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Raman Spectroscopy for Detection of the Boundary Zone of Tumor in Colorectal Cancer

A Precision Tool for Real-Time Definition of Surgical Margins

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1. Background-Aim

Colorectal cancer (CRC)

is the fourth most common and the third deadliest cancer worldwide. Colon cancer treatment often involves open surgical resection as the primary treatment for localized disease. Accurate detection of tumor-positive margins is crucial to minimize the removal of healthy tissue during surgery.

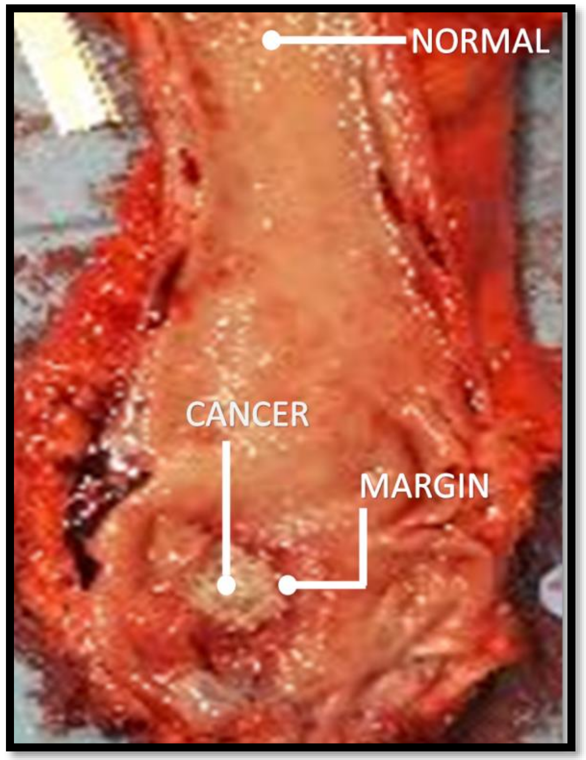
Raman spectroscopy (RS)

is an advanced analytical optical technique that has shown significant promise in the detection and diagnosis of colorectal cancer (CRC). Raman spectra can be used as a fingerprint for colorectal tissues, with patterns indicating anatomical and molecular changes. Machine Learning (ML) algorithms are commonly used in the analysis of spectral data for cancer prediction.

This study focuses on

detecting the tumor boundary zone of CRC using Raman spectroscopy, with the goal of making RS a future clinical tool for surgeons.

2. Materials & Methods



Patients & Samples:

- In this work, human colorectal specimens were collected from 23 patients who underwent open surgery.
- Three different types of samples were used: normal colon area, cancerous area, and tumor boundaries.

Raman spectroscopic measurements:

Micro-Raman spectra were recorded using a Renishaw Invia micro-Raman system operating at 785 nm.

Data analysis:

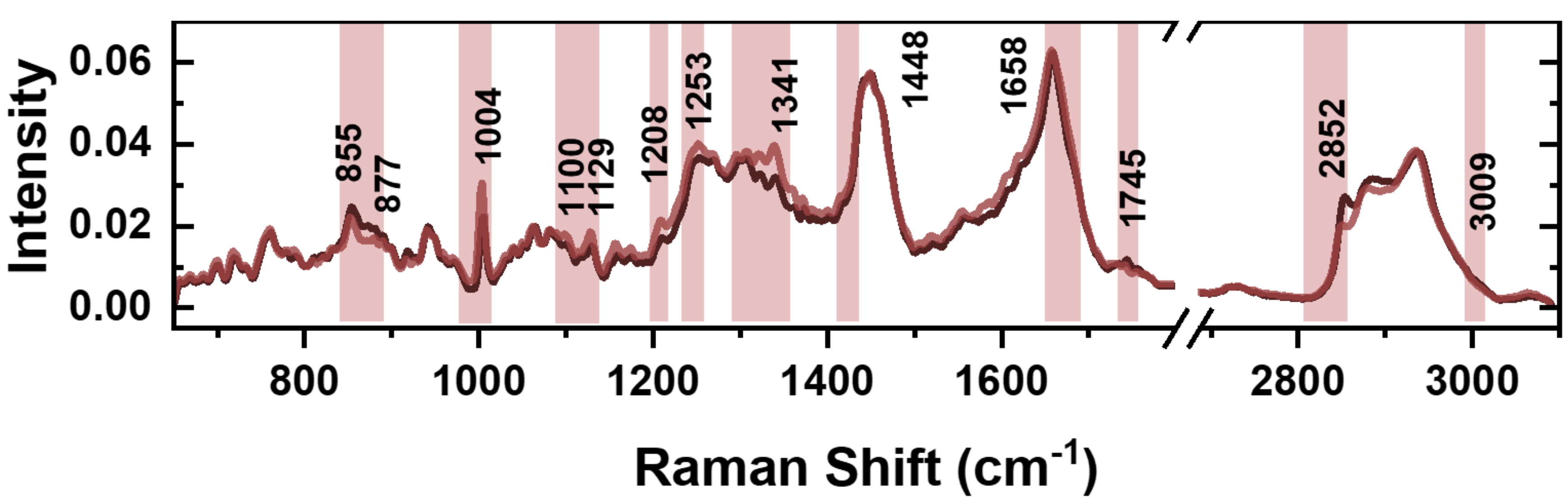
The extracted data were analyzed spectroscopically and utilized for a classification task using machine learning algorithms. Specifically, we used Partial least squares-discriminant analysis (PLS-DA) that is a supervised dimensionality reduction method.

Comparison study between normal and cancerous spectral data

- Numerous significant differences between normal (black line) and cancerous (red line) spectra.
- Collagen and lipid spectral features could serve as markers for the spectroscopic detection of cancer.
- Raman spectra reveal protein overexpression in cancer tissues.
- The table includes the attributions of the significant Raman bands.

Frequencies	Assignments of Raman bands
855 cm^{-1}	Tyrosine, collagen (proline)
877 cm^{-1}	Tryptophan, hydroxyproline
1004 cm^{-1}	Phenylalanine
1100 cm^{-1}	Lipids, DNA
1129 cm^{-1}	Proteins
1208 cm^{-1}	Proteins (phenylalanine)
1253 cm^{-1}	Proteins (Amide III), collagen
1341 cm^{-1}	Lipids, proteins (aliphatic amino acids, tryptophan)
1448 cm^{-1}	Lipids, proteins (aliphatic amino acids)
1657 cm^{-1}	Proteins (Amide I), lipids (unsaturated fatty acids)
1745 cm^{-1}	Lipids (esters)
2852 cm^{-1}	Lipids, fatty acids
3009 cm^{-1}	Lipids

MEAN SPECTRA:



Shaded regions of the spectrum indicate statistically significant differences. The frequencies of the corresponding peaks are also marked above the spectrum.

Comparison study between spectra of normal tissues and tumor boundaries

- The samples of boundaries (green line) also present several significant differences in comparison with the normal samples (black line).

