Towards a Real-Time Raman-Based Lactate Sensor for Acute Fetal Hypoxia Detection

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1. Introduction and Motivation

Enabling safe childbirth remains a major global challenge. **Fetal hypoxia** arises when oxygen delivery to fetal tissues is restricted and remains a major contributor to stillbirth worldwide. Current methods for detecting fetal hypoxia are indirect, such as **cardiotocography** (CTG), or invasive, such as **fetal blood sampling** to assess lactate levels.

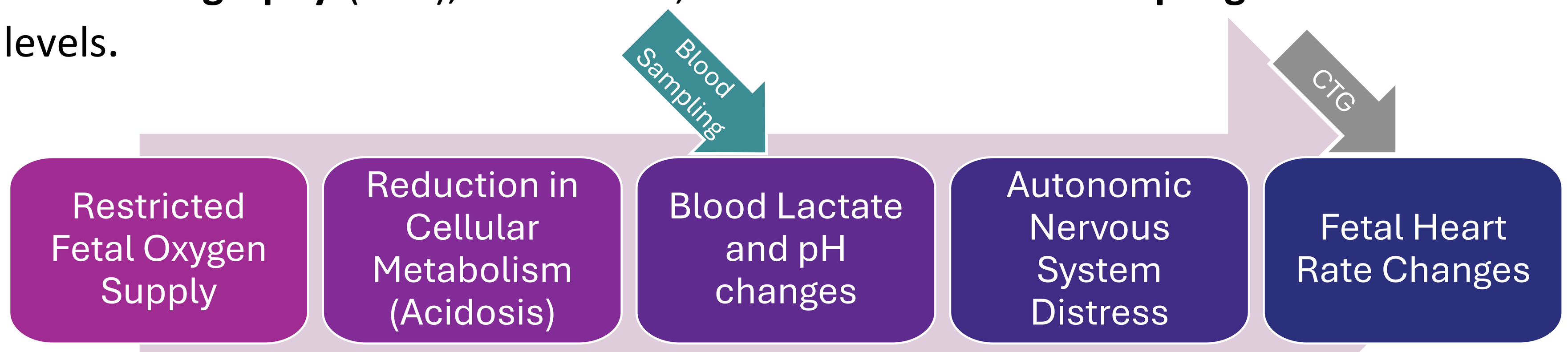

Figure 1 – Pathophysiology of Fetal Hypoxia. Blood lactate measurements can be used to diagnose fetal hypoxia and acidosis

We propose using Raman spectroscopy as a non-invasive optical method to quantify lactate for diagnosing fetal hypoxia.

2. Raman Spectroscopy and Fluorescence

Raman Spectroscopy is a label-free optical technique that measures molecular composition and concentration through inelastic light scattering.

- Lactate peak located at 841.5 nm (848 cm^{-1})
- < 2% is Raman Signal
- > 90 % is fluorescence background
- Dark current, cosmic rays, and others also need to be removed

