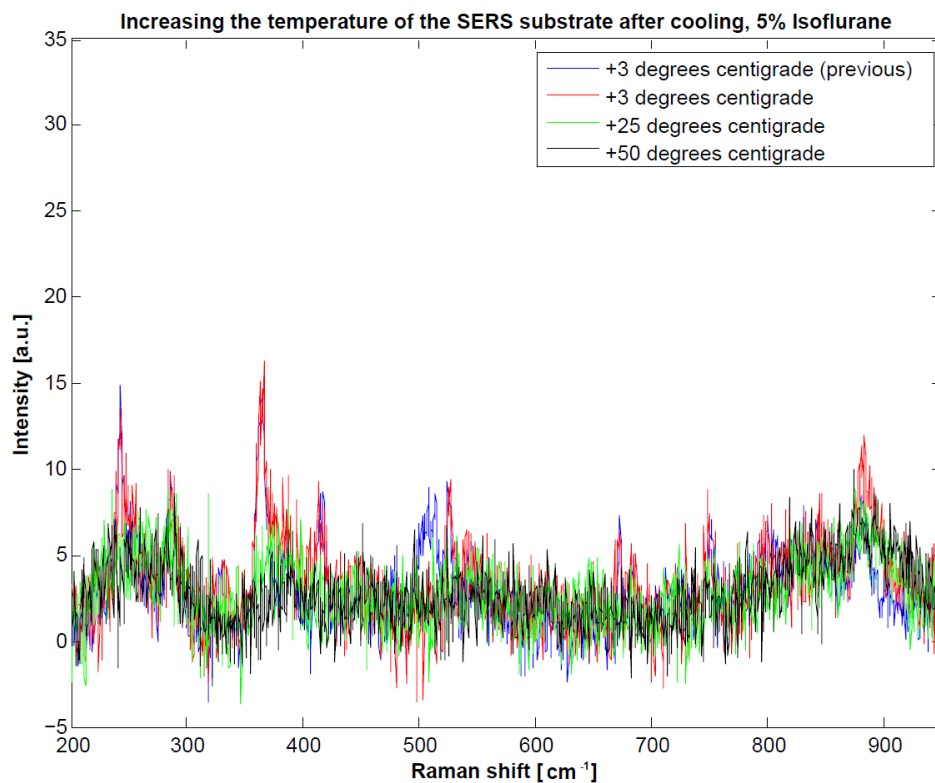


Figure 24. "Dampened" spectra compared to a spectrum previously collected at +3 °C.

The signal apparently decreases when the temperature of the SERS substrate is increased, which can be seen in figure 25. At +25 °C, the ambient temperature, the signal seemed to have disappeared implying that the SERS substrate is fully regenerated.

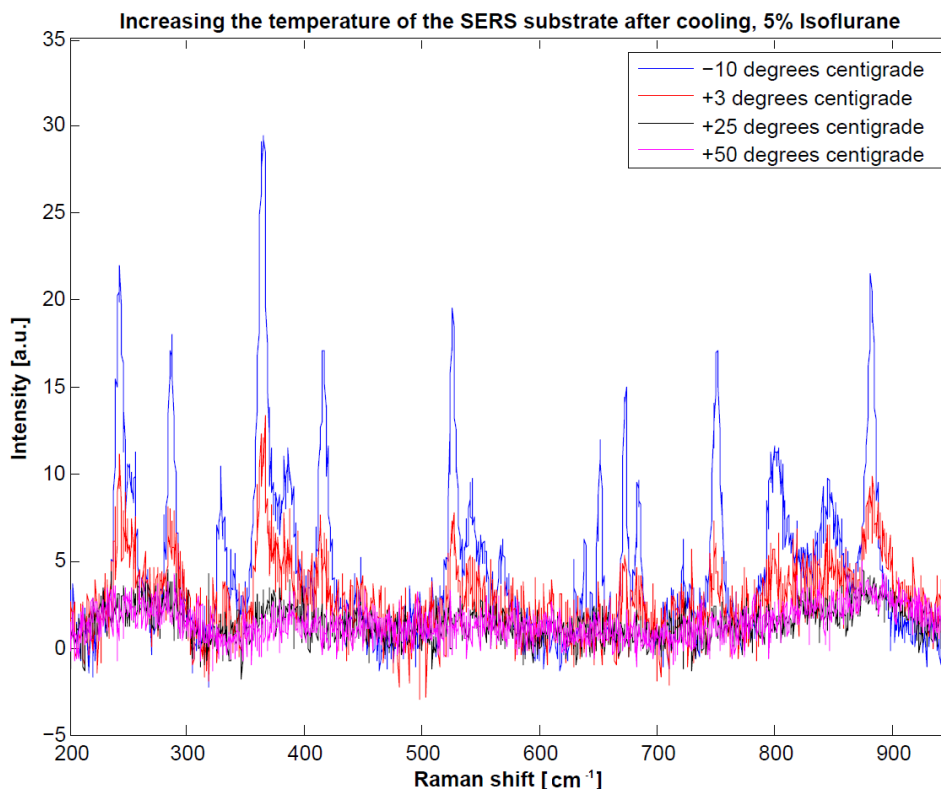


Figure 25. Regeneration of the SERS surface. The intensities are adjusted to enhance visualization.

