EARLY DETECTION OF ORAL CAVITY CANCER BY OPTICAL SPECTROSCOPY

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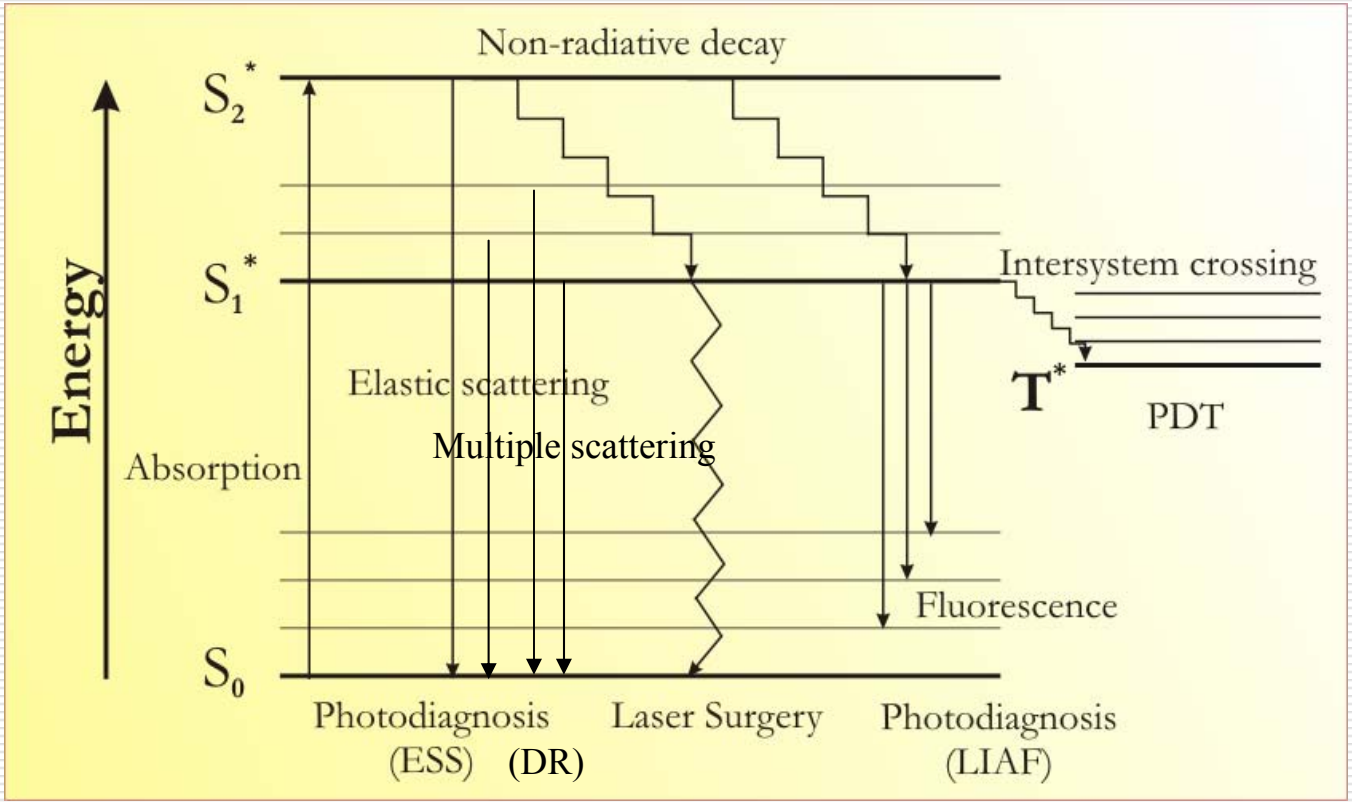
Motivation

- Oral cancer - a significant and growing concern
- What causes oral cancer – smoking, tobacco, alcohol, and chewing of pan, *Pan Parag* etc in India
- Presentation – Leuokoplakia and Erythroplakia
- Challenge for a diagnostic system – distinguish between benign and pre-malignant tissues *in vivo*
- Current clinical procedure and its demerits
- Need for fast and non-invasive detection systems
- Optical spectroscopy – a potential tool
- Autofluorescence and diffuse reflectance spectroscopy

Light tissue interaction: Potential medical applications



Energy level diagram of an exited molecule showing various de-excitation mechanisms and medical applications of lasers

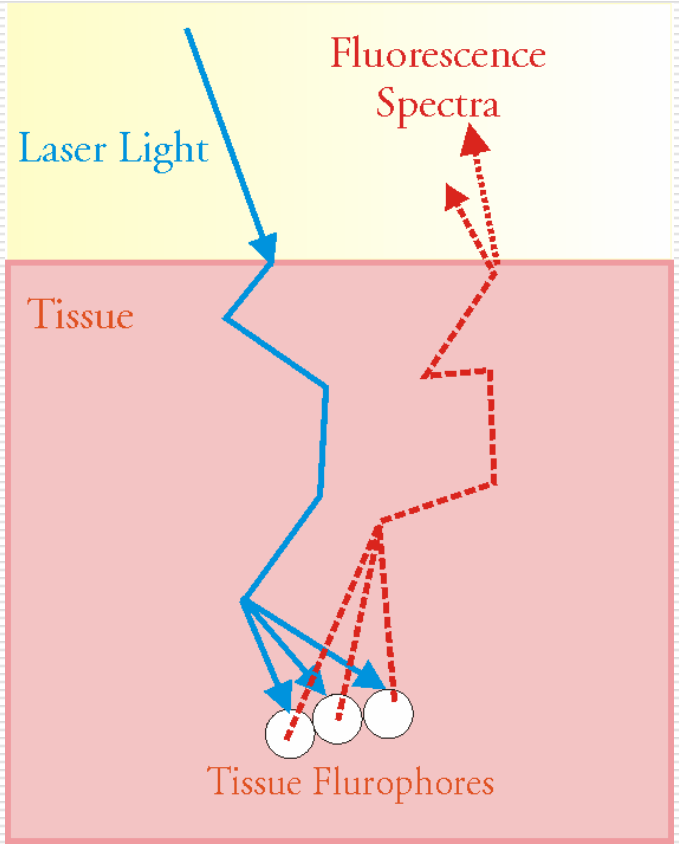
Photodiagnosis of Cancer

- Autofluorescence of Tissues
- Diffuse Reflectance of Tissues

Detection Principles

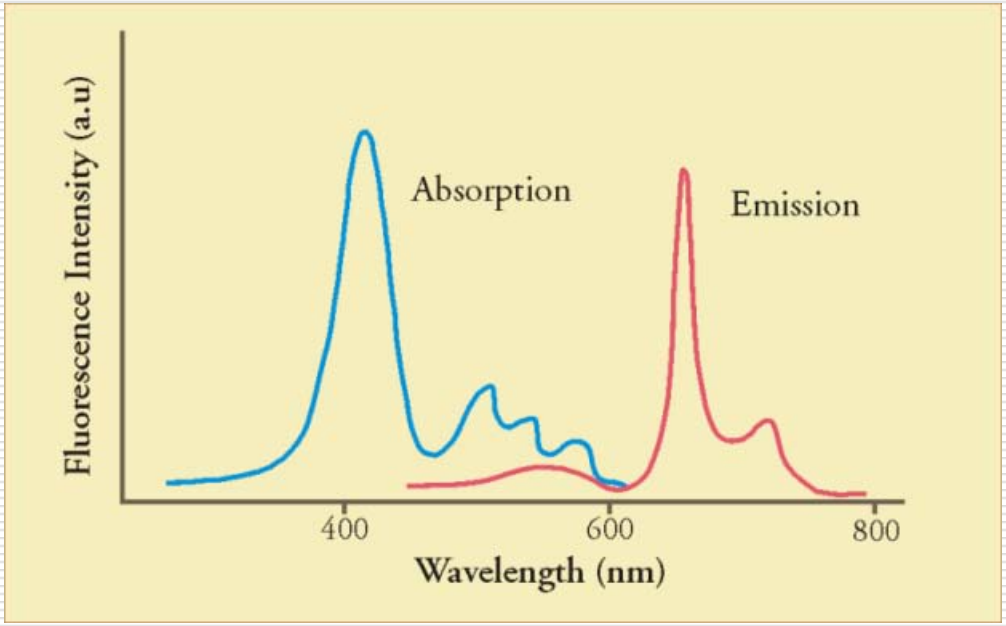
Laser- Induced Autofluorescene (LIAF) Spectroscopy

- Light impinging on the tissue is absorbed by various tissue fluorophores.
- The absorbed fluorophores emits a fingerprint spectra.
- During tissue transformation towards malignancy, tissue morphology as well as composition gets altered thereby changing the fingerprint.