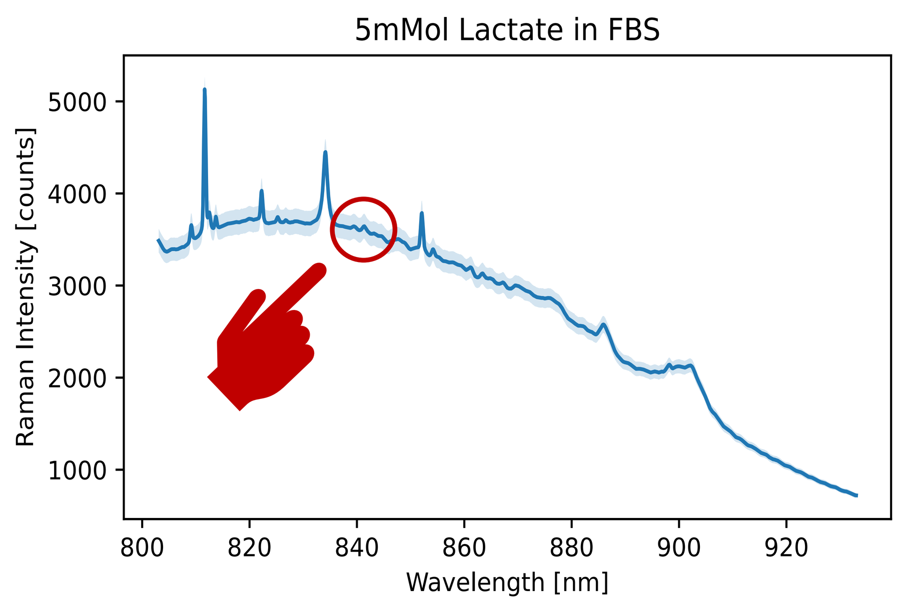

Figure 2 – Raw Raman spectra of FBS spiked with 5 mMol of lactate. Shaded area denotes standard deviation of 500 individual measurements. Circled region shows location and magnitude of lactate peak at 841.5 nm

Accurate background removal is essential for reliable lactate concentration measurements. Background can be estimated and removed algorithmically via ALS or Bubble-Fill. Errors in estimation lead to inaccuracy in Raman reconstruction.

3. SERDS Approach

Shifted Excitation Raman Difference Spectroscopy (SERDS) uses two detuned lasers spaced 0.5 nm apart to shift the Raman spectra. Fluorescence does not shift, thus subtracting the two spectra removes the fluorescence background.

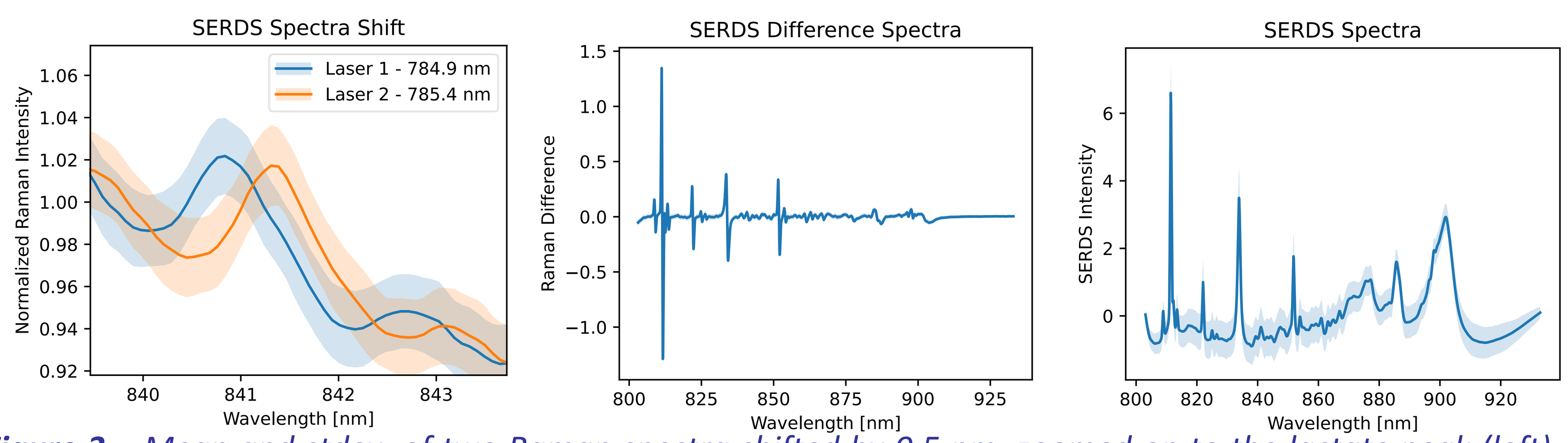

Figure 3 – Mean and stdev. of two Raman spectra shifted by 0.5 nm, zoomed on to the lactate peak (left). The difference between the two spectra (center). The integral of the difference spectra to reconstruct the Raman spectra (right).

