All fiber optic multi-modality imaging endo-speckle-fluoroscope for disease diagnosis in body cavities

Sujatha Narayanan Unni

2005

Sujatha, N. U. (2005). All fiber optic multi-modality imaging endo-speckle-fluoroscope for disease diagnosis in body cavities. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/6409>

<https://doi.org/10.32657/10356/6409>

Nanyang Technological University

Downloaded on 26 Apr 2023 21:28:22 SGT

**ALL FIBER OPTIC MULTI-MODALITY IMAGING
ENDO-SPECKLE-FLUOROSCOPE
FOR DISEASE DIAGNOSIS IN BODY CAVITIES**

Sujatha Narayanan Unni

**School of Mechanical and Aerospace Engineering
Nanyang Technological University
Singapore**

2005

**ALL FIBER OPTIC MULTI-MODALITY IMAGING
ENDO-SPECKLE-FLUOROSCOPE
FOR DISEASE DIAGNOSIS IN BODY CAVITIES**

Sujatha Narayanan Unni

SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

A thesis submitted to the Nanyang Technological University
in fulfillment of the requirements for the degree of
Doctor of Philosophy

2005

ABSTRACT

Cancer growths found in body cavities, such as colon, are a leading cause of death all over world. Medical intra cavity inspection techniques facilitate the diagnosis of cancers and other diseases, found in the different body cavities, such as nasal cavity, esophagus, colon, bladder, rectum etc. The cancer in the colorectal region of the body, which represents one of the most common malignant tumors, has become the second leading cause of death around the world, requiring urgent attention for its diagnosis and follow-up treatment. Also, to optimize patient management, precise detection or exclusion of metastases as well as assessment of their number and extent is indispensable. As colon cancer is highly curable if detected at a very early stage, diagnostic procedures based on early detection of the same is having important relevance. This early stage of cancer development is indicated by the presence of polyps of very small size ($\approx$ mm) originating from the inner mucosal layer of the colon wall. The diagnostic techniques, which can identify small abnormalities such as polyp growth present at either the surface or subsurface of the mucosal layer, will be of much help in the early detection of cancer. The currently available methods for colon cancer diagnosis are still at the research and development stages within the context of early detection capability. The relevance and potential of the proposed research comes in this context.

To ensure a complete and reliable diagnosis of the colon cancer, a multi-modality endoscope named as “ENDO-SPECKLE-FLUOROSCOPE”, is proposed in this thesis, which operates

in three different modes of diagnosis namely normal imaging mode, speckle correlation mode and fluorescence spectrum analysis mode. Normal imaging mode is useful in detecting polyps of visible size from the mucosal surface. Speckle correlation mode enables detection of polyps / abnormalities of very small size ($\approx 1\text{mm}$) from the surface /subsurface of the colon mucosa. Since all of the detected growths may not be cancerous, a distinction between cancerous and non-cancerous growths is made using the fluorescence spectrum analysis mode. This is done by looking at the autofluorescence spectrum collected from the colon. The growth in the colon with cancerous tissue has less fluorophore contents and hence only reduced fluorescence intensity can be observed as compared to that of a normal tissue.

An all fiberoptic based approach for the illumination and imaging of the colon wall is adopted here for making the proposed multi-modality endoscope and its associated system more compact. This design also helps for allowing maximum flexibility for the endoscope in order to provide increased accessibility inside the colon pathway. The concept of dual illumination / excitation channels employing a single laser source is presented here. A novel image collection unit, consisting of ball lens (imaging element) - image fiber bundle (image guiding element) - objective lens (collection element), is employed in the endoscope design. The imaging scheme adopted allows all the detection electronics to remain outside the body, thus ensuring the easy switching between diagnostic modalities. The switching between various imaging modes by introducing an optical component selection unit and associated detection systems is given in detail.

The thesis contains the detailed research work carried out on various theoretical aspects regarding the interaction between the proposed endo-speckle fluoroscope and the curved colon surface during different modes of diagnosis. In speckle correlation mode, the established speckle fringe formation equations for flat surfaces are modified in order to

apply in the case of curved inner surfaces. The problems of illumination beam divergence and the effect of image fiber in transmitting the speckle patterns are carefully studied to employ in endoscopic applications and modifications in the fringe equations are done accordingly. The working of the probe in the fluorescence spectrum analysis mode is carefully analyzed and a theoretical analysis is presented to optimize different parameters related to excitation and collection of emitted fluorescence from the colon, using the developed probe.

The experimental results with the endo-speckle fluoroscope in different imaging modes and their analyses involving normal and abnormality introduced colon phantoms (depicting cancerous colon) as the test specimens are presented. The diagnosis of cancer by observing the normal images, speckle fringes, and the fluorescence spectrum obtained from normal as well as cancerous colon is illustrated. Such an approach with multiple modality imaging, as proposed in this thesis, can facilitate the early diagnosis of cancer growth as well as it may have the potential of limiting the need for current biopsy approaches in the long run.

The potential applications of the proposed system are not just limited to medical uses, *in vitro* and *in vivo*, but also can be extended for engineering intra cavity inspection such as the evaluation of pressure vessels, some illustrations of which are given in the appendix. Suggestions for extending the applications of the proposed endoscope system by incorporating additional elements are mentioned as future research guidelines at the end of the thesis.

ACKNOWLEDGEMENT

- ❖ First and foremost, I would like to express my sincere thanks and appreciation to my supervisor, Assistant Professor V. M. Murukeshan for his continuous guidance, suggestion, support and constant encouragement throughout the course of this research work.
- ❖ I wish to extend my sincere thanks to Associate Professors Dr. Ong Lin Seng, Dr. Seah Leong Keey and Prof. Anand Krishna Asundi for being a constant source of inspiration and encouragement during discussions of the project work.
- ❖ I would like to express my thanks and appreciation to Associate Professor Dr. Lawrence Ho (Endoscopic surgeon of National University Hospital) who is collaborating with this project for sharing his experience and knowledge in this field.
- ❖ I sincerely appreciate and extend my thanks to Ast/P Dr. Suresh and Dr. Sindhu Swaminathan (Biochemistry lab, NUS) for their help during the preparation of the test sample.

Acknowledgement

- ❖ I am also thankful to the lab staff of Photonics and Microsciences Lab, SOM lab, RRC, Tissue Engineering Lab, CNC lab and Materials Lab-A for arranging the lab facilities for the project work.
- ❖ I acknowledge gratefully the financial support from the university for the project and for my research studentship.
- ❖ I am deeply indebted to my family for giving their blessings and support for my higher studies.
- ❖ Finally, many thanks to my friends in the research circle for their help and encouragement during the course of the project.

CONTENTS

ABSTRACT	I
ACKNOWLEDGEMENT	IV
CONTENTS	VI
LIST OF FIGURES	XI
LIST OF TABLES	XVII
LIST OF SYMBOLS	XVIII
LIST OF ABBREVIATIONS	XXIII

CHAPTER 1 INTRODUCTION 1

1.1 Motivation.....	1
1.2 Colon Cancer: A brief history.....	2
1.3 Limitations of current diagnostic procedures.....	5
1.4 The project objectives.....	6
1.5 The project scope.....	7
1.6 Organization of the report.....	11

CHAPTER 2 LITERATURE REVIEW 14

2.1 Classification of currently available colon cancer diagnostic methods.....	15
2.1.1 Diagnostic methods based on external imaging techniques.....	15
2.1.2 Diagnostic methods based on internal imaging techniques.....	16
2.1.3 Medical endoscopic procedures.....	17

2.1.3.1	Classification of endoscopes.....	18
2.1.3.2	White light endoscopy for colon cancer diagnosis.....	23
2.1.3.3	Endoscopic techniques based on indirect imaging for colon cancer diagnosis.....	24
2.2	Speckle techniques.....	26
2.2.1	The speckle effect	26
2.2.2	Speckle pattern.....	26
2.2.2.1	Objective speckle pattern.....	27
2.2.2.2	Subjective speckle pattern.....	27
2.2.3	Speckle correlation analysis	28
2.2.4	Speckle photography.....	28
2.2.5	Speckle intrefrometry.....	28
2.2.6	Electronic / Digital Speckle Pattern Interferometry (ESPI / DSPI).....	29
2.2.6.1	DSPI configuration.....	29
2.2.6.2	Different basic configurations of DSPI.....	31
2.2.7	Digital speckle pattern shearing interferometry (DSPSI).....	32
2.2.8	DSPI and Shearography for NDT.....	33
2.2.9	Applications of speckle techniques.....	34
2.2.10	Speckle methods for biomedical applications.....	34
2.3	Fluorescence spectroscopy and cancer diagnosis.....	37
2.3.1	The Fluorescence phenomenon.....	37
2.3.2	Fluorescence spectroscopy in cancer detection	40
2.3.3	Fluorescence spectrum collection and analysis for colonic tissues: devices and methods.....	45

2.3.4	Different endoscope configurations for fluorescence analysis.....	45
2.3.5	Monochromators and spectrometers.....	50
2.3.6	Detectors.....	51
 CHAPTER 3 INTRACAVITY INVESTIGATIONS USING SPECKLE		
INTERFEROMETRY		53
3.1	Speckle pattern interferometry for curved surfaces	53
3.2	DSPI fringe formation on curved phantom tissue surfaces – modified theory and experimental analysis	55
3.3	DSPI configurations for curved surfaces	59
3.3.1	In-plane configuration	60
3.3.2	Out-of-plane configuration	64
3.3.3	Shear configuration.....	67
3.4	Effect of random loading in the interferometric fringes	71
3.5	Effect of illumination beam divergence.....	71
3.6	DSPI and Shearography for NDT of curved tissue surfaces.....	78
 CHAPTER 4 THE ENDO-SPECKLE-FLUOROSCOPE SYSTEM: DESIGN AND		
ANALYSIS.....		84
4.1	Design considerations of the E-S-F system	85
4.1.1	The E-S-F probe	86
4.1.1.1	The selection of source.....	88
4.1.1.2	Design aspects of the E-S-F probe distal end.....	88
4.1.1.3	Selection of image fiber.....	90
4.1.1.4	Selection of imaging lens.....	91

4.1.2	Selection of collection lens	92
4.1.3	The OCSU	92
4.1.5	Normal imaging and speckle correlation analysis system	93
4.1.6	Fluorescence spectrum analysis system.....	93
4.2	Preparation of the phantom colon tissue model	94
4.3	E-S-F system in the normal imaging modality	96
4.4	E-S-F system in the speckle correlation modality	97
4.4.1	Image fiber effect in the visibility of correlation fringes obtained using the E-S-F system	97
4.4.2	Concept of divergent illumination beams in the E-S-F system.....	101
4.4.3	E-S-F system in the in-plane configuration	102
4.4.4	E-S-F system in the out-of-plane configuration.....	108
4.4.5	E-S-F system in the shear configuration.....	111
4.5	E-S-F system in the fluorescence spectrum analysis modality.....	115
4.6	Simultaneous operation of multiple diagnostic modalities with the E-S-F system.....	127
CHAPTER 5	CONCLUSIONS	130
5.1	Important contributions.....	130
5.2	Main contributions and conclusions	135
CHAPTER 6	FUTURE RESEARCH DIRECTIONS	137
6.1	Steering and locomotion aspects of the endo-speckle-fluoroscope	137
6.2	Addition of auxiliary channel in the endo-speckle-fluoroscope	138
6.3	Miniaturization of the entire system	139
6.4	Enhancement of diagnostic accuracy	139
6.5	Application of the developed system in clinical environments	140

6.6	Extension of application areas	141
APPENDICES.....		142
APPENDIX A	EDC 2000N COMPUTER CAMERA SPECIFICATIONS	143
APPENDIX B	PHANTOM TISSUE MECHANICAL PROPERTIES	144
APPENDIX C	DSPI INVESTIGATIONS ON CURVED TECHNICAL SPECIMENS	146
APPENDIX D	EFFECT OF RANDOM LOADING IN DSPI	150
	INVESTIGATIONS ON CURVED TECHNICAL SPECIMENS	
APPENDIX E	COMPARISON OF FRINGE PATTERNS WITH DIVERGENT AND COLLIMATED ILLUMINATION UNDER SIMILAR LOADING CONDITIONS	153
APPENDIX F	NON-DESTRUCTIVE TESTING OF TECHNICAL CAVITY SURFACES USING DIGITAL SPECKLE SHEAROGRAPHY	159
APPENDIX G	DONGWOO OPTRON DM150 MONOCHROMATOR SPECIFICATIONS	164
APPENDIX H	SPECIFICATIONS OF PMT (R928)	165
APPENDIX I	PHOTOGRAPH OF THE PHANTOM COLON MODEL	166
APPENDIX J	STAINING PROCEDURES FOR THE PHANTOM TISSUE	167
APPENDIX K	PHOTOGRAPHS OF THE PHANTOM TISSUE	168
APPENDIX L	EXPERIMENTAL SETUP FOR E-S-F SYSTEM (OUT-OF-PLANE CONFIGURATION)	169
APPENDIX M	EXPERIMENTAL SETUP FOR E-S-F SYSTEM (SHEAR CONFIGURATION)	170
APPENDIX N	EXPERIMENTAL SETUP FOR E-S-F SYSTEM (FLUORESCENCE SPECTRUM ANALYSIS MODALITY)	171
LIST OF ORIGINAL PUBLICATIONS.....		172
LIST OF REFERENCES.....		175

LIST OF FIGURES

FIGURE NO.	DESCRIPTION	PAGE NO.
Figure 1.1	The colon image	3
Figure 1.2	Schematic diagram representing various stages of colon cancer development	4
Figure 1.3	The block diagram representation of the multi-modality imaging methodology	10
Figure 2.1	Typical rigid endoscope	18
Figure 2.2	Schematic diagram of an electronic video endoscope	21
Figure 2.3	Schematic diagram of a fiberscope	23
Figure 2.4	Schematic of speckle pattern	27
Figure 2.5	Basic constituents of a DSPI system	29
Figure 2.6	Schematic of basic DSPI configurations	32
Figure 2.7	Energy level diagram illustrating the phenomena of absorption and fluorescence of a molecule	38
Figure 2.8	Fluorescence spectrum for excitation and emission	39
Figure 2.9	Fluorescence analysis with single fiber excitation and emission collection	46
Figure 2.10	Fluorescence analysis with single fiber excitation and multiple fibers for emission collection	47
Figure 2.11	Fluorescence analysis with excitation and emission	48

	collection using fiber bundles	
Figure 2.12	Schematic drawing of a monochromator	51
Figure 3.1	Representation of maximum point of displacement in the curved object surface	57
Figure 3.2	Different displacement components for a cylindrical specimen	58
Figure 3.3	Experimental setup for in-plane displacement analysis of curved phantom tissue	60
Figure 3.4	In-plane displacement contours for a cylindrical tissue phantom specimen	63
Figure 3.5	In-plane displacement contours for a rectangular tissue phantom specimen	63
Figure 3.6	Experimental setup for out-of-plane displacement analysis of curved phantom tissue	64
Figure 3.7	Out-of-plane displacement contours for a cylindrical tissue phantom specimen	65
Figure 3.8	Out-of-plane displacement contours for a rectangular tissue phantom specimen	66
Figure 3.9	Experimental setup for out-of-plane displacement derivative analysis of curved phantom tissue	67
Figure 3.10	Representation of sheared points in the object plane	68
Figure 3.11	Displacement derivative contours for a cylindrical tissue phantom specimen	69
Figure 3.12	Displacement derivative contours for a rectangular tissue phantom specimen	70

List of figures

Figure 3.13	Representation of angle of divergence in a spherical wavefront	72
Figure 3.14	Interrogation of a divergent illumination beam with a flat object surface	74
Figure 3.15	Interrogation of spherical divergent beam with curved object surface	76
Figure 3.16	In-plane displacement contours for the curved abnormal phantom tissue specimen	80
Figure 3.17	Out-of-plane displacement contours for the curved abnormal phantom tissue specimen	81
Figure 3.18	Out-of-plane displacement derivative contours for the curved abnormal phantom tissue specimen	82
Figure 4.1	Schematic of the E-S-F system	85
Figure 4.2	The E-S-F probe	87
Figure 4.3	Dimensions of the endoscope probe distal end	89
Figure 4.4	End face of the image fiber bundle (Fujikura FIGH-15-600N)	91
Figure 4.5	Effect of imaging lens diameter in the transmitted USAF resolution chart image	92
Figure 4.6	Schematic of the OCSU	93
Figure 4.7	Images of the colon wall obtained using the E-S-F system in the normal imaging modality	96
Figure 4.8	Schematic representation of object points as imaged by image fiber pixels	100
Figure 4.9	E-S-F system in the in-plane configuration	102

Figure 4.10	Interrogation of E-S-F probe diverging illumination beams with curved colon surface	104
Figure 4.11	In-plane fringes obtained from the normal phantom colon surface using the E-S-F system	107
Figure 4.12	In-plane fringes obtained from the cancerous phantom colon surface using the E-S-F system	107
Figure 4.13	E-S-F system in the out-of-plane configuration	108
Figure 4.14	Out-of-plane fringes obtained from the normal colon phantom model	110
Figure 4.15	Out-of-plane fringes obtained from the cancerous colon phantom model	110
Figure 4.16	E-S-F system in the shear configuration	111
Figure 4.17	Shear fringe pattern obtained for a normal colon phantom	112
Figure 4.18	Shear fringe patterns obtained for cancerous colon phantom	113
Figure 4.19	E-S-F probe distal end oriented normal to the colon wall	116
Figure 4.20	Interaction of colon tissue with laser excitation	120
Figure 4.21	Relationship between the total number of collected photons from the probe and the distance of the probe from the tissue surface	124
Figure 4.22	Relation between the normalized value of collected number of photons and the distance of the tissue	125

	surface points from the optical axis	
Figure 4.23	E-S-F system in the fluorescence spectrum analysis modality	126
Figure 4.24	Excitation emission spectra collected using the E-S-F probe from simulated normal and cancerous tissues	127
Figure 4.25	E-S-F system operating in multiple diagnostic modalities	128

APPENDIX

Figure B. 1	Stress-Strain relationship for the phantom tissue	144
Figure B. 2	Relation between linear and lateral extensions of the phantom tissue	145
Figure C. 1	In-plane displacement contours for the half-cylindrical aluminium specimen	146
Figure C. 2	Out-of-plane displacement contours for the half-cylindrical aluminium specimen	148
Figure C. 3	Out-of-plane displacement derivative contours for the half-cylindrical aluminium specimen	149
Figure D. 1	Displacement contours for the half-cylindrical aluminium specimen with fixed side and bottom edges (applied displacement- $5\ \mu m$)	150
Figure D. 2	Displacement contours for the half-cylindrical aluminium specimen with fixed side and bottom edges (applied displacement- $10\ \mu m$ (out-of-plane) and $5\ \mu m$ (in-plane))	151
Figure D. 3	Displacement contours for the half-cylindrical aluminium specimen with fixed side and bottom edges (applied displacement- $5\ \mu m$ (out-of-plane) and $10\ \mu m$ (in-plane))	152

Figure E. 1	Out-of-plane fringe patterns employing divergent or collimated illumination beams at different applied loads (for flat specimen)	154
Figure E. 2	Relationship between fringe width and applied load while using divergent or collimated illumination beams (flat surface)	155
Figure E. 3	Out-of-plane fringe patterns employing divergent or collimated illumination beams at different applied loads (for curved specimen)	157
Figure E. 4	Relationship between fringe width and applied load while using divergent or collimated illumination beams (curved surface)	158
Figure F. 1	Displacement derivative contours obtained for a normal aluminium half- cylindrical specimen	159
Figure F. 2	Shearographic NDT on aluminium half-cylindrical specimen	160
Figure F. 3	Displacement derivative contours obtained for a normal aluminium-CFRP sandwiched half- cylindrical specimen	161
Figure F. 4	Shearographic NDT on aluminium-CFRP sandwiched half-cylindrical specimen with single defect	162
Figure F. 5	Shearographic NDT on aluminium-CFRP sandwiched half-cylindrical specimen with double defect	163
Figure I. 1	The phantom colon model	166
Figure K. 1	Normal phantom tissue stained with 1mg Rhodamine 6G in 10ml acetone	168
Figure K. 2	Phantom tissue with simulated cancerous growth (size 1mm) stained with 1mg Rhodamine 6G in 20ml acetone	168
Figure L. 1	Experimental setup for the E-S-F system to operate in the out-of-plane configuration	169
Figure M. 1	Experimental setup for the E-S-F system to operate in the shear configuration	170
Figure N. 1	Experimental setup for the E-S-F system to operate in the fluorescence spectrum analysis modality	171

LIST OF TABLES

TABLE NO.	DESCRIPTION	PAGE NO.
Table 2.1	Autofluorescence analysis of human colon tissue- a brief review of previous research	44
Table 4.1	Comparison of endoscopic DSPI configurations for abnormality detection	115
Table 4.2	Numerical values of the parameters used in the theoretical model for fluorescence analysis	123

LIST OF SYMBOLS

SYMBOL	DESCRIPTION	PAGE NO.
λ	Wavelength of illumination	28
L	Image distance from object to the observation plane	28
f	Focal length of the lens	28
a	Aperture of the lens	28
D_o	Diameter of the object	28
S_o	Ground electronic state in the energy level diagram	37
S_1	Excited electronic state in the energy level diagram	37
I_1	Intensity of interfering beam 1	55
I_2	Intensity of interfering beam 2	55
ϕ	Phase change between two interfering beams	55
$\Delta\phi$	Phase change due to object displacement	55
I	Resultant intensity	55
K	Constant of proportionality	56
V_s	Subtracted signal displayed on the monitor	56
B	Brightness of the fringes on the monitor	56
$\vec{L}$	Displacement vector for point P	57
w_r	Component of displacement in the radial direction	57
w_θ	Component of displacement in the tangential direction	57
w_a	Component of displacement in the axial direction	57
$\mathbf{e}_r$	Unit vector in the radial direction	57
$\mathbf{e}_\theta$	Unit vector in the tangential direction	57
$\mathbf{e}_a$	Unit vector in the axial direction	57

List of symbols

ds	Elemental displacement along the curved surface	58
$d\phi$	Angle between the tangents at points P and Q	58
W_T	Vector sum of three displacement components	58
v	Y-direction displacement of flat specimen	58
W	Resultant displacement along the curved trajectory of the specimen	59
K	Curvature of the curved object (cylinder)	59
$R(o)$	Radius of curvature of the curved object	59
$\vec{k}_1$	Direction vector of illumination 1	61
$\vec{k}_2$	Imaging vector	61
$\vec{k}_3$	Direction vector of illumination 2	61
θ_i	Angle of illumination (for collimated beam)	61
$\Delta\phi'$	Phase change due to object deformation caused by illumination 1 (in-plane configuration)	61
$\Delta\phi''$	Phase change due to object deformation caused by illumination 2 (in-plane configuration)	61
θ_{i1}	Angle of illumination for illumination 1 in non-symmetric in-plane configuration	62
θ_{i2}	Angle of illumination for illumination 2 in non-symmetric in-plane configuration	62
u	Flat specimen deformation component in the in-plane direction	66
w	Flat specimen deformation component in the out-of-plane direction	66
$\Delta\theta_{p0}$	Shear amount in the tangential direction	67
ϕ_1	Phase corresponding to point P	68
ϕ_2	Phase corresponding to point P'	68
$\Delta\phi_1$	Phase change due to object deformation of point P	68
$\Delta\phi_2$	Phase change due to object deformation of point P'	68
$\vec{L}'$	Deformation vector for point P'	68
M	Magnification of the imaging scheme	68
Δx_0	Shear amount in the x-direction	70
β	Divergence angle of the illumination beam	72
θ_n	Angle of illumination at W_n (point on the wavefront)	72

