Breast tissue diagnosis by Raman spectroscopy

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GOAL

Some substances are produced by the organism in response to the cancer's presence. They are called tumor markers.

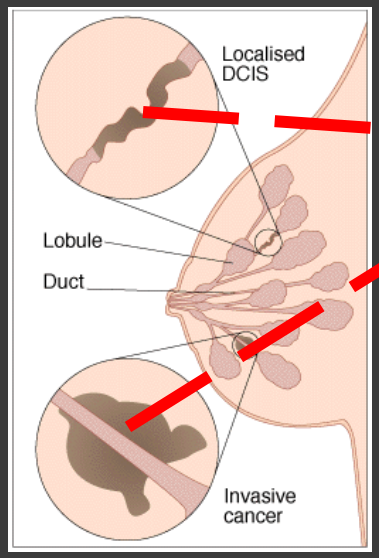
Tumor markers are molecules occurring in blood or tissue that are associated with cancer and whose measurement or identification is useful in patient diagnosis or clinical management. The ideal marker would be a "blood test" for cancer in which a positive result would occur only in patients with malignancy, one that would correlate with stage and response to treatment and that was easily and reproducibly measured. No tumor marker now available has met this ideal.

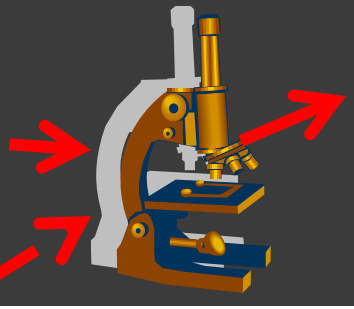
We believe that optical methods including Raman spectroscopy will provide such a marker. Possibly it will help to find the hallmarks of cancer.

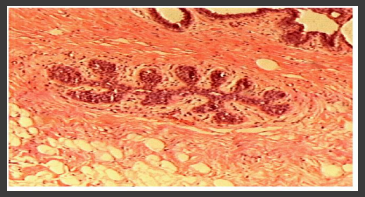
Why ?

- immediate in vivo diagnosis
- reduction the number of biopsies
- combination of biochemical and histopathological diagnosis provides more information because pathology is intimately related to biochemistry
- method has a potential to remove human interpretation
- non-invasive,non-ionizing method that probes with chemical specificity –vibrations and fluorescence (not just structure)
- extremaly high spatial resolution (optical imaging)