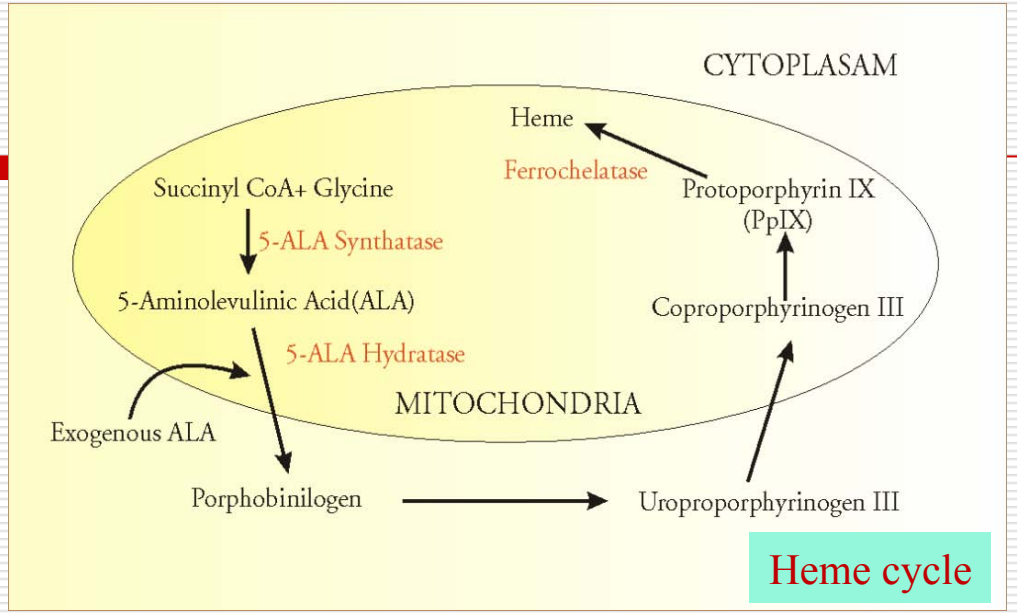
Tissue Absorbers and their characteristic emissions

Tissue Flurophores	λmax excitation (nm)	λ peak emission (nm)
Tryptophan	275	350
Collagen	340 270 285	395 395 310
Elastin	460 360 425 260	520 410 490 410
NADH	350	460
Endogenous porphyrins	> 400	635, 705



Absorption and emission spectra of porphyrins (PpIX)

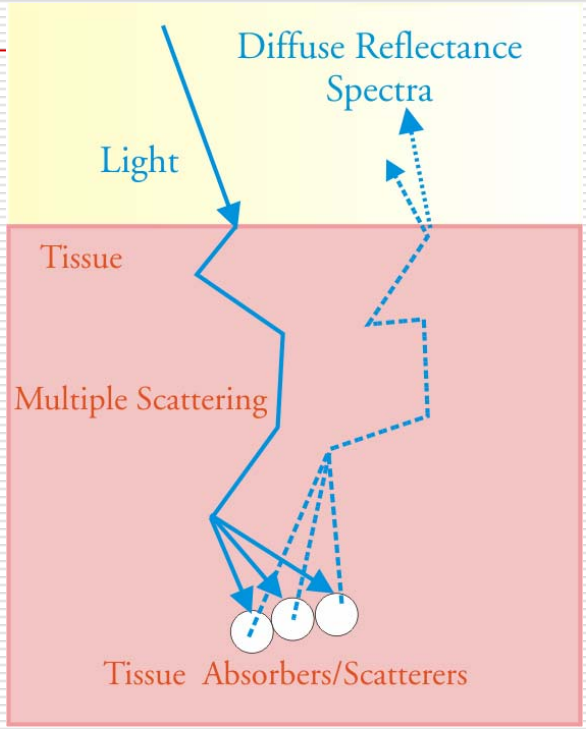
Selective accumulation of PpIX in malignant tissues



- **All nucleated mammalian cells synthesize Heme.**
- **In the mitochondria of cells, Glycine and Succinyl CoA combine to form ALA which gets converted to Protoporphyrin IX (PpIX)- a flurophore, through a number of bio-chemical reactions activated by various enzymes.**
- **Lack of the enzyme Ferrochealtase in abnormal tissues reduces Heme production thereby enhancing the accumulation of PpIX in abnormal tissues and reducing Heme production (inhibiting Oxygenated Hemoglobin presence).**
- **When ALA is given exogenously (for PDD or PDT) it bypasses into the Heme pathway and enhances the PpIX production.**

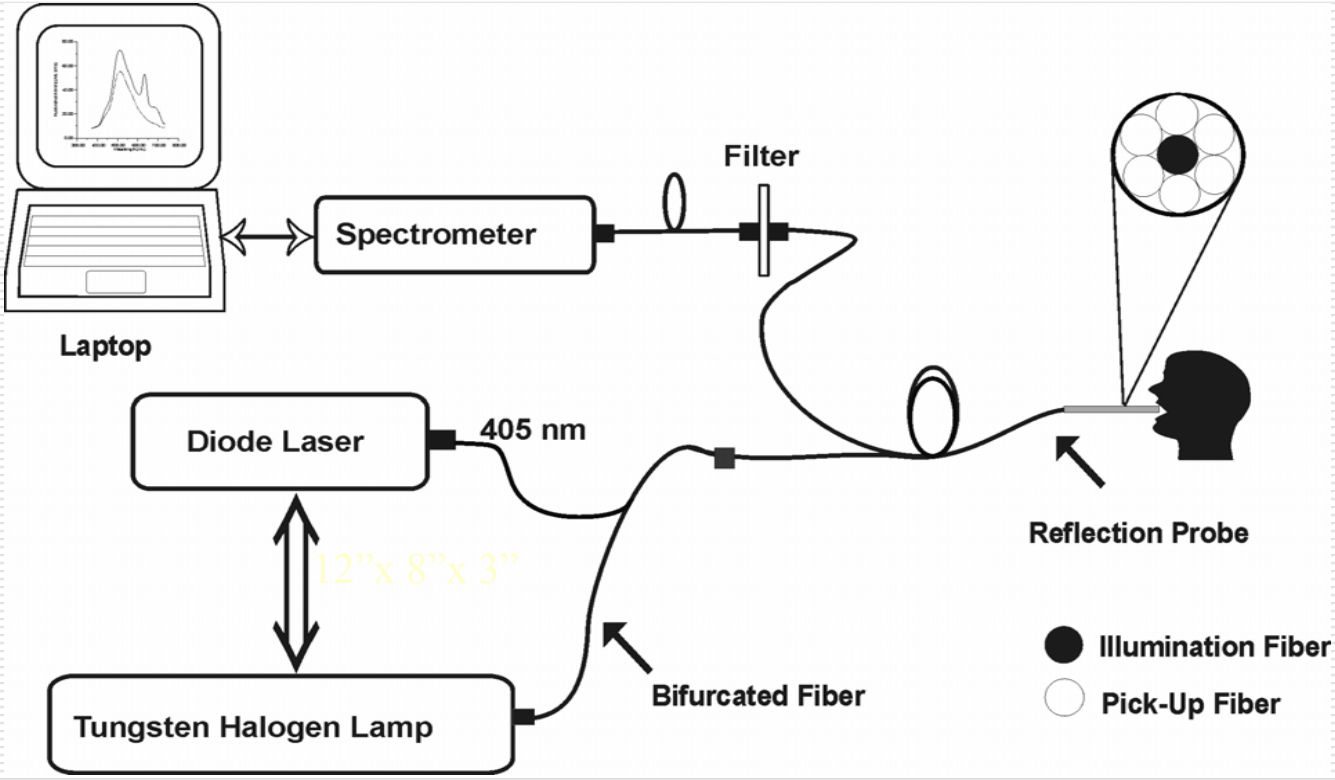
Diffuse Reflectance (DR) Spectroscopy

- During tissue transformation towards malignancy, its composition, morphological properties (vascularity, metabolic rate, intravascular oxygenation, etc) changes affecting the elastic scattering properties.
- Major absorbers in tissue - Water, Oxy- and de-oxygenated hemoglobin
- DR spectra from spatially distant points with respect to the incident beam will show effects of multiple scattering, absorption and tissue vascularity.