List of symbols

$R(w)$	Radius of curvature of the illumination beam	72
ds	Elemental distance between wavefront points	72
$d\theta$	Angular displacement between wavefront points	72
θ_{n-m}	Angle of divergence at the wavefront point W_{n-m}	73
θ'_n	Angle of illumination at the object point O_n	74
L	Distance between the object center and the beam center of curvature	74
C_w	Illumination beam center of curvature	74
θ'_{n-m}	Angle of illumination at the object point O_{n-m}	75
C_o	Object center of curvature	76
I''	Ensemble average of total intensity distribution	97
$\langle I \rangle$	Average intensity of the speckle fields before or after object deformation	98
μ	Normalized cross correlation function of the speckle field at detector plane before and after object deformation	98
$I''_{\min}$	Minimum resultant intensity at maximum correlation	98
$I''_{\max}$	Maximum resultant intensity at zero correlation	98
$\mu_{\max}$	Maximum cross correlation function of the speckle field at detector plane before and after object deformation	98
V	Visibility of fringes in the conventional DSPI configuration	98
$s(x)$	Intensity transmittance function of individual fiber in a fiber bundle	99
A	The effective core area of an elementary fiber	99
$I_o(X)$	The average intensity distribution at the output end of elementary fiber	99
$F(X)$	Aperture function across the output face of elementary fibers in the bundle	99
x_i	Elementary fiber position corresponding to object point position x_1	99
x_j	Elementary fiber position corresponding to object point position x_2	99
N	Number of elementary fibers / pixels of the image fiber	99
$\mu(X)$	Normalized cross correlation function of the speckle fields at the image fiber output before and after object displacement	100
V'	Visibility of fringes at the image fiber output	101
$\Delta\phi_{e-s-f(in)}$	Phase change due to object deformation calculated in the e-s-f probe system in in-plane configuration	103

List of symbols

$\theta_{fi(in)}$	Effective illumination angle at object point $\mathbf{O}_{n-m}$ (in-plane configuration)	103
w_{cma}	Phantom colon model deformation in the axial direction	103
l	Distance of illumination fiber center to image fiber center	104
C_{cm}	Center of curvature of the phantom colon model	104
C_{w1}	Centre of curvature of illumination beam 1 from the e-s-f probe	104
C_{w2}	Centre of curvature of illumination beam 2 from the e-s-f probe	104
r_1	Radial distance of illumination fiber 1 to tissue surface point $\mathbf{O}_{n-m}$	104
r_2	Radial distance of illumination fiber 2 to tissue surface point $\mathbf{O}_{n-m}$	104
C	Center of the e-s-f probe	104
$\theta'_{1(n-m)}$	Angle of illumination at $\mathbf{O}_{n-m}$ due to illumination 1	104
$\theta'_{2(n-m)}$	Angle of illumination at $\mathbf{O}_{n-m}$ due to illumination 2	104
$R_{cm}(o)$	Radius of curvature of the phantom colon model	105
$\Delta\phi_{e-s-f(out)}$	Phase change due to object deformation calculated in the e-s-f probe system out-of-plane configuration	109
$\theta_{fi(out)}$	Effective illumination angle at object point $\mathbf{O}_{n-m}$ (out-of-plane configuration)	109
W_{cm}	Resultant deformation along the curved trajectory of the phantom colon model	109
ds_{cm}	Elemental deformation along the curved surface of the phantom colon model	109
$\Delta\phi_{e-s-f(shear)}$	Phase change due to object deformation calculated in the e-s-f probe system in the shear configuration	112
P	Incident power of excitation 1 / Incident power of excitation 2	117
θ'_1	Angle of excitation at point $\mathbf{O}$ due to excitation 1	117
Δt	Integration time for excitation 1 / Integration time for excitation 2	117
Ω	Solid angle of excitation 1 / Solid angle of excitation 2	117
F_1	Incident fluence of excitation 1	117
d	Distance of the e-s-f probe from the colon surface	117
Δx	Vertical distance of colon surface point $\mathbf{O}$ from optic axis of the e-s-f probe	117
δ	Horizontal distance of colon surface point $\mathbf{O}$ from the colon surface point crossing optic axis	117

List of symbols

θ'_2	Angle of excitation at point O due to excitation 2	118
F_2	Incident fluence of excitation 2	118
γ	Sector angle including the colon surface point O and the colon surface point which crosses the probe axis	118
F	Total fluence delivered to the colon tissue	118
E_r	Return energy from the colon per pixel of the image fiber	119
η	Energy conversion efficiency of the colon tissue	119
C_L	Collection efficiency of the imaging lens	120
r_L	Effective radius of ball lens	120
r	Radial distance of ball lens center to the tissue surface point O	120
α	Collection angle of the imaging unit with respect to point O	120
i	Image distance of the ball lens	120
α_f	Half the collection angle of the image fiber	120
n	Refractive index of ball lens	121
D	Diameter of ball lens	121
E_c	Collected energy using the imaging unit per pixel of the image fiber	121
λ_e	Peak wavelength of emitted light	121
c	Velocity of light	121
h	Planck's constant	121
T_o	Transmission efficiency of the image fiber bundle	122
f_p	Image fiber bundle packing fraction	122
N_p	Number of photons collected by the endo-speckle fluoroscope probe per pixel of the image fiber	122
N_p	Total number of photons reaching the PMT	122

LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPLANATIONS	PAGE NO.
DSC	Digital speckle correlation	10
LIFS	Laser induced fluorescence spectroscopy	10
NDT	Non destructive testing	12
CT	Computed tomography	15
MRI	Magnetic resonance imaging	16
PET	Positron emission tomography	16
CCD	Charge coupled device	20
OCT	Optical coherence tomography	24
ESPI	Electronic speckle pattern interferometry	29
DSPI	Digital speckle pattern interferometry	29
PM	Phase modulation	30
DSPSI	Digital speckle pattern shearing interferometry	32
EEM	Excitation-emission matrix	39
ALA	5-aminolevulinic acid	40
PpIX	Protoporphyrin IX	40
UV	Ultraviolet	41
NADH	Nicotinamide adenine dinucleotide	41
CFM	Confocal microscopy	44
FI	Fluorescence intensity	44
S	Spectroscopy	44
I	Imaging	44
PA	Probability based algorithm	44
NA	Numerical aperture	47
RLD	Reciprocal linear dispersion	50
VIS	Visible	50

List of abbreviations

PMT	Photo multiplier tube	51
QE	Quantum efficiency	51
F	Optical fiber	60
FH	Fiber holder	60
CL	Collimating lens	60
FEA	Finite element analysis	62
FEM	Finite element modeling	62
BC	Beam combiner	64
CFRP	Carbon fiber reinforced plastics	78
E-S-F	Endo-speckle-fluoroscope	84
OCSU	Optical component selection unit	85
FS	Finger splice	86
USAF	United states air force target resolution chart	91
THF	Tetra hydro furan	95
ND	Neutral density	109

CHAPTER 1

INTRODUCTION

The chapter begins with a motivation for undertaking this project followed by a brief review, on the colon cancer and its various stages of development, which will be discussed in detail. A short note on the limitations of current diagnostic methods will be given followed by the new proposed method, which is capable of subduing the current diagnostic limitations. The scope and objectives of this thesis will be discussed followed by a block diagram representation of the whole research methodology adopted for the project. The chapter concludes with the organization of the whole thesis.

1.1 Motivation

Colon cancer is the second leading cause of death around the world, especially in United States and Singapore and its incidence is increasing very rapidly with about 1000 new cases diagnosed annually in Singapore [1, 2]. In 2004, an estimated 106370 new cases will be diagnosed in the United States [3]. Reports from all over the world support the need for a fruitful diagnostic method in the very early stages of its development, as this particular type

of cancer is completely curable if detected early [1,2]. None of the currently available diagnostic methods are able to detect the presence of cancer at a very early stage. The important limitations of the current diagnostic methods are given in section 1.3 of this chapter.

The main motivation for this project idea was the prevailing situation of colon cancer in the world and the limitations of present diagnostic tools for early detection of the same, with which it is completely curable.

1.2 Colon Cancer: A brief history

Colon cancer is the cancer found in the colon. Like all forms of cancer, colon cancer begins to develop when cells start dividing uncontrollably. The cells then form tumors that can spread to other parts of the body [4, 5]. Colon cancer is completely curable if detected at the very early stage.

How early is early?

Most colon cancers arise from small growths called polyps. Polyps can be divided into two, (1) benign or hyperplastic (2) adenomatous [4, 5]. Benign polyps of size of the order of millimeters are not generally dangerous, but may develop into adenomatous as years pass by and once they are adenomatous, can be dangerous, depending largely on size. At this stage, polyps are referred to as tumors and should be removed and sent to the pathology lab for study. The diagram showing the presence of a polyp and tumor inside the colon wall is given below (Fig. 1.1) [6].

Fig. 1.1 The colon image

The colon wall consists of 4 tissue layers. The severity of the cancer is described by different stages. There are five different stages for the development of colon cancer [7]. A schematic representation of various stages of cancer development is shown in Fig. 1.2. When the cancer is confined to the inner layer of the colon, it is called stage 0 or carcinoma in situ (Fig. 1.2 (a)). The survival rate at this stage is greater than 90%. When the cancer is grown through the inner lining of the colon and underlying layers, but is not penetrated beyond the colon wall into adjoining tissue, it is called stage 1 (Fig. 1.2 (b)). The survival rate at this stage is about 70-85%. When cancer is grown through the colon wall but hasn't spread to nearby lymph nodes, the condition is depicted as stage 2 (Fig. 1.2 (c)). The survival rate is about 55-65%. In the next stage (stage 3) as shown in Fig. 1.2 (d), cancer is spread from the colon to nearby lymph nodes but not to other parts of the body. The survival rate is 20-30%. In the last stage (stage 4), as shown in Fig. 1.3 (e), cancer is spread to distant organs. Most common sites for metastasis are the liver, lungs and brain. The survival rate is around 1%.

Fig. 1.2 Schematic diagram representing various stages of colon cancer development

Early detection of colon cancer refers to the detection of polyps when they are of size of millimeter order. Some polyps of these sizes may not grow cancerous, but it is advisable to check for its growth once detected. In the case of flat abnormalities, the thickness of the growth may be even small, but it represents a worse scenario that requires urgent attention. Also for polyps that grow from the inner layer to the outer colon layers (polyps present in the subsurface colon layers); the diagnosis is difficult using the current techniques. A detailed description of the limitations of the currently available diagnostic techniques is given in the next section.

1.3 Limitations of current diagnostic procedures

The diagnosis of colon cancer is based on minimally invasive procedures, which are becoming central to medicine, both improving results and reducing recovery time and costs. The currently available methods make use of external or internal imaging techniques. A detailed description of the commonly used imaging technologies for the colon cancer diagnosis is given in section 2.1 of Chapter 2. Internal imaging techniques make use of flexible endoscopic technologies and provide the most accurate method among the various minimally invasive diagnostic procedures. All the current methods facilitate single imaging modality, i.e., the incorporation of a single technique for the disease diagnosis. Electronic video endoscope, which is used as the standard tool for colon cancer diagnosis, is having a disadvantage that all the detection electronics goes inside the body. So, in case of any changes required, each time the instrument is to be taken out of the body. Another major drawback of the current diagnostic methods is that, they cannot provide the disease diagnosis at a very early stage. Also, they cannot provide accurate diagnostic results when the polyps are in a subsurface layer or when polyps start growing from inner to the outer colon wall layers. Colon cancer is completely curable if detected very early and hence the need for a

fruitful diagnostic method for the above-mentioned cases is of much research interest and potential. This can make an effective diagnosis of the same at a very early stage where the currently available methods fail.

Incorporation of two or more modalities in a single endoscope will definitely increase the diagnostic accuracy and will provide more favorable results. In this context, this research project focuses on the development of a multiple modality endoscope system for the disease diagnosis. A detailed description regarding the objectives and scope of the project is given in the next section.

1.4 The project objectives

In view of the prevailing situation of colon cancer, an early detection technique for the cancer diagnosis is proposed in this thesis, which can make the disease diagnosis possible at the beginning stage of the polyp or growth itself thus ensuring maximum survival rate. To achieve this objective, the concept for an all fiber optic endoscope system named as “end-speckle-fluoroscope system” is realized and designed to work in different imaging modalities. Besides the experimental analysis using the designed probe in colon models, the project also focuses on the development of necessary theoretical formulation and subsequent analysis for each of the imaging modalities. Some of the theoretical expressions derived here are applicable to all kinds of cavity structures such as medical and technical cavities. The designed diagnostic tool and novel concepts and methodology are employed to interrogate both the normal and abnormal colon phantom models (resembling the properties of real colon) in order to validate the usage of the probe in biological cavities for disease diagnosis. The main research objectives are listed below.

The main objectives of the project can be summarized as:

1. Investigations into the research and development of an all fiber optic illumination and multiple modality imaging based endoscopic diagnostic methodology and concepts for cancer diagnosis in body cavities specifically, human colon.
2. Development of theoretical formulation and subsequent analysis for various imaging modalities so as to apply the probe optics concepts in body cavities.
3. Design and fabrication of the endo-speckle-fluoroscope probe for illumination and imaging as per the proposed concepts (mentioned in objective number: 1), which can work in three modalities such as normal imaging, speckle correlation analysis and fluorescence spectrum analysis.

The scope of the whole project to achieve these desired objectives is described below.

1.5 The project scope

The early stage for colon cancer is represented by an unusual growth of cells, resulting the thickening of the colon tissue wall, indicating a difference in elasticity between normal and abnormal tissue parts. Also, there will be a decrease in the emitted fluorescence intensity from the natural fluorophores present in the cancerous colon tissue layers compared to a healthy one. The most important part of the proposed system configuration is the design and development of an endoscope probe, which can work in multiple modalities, utilizing the above mentioned difference in properties between normal and cancerous colon. This approach facilitates normal imaging, surface and subsurface abnormality detection as well as the tissue fluorescence spectroscopy. In the normal imaging modality, surface abnormalities

of visible size can be imaged as in a normal colonoscopic procedure. While white light is used as the illumination source for currently employed endoscopic schemes or systems, the illumination source used in the proposed scheme will be the coherent laser. The selection of the single source is critical and is made in order to incorporate all the three modalities of diagnosis and at the same time to provide a simplified setup. Laser speckle correlation is proposed as the technique for abnormality detection from surface and subsurface layers of the colon wall, by locating the speckle fringe anomaly due to the tissue elasticity difference. This gives a promising way to detect the abnormalities at the very beginning stage. Moreover certain polyps, which grow only into the outer layers of colon wall and do not show any growth inside the colon under normal colonoscopy examination, can also be detected by the introduction of the proposed technique with its subsurface defect or abnormality detection capability. The designed probe is capable of analyzing tissue autofluorescence spectrum also, whereby the presence of cancer can be confirmed, if any. Here the potentials of the use of laser are exploited for providing illumination for normal imaging, biospeckle (here biospeckle is referred to the laser induced speckle pattern formed at the biological surfaces, such as colon) generation and for providing necessary excitation for tissue autofluorescence generation. Since the existing biopsy collection and examination methods are really harmful to the tissues causing injuries, a non-destructive evaluation of them as proposed here would be of great diagnostic value.

The scope of the project can thus be summarized as:

1. Extended theoretical formulation of in-plane, out-of-plane and out-of-plane shear speckle fringe formation for curved inner (such as inner cylindrical) surfaces.
2. Development of a theoretical model to determine the interrelationship between various parameters affecting the collected fluorescence intensity using the designed probe.
3. Experimental investigations involving different configurations of speckle interferometry in biological cavities, and the comparison of results with the theoretical formulation and corresponding finite element models.
4. Design and fabrication of endoscope probe for multiple-modality imaging.
5. Studies on the effect of illumination beam divergence in endoscopic speckle correlation interferometry.
6. Experimental investigations with the designed and fabricated probe on normal and abnormal phantom colon models for early detection of colon cancer using the incorporated imaging modalities.

A block diagram describing the technical R & D approach on the proposed imaging methodology is given below (Fig. 1.3). The different techniques adopted for the development of the final diagnostic tool are listed here, describing the various steps carried out in between.

Fig. 1.3 The block diagram representation of the multi-modality imaging methodology

The key element in the proposed minimally invasive system for early colon cancer diagnosis is the multi-modality endoscope. The three main functions that can be performed by the endoscope is normal imaging for detection of abnormalities of visible size, non-destructive inspection of colon inner surface or subsurface for detection of early abnormalities (of size~mm) and the biochemistry analysis of the colon tissue. The subsurface detection modality makes use of digital speckle correlation technique, where as the biochemistry analysis modality makes use of the fluorescence spectrum analysis technique. Another important aspect in the project development is the theoretical analysis for the endoscopic applications of DSC and LIFS techniques. A detailed analysis of various stages of the

project hierarchy structure (shown in Fig. 1.3) and related issues are given in Chapter 4 of this thesis. The organization of the whole thesis is detailed below.

1.6 Organization of the report

The whole thesis is divided into six chapters. Each chapter begins with a short note, which reflects on the main contents of that chapter.

Chapter 1 is an introduction chapter, which gives a brief overview about the present status of the problem. The main motivation, scopes and objectives of the project are discussed here. A summary regarding the limitations of current diagnostic methods for colon cancer is given emphasizing the need for a fruitful diagnostic system subduing the current limitations. A block diagram representation of the proposed project explaining its methodologies is presented. The organization of the whole thesis is given in the last section of the chapter.

Literature review regarding the current colon cancer diagnostic methods and the methodologies are given in **Chapter 2**. Speckle concepts and different configurations of the correlation interferometry are summarized here. Review of previous works on the applicability of speckle techniques in biomedical area is discussed. Beginning with a brief theory of fluorescence, a detailed literature survey regarding the applicability of fluorescence concept in cancer detection of various parts of the body, specifically colon is given. Autofluorescence techniques are mentioned and compared with that of photodynamic therapy techniques. Different types of endoscope probes, previously reported in literature, for their use in fluorescence spectroscopy are discussed and their limitations are presented. The proposal for the novel endoscope system subduing the limitations of previously mentioned configurations is given. A brief review of the instrumentation aspects for fluorescence spectroscopy is given towards the end of the chapter.

In **Chapter 3**, speckle interferometry techniques for deformation analysis as well as non-destructive testing (NDT) of curved surfaces are introduced. Theoretical and experimental investigations on curved biological surfaces are investigated, employing curved phantom tissue surfaces as the test specimens. Fringe formation equations for in-plane, out-of-plane and shear configurations are formulated and compared with the experimentally obtained results. The experimental fringes are also compared with the simulated displacement and displacement derivative contours generated using finite element analysis. Modifications in the fringe formation theory while involving a divergent beam illumination in speckle interferometric configurations are presented.

In **Chapter 4**, different design aspects of the proposed “Endo-speckle-fluoroscope” which can work in multiple modalities such as normal imaging, speckle correlation analysis and fluorescence spectrum analysis are given. Selection criteria of different constituent elements of the endoscope system are given. Methods of simulation of abnormalities and fluorophores in a phantom model of colon are described. The results of experimental investigations on the phantom model using the multiple modality endo-speckle fluoroscope are presented and analysed for each of the modalities. In speckle correlation modality, the image fiber effect in the transmission of speckle pattern is described. Modifications in the fringe formation equations are carried out in accordance with the orientation of illumination fibers at the endoscope distal end. A theoretical analysis on the fluorescence emission collection from the curved colon surface using the proposed endo-speckle-fluoroscope is given.

A conclusion for the whole thesis is given in **Chapter 5**, which describes the important original contributions made during the whole research work. All the theoretical and experimental works related to the proposed research for fulfilling the desired objectives are concluded here.

Chapter 6 is the last chapter of the thesis, which contains the guidelines for future research. Recommendations for modifications in the probe design, in order to apply the proposed system for real clinical environments are given. Also, certain suggestions are provided for extending the application areas of the proposed system by incorporating additional elements.

Although the main contents of the thesis are aiming at the biomedical applications of the proposed system, the application areas can be extended to the nondestructive testing of technical cavities, such as pipes and pressure vessels, employing the speckle correlation concepts. Most of the speckle concepts are analyzed initially using known standard technical specimens, for proof of concept and for the validation of theoretical formulations. Investigations from this perspective are carried out as part of the project, and the results are given in the appendix section, which is provided at the end of the thesis.

As evident from the scope of the project, the research work will be mainly concentrating on the proposed flexible endo-speckle-fluoroscope, which can work in three different modalities to ensure the early diagnosis of colon cancer and which could overcome the limitations of current methods to provide a more accurate disease diagnosis. Next few chapters of the report will be dealing with a comprehensive review and analysis of the techniques incorporated in the proposed system design such as speckle correlation techniques and fluorescence spectrum analysis for curved biological pathways, such as colon.

CHAPTER 2

LITERATURE REVIEW

This chapter on the literature review will be divided into three main sections A, B and C based on the previous works done in related areas, which are relevant to the proposed project. The first section will be starting with a brief review of various endoscopic methods, which are developed previously / in clinical use / in the research stage for cancer detection in the colon path. The second section on speckle interferometry will be giving an understanding on the concept of speckles, a brief outlook regarding different interferometric configurations of speckle correlation and their non-destructive testing applications. The third and last section of this chapter will be dealing with the early works of spectroscopic techniques for cancer diagnosis, in various parts in the human body in vivo and in vitro. The difference between photodynamic diagnosis and autofluorescence analysis will be analyzed. The excitation and emission wavelength range of different endogenous fluorophores for the autofluorescence analysis of the colon tissue will be illustrated in this chapter. Finally, a brief description on the instrumentation aspects of fluorescence spectrum analysis will be given.

SECTION - A

2.1 Classification of currently available colon cancer diagnostic methods

The currently available methods for the colon cancer diagnosis are classified into two based on the methods adopted for imaging. Some diagnostic methods make use of external imaging means while some others are based on internal imaging means. Some screening tests are also available to diagnose the presence of cancer by detecting the blood traces in the stool samples (Fecal Occult Blood Test) [8]. The disadvantage of this method is that the blood traces in the stool samples can also be caused by other gastric diseases or wounds and so the diagnosis of cancer made by the method need not be accurate.

2.1.1 Diagnostic methods based on external imaging techniques:

- ***Virtual colonoscopy* [9]:** A non-invasive examination of the colon, where the colon is inflated with air and the patient is imaged in a CT scanner. It is then possible to generate a similar view to the endoscope by using virtual reality techniques to fly through the resulting 3-dimensional image
- ***Barium X-ray* [10]:** This technique is based on inflating the colon with radioactive barium and then subjecting the patient to x-ray examination. From the x-ray report, the presence of abnormalities is found out.
- ***Computed Tomography (CT)* [11]:** This technique involves the inflation of the colon with air, succeeded by the x-ray examination using a CT scanner. The

abnormalities in the colon wall (if any) will be detected by the looking at the x-ray report.

- ***Magnetic Resonance Imaging (MRI) [12]:*** The patient will be allowed to lay down inside a huge magnet with strong magnetic field and radio waves of very high intensity are allowed to pass through the patient. The nuclei of hydrogen atoms inside the water content in the body tissues will align in a particular direction in the presence of this magnetic field, and this will give rise to picture of the tissue wall structure, from which the presence of abnormalities is found out.
- ***Positron emission tomography (PET) [13]:*** Here, some radioactive elements are injected into the patient's body and due to their reaction with the electrons of the atoms inside the body, positrons are emitted. The positrons are captured by a scanner placed outside the body and thus forming a pictorial representation of the examined cavity structure from which the presence of abnormalities is found out.

