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Comparison of ex vivo with in vivo Spectroscopic fingerprints of colorectal cancer tissues in experimental mouse model SCID

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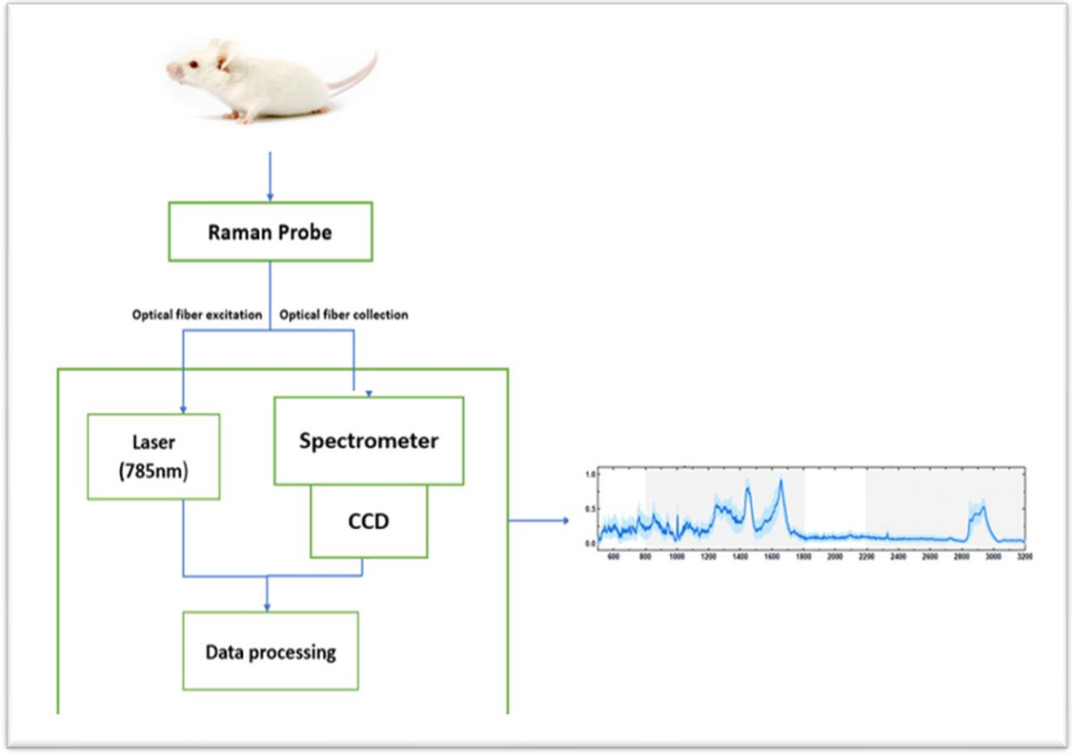
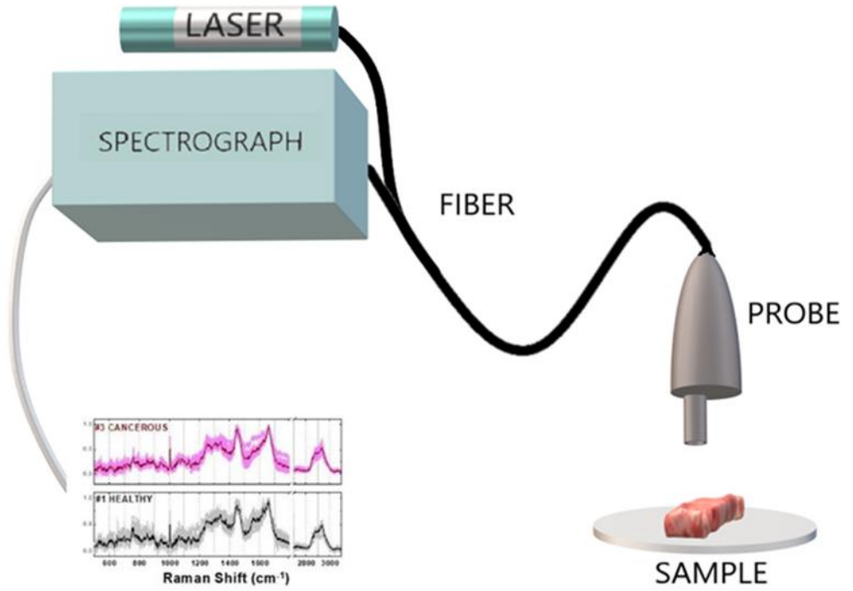
1. Background-Aim