Due to variations in the spectra between the two lasers, some residual background remains which can be removed analytically via ALS or Bubble-Fill [1].

Can SERDS be used to improve background removal and increase lactate detection sensitivity in fluorescent media?

4. Experimental Setup

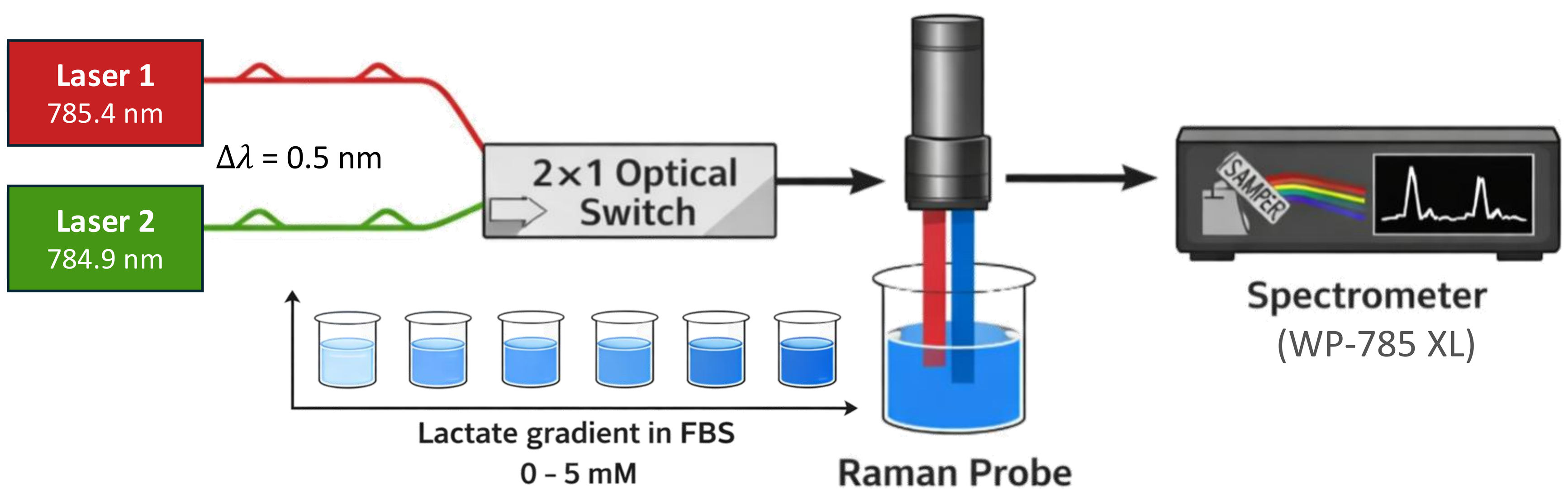

Figure 4 – Hardware experimental setup. Two lasers fiber lasers (784.5 nm, 785.4 nm) are time-multiplexed using a synchronized optical switch. Laser light is then fiber coupled into a Raman probe placed in fetal bovine serum (FBS). Raman backscattered light is then recorded by a spectrometer

- Spectra collected using a Wasatch XL Raman system with Andor CCD
- 10x 3mL FBS samples spiked with NaLac (0–5 mMol, 0.5 mMol steps)
- Probe submerged in sample.
- 2 sec per spectra, 500 spectra per laser
- Alternated laser in between exposures to reduce background fluctuations
- Samples were photobleached prior to measurement to stabilize fluorescence background