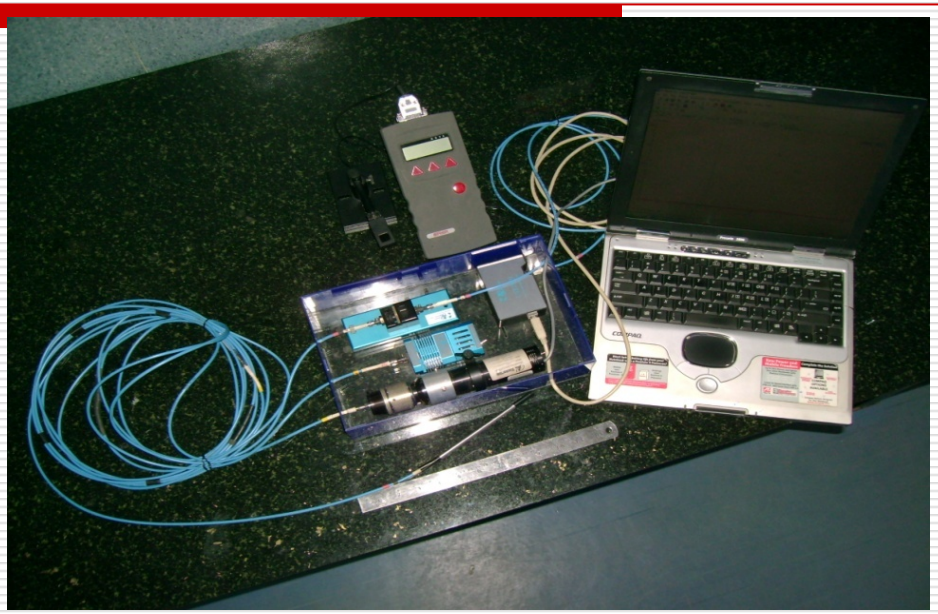
Tissue Absorbers	Absorption maxima (nm)
Water	Above 1300, below 200
Oxygenated Hemoglobin (HbO_2)	418, 542, 577
Deoxygenated Hemoglobin	750

Laser-Induced Fluorescence and Diffuse Reflectance Spectroscopy (LIFRS) system



Schematic of LIFRS system for Oral Cavity Cancer Detection

LIFRS system developed for clinical studies

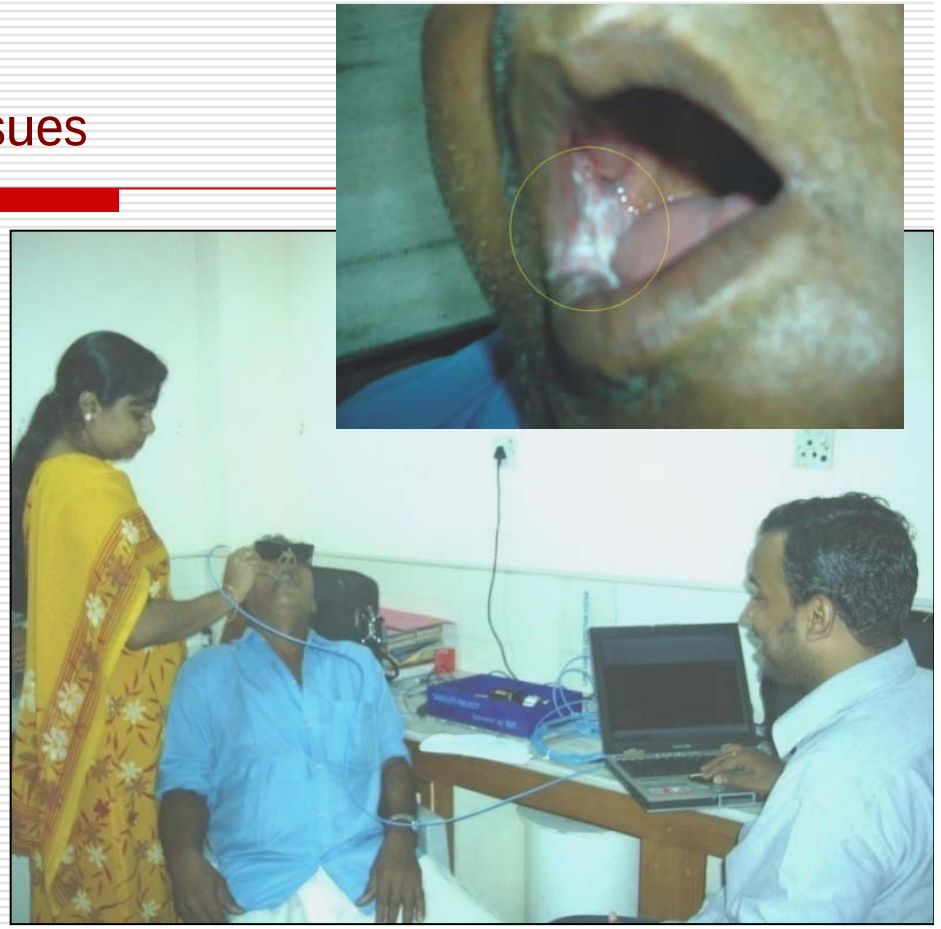


A closer view

Fiber tip in stainless ferrule with plastic sleeve

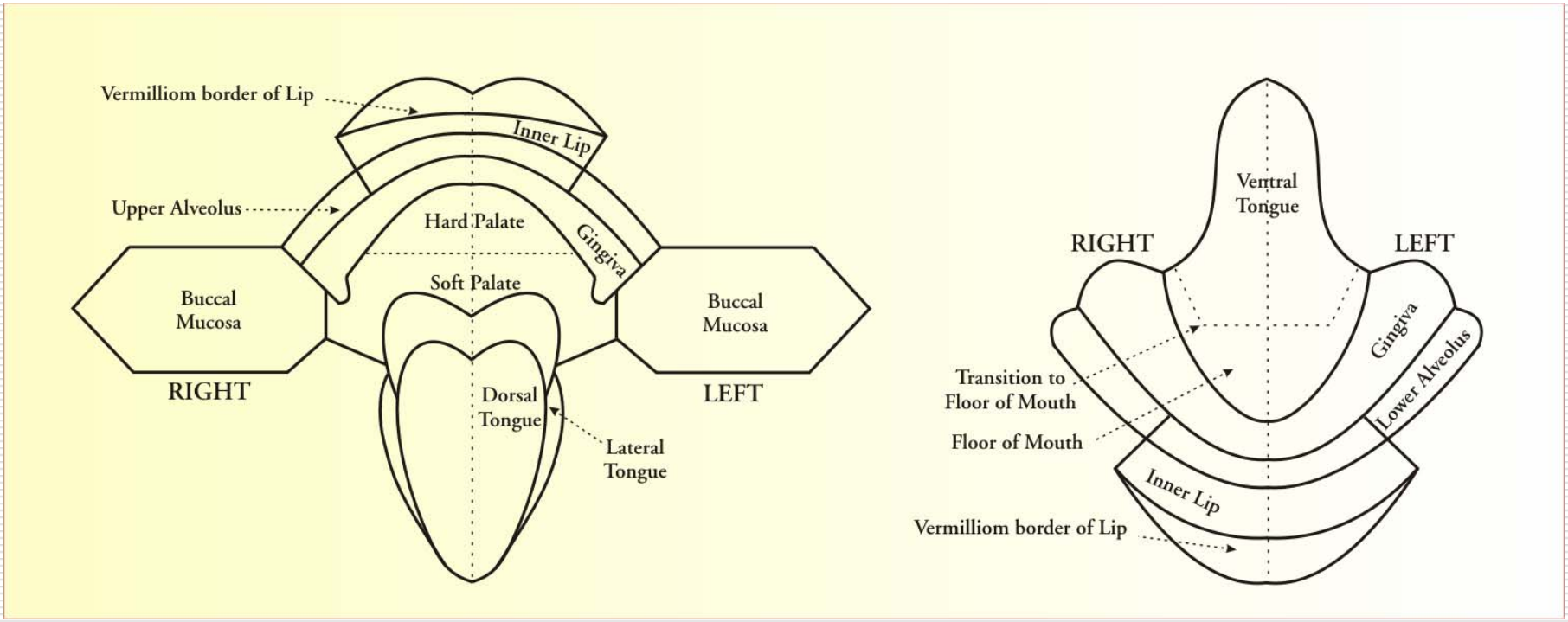
Clinical study protocol and ethical issues

- Ethical Clearance for trials obtained from Ethics Committee of Regional Cancer Centre (RCC), Trivandrum.
- Subjects included healthy volunteers and patients with oral cavity cancer.
- The study protocol consisted of recording the visual imprint by clinician followed by Clinical Trails on consenting patients.
- 0.9% saline wash given prior to spectral measurements.
- 15 sets of LIAF and DR measurements taken from each site.
- Biopsy from measurement sites taken for pathological analysis and correlation with spectral data.