Kinetics - Time mapping

The time mapping shows that it is possible to monitor the kinetics of the substance binding to, or being released from, the substrate. The result of the mapping can be seen in figure 26. The signal was calculated as the area of the 365 cm^{-1} peak. The signal increases rapidly, rising immediately from the first spectrum, begins to level out after 20 s and decreases after the vaporizer were set to 0 %, indicated by the red vertical line in figure 26. Notice the sudden drop after about 140 s. This drop could also be seen for the 243 cm^{-1} peak as well as for the 416 cm^{-1} peak. In hindsight a better baseline could be attained by letting the kinetic mapping to go on a longer time without flowing isoflurane.

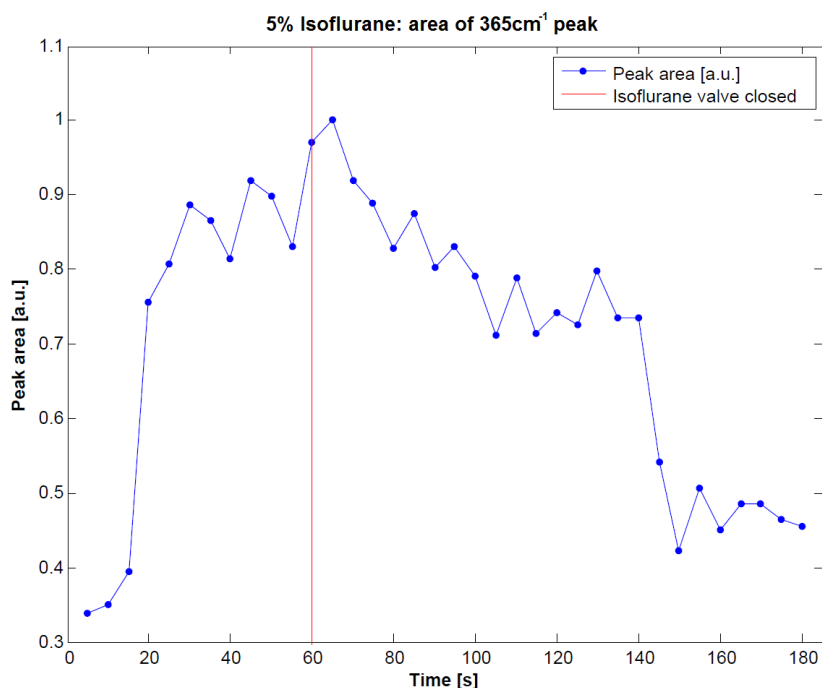


Figure 26. Area of the 365 cm^{-1} peak vs time plot. The data is from the 4th time mapping. The red vertical line is indicating when the isoflurane vaporizer was set to 0 %.

5 Conclusion

The described tests show that the temperature control cell is working well; by cooling, and thereby condensing analyte substances onto the SERS substrate, volatile substances can be detected easily in-situ. By increasing the temperature of the SERS substrate, the volatile analyte is released and the SERS substrate is fully regenerated. It can also be concluded by the results of this project that reproducible SERS measurements are possible. Isoflurane is just an example of many volatile substances, that weakly interact with gold surfaces, but shown possible to detect using the temperature control of the SERS substrate.

The choice of; cooler/heater, temperature sensor, and thermally conductive adhesive etc. implies that the working temperature range is narrower than the originally desired -20°C to $+200^{\circ}\text{C}$. Instead it ranges approximately from -15°C to $+110^{\circ}\text{C}$ if the temperature of the water reservoir of the water cooling system is at room temperature ($\sim 23^{\circ}\text{C}$) as it was in the tests conducted in the project. Due to the properties of the Peltier device, one way to expand the temperature range would be to use a temperature controlled water reservoir for the water cooling system. In that way, one would be able to lower the temperature of the circulating water and thereby achieving a lower temperature of the cold side of the Peltier element. Conversely heating the circulating water would yield higher temperatures, however the temperature limits of individual components must be taken into consideration since they could be damaged.

To simplify for the user it would be convenient to have an automated set point (or controller output) ramp function, in which the set point (or controller output) can be changed at a suitable rate preferably chosen by the user. In the present case, the user makes a manual set point “ramp” by

selecting target temperatures in reasonable steps, and in this way undue thermal stress on the substrate and other equipment is avoided.