5. Data Postprocessing

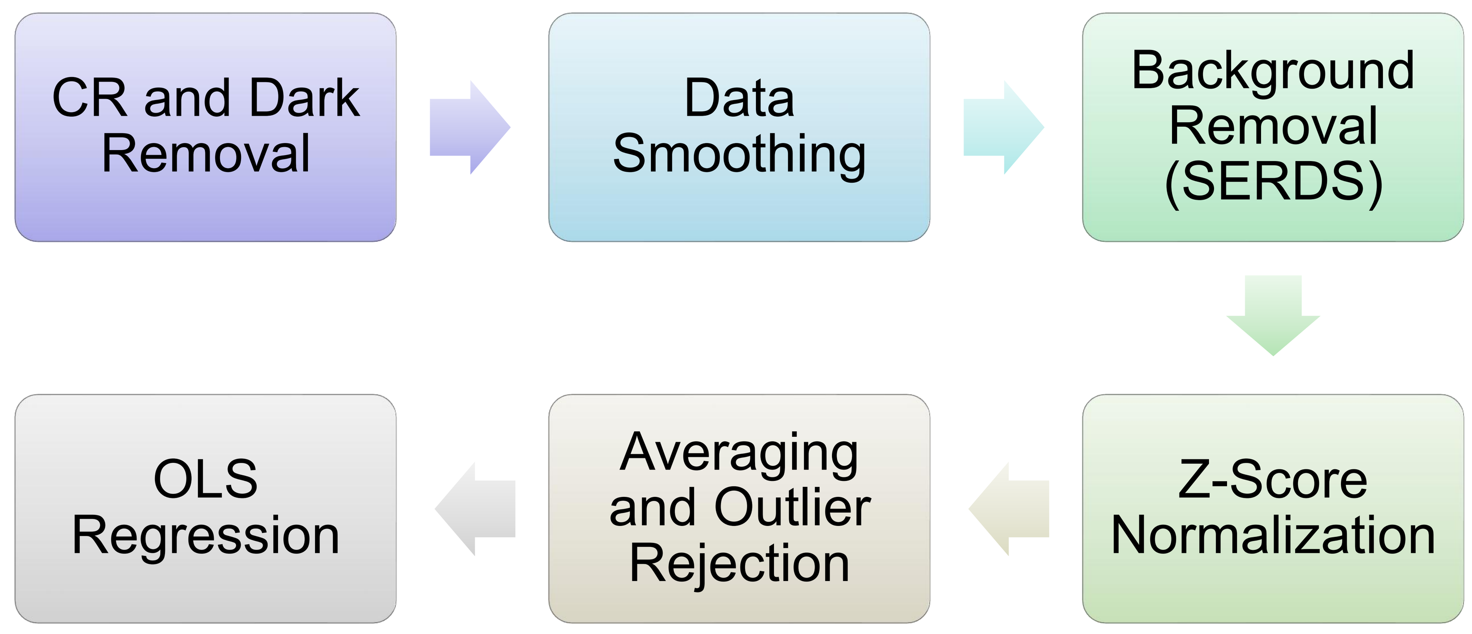

Figure 5 – Data postprocessing flowchart. Each sample's 500 spectra were cleaned (cosmic rays and dark counts removed), smoothed with a Gaussian filter, and fluorescence background corrected (ALS, bubble-fill, or SERDS). Data were then z-score normalized, averaged per sample, and analyzed using ordinary least squares (OLS) regression across all samples.

Background removal repeated using ALS, bubble-fill and SERDS (with Bubble-Fill) to compare approaches.