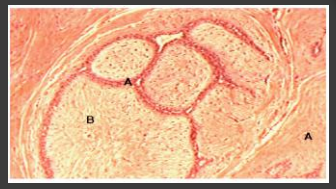
Tissue Preparation



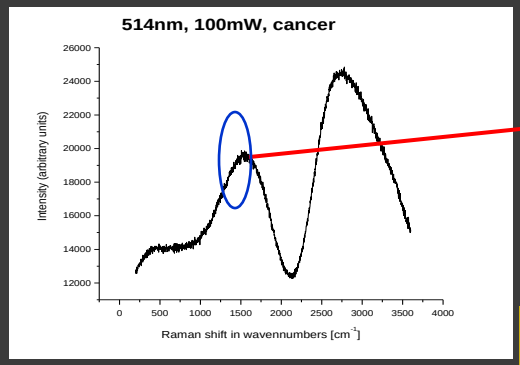


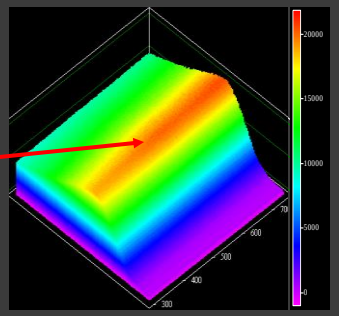


carcinoma mammae



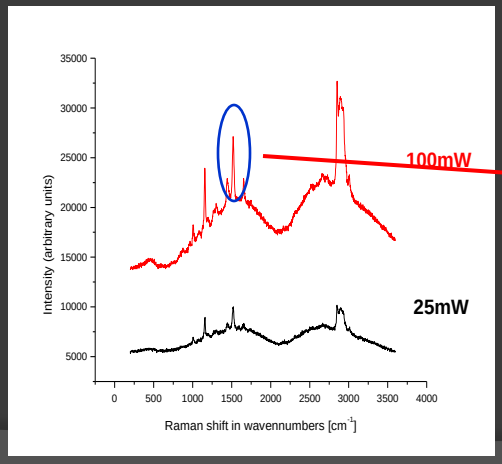
fibroadenoma mammae

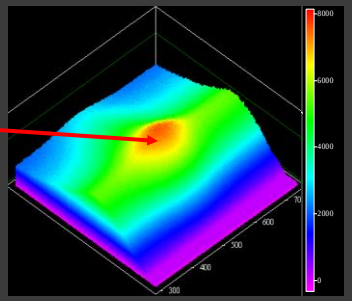




maligant tissue

normal tissue





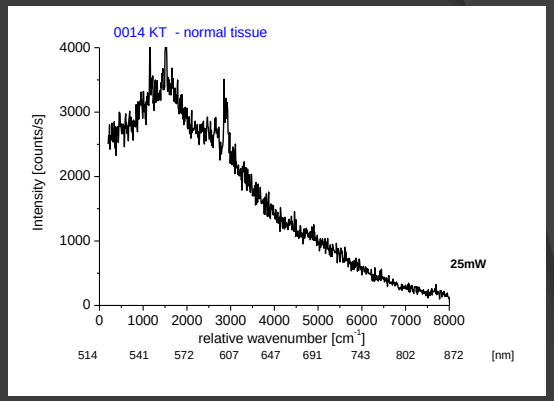
- ❖Human speciemens obtained from surgery. Upon removal during the operation, the ex vivo sample is devided by a doctor into two parts, one goes to our lab the second goes to the pathology examination
- ❖The ex vivo samples are neither frozen in liquid nitrogen for storage nor fixed in formalin, the fresh tissue is measured immeditely after delivering from the hospital
- ❖The samples for pathology measurements are passively thawed at room temperature and kept moist with PBS fixed in formalin
- ❖Cut through the marked locations into 5-µm-thick sections, and stained with eosin

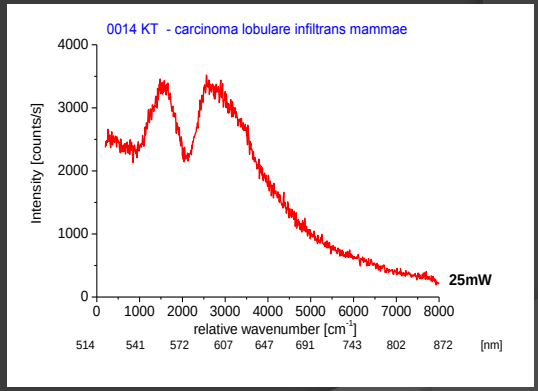
Measurements Parameters

- The laser excitation is 514 nm, the spot is d=500 µm in diameter
- Light diffusion in the tissue results in a spot of v≈1mm
- Integration time 0.5 s
- Spectral resolution 2 and 8 cm^{-1}
- The laser excitation power: 25 mW, 100mW