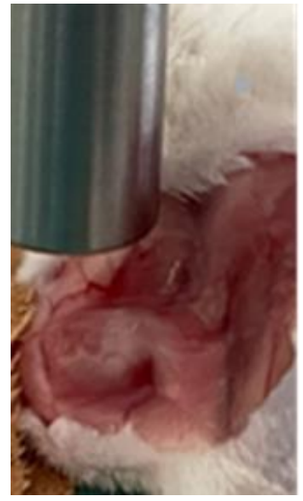
- **Raman spectroscopy (RS**) has emerged as a powerful tool in medicine with high specificity, sensitivity, spatial and temporal resolution.
- Advanced **portable Raman systems** have been developed which allow the collection of **spectral fingerprints of biostructures *in vivo***.
- The integration of **artificial intelligence (AI) algorithms with RS** has significantly enhanced its ability to accurately classify spectral data in real time.
- However, the application of RS as a clinical diagnostic tool *in vivo* remains a challenge.
- **This study aims to compare *ex vivo* spectral fingerprints of colorectal cancer tissues with spectral fingerprints which were recorded *in vivo, in mice*.**



2. Materials & Methods

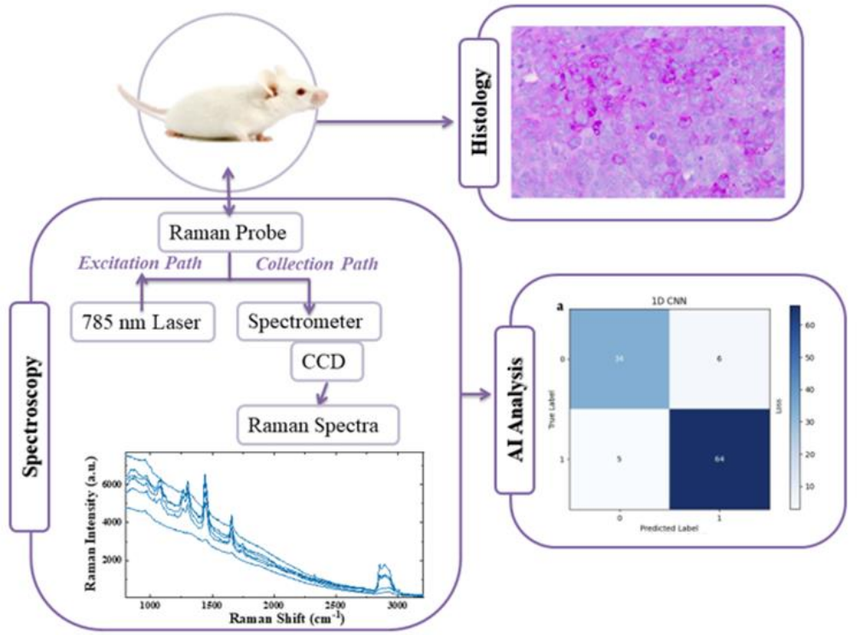
- **20 immunocompromised SCID mice** were used. The animals were divided in two groups. **Group C, where no cancer cells** were injected and **Group Ca, where HCT cancer cells were injected.**
- When the tumors approached the size of 100 mm^3, **a surgical incision** was made at the site of tumor growth and Raman spectra were recorded ***in vivo*** **using a portable Raman system with a fiber-optic probe.**
- Then, **the tumors were surgically excised** and divided into two pieces: ex vivo spectra were recorded from one piece, while histological analysis was performed on the other.
- ***Ex vivo*** **spectra were compared with** ***in vivo*** **spectra** using **AI algorithms** trained by **spectral libraries created by ex vivo colorectal spectra.**

