Most of the following questions refer to the attached paper:

**Development of an in situ fiber optic Raman system to monitor hydrothermal vents**

There are also a few questions interspersed which will probe your general analytical knowledge.

Let the points guide you how long of an answer is expected.

**A. The “Big Picture” (15 points)**

1. Describe in a few sentences the research objective – what chemical system is studied and why? (3 points)

see p1, left column, 1st paragraph

2. Briefly describe two major experimental challenges the researchers were facing. (2 points)

location (sea floor – how do you get there?), pressure, temperature difference, corrosive environment

3. The conclusion is “off aim” - rewrite a short conclusion that outlines the research objective, the major challenges, the experimental setup and outlines the major successes and limitations. (10 points)

I liked one of the answers so it will -somewhat adapted- serve as benchmark what has been expected:

A field portable, battery operated fiber optic Raman system was developed to examine the metabolic pathways of environmental systems in deep sea hydrothermal vents. These vents might be similar to the origin of life on Earth and thus could help to understand those past processes.

The requirements for standalone system with the capability to monitor these vents for several minerals are challenging and have not been met yet. Major challenges for instrumental development are: strong corrosion near hydrothermal vents, temperature differences, pressure and the remote location itself.

In order to keep instrument costs down and to enable straightforward adaptations to similar tasks the Raman system was developed utilizing commercial components. It included a sapphire lens focusing the laser light to a small sample volume; this lens is connected to a stainless steel sheath which will reach into the vent and thus keeps the main part of the instrument away from high temperatures. In order to be able to cope with the extreme pressures at the sea floor, the system was encapsulated in a rugged stainless steel housing.

The system was tested at ambient conditions and at conditions mimicking those at the measurement location. Pressure effects upon the analysis of nitrates, carbonates and sulfates were examined. It was found that sulfate and nitrate could be quantified at room temperature. Carbonates, however, which are weaker Raman scatterers were at the detection limit. To test the system at -2°C, what correspond to real-world conditions, the system was placed into a fridge. Compared to room temperature the Raman spectra changed significantly and detection limits were worse. Thus, temperature imposed limitations to detection limits; pressure impacts, however, were found to be negligible. In seawater condition it was found that sulfate can be detected down to 5% of the concentration present in sea water; in artificial black smoker fluid worse detection limits were found. Nonetheless, 10% of the concentration naturally present in seawater could be detected. Carbonate showed a too weak of a signal and can presently not be analyzed at relevant concentration. Further optimizations of our novel Raman system are required in order to be able to include carbonate into analyses.

**B. Raman Spectroscopy - General Principle (20 points)**

1. Describe the principle of Raman spectroscopy; a drawing might be helpful. (10 points)

see Skoog “Principles of Instrumental Analyses” chapter 18A, B – (Resonance Raman Spectroscopy has not been applied and was not expected in the answer)

2. Which physical property of the target analyte needs to change for a molecule to be Raman active? (4 points)

polarizability of the molecule; see Skoog chapter 18A-3
In a Raman spectrum three different types of lines are found. Which ones and what is their physical/chemical origin? (6 points)

- Elastic Rayleigh scattering (excitation to virtual state returns to same original state).
- Stokes lines (starts from a electronic ground state, excited to virtual state, returns to excited vibrational, electronic ground state).
- Anti-Stokes (excited from vibrationally excited, electronic ground state to virtual state, returns to electronic ground state at lower vibrational excitation).

Rayleigh line has same frequency as laser emission.
Stokes lines have a positive Raman shift (to longer emission wavelength) because energy is “left in the system”.
Anti-Stokes lines have negative Raman shifts (to shorter emission wavelength) because they carry away energy that had been “stored” in the molecule in an excited vibrational mode before scattering happened.

C. Raman Spectroscopy - Experimental Setup (25 points)

(1) Fig. 5 shows a schematic of the setup. However it is not very clear how exactly Raman spectra are acquired. Augment the discussion and clarify the spectra acquisition. A drawing might be helpful; focus on instrumentation. (5 points)

The spectrometer + CCD camera setup a so-called imaging spectrometer: collected light from (anti-) Stokes and Rayleigh lines is guided by a fiber to a grating, which splits up the light into the different wavelengths; typically a reflection grating would be used which reflects incoming light under wavelength-dependent angles (see Skoog figure 7-16); this dispersed light is measured by the CCD.

