Evaluation and Validation of Raman Spectroscopic Diagnostic Methodology of Cervix cancers by Blind Studies

*K. Maheedhar[1,2], B.M.Vadhiraja[2], Rani .A .Bhat[3], Donald .J. Fernandes[2], Pralhad Kushtagi [3],M.S.Vidyasagar[2], V.B. Kartha[1] and *C. Murali Krishna[1]*

[1]Centre for Laser Spectroscopy, MLSC, Manipal University, Manipal – 576 104

[2] Department of Radiation Oncology, Shirdi Saibaba Cancer Hospital, KMC, Manipal University, Manipal – 576104

[3] Department of Obstetrics and Gynecology, KMC, Manipal Univeristy, Manipal - 576104

Introduction

One of the leading cause of cancer death among Gynecological cancers [1] - one fourth of global total are reported in India.
Primary screening tools – Pap test and Colposcopy, Diagnosis – Histopathology [2]

- Morphology
- Subjective Interpretation
- High false positive / negative results
- Requires skilled Pathologist

Promising alternative - Optical spectroscopy (Fluorescence [3], FTIR and Raman [4,5])

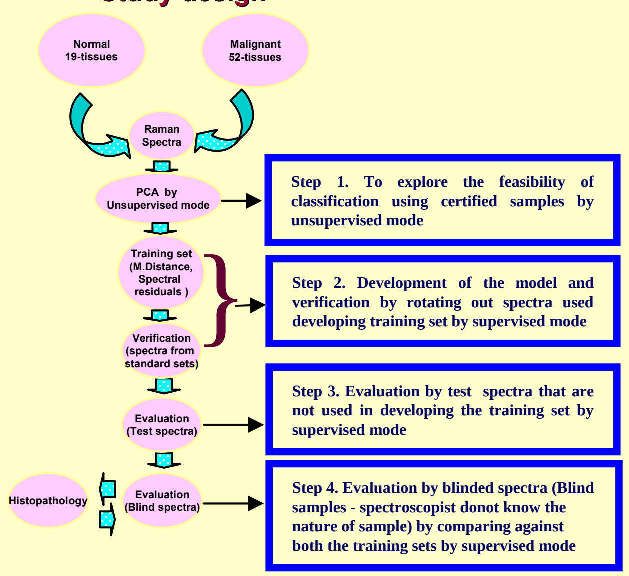
- ☑ Biochemical composition
- ☑ Interpretation is objective
- ☑ Minimally skilled technician

AIM

☞ To evaluate the discriminating model developed by us [6] for diagnosis of cervical cancer by blind studies

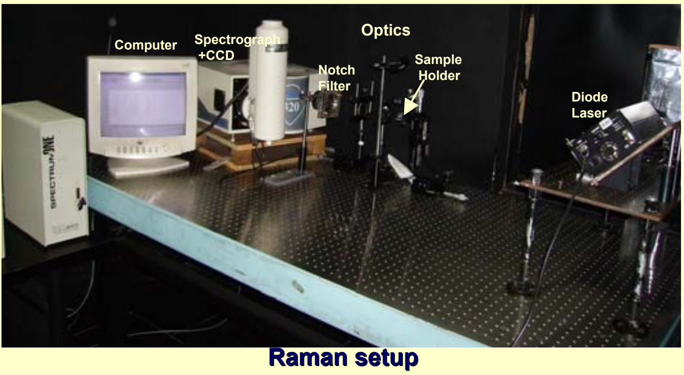
Materials and methods

Study design



Experimental Setup

Sir C.V. Raman



Raman setup

Data Analysis

Data pretreatment: Spectra were baseline corrected, smoothened, calibrated and normalized to δCH_2 band. Pretreated spectra are subjected to Principal Components Analysis (PCA).
Classification of tissue types by PCA: PCA was carried out in unsupervised and supervised modes
Unsupervised analysis : Score of factor 1 was discriminating parameter.
Supervised analysis: Mahalanobis distance and spectral residuals were discriminating parameters and match / mis-match 'limit test' methodology was also employed

Results and Discussion

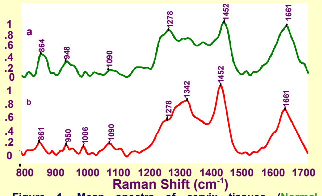


Figure 1. Mean spectra of cervix tissues (Normal, Malignant)

Mean normal spectrum characterized by broader amide I, peaks at 1275,948 and 864 cm^{-1} - indicate collagen and structural protiens. Mean malignant spectrum show sharper features in amide I and amide III region and minor blue shift in δCH_2 band – indicate lipids, DNA

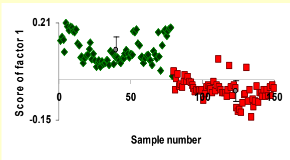


Figure 2. Unsupervised classification - Cluster analysis of certified tissue spectra (■ Malignant, ♦Normal)