Clinical Trials being conducted at the Out-Patient Clinic of Regional Cancer Centre, Trivandrum.

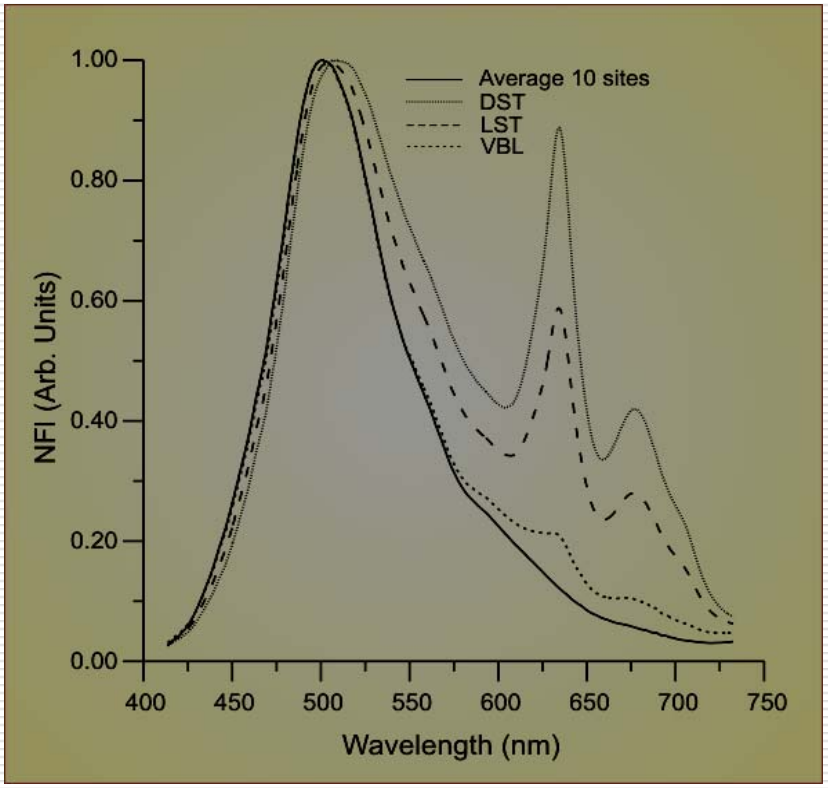
Control Measurements on Healthy Volunteers



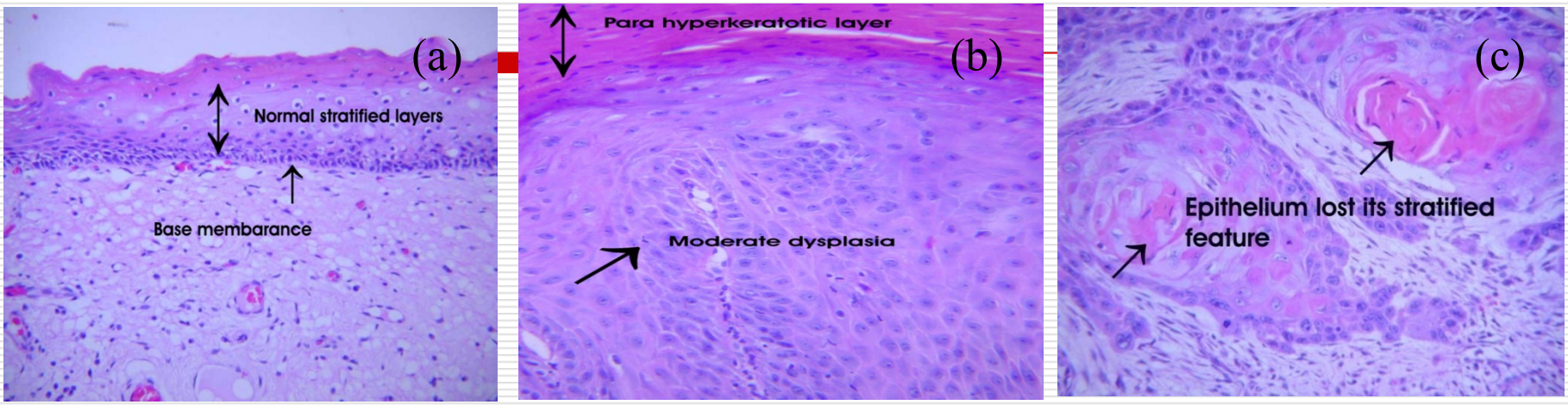
LIF and DR spectra were measured from 14 anatomical sites of oral cavity in 36 healthy volunteers and used as control during *in vivo* clinical trials.

Spectral features of healthy oral mucosa

- Broad autofluorescence emission at 500 nm
- Out of 14 anatomical sites, 11 have similar spectral features
- Exceptions are - Dorsal side of tongue (DST), Lateral side of tongue (LST) and Vermillion border of lip (VBL)
- Abnormal emissions at 635 and 705 at these 3 sites could be from porphyrins, bacteria or chlorophyll.