The round bead shape geometry of the Omega 44004 thermistor element is not optimal for the cell. By decreasing the temperature sensor size and/or using a flat sensor (specifically designed for surface temperature measurements) one could increase the thermal contact between the temperature sensor and the substrate surface and thereby increase the *reliability* of the temperature values from the sensor. Note, however, that this does not increase the *accuracy* of the sensor. The ideal would be finding a way to have four-wire sensor connection (not using the thermocouple connector which is the present case) and a platinum RTD with small size and short response time, since platinum RTDs have better long term stability and have rather linear response in the temperature range of the application. Since the Pt-100 sensors are almost linear in the temperature range for this application, one could perhaps replace the thermistor with a Pt-100 sensor and connect it with the thermocouple connector. Then one can compensate for the added resistance at one temperature in the software and due to the approximate linearity of the Pt-100 it would probably work well through the temperature range.

One must bear in mind that the substance to be analyzed can interact with the cooler, sensor and/or thermal compound (which was the case in the acetone test) etcetera, which affect performance of the temperature control system; and therefore one should investigate if this is the case prior to tests.

Additionally, by reducing the volume of the cell interior, one could reduce the time it takes for the gas to fill the cell, as well as directing the flow over the SERS substrate surface.

6 Acknowledgements

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7 Appendix:

Appendix A: Source Code

```
/* PROGRAMKOD FÖR TEMPERATURKONTROLL AV MÄTCELL
 * Arduino Uno med Pololu SMC05a Low Voltage Dual Serial Motor Controller
 * och PID-kontroll. Spänningsregulator LM1084 3.3V, Nätadapter 5V, 3A.
 * Peltierkylare(TE Technology VT-31-1.0-1.3) och termistor (Omega 44004)
 */

/* Följande PID-bibliotek implementeras i koden:
 * Arduino PID Library, PID_v1 (Beaugard 2014)
 * Brett Beaugard <br3ttb@gmail.com>
 */

/*
 * Obs! Funktionerna för styrning av och kommunikation med
 * SMC05A-kortet är inspirerade av följande länk:
 * http://www.psurobotics.org/wiki/index.php?title=Pololu_Dual_Serial_Motor_Controller
 * SMC05A Pololu Dual Serial Motor Controller
 * Jeremy Bridon <jbridon@psu.edu>
 * Anthony Cascone <acascone@psu.edu>
 */

//IMPORTERA BIBLIOTEK
#include <SoftwareSerial.h> //Mjukvaruseriekomm.-bibliotek
#include <PID_v1.h> //PID-kontrollbibliotek
#include <math.h> //Matematikbibliotek

//SKAPA/DEFINIERA KONSTANTER OCH VARIABLER:
#define rxPin 6 // Definierar "Recieve"-ingången för mjukvaruserieport för drivkretsen
#define txPin 7 // Definierar "Transmit"-utgången för mjukvaruserieport för drivkretsen
#define resetPin 8 // Definierar "Reset"-utgången för drivkretsen
#define inputPin A0 // Definierar temperatursensoringång
double temperature; //Temperaturvärde Celsius
double Rt; //Termistormotstånd
double E_Uab; // E - Uab
double logRt; // log(Rt)
const double R0 = 2252.3; //Fixt referensmotstånd i spänningsdelare
float Rsp_R0; //"Förenkling"
int i; //Räknevariabel
int antalprov=10; //Antal prov att ta medel av
int provvektor[10]; //Vektor/array för prov för medelvärde
double Setpoint, Input, Output; //Variabler
double Tsp = 25; //Default måltemperatur
double TspK; //Måltemperatur i Kelvin
double Rsp; //Motstånd vid måltemperatur
float alpha; //Alphavärde
float beta; //Betavärde
float b3c; //"Förenkling"

//Termistorspecifika konstanter till
//Steinhart-Harts ekvation
//(Thermistor element 44004, Omega Engineering Ltd)
const float a = 0.001468;
const float b = 0.0002383;
const float c = 0.0000001007;

// Tuning-parametrar för PID-loop
//(Kylning:)
float Kp=1.5; //Proportional gain för kylning, använd 1.5
float Ki=0.5; //Integral gain för kylning, använd 0.5
float Kd=0; //Derivative gain för kylning, använd 0
//(Värmning:)
float hKp=1; //Proportional gain för värmning, använd 1
float hKi=0.3; //Integral gain för värmning, använd 0.3
float hKd=0; //Derivative gain för värmning, använd 0
```

```
//Skapa PID-loop med namnet peltierPID
PID peltierPID(&Input, &Output, &Setpoint, Kp, Ki, Kd, DIRECT);

// Skapa mjukvaruserieport (Rx, Tx -kommunikationsut-/ingångar):
SoftwareSerial drivkretsSerial(rxPin, txPin);

//KONFIGURERINGSDEL (körs en gång):
void setup(){
  Serial.begin(9600); //Starta seriekommunikation
  delay(50);

  //VIKTIGT!!! Följande kodrad måste finnas om extern referensspänning används!!!
  //Annars kortsluts den interna referensspänningen med den externa vilket kan
  //skada arduinokortet!
  analogReference(EXTERNAL);

  startDrivkrets(); //Starta kommunikation och styrprotokoll för drivkretsen
  peltierPID.SetMode(AUTOMATIC); //Läge för PID-kontrollern, AUTOMATIC = "på"
  //Sätt ingångsläge för temperatursensoringången:
  pinMode(inputPin, INPUT);
}

//HUVUDLOOP (körs om och om igen):
void loop(){
  //Läs målvärdestemperatur vid inmatning
  if(Serial.available() > 0){
    Tsp = Serial.parseFloat();
  }
  TspK = Tsp + 273.15; // Konvertera till Kelvin
  alpha = (a - 1/TspK)/c; //Alfavärde
  b3c=b/(3*c); //Förenkling"
  beta = sqrt(b3c*b3c*b3c + alpha*alpha/4); //Betavärde
  Rsp = exp(pow((beta - 0.5*alpha),1.0/3.0) - pow((beta + 0.5*alpha),1.0/3.0)); //Motstånd vid måltemperatur
  Rsp_R0 = Rsp / R0; //Förenkling"

  //Räkna om till värde motsv. spänning i spänningsdelare (0-1023)
  //och sätt Setpoint-variabeln till detta värde:
  Setpoint = 1023*(Rsp_R0/(1 + Rsp_R0));

  //Läs antalprov sensorvärden (0-1023) med liten fördröjning
  float average;
  for (i=0; i< antalprov; i++) {
    provvektor[i] = analogRead(inputPin);
    delay(20);
  }
  //Ta medelvärde av lästa sensorvärden
  average = 0;
  for (i=0; i< antalprov; i++) {
    average += provvektor[i];
  }
  average /= antalprov;

  //Sätt Input-variabeln till detta medelvärde
  Input = average;

  //Räkna ut temperaturvärde från mätt värde
  E_Uab = 1023-average;
  Rt = R0*(average/E_Uab);
  logRt= log(Rt); //log = naturliga logaritmen (ln(X)) i Arduino

  //Steinhart-Harts ekvation
  temperature = 1/(a + b*logRt + c*logRt*logRt*logRt) - 273.15;

  float Time = millis(); //Millisekunder sedan programmet startades
  float Timeseconds = Time/1000; //Konvertera till sekunder

  //Skriv tid sedan start och temperatur till serieporten.
  Serial.print(Timeseconds);
  Serial.print(",");
  Serial.print(temperature);
  Serial.print("\n");
}
```

```
delay(50);

if (Setpoint <= 510){
  //Om måltemperaturen överstiger (ca) 25 grader
  //ställ om PID-kontrollern för värmning.
  peltierPID.SetControllerDirection(REVERSE);
  peltierPID.SetTunings(hKp,hKi,hKd);
}
else {
  //Annars ställ in PID-kontrollern för kylning.
  peltierPID.SetControllerDirection(DIRECT);
  peltierPID.SetTunings(Kp,Ki,Kd);
}

delay(10);

//Kör PID-algorithm (P, PI eller PID beroende på hur konstanterna valts)
peltierPID.Compute();
delay(50);

Output=map(Output, 0, 255, 0, 127); //Skala om till 6bit

if (temperature >= 120) {
  Output=0; //Sluta värma om temperaturen överstiger 120 grader.
  //Detta för att skydda temperatursensorn och peltierelementet.
}

if (Setpoint > 510) {
  SetPeltier(0, 128 - Output); //Kylning
}
else
{
  SetPeltier(0, 128 + Output); //Värmning
}
}

// NEDAN FÖLJER DEFINITIONER AV FUNKTIONER FÖR STYRNING AV DRIVKRETS

// Initiering av drivkretsen
void startDrivkrets(){
  //Ingångs-/Utgångskonfiguration
  pinMode(rxPin, INPUT);
  pinMode(txPin, OUTPUT);
  pinMode(resetPin, OUTPUT);

  drivkretsSerial.begin(9600); //Påbörja seriekommunikation (drivkrets)
  delay(50); //Vänta X millisekunder
  digitalWrite(resetPin, LOW); //Stäng drivkretsen
  delay(10); //Vänta X millisekunder
  digitalWrite(resetPin, HIGH); //Slå på drivkretsen
  delay(100); //Vänta X millisekunder för uppstart
}

// Funktion för att välja effekt och riktning
void SetPeltier(int peltierIndex, int power)
{
  // Begränsa till 0-255
  if(power < 0)
    power = 0;
  else if(power > 255)
    power = 255;

  // Konvertera till riktning
  boolean Cool;
  if(power >= 128)
  {
    Cool = true; //Kyl
    power -= 128; // Power=Power-128
  }
  else
  {
    Cool = false; //Värm
  }
}
```

```

        power = 127 - power;
    }
    // Skicka vidare till grundfunktionen av "SetPeltier()", se nedan
    SetPeltier(peltierIndex, power, Cool);
}
// Grundfunktion SetPeltier()
void SetPeltier(int peltierIndex, int power, boolean GoCool)
{
    // Skapa paketbuffer
    unsigned char buffer[4];
    // Start-byte och ID-byte
    buffer[0] = 0x80; //Startbyte
    buffer[1] = 0x00; //Drivkrets-ID
    unsigned char index = 0; // Peltierindex (vilka utgångar på drivkortet ska användas)
    if(peltierIndex > 0 || peltierIndex < 0)
        index = 2;
    // Lägg till riktning och skapa byte 3 i protokollet.
    if(GoCool)
        buffer[2] = 0x00 | index; //Bitwise OR
    else
        buffer[2] = 0x01 | index; //Bitwise OR
    // Energiflöde till Peltierelement
    unsigned char targetpower = 0;
    if(power >= 128)
        targetpower = 127;
    else if(power < 0)
        targetpower = 0;
    else
        targetpower = (unsigned char)power;
    buffer[3] = targetpower;
    // Skicka paketet till drivkrets
    Kommunicera(buffer, 4);
}
//Skriv till drivkretsen via den virtuella serieporten
void Kommunicera(unsigned char *buffer, int bufferCount){
    for(int i = 0; i < bufferCount; i++)
        drivkretsSerial.write(byte(buffer[i]));
}

