**ABSTRACT**

Nearly 80,000 new cases of primary brain tumors will be diagnosed in the US this year and approximately one-third of brain and central nervous system (CNS) tumors are malignant. Of all brain tumors, gliomas which includes all tumors arising from the glial or supportive tissue of the brain, represent 24.7% of all primary brain tumors and 74.6% of all malignant tumors. Currently, surgery is the primary treatment for brain tumors, and the tumors can be removed without causing severe damage. During the surgery, accurately distinguishing the cancer from the surrounding normal tissue and identifying cancer margins is important but a major challenge. Sufficient removal of cancer can lead to lower chance of recurrence, but on the other hand, accidently removing healthy tissue can cause neurologic problems. In this study, we investigated a new optical molecular histopathology method based on visible resonance Raman (VRR) spectroscopy for diagnosing human brain tissues and distinguish glioma tissues of grades I through IV from normal brain tissues. Optical biopsy: fluorescence spectroscopy, Raman spectroscopy, multiphoton microscopy, SRS, CARS ... Rapid, Non-invasive or minimally invasive, Diagnostic information for early detection (biochemical, morphological), Avoid subjectivity.

**VISIBLE RESONANCE RAMAN SPECTROSCOPY**

- Signal enhancement due to resonance or pre-resonance.
- Signal enhancement due to use of shorter wavelength: Raman scattering cross section is inversely proportional to the fourth power of the excitation wavelength.
- Resonance large biomolecules: flavins, lactate, NADH, NAD+, collagens, elastin, carotenoids, tryptophan, heme proteins, mitochondrial cytochromes.

**SAMPLES AND EXPERIMENTS**

- We collected 241 VRR spectra from 97 subjects.
- 3 spectra for each patient on average.
- Samples include ex vivo normal human brain tissues and glioma tissues at different grades ranging from grade I through grade IV.
  - Grade I and Grade II is considered low grade.
  - Grade II-III, Grade III, Grade III-IV and Grade IV are considered high grade.
- The measurements were performed using a HORIBA HR-800 confocal micro-Raman spectrometer with an excitation wavelength of 532 nm over a spectral scan region of 500 to 3500 cm⁻¹.

**RESULTS**

- Typical VRR spectra
- Three PC spectra
- ANOVA: log(p) of first 20 PCs

**ANALYSIS METHODS**

- Dimensional Reduction and Feature Extraction:
  - Principal Component Analysis (PCA)
- Classification:
  - Support Vector Machine (SVM) with Gaussian Kernel
  - Leave One Out Cross Validation (LOOCV)
  - Patient-based and spectra-based analysis

**Principal Component Analysis (PCA)**

\[ X = WH \]

- PCA is a unsupervised machine learning method to maximizing variance explained in X.
- PCs are the components that characterize the data most strongly.
- W: columns are PC spectra
- H: rows are PC scores used for classification

**Support Vector Machine (SVM)**

-leave one out cross validation (LOOCV)

**RESULTS**

- AUROC: 0.9412
- AUROC: 0.9978

**CONCLUSIONS AND DISCUSSIONS**

- VRR along with PCA-SVM may be used to effectively distinguish normal and glioma tumors as well as distinguishing different grades of glioma tumors.
- The high grade can be distinguished from normal tissue very accurately.
- More analysis will be performed further to improve the results, especially identifying the low-grade glioma tissues, which will be potentially important for margin detection in malignant tumors.
- Sources of errors include presence of mixed features, e.g. grade II-III has both features of grade II and grade III.

**REFERENCES**