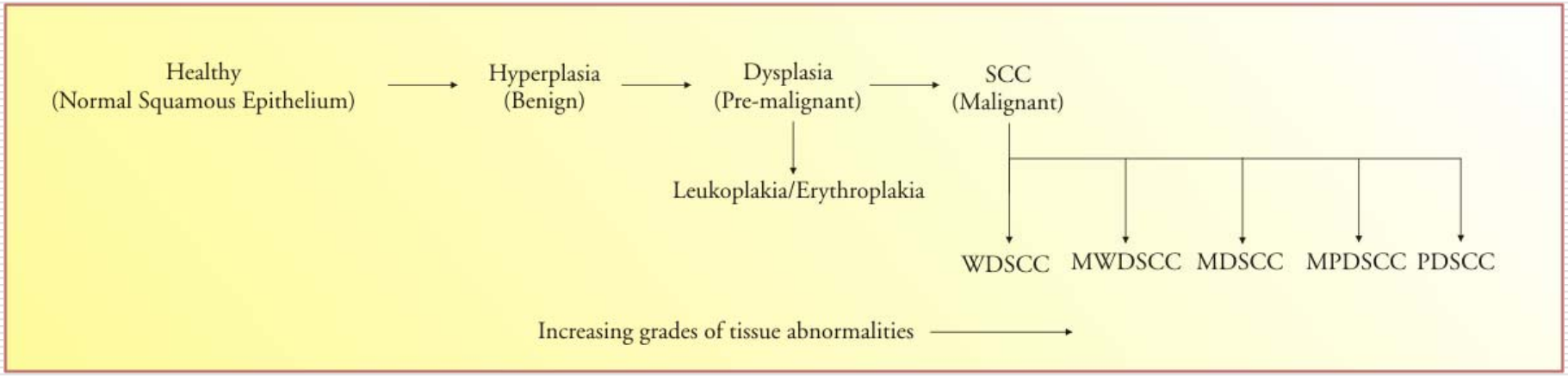


Histopathological grading of tissues



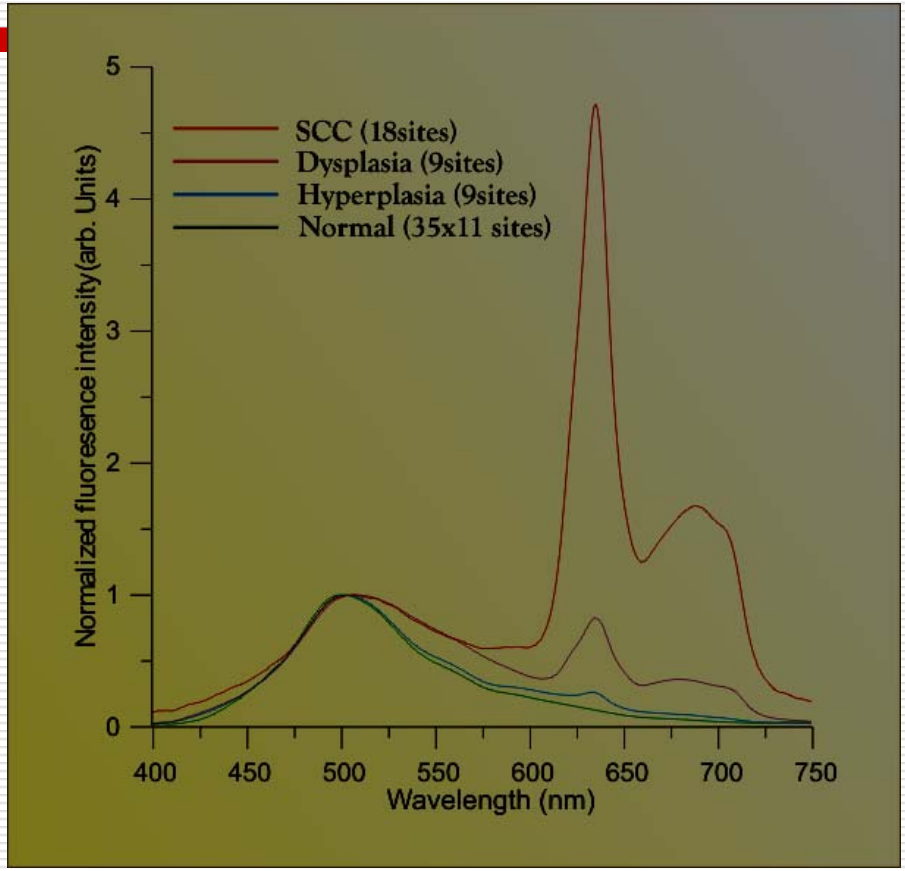
Eosin stained histopathologic images of (a) Normal (b) Dysplastic and (c) SCC

Tissue grouping for early detection of cancer



LIAF Spectra of Malignant Tissues

- Study excludes DST, LST and VBL
- 500 nm band broadens/shifts with increase in malignancy
- Three peaks appear at 635, 685 and 705 nm in malignant/pre-malignant tissues owing to emission from PpIX and its pre-cursor (Coproporphyrinogen III) in Heme cycle.
- Peak intensities at 635, 685 and 705 nm varies with extent of tissue transformation.
- Peak intensity ratios F500/F635, F500/F685 and F500/F705 used for tissue discrimination.



Normalized LIAF spectral of different tissue groups

Rupananda et al, *Oral Oncology, 2(1), 259-260, 2007*

Variation in LIAF intensity ratios with tissue abnormality

Histological Diagnosis	Population (n)	F500/F635	F500/F705	F500/F685
Healthy	35	8.00±0.66	33.08±5.71	27.61±3.74
Hyperplasia	9	6.16±1.00 (23)	21.57±7.26 (35)	15.10±4.26 (45)
Dysplasia	9	2.78±0.80 (55)	7.61±3.17 (65)	4.82±1.33 (68)
Squamous Cell Carcinoma (SCC)	18	0.80±0.74 (71)	1.96±1.46 (75)	1.6±1.20 (67)

- Fluorescence Intensity Ratios F500/F635, F500/F705 and F500/F685 grouped into Normal, Hyperplastic, Dysplastic and Squamous cell carcinoma (SCC), as per histology
- All ratios show a decreasing trend with tissue abnormality
- Values shown in parenthesis is the % variance with respect to the lower grade

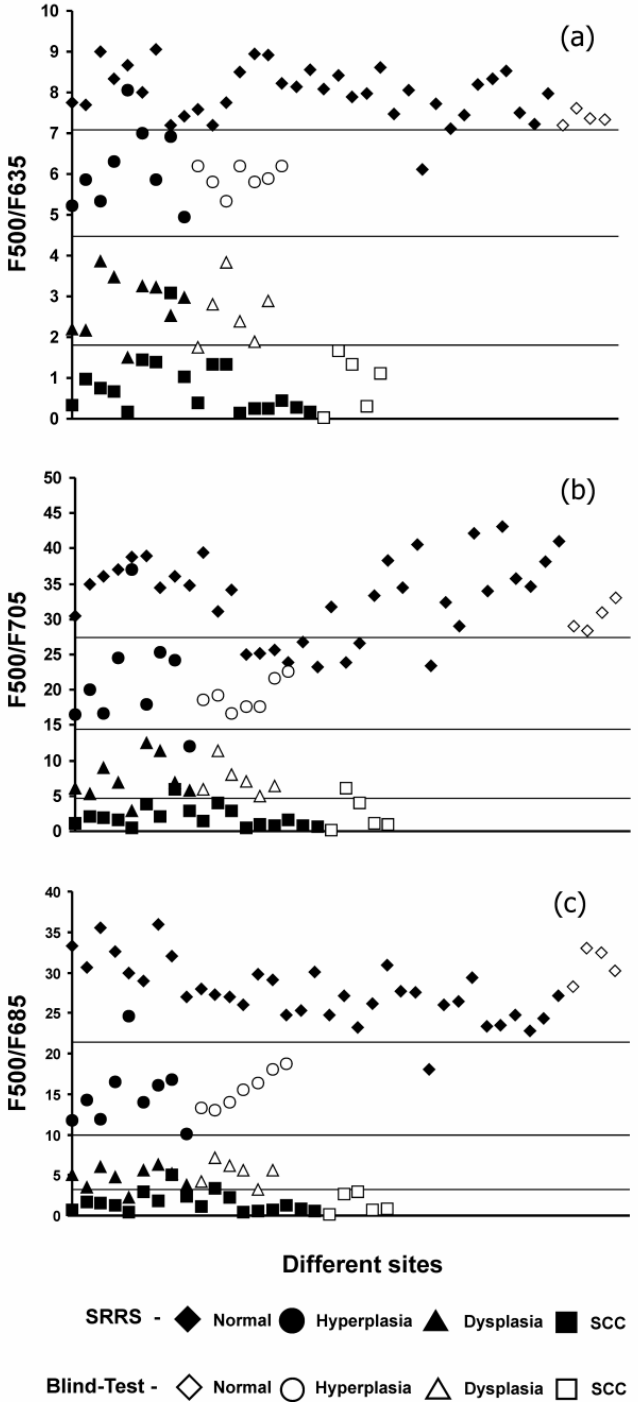
Rupananda Mallia et al, CANCER, 112(7), 1503-1512, 2008

SRRS for oral tissue discrimination and grading

- Spectral Ratio Reference Standard (SRRS) discriminates between normal, hyperplasia, dysplasia and SCC.
- Cut- off line represents the average value of adjoining group data points.
- Classification sensitivity and specificity for each categories are determined based on cut-off line by validation with gold standard (histopathological results).
- Blind test (Open symbols) in a different subject group (21 sites) tests the reliability of SRRS.

Rupananda Mallia et al., CANCER, 112(7), 1503-1512, 2008.