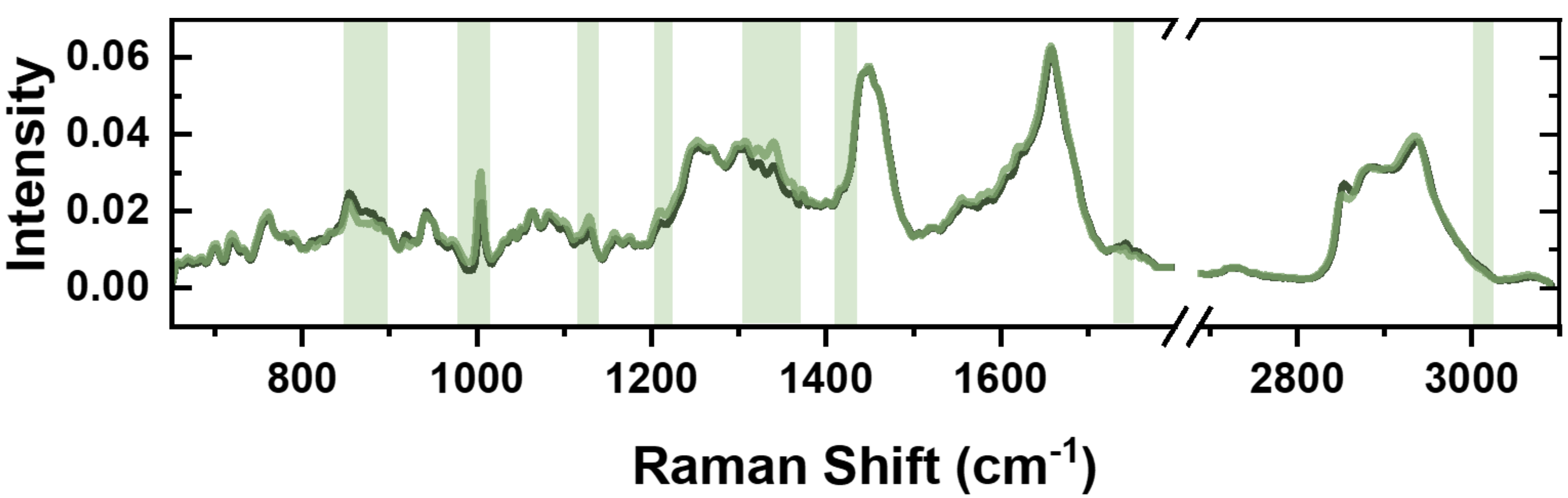
- Fewer differences than in the comparison between normal and cancerous spectrum: In the boundary zone of the tumor, malignant and non-malignant cells coexist.

- The table shows the types of differences:

↓: reduction of peak intensity, ↑: increase of peak intensity, ↔: peak broadening, →: peak shift, –: not a significant difference.

Frequency positions (cm^{-1})	NORMAL vs CANCER	NORMAL vs MARGIN
855	↓	↓
877	↓	↓
1004	↑	↑
1100	↑	–
1129	↑	↑
1208	↑	↑
1253	↑	–
1341	↑	↑
1448	→	→
1658	↔	–
1746	↓	↓
2852	↓	–
3009	↓	↓

MEAN SPECTRA:



Shaded regions of the spectrum indicate statistically significant differences.

Machine learning analysis on cancer and margin spectral datasets

- Two classification tasks were performed, the first for cancer discrimination and the second for tumor boundary discrimination.
- The experiments yielded an accuracy of 86% for the cancer data and 84% for the tumor boundary data, which is comparable to other state-of-the-art results.
- The classification accuracy for the tumor boundary data was found to be similar to that of the cancer data, suggesting that Raman spectroscopy can efficiently discriminate even heterogeneous tissue regions.

	Classification Metrics for PLS Model		
	Accuracy	Sensitivity	Specificity
NORMAL vs CANCER [cancer detection]	86%	87%	86%
NORMAL vs MARGIN [margin detection]	84%	74%	89%

4. Conclusions

Summary:

- Spectral differences between cancerous and healthy tissues were identified.
- The study demonstrated that Raman spectroscopy can effectively differentiate between healthy and cancerous tissues, and the statistical classification results were highly satisfactory (Accuracy: 86%, Sensitivity: 87%, Specificity: 86%).
- The coexistence of healthy and cancerous cells in the marginal region of the tumor is evident in the spectral results.
- The classification accuracy between normal spectra and spectra derived from boundaries achieved 84%, demonstrating the technique's capability to distinguish cancer cells within heterogeneous regions.

Conclusion:

This study demonstrates that RS is a technique that could not only be utilized in CRC diagnosis but also shows potential as a valuable tool for confirming clear margins in CRC in real-time during open surgery.

5. References

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