In other words: a spectrum is generated and then projected across one or several rows (or columns - depending on the setup) of the CCD camera. Thus, an entire spectrum is captured by the CCD's rows (or columns) in one read out circle. The integration time the authors mention determine how LONG light is allowed to illuminate the CCD – for every pixel individually the CCD then integrates the pixels' signals over time. In this way the signal level and (hopefully) the signal-to-noise ratio is enhanced.

This integration time is the reason for implementing a notch filter (see below).

(NOTE: If several collection fibers would have been implemented, the scattered light from different sample location would be dispersed and projected onto different rows (or columns) of the CCD. Then Raman spectra from different sample locations could be measured at the same time.)

(2) On the first page (top of right text column) the authors discuss the use of a notch filter. What is the purpose of the notch filter? How does “reducing noise” work – or could you describe in a better way what this notch filter is needed for? Discuss in more detail what the problem is. On the other hand, a notch filter introduces a limitation - which? (15 points)

The Rayleigh line we are not interested in is orders of magnitude stronger than the (anti-) Stokes lines. Further, the integration time of a CCD chip is defined by the user and (in almost all cases) has to be the same for ALL pixels. Here comes the problem: one either cannot integrate the (anti-) Stokes lines long enough to get “out of the noise” or one has to deal with “blooming” (see Fig. 1 below). Blooming is in almost all cases not acceptable because it would bury weaker Stokes lines in the blooming area. Thus, the Rayleigh lines determines how long integration times can be without causing blooming. This for sure prohibits measurable (anti-) Stokes lines which are so much weaker. Solution: Block the Rayleigh lines which does not contain chemical information to begin with and let the Stokes lines (usually stronger than anti-Stokes because at room temperature excited vibrational levels of the electronic ground state are less populated) define how long we can integrate without risking blooming. Thus: removing the Rayleigh line by means of a notch filter enables longer signal integration times, which will help to enhances SNR. The problem with using a notch filter is that the filter has a certain width, i.e. (anti-) Stokes lines of interest that are very close to the Rayleigh lines are also blocked by this notch filter.

(3) Do you think this project could have been accomplished with mid-infrared spectroscopy (yes/no/in a certain mode of operation) – give a reason for your answer. (5 points)

The analytes of interest are dissolved in water – one of the stronger IR absorbers over wide wavelength ranges. Thus, water as the chemical matrix will absorb the mid-IR radiation unless we make the absorbance pathlength very small. This, however, would most likely prohibit sufficient detection limits. One alternative might be using “attenuated total reflection” (ATR) mid-infrared spectroscopy (for ATR see Skoog chapter 17B-3). So, most likely mid-IR spectroscopy -at least in
conventional transmission mode—would not be an alternative. The authors also mention that water is not Raman active and thus does not cause a large signals limiting the spectrometers signal resolution.

D. Results (40 points)

(1) Fig 1A: to which compound does the peak at \( \approx 750 \text{cm}^{-1} \) Raman shift most likely belong to? (5 points)

see chapter 3.1.2: it is apparently sapphire

(2) Fig. 2B: temperature apparently has an impact on the spectra. What do you have to make sure of when preparing the spectrometer for a measurement series? For answering this question you do not need to know which spectrum belongs to which temperature setting. (5 points)

If calibration and measurements are performed at the same temperature, temperature impacts can be prevented or at least significantly reduced. BTW: the temperature all measurements should be done at is defined by the temperature of the future measurement environment, i.e. the sea floor.

(3) Chapter 3.2: The authors mention two reasons for a wavelength shift. What else could cause such drifts? (5 points)

This paper reports on instrumental development and most instrumentations have temperature drifts like: (plus we are measuring at the detection limit and thus very sensitive to drifts!)