Study population : 35 healthy volunteers and 43 patients



Diagnostic accuracies - SRRS and Blind-test

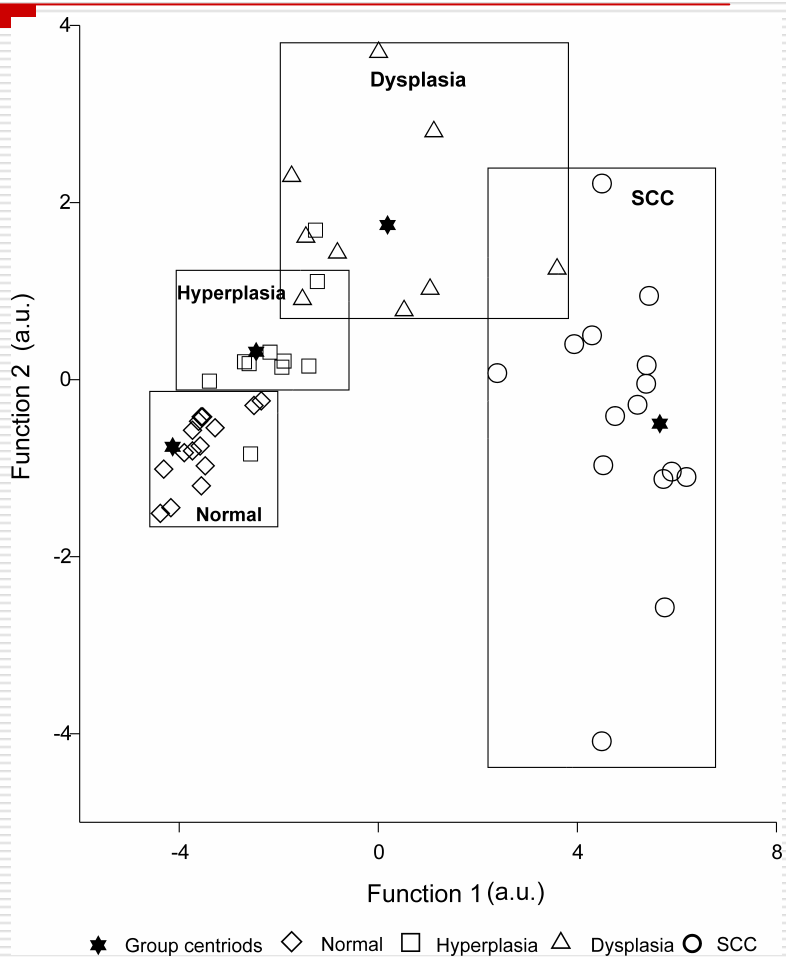
Test Results	LIAF Ratios	Normal Vs Hyperplasia		Hyperplasia Vs Dysplasia		Dysplasia Vs Squamous Cell Carcinoma	
		*Sensitivity	*Specificity	Sensitivity	Specificity	Sensitivity	Specificity
SRRS (20)	F500/F635	89	97	100	100	95	86
	F500/F685	89	97	100	100	95	86
	F500/F705	89	74	100	89	95	86
	Over all	89	89	100	96	95	86
Blind-test (17)	F500/F635	100	100	100	100	100	83
	F500/F685	100	100	100	100	100	100
	F500/F705	100	100	100	100	80	100
	Over all	100	100	100	100	93	94
Independent Student's t-test, $p<0.0005$ for each discrimination groups							

* Sensitivity= (Spectra abnormal/Histopathological abnormal)%
* Specificity= (Spectra normal/Histopathological normal)%

Rupananda et al, *Cancer, 112(7), 1503-1512, 2008.*

Linear Discriminant Analysis of LIAF spectral data

- LIAF spectra preprocessed by normalization and mean scaling.
- LDA based on leave one out (LOO) method is used.
- Sensitivity and specificity determined from scatter plot of discriminant function scores for different lesion pairs.



Scatter plot of the first two discriminant functions by LDA in 15 healthy volunteers and 34 patients. The four categories of oral tissues were located at four distinct areas.

Cross Validation using Linear Discriminant Analysis

Overall diagnostic accuracies for different lesion pairs -

Lesion Pairs	Normal Vs Hyperplasia		Hyperplasia Vs Dysplasia		Dysplasia Vs SCC	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Training set (29 sites)	83 (89)	100 (89)	80 (100)	100 (96)	89 (95)	80 (86)
Blind test (20sites)	100 (100)	100 (100)	75 (100)	100 (100)	83 (93)	100 (94)
Overall	92	100	78	100	86	90

Value in parenthesis are the overall sensitivity and specificity obtained with SRRS method

Sensitivity = True Positive / (True Positive + False Negative)

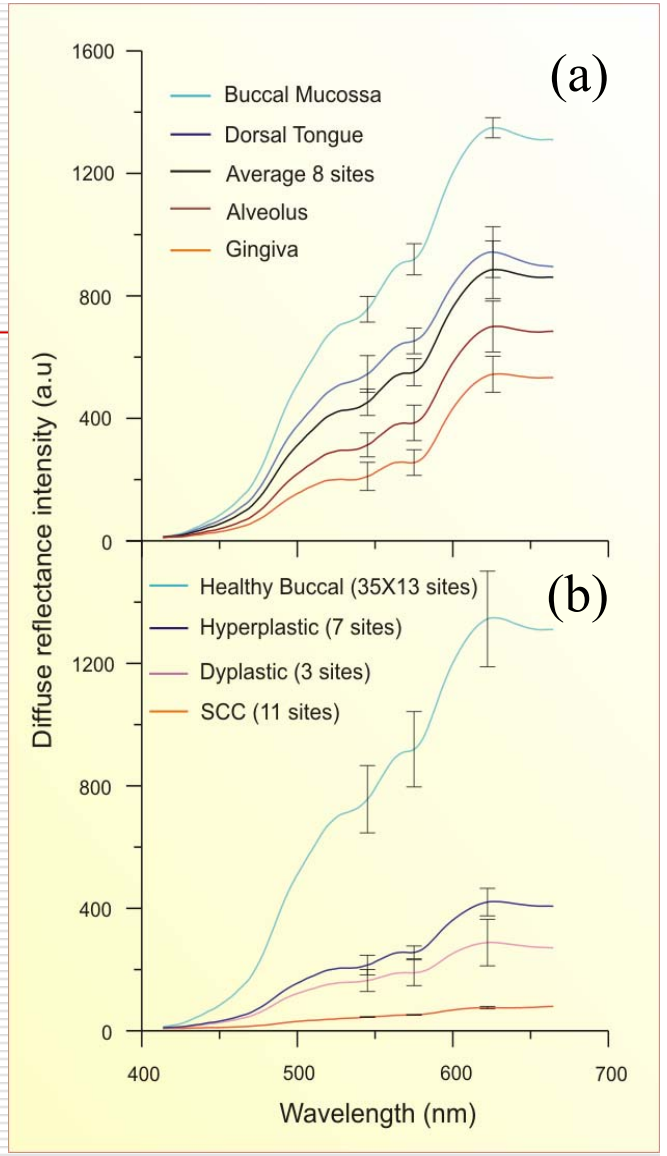
Specificity = True Negative / (True Negative + False Positive)

Diffuse reflectance spectral features

- Dips at 545 and 575 nm due to oxygenated hemoglobin absorption.
- DR intensity of healthy subjects (a) show different levels of absorbance at various anatomical sites
- Absorption maxima and spectral intensity decreases with tissue progression towards malignancy
- Reflectance intensity ratio R545/R575 shows a increasing trend with malignancy.

Histological diagnosis	Population (n)	R545/R575
Healthy	35	0.782±0.009
Hyperplasia	11	0.795±0.020
Dysplasia	10	0.822±0.012
SCC	28	0.871±0.023

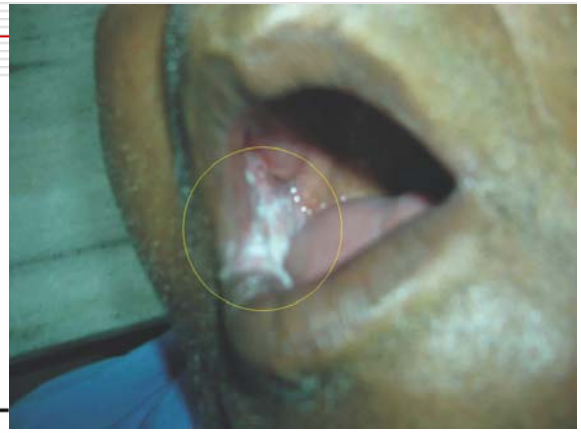
Study population: 35 healthy volunteers and 29 patients