2.1.2 Diagnostic methods based on internal imaging techniques

All the techniques based on the external imaging means as described above, suffer from the disadvantage of missing polyp [1, 12], i.e., the percentage chance of detection of abnormalities is low. Since they are not based on the real internal view of the cavity, the abnormalities often detected may not be the actual polyps, but can be some other protrusions. Virtual colonoscopy has a bigger risk of failing to identify precancerous polyps. Techniques such as MRI are more efficient in detecting distant metastases, in other organs such as liver [14]. Moreover, CT, Barium-x ray and PET involve inspection with x-rays,

which can be harmful to the body tissues. Barium x-ray and PET suffer from the additional disadvantages of the intake of radioactive elements, which is also not advisable. Also, the sensitivity of Barium Enema is far from optimal and the examination procedure is uncomfortable for both the patient and the examiner.

For all the above reasons, for a more accurate cancer diagnosis, it is critical to have internal view provided by an endoscope. Some of the endoscopic procedures (ultrasound [15] and optical coherence tomography [16]) make use of indirect imaging means, while some others (sigmoidoscopy [17] and colonoscopy [18]) make use of direct visualization of the inner cavity wall. A review on types and classification of endoscopes is given in the next section, followed by a brief description of endoscopic methods used in this field of colon cancer diagnosis.

2.1.3 Medical endoscopic procedures

Endoscopy is a very promising and advanced technique used for the visualization of technical or medical intra cavities [19-23] through natural body openings. The name endoscope is derived from two Greek words, which are *endom* (within) and *skopein* (view) [24]. The endoscope is an optical instrument used for viewing internal organs through natural openings (ear, throat, rectum, etc.) or through a small incision in the skin. Usual endoscopic procedures involve the intrusion of an endoscope into the cavity under inspection. Medical endoscopy has got a variety of applications over different parts of the body [19 -23], such as laparoscopy, laryngoscopy, rhinoscopy, pharyngoscopy, bronchoscopy, gastroscopy, arthroscopy, sinuscopy, cystoscopy, hysteroscopy, sigmoidoscopy and colonoscopy.

2.1.3.1 Classification of endoscopes

Most endoscopes are divided into three distinct subsystems: the objective, the relay system and the eyepiece. Of the three, the relay system, which starts from the tip of the endoscope to transfer the image and whose length varies according to the application, is used in some classifications of the entire endoscope system [23, 25]. A very basic classification of medical endoscopes is based on their structural rigidity namely (a) rigid and (b) flexible endoscopes.

(a) Rigid endoscopes

As the name indicates, rigid endoscopes are having a rigid structure, and are useful for industrial as well as medical inspection. These endoscopes make use of lens relay systems for image collection. Fig. 2.1 [23] shows a typical rigid endoscope consisting of an object lens at the distal end, eye lenses at the proximal end and an optical relay system located between them. The optical relay system passes the image from the distal end to the proximal end.

Fig. 2.1 Typical rigid endoscope

Commonly used rigid endoscopes for medical intra cavity inspection include laparoscopes, arthroscopes, dental scopes, laryngoscopes, nasal scopes, sinusscopes etc [23].

However, these types of endoscopes suffer from certain disadvantages, which are considered as their limitations and are given below:

- ***Limitations of rigid endoscopes***

The limitations of rigid endoscopes can be summarized to:

1. The lens relay system used in some cases can be as many as 40-50 lenses that can cause considerable optical aberration, light loss and ghost images. In addition, off centering of lenses can cause significant vignetting and image deterioration unless great care is taken in system design [23].
2. Rigid endoscopes cannot bend around corners to access hidden areas because of its structure [23, 25].

Adopting a different method, such as using an optical fiber bundle for transmitting the image, the aberration present in the transmitted image due to the relay lenses can be eliminated. Also, the incorporation of a flexible fiber bundle in the endoscope can facilitate its bending along the cavity structures. Incorporation of these concepts gave rise to a new class of endoscopes, namely flexible endoscopes, which will be detailed, in the next section.

(b) Flexible endoscopes

Flexible type of endoscopes are highly useful in medical intra cavity inspection where, most of the body parts under inspection involve curved structures. Here, the endoscope will be directed along a complicated path to enable the physician to view the body parts in any

direction. A flexible endoscope is considered to be a more appropriate tool for minimally invasive inspection and surgery as it enters the body through natural openings [24]. In such cases, more care should be taken in selecting appropriate biocompatible materials, which are considered to interact with living tissue without any kind of conflict or collateral effect for the endoscope outer covering, to ensure seldom hazards to the body tissue sites [26].

The first flexible endoscope developed was a gastroscope developed by Basil Hirschowitz in 1957 [24]. A brief history of the further developments in the field of flexible endoscopy can be seen in the literature elaborating their working principles and technologies [24]. Today, flexible endoscopy has been considered as a reliable diagnostic and treatment tool in various branches of medical science.

Flexible endoscopy can be divided into two important categories, according to the way in which the image is collected and transmitted to the proximal end of the endoscope, namely electronic video endoscopy and fiber optic endoscopy.

(i) Electronic video endoscopy

In electronic endoscopy, the image is captured by an image sensor at the distal end, which is the key element of the whole system. This compact microelectronic device (miniaturized charge coupled device (CCD) converts an image into a time sequence of electronic signals, which after appropriate processing, are reconverted into an image on the monitor screen. The concept of all solid-state photo sensors in electronic endoscopy is based on the fundamental property of silicon of transforming light into negatively charged electrons.

Below given is a schematic of a typical electronic video endoscope (Fig. 2.2) [19]

Fig. 2.2 Schematic diagram of an electronic video endoscope

An image sensor comprises of an array of a large number of separate photosensitive elements, which “break down” an image into a given number of picture elements, each representing a spatial sample of the image [27]. After a given exposure time, typically of the order of 10 to 50 ms, the charge distribution produced by the light pattern impinging on the sensor has to be read out. This is accomplished by a method called charge coupling. Currently available endoscopes used for the examination of colon (colonoscopes) adopt the above-mentioned imaging scheme, i.e., imaging through miniaturized CCD cameras [28]. Each existing camera is adapted to one imaging scheme. But, the presence of the entire electronic detection system inside the human body makes it difficult to change any imaging configuration if necessary. In other words, such systems do not encourage more than one imaging modality, although some features such as shape of the inspected organ could be understood after executing several image processing routines. A change requires, pulling out of the entire device and reconfiguration of its internal component circuitry, which eventually leads to more efforts and cost. Moreover, size limitations on a tip camera present a problem, particularly for new capabilities such as fluorescence imaging. Also, current methods require

intensified CCD cameras to handle the low ratio of fluorescence signal to diffuse-white-light noise. This adds to the bulk and complexity of the camera.

In this context, use of fiber bundles, for imaging can subdue the above stated problems to a great extent. The concept of fiber optic endoscopy will be explained in the next section. Generally, a flexible fiber optic endoscope is referred to as a fiberscope. The major advantage of the fiberscopes is that it permits the detection electronics to remain outside the body, facilitating the miniaturization of endoscopes and the possibility of multiple imaging modalities using a single instrument.

(ii) Fiber optic endoscopy

Demands on design and performance of optical endoscopic instruments, including very small diameters and extreme flexibility have increased over the last thirty years. Today, optical fibers are having an important role in almost all optical systems. Due to the system compatibility with the use of optical fibers, such configurations are most relevant in the current world of miniaturization [29]. In a fiberscope, the illumination is provided by either a single fiber or a bundle of fibers. For imaging, a fiber bundle of precisely aligned flexible optical fibers (the bundle is often referred to as image fiber bundles or simply image fiber) is employed. Fiberoptic endoscopes permit visualization of normally inaccessible areas within the body and with minimum pain or discomfort for patients. Besides the light guide and image guide, ancillary channels may be present in a fiberscope, for the passage of air and water as well as for remote control implements such as biopsy forceps. Fiber optic endoscopes can be divided into two distinct categories according to their medical application, even though principles of operation and basic features are similar for both of them. The first category includes regular endoscopes such as the gastroscope, colonoscope,

and bronchoscope, etc. The second category includes ultrathin endoscopes for use as ophthalmic endoscopes [29]. Ophthalmic endoscopes allow a direct viewing of certain internal structures of the eye including the backside of the iris and posterior chamber [29]. Fiber optic endoscope can also be used for intracanal visualization [19]. A schematic of a typical fiberscope that uses fiber bundles for imaging is given below (Fig. 2.3) [19].

Fig. 2.3 Schematic diagram of a fiberscope

2.1.3.2 White light endoscopy for colon cancer diagnosis:

Sigmoidoscopy and colonoscopy [17, 18] are the commonly used endoscopic procedures for the colon cancer diagnosis. They are defined as screening and diagnostic procedures, used to examine the colon, utilizing a flexible viewing tube with a small camera and lighted end to visualize the inside of the colon for identifying the abnormalities. All currently available colonoscopes have their detection electronics scheme inside the body, which makes the diagnosis process more difficult. Flexible sigmoidoscope can only see a small portion of the large bowel (colon), and is considered as an inferior test. Even though biopsy procedures followed by colonoscopy is considered as the gold standard for today's colon cancer diagnosis, it has the disadvantages of causing injuries inside the colon during sample collection [30].

Besides, colonoscopy and sigmoidoscopy, endoscope probes are being developed for colon cancer diagnosis to incorporate indirect imaging technologies, such as optical coherence tomography (OCT), ultrasound etc. which are briefly described below.

2.1.3.3 Endoscopic techniques based on indirect imaging for colon cancer diagnosis:

- **Ultrasound [15]:** In ultrasound probes, sound waves of suitable frequency are emitted from the probe end to hit the tissue surface and the return waves are used to generate a picture of the layers of the tissue wall. Although this technique has been used to identify how much the cancer has grown through the layers, it suffers from a severe disadvantage of low resolution. The resolution highly depends on frequency, and is inversely proportional to the depth of penetration. So, for a sufficient penetration depth, one has to definitely compromise the resolution. Moreover, endoscopic ultrasound is used to identify solid tumors only and so is not an early detection technique.
- **Optical coherence tomography (OCT) [16]:** This new emerging technology in the field of cancer diagnosis makes use of an endoscope probe that directs laser light in the infrared region to hit the tissue wall and the reflected return light is collected and used to produce images of the colon wall. Even though the tissue wall can be imaged in a layer-by-layer basis, the effect of biospeckle formed due to the interaction of the laser with the tissue, OCT suffers from severe degradation of the optical image quality.

A more recent development in the field of endoscopy is the introduction of a miniaturized telemetry module used for wireless endoscopy [31]. With the intention of monitoring gastrointestinal diseases, a new type of video telemetry capsule is developed that can be swallowed and guided down the gastrointestinal tract while transmitting images [30]. This

single channel, unidirectional telemetric instrument has got several limitations such as its inability to transmit more than one signal from the body and the inability to control the camera behavior including the on-off functions. This was because the capsule can be operated only in one direction, i.e., the biomedical signals from the body to the outside circuit [31]. To overcome these limitations, a new type of video capsule is designed and proposed, which has multiple channels to communicate various body signals such as pH, temperature and pressure in both directions [32]. Research works are still going on in the field of wireless endoscopy to enhance its current features and to overcome the current limitations [33]. Again, this type of wireless endoscopy supports only one imaging modality, i.e., normal imaging.

The literature review on the present status of colon cancer in the world and the deficiencies of the presently available diagnostic methods to detect abnormalities at a very early stage clearly reveals the need for an early detection technique with which it is highly curable. The project aims at this objective and the concepts and methodologies adopted here to fulfill this objective is detailed in section 1.5 of Chapter 1. The next two sections of this chapter will be dealing with a brief overview of the techniques incorporated in the proposed diagnostic tool and their present status in the biomedical field. The concepts and techniques of speckle correlation interferometry are given below. Fluorescence techniques for cancer detection will be dealt with in Section C.

SECTION - B

2. 2 Speckle techniques

This section on speckle techniques is intended to have an understanding on the concept of speckles and to have a brief outlook of different interferometric configurations of speckle correlation. The application of speckle interferometric techniques in the NDT of specimens will be described. A review on the application of speckle concepts in biological structures will be presented.

2.2.1 The speckle effect

“**The speckle**” refers to the granular appearance on an optically rough surface when such a surface is illuminated by coherent radiation. The formation of speckles can also be due to the effect of spatial phase variations at the illumination beam wavefront. Speckle effects can generally be seen with laser, radar and ultrasonic imaging [34]. This project only concentrates on laser speckle and hereafter the “speckle” refers to “laser speckle” unless otherwise specified.

2.2.2 Speckle Pattern

The general criteria for the speckle pattern generation are

- The surface should be optically rough, i.e. its height variation is of the order of, or greater than the wavelength of the illuminating light
- The illuminating light should be coherent i.e., monochromatic.

When the surface is illuminated, each point on the surface can be considered to be acting as a source of spherical waves. The complex amplitude of the scattered light at any point in space is given by the sum of the amplitudes of the contributions from each point on the surface.

Since the surface height is of varying nature the total amplitude of light reaching any point in space can be described by a set of vectors of random phase, which when added together give a random resultant amplitude. The total amplitude has a value, which varies between zero and maximum determined by the magnitude and phase of the individual amplitudes. So, as the points in space are varied, the resultant amplitude and hence the intensity will have a different random resultant value. The speckle pattern originates from this random intensity variation. Generally observed speckle patterns can be put into two categories namely objective and subjective speckle patterns.

2.2.2.1 Objective speckle pattern

When an optically rough surface is illuminated by a laser beam, the intensity of the scattered light is found to vary randomly with position [34]. This is referred as the objective speckle pattern (Fig.2.4 (a)).

2.2.2.2 Subjective speckle pattern

When the surface is illuminated by laser light and an image of the surface is formed, the image shows a similar random intensity variation and is referred as the subjective speckle pattern [34] (Fig.2.4 (b))

(a)

(b)

Fig. 2.4 Schematic of speckle pattern ((a): Objective speckle pattern, (b) Subjective speckle pattern)

The average 'size' of an objective speckle is $1.22\lambda L/D_o$ where λ, L, D_o are the wavelength of illumination, image distance and diameter of the object respectively. The average 'size' of a subjective speckle is $1.22\lambda f/a$ where λ, f, a are the wavelength of incident illumination, focal length of the lens and the aperture of the lens, respectively.

2.2.3 Speckle correlation analysis

Speckle effect is considered to be an important tool for deformation analysis in engineering metrology [35]. Speckle analysis can be broadly classified into 3 categories; speckle photography, speckle interferometry and speckle shear interferometry.

2.2.4 Speckle photography

Speckle photography is one of the techniques to setup and operate in speckle metrology. The method permits one to measure displacement components [35]. The general procedure for recording a speckle pattern (alternatively known as a specklegram) is to expose a photographic plate to two displaced speckle patterns, once before and once after the object has been moved (deformed). The double exposed plate is developed and processed to get the required data.

2.2.5 Speckle interferometry

While speckle photography concentrates on the movements of individual speckles, speckle interferometry emerges as a whole field evaluation technique using speckle pattern correlation analysis. Speckle interferometry is a technique, which allows coherent addition of a speckle field with another speckle field or a smooth reference wave. Different configurations can be set up that make use of speckle interferometry to measure displacements sensitive to specific directions [35, 36]. Apart from the deformation analysis, speckle interferometry is also useful in the non-destructive testing of materials. Speckle

interferometry differs from speckle photography in the recording and post processing procedures of the resulting specklegram.

2.2.6 Electronic / Digital Speckle Pattern Interferometry (ESPI / DSPI)

Speckle interferometry in which normal and cumbersome photographic recording is completely replaced by advanced electronic or digital recording and subsequent post-processing is referred to as electronic or digital speckle pattern interferometry (ESPI or DSPI). Hereafter we will be using the term DSPI for denoting either DSPI or ESPI throughout the report. Here the speckle fringes can be seen in real time in a display monitor. The main feature of DSPI is its ability to present correlation fringes in real time. Intensity correlation in DSPI is observed by the process of either video signal addition or subtraction [34]. A review on the development of DSPI technique can be seen in [37].

2.2.6.1 DSPI Configuration

The basic constituents of DSPI system are shown in Fig 2.5. The commonly used sources of coherent light are He-Ne, Ruby, Argon ion, frequency doubled Nd: YAG and diode lasers. Like in other interferometers, the light from the source is split into object and reference branches.

Fig. 2.5 Basic constituents of a DSPI system –EP (electronic processing, DP-Digital processing)

In the object branch, the illuminated test-object is imaged by a lens system onto the light sensitive surface of the video camera. The reference branch produces a uniform reference wave, which impinges on the sensitive surface from the same direction as the object wave [34]. Phase modulation (PM) is introduced in the reference branch to change the phase of the wave in a controlled manner. The video camera records the image speckle pattern created by the combination of the object and reference waves by transforming its spatial content into an equivalent video signal. The video camera should have good sensitivity, large dynamical range and high resolution in order to have an optimum performance. These requirements are met by the present generation of CCD cameras. Also, a CCD camera is useful in industrial environment due to its robustness, small size, and immunity towards external magnetic fields. The video signal from the camera is fed to a digital-processing unit, which essentially performs the speckle correlation, filtering and rectification. The processed image is displayed on the display monitor, where the correlation fringes are observed in real time. The system records 25 speckle patterns per second [European CCIR standards] at a normal exposure time of 40 ms. The high framing rate provides for great stability and tolerances to external disturbances. Further, the digitization of the speckle pattern opens up a variety of signal manipulation possibilities like phase stepping and eventually provide full automation of the desired system.

The DSPI video system consists of a CCD camera, display monitor, video store and signal-processing unit. The main objective is to design the system such that the speckle can be suitably resolved by the video system. When the speckle is not fully resolved, the output voltage is a function of the intensity of the image averaged over the resolution area of the camera. The effect of this averaging is to reduce the contrast of the correlation fringes.

The ability of the video system to resolve the speckle pattern is limited. In order to get proportionality between the camera signal from a given point and the intensity at the same point, it is necessary that the intensity of the speckle pattern falling on the CCD faceplate should lie within the linear region of the camera signal response [34].

For a given CCD camera, a certain minimum intensity is required to generate an output voltage. This voltage must be detectable above the background electronic noise. There is also a maximum intensity beyond which the output voltage reaches. After this saturation level, the output voltage remains constant for any further increase in the intensity. When two speckle beams are used in subtraction correlation system, the mean intensities of the two beams at the image plane should be equal and the combined peak intensities should be approximately equal to the camera saturation intensity. By the aperturing of the imaging lens, the contrast of the fringes dramatically increases, and multiplexing of the image is possible [34, 36, 38].

2.2.6.2 Different basic configurations of DSPI

The advantage of DSPI to sense the displacement direction is already mentioned in section 2.2.5 of this chapter. DSPI is able to qualify and quantify the displacements in out of plane as well as in-plane directions. In addition, shearographic techniques make it possible to get the derivative of the displacements both static and dynamic. Below given is the simplified schematic of in-plane, out-of-plane and shear configurations. A single fiber port is used the object illumination for out-of-plane (Fig. 2.6 (b)) as well as out-of-plane shear configurations (Fig. 2.6(c)) whereas dual equal illumination is required for in-plane configuration (Fig. 2.6(a)). In out-of-plane configuration, one of the illumination fiber outputs is used as the clear beam reference. In shear configuration, a shearing element placed in the imaging path shears the object image into two.

Fig. 2.6 Schematic of basic DSPI configurations

2.2.7 Digital speckle pattern shearing interferometry (DSPSI)

In speckle shear interferometry, correlation fringes are generated which are contours of constant slope of the out-of-plane displacement of an object under study. In this configuration, called TV Shearography (Shear DSPI), a point in the CCD obtains contributions from two object points, and hence there is no need for a reference wave, as in the other speckle interferometric geometries. In normal shearography, the shearing of object images is done optically. In DSPSI, the slope (displacement derivative information) is obtained by shearing the images of individual object state, or by performing a numerical differentiation in the displacement map. In the first method, speckle image of the undeformed object state is sheared laterally by computer and superimposed with the original (unsheared) speckle image. The same is also performed to the speckle image of the deformed object state. The subtraction of the sheared images, which are generated in two different states of the object, results in a shear fringe pattern that gives slope information [39]. With this method, sensitivity can be changed by adjusting the amount of lateral shear introduced to images. Thus, measurement sensitivity is adjustable after measurement takes place. Another work studied the effect of shearing amount on the fringe pattern [40] and the

observation was that a smaller shearing amount will reduce the relative phase error but both contrast and the sensitivity of the fringes will be reduced. A more detailed description regarding the limitations of numerical differentiation technique can be seen in the literature [41].

Besides analyzing the displacement derivatives, the DSPI and shearography are having another important and relevant application, which is for the NDT of specimens. Several works have been reported in related areas. A brief review of them is given below.

2.2.8 DSPI and Shearography for NDT

In speckle interferometry, the fringe pattern represents the loci of strains. Since flaws in object usually induce strain concentrations, it can be determined by identifying the fringe anomalies due to these strain concentrations. Both surface and subsurface (internal) flaws can be detected using DSPI and shearography. Several studies have been carried out proving the efficiency of speckle metrology techniques as an optical NDT method [42- 44]

When compared with the conventional NDT techniques like dye penetrant, liquid crystal, ultrasonic, acoustic emission, x-rays and radiography, shearography is more superior because it is able to determine the criticality of defects directly. Conventional NDT techniques reveal flaws by identifying surface anomalies through enhanced visual means (for example, dye penetrant) and reveal internal flaws by detecting material inhomogeneties (for example, ultrasonic, x-ray). These techniques provide no direct information on whether the flaws will weaken the structure.

Shearography identify the abnormal strains induced by the flaws directly. If the components are loaded in a stress similar to actual loading in service, only critical defects will be revealed. Cosmetic defects can be ignored and false rejects can be avoided. Furthermore, shearography does not require scanning or contact with the object and its inspection

efficiency is qualitatively higher than the other techniques. Some of the application examples include the identification of missing cells in a honeycomb panel, the detection of cracks in the weldment of metal plates and finding out the inter-ply delaminations in composite structures which in turn find applications in aircraft structures, spacecraft structures, rocket motor casings, missiles, satellites, boats, ships, automobiles, chemical tanks, electrical insulation etc [35].

2.2.9 Applications of speckle techniques

The applications of speckle techniques for practical purposes can be broadly divided into two categories, namely industrial and biomedical [35]. Industrial applications are well defined and proven over many years. Some of them include the study of static and dynamic deformation of complex structures, non-destructive testing of various parts of engineering structures, investigations of material properties, surface profile measurements and vibration studies [34, 35]. Although the biomedical applications of speckle techniques have been studied in previous years, the application of speckle interferometry for studies in biological specimens is a budding concept, the details of which will be discussed in detail in the following section.

2.2.10 Speckle methods for biomedical applications

The potential of laser speckle analysis in biomedical field is receiving great attention among researchers. There are several applications of laser speckles in biomedical area.

One important field of application is in *optometry*. Speckle methods have been used for the diagnosis of long-sight, short-sight etc. and also for the study of chromatic aspects of vision [45, 46]. The significance of speckle analysis in *Retinal blood flow studies* [45, 47] has been noted in previous years. Studies were extended to *skin blood flow analysis* for early diagnostics of some diseases and healing processes [45, 48, 49]. Theoretical aspects of the

scattered field and the *blood flow in vessels* can be seen in previous works [50, 45]. Also, the study of *blood flow in the skin surface, internal organs, gastric mucosa and ocular fundus* has been carried out by laser speckle method [51]. *Blood flow within the subchondral bone* of the femoral head was evaluated using laser speckle method [52]. The authors claim that the method was able to distinguish between the ischemic areas and the normal areas of femoral heads. The application of speckle methods in tooth deformation studies is reported [53, 45]. The usefulness of speckle metrology in *orthopedic biomechanics* was demonstrated [54, 45] by making use of double-exposure laser speckle photography of broken leg bones. The possibility of applying a Michelson speckle interferometer for the investigation of cardio vibrations and the detection of pulse waves was substantiated [55, 56]. A laser differential speckle interferometer with two beams focused onto the surface of an object has been successfully employed for the detection of human pulse at various points of skin surface in the waist area by the same group [56].