```

Appendix B: Introductory SERS Experiment using BPE

Experimental

As an introduction to SERS measurements, a test was made to measure the response from BPE (*trans*-1-(2-Pyridyl)-2-(4-pyridyl)-ethylene on a Klarite® substrate at different concentrations (200 nM, 500 nM, 1 µM, 5 µM, 10 µM, 100 µM, 500 µM and 1mM).

The Raman instrument setup was a Horiba Jobin Yvon LabRam 800 HR confocal Raman microscope with LabSpec 5 software equipped with an Andor Newton back-illuminated EMC CDD and a 785 nm Sacher Lasertechnik Tiger diode laser. A 5x lens was used in backscatter geometry and the confocal hole was set to 500µm. The grating used in the experiment was a 600 gr/mm blazed at NIR wavelengths. Due to etalon effects in the detector all spectra were corrected according to NIST Standard Reference Material 2241 using 10s acquisitions and 75 cycles.

The experimental procedure follows roughly the same experimental procedure as in:

Jason A. Guicheteau, Mikella E. Hankus, Steven D. Christesen, Augustus W. Fountain III, Paul M. Pellegrino, Erik D Emmons, Ashish Tripathi, Phillip Wilcox, Darren Emge, *Standard method for*

characterizing SERS substrates, Proceedings of SPIE Vol. 8373 837320, p1-8. Downloaded on 20140306 from <http://proceedings.spiedigitallibrary.org/>

After warming up the 785 nm diode laser output was stable and the Raman microscope was calibrated against the 520.7 cm^{-1} peak of Si. The Klarite® SERS substrate was first placed in a clean Petri dish and analyzed dry using a 5x lens and 10 s acquisition time. Then spectroscopic grade ethanol was added to the Petri dish and the substrate was observed to see if the ethanol was causing any visible change to the substrate surface. If no changes in the surface were observed, the substrate was then placed in the lowest concentration solution and was then analyzed every 2 minutes for a 20 minute time period as a kinetic study to see that the spectral response had stabilized before the next step. After the 20 minutes of the kinetic study, a few minutes passed as the Raman instrument was adjusted for duoscan (i.e., “sweeping”) mode. A mapping was then made by analyzing at 5 “spots”, sweeping a $50 \times 50\ \mu\text{m}$ rectangle at each spot, on an array over the substrate taking two spectra at each spot for averaging. The substrate was then placed in the next concentration and the procedure is repeated for every concentration.

The signal was then calculated as the ratio of the area of the 1200 cm^{-1} BPE peak and the area of the 880 cm^{-1} ethanol peak from the mapping. The data was plotted as the signal as a function of concentration.

Results and Discussion

All concentrations were covered for the Klarite® substrate. Figure B1 below shows a spectrum for the $500\ \mu\text{M}$ concentration as an example. The ratio of the area of the 1200 cm^{-1} BPE peak and the 880 cm^{-1} ethanol peak from the mappings for the different concentrations is plotted in figure B2 with error bars showing the standard deviation. Notice the large standard deviations due to large variations in signal between the mapping spots on the substrate.