Patients Statistics

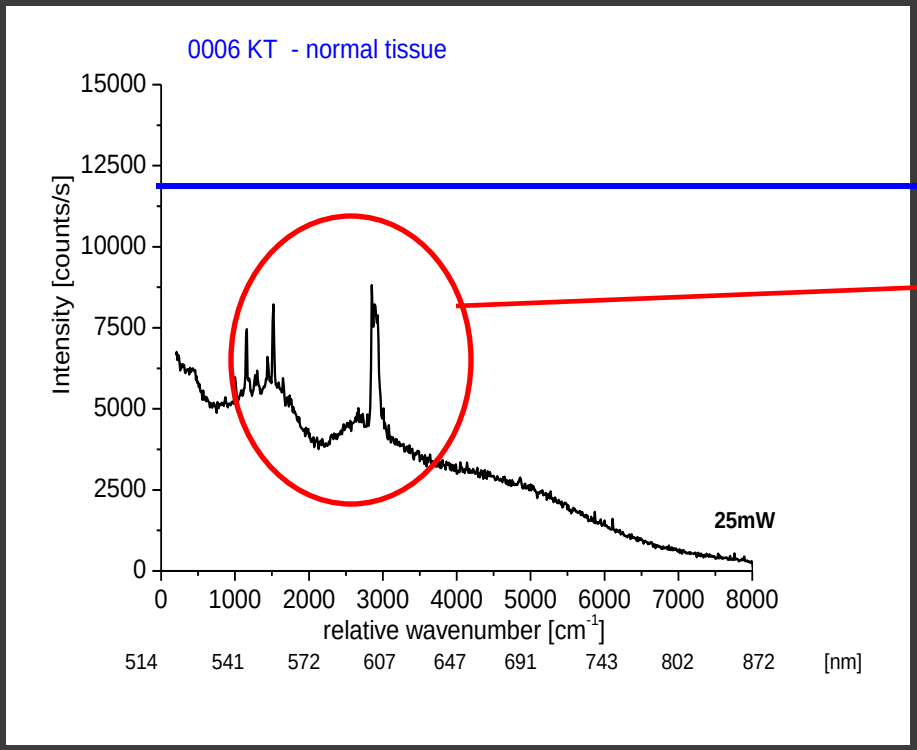
❖ **1100 spectra from 100 patients**

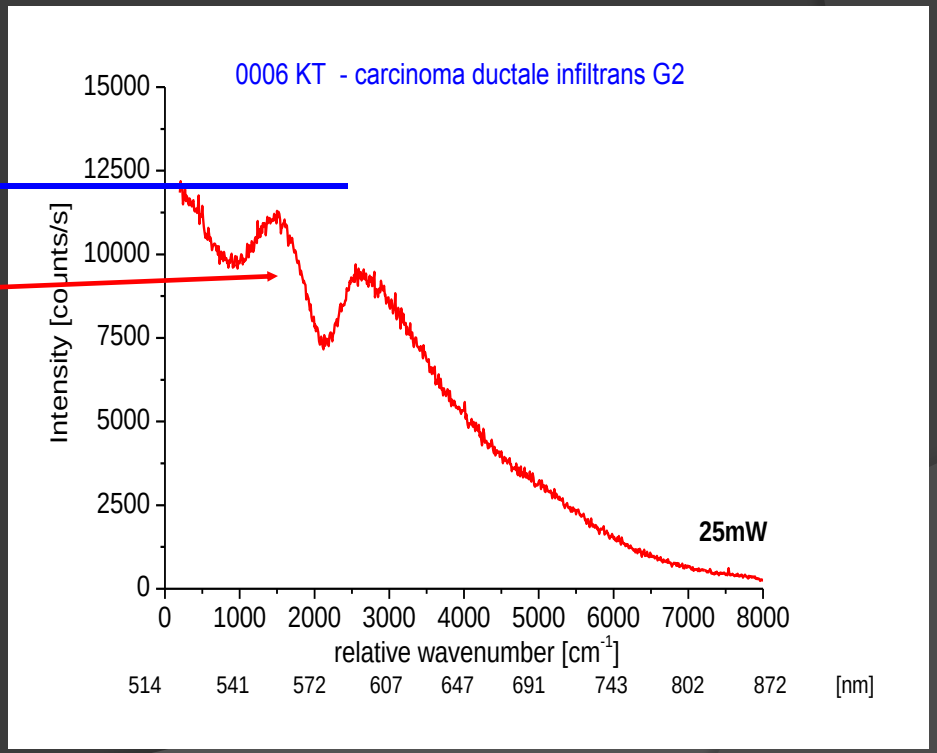




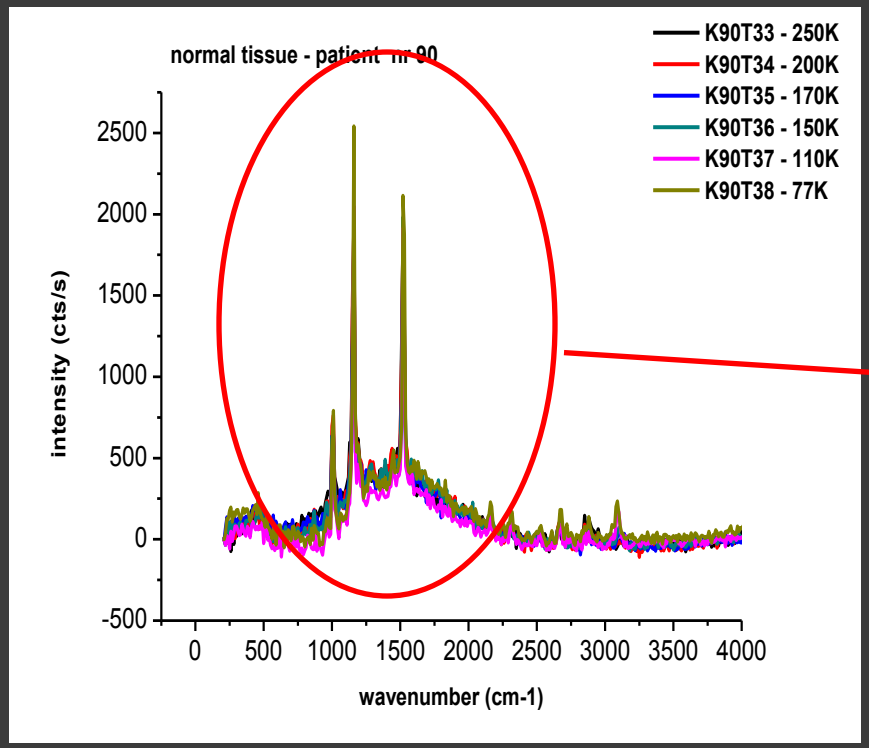
- **fibroadenomas**
- **infiltrating carcinoma**
- **infiltrating ductal carcinoma (IDC)**
- **infiltrating lobular carcinoma (ILC)**
- **IDC+ILC**
- **multifocal carcinoma**
- **carcinoma microinvasive**
- **intracystic papillary carcinoma (noninvasive)**
- **carcinoma mucinosum**
- **carcinoma intraductal microinvasive**
- **benign dysplasia**
- **dysplasia benign dysplasia (cystes, apocrinal metaplasia, adenosis)**
- **ductal-lobular hyperplasia**
- **cystic mastopathy**