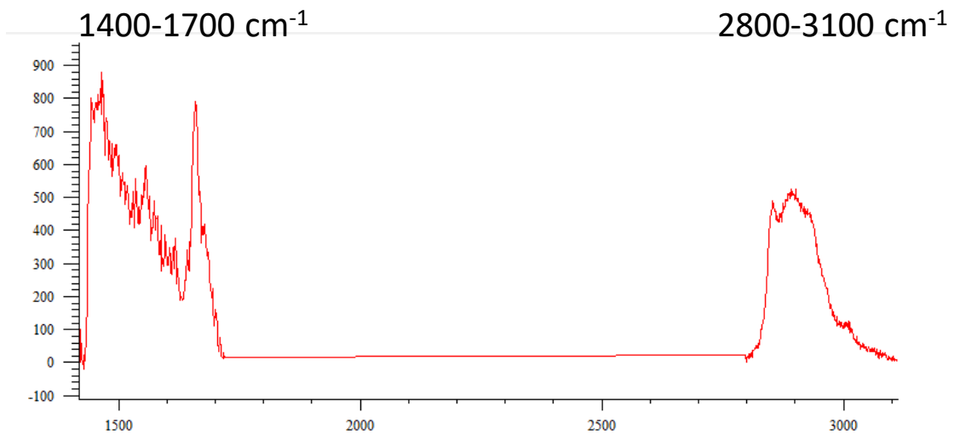




2. Materials & Methods

- The acquired Raman spectra were preprocessed following three steps: (1) removal of spectral regions that are dominated by parasitic signals, (2) background subtraction and (3) normalization.
- Two spectra region alternations were selected: the medium region where the frequencies was ranging from 1400–1700 cm^{-1} and the high region where the frequencies from 2800–3100 $cm^{-1.}$
- An 1D-CNN model with transfer learning technique was employed to classify the Raman spectra.
- In the pretraining phase, we employed an extended dataset from our previous work of 442 Raman spectra in human tissues, 221 from healthy and 221 from cancerous colorectal tissue samples