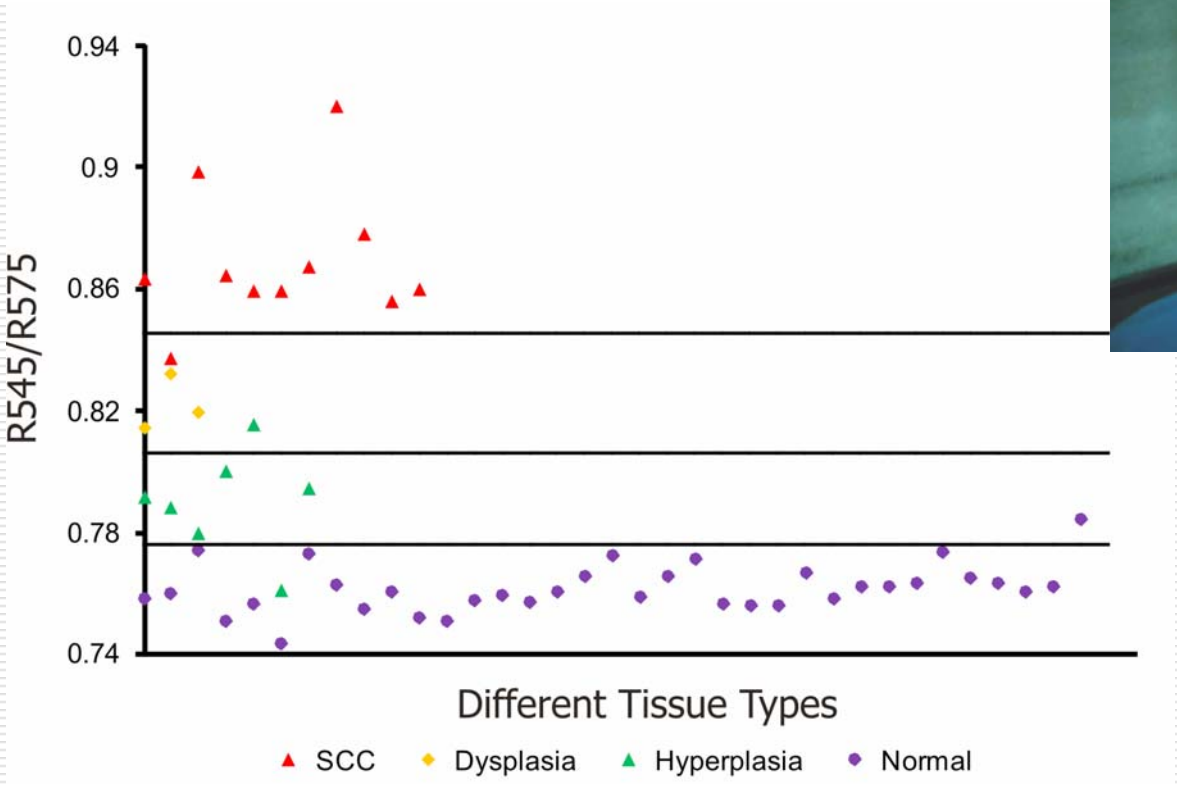


DR spectra of (a) healthy population and (b) patients with buccal cancer

R. Mallia et al., J.Biomed. Opt.,13(4), 041306, Jul/Aug, 2008

DR –SRRS plot for buccal cancer grading

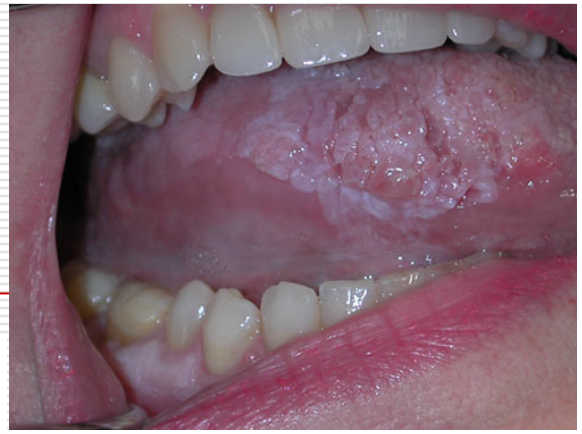


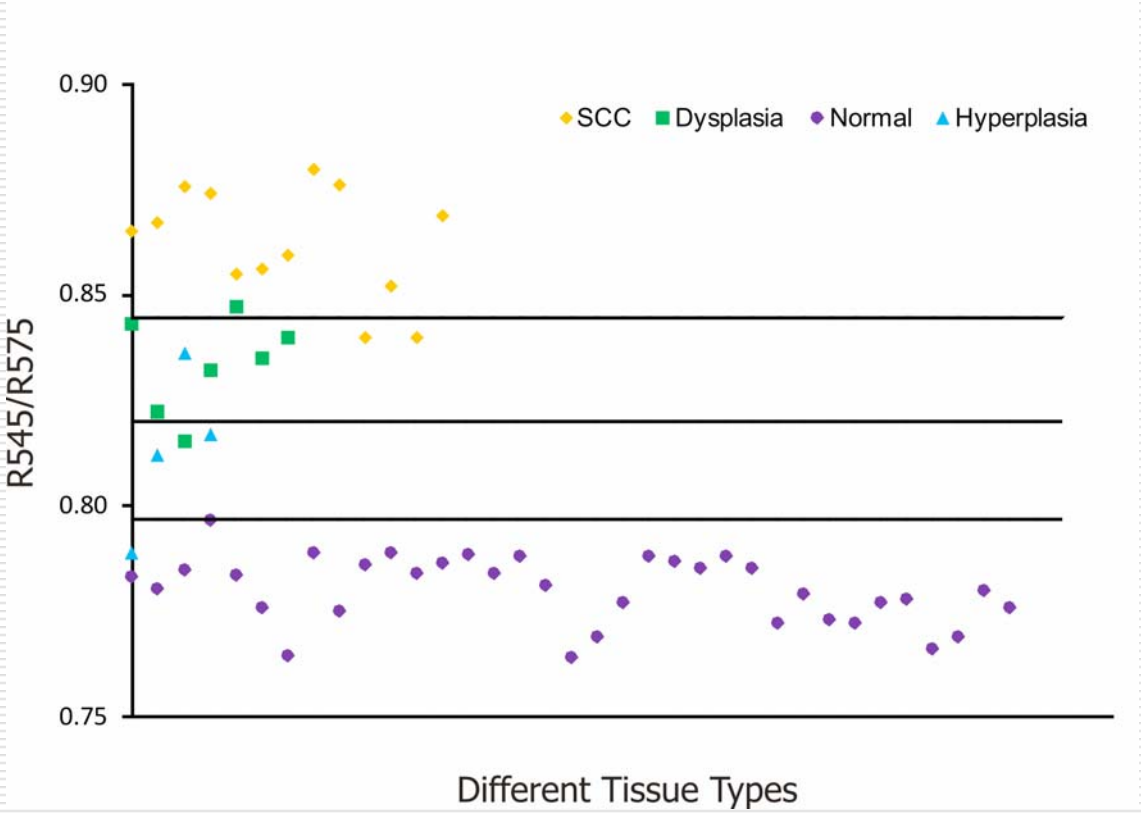


Site-specific R545/R575 scatter plot discriminating different grades of cancer from buccal mucosa (21 sites) in 12 patients and 35 healthy volunteers.