Speckle interferometric methods have been applied for bio-related applications. Vibration measurement in *human ear drum (in vivo)* was conducted in which the vibration amplitude, frequency response and phase of the vibration are measured using electronic speckle interferometry [57]. DSPI has been performed to study the mechanical behavior of *human jaws* during chewing [58]. Works are also done to conduct DSPI investigations on the *thermal responds in human teeth* [59]. The application of speckle interferometric techniques on soft tissues is a budding concept in the field of biomedical applications of speckle interferometry. The related research works carried out in the area deals with single illumination technique [60].

From the above discussions, it is evident that the application of speckle techniques for deformation studies in biological systems is feasible. Studies on the effect of random

loading direction become important, as there can be components of displacement in all directions for an object like tissue upon random loading. The applications can be extended to the investigations on the displacement derivative components on such tissue surfaces, which can yield fruitful information regarding the abnormal growths in the surface / subsurface layers and thus provide a non-destructive, non-contact method for the abnormality detection, such as cancer growth.

SECTION - C

2.3 Fluorescence spectroscopy and cancer diagnosis

Another diagnostic technique incorporated in the proposed probe is fluorescence spectrum analysis. This section will be dealing with the review of previous literature regarding the present status of this technique in the diagnosis of cancer.

2.3.1 The fluorescence phenomenon

Fluorescence refers to a sort of luminescence where the emission of light at certain wavelengths occurs from molecules at singlet excited states. Fluorescence monitors changes in electronic energy levels, to provide biochemical information from biological molecules [61-63]. In excited singlet states, the electron in the excited orbital is paired (of opposite sign) to the electron in the ground state orbital. Consequently, returning to the ground state is spin allowed and occurs rapidly by the emission of a photon [62].

Fig. 2.7 displays an energy level diagram with ground (S_0) and excited (S_1) electronic states as well as the vibrational energy levels within electronic state of a molecule [62]. When a molecule is excited at a wavelength that lies within the absorption spectrum of that molecule, it will absorb the energy and be activated from its ground state (S_0) to an excited singlet state (S_1), with the electron in the same spin as its ground state. The molecule can then relax back from the excited state to the ground state by generating energy either nonradiatively or radiatively, depending on the local environment. In a non-radiative transition, relaxation occurs by thermal agitation (dashed arrows). In a radiative transition, relaxation occurs via fluorescence at specific emission wavelengths (solid arrow). Fluorescence generation occurs

in 3 steps: (i) thermal equilibrium is achieved rapidly as the electron makes a non - radiative transition to the lowest vibrational level of the first excited state (ii) the electron then makes a radiative transition (emission) to the vibrational level of the ground state (iii) the electron makes a non - radiative transition to the lowest vibrational level of the ground state.

The phenomenon of fluorescence displays several general characteristics for a particular biological molecule [62]. Due to the losses in energy between absorption and emission, fluorescence occurs at emission wavelengths that are always red-shifted relative to the excitation wavelength. The emission wavelengths are independent of the excitation wavelength and the fluorescence spectrum of a biological molecule is generally a mirror image of the absorption spectrum.

Fig. 2.7 Energy level diagram illustrating the phenomena of absorption and fluorescence of a molecule

Fluorescence spectroscopy is the measurement and analysis of various features that are related to the fluorescence quantum yield (ratio of the number of photons emitted to the number absorbed) and / or lifetime (average time the biological molecule spends in the

excited state prior to return to the ground state) of a biological molecule. The fluorescence intensity of a biological molecule is a function of its concentration, its extinction coefficient (absorbing power) at the excitation wavelength and its quantum yield at the emission wavelength [61, 62]. The amount of fluorescence emission from a biological sample depends on its fluorescence efficiency, which is the quantitative relationship between the excitation and emission energy [62]. A fluorescence emission spectrum represents the fluorescence intensity measured over a range of emission wavelengths at a fixed excitation wavelength. A fluorescence excitation spectrum is a graphical representation of fluorescence intensity at a particular emission wavelength for a range of excitation wavelengths. A fluorescence excitation emission matrix (EEM) is a two dimensional contour plot that displays the fluorescence intensities as a function of a range of excitation and emission wavelengths [62]. Each contour represents points of equal fluorescence intensity. Fig.2.8 illustrates a fluorescence emission spectrum (a), a fluorescence excitation spectrum (b), and a fluorescence EEM (c) [62].

Fig. 2.8 Fluorescence spectrum for excitation and emission

Fluorescence studies have gained great importance and relevance in biomedical field. Most of the endoscopic configurations used for fluorescence analysis are dealing with the detection of cancerous regions in different parts of human body. The following section gives a brief outlook of the relevance and importance of fluorescence analysis in the diagnosis of cancer present in different body parts. From the project point of view, the application of this method in the diagnosis of colon cancer is detailed with special reference.

2.3.2 Fluorescence spectroscopy in cancer detection

It is well recognized in *conventional white light endoscopy* that abnormalities (dysplastic lesions) may not be visually recognized unless there is some topographic alteration such as nodules or raised plaques [64]. One interesting report from Japan [65] screened patients with head and neck cancers for associated esophageal cancer detection using conventional white light endoscopy. Out of 13 lesions detected in these patients, nine were not seen at white light endoscopy. Sometimes, an endoscopist may be relying on *random biopsies*. Tissue removal is subjected to sampling errors, particularly when the lesions are not visible to the eye. Also the multiple stage sample preparation process is time consuming, labor intensive, and can introduce undesirable results due to cutting, freezing and staining of the tissue [64]. Both methodologies mentioned above are non - desirable where the abnormalities start from non - visible layers in biological systems. In this context, the need for a technique that could identify and locate dysplasia with high sensitivity and specificity becomes important.

An experimental attempt is described in the literature, in chronic ulcerative colitis rat model, to enhance visualization of (low-grade) dysplastic lesions by fluorescence using an exogenous drug, 5-aminolevulinic acid (ALA) and protoporphyrin IX (PpIX) and their accumulation was detected by directly viewing its characteristic orange red fluorescence

when activated by ultraviolet (UV) light source (*Photodynamic diagnosis*) [66]. Spectroscopy can provide information in real time and not affected by sampling errors. It can provide quantitative information that is largely free of subjective interpretation. Lot more studies have been done related to photodynamic diagnosis in the field of cancer detection in different parts of the body such as bladder [67, 68], oral cavity [69], esophagus [70] etc. Additional costs and possible toxicity (hepatotoxicity and phototoxicity) as well as regulatory approval complicate the use of exogenous fluorophores (photosensitizers) [64]. These limitations have reduced the method of inducing fluorescence by external means to a minimum.

- ***Autofluorescence vs photodynamic diagnosis***

To overcome the limitations of photodynamic diagnosis as described above, a diagnostic method based on the *autofluorescence* of tissues has been suggested. The term Autofluorescence refers to the fluorescence emitted by the natural fluorophores inherent in the considered tissue. A significant advantage of autofluorescence is that it is not necessary to administer a drug. Significant differences in the tissue fluorescence emission, in terms of both signal shape and spectral amplitude have been reported [64, 71-79] in the case of colonic neoplastic lesions with respect to the surrounding normal tissue, suggesting that autofluorescence could provide a useful aid in distinguishing normal and abnormal tissue sites. Autofluorescence phenomenon is exhibited by naturally occurring fluorophores, such as collagen, elastin, nicotinamide adenine dinucleotide (NADH), flavins and porphyrins [71, 72, 80]. Changes in the intrinsic fluorescence spectrum with disease are due to the altered biochemical composition of the tissue. The field of autofluorescence in gastrointestinal endoscopy is less than twelve years old, and *in vivo* imaging has only begun

to appear less than six years back. In the first *in vitro* studies [73] excised colonic polyps were examined with UV excitation (325nm). A multivariate analysis of the fluorescence emission spectrum (350-600nm) distinguished normal colon from adenomatous polyps and hyperplastic polyps with accuracy of 100 and 94% respectively. Related works in the bronchus [81-83] showed that, autofluorescence was more accurate in detecting early lesions than fluorescence based on a porphyrin drug, succeeded by the development of lung imaging fluorescence endoscope. Application of this technology in the gastrointestinal tract [84, 85], with parallel preclinical equipment was explored in the studies using animal models with induced colonic and esophageal dysplasia or carcinoma. The study carried out *in vivo*, using point spectroscopy and a probability based algorithm to detect colonic dysplasia, [75] determined the correct tissue type in 88% of cases with a sensitivity of 90% and a specificity of 95%.

Point spectroscopic studies with 410nm excitation, applied a stepwise discriminative analysis to select emission wavelength to differentiate between normal tissue and malignant esophageal tissue with a sensitivity of 100% and specificity of 98% [86]. The group also studied selected patients with Barrett's esophagus, and was able to distinguish high-grade dysplasia in 90% of the cases. This was the first study to identify endoscopically, occult premalignant lesions based on tissue autofluorescence.

Studies on endoscopic fluorescence spectroscopy in the upper GI tract for *in vivo* diagnosis of GI cancer using light induced fluorescence spectroscopy (short arc xenon lamp with 375-478nm band pass filter) showed significant difference between normal and cancerous tissue [87].

More research work based on autofluorescence in various parts of the human body such as arterial tissue [88, 89], bronchus [81-83], stomach [90], ocular [91], esophagus [92, 93], skin [94], colon tissue [64, 71-80, 95-101] etc. both *in vitro* and *in vivo* have been reported in literature, supporting strongly the potential of the technique to distinguish malignant tissue from normal one.

Focusing again to the *autofluorescence analysis of human colon tissue*, a morphological model was developed, which connects the clinically observed data with their underlying microscopic origins [71]. The fluorescence intensity difference between normal and adenomatous tissue was attributed to the increased hemoglobin absorption, reduced mucosal fluorescence intensity and absence of submucosal fluorescence in adenomatous polyps. The group claims the potential of the model to detect flat dysplasia, which cannot be detected with conventional colonoscopy.

Laser Induced Fluorescence Spectroscopy (LIFS) studies using He-Cd laser (325nm) for the detection of adenomatous transformation of human colonic mucosa are described in the literature [73]. In such studies, the emission spectrum was found to be of the range 350-600nm. A quantitative LIF score to discriminate adenomatous from normal tissue was also developed using regression analysis of the spectral data. Few more groups have studied LIF regarding human colon tissue [85] which deals with a simplified Monte Carlo model to study the spectrum dependence on histological and histochemical properties of colon tissue.

Studies regarding the intensity of emission at different emission wavelengths are described in the literature [96]. This study shows that colon tissues can emit in the green and red wavelength region and in the emission wavelength region of 500-580nm, the adenomatous polyps show a considerable decrease in intensity as compared to normal mucosa.

Chapter 2

Literature Review

To put it in a nutshell, the following table (Table 2.1) summarizes some of the important works done so far in the field of autofluorescence spectroscopy of colon tissue.

First author (Ref.)	Year	Technique	Excitation wavelength (nm)	Emission Wavelength (nm)	Evaluation
C. R. Kapadia [73]	1990	S	325	350-600	6 λ multivariate linear regression
R. Marchesini [98]	1994	S	410	450-800	6 λ stepwise discriminant function
G. S. Fiarman [99]	1995	CFM and S	488	>515	Integral FI
T. J. Romer [72]	1995	S and I	351-364	420-610	Fluorescence
G. I. Zonios [71]	1996	S and I	<380	<700	Various algorithms
R. M. Cothren [75]	1996	S and PA	370	400-700	Various algorithms
T. D. Wang [76]	1996	S and I	351-364	400-700	Fluorescence
R. S. DaCosta [85]	1997	CFM	442	480-700	Monte Carlo Simulation
Y. Yuanlong [97]	1997	S	365	550-900	Spectral Shape
A. F. Zuluga [100]	1999	S	330-500	400-700	EEM analysis
N. Ramanujam [62]	2000	FI and S	UVIS	400-700	Fluorescence
P. M. Gregory [101]	2002	FI	UVIS	300-700	Fluorescence
B. Mayinger [96]	2003	S	Visible	500-700	Fluorescence

Table 2.1 Autofluorescence analysis of human colon tissue - A brief review of previous research

CFM- confocal microscopy, FI- fluorescence intensity, S-Spectroscopy, I – imaging, PA- Probability based algorithm and EEM- excitation emission matrix.

In fluorescence studies, the devices and methods used for the spectrum analysis play an important role. The collection of fluorescence emission from a biological tissue can be done *in vitro* or *in vivo*. A real time fluorescence analysis on body cavities, as proposed in this project, needs in-vivo investigations. The following section deals with the devices and methods used for the real time fluorescence in body cavities, with a special reference to the colon path.

2.3.3 Fluorescence spectrum collection and analysis for colonic tissues: devices and methods

Collection of fluorescence emission from body cavities requires an endoscopic instrument, through which the excitation of biological tissues is also done. The detector system placed at the proximal end of such fluoroscopes also play a key role, in determining the amount of collected photons emitted from the concerned biological tissue. Previous literature discusses various endoscopic configurations, used for fluorescence emission collection from bio-specimens [73, 75, 96]. The advantage and disadvantage of the above-mentioned configurations are discussed and suggestions for improving previous configurations for achieving greater performance are proposed.

2.3.4 Different endoscope configurations for fluorescence analysis

Fluorescence techniques in endoscopy are of great diagnostic value, as the qualitative differences in tissue fluorescence intensity are used to identify the presence of cancer during endoscopy. Fluorescence images of cancerous tissue will be having reduced intensity as compared to that of normal tissue and thus such images can normally highlight regions of suspicious tissue providing biopsy guidance.

Normally, the reported and commercial fluoroscopes make use of a light source (coherent and incoherent) in order to provide the necessary excitation for inducing fluorescence. In the fiberscopes, this excitation is delivered to the tissue through single mode/ multimode fibers. The collection of the emitted signal is also done using optical fibers. Different arrangements of the excitation and collection fibers for the fluorescence analysis of colonic tissues have been reported in the literature that makes use of the following geometries:

(i) Single fiber for excitation and collection

In this configuration, a single optical fiber is used to deliver the excitation light, as well as to collect the emitted fluorescence. An example of such a system is given in Fig. 2.9 [73].

Fig. 2.9 Fluorescence analysis with single fiber excitation and emission collection

In this setup, the excitation source is coupled to a 400 μ m optical fiber, which in turn induces endogenous tissue fluorescence. By the use of a custom beam splitter, the simultaneous excitation and emission collection are carried out. Here the fiber was positioned perpendicular to and in contact with the tissue surface. This type of contact methods suffer from the disadvantage of distortion in collected signal by the variable pressure on the tissue

[62]. Also, the area of tissue from which the emission is collected will be small. Again, above mentioned setup is used for biopsy examinations outside the body and not a real endoscope setup.

(ii) Single fiber for excitation and multiple fibers for collection

An example of fluorescence analysis with single fiber excitation and multiple fibers for emission collection is reported in literature [75]. In this setup shown in Fig. 2.10, the fluorescence analysis probe consisted of a circular array of nine 100 μm -core 0.22NA collection fibers surrounding a single 200 μm -core 0.22 NA excitation fiber.

Fig. 2.10 Fluorescence analysis with single fiber excitation and multiple fibers for emission collection

In this system also, the collection fibers are in contact with the tissue and thus suffers from the disadvantage of signal distortion. Also, as the collection fibers are less in number and arranged surrounding the excitation fiber, such an arrangement may not be maximized for

emission collection and definitely cannot be used for fluorescence imaging. The concept of fluorescence imaging will be discussed later in this chapter.

(iii) Fiber bundles for excitation and collection

Fluorescence analysis using a standard colonoscope is reported in literature [96] where the excitation is done with the illumination optical fiber bundle of the endoscope (Fig. 2.11). The collection of the emission is done through an optical probe inserted through the accessory channel of the same endoscope. The collection probe consisted of a bundle of 16 glass fibers each 200 μm in diameter.

Fig. 2.11 Fluorescence analysis with excitation and emission collection using fiber bundles

The above configuration also allowed white light imaging modality, like standard colonoscopes. Although, the use of collection fiber bundle increased the examined tissue area as compared with collection using a single fiber, the above method is definitely complicated and involves two separate probes (commercial endoscope and custom made collection probe) for the fluorescence analysis. Also, the study mainly concentrated on biopsy samples, which were taken outside from the human body.

All the fluoroscope designs discussed above, deal with a single fiber excitation. A symmetrical configuration of two-illumination fiber ports can give flexibility in enlarging the interrogating area providing uniform specimen excitation at the desired angle. Also, the collection of emission depends on the imaging lens used, imaging lens-collection fiber coupling and the collection fiber transmission efficiency. A single collection fiber can only transmit the emission, whereas with the use of a collection fiber bundle, the fluorescence image of the tissue could be transmitted, which can provide an alternative to the spectrum analysis. An attempt to collect such fluorescence images using standard white light colonoscope with laser excitation provided by an external fiber has been reported in literature [76]. The study involved examination of biopsy samples as the fluorescence excitation and image collection were not carried out using a single probe, and thus was not able to take the setup inside body cavities. Since fluorescence spectrum of cancerous tissue shows a decrease in intensity compared to normal tissue, in a fluorescence image, the cancerous regions are seen with decreased intensity.

In autofluorescence studies, as the emitted fluorescence intensity from the live tissue is weak, the selection and use of proper imaging elements in the endoscope for fluorescence analysis is very important to collect sufficient amount of the emitted energy. Also, the emitted signal from the endo-fluoroscope passes through the spectrometer or

monochromator where the reflected and emission wavelengths are scanned. A brief description of a monochromator configuration used for scanning the endo-fluoroscope output is given below.

2.3.5 Monochromators and spectrometers

A monochromator scans one wavelength of the input light at a time and presents from its exit slit, whereas a spectrometer presents a range of wavelengths simultaneously at the exit focal plane. The key components of a monochromator or spectrometer are: an entrance slit, a collimator (mirror M1), a grating (dispersing unit) for wavelength selection, a focusing element (mirror M2) and an exit slit (see Fig. 2.12). For a fluoroscope system, the input to the monochromator will be the output light from the endoscope proximal end as shown in Fig. 2.12. The grating can be either ruled (where the groove density will be from 50 to 3600 grooves/mm) or holographic (where the groove density will be higher than ruled one). An important parameter relevant to the selection of a monochromator or spectrometer is the reciprocal linear dispersion (RLD), which is inversely related to the grating groove density, diffraction order and focal length of the focusing element. RLD when multiplied by the entrance slit width provides the spectral resolution. For fluorescence studies, generally a spectral resolution of 10nm is sufficient [62]. Stray light rejection is another important factor in the selection of monochromators. Stray light is reduced by using holographic gratings compared to ruled gratings. Typical stray light rejection for UV and Visible (VIS) fluorescence spectroscopy should be specified to five orders of magnitude lower than the signal. Transmission efficiencies of both holographic and ruled gratings are comparable (~40%) in the VIS spectral region, with a maximum efficiency at the blazed wavelength.

Fig. 2.12 Schematic drawing of a monochromator

2.3.6 Detectors

A photodetector system is placed after the spectrometer or monochromator to measure the emitted fluorescence intensity for all emission wavelengths. If measurements at only one or several wavelengths are being made, a single channel photoemissive tube, i.e., a photomultiplier tube (PMT) can be used [62]. It is important to note that the detector (PMT) must detect emitted photons of all wavelengths with equal efficiency [61]. Fluorescence spectroscopy can be performed using a monochromator coupled to a PMT [62].

As far as the detector performance is concerned, other considerations include quantum efficiency and sources of noise. The detector quantum efficiency (QE) is defined as the ratio of induced current to the induced flux (often measured in electrons/photon). QE depends on the wavelength of light used, the material type and shape and other physical parameters. Different types of noises during the detection of light can be classified as shot noise, dark noise /dark signal and readout noise [62]. A proper selection of the PMT used for fluorescence analysis should ensure maximum QE and minimum noise factors so as to ensure a higher signal to noise ratio.

A review of previous literature regarding the currently available diagnostic methods for colon cancer and their limitations is given in section A of this chapter. From the discussions, given in section 2.1, it is clear that none of the available methods are proven to be an early detection technique. In section B, the concept of speckle and speckle interferometric techniques for non-destructive inspection of specimens has been analyzed theoretically and experimentally. From the detailed literature survey on the fluorescence spectroscopic techniques especially autofluorescence techniques for cancer diagnosis, discussed in section C, potential of this technique in distinguishing normal and abnormal colon tissue samples has become evident.

Regarding the speckle interferometric configurations, the literature survey given in this chapter deal with flat surfaces. In view of the research project, this thesis is dealing with speckle fringe formation on biological cavity surfaces, specifically the colon. Analysis of fringe formation in colon, requires a thorough theoretical formulation on speckle fringe formation on such surfaces, which will be discussed in the next chapter. Also, in Chapter 3, DSPI configurations involving colon-like curved surfaces are analyzed experimentally.

CHAPTER 3

INTRACAVITY INVESTIGATIONS USING SPECKLE INTERFEROMETRY

In this chapter, application of speckle interferometry techniques for intra cavity inspections will be described. The potential of the speckle interferometric techniques in medical intra-cavities will be brought into attention within the context of the proposed project. The well-known speckle fringe formation theory applied to flat surfaces is modified for the analysis of speckle fringe formation at the curved biological surfaces. Also, detailed theoretical analysis regarding the effect of illumination beam divergence on DSPI fringe formation will be given. Finally, the experimental analysis regarding the non-destructive testing of curved tissue specimens will be discussed, using tissue phantoms as the test specimen.

3.1 Speckle pattern interferometry for curved surfaces

Although experimental and theoretical analysis on different speckle interferometric configurations with flat surfaces are well reported in literature [35, 36], the same in the case of curved object surfaces are very few till to date. Studies on the deformation of curved surfaces are having wide range of applications in engineering as well as in biomedical areas and thus having much research scope. One of the earlier reported works in this area concentrated mainly on the recording of far-field objective speckles for three-dimensional

surface displacement measurement of photosensitive-coated surfaces [102]. A study regarding a mirror concept for the simultaneous measurement of in-plane and out-of-plane deformation components of a thermally loaded piston is reported in literature [103]. DSPI investigations on curved parts of spacecraft structural components are reported in the literature [104]. Investigations into the defect or crack identification in pipes and pressure vessels are also reported recently [105-107].

Theoretical formulation of the fringes formed on curved surfaces is very crucial for the quantitative and qualitative analysis of fringe patterns on such surfaces to extract the exact displacement information. This also helps to have a better insight into the difference in the nature of fringes of a curved specimen from that of a flat specimen upon the application of loads. A thorough analysis of the previous works in the area of curved surface DSPI shows, the deficiency of a fruitful theoretical explanation to the experimental results. In this context, an extended theoretical formulation followed by experimental analysis of the curved specimens related to their displacement (in-plane and out-of-plane) as well as displacement derivative (shear) components under different loading conditions is presented here. The above-mentioned formulation will be helpful for the non-destructive evaluation of such curved parts of various engineering and medical structures.

Generally, curved surfaces can be cylindrical or spherical. In the context of the current application, where the object (the colon) is a cylindrical cavity, the existing speckle fringe theory has been extended to apply on such surfaces.

As part of the intended research, the application of speckle interferometry for the NDT analysis on curved biological phantom specimens will be described along with necessary theoretical formulations in the following section of this chapter. The specimen phantom

Chapter 3 Intra cavity investigations using speckle interferometry

tissue description, which resembles the real colon tissue in its properties relevant to the scope, is given in section 4.2 of Chapter 4.

3.2 DSPI fringe formation on curved phantom tissue surfaces – modified theory and experimental analysis

The fringe formation in DSPI is based on the intensity correlation of the speckle patterns before and after object loading [34-36]. Let I_1 and I_2 be the intensity of the two beams, object beam and reference beam respectively. Then the total intensity of the two beams, before applying object displacement, is given by [35]:

$$I = I_1^2 + I_2^2 + 2 (I_1 I_2)^{1/2} \cos \phi \quad (3.1)$$

where, ϕ is the phase difference between the two beams.