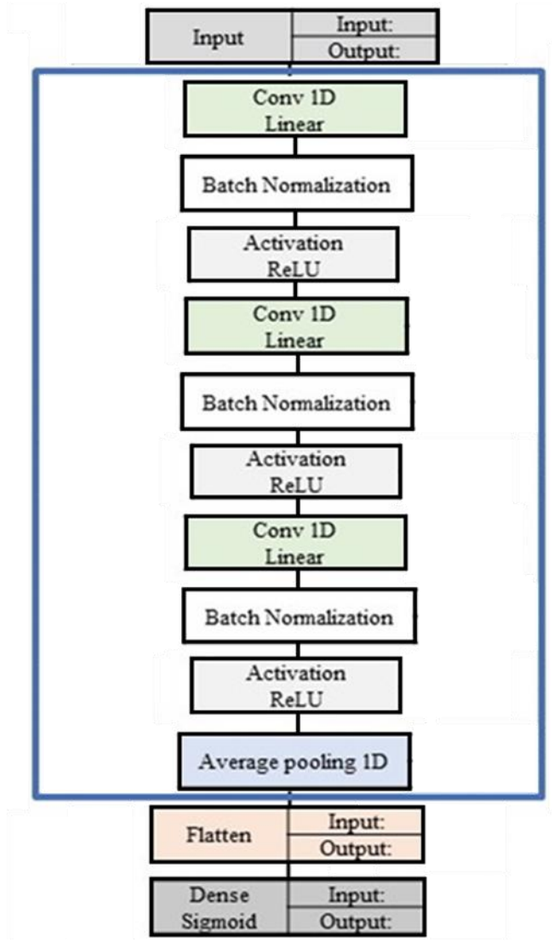




3. Results

Transfer Learning μοντέλο

1D-CNN



Pre-train dataset

442 ***ex-vivo*** Human colorectal

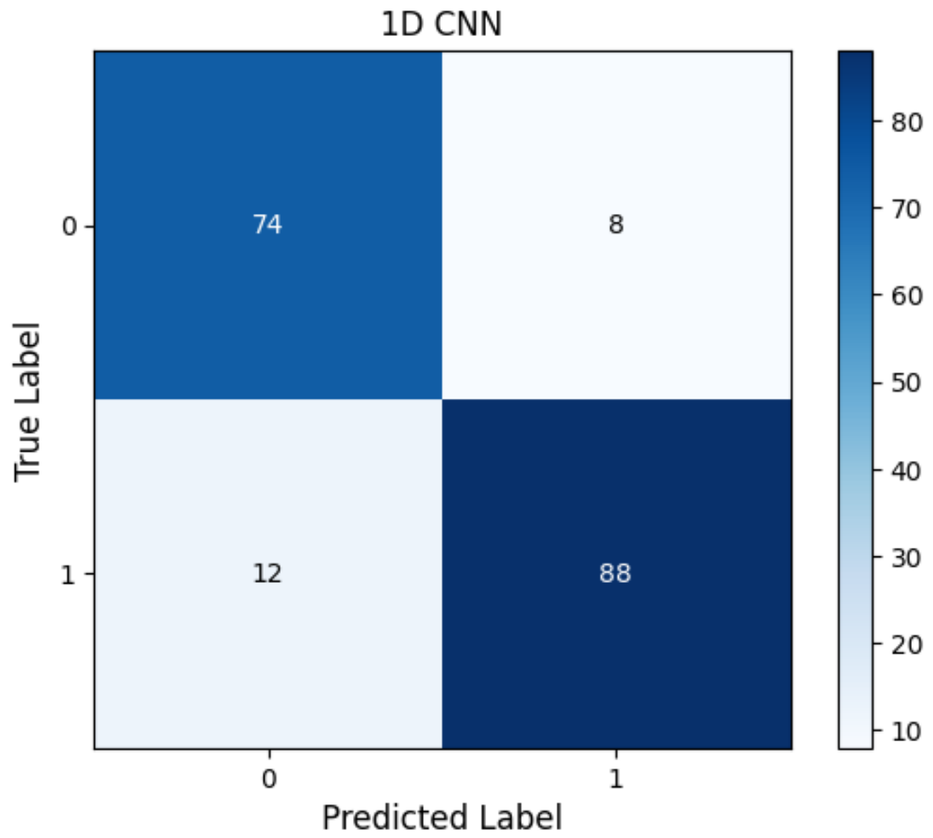
Fine-tuning

182 ***ex-vivo*** Mice colorectal

(**82** υγιή/**100** καρκινικά)

Classification results for ex vivo spectra data

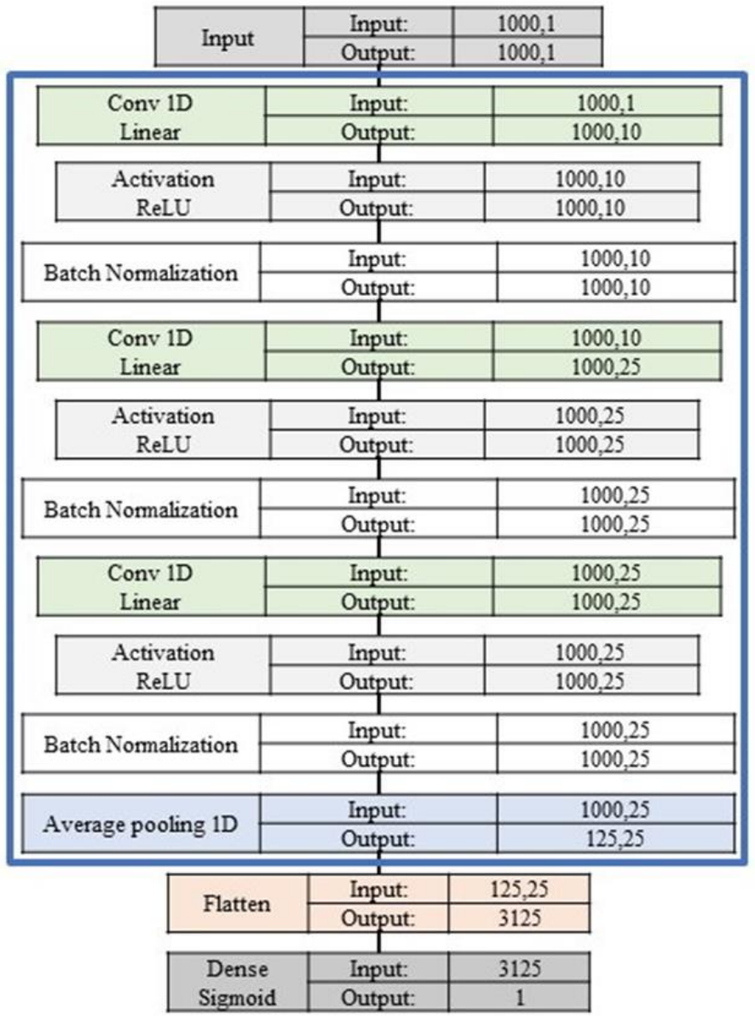
	accuracy	precision	recall	F1-score	Support
Healthy	89	86	90.2	88.1	82
Cancerous		91.7	88	89.8	100