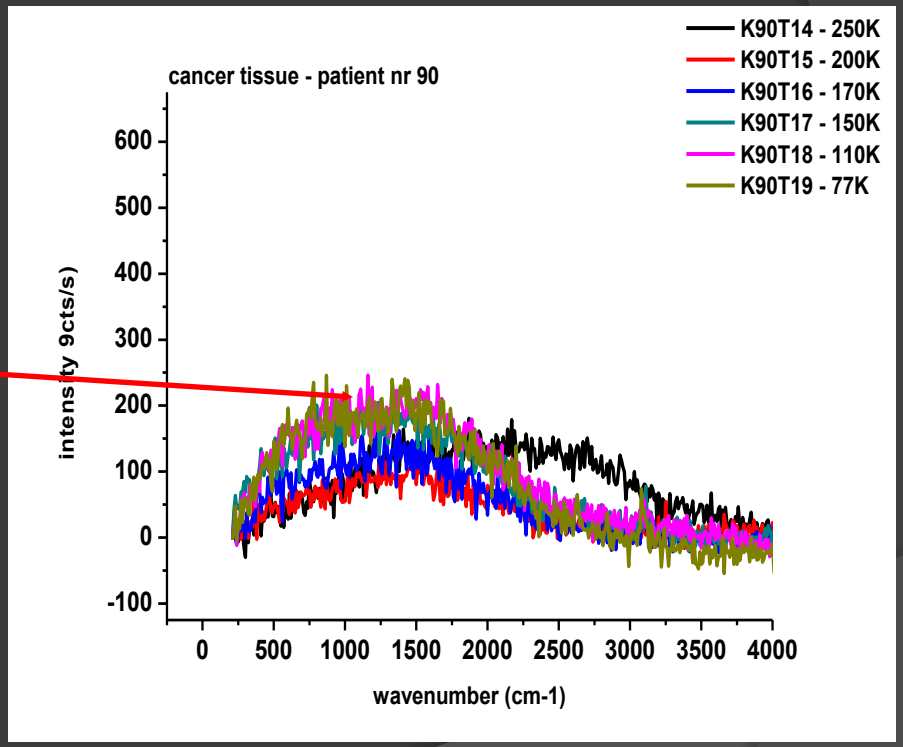
Comparison between the Raman spectra for normal and malignant tissue (infiltrating ductal carcinoma (IDC))