6. Results

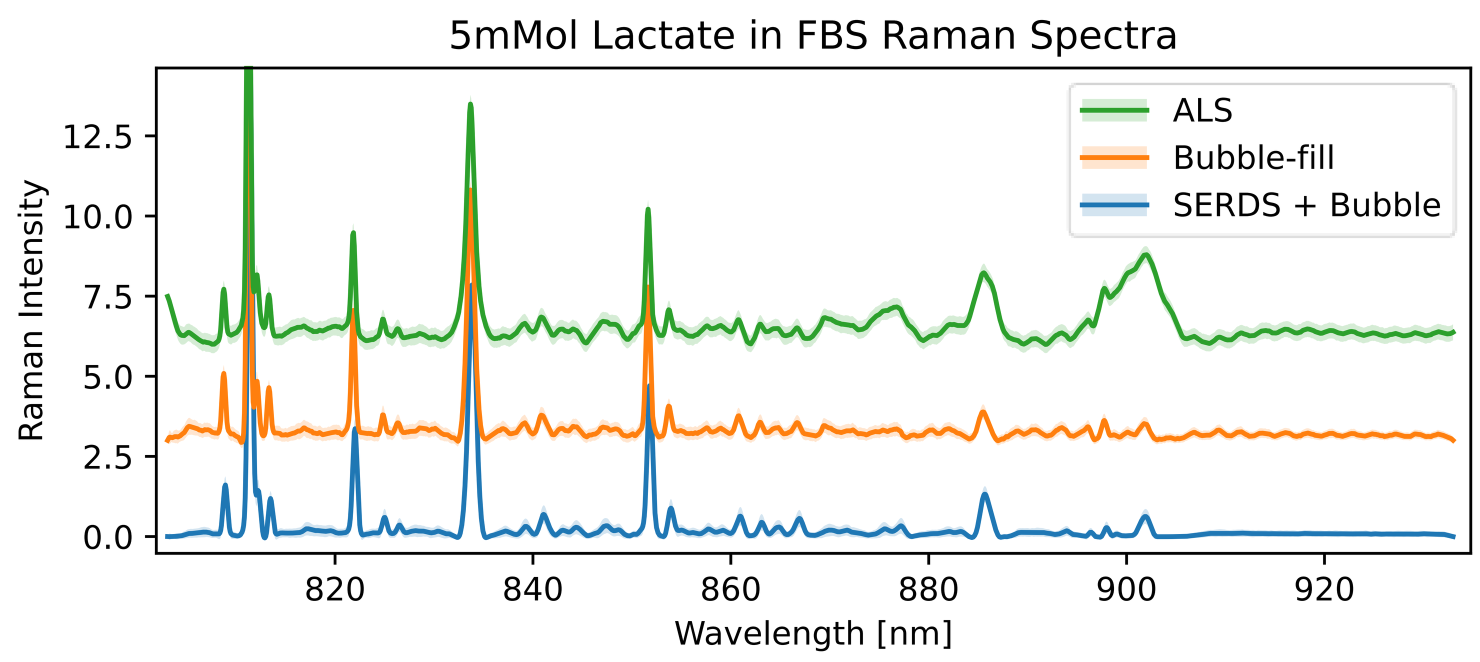

Figure 6 – Raman spectra of FBS spiked with 5 mMol of NaLac using each background removal technique. Each curve is the mean of 500 individual spectra. Shaded area denotes standard deviation from the mean.