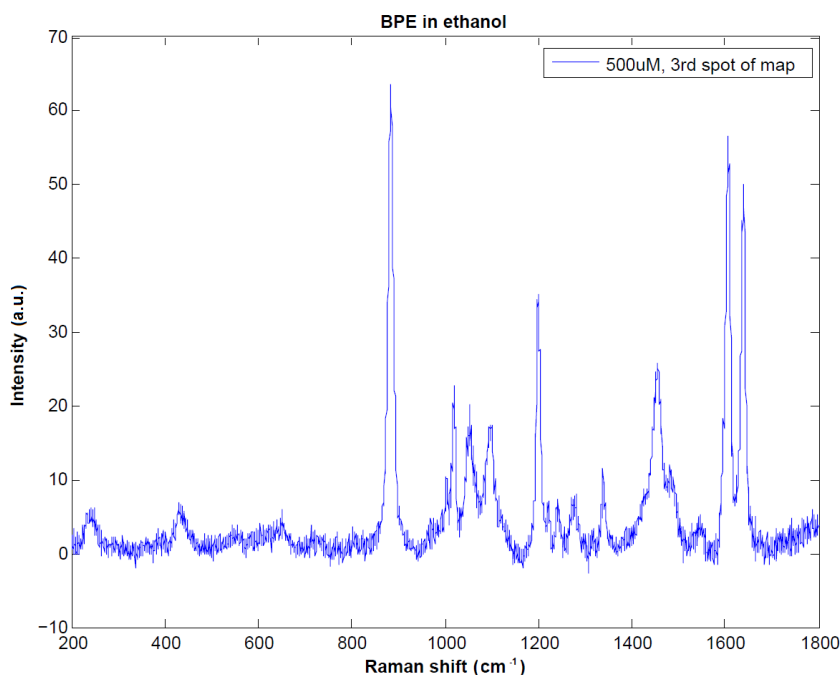


Figure B 1. A SERS spectrum for $500\ \mu\text{M}$ BPE/Ethanol using a Klarite® substrate.

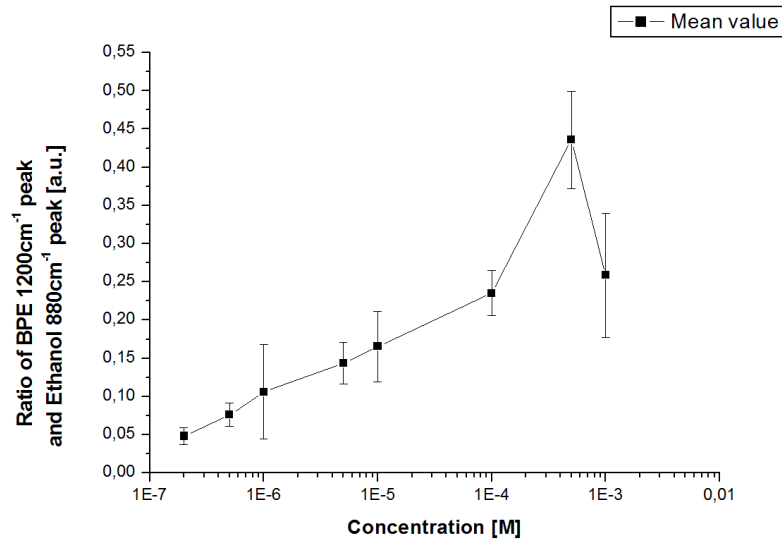


Figure B 2. Mean value of mapping of each concentration. Error bars show standard deviation. The horizontal axis has logarithmic scale.

Figure B3 below shows the result of the kinetic measurements of four concentrations as an example. The fluctuations in signal made it difficult to establish when equilibrium was reached. It seems that equilibrium was reached within the 20 minute time period for all concentrations except 10 μ M and 1 mM where the signal curve had not leveled out. However, as a few minutes went by between the kinetic study and the mapping was started equilibrium was probably reached before the mapping was started for these concentrations as well.

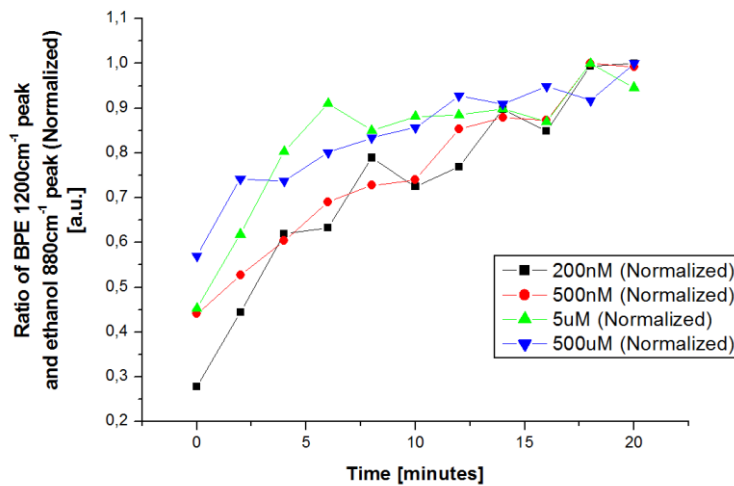


Figure B 3. Kinetic analysis at four concentrations.