The total intensity after object displacement is given by [35]:

$$I' = I_1^2 + I_2^2 + 2 (I_1 I_2)^{1/2} \cos(\phi + \Delta\phi) \quad (3.2)$$

where $\Delta\phi$ is the additional phase change introduced due to the specimen displacement.

Though the calculation of $\Delta\phi$ in the case of flat surfaces are well reported in literature [34, 35], it is not the same case as far as curved surfaces are concerned. In this context, this extended theory is proposed to explain the speckle fringe formation on curved surfaces.

In the subtraction correlation process, the camera (video) signals corresponding to the image plane speckle pattern of the undisplaced object is stored electronically or digitally. The object is then displaced and the live video signal, as detected by the camera, is subtracted from the stored image, processed and displayed on a monitor where live correlation fringes are observed. By applying subtractive speckle correlation to I and I' , the total intensity, $\mathbf{I}$ is given by [35],

$$\mathbf{I} = 4(I_1 I_2)^{1/2} \sin\left[\phi + \left(\frac{\Delta\phi}{2}\right)\right] \sin\left(\frac{\Delta\phi}{2}\right) \quad (3.3)$$

It is assumed that the voltage signals from the CCD camera are proportional to the irradiances and are subtracted in a pixel-by-pixel basis. The subtracted signal is displayed on the TV or PC monitor and is given by

$$V_s = 4I_1 I_2 \sin\left[\phi + \left(\frac{\Delta\phi}{2}\right)\right] \sin\left(\frac{\Delta\phi}{2}\right) \quad (3.4)$$

This signal has both positive and negative values. Since negative areas are displayed as dark areas on the monitor, the difference signal is rectified before it reaches the monitor. Hence the brightness B on the monitor is proportional to and is expressed as

$$B = 4\mathbf{K}I_1 I_2 \sin\left[\phi + \left(\frac{\Delta\phi}{2}\right)\right] \sin\left(\frac{\Delta\phi}{2}\right) \quad (3.5)$$

where $\mathbf{K}$ is a constant of proportionality. The brightness on the monitor will be zero when $\Delta\phi = 2m\pi$ and maximum when $\Delta\phi = (2m+1)\pi$. It can be seen that the brightness on the monitor is modulated by $\sin\left(\frac{\Delta\phi}{2}\right)$, which represents the fringe pattern.

The phase change at the image plane due to the curved object surface displacement ($\Delta\phi$) can be calculated with respect to the initial undeformed point of the specimen surface, which is subjected to maximum deflection upon loading. The maximum deflection point after loading is the meeting point (P) of the three co-ordinate axes as shown in Fig. 3.1.

Fig. 3.1 Representation of maximum point of displacement in the curved object surface

The object surface considered is the inner surface of a cylindrical specimen. In this case, the displacement vector is derived in cylindrical co-ordinates. Depending on the type of loading, the displacement $\vec{L}$ of any point in the specimen can be resolved along 'r' (radial), ' θ ' (tangential) and 'a' (axial) directions and are denoted as w_r , w_θ and w_a (see Fig. 3.2). The unit vectors in cylindrical coordinates are denoted as $\mathbf{e}_r$, $\mathbf{e}_\theta$ and $\mathbf{e}_a$. Therefore, the displacement vector of the specimen point P as being moved to point Q upon random loading is given by

$$\vec{L} = w_\theta \mathbf{e}_\theta + w_a \mathbf{e}_a - w_r \mathbf{e}_r \quad (3.6)$$

Fig. 3.2 Different displacement components for a cylindrical specimen (ds : Elemental displacement along the curved surface, $d\phi$: angle between the tangents at points P and Q. w_r : displacement component along radial direction, w_θ : displacement component along tangential direction, w_a : displacement component along axial direction)

Now, let the total displacement of the point P (Fig. 3.2) be represented by W_T , which is the vector sum of the three displacement components. i.e.,

$$W_T = ((w_\theta)^2 + (w_r)^2 + (w_a)^2)^{1/2} \quad (3.7)$$

The axial component w_a does not contain any curvature information and can be considered as similar to the Y-direction displacement component of the flat specimen (v). The radial and tangential displacement components containing the specimen curvature information can be derived as below.

❖ **Calculation of w_θ and w_r :**

Consider two points P and Q on the curved surface where the point P moves to point Q due to the application of external perturbation. Let the tangents at points P and Q makes an angle

$d\phi$ with each other (Fig. 3.2). Then, the tangential and radial components of displacement for the curved specimen can be written as:

$$w_r = W \sin\left(\frac{d\phi}{2}\right) \quad (3.8)$$

$$w_\theta = W \cos\left(\frac{d\phi}{2}\right) \quad (3.9)$$

where W is the resultant displacement along the curved trajectory of the specimen and is

$$\text{given by, } W = ((w_r)^2 + (w_\theta)^2)^{1/2}$$

Now, $d\phi$ can be written as:

$$d\phi = ds K \quad (3.10)$$

where K is curvature of the cylinder and ds is the elemental displacement from P to Q [108].

Assuming the curve to be circular with radius of curvature $R(o)$,

$$K = \left(\frac{1}{R(o)}\right) \quad (3.11)$$

$$\therefore d\phi = \left(\frac{ds}{R(o)}\right) \quad (3.12)$$

Hence, from equation (3.6), the resultant displacement vector,

$$\vec{L} = W \cos\left(\frac{ds}{2R(o)}\right) \mathbf{e}_\theta + w_a \mathbf{e}_a - W \sin\left(\frac{ds}{2R(o)}\right) \mathbf{e}_r \quad (3.13)$$

3.3 DSPI configurations for curved surfaces

The calculation of $\Delta\phi$ depends on the displacement vector and the orientation of illumination and imaging directions and is different for different configurations of speckle interferometry. The following sections deal with speckle interferometric configurations for curved surfaces, which sense in-plane and out-of-plane components of displacement as well as out-of-plane displacement derivative component.

3.3.1 In-plane configuration

In the in-plane setup (Fig 3.3), the object surface was illuminated using two equal intensity collimated monochromatic beams. Light from the laser (532nm) was coupled into a single mode fiber, which was then split into two using a 1x2 fiber beam splitter. The introduction of fiber optics for beam delivery, makes the entire setup compact and light weight [109]. The light from the two fiber ports was allowed to pass through collimator lenses so as to obtain collimated beams. The beams were then allowed to interrogate the diffuse phantom tissue surface. The test object was the curved phantom tissue (50mm length, 45mm outer diameter, and 1mm thickness), fixed from the top and bottom edges. The light from the two fiber ports was allowed to pass through collimator lenses so as to obtain collimated beams. The beams were then allowed to interrogate the diffuse phantom tissue surface. The test object was the curved phantom tissue (50mm length, 45mm outer diameter, and 1mm thickness), fixed from the top and bottom edges.

Fig. 3.3 Experimental setup for in-plane displacement analysis of curved phantom tissue (F: Optical fiber, FH: Fiber holder, CL: collimating lens)

Chapter 3

Intra cavity investigations using speckle interferometry

In-plane displacement ($\approx \mu m$) in the axial direction was given to the specimen using a custom fabricated loading jig. Speckle patterns before and after object loading are captured by a zoom lens attached EDC 2000N computer camera system (Pls. see Appendix A for details). By optimizing the intensity of the illuminating laser beam and the brightness of the PC monitor, and by controlling the zoom lens aperture attached to the CCD the maximum fringe contrast is obtained. The speckle pattern corresponding to the undeformed state of the specimen was considered to be the reference frame and frames corresponding to the deformed state of the object were subtracted from the reference frame at a rate of 25 frames per second. The evolution of fringes upon loading is observed on a PC monitor in real-time, using the developed software and EDC 2000N image-processing card.

Let the two illumination vectors be represented by $\vec{k}_1$ and $\vec{k}_3$ and the imaging vector is represented by $\vec{k}_2$.

$$\vec{k}_1 = -\mathbf{e}_a \sin \theta_i + \mathbf{e}_r \cos \theta_i, \quad \text{where } \theta_i \text{ is the illumination angle} \quad (3.14)$$

$$\vec{k}_2 = -\mathbf{e}_r \quad (3.15)$$

$$\vec{k}_3 = \mathbf{e}_a \sin \theta_i + \mathbf{e}_r \cos \theta_i \quad (3.16)$$

Considering the phase change caused by object displacement and treating the illuminations individually,

The phase change due to object displacement $\Delta\phi = \Delta\phi' - \Delta\phi''$, where $\Delta\phi'$ and $\Delta\phi''$ represent the phase change due object displacement with respect to illumination 1 and 2.

$$\Delta\phi' = \left(\frac{2\pi}{\lambda} \right) \left(\vec{k}_2 - \vec{k}_1 \right) \cdot \vec{L} \quad (3.17)$$

where λ represents the wavelength of illumination.

$$\Delta\phi'' = \left(\frac{2\pi}{\lambda} \right) \left(\vec{k}_2 - \vec{k}_3 \right) \cdot \vec{L} \quad (3.18)$$

$$\Delta\phi' - \Delta\phi'' = \left(\frac{2\pi}{\lambda} \right) \left(\vec{k}_3 - \vec{k}_1 \right) \cdot \vec{L} = \left(\frac{2\pi}{\lambda} \right) \left(2\mathbf{e}_a \sin \theta_i \right) \cdot \vec{L} \quad (3.19)$$

$$\Delta\phi = \left[\left(\frac{2\pi}{\lambda} \right) (2\mathbf{e}_a \sin \theta_i) \right] \left[W \cos \left(\frac{ds}{2R(o)} \right) \mathbf{e}_\theta + w_a \mathbf{e}_a - W \sin \left(\frac{ds}{2R(o)} \right) \mathbf{e}_r \right] \quad (3.20)$$

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left(2w_a \sin \theta_i \right) \quad (3.21)$$

If the two illumination angles are different, (θ_{i1} and θ_{i2}) then

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left(w_a (\sin \theta_{i2} + \sin \theta_{i1}) - w_r (\cos \theta_{i2} - \cos \theta_{i1}) \right) \quad (3.22)$$

Considering pure in-plane displacement in the axial direction, without any out-of-plane displacement component for the cylindrical tissue specimen surface,

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left(w_a (\sin \theta_{i1} + \sin \theta_{i2}) \right), \text{ where } \theta_{i1} \neq \theta_{i2} \quad (3.23)$$

Fig. 3.4 (a) represents the in-plane fringes obtained for the curved tissue phantom and Fig. 3.4(b) represents the corresponding simulated contours using finite element analysis (FEA). The tissue mechanical properties such as Young's modulus and Poisson's ratio used for the FEA are determined experimentally, the details of which are given in Appendix B.

Here the fringes are having horizontal straight line shape, and are similar to in-plane displacement fringes on a flat surface. (See Fig. 3.5 (a) for in-plane fringes from a flat tissue and Fig. 3.5 (b) for its FEM (Finite element modeling) simulated contours)

Fig. 3.4 In-plane displacement contours for a cylindrical tissue phantom specimen:
(a) experimental fringe pattern (b) Simulated contours using FEM

Fig. 3.5 In-plane displacement contours for a rectangular tissue phantom specimen:
(a) experimental fringe pattern (b) Simulated contours using FEM

Considering a flat specimen, the phase change due to object displacement is [35, 36]

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left(v (\sin \theta_{i2} + \sin \theta_{i1}) \right), \text{ where } \theta_{i1} \neq \theta_{i2} \quad (3.24)$$

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) (2v \sin \theta_i), \text{ where } \theta_{i1} = \theta_{i2} = \theta_i \quad (3.25)$$

where v is the y-direction displacement of the flat specimen.

Comparing equations (3.23) and (3.25), it is noted that, for the in-plane fringe formation, the theoretical formulations for the cylindrical and flat specimen are the same except for the magnitude of the axial or y-direction displacement. This explains the similarity in fringe

shape seen with the curved (cylindrical) and flat (rectangular) specimens. The loading given to the curved and flat tissue specimens was uniform tensile extension which resulted the elongation of the specimens in axial direction (for curved specimen) and y-direction (for flat specimen). As there is no difference in the displacement components along the above-mentioned directions for a flat and cylindrical specimen, as inferred from the theoretical formulations, the fringes showed similarity in shape.

3.3.2 Out-of-plane configuration:

In the out-of-plane setup (Fig 3.6), light from the laser is coupled into a single mode fiber, which is then split into two using a 1 x 2-fiber beam splitter. One of the beams illuminates the object surface (object beam) while the other beam acts as the reference beam, the intensity of which is controlled using the attenuator. The reflected beam from the object surface combines with the reference beam at the beam combiner BC and is then captured by the CCD camera-zoom lens combination. The captured fringes are processed by the same system used for in-plane configuration. The tissue specimen is clamped along the top and bottom edges and subjected to loading in the out-of-plane direction.

Fig. 3.6 Experimental setup for out-of-plane displacement analysis of curved phantom tissue

(F: Optical fiber, FH: Fiber holder, BC: Beam combiner)

Let $\vec{k}_1$ and $\vec{k}_2$ be the illumination and imaging vectors.

$$\vec{k}_1 = -\mathbf{e}_a \sin \theta_i + \mathbf{e}_r \cos \theta_i \quad (3.24)$$

$$\vec{k}_2 = -\mathbf{e}_r \quad (3.25)$$

$$\begin{aligned} \Delta\phi &= \left(\frac{2\pi}{\lambda} \right) \left(\vec{k}_2 - \vec{k}_1 \right) \cdot \vec{L} \\ &= \left(\frac{2\pi}{\lambda} \right) \left[-(\mathbf{e}_r) - (-\mathbf{e}_a \sin \theta_i + \mathbf{e}_r \cos \theta_i) \right] \cdot \vec{L} \\ &= \left(\frac{2\pi}{\lambda} \right) \left[\mathbf{e}_a \sin \theta_i - \mathbf{e}_r (1 + \cos \theta_i) \right] \cdot \vec{L} \end{aligned} \quad (3.26)$$

$$\begin{aligned} \Delta\phi &= \left(\frac{2\pi}{\lambda} \right) \left[\mathbf{e}_a \sin \theta_i - \mathbf{e}_r (1 + \cos \theta_i) \right] \cdot \left[W \cos \left(\frac{ds}{2R(o)} \right) \mathbf{e}_\theta + w_a \mathbf{e}_a - W \sin \left(\frac{ds}{2R(o)} \right) \mathbf{e}_r \right] \\ &= \left(\frac{2\pi}{\lambda} \right) \left[w_a \sin \theta_i + W \sin \left(\frac{ds}{2R(o)} \right) (1 + \cos \theta_i) \right] \end{aligned} \quad (3.27)$$

which represents the phase change introduced due to curved surface displacement upon the application of an out-of-plane load.

Fig. 3.7 (a) represents the out-of-plane fringes obtained for the curved tissue phantom and

Fig. 3.7 (b) represents the simulated contours using finite element analysis.

Fig. 3.7 Out-of-plane displacement contours for a cylindrical tissue phantom specimen: (a) experimental fringe pattern (b) Simulated contours using FEM

Here the fringes are having concentric oval shape of varying thickness along axial direction at the specimen center region. This out-of-plane fringe pattern for the cylindrical tissue surface shows a significant difference in its shape compared to the out-of-plane fringes from a flat tissue surface, which has concentric circular shape (see Fig. 3.8 (a) for out-of-plane fringes obtained for a flat tissue surface and Fig. 3.8 (b) for its FEM simulated contours)

Fig. 3.8 Out-of-plane displacement contours for a rectangular tissue phantom specimen: (a) experimental fringe pattern (b) Simulated contours using FEM

Now, the phase change due to the introduction of displacement of a flat specimen, for the out-of-plane configuration is given by [35, 36]:

$$\Delta\phi = (2\pi / \lambda)[u \sin \theta_i + (1 + \cos \theta_i)w] \quad (3.28)$$

where w and u represent the specimen displacement component in the out-of-plane and in-plane direction.

Comparing equations (3.27) and (3.28) it is clear that the specimen curvature affects $\Delta\phi$ for the curved specimen and hence will affect the fringe shape. For the curved specimen, we concentrated only on the central region as the interrogation area because of the difficulty in illuminating the whole specimen.

3.3.3 Shear configuration:

For shear configuration, the experimental setup used is shown in Fig. 3.9. The laser light is coupled to a single mode fiber and is then allowed to illuminate the object. As a result of the laser interrogation with the diffuse object, a speckle pattern is formed. The speckle pattern formed in the object plane is sheared into two by means of a shearing element (Michelson interferometric configuration or Fresnel's biprism). The captured fringes are processed by the same system used for in-plane configuration. The combined speckle pattern (original and sheared one) is captured by the CCD camera, for the processing using the image processing card and the results are displayed on the PC monitor in real-time.

Fig. 3.9 Experimental setup for out-of-plane displacement derivative analysis of curved phantom tissue (F: Optical fiber, FH: Fiber holder)

In this configuration, one image plane point receives contributions from two object points and vice versa. Let the speckle pattern before object displacement is sheared by an amount $\Delta\theta_{p0}$ in the object plane as shown in Fig. 3.10.

Fig. 3.10 Representation of sheared points in the object plane

The shear in the image plane will be $M\Delta\theta_{p0}$ where M is the magnification of the imaging scheme. The co-ordinates of the two sheared points at the object plane are (θ_p, a_p, r_p) and $(\theta_p + \Delta\theta_{p0}, a_p, r_p)$.

The intensity of the resultant beam before object displacement, $I = I_1^2 + I_2^2 + 2(I_1 I_2)^{1/2} \cos \phi$ where $\phi = \phi_1 - \phi_2$, ϕ_1 and ϕ_2 being the phase corresponding to the sheared points. The intensity of the resultant beam after object displacement, $I_1^2 + I_2^2 + 2(I_1 I_2)^{1/2} \cos(\phi + \Delta\phi)$, where, $\Delta\phi = \Delta\phi_1 - \Delta\phi_2$, $\Delta\phi_1$ and $\Delta\phi_2$ being the phase change due to object displacement of two sheared points.

$$\Delta\phi_1 = \left(\frac{2\pi}{\lambda} \right) \left(\vec{k}_2 - \vec{k}_1 \right) \cdot \vec{L} \quad (3.29)$$

$$\Delta\phi_2 = \left(\frac{2\pi}{\lambda} \right) \left(\vec{k}_2 - \vec{k}_1 \right) \cdot \vec{L}' \quad (3.30)$$

where, $\vec{L}$ and $\vec{L}'$ are the displacement vectors at the sheared points.

$$\Delta\phi = \Delta\phi_1 - \Delta\phi_2 = \left(\frac{2\pi}{\lambda}\right) \left(\vec{k}_2 - \vec{k}_1 \right) \left(\vec{L} - \vec{L}' \right) \quad (3.31)$$

$$\Delta\phi =$$

$$\left(\frac{2\pi}{\lambda}\right) \left\{ [(-\mathbf{e}_r) - (-\mathbf{e}_a \sin \theta_i + \mathbf{e}_r \cos \theta_i)] \cdot \left[\left(\frac{dW}{d\theta}\right) \cos\left(\frac{ds}{2R(o)}\right) \mathbf{e}_\theta + \left(\frac{dw_a}{d\theta}\right) \mathbf{e}_a - \left(\frac{dW}{d\theta}\right) \sin\left(\frac{ds}{2R(o)}\right) \mathbf{e}_r \right] \cdot \Delta\theta_{p0} \right\} \quad (3.32)$$

$$\Delta\phi = \left(\frac{2\pi}{\lambda}\right) \left[\left(\frac{dw_a}{d\theta}\right) \sin \theta_i + \left(\frac{dW}{d\theta}\right) (1 + \cos \theta_i) \sin\left(\frac{ds}{2R(o)}\right) \right] \cdot \Delta\theta_{p0} \quad (3.33)$$

which gives a combined in plane and out of plane shear component expression.

Fig. 3.11 (a) represents the shear fringe pattern for the curved tissue phantom and Fig. 3.11(b) represents the corresponding simulated contours using FEM. The shear fringe pattern obtained with cylindrical tissue specimen resembled a “butterfly” in its shape. The simulated contours are found to be in agreement with the experimental fringe pattern.

(a)

(b)

Fig. 3.11 Displacement derivative contours for a cylindrical tissue phantom specimen: (a) experimental fringe pattern (b) Simulated contours using FEM

Now, the phase change due to the introduction of displacement of a flat specimen, for the shear configuration is given by [35, 36]:

$$\Delta\phi = \left(\frac{2\pi}{\lambda}\right) \left[\sin\theta_i \frac{du}{dx} + \frac{dw}{dx}(1 + \cos\theta_i) \right] \cdot \Delta x_0 \quad (3.34)$$

where Δx_0 is the amount of shear in the x direction and $\frac{dw}{dx}$ is the derivative of out-of-plane

displacement and $\frac{du}{dx}$ is derivative of the in-plane displacement for the flat specimen. Fig.

3.12(a) represents the shear fringe pattern for the flat specimen and Fig. 3.12(b) represents the corresponding FEM contours.

Fig. 3.12 Displacement derivative contours for a rectangular tissue phantom specimen: (a) experimental fringe pattern (b) Simulated contours using FEM

The qualitative difference observed in the fringe patterns between the shearographic fringes, obtained for the flat specimen and cylindrical specimen, could be explained using equations (3.33) and (3.34). The “butterfly type” shear fringes obtained for the cylindrical specimen (see Fig 3.11(a)) differ from the “double bull’s eye type” shear fringes (see Fig. 3.12(a)) obtained for flat specimen. The difference in the fringe shape was due to the curvature of the specimen satisfying equation (3.33).

The phantom colon had low stiffness value compared to normal composite or metal specimens, and so application of loads $< 5\mu\text{m}$ give rise to large number of fringes due to large displacement in the specimen. Here the load was given in terms of displacement ($\approx \text{few } \mu\text{m}$) to the phantom specimen. Similar experiments were also carried out for technical

cavities such as an aluminium cylinder to have a quantitative analysis regarding the number of fringes in cavity surfaces and the results are given in Appendix C.

3.4 Effect of random loading in the interferometric fringes

Considering the case of random loading, the shape of the interferometric fringes will change. In the case of medical cavities such as colorectal region of the human body, there may be natural stretching and compression effects, which give rise to such combined loading effects. A quantitative analysis of the random loading given to the phantom tissue was difficult because of the low stiffness value of the specimen. To illustrate the phenomenon, experiments were carried out on curved technical specimens, under combined in-plane and out-of-plane loading, yielding a quantitative analysis of the obtained fringes and the results are given in Appendix D.

3.5 Effect of illumination beam divergence

The theoretical formulation for the speckle interferometric fringes described in section 3.2 applies to a collimated beam illumination. In order to perform interferometry for the real time inspection of cavities, the interferometric configurations must be confined in an endoscope form. In this case, the illumination will be provided by fiber optic ports in the endoscope which are sources of divergent illumination (a more detailed analysis in this direction is discussed in Chapter 4). So, a modification of the above mentioned theory is required for incorporating divergent illumination concept, which is discussed below.

The illumination beam from an optical fiber end will be divergent. In the case of a divergent beam, the angle of illumination will no more remain a constant, as different object points are illuminated at different angles from the source. i.e., the illumination angle will be varying depending upon the object point under consideration. Consider a spherical wavefront as

shown in Fig. 3.13. Let the spherical wavefront be divided into '2n' number of points and the total divergence angle is β . Now, let W_0 represents the wavefront point which is lying in the optic axis where the illumination angle is zero and let W_n be the point in the wavefront, where the illumination angle is given by θ_n , which is equal to the half divergence angle of the beam. W_{n-1} is another point which is adjacent to W_n and the angular deviation of these two points is represented by $d\theta$. The illumination angle at W_{n-1} is given by θ_{n-1} .