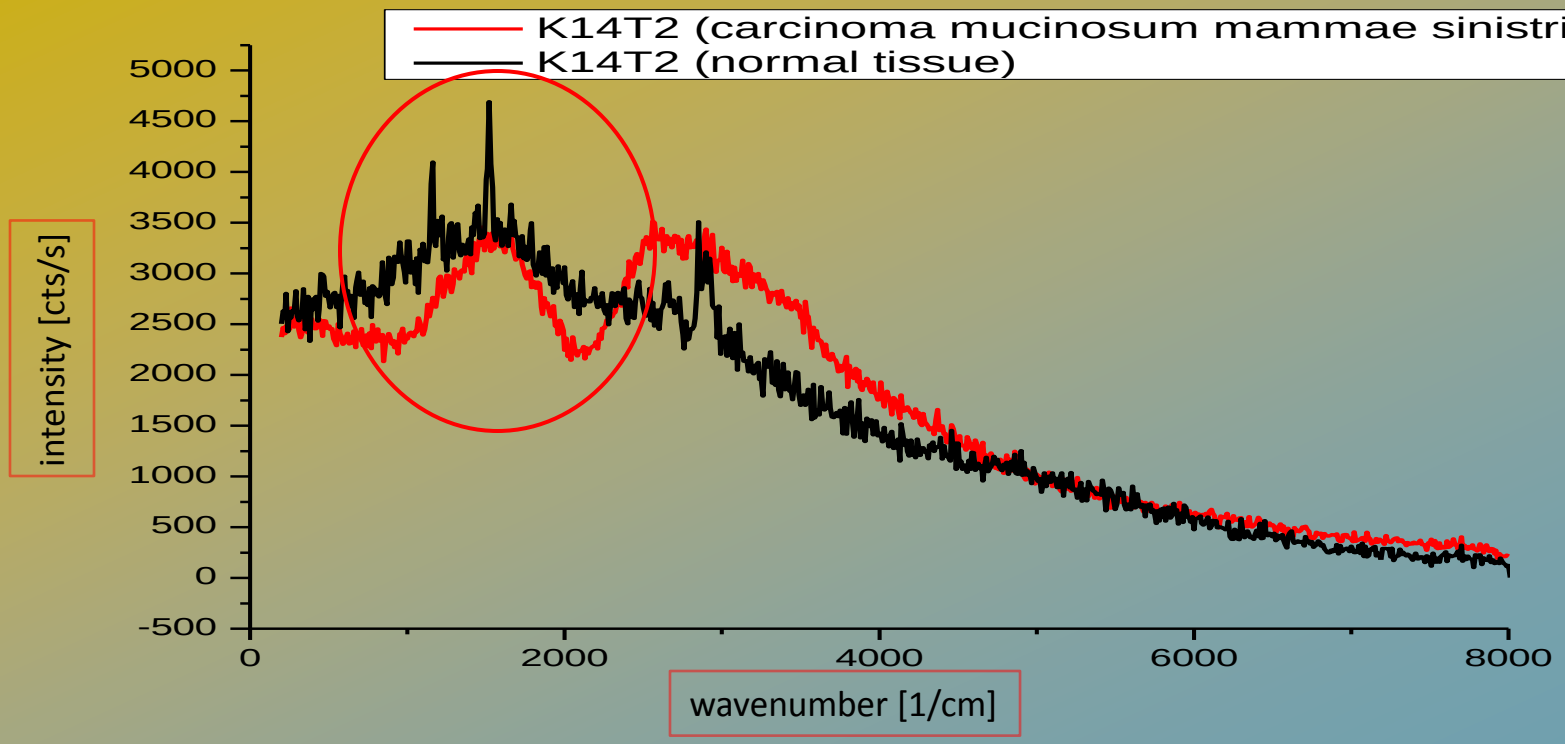


Quenching of autofluorescence by low temperature Raman spectroscopy

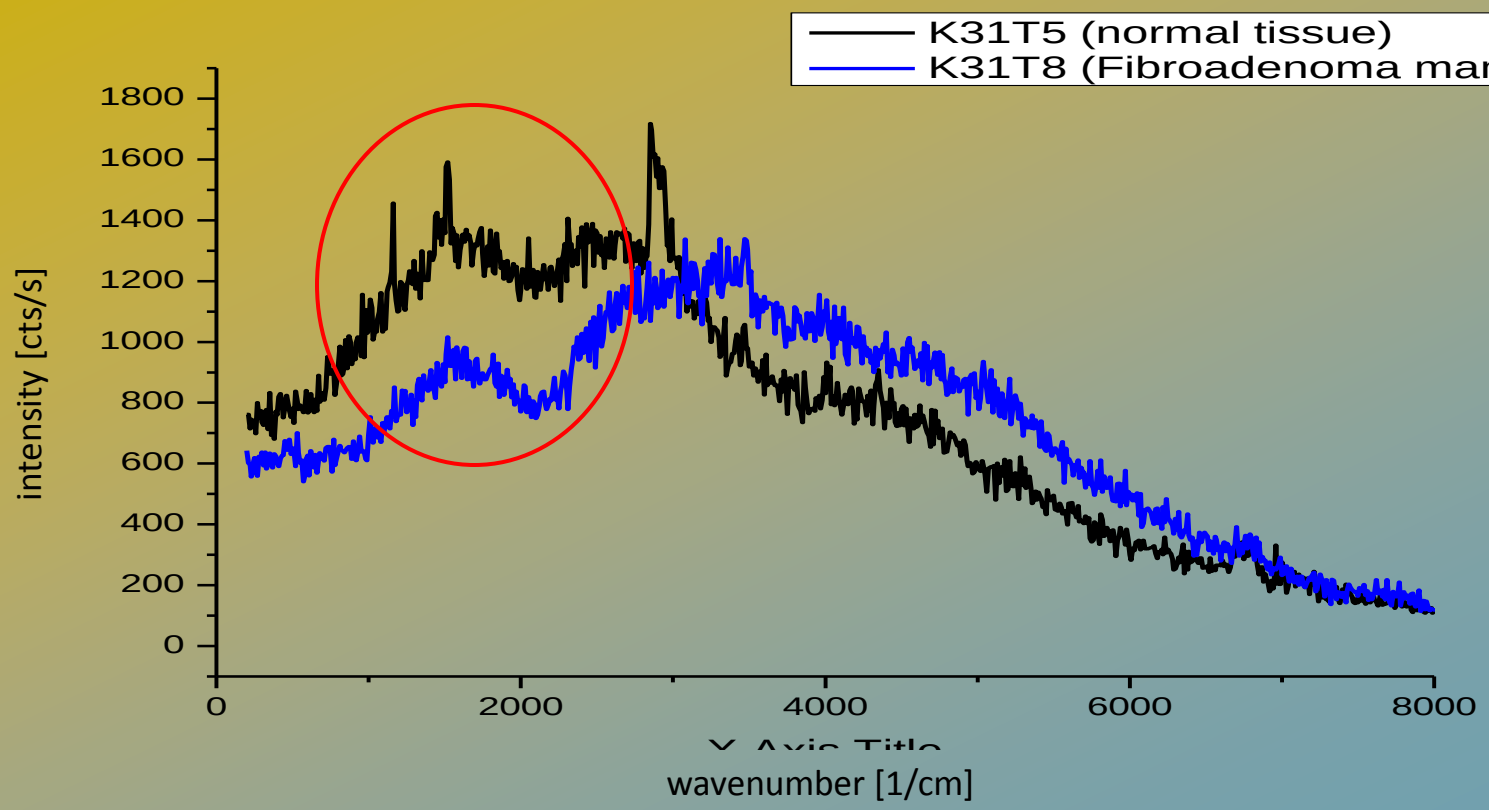