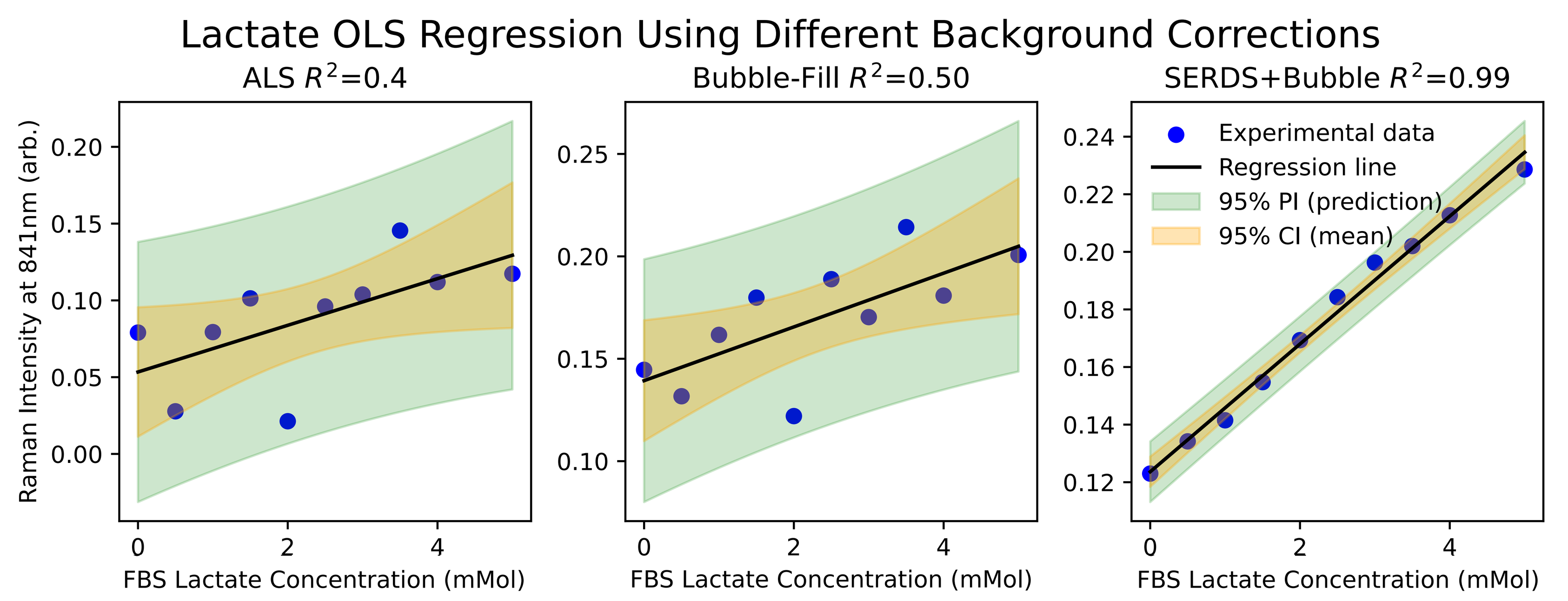

Figure 7 – OLS Regression of Raman signal intensity at 841 nm and FBS Lactate concentration using different background removal techniques. Green shaded area denotes the 95% prediction interval. Yellow denotes the 95% confidence interval from the regression line. R^2 values for the regressions are 0.4, 0.5, and 0.99 for ALS, Bubble-fill, and SERDS, respectively.

- Raman spectra processed with ALS and Bubble-fill show high-pass filter artifacts between 910–930 nm.
- SERDS eliminates filter artifacts and retains Raman spectra morphology
- Uncertainty in ALS and Bubble-fill background removal algorithms result in wider prediction and confidence intervals and lower R^2 regression coefficients.

7. Discussion and Conclusions

Discussion and Limitations:

- SERDS assumes identical fluorescence backgrounds for both laser acquisitions; any difference leaves residual background.
- Longer integration times reduce SERDS effectiveness due to background differences between lasers caused by temporally fluctuating fluorescence.
- Residual fluorescence can be approximated and removed using ALS or Bubble-Fill algorithms.

Conclusions:

- SERDS physically discriminates Raman signals from non-Raman background.
- Combining SERDS with approximation algorithms improves Raman reconstruction accuracy compared to using algorithms alone.
- SERDS-enhanced Raman reconstruction enables more accurate lactate quantification in FBS.

This approach could be adapted for in-vivo use, enabling optical lactate measurement in fetuses and advancing non-invasive fetal hypoxia monitoring.

8. Acknowledgements and References

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[1] Sheehy G, et al. Open-sourced Raman spectroscopy data processing package implementing a baseline removal algorithm validated from multiple datasets acquired in human tissue and biofluids. J Biomed Opt. 2023 Feb;28(2):025002. doi: 10.1117/1.JBO.28.2.025002. Epub 2023 Feb 21. PMID: 36825245; PMCID: PMC9941747.

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