Fig. 3.13 Representation of angle of divergence in a spherical wavefront

As stated above, if the total beam divergence angle is β , then

$$\theta_n = \frac{\beta}{2} \text{ and } \theta_0 = 0;$$

and

$$\theta_n - \theta_{n-1} = d\theta;$$

Now

$$d\theta = \frac{ds}{R(w)};$$

where $R(w)$ is the beam radius of curvature and ds is the elemental distance between W_n and W_{n-1} .

Now,

$$\theta_{n-1} = \theta_n - d\theta = \left(\frac{\beta}{2}\right) - \left(\frac{ds}{R(w)}\right);$$

Similarly,

$$\theta_{n-2} = \theta_n - 2d\theta = \left(\frac{\beta}{2}\right) - 2\left(\frac{ds}{R(w)}\right);$$

$$\therefore \theta_{n-m} = \left(\frac{\beta}{2}\right) - (m)\left(\frac{ds}{R(w)}\right) \quad (3.35)$$

where m is any arbitrary point in the wavefront.

Now, for all points on the wavefront that lies below the optic axis (from W_0 to W_n), the

beam angle at m^{th} point will be $-(\theta_{(n-m)}) = -\left[\left(\frac{\beta}{2}\right) - (m)\left(\frac{ds}{R(w)}\right)\right]$ (3.36)

If the object is a curved surface, which is having exactly the same curvature as the interrogating spherical wavefront, the expression of angle of illumination at different object points will be the same as equation 3.35 and 3.36. In this case, the expression for phase change due to object displacement represented by equations 3.27 and 3.33 will be modified respectively as:

$$\Delta\phi = \left(\frac{2\pi}{\lambda}\right) \left\{ w_a \sin \left[\left(\frac{\beta}{2}\right) - m \left(\frac{ds}{R(w)}\right) \right] + W \sin \left(\frac{ds}{2R(o)} \right) \left\{ 1 + \cos \left[\left(\frac{\beta}{2}\right) - m \left(\frac{ds}{R(w)}\right) \right] \right\} \right\} \quad (3.37)$$

(for out-of-plane configuration)

and

$$\Delta\phi = \left(\frac{2\pi}{\lambda}\right) \left\{ \left[\frac{dw_a}{d\theta} \right] \sin \left[\left(\frac{\beta}{2}\right) - m \left(\frac{ds}{R(w)}\right) \right] + \left[\frac{dW}{d\theta} \right] \left[1 + \cos \left[\left(\frac{\beta}{2}\right) - m \left(\frac{ds}{R(w)}\right) \right] \right] \sin \left(\frac{ds}{2R(o)} \right) \right\} \Delta\theta_{p0} \quad (3.38)$$

(for shear configuration)

If the object is curved and the object curvature is different from that of the beam, or if the object is flat, the above theoretical formulation will not be applicable. The fringe formation theory is modified for the above mentioned cases and is presented as below.

❖ Flat object and divergent illumination beam

If the object is flat, then the interrogation between the divergent spherical beam and the object will be as shown in Fig. 3.14. Let O_n be the point in the object which is in line with W_n . The object illumination angle at O_n is given by θ'_n . Let L be the distance between the object center from the beam center of curvature.

Fig. 3.14 Interrogation of a divergent illumination beam with a flat object surface

Now, from Fig. 3.14,

$$\tan \theta'_n = \frac{R(w) \sin(\frac{\beta}{2})}{L};$$

$$\therefore \theta'_n = \tan^{-1} \left(\frac{R(w) \sin(\frac{\beta}{2})}{L} \right);$$

Similarly,

$$\theta'_{n-1} = \tan^{-1} \left(\frac{R(w) \sin \theta_{n-1}}{L} \right);$$

$$\therefore \theta'_{n-m} = \tan^{-1} \left(\frac{R(w) \sin \theta_{n-m}}{L} \right); \text{ where } m \text{ is an arbitrary point on}$$

the object surface.

Substituting the value of θ_{n-m} for equation (3.35),

$$\therefore \theta'_{n-m} = \tan^{-1} \left(\frac{R(w) \sin \left[\left(\frac{\beta}{2} \right) - (m) \left(\frac{ds}{R(w)} \right) \right]}{L} \right) \quad (3.39)$$

In this case, the expression for phase change due to object displacement represented by equations (3.28) and (3.34) can be modified respectively as:

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left\{ u \sin \left[\tan^{-1} \left(\frac{R(w) \sin \phi}{L} \right) \right] + w \left[1 + \cos \left[\tan^{-1} \left(\frac{R(w) \sin \phi}{L} \right) \right] \right] \right\} \quad (3.40)$$

(for out-of-plane configuration)

and

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left\{ \left[\frac{du}{dx} \right] \sin \left[\tan^{-1} \left(\frac{R(w) \sin \phi}{L} \right) \right] + \left[\frac{dw}{dx} \right] \left[1 + \cos \left[\tan^{-1} \left(\frac{R(w) \sin \phi}{L} \right) \right] \right] \right\} \Delta x_0 \quad (3.41)$$

(for shear configuration)

$$\text{where } \phi = \left(\frac{\beta}{2} \right) - (m) \left(\frac{ds}{R(w)} \right)$$

❖ Curved object and divergent illumination beam

Consider a spherical beam interacting with a curved object surface as shown in Fig. 3.15 where the radius of curvature of the object is different from the radius of curvature of the spherical wave ($R(o) > R(w)$).

Let C_o and C_w be the center of curvatures of the object and the spherical wavefront respectively.

From Fig.3.15,

$$\sin \theta_o = \frac{R(w) \sin(\frac{\beta}{2})}{R(o)}$$

(3.42)

Fig. 3.15 Interrogation of spherical divergent beam with curved object surface ($R(o) > R(w)$)

Considering the triangle with sides $R(o)$, $(R(o) - L)$ and included angle (θ_o) , the unknown length x , which is the distance between the centre of curvature of the illumination beam and the object point under consideration (O_n) is given by,

$$x = \left[(R(o))^2 + (R(o) - L)^2 + 2R(o)(R(o) - L) \cos \left(\sin^{-1} \left(\frac{R(w) \sin(\frac{\beta}{2})}{R(o)} \right) \right) \right]^{1/2} \quad (3.43)$$

$$\text{Now, } \theta'_n = \sin^{-1} \left[\frac{R(w) \sin(\frac{\beta}{2})}{x} \right] \text{ or } \theta'_n = \sin^{-1} \left[\frac{R(w) \sin \theta_n}{x} \right]$$

$$\therefore \theta'_{n-m} = \sin^{-1} \left[\frac{R(w) \sin \theta_{n-m}}{x} \right], \text{ where m is any integer.}$$

Substituting the value of θ_{n-m} from equation (3.35),

$$\therefore \theta'_{n-m} = \sin^{-1} \left[\frac{R(w) \sin \left(\left(\frac{\beta}{2} \right) - (m) \frac{ds}{R(w)} \right)}{x} \right] \quad (3.44)$$

$$\text{where } x = \left[(R(o))^2 + (R(o) - L)^2 + 2R(o)(R(o) - L) \cos \left(\sin^{-1} \left(\frac{R(w) \sin(\theta_{(n-m)})}{R(o)} \right) \right) \right]^{1/2}$$

In this case, the expression for phase change due to object displacement represented by equations (3.27) and (3.33) can be modified respectively as:

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left\{ w_a \sin \left[\sin^{-1} \left(\frac{R(w) \sin \phi}{x} \right) \right] + W \sin \left(\frac{ds}{2R(o)} \right) \left[1 + \cos \left[\sin^{-1} \left(\frac{R(w) \sin \phi}{x} \right) \right] \right] \right\} \quad (3.45)$$

(for out-of-plane configuration)

and

$$\Delta\phi = \left(\frac{2\pi}{\lambda} \right) \left\{ \left[\frac{dw_a}{d\theta} \right] \sin \left[\sin^{-1} \left(\frac{R(w) \sin \phi}{x} \right) \right] + \left[\frac{dW}{d\theta} \right] \left[1 + \cos \left[\sin^{-1} \left(\frac{R(w) \sin \phi}{x} \right) \right] \right] \sin \left(\frac{ds}{2R(o)} \right) \right\} \Delta\theta_0 \quad (3.46)$$

(for shear configuration)

From the above theoretical formulations, it is evident that the beam divergence will definitely affect the nature of fringes in interferomeric configurations. Considering the

fringe formation in DSPI configurations, the use of divergent beam can give rise to an error factor due to the lack of a constant angle of illumination. Due to the change in illumination angle at different object points, the calculated $\Delta\phi$ for a divergent beam will differ from that for a collimated beam, which eventually give rise to a change in fringe width (fringe width obtained with divergent beam > fringe width obtained with collimated beam) under similar loading conditions. Thus with a divergent illumination beam, for a constant interrogation area, the resultant fringe pattern will consist of reduced number of fringes as compared with a collimated illumination beam. An illustrative demonstration of this phenomenon with out-of-plane configuration has been carried out. The studies are conducted using flat and curved technical specimens in order to yield the accurate load amount and the results are given in Appendix E.

3.6 DSPI and Shearography for NDT of curved tissue surfaces

In curved biological specimens, DSPI and shearography can offer a non-contact, non-destructive method for identifying the abnormal growths, which can be on the specimen surface or specimen subsurface and thus be an effective tool in the diagnosis of cancerous growths. This becomes feasible as the applications of speckle interferometric techniques on curved technical surfaces are already proven, which are referred in section 3.1. Works are also done as part of this project for analyzing the applicability of speckle shearographic methods for NDT of aluminium and aluminium-CFRP sandwiched composite cylindrical structures, results of which are given in the Appendix F.

However, little work has been reported regarding the applications of DSPI and shearography in biological specimens. In this context, investigations have been carried out in this project with such an approach employing a phantom tissue sample as the test specimen.

Abnormal growths were simulated in different layers of the phantom sample, the details of which are described in section 4.2 of Chapter 4. The experiments were carried out with in-plane, out-of-plane and shear configurations with the abnormal tissue specimen as the test target and the obtained results are analysed. Figures 3.16 – 3.18 represent the fringe patterns obtained for the phantom specimen with simulated abnormality, for in-plane, out-of-plane and shear configurations. A schematic representation of the abnormal tissue layers indicating the position of abnormality is shown along with the respective fringes as well as simulated results. In all the cases, the position of the abnormality can be identified by a kink in the respective fringe patterns. In simulated contours, the presence of abnormality is indicated by the delineation (deviation from normal) of the contours at the site of interest.

Fig. 3.16 In-plane displacement contours for the curved abnormal phantom tissue specimen (defect position encircled) ((a): Fringe pattern, (b) Simulated contours, (c): defect location)

Fig. 3.17 Out-of-plane displacement contours for the curved abnormal phantom tissue specimen (defect position encircled) ((a): Fringe pattern, (b) Simulated contours, (c): defect location)

Fig. 3.18 Out-of-plane displacement derivative contours for the curved abnormal phantom tissue specimen (defect position encircled) ((a): Fringe pattern, (b) Simulated contours, (c): defect location)

The concept of speckle interferometric techniques for inspection of cavity surfaces has been analyzed theoretically and experimentally. Although the fringe formation theory for curved object surfaces derived here is based on the conventional DSPI system, the theory will almost be the same with endoscopic interferometric configurations for curved surfaces. The only difference will be in considering the image fiber effect on the speckle pattern transmission which will be discussed in Chapter 4. The present study is concentrated on the NDT of phantom colon curved surfaces, which resembles the real sample in its surface and fluorophore content properties.

All the above-mentioned configurations are making use of bulk optics for imaging, thus making the whole system size large. The use of fiber optics finds exploring possibilities in the current world of miniaturization. The following chapter will be dealing with the miniaturization issues in speckle interferometric system by introducing an all fiber optic concept for illumination as well as for imaging. Also, such compact systems are important while considering their applications in intra cavity investigations of the otherwise normally inaccessible areas. While considering the endoscopic applications of DSPI, transmission of speckle patterns through fiber bundles and their associated issues become important. Next chapter will be dealing with all these topics with the proposed endoscope (endo-speckle-fluoroscope) which can function in multiple modalities for disease diagnosis.

CHAPTER 4

THE ENDO-SPECKLE-FLUOROSCOPE SYSTEM: DESIGN AND ANALYSIS

In this chapter, the design of the endo-speckle-fluoroscope (E-S-F) system incorporating the proposed concepts and techniques for the multiple modalities of imaging of colon such as normal imaging, speckle correlation analysis and fluorescence spectrum analysis for the diagnosis of cancer, will be discussed. The design details and dimensions of the E-S-F probe distal end and the selection of its constituent elements will be dealt in detail. A comprehensive theoretical analysis regarding the effect of illumination beam divergence in the fringe formation and the image fiber effect on the transmitted speckle patterns through the E-S-F probe, while using the system in the speckle correlation modality will be carried out. In the fluorescence spectrum analysis modality, a theoretical model involving the parameters of the curved colon surface and the developed E-S-F system will be discussed for analyzing the system efficiency for collecting the emitted fluorescence. Finally, the use of the E-S-F system in identifying the presence of growth or cancer employing all diagnostic modalities will be illustrated by using a phantom model of colon as the test specimen.

4.1 Design considerations of the E-S-F system

The design of the proposed E-S-F system was done based on the concept of multiple modality imaging which can provide a more accurate diagnosis of colon cancer. The whole system consists of 4 main elements namely, (i) the E-S-F probe (ii) the collection lens (iii) the Optical Component Selection Unit (OCSU) and (iv) the associated image processing and detection systems (the details of which are given in the following sections). Fig. 4.1 represents the schematic of the E-S-F system.

Fig. 4.1 Schematic of the E-S-F system

Light from the laser source is coupled into a sheathed single mode fiber (standard single mode fiber with protective covering), which is then split into two sheathed single mode

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

fibers via a 1 x 2 fiber splitter. The two sheathed fibers are connected to two bare fibers (with 1mm removable sheath) via connectors known as Finger Splices (FS). These bare fibers are then fed into the illumination channels of the E-S-F probe. This type of arrangement allows the easy plug-in and plug-out of the E-S-F probe and the source. The source light is delivered to the colon surface from the fiber ports at the distal end of E-S-F probe. The return light from the colon surface is collected by an imaging lens and is transmitted through an image fiber (Fujikura 15-600N). An objective lens placed at the proximal end of the probe collects the return image or light from the image fiber and directs them to the OCSU, where different constituent elements are employed to facilitate the multi-modality imaging. The output from the OCSU is directed to a normal imaging - speckle correlation analysis unit or fluorescence spectrum analysis unit depending on the employed imaging modality. Initially, the colon walls will be checked for visible abnormalities on the surface (normal imaging modality). Secondly, the colon walls will be subjected to speckle correlation analysis modality where surface abnormalities of millimeter order and subsurface abnormalities are diagnosed. If such abnormalities were found, the system will be switched to the final modality of fluorescence analysis where the intensity of the emitted light will be checked to diagnose the presence of cancer. The design of the 3 main sections of the system which is very important (as mentioned in the earlier portion of this section) for the proper working of the entire system is given below.

4.1.1 The E-S-F probe

An all fiber optic approach is adopted here for the E-S-F probe in order to facilitate the working of the system in multiple modalities. Single mode optical fibers were used to guide

the light from the laser source through the E-S-F body. The E-S-F probe and its constituent elements are shown in Fig. 4.2.

Fig. 4.2 The E-S-F probe

The probe consists of two illumination fibers for providing necessary illumination or excitation. An imaging lens-image fiber bundle combination is used as the imaging unit in the probe. The probe has rigid distal and proximal ends and a flexible part to facilitate the inspection inside body cavities, such as colon. The total length of the probe is designed to be of 140 cm to make sure the inspection of the colon up to cecum. In order to facilitate the complete inspection of the inner colon walls, a certain length of the probe can be bent. This helps to keep the probe to be located far and near from the colon tissue wall. When the probe is located far from the colon wall, the interrogated and imaged area will be more as compared to a probe position, which is near to the tissue wall. The latter will help for a close view of the colon and for a more accurate inspection. The total diameter of the end face is 10 mm excluding the biocompatible sleeve. The main considerations while designing the E-S-F probe were the selection of source, the design aspects of the probe distal end, the choice of the image fiber, and the selection of imaging lenses which will be discussed in the sections below.

4.1.1.1 The selection of source

The concept of a single source to provide necessary illumination or excitation of the colon tissue was crucial in order to have a miniaturized and less complicated system. Reported literature shows a history of fluorescence emission from the colon tissue in the UVIS region, a detailed survey on this is already given in Chapter 2. Lasers having wavelength $>700\text{nm}$ are not found to produce any significant fluorescence from the colon tissue. In the case of speckle correlation modality, the illumination source chosen should be able to produce necessary biospeckle, which depends mainly on the source wavelength and the tissue surface roughness. Lasers of wavelength below 500nm were not found to produce speckles from the tissue surface, while lasers of wavelength in the green-red region were found to interact with tissue to produce biospeckles. Also, the low-cost CCD detector that was integrated in the system had spectral sensitivity in the visible wavelength range.

Hence, a green diode pumped solid state (DPSS) laser ($\lambda = 532\text{nm}$) was selected as the source for the E-S-F system in order to generate biospeckles from the colon tissue as well as to excite some of the fluorophores contained in the colon tissue. With the difference in emitted intensities of these fluorophores, a diagnosis of presence of cancer was possible as is evident from the literature described in Chapter 2.

4.1.1.2 Design aspects of the E-S-F probe distal end

The E-S-F probe distal end consists of optical elements selected and integrated to meet the target objectives such as normal imaging, analysis with different speckle interferometric configurations and fluorescence emission collection and subsequent analysis. The dimensions of the distal end are as shown in Fig 4.3.

Fig. 4.3 Dimensions of the endoscope probe distal end

The distal end of the E-S-F probe consists mainly of two single mode fiber ports (1mm diameter) and an imaging port (3.1mm diameter) for carrying an imaging unit, which consists of an imaging lens-image fiber combination. The two fiber ports are intended to deliver the source light to the test target. In normal imaging modality, the use of dual illumination ports will enhance the total illumination light, thus resulting a brighter image. For speckle correlation modality, the use of dual illumination ports will enable the probe for in-plane sensitive configuration. For out-of-plane and shear configurations, illumination from one of the fiber ports is required, where the other one is disconnected accordingly. For the fluorescence spectrum analysis modality, the use of double illumination will enhance the interrogation area and also the excitation light delivered to the tissue so as to get considerable amount of emission from the concerned tissue. The two illumination fibers are oriented at equal angles from their respective optic axis, so as to make the center of the

illuminated or excited area in line with the imaging unit. The fibers are located at 1 mm from the probe outer covering. The distance of each illumination fiber center to the image fiber center at the probe distal end was fixed at 2.5mm.

4.1.1.3 Selection of image fiber

The choice of imaging elements forms the important part in an endoscope probe design. The design of the all fiber optic probe requires the images or return light to be guided through an image fiber to the probe proximal end. The selection of image fiber in the scheme is to increase the flexibility of the probe and to miniaturize the whole image collection scheme. More information on the image fibers and their applications can be seen in the literature [110,111]. The image fiber bundle has a double role to perform here, i.e. that of an image-conveying device and also as a fluorescence signal transmitting element. Image fibers differ in the number of individual fibers (fiberlets) and, and thus differ in their image resolution. An image fiber consisting of more number of pixels has better resolution compared to an image fiber with lesser number of pixels. However, the flexibility of the image fiber decreases as the number of individual fibers increase. So, a compromise between the flexibility and the image resolution is done while selecting the most appropriate image fiber for flexible fiberscopes. As a result of studies in this direction, a Fujikura image fiber bundle with 0.6mm diameter and 15,000 pixels is selected for the image and light transmission in the E-S-F probe. The selected image fiber is having sufficient flexibility to bend around. Below given is the microscopic image of the end face of the selected image fiber bundle (Fujikura FIGH-15-600N) taken under the microscope (Axiotech Reflected Light Microscope with SONY Hyper HAD camera) with a magnification of 100X (Fig. 4.4 (a)) and 200X (enlarged view- Fig 4.4 (b)).

Fig. 4.4 End face of the image fiber bundle (Fujikura FIGH-15-600N)

4.1.1.4 Selection of imaging lens

The imaging lens located at the distal end of the E-S-F probe helps in collecting the reflected image for normal imaging modality and speckle correlation modality as well as for collecting the emitted fluorescence in the fluorescence spectrum analysis modality. Among the different types of lenses, a ball lens with proper diameter was chosen as the imaging lens for the E-S-F probe, which gave advantages such as

- (i) Since the ball lens has perfect symmetry with the probe axis, the assembly of the imaging lens inside the probe was easy.
- (ii) Ball lenses possessed concentricity and excellent uniformity.

The selection of ball lens diameter depended on the diameter of the image fiber selected in the scheme. Use of lenses having same diameter as the image fiber resulted in barrel distortion of the image. An example of the above phenomenon is shown in Fig. 4.5. Figure 4.5 (a) represents the USAF resolution chart image taken employing an imaging lens with comparable size (0.7mm diameter) to the image fiber (0.6mm diameter) and Fig. 4.5(b) shows the USAF resolution chart image taken employing an imaging lens with bigger diameter (3mm) than the image fiber diameter (0.6mm).

(a) LD= 0.7mm

(b) LD=3mm

Fig. 4.5 Effect of imaging lens diameter in the transmitted USAF resolution chart image (LD: lens diameter)

It is to be mentioned that we have tried lenses with different diameters and found that bigger diameter lenses certainly increased the probe overall diameter. Hence after carefully considering these two aspects, a ball lens of 3mm diameter was chosen as the imaging lens in the E-S-F system.

4.1.2 Selection of collection lens

The reflected image and the emitted light were allowed to pass through a collection lens located outside the E-S-F probe. The choice of this lens was extremely important in the case of speckle correlation analysis, as all the pixels of the image fiber were to be resolved by this lens for maximum fringe contrast. The collection lens used here was Mitutoyo infinity corrected 20X objective with 20mm working distance.

4.1.3 The OCSU

The OCSU that is positioned at the output of the E-S-F probe after the collection lens is meant for the speckle correlation analysis modality of diagnosis (for switching between out-of-plane and shear configurations of speckle interferometry). Two optical elements (a biprism and a beam combiner) are fixed inside the holder (see Fig. 4.6) which is provided with a rotating mechanism. The selection of beam combiner is meant for out-of-plane configuration of speckle interferometry and the choice of biprism is for the shear configuration. A detailed description about the function of OCSU in the E-S-F system during

different modalities of diagnosis is given at the respective pages. It is to be mentioned that at the moment, OCSU is in the manual mode and the automation of the same will be the future work.

Fig. 4.6 Schematic of the OCSU

4.1.5 Normal imaging and speckle correlation analysis system

For normal imaging modality, the OCSU output is directed towards a CCD camera, which is connected to a PC installed with a framegrabber (EDC-2000N computer camera system: see Appendix A). The images of the test target (phantom colon surface) were seen on the PC monitor with the help of developed windows based software. For speckle correlation analysis, the software allowed subtractive correlation of frames corresponding to the deformed and undeformed state of object.

4.1.6 Fluorescence spectrum analysis system

The E-S-F system was first operated in the normal and speckle correlation analysis modality to detect the presence of abnormalities or suspicious cancerous growths. In case of such findings, the whole system was switched to the final diagnostic modality, i.e., fluorescence spectrum analysis. In this modality, the collection lens output is directed towards a monochromator system (Dongwoo Optron, DM 150, details are given in Appendix G),

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

where the OCSU output is scanned according to the set resolution of the monochromator. This scanned output is allowed to pass through a PMT (Hamamatsu R928, for details, pls. refer Appendix H). The PMT output signal is connected to the system interface card installed in a PC. The fluorescence spectrum (emission intensity Vs emission wavelength) is displayed on the PC monitor with the help of related software. The diagnosis of cancerous regions in the colon tissue was made by looking into the difference in emitted intensity of normal and cancerous regions. The experimental investigation using the developed probe as per the design (described in section 4.1) is carried out on the phantom colon model, the preparation of which is discussed below.