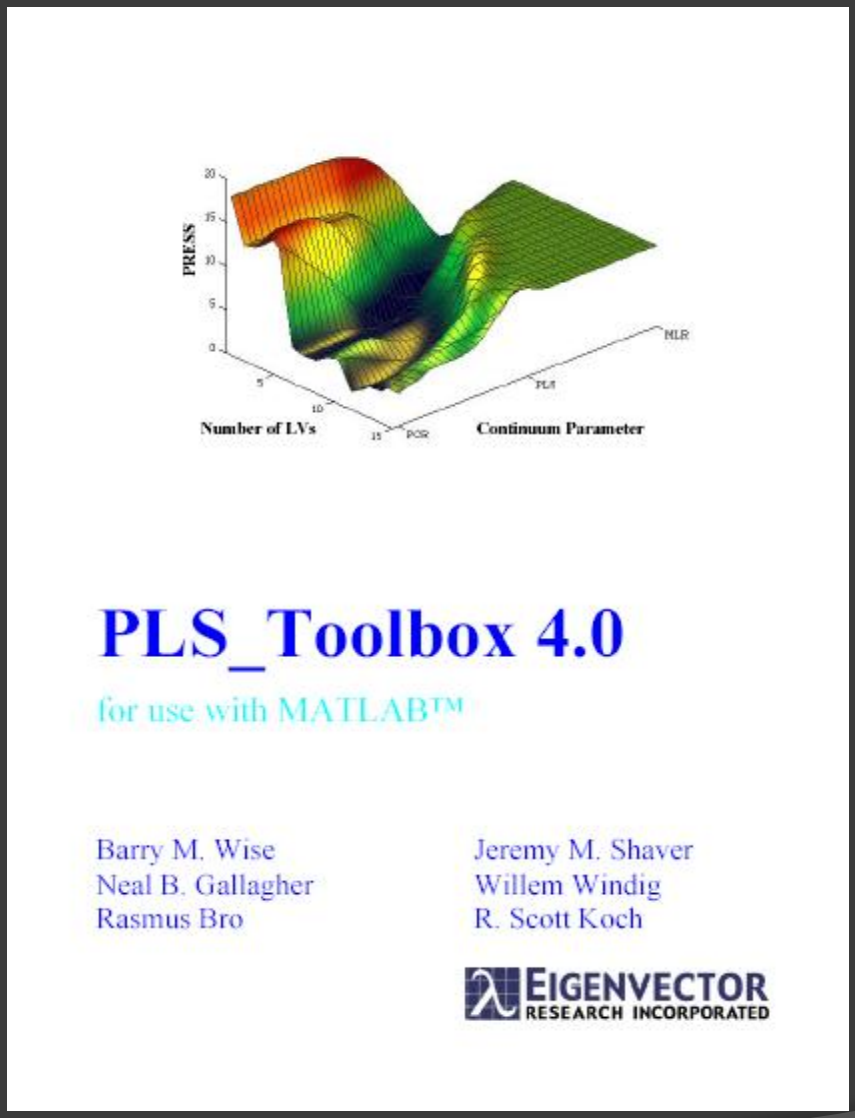
Comparison between the Raman spectra for normal and malignant tissue (carcinoma mucinosum)