* mechanical expansion of optical components might cause a slight change in the alignment resulting in small changes in the amount of light reaching the CCD — and light levels are apparently very low since integration times are tens of seconds.

* if the optic's alignment changes, the image projected by the spectrometer onto the CCD might shift, too (say in X direction = wavenumber axis). Because data analysis assumes the wavelength axis to be stable, shifts of images would also translate into wavelength shifts.

* all electronic components like operational amplifiers have temperature drifts (that are usually listed in the data sheet; output voltage changes in the range of ppm/°C)

* less likely a problem: temperature effects could change the refractive indices of fiber and sample

(4) Fig. 1B and chapter 3.1.3: Do you agree with the authors' discussion? Yes/no/partially give reasons. In this chapter the authors state that for sulfate 4% of concentration change can be detected. Do you think this is a representative result? Base your answer on the figures. (15 points)

Just a few ideas:

- all Raman spectra shown contain a high noise level; that raises some daubs about sufficient limits of detects

- many experiments done for proof of principle were done under lab conditions and further results indicate that limits of detections get considerably worse at lower -more realistic-temperatures.

- In overall it is not obvious that the shown results actually prove a successful deep-sea measurements; the authors are somewhat short of good data.

- The way the paper is written sounds somewhat boring: we did this then this then this ... the reader wants a clear motivation and outline were we are going and needs to get some feedback like “and this is how we achieve the aforementioned goal and this fits so-and-so into the big picture presented in the beginning”

- it was NOT expected that you verify that 4% of the seawater sulfate concentration can be determined given the shown data – you were supposed to discuss these numbers!

- Given the data in Fig. 3 the repeated statements that give or take LODs of 0.1g/l are feasible is questionable at room temperature; going to lower temperatures limits of detections gets worse according to the authors and giving a graph similar to Fig. 3 determined at low temperatures would be helpful for assessments

- a paragraph outlining the future realization, i.e. bringing it down, would have been nice

- the discussion of the capillary waveguide is highly incomplete and hardly comprehensible

- Fig. 3B is contradicting the other figures: the conc is >3g/L and the Raman peaks are barely visible – how does this support the remaining data especially limits of detection around 0.1-0.3g/L
(5) What is your overall impression of this manuscript? Will the authors easily succeed or do you see major challenges? (10 points)

Just a few ideas:

- the science behind the project is great (studying hydrothermal vents on the ocean floor to learn about the origin of life)
- the analytical challenges are great and working towards solutions is demanding and from an instrumental development perspective really exciting!

But:

- plugging together an instrument from off-the-shelf products might not be the way to achieve the required breakthroughs in sensitivity
- nonetheless, making this instrument rugged enough for deep-sea measurements in a corrosive environment IS a strong achievement
- the discussion of the experimental setup and data analysis (they just mention principal component regression) appears to be insufficient
- only sulfate out of a number of analytes could be measured (in the lab mind you) in the required concentration range; one analyte (carbonate) seems to be hopeless without really major breakthroughs in instrumentations
- nitrates appears to be a promising case, however, the authors admit that nitrates are not present at the vents – this only serves as outlook “there are more applications”.
- In conclusion: a successful application might be possible but the instrument needs major improvements and more realistic data shown be given to support the research results.

General comment about the grading:

The cut-off values were chosen based on the overall performance in your discussion about Raman spectroscopy (basic knowledge) and assessing the manuscript (D4 and D5). Almost all of the students who took this cume show clear deficits regarding this important analytical technique but could gain over-proportionally many points in mostly well-written (!) assessments of other folks results. The cut-off would have been different, if this would have been vice versa!
Fig. 1: example of the blooming effect (image was acquired with a digital camera): the flash light reflected in a window is much stronger than the ambient light level and causes over-saturation of some pixels; principle of CCD: incoming photons generate electron-hole pairs in the CCD's pixels and the electrons are stored under the pixels in so-called (potential) wells (see Skoog Fig. 7-35). If a pixel is over-saturated, the large number of electrons cannot be stored in a particular well anymore and literally over-flow into surrounding wells causing a wrong, too strong signal there; the halo shown above is a measurement error!