4.2 Preparation of the phantom colon tissue model

The phantom colon model (purchased from Buy-A-Mag Corporation [112]) which resembles the human colon was used as the test specimen. Photograph of the phantom colon model is given in Appendix I. At different locations inside this phantom colon, layers of phantom tissues were pasted, which were obtained from Simulab corporation [113]. The tissue layers were having a thickness, each equal to the layer thickness of the real colon (approximately 1mm) [114].

❖ Simulation of fluorophores in the normal colon phantom

Each layer of the tissue was stained with fluorescent dye (Rhodamine 6G, Biochemica for fluorescence from Sigma-Aldrich) that has emission wavelength in the range of 540-550nm for a 532nm excitation. This emission range was exhibited by natural fluorophores contained in the real colon, the details of which are already given in Chapter 2. The tissue was stained using the respective dyes as per the standard procedures. Care has been taken that the surface is not changed due to staining. To summarise, the staining of tissue layers with the selected

Chapter 4
The endo-speckle-fluoroscope system: Design and analysis

fluorescent dye was done based on 3 criterions, (i) The solubility of the fluorescent dye should be maximum in the selected solvent, (ii) the reaction of the tissue with the solvent should be minimum so that tissue surface properties should not be changed after the staining procedure and (iii) the dye should be adsorbed into the tissue. The interaction of the tissue with different solvents such as Chloroform, Ethyl Acetate, Aceto Nitrile, Tetra Hydro Furan (THF), Acetone, Hexane, Ethanol, Toluene, Methanol and H₂O was tested. From the experimental results, Acetone was selected as the solvent for the fluorescent dye (Rhodamine 6G) satisfying the above 3 conditions. A detailed explanation of the experiments carried out for the selection of the appropriate solvent from the number of different solvents is given in Appendix J.

Each tissue layer was dipped into the staining solution (1mg Rhodamine 6G in 10ml Acetone) for necessary staining before pasting them together. The pasted tissue layers are introduced into the phantom colon model as explained in the beginning portion of this section.

❖ Simulation of cancerous growths

Phantom tissue material representing cancerous growth was introduced between the 1st and 2nd tissue layers. The size of the tissue material to represent the cancerous growth was chosen to be either 1mm (to represent small growth) or 3mm (to represent larger growth). The 1st and 2nd layers were chosen to simulate abnormality because the cancer at the early stage is confined to these layers.

For experiments with E-S-F probe, after introducing the abnormality, the layers are pasted together, and introduced into the phantom model. The abnormal tissue parts were dipped in a staining solution with less fluorescent dye concentration (1mg Rhodamine 6G in 20ml Acetone) to represent real cancerous tissues having emission in the same wavelength range.

Photograph of normal and cancerous tissue layer, stained with fluorescent dye as described above, are provided in appendix K.

The experimental analysis with the developed probe working in multiple modalities is discussed the next section. Also, given are the theoretical aspects of speckle correlation analysis and fluorescence emission collection analysis with the developed probe when the curved colon model was used as the test specimen.

4.3 E-S-F system in the normal imaging modality

In the normal imaging modality, the two illumination fibers in the E-S-F probe are used for providing necessary illumination for the inner walls of phantom tissue model, and the imaging unit in the probe images different parts of the phantom colon model, to check for the presence of cancerous growths. In this process, all the visible cancerous growths protruding from the colon wall (inherent in the phantom model) were identified. Fig. 4.7 (a) shows the image of the normal phantom colon taken using the developed E-S-F system. The phantom colon image with cancerous growth taken using the probe is illustrated in Fig. 4.7 (b). Here, the protrusions from the colon wall image indicate the presence of cancerous growths (polyps).

Fig. 4.7 Images of the colon wall obtained using the E-S-F system in the normal imaging modality

4.4 E-S-F system in the speckle correlation modality

The E-S-F system, which was developed as per the design was able to perform all the three configurations of speckle interferometry such as, in-plane, out-of-plane and shear configurations. In this way, the analysis of deformation of the phantom colon wall occurring in the in-plane as well as the out-of-plane directions was made possible. Also, the abnormality growth detection was possible in all the configurations. For all the aforementioned configurations, the reflected speckle pattern from the tissue wall is to be transmitted through the image fiber confined within the E-S-F probe, before it is fed to the CCD for further processing.

Transmitting speckle pattern through the image fiber involves quite number of research issues such as the integration of transmitted speckle patterns, visibility of fringes etc [115-117]. While considering speckle correlation interferometry using fiber bundles, a theoretical analysis on the statistics of transmitted speckle patterns through the image fiber becomes important. Also, the spatial resolution of the image fiber is directly related to the quality of correlation fringes and influences their visibility.

4.4.1 Image fiber effect in the visibility of correlation fringes obtained using the E-S-F system

It has been mentioned earlier in Chapter 3 that the brightness of DSPI fringes is proportional to the intensity and phase of two interfering beams and also the phase change introduced due to the object displacement (refer equation 3.5). In this case, the ensemble average intensity distribution of the correlation fringes (using subtractive correlation) can be represented as:

$$I'' = \left\langle (I - I')^2 \right\rangle^{1/2} \quad (4.1)$$

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

Assuming a circular Gaussian distribution to the speckle field intensities at the detector plane [116],

$$\langle I^2 \rangle = 2\langle I \rangle^2 \quad (4.2)$$

$$\langle I'^2 \rangle = 2\langle I' \rangle^2 \quad (4.3)$$

$$\langle I I' \rangle = \langle I \rangle \langle I' \rangle (1 + |\mu|^2) \quad (4.4)$$

where μ is the normalized cross correlation function of the speckle fields at the detector plane before and after the object displacement. So, from equation (4.1),

$$I'' = \sqrt{2} \langle I \rangle (1 - |\mu|^2)^{1/2} \quad (4.5)$$

Assume equal average intensity of speckle fields before and after object displacement, i.e.,

$$\langle I \rangle = \langle I' \rangle = \langle I \rangle \quad [117].$$

So, from equation (4.5), it can be seen that the intensity maxima is obtained at points where there is no correlation ($\mu = 0$) between the speckle patterns.

i.e.,
$$I''_{\max} = \sqrt{2} \langle I \rangle \quad (4.6)$$

Intensities lower than $I''_{\max}$ appear at points having greater correlation values. Minimum intensity will occur when the correlation is maximum ($\mu_{\max}$).

i.e.,
$$I''_{\min} = \sqrt{2} \langle I \rangle (1 - |\mu_{\max}|^2)^{1/2} \quad (4.7)$$

Speckle fringes are obtained, by applying subtractive correlation to the intensities before and after object displacement. Now the fringe visibility, V can be expressed as:

$$V = \left(\frac{I''_{\max} - I''_{\min}}{I''_{\max} + I''_{\min}} \right) \quad (4.8)$$

From equations (4.6 - 4.8),

$$V = \frac{\left(1 - \left(1 - |\mu_{\max}|^2\right)^{1/2}\right)}{\left(1 + \left(1 - |\mu_{\max}|^2\right)^{1/2}\right)} \quad (4.9)$$

In the case of transmission of speckled images through the image fiber, the intensity averaged over the input face of an elementary fiber located at x_i can be expressed as [115]:

$$I_{x_i} = \frac{1}{A} \int s(x - x_i) I(x) dx \quad (4.10)$$

where $s(x)$ is the intensity transmittance function and A is the effective core area of an elementary fiber, which is given by:

$$A = \int s(x) dx \quad (4.11)$$

The average intensity distribution at the output end of the elementary fiber can be expressed as [115]:

$$\langle I_o(X) \rangle = \frac{1}{A} \sum_{i=1}^N F(X - x_i) \int s(x - x_i) \langle I(x) \rangle dx \quad (4.12)$$

where N is the number of elementary fibers in the image fiber bundle and $F(X)$ is the aperture function across the output face in a single elementary fiber.

Let x_1 and x_2 represent the respective positions of a considered object point before and after object displacement. Let x_i be the elementary fiber position corresponding to object point position x_1 and let x_j be the elementary fiber position corresponding to object point position x_2 . Schematic representation of x_1 , x_2 , x_i and x_j are given in Fig. 4.8.

Fig. 4.8 Schematic representation of object points as imaged by image fiber pixels

Now the cross correlation function of the intensities observed at the image fiber output before and after the object displacement is given by:

$$\langle I_{o1}(X)I_{o2}(X) \rangle = \left(\frac{1}{A} \right)^2 \sum_{i=1}^N \sum_{j=1}^N F(X - x_i)F(X - x_j) \iint s(x_1 - x_i)s(x_2 - x_j) \langle I_1(x_1)I_2(x_2) \rangle dx_1 dx_2 \quad (4.13)$$

Now, the ensemble average distribution of the correlation fringes (with the use of image fiber) can be expressed as

$$I_o(X) = \left(\langle (I_{o1}(X) - I_{o2}(X))^2 \rangle \right)^{1/2} \quad (4.14)$$

Following equation (4.4) and by assuming a circular Gaussian distribution to the speckle field intensities at the detector plane,

$$\langle I_{o1}(X)I_{o2}(X) \rangle = \langle I_{o1}(X) \rangle \langle I_{o2}(X) \rangle \left(1 + |\mu(X)|^2 \right) \quad (4.15)$$

where $\mu(X)$ is the normalized cross correlation function of the speckle fields at the image fiber output before and after the object displacement.

Assuming equal average intensity of speckle fields before and after object displacement, and following equations (4.5 – 4.8),

The visibility of fringes at the image fiber output can be expressed as

$$V' = \frac{\left(1 - \left(1 - |\mu_{\max}(X)|^2\right)^{1/2}\right)}{\left(1 + \left(1 - |\mu_{\max}(X)|^2\right)^{1/2}\right)} \quad (4.16)$$

where $\mu_{\max}(X)$ is the maximum value of the normalized cross correlation function of the speckle fields before and after object displacement while employing an image fiber for speckle pattern transmission. The visibility of fringes in this case depends on $\mu_{\max}(X)$ which in turn depends upon several parameters of the image fiber such as the number of elementary fibers, aperture function and position of the individual elementary fibers etc according to equations (4.12) and (4.13). Thus, equation (4.16) indicates that the image fiber does affect the interference fringe visibility, due to the speckle pattern transmission through the image fiber.

4.4.2 Concept of divergent illumination beams in the E-S-F system

To have a complete theoretical formulation on the fringes formed on the curved phantom tissue wall using the developed E-S-F system, the effect of the illumination beams and the geometry of the object surface are also needed to be considered. An analysis in this direction will be presented below for each speckle interferometric configuration.

As mentioned earlier in Chapter 3 (section 3.5), when the DSPI configurations are confined in endoscope form, to illuminate a large object area, the illuminations should be divergent. Even for oblique illumination, the use of small collimating lenses (restricted by the probe maximum diameter) will result in illuminating only a small area. Thus the area imaged under normal imaging will be small, which makes the process of diagnosis much time-consuming. Also, in the case of speckle correlation analysis, only fractions of a fringe could be seen with this reduced imaged area, which will again make the process of diagnosis difficult. Hence, the derived theory will be concentrating on divergent illumination beams only.

4.4.3 E-S-F system in the in-plane configuration

The schematic diagram of the E-S-F system in the in-plane configuration is shown in Fig. 4.9 (a).

Fig. 4.9 (a) E-S-F system in the in-plane configuration

In this configuration, the laser (at $\lambda=532\text{nm}$) is split into two using a cube beam splitter attached to the laser unit. Each of the split beams is coupled into a single mode fiber. These single mode fibers are connected to the illumination fiber ports of the E-S-F probe using finger splices, as discussed in section 4.1. Illumination directions of the two illumination ports of the E-S-F probe are shown in green color (see Fig. 4.9). The reflected speckle pattern is collected by the imaging lens (ball lens) and transmitted through the image fiber. A collection lens (Mitutoyo infinity corrected 20X objective) after the probe proximal end collects the speckle pattern transmitted through the image fiber. This collected speckle pattern is allowed to fall on a CCD placed behind the collection lens. A photograph of the experimental setup is given in Fig. 4.9 (b). The photograph of the probe is shown separately in Fig. 4.9 (c).

Fig. 4.9 (b) Experimental setup for the E-S-F system to operate in the in-plane configuration (c) A complete view of the probe

The image or speckle pattern is digitized by the CCD and fed to the computer installed with the frame grabber. The speckle pattern is stored in a frame buffer and displayed in the PC monitor in real-time with the help of developed software.

Frame corresponding to the undeformed state of the phantom colon model is stored as the reference frame. The object was subjected to minute in-plane displacement and the frames corresponding to the deformed state of the object were subtracted from the first frame at the rate of 25 frames per second. In this process, straight line fringes are seen to be evolving in real time.

As the orientation of the illumination fibers are symmetrical, the phase difference introduced due to the object displacement can be written in this case (Pls. refer equation 3.21) as

$$\Delta\phi_{e-s-f(in)} = \left(\frac{2\pi}{\lambda} \right) \left(2w_{cma} \sin \theta_{fi(in)} \right) \quad (4.17)$$

where $\theta_{fi(in)}$ is the effective illumination angle at the considered object point, with the use of the divergent illumination beam. The phantom colon model deformation in axial direction is given by w_{cma} . The expression for $\theta_{fi(in)}$ can be derived as below.

Consider the distal end of the E-S-F probe oriented normal to the colon wall (Fig. 4.10).

Fig. 4.10 Interrogation of E-S-F probe diverging illumination beams with curved colon surface

Let the object be divided into $2n$ number of points, within the interrogation area. Consider an arbitrary point O_{n-m} on the curved colon. From Fig. 4.10, the angle of illumination at O_{n-m} due to illumination 1,

$$\theta'_{1(n-m)} = \sin^{-1} \left[\frac{R(w) \sin\left(\frac{\beta}{2}\right)}{r_1} \right] \quad (4.18)$$

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

where r_1 is the distance of illumination port 1 and the considered point $\mathbf{O}_{n-m}$.

From triangle $C_{w1} C_{cm} \mathbf{O}_{n-m}$,

$$r_1 = \left[(R_{cm}(o))^2 + (C_{cm} C_{w1})^2 + 2R_{cm}(o)(C_{cm} C_{w1}) \cos(\angle C_{w1} C_{cm} \mathbf{O}_{n-m}) \right]^{1/2} \quad (4.19)$$

where C_{w1} is the center of curvature of illumination is beam 1 and C_{cm} is the phantom colon center of curvature. Here, $R_{cm}(o)$ represents the radius of curvature of the colon.

Now, $\angle C_{w1} C_{cm} \mathbf{O}_{n-m} = \angle C_{w1} C_{cm} C - \angle \mathbf{O}_{n-m} C_{cm} M$ where C is the center of the probe and M is the point of intersection between the E-S-F probe axis and the curved colon surface.

$$\text{Now, } \angle C_{w1} C_{cm} \mathbf{O}_{n-m} = \tan^{-1} \left(\frac{C_{w1} C}{C_{cm} C} \right) - \sin^{-1} \left(\frac{l - R(w) \sin\left(\frac{\beta}{2}\right)}{R_{cm}(o)} \right) \quad (4.20)$$

where l is the distance between one illumination port and the center of the probe.

$$\theta'_{l(n-m)} = \sin^{-1} \left[\frac{R(w) \sin\left(\frac{\beta}{2}\right)}{\left[(R_{cm}(o))^2 + (C_{cm} C_{w1})^2 + 2R_{cm}(o)(C_{cm} C_{w1}) \cos(\angle C_{w1} C_{cm} \mathbf{O}_{n-m}) \right]^{1/2}} \right] \quad (4.21)$$

Or in a more generalized form, considering m^{th} point in the colon surface,

$$\theta'_{l(n-m)} = \sin^{-1} \left[\frac{R(w) \sin\left(\theta_{(n-m)}\right)}{\left[(R_{cm}(o))^2 + (C_{cm} C_{w1})^2 + 2R_{cm}(o)(C_{cm} C_{w1}) \cos(\angle C_{w1} C_{cm} \mathbf{O}_{n-m}) \right]^{1/2}} \right] \quad (4.22)$$

Now, $\theta_{n-m} = \left(\frac{\beta}{2}\right) - (m) \left(\frac{ds}{R(w)}\right)$, according to equation 3.35 (Chapter 3).

Similarly, the illumination angle at $\mathbf{O}_{n-m}$ due to illumination 2, $\theta'_{2(n-m)}$ can be expressed as

$$\theta'_{2(n-m)} = \sin^{-1} \left[\frac{2l - R(w) \sin(\theta_{(n-m)})}{r_2} \right] \quad (4.23)$$

where r_2 is the distance of illumination port 2 and the considered point $\mathbf{O}_{n-m}$.

$$\theta'_{2(n-m)} = \sin^{-1} \left[\frac{2l - R(w) \sin(\theta_{(n-m)})}{\left[(R_{cm}(o))^2 + (C_{cm}C_{w2})^2 + 2R_{cm}(o)(C_{cm}C_{w2}) \cos(\angle C_{w2}C_{cm}\mathbf{O}_{n-m}) \right]^{1/2}} \right] \quad (4.24)$$

where $\angle C_{w2}C_{cm}\mathbf{O}_{n-m} = \tan^{-1} \left(\frac{C_{w2}C}{C_{cm}C} \right) + \sin^{-1} \left(\frac{l - R(w) \sin(\theta_{(n-m)})}{R_{cm}(o)} \right)$ (4.25)

The effective illumination angle,

$$\theta_{fi(in)} = \theta'_{1(n-m)} + \theta'_{2(n-m)} \quad (4.26)$$

Thus the modified equation for $\Delta\phi$ will be $\Delta\phi_{e-s-fi(in)} = \left(\frac{2\pi}{\lambda} \right) \left(2w_{cma} \sin \theta_{fi(in)} \right)$ as stated in

equation (4.17).

Experiments were carried out with the same setup for normal and cancerous colon phantoms (the methods of preparation are given in section 4.2) and the obtained results are shown in Fig. 4.11 and Fig.4.12. Fig. 4.11 represents the fringe pattern for a normal colon phantom, where the fringes followed straight line nature.

Fig. 4.11 In-plane fringes obtained from the normal phantom colon surface using the E-S-F system

Cancerous growths of different sizes were simulated as explained in section 4.2. Fig. 4.12 shows the fringe patterns obtained for the cancerous colon phantom (simulated growth size 3mm).

Abnormality

(a)

Abnormality

(b)

Fig. 4.12 In-plane fringes obtained from the cancerous phantom colon surface using the E-S-F system

Fig. 4.12(a) and Fig. 4.12 (b) show the in-plane fringe patterns obtained using E-S-F system, where Fig. 4.12 (b) is a closer view obtained as the endoscope is moved towards the colon

surface. In this configuration, growth size less than 3mm was difficult to identify. Smaller abnormalities required more number of in-plane fringes to be formed to get them identified as a kink, upon increased loading, which eventually decorrelated the whole interference fringes.

4.4.4 E-S-F system in the out-of-plane configuration

The schematic diagram of the E-S-F system in the in-plane configuration is shown in Fig. 4.13.

Fig. 4.13 E-S-F system in the out-of-plane configuration

In this configuration, only one of the fiber ports is used for illuminating the curved colon phantom surface. The reflected speckle pattern is collected by the ball lens and transmitted through the image fiber. The collection lens after the probe proximal end collects the speckle pattern transmitted through the image fiber. This collected speckle pattern is combined with the reference beam (supplied by the other illumination fiber connected to the laser) at the

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

beam combiner fixed in the OCSU. The reference beam is attenuated before it reaches the beam combiner using an attenuator (Variable ND filter). The reference beam combined with the reflected image is then captured by the CCD placed behind the beam combiner. The rest of the image processing procedures were exactly similar to that explained for the in-plane configuration, in section 4.4.3. The experimental setup is similar to the one shown in Fig. 4.9(b) except for change of elements after the collection lens and is given in Appendix L.

In this case, the phase change due to the object displacement can be represented (refer to equation 3.27) as:

$$\Delta\phi_{e-s-f(out)} = \left(\frac{2\pi}{\lambda} \right) \left[w_{cma} \sin \theta_{fi(out)} + W_{cm} \sin \left(\frac{ds_{cm}}{2R_{cm}(o)} \right) (1 + \cos \theta_{fi(out)}) \right] \quad (4.27)$$

where $W_{cm} \sin \left(\frac{ds_{cm}}{2R_{cm}(o)} \right)$ is the deformation of the phantom colon model in the radial direction. Since, the illumination beam is divergent, as explained in section 4.3.3, $\theta_{fi(out)}$ is no more a constant, and can be expressed as

$$\theta_{fi(out)} = \sin^{-1} \left[\frac{R(w) \sin(\theta_{(n-m)})}{\left[(R_{cm}(o))^2 + (C_{cm}C_{w1})^2 + 2R_{cm}(o)(C_{cm}C_{w1}) \cos(\angle C_{w1}C_{cm}O_{n-m}) \right]^{1/2}} \right] \quad (4.28)$$

where, $\theta_{n-m} = \left(\frac{\beta}{2} \right) - (m) \left(\frac{ds}{R(w)} \right)$ as per equation (3.35).

Experimental results of E-S-F system in the out-of-plane configuration with normal and abnormal colon phantoms are shown in Fig. 4.14 and Fig. 4.15. Fig. 4.14 represents the fringe pattern for a normal colon phantom with the E-S-F system in the out-of-plane configuration, where the fringes followed concentric oval shape.

Fig. 4.14 Out-of-plane fringes obtained from the normal colon phantom model

Fig. 4.15 shows the fringe pattern obtained for the cancerous tissue prepared as per section 4.2. The size of the abnormality used was 3mm. Fig. 4.15(b) shows the out-of-plane fringes obtained when the probe was kept closer to the object. In Fig. 4.15(a) and Fig. 4.15(b), the presence of simulated cancer growth is indicated by a kink in the fringe pattern.

Fig. 4.15 Out-of-plane fringes obtained from the cancerous colon phantom model

In this configuration, growth size less than 3mm was difficult to be identified. Similar to the case of in-plane configuration, here also, smaller abnormalities required more number of out-of-plane fringes to be formed to get them identified as a kink, upon increased loading, which eventually decorrelated the whole interference fringes.

4.4.5 E-S-F system in the shear configuration

The schematic diagram of the E-S-F system in the in-plane configuration is shown in Fig. 4.16.

Fig. 4.16 E-S-F system in the shear configuration

In this configuration, one of the fiber ports is used for illuminating the curved phantom surface, similar to that for the out-of-plane configuration. The reflected speckle pattern is collected by the ball lens and transmitted through the image fiber. The collection lens after the probe proximal end collects the speckle pattern transmitted through the image fiber. This collected speckle pattern is sheared into two by means of a biprism (1 degree wedge angle) fixed in the OCSU. The sheared speckle patterns are allowed to fall on a CCD placed directly behind the biprism. The rest of the image processing procedures were exactly similar to that explained for the in-plane configuration, in section 4.4.3. The experimental setup is

similar to the one shown in Fig. 4.9(b) except for change of elements after the collection lens and is given in Appendix M.

In this case, the phase change due to the object displacement can be represented (refer equation (3.33) as:

$$\Delta\phi_{e-s-f(\text{shear})} = \left(\frac{2\pi}{\lambda}\right) \left[\left(\frac{dw_{\text{cma}}}{d\theta_{\text{cm}}}\right) \sin\theta_{\text{fi(out)}} + \left(\frac{dW_{\text{cm}}}{d\theta_{\text{cm}}}\right) \sin\left(\frac{ds_{\text{cm}}}{2R_{\text{cm}}(o)}\right) (1 + \cos\theta_{\text{fi(out)}}) \right] \cdot \Delta\theta_{\text{cm0}} \quad (4.29)$$

where $\Delta\theta_{\text{cm0}}$ represents the object shear in the tangential direction. The illumination angle $\theta_{\text{fi(out)}}$ is given by equation (4.28).