Confusion matrix of 1D-CNN

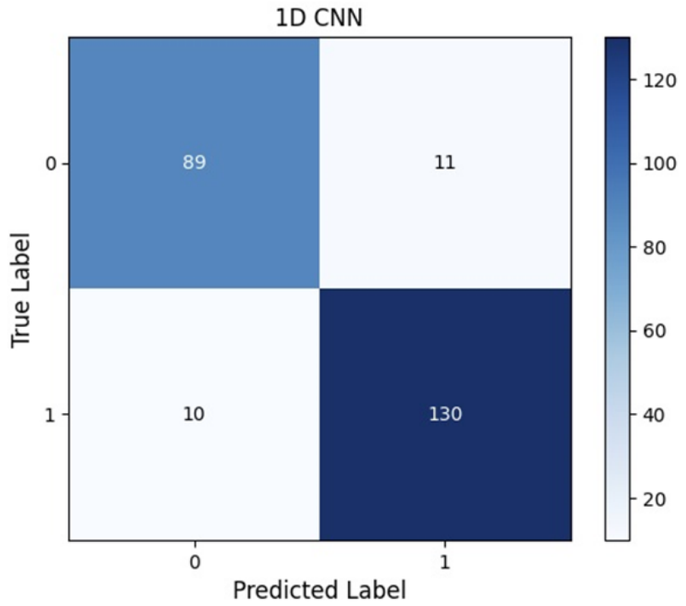
3. Results

Transfer learning model



Classification results for in vivo spectral data

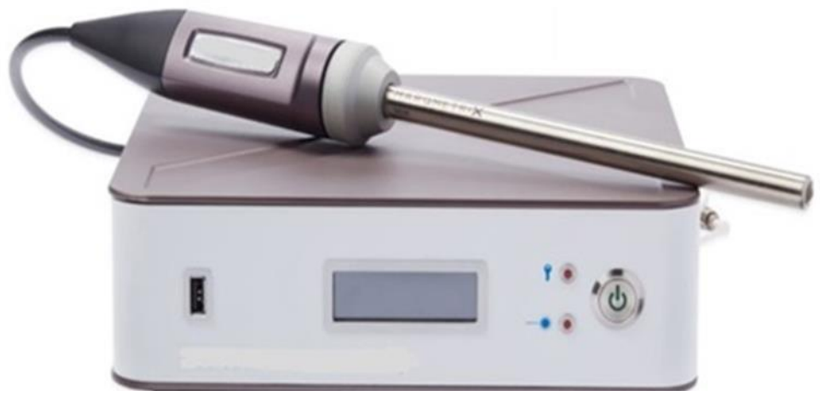
	accuracy	precision	Recall	F1-score	support
Healthy	91.2	89.9	89	89.4	100
Cancerous		92.2	92.9	92.5	140



Confusion matrix of 1D-CNN

4. Conclusions

- The models overcome the limitations of the large data collection and demonstrate their effectiveness in *ex-vivo* and *in-vivo* settings.
- The 1D-CNN deep learning model demonstrated high percentages of accuracy for both ex vivo and in vivo spectra in distinguishing cancerous tissues from normal ones.
- However, 1D-CNN deep learning model exhibited higher accuracy, the precision and the recall for in vivo spectral data highlighting the potential of the AI model and the portable Raman system in case of a colorectal open surgery.
- Overall, all results brought RS one step closer to clinical application as an auxiliary tool for real-time biopsy and so surgical guidance.



5. References

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2. Bergholt, M.S.; Zheng, W.; Lin, K.; Wang, J.; Xu, H.; Ren, J.L.; Ho, K.Y.; The, M.; Yeoh, K.G.; Huang, Z. Characterizing variability of in vivo Raman spectroscopic properties of different anatomical sites of normal colorectal tissue towards cancer diagnosis at colonoscopy. Anal. Chem. **2015**, 87, 960–966.

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