Comparison between the Raman spectra for normal and benign tumour tissue (fibroadenoma)



PRINCIPAL COMPONENT ANALYSIS (PCA)



PLS_Toolbox Version 4.0
for use with MATLAB™

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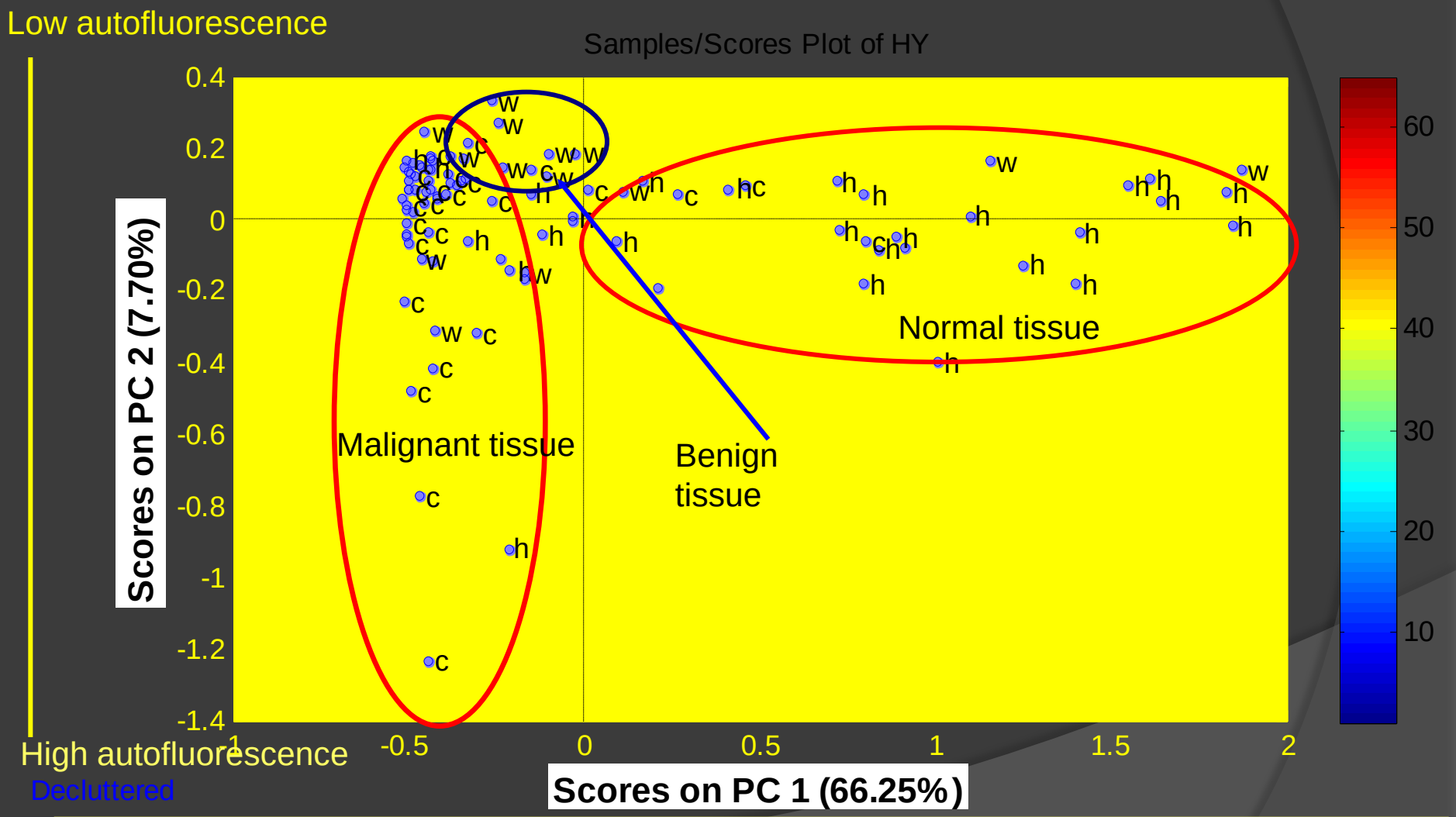
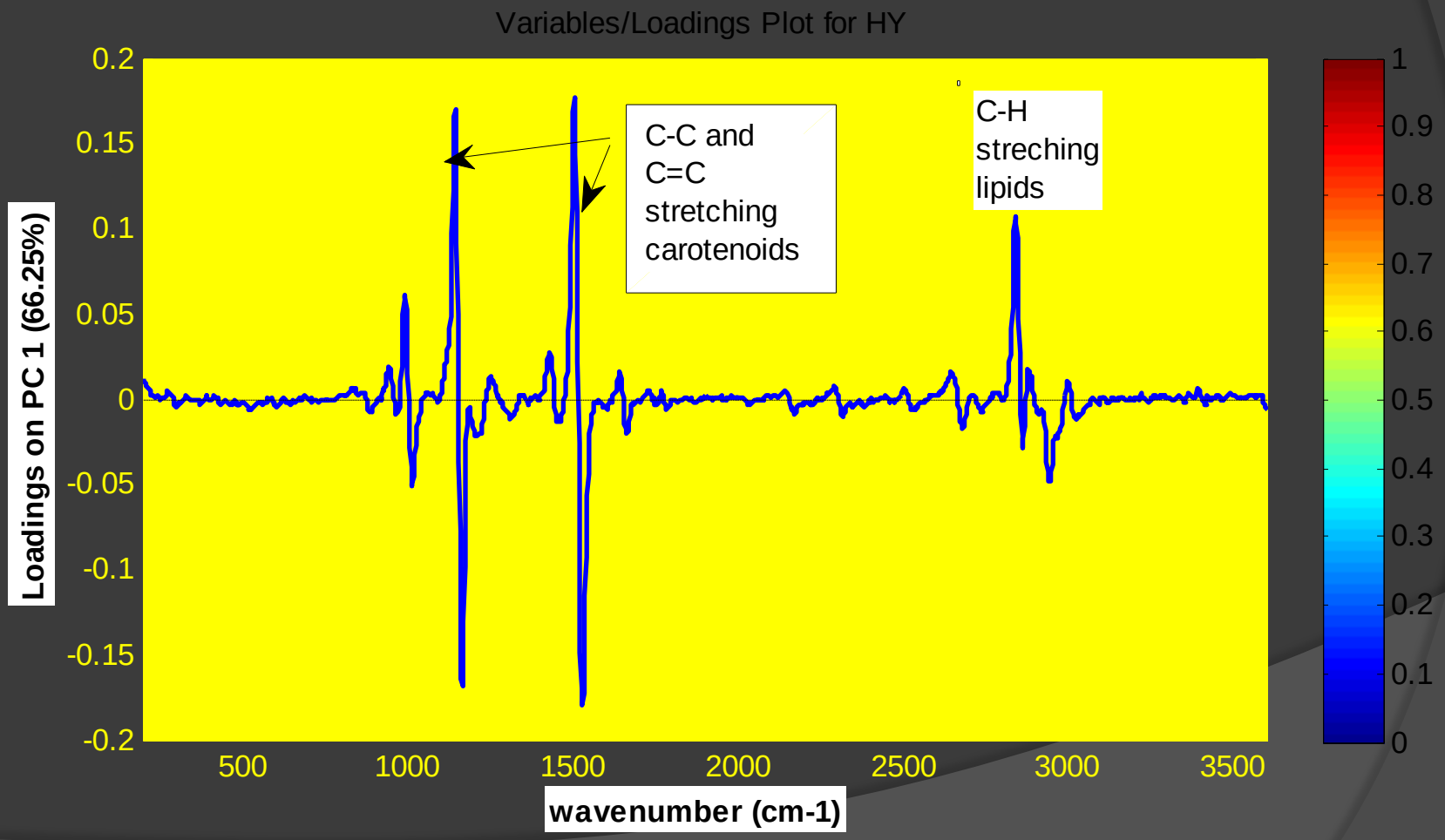
PCA score plot – mean center, SNV, 1-st derivative



PCA loading plot – mean center, SNV, 1-st derivative



CONCLUSIONS

- **The results clearly illustrates the ability of Raman spectroscopy to accurately diagnose breast cancer and demonstrates how the diagnostic scheme can be adjusted to obtain the desired degree of sensitivity and specificity (88%,72%)**
- **The normal tissue has a characteristic bands: C-C (1110 cm^{-1}) and C=C (1520 cm^{-1}) stretching bands of carotenoids and at 2840-2900 cm^{-1} for the C-H symmetric and asymmetric bands of lipids (fat) which are not visible in the malignant tissue and in benign tumor tissue. Moreover, the fluorescence is much higher in the malignant tissue.**
- We belive that in a very near future a good quality Raman signal will be obtained with the optical fibers coupled with a biopsy needle and incorporated into Raman spectrometer for breast tissue measurements in vivo.