Score of factor 1 produced clear classification between normal and Malignant tissue spectra

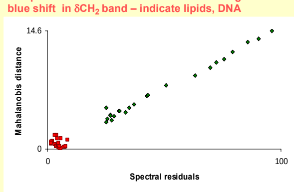


Figure 3. Supervised classification - Verification of training sets (■ Malignant, ♦Normal)

Training sets were developed by randomly selected 24 normal and 28 malignant spectra based on score of factor 1 and histopathological certification. These training sets were verified by matching the spectra against both the training sets. Clear classification observed between normal and malignant training sets, which indicates representative of normal and malignant conditions

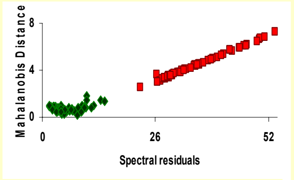


Figure 4. Supervised classification - Evaluation of training sets (■ Malignant, ♦ Normal)

Test spectra (that were not used in developing the training sets) 44 normal and 54 malignant spectra were matched against both the training sets. Once again clear classification among the two tissue types is observed

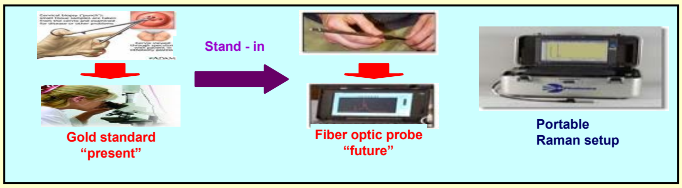
S.NO	Spectra Analyzed	Match status against Normal standard set	Match status against Malignant standard set	Histopathology Report	Raman Spectroscopy Report
1	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
2	a,b,c,d,e,f ,g,h,l,j (10)	Y,Y,P,Y,Y,Y,Y,Y,P,Y	N,N,N,N,N,N,N,N,N,N	Normal	Normal
3	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
4	a,b,c,d,e,f (6)	N,N,N,N,N,N	P,P,P,P,P,N	Malignant	Malignant
5	a,b,c,d,e,f ,g,h,l (8)	Y,Y,Y,Y,P,N,Y,Y	N,N,N,N,N,N,N,N,N,N	Normal	Normal
6	a,b,c,d,e,f (6)	P,P,P,P.N,N	N,N,N,N,P,P	Normal	Malignant??
7	a,b,c,d,e,f (6)	N,N,N,N,N,P	Y,Y,Y,Y,Y,N	Malignant	Malignant
8	a,b,c,d,e,f ,g,h,l,j (10)	P,P,P,P,P,P,P,N,P,P	N,N,N,N,N,N,N,N,N,N	Normal	Normal
9	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
10	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
11	a,b,c,d,e,f ,g,h,lj (9)	P,P,P,P,P,P,P,P,P	N,N,N,N,N,N,N,,N,N	Normal	Normal
12	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
13	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
14	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant
15	a,b,c,d,e,f (6)	N,N,N,N,N,N	Y,Y,Y,Y,Y,Y	Malignant	Malignant

Table 1. Limit test approach of the blinded spectra.

Blinded tissue spectra are matched against both the training sets to know the match / mismatch status of each spectrum against the training sets. All spectra in samples 1,2,3,9,10,11,12,13,14,15 match with one standard set and does not match with the other. Hence they can be diagnosed straight forwardly as normal and malignant respectively. In case of samples 4 and 7 at least one of the spectrum match with malignant training set. Thus these tissues were treated as malignant as in the lines of histopathology. In case of samples 5 and 8 few spectra does not match with either of the training sets. Since most of the spectrum match against normal training set they were treated as normal. In case of sample 6 though histopathology report shows normal since spectra 'e' and 'f' match against malignant training set it was treated as malignant.

Conclusions and Prospective

- This study demonstrates good correlation between Raman spectroscopy and histopathology (70 out of 71 tissues) in diagnosis of cervical caner
- Prospectively, with rigorous validation of the models, inclusion of training sets for other pathological conditions such as premalignant, different stages of cancer and inflammatory conditions and with development of fiberoptic probe, *in vivo* Raman spectroscopic diagnosis of cervical cancers can be a reality.



References

1. Parking D.M, et al, CA Cancer J Clin 2005; 55:74.
2. Koss L.G., J. Am. Med. Assoc. 1989; 261: 737.
3. Mitchell M.F., Consult. Obstet. Gynecol. 1994; 6: 70.
4. Mahadevan Jasen, A.et al, Photochem Photobiol. 998, 68: 427.
5. Lyng, F.M., et al, Exp Mol Pathol. 2007; 82:121.
6. Murali Krishna, C. et al, Vib. Spectrosc., 2006; 41:136.

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•Dr. Murali Krishna Chilakapati, Scientific officer 'E' and Principal Investigator, Chilakapati laboratory, ACTREC, TMC, Khargar, Sector '22', Navi Mumbai – 410208,, INDIA