R. Mallia et al., J.Biomed. Opt.,13(4), 041306, Jul/Aug, 2008

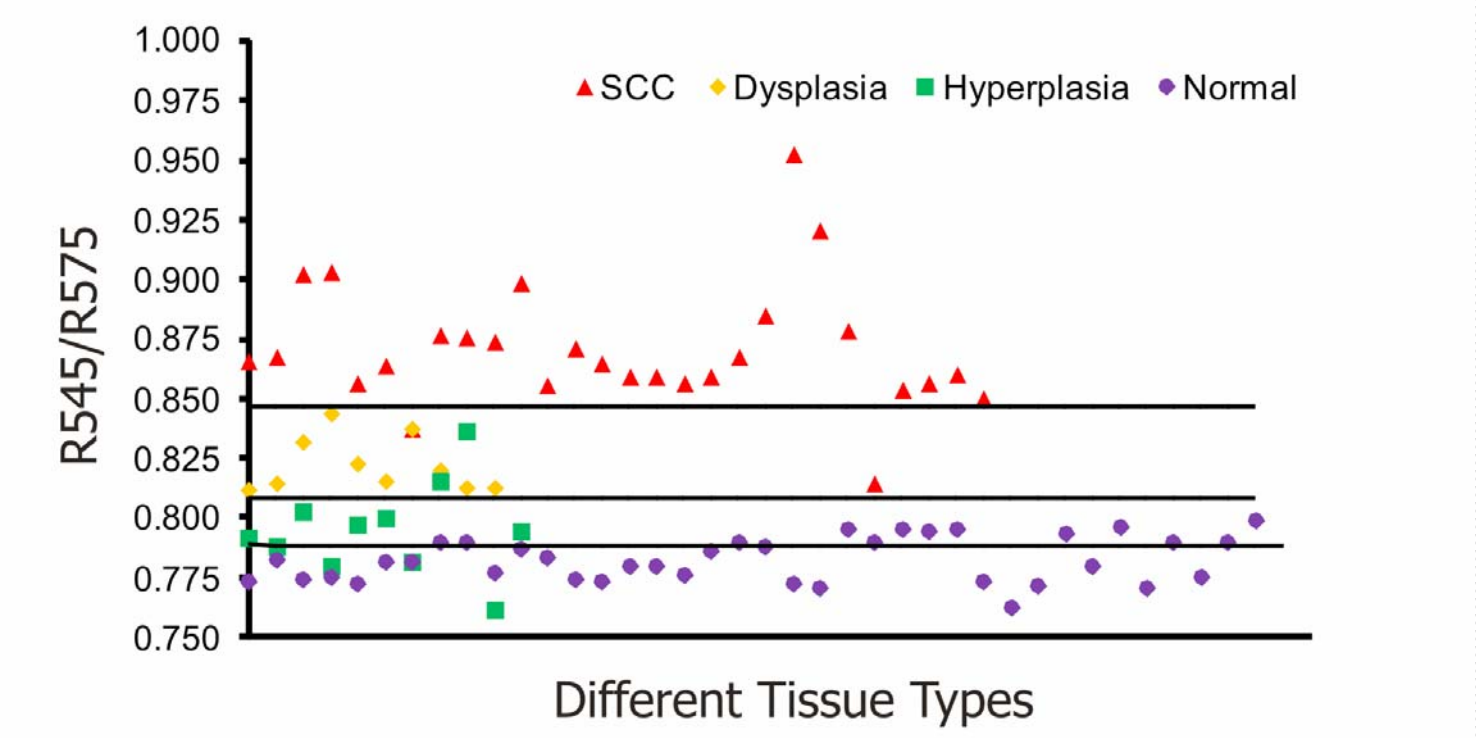
DR-SRRS plot for tongue cancer grading





Site-specific DR spectral ratio (R545/R575) scatter plot developed for discriminating different grades of DST tissues (21 sites) in 13 patients and mean control from 35 healthy volunteers.

Combined scatter plot R545/R575 ratio for grading oral cancer



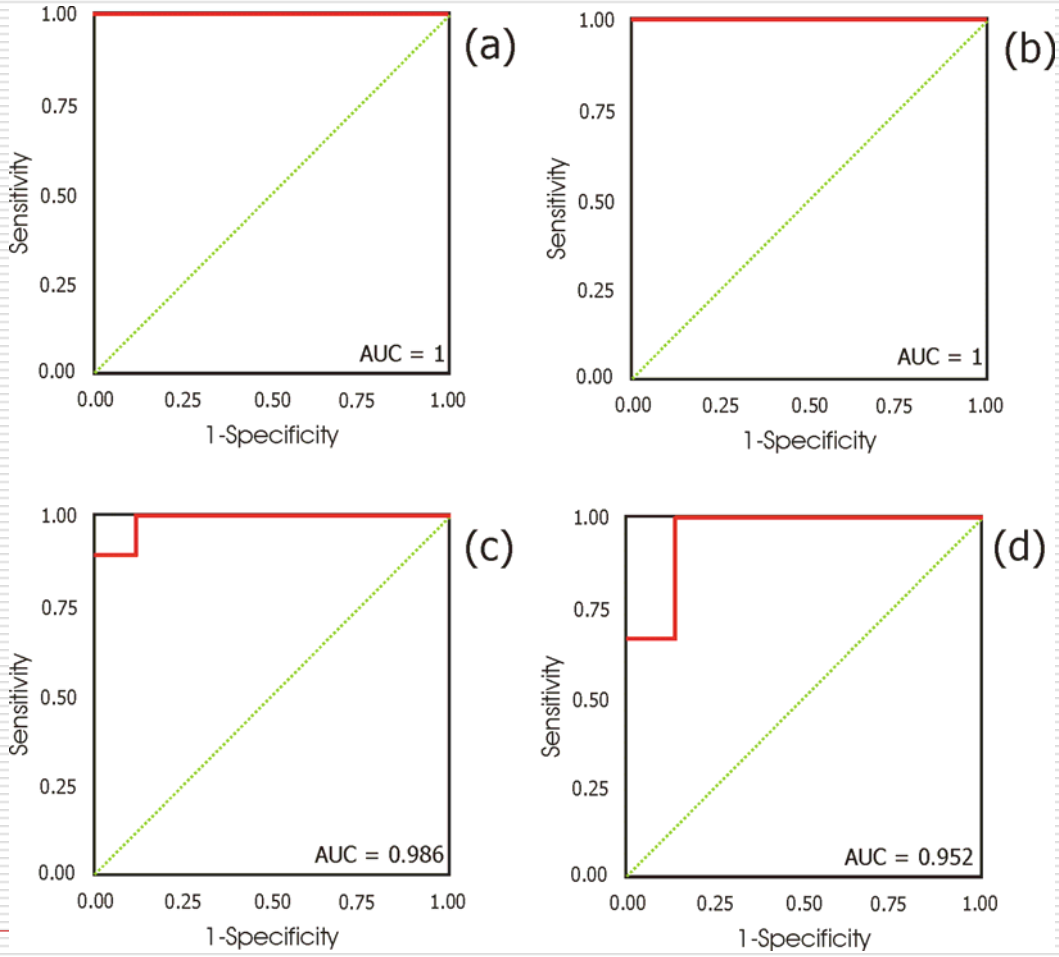
Combined scatter plot discriminating 48 sites in 29 patients using the R545/R575 ratio from 35 healthy volunteers

Rupananda Mallia et al., J. Biomedical Optics, 13(4), 041306, July/August 2008.

LIAF Vs DR spectroscopy for detection of pre-malignancies

Receiver Operating Characteristic (ROC) curve and area under curve (AUC) for classifying hyperplasia from dysplasia:
(a) F500/F635 (b) F500/F685
(c) F500/F705 (d) R545/R575

(a-c) represent the mean in vivo LIAF ratio of all sites in 43 patients, excluding DST, LST and VBL sits.
(d) represents mean in vivo DR ratio from all the sites in 29 patients



Diagnostic accuracies for R545/R575 ratio in vivo

Diagnostic Accuracies	Normal Vs Hyperplasia		Hyperplasia Vs Dysplasia		Dysplasia Vs SCC	
	All sites	Buccal	All sites	Buccal	All sites	Buccal
*Sensitivity (%)	70	97	100	100	91	96
*Specificity (%)	63	86	80	86	100	100
P value	<0.005	<0.001	<0.005	<0.01	<0.0005	<0.005

* Sensitivity= (Spectral abnormal/Histopathological abnormal)%

* Specificity= (Spectral normal/Histopathological normal)%

Where we stand?

Comparison of diagnostic accuracies achieved by different groups in discriminating hyperplastic from dysplastic tissues

Research Group	Spectroscopy/ Methodology	Measurement site	Sensitivity (%)	Specificity (%)
CESS	LIAFS, Spectral ratio reference standard (SRRS) using scatter plots (LDA)	Oral	100 (78)	100 (100)
CESS	DRS, HbO_2 absorption intensity ratios	Oral	100	86
Lovat et al, 2006	ESS, Classification by leave one out and block validation statistical approach.	Esophagus	79	79
Anjan Dhar et al, 2006	ESS, Statistically validated model using PCA and linear discriminant analysis (LDA)	Colon	85	88
de Veld et al, 2005	LIAF and DRS, PCA with various classifiers	Oral	77	76
Feld et al, 2003	Tri-modal spectroscopy	Oral	64	90
Nordstrom et al, 2001	DRS and UV excited fluorescence, Multivariate algorithm	Cervical	77	76
Ge et al,1998	DRS, Pattern recognition algorithms including multiple linear regression (MLR), LDA, and back-propagating neural network (BNN)	Colon	89	75

Conclusions

- Results demonstrate that LIAF and DR spectroscopy has the ability to diagnose early stages (dysplasia) of oral cancer from benign tissues in vivo.
- LIAF-SRRS ratios F500/F635 and F500/F685 gave 100% sensitivity and specificity to discriminate oral pre-malignancies (except at VBL, LST and DST) in a blind test without the use of photosensitizers.
- DR-SRRSratio R545/R575 discriminates all oral cavity pre-malignancies (dysplasia) of all sites with 100% sensitivity and 80% specificity.
- The Spectral Ratio Reference Standard (SRRS) criteria developed based on LIAF and DR discerns various tissue types in short time for immediate follow up.
- DR spectroscopy being cost-effective and diagnoses all types of lesions, provides the best alternative for pre-cancer screening through community centers.

Potential Applications

- ***As an Optical biopsy tool:*** Head and Neck Cancer and Cervical cancer screening
- ***Fiber-optic endoscopy:*** The can be adapted and coupled to endoscopes for detection of malignancies of internal organs (colon, GI tract, stomach, etc).
- ***Adjunct in surgery:*** Assistance to determine tumor margins during surgical intervention.
- ***Lesion imaging :*** Multi-spectral fluorescence imaging with CCD camera can be used to generate a 2D map of the entire lesion.

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