Appendix C: Introductory SERS Experiment using Acetone

Experimental

To investigate if the temperature control cell is working as planned, a test was made using a not so harmful volatile substance. Acetone was chosen as analyte substance as it is volatile and commonly available, and a Klarite® SERS substrate was used. The substrate was placed on top of the Peltier element, i.e., between the thermistor and the Peltier element.

The Raman instrument setup was a Horiba Jobin Yvon LabRam 800 HR confocal Raman microscope with LabSpec 5 software equipped with an Andor Newton back-illuminated EMC CDD and a 785 nm Sacher Lasertechnik Tiger diode laser. A 5x lens was used in backscatter geometry and the confocal hole was set to 500µm. The grating used in the experiment was a 600 gr/mm blazed at NIR wavelengths. Due to etalon effects in the detector all spectra were corrected according to NIST Standard Reference Material 2241.

Nitrogen gas (N₂) is used as carrier gas, and the N₂ gas is bubbled through a liquid phase of the Acetone to generate the analyte gas. The gas was then led to the temperature control cell where the SERS substrate had been placed on the Peltier cooler.

After warming up the 785 nm diode laser output was stable and the Raman microscope was calibrated against the 520.7 cm⁻¹ peak of Si. First the temperature was set to ambient/room temperature (in this case 22 °C) and a flow of 20 mln/min of clean N₂ gas, i.e., no acetone, was applied. A spectrum is collected after the cell had been completely filled with N₂. Then the acetone was introduced and after four minutes a spectrum was collected. More spectra were collected every second minute until equilibrium has been reached. Then the target temperature was set to 5 °C and, after the temperature had stabilized, spectra were collected every second minute until equilibrium is reached. The temperature was then set to +10 °C and spectra were collected every second minute for 6 minutes. The target temperature was then again set to 22 °C and the temperature was allowed to stabilize. Spectra were then collected every second minute for 8 minutes so one could observe that the signal is decreasing. Then the target temperature was set to 45 °C and a few spectra were collected after the temperature had stabilized to ensure that the acetone signal has disappeared.

Results and Discussion

It seemed impossible to cool the substrate lower than +5 °C during the test, which was surprising. It was also surprising that the signal had increased significantly between ambient temperature and +5 °C; it was expected to require lower temperatures than +5 °C. However, after the test was completed it was observed that the thermal compound between the thermistor and the surface had been destroyed by the acetone, causing a poor thermal connection between the sensor and the surface. This probably means that the substrate surface was at a considerably lower temperature than +5 °C, perhaps as low as -15 °C since it was impossible to decrease the temperature any further. This would explain that the sensor was indicating +5°C as minimum temperature. The temperature could also have been lower than +22 °C when the sensor was reading “22 °C” and higher than +45 °C when the sensor was reading “+45 °C”⁶.

⁶ The cooling system is set to cooling for temperatures less than +25 °C and heating above +45 °C.

Figure C1 shows SERS spectra collected at ambient temperature (+22 °C) and at a lower temperature (sensor reading "+5 °C").

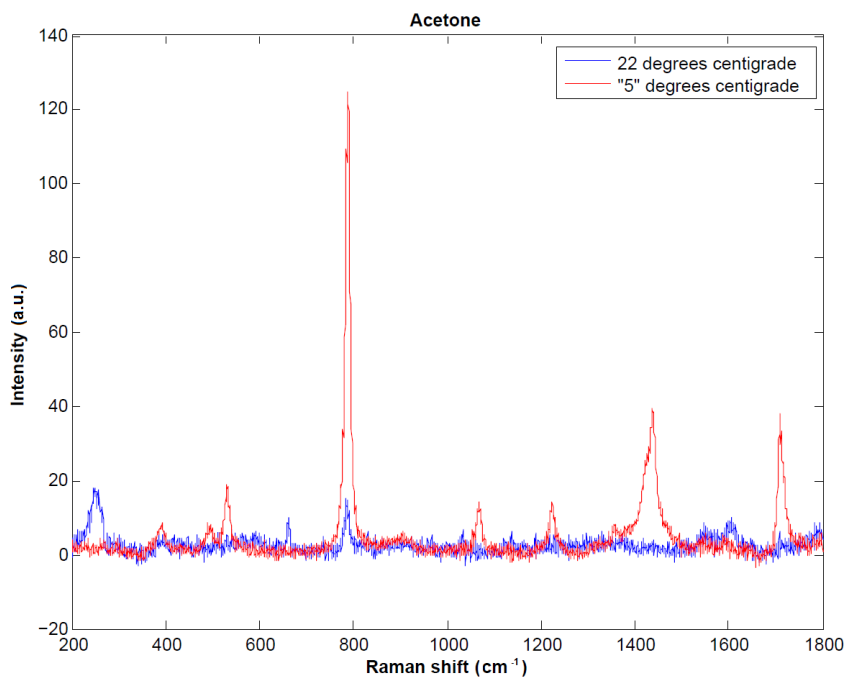


Figure C 1. Spectra collected at 22 °C (blue) and "5 °C" (red) for comparison.

Plots of analyses, at "+5 °C" and at +22 °C after cooling, can be found in figure C2 below. The 790 cm⁻¹ peak was chosen since it was the dominating one of the acetone peaks. Equilibrium is reached after approximately 20 minutes (plots after 2, 4, 8, 10, 12 and 18 minutes are not shown). The signal decreases when the Klarite SERS substrate is heated to "+22 °C" (the sensor was reading 22 °C). It is decreasing slower than expected which is probably due to the poor thermal connection between the temperature sensor and SERS substrate which means that the temperature of the substrate was probably below +22 °C.

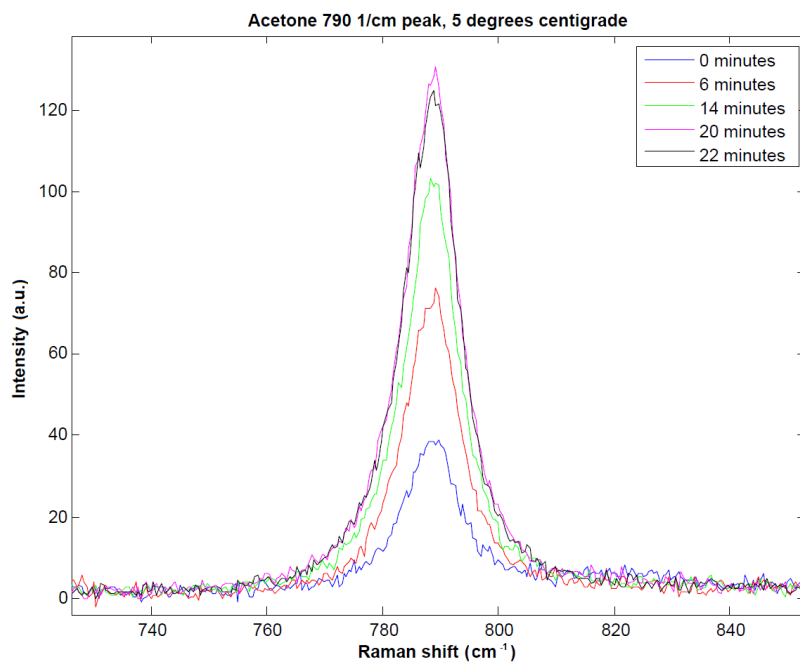


Figure C 2. Analyses at "5 °C".

Figure C3 below shows a spectrum collected at "+5 °C" and after the SERS substrate has been heated to +45 °C for comparison. It can be seen that the acetone has been released from the substrate surface, i.e., the characteristic acetone peaks are no longer visible, perhaps with the exception of the 790 cm⁻¹ peak which can still be discerned.

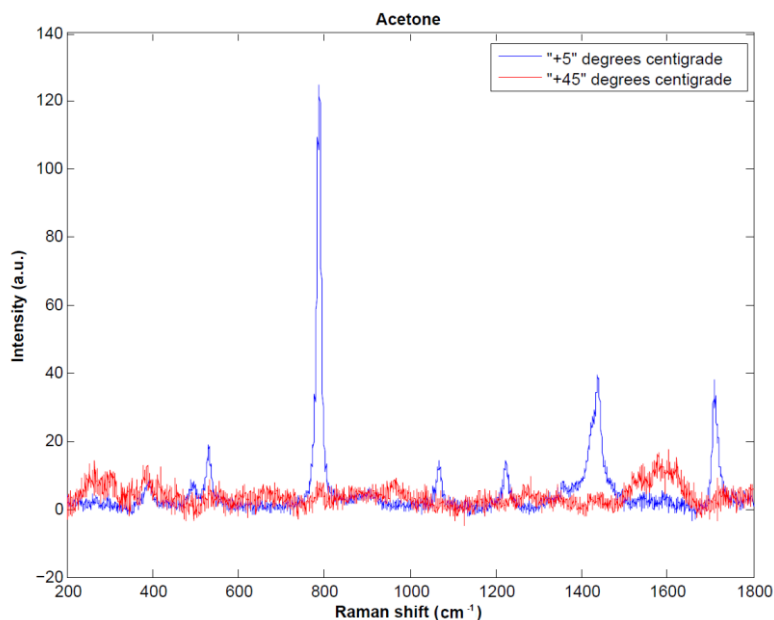


Figure C 3. Spectra at "5 °C" and at "45 °C".

Although the temperature readings are not to be trusted due to the thermal contact problem, it is clear that the cooling increases signal strength significantly, and that heating releases the volatile substance from the SERS substrate, which is showing that the temperature control cell is working as planned.