Experimental results of E-S-F system in the shear configuration with normal and cancerous colon phantoms are shown in Fig. 4.17 and Fig. 4.18. Fig. 4.17 (a) represents a full butterfly shear fringe pattern (simulated contours). Fig. 4.17 (b) represents the center region of the butterfly pattern as shown zoomed in for better clarity. This central region is imaged by the E-S-F probe, and the shear fringe pattern obtained corresponding to the central region is shown in Fig. 4.17 (c).

Fig. 4.17 Shear fringe pattern obtained for a normal colon phantom ((a): Finite element model representing the whole butterfly pattern, (b): Finite element model representing the central region; (c): Fringe pattern obtained for the central region)

The E-S-F system in the shear configuration was tested with the cancerous tissue prepared as per section 4.2. Fig. 4.18 shows the shear fringe pattern obtained for the cancerous colon phantom (cancer growth size 1mm).

Fig. 4.18 Shear fringe patterns obtained for cancerous colon phantom shown successively in the direction of increased loading (c) fringe pattern with less number of contours, (d) fringe pattern with more number of contours, (e) development of kink at the defect site (f) the transformation of the kink into a separate shear fringe pattern, (g) a fully developed shear fringe pattern at the defect site.

Here also, the image area of the E-S-F probe is the center region of the whole butterfly pattern as indicated by Fig. 4.18 (a). A zoomed image of this center portion along with the position of the simulated cancerous growth is shown in Fig. 4.18 (b). The corresponding shear fringe patterns are shown in Fig. 4.18 (c) - (h) in the order of increase of applied load. Here the presence of abnormality is indicated either by a kink (Fig. 4.18 (f) and Fig. 4.18 (e)) or separate fringe system (Fig. 4.18 (h)) obtained by increasing the applied load. The various stages of development of the separate fringe system at the defect site are indicated in Fig. 4.17(c)-(h).

In the shear configuration, the presence of abnormality created a separate fringe system upon the decorrelation of the original fringe system, as the applied load is kept increasing, irrespective of the defect size. In this way, identification of smaller abnormalities ($\approx 1\text{mm}$) were possible with the E-S-F system in the shear configuration. It is to be noted that, the increase of load resulted decorrelation of fringe patterns in out-of-plane as well as in-plane configurations. Since the defect was represented as a kink in the original fringe system, decorrelation of the same made the defect identification impossible in these configurations.

Now, regarding the thickness of abnormalities, abnormalities of very small thickness such as 1mm representing an early growth were detectable by all the endoscopic DSPI configurations. As early stages of flat adenomas, are of small thickness and large sizes, the identification of them can be done by using either of the three DSPI configurations. The identification of initial stages of development of a polyp will be enhanced by employing a shear configuration, as it can identify abnormalities of smaller sizes as compared to in-plane and out-of-plane configurations.

A comparison of in-plane, out-of-plane and shear configurations based on their abnormality detection capability is given in Table 4.1.

DSPI configurations	In-plane	Out-of-plane	Shear
Representation of abnormality	Kink in the fringe system	Kink in the fringe system	Kink in the fringe system or Separate fringe system at the defect site
Surface or subsurface defect (size $\geq 3\text{mm}$; thickness 1mm)	●	●	●
Surface or subsurface defect (size $< 3\text{mm}$; thickness 1mm)	●	●	●
Diagnosis	Early stages of flat adenoma	Early stages of flat adenoma	Early stages of flat adenoma and polyp

Table 4.1 Comparison of endoscopic DSPI configurations for abnormality detection

(● Detectable ● Not-detectable)

It is to be noted that, all the experiments have been conducted here using the phantom tissues and phantom colon models that resemble the real colon cavity. However, in real clinical trials, any possible effects due to the presence of wet tissues should be analyzed. These aspects will be discussed in detail as part of the future work in Chapter 6.

If the presence of an abnormality is detected in any of the in-plane, out-of-plane or shear fringes, the E-S-F system is switched into the 3rd and final diagnostic modality, fluorescence spectrum analysis modality. In the next section, a comprehensive theoretical analysis on the performance of the E-S-F system in fluorescence spectrum analysis modality is discussed.

4.5 E-S-F system in the fluorescence spectrum analysis modality

The analysis on the performance of the E-S-F system in the fluorescence spectrum collection modality requires information regarding the parameters such as excitation energy, distance between the E-S-F distal end to the tissue surface, spatial distribution of the image intensity etc. for better quantitative imaging of the biological tissue. Exact information and knowledge regarding the excitation energy is needed for deciding its optimum level as too much energy causes tissue damage and too little energy gives only undetectable signals. This also determines the closeness of the distal end to the tissue wall. The power density at the output illumination ports of the E-S-F probe are $< 10 - 15\text{mW per cm}^2$ (variable), which is

well below the reported power density for the in-vivo diagnostics of the colon [96]. As absorption coefficient is a dependant parameter with wavelength, use of different excitation wavelengths requires different optimum excitation energy level for better performance. By considering all these points, a theoretical model is developed here for the E-S-F system to determine the relationship between various fluorescence imaging parameters.

Let the distal end of the E-S-F probe is oriented at normal incidence to the curved tissue wall at a distance d as shown in Fig 4.19. The necessary excitation light for generating fluorescence emission from the tissue is delivered through the two fiber ports in the E-S-F distal end.

Consider the points in the curved wall each in different planes, having a fixed distance from the tissue surface point intersecting the optic axis of the E-S-F probe. Let $\mathbf{O}$ be such a point with distance δ from the plane containing the curved tissue point that lies in the optic axis of the E-S-F probe as shown in Fig. 4.19.

Fig. 4.19 E-S-F probe distal end oriented normal to the colon wall (■ parameters related to excitation, ■ parameters related to imaging, ■ parameters related to colon)

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

Let the excitation provided by the two fiber ports of the E-S-F probe are identical with respect to the power P , integration time Δt and maximum angle of excitation β . The excitation fluence and the collected emission with respect to the point **O** on the curved tissue surface can be analyzed as follows:

The incident energy on the tissue surface for excitation 1 $= P\Delta t \cos \theta'_1$, where θ'_1 the angle of excitation from port 1 for the point **O**. This energy from the illumination fiber port end is delivered over a solid angle $\Omega = 2\pi(1 - \cos \beta)$ [Here, it is assumed that the angular distribution of the light intensity is Lambertian].

Now, the excitation fluence for fiber port 1 is given by,

$$F_1 = \left(\frac{P\Delta t \cos \theta'_1}{2\pi (1 - \cos \beta) r_1^2} \right) \quad (4.30)$$

where r_1 is the radial distance of excitation port 1 and the referred point (**O**) on the tissue surface [118].

From Fig. 4.19, $r_1 = \frac{(d - \delta)}{\cos \theta'_1}$; $\cos \theta'_1 = \frac{1}{(1 + \tan^2 \theta'_1)^{1/2}}$ and $\tan \theta'_1 = \frac{(l - \Delta x)}{(d - \delta)}$ where d , Δx , δ and

l are the probe distance from the colon surface, vertical distance of point P from the E-S-F probe optical axis, horizontal distance of the point **O** on the curved surface to the surface point, which lies in the probe axis, and the distance of imaging unit center to the center of the excitation port 1 or excitation port 2 respectively.

$$\therefore F_1 = \frac{P\Delta t}{2\pi (1 - \cos \beta)(d - \delta)^2 \left(1 + \left(\frac{(l - \Delta x)}{(d - \delta)} \right)^2 \right)^{3/2}} \quad (4.31)$$

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

The incident energy on the tissue surface for excitation 2 will be equal to $P\Delta t \cos \theta'_2$ where θ'_2 is the angle of excitation from port 2 for point **O**. This energy from the second fiber port end is also delivered to the tissue over the same solid angle Ω .

Now, the excitation fluence for fiber port 2 is given by

$$F_2 = \left(\frac{P\Delta t \cos \theta'_2}{2\pi (1 - \cos \beta) r_2^2} \right) \quad (4.32)$$

where r_2 is the radial distance of excitation port 2 to the referred point (P) on the tissue surface.

$$\text{Again from Fig. 4.19, } r_2 = \frac{(d - \delta)}{\cos \theta'_2}; \cos \theta'_2 = \frac{1}{(1 + \tan^2 \theta'_2)^{1/2}}; \tan \theta'_2 = \frac{(l + \Delta x)}{(d - \delta)}$$

$$\therefore F_2 = \frac{P\Delta t}{2\pi(1 - \cos \beta)(d - \delta)^2 \left[1 + \left(\frac{(l + \Delta x)}{(d - \delta)} \right)^2 \right]^{3/2}} \quad (4.33)$$

$$\text{Again, from Fig. 4.19, } \delta = R_{\text{cm}}(o) - R_{\text{cm}}(o) \cos \gamma = R_{\text{cm}}(o) - ((R_{\text{cm}}(o))^2 - (\Delta x)^2)^{1/2}$$

where $R_{\text{cm}}(o)$ is the radius of curvature of colon and γ is the sector angle including the point **O** and the colon surface point, which crosses the optic axis of the E-S-F probe, and Δx the vertical distance of the referred point (**O**) from the optic axis of the probe.

Thus the total fluence (F) delivered to the colon tissue is given by

$$F = F_1 + F_2 \quad (4.34)$$

i.e.,

$$F = \frac{P\Delta t}{2\pi(1 - \cos \beta)(d - \delta)^2 \left[1 + \left(\frac{(l - \Delta x)}{(d - \delta)} \right)^2 \right]^{3/2}} + \frac{P\Delta t}{2\pi(1 - \cos \beta)(d - \delta)^2 \left[1 + \left(\frac{(l + \Delta x)}{(d - \delta)} \right)^2 \right]^{3/2}}$$

$$= \frac{P\Delta t}{2\pi(1-\cos\beta)(d-\delta)^2} \left\{ \frac{1}{\left(1 + \left(\frac{(l-\Delta x)}{(d-\delta)}\right)^2\right)^{3/2}} + \frac{1}{\left[1 + \left(\frac{(l+\Delta x)}{(d-\delta)}\right)^2\right]^{3/2}} \right\} \quad (4.35)$$

Consider a small area A with $\mathbf{O}$ as the center point, viewed by an arbitrary pixel of the image fiber, which is the ratio of the total area imaged to the total number of pixels and is given by

$$A = \frac{\pi((d-\delta)\tan\alpha_{\max})^2}{N} \quad (4.36)$$

$\alpha_{\max}$ represents the maximum collection angle of the ball lens-image fiber combination (imaging unit) and N represents the number of picture elements in the image fiber.

The return energy per pixel (E_r) depends on the absorptivity, scattering and internal transmittance of the tissue.

The return emission energy can be expressed as:

$$E_r = \left(\frac{\pi((d-\delta)\tan\alpha_{\max})^2}{N} \right) F \eta \quad (4.37)$$

where η is the fluorescence energy conversion efficiency which depends on the absorption, scattering and transmitting properties of the tissue. The typical value for η is taken to be 10^{-5} corresponding to the wavelength of the excitation laser. A representation of the interaction of tissue with the excitation laser is shown in Fig. 4.20.

Fig. 4.20 Interaction of colon tissue with laser excitation

This return emission energy is collected by the E-S-F probe. The amount of collected emission is purely dependant on the imaging lens, and the coupling aspects of the lens and the image fiber. The collection efficiency of the imaging lens, C_L is considered to be the ratio of the effective collection area to the total area of the hemispherical region, i.e., $(\pi r_L^2) / (2\pi r^2)$ where r_L and r represents the effective radius of the imaging lens and the radial distance of the imaging port from the referred point on the curved surface.

From Fig. 4.19, $d - \delta = r \cos \alpha$; $\tan \alpha = \Delta x / (d - \delta)$ where α is the collection angle of the imaging unit with respect to point O.

$$\therefore C_L = \left(\frac{\pi r_L^2}{2\pi (d - \delta)^2 [1 + (\Delta x / (d - \delta))^2]} \right) \quad (4.38)$$

Now, the effective radius of the ball lens, r_L is given by,

$$r_L = i \tan \alpha_f \quad (4.39)$$

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

where ‘i’ is the image distance of the ball lens and α_f is half the collection angle of the image fiber. The image distance for a ball lens is given by [119]:

$$i = \left(\frac{nD}{4(n-1)} \right) \quad (4.40)$$

where n and D represent the refractive index and diameter of the ball lens respectively.

Now, the numerical aperture of the imaging unit (NA_{imu}) can be written as:

$$NA_{imu} = 4r_L \frac{(n-1)}{nD} \quad (4.41)$$

Therefore the maximum collection angle of the imaging unit,

$$\alpha_{max} = 2 \times \sin^{-1}(NA_{imu}) \quad (4.42)$$

Now, the collected energy using the ball lens,

$$E_c = E_r C_L \quad (4.43)$$

$$E_c = \left(\frac{(\tan \alpha_{max})^2 F \eta \pi i^2 \tan^2 \alpha_f}{2 N \left[1 + \left(\frac{\Delta x}{(d - \delta)} \right)^2 \right]} \right) \quad (4.44)$$

The approximate number of photons collected per pixel can be determined from E_c (given in equation (4.44)) by the use of energy per photon (hc/λ_e) where h, c and λ_e are the Planck’s constant, velocity of light and the emission wavelength respectively. Considering

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

the transmission coefficient (T_0) and the packing fraction (f_p) of the image fiber, the total number of photons collected per pixel of the image fiber is given by,

$$N_p = \frac{\lambda_e T_o f_p E_c}{hc} \quad (4.45)$$

The total number of photons at the E-S-F probe output, which will be reaching the PMT, is given by (assuming 100% efficiency for the collection lens)]

$$N_p = \left(\frac{\lambda_e T_o f_p E_c}{hc} \right) N \quad (4.46)$$

The displayed spectrum intensity will be proportional to the signals from PMT which is in turn proportional to N_p . From the above theoretical analysis, it is inferred that the total number of collected photons will change as the distance of the E-S-F probe from the tissue wall is changed. A study on the change in collected emission intensity (the number of photons) with the variation in the distance of the probe from the tissue inner wall surface is carried out. The numerical values of the parameters used for the study are given in Table 4.2.

PARAMETER	VALUES
P	0.2mW
$R_{cm}(o)$	31.25mm
l	2.5mm
λ_e	550nm
Δt	5ms
N	15,000
α_f	23.578^0
α_{max}	25.857^0
D	3mm
n	1.5
η	10^{-5}
T_o	0.82
f_v	0.417
c	3.08×10^8 m/s
h	6.634×10^{-34} Jsec

Table 4.2: Numerical values of the parameters used in the theoretical model for fluorescence analysis

Using the input parameters given above, a quantitative analysis regarding the total number of collected photons through the probe at varying probe distance from the tissue wall was carried out. The total number of photons collected using the E-S-F probe was calculated using equation (4.45), and the obtained numerical plot is shown in Fig. 4.21. In the analysis, the probe distance was varied from 0.1mm to 50mm from the specimen surface. The number of collected photons found to be less when the probe was in close proximity with specimen surface and found to increase and reach maximum at a certain probe distance and then found

to decrease. The optimum distance of the probe at which maximum number of photons collected was calculated to be 2mm.

Fig. 4.21 Relationship between the total number of collected photons from the probe and the distance of the probe from the tissue surface

A quantitative analysis regarding the amount of light collected from the various points in the colon surface at fixed probe distance was also carried out. N_p is calculated with different values of Δx (from -5mm to 5mm) for 10mm probe distance and the obtained graph is shown in Fig. 4. 22. Maximum intensity distribution was found to be along optic axis, in direct line with the image fiber axis or probe axis. The values of the number of photons collected from other tissue points were calculated and compared with the maximum value. It was concluded that the number of collected photons decreased as the respective tissue points

are farther from the probe axis in either direction. The decreasing tendency of the photon distribution at both sides was observed to be symmetrical in nature.

Fig. 4.22 Relation between the normalized value of collected number of photons and the distance of the tissue surface points from the optical axis

As part of the experimental studies, the E-S-F system was operated in the fluorescence spectrum analysis modality for collecting the fluorescence emission from the test tissue phantom. The schematic diagram of E-S-F system in the fluorescence spectrum analysis modality is given in Fig. 4.23.

Fig. 4.23 E-S-F system in the fluorescence spectrum analysis modality

In this setup, the excitation laser light to the test tissue is delivered by the two fiber optic ports in the distal end of the E-S-F probe. The emission from the test tissue is imaged by means of the ball lens-image fiber combination. The collected emission is transmitted to the proximal end of the E-S-F probe via the same image fiber. The transmitted emission is collected by a collection lens placed after the probe proximal end. The collection lens output is directed to the fluorescence spectrum analysis unit (Monochromator integrated with PMT) the details of which are explained in section 4.1.6. The experimental setup is similar to the one shown in Fig. 4.9(b) except for change of elements after the collection lens and is given in Appendix N.

The test tissue used here was the simulated normal and abnormal phantom tissues in the colon model. Here, abnormal phantom tissues depicting cancerous tissues were prepared by staining the tissue with less fluorescent dye concentration (as explained in section 4.2) than

the normal phantom tissue. Fig. 4.24 shows the excitation-emission spectrum from normal and cancerous phantom tissues collected using the E-S-F system. In the case of simulated cancerous tissue, the collected fluorescence emission showed a decrease in emission intensity, for similar emission wavelength range, as expected.

Fig. 4.24 Excitation emission spectra collected using the E-S-F probe from simulated normal and cancerous tissues

4.6 Simultaneous operation of multiple diagnostic modalities with the E-S-F system

All the sections described above were dealing with the working of different diagnostic modalities individually. Incorporation of a splitting or switching device in between the CCD and the monochromator helped the simultaneous operation of the E-S-F system in normal

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

imaging, speckle correlation configurations and fluorescence spectrum analysis modality. A schematic diagram for the simultaneous operation of the E-S-F system in multiple modalities is shown in Fig. 4.25.

Fig. 4.25 E-S-F system operating in multiple diagnostic modalities (A: attenuator)

A detailed description of the functioning of the E-S-F system in each diagnostic modalities is given in sections 4.3, 4.4 and 4.5. While one arm of the splitting unit is fed into the normal imaging or speckle correlation analysis unit, the other arm is fed into the fluorescence spectrum analysis unit, making the simultaneous analysis possible.

The design details of the “Endo-speckle fluoroscope system” working in multiple modalities for the diagnosis of colon cancer have been discussed. The concept of dual illumination

Chapter 4

The endo-speckle-fluoroscope system: Design and analysis

fibers oriented at equal angles from the respective optic axes was helpful in configuring speckle correlation setup for sensing the displacement of the tissue in the in-plane directions as well as for getting maximum interrogation area for fluorescence emission generation. The reflected image or emitted light was collected and guided through a novel imaging scheme consisting of a ball lens and image fiber bundle. The research issues associated with transmitting the speckle pattern through a fiber bundle is discussed along with a detailed theoretical analysis on the speckle fringe formation for curved surfaces with the use of the developed system. Theoretical analysis incorporating different imaging parameters, that determine the amount of collected emission intensity, is carried out. The numerical plots obtained as per the theoretical formulation showed the relationship between the probe distance with the tissue wall to the amount of collected intensity. The developed probe was tested in all the diagnostic modalities and the obtained results were analyzed. Phantom tissues with simulated fluorophores (to represent normal colon tissue) and simulated abnormalities having fluorophore contents (to represent cancerous colon tissue) were used throughout the study. The developed E-S-F probe was able to detect visible abnormalities in the normal imaging modality and subsurface abnormalities of millimeter order in the speckle correlation modality. In fluorescence spectrum analysis modality, the E-S-F system was able to collect spectrums from normal and abnormal phantom tissues. A comparison of both spectrums were done where it is found that the fluorescence intensity collected from normal tissue was greater than that collected from cancerous tissue with fixed probe distance from the colon tissue wall.

CHAPTER 5

CONCLUSIONS

This chapter of the thesis concludes the whole research work done towards the achievement of the desired project objectives. The significant contributions made during the course of research are pointed out in sequence.

5.1 Important contributions

In order to have a thorough understanding of the research problem, an extensive literature survey was carried out in various areas relevant to the main project objectives. The research project was focused on the investigations of biological cavities (specifically human colon) for the early diagnosis of cancer using a specially designed multi-modality endo-speckle-fluoroscope. From a detailed analysis of the previous literature, the diagnostic techniques that are currently available in the medical industry are found to be insufficient for the early detection of colon cancer. Also, most of them caused physical discomfort to the patient. In this context, a non-contact, minimally invasive, multimodality imaging diagnostic system was proposed, which can identify the presence of cancer at its very early stage. The incorporation of different diagnostic modalities into a unique system will help to increase the diagnostic accuracy to a maximum and thus subduing the errors to a minimum. The system

proposed here may provide an answer to the replacement of current biopsy approaches in the long run.

Comprehensive review and analysis of the techniques incorporated in the proposed system design such as speckle correlation techniques and fluorescence spectrum analysis are carried out. The concept of speckle and speckle interferometric techniques for non-destructive inspection of specimens has been analyzed theoretically and experimentally. The feasibility of speckle pattern formation on biological surfaces was understood, and it was concluded that the application of speckle correlation techniques on such surfaces could open up research possibilities in the non-destructive testing of bio-specimens. Thus, speckle interferometry technique was taken as a proper choice for the detection of cancerous growths inside the colon cavity.

The application of fluorescence spectroscopic techniques especially autofluorescence techniques were found to be an appropriate tool for differentiating normal and cancerous tissues. For colon tissue fluorophores, the excitation wavelength ranged from UV to Visible for emission in the range of 400nm-700nm. It was also noted that emitted fluorescence intensity of the colon tissue fluorophores was considerably less for adenoma as compared to that of normal mucosa in the wavelength region $< 600\text{nm}$. The researches that have been carried out previously for developing an endoscopic tool for the real-time cancer diagnosis, incorporating fluorescence spectroscopic techniques were found to have certain limitations. In this context, the design of the proposed endo-speckle-fluoroscope was carefully done for subduing such limitations of current fluoroscopes. This is done by the incorporation of the concept of dual excitation and a novel “imaging lens-high resolution imaging fiber bundle” combination for emitted fluorescence collection.

Thus, the incorporation of speckle interferometry and fluorescence spectroscopy in a single endoscopic tool was found to be a promising way for an accurate diagnosis of cancer. Additionally, the proposed endoscopic tool was also able to perform in the normal imaging modality, so that any visible abnormalities present in the colon pathways could be found out. The proposed probe consisted of two illumination or excitation channels, oriented at equal angles from the probe axis, which helped to realize all the speckle interferometric configurations as well as to maximize the interrogation area for fluorescence excitation. The introduction of ball lens as the imaging lens helped for the easy orientation of the lens inside the probe due to its symmetry and uniformity in all directions. The choice of the image fiber was made in order to maintain the flexibility of the probe and correspondingly, a suitable collection lens was chosen in order to resolve the entire pixels present in the image fiber. The choice of optical fibers for illumination as well as imaging helped to miniaturize the whole diagnostic system, with increased flexibility. This was also essential for the simultaneous operation of the endo-speckle-fluoroscope in multiple imaging modalities. The concept of optical component selection unit along with the splitting unit facilitated the respective speckle interferometric configurations and the simultaneous operation of the different imaging modalities incorporated in the endoscopic tool.

Experimental investigations using the endo-speckle-fluoroscope for the diagnosis of pathologic lesions such as cancer were carried out using a phantom model of colon as the test specimen. Cancerous growths of different sizes were simulated inside the phantom model. Fluorescence spectrum collected from the normal and simulated cancerous region of the phantom model showed difference in the collected intensity at a particular wavelength. The fluorescence intensity collected from cancerous colon was found to be less compared to that collected from a normal colon. This is because, the colon fluorophores that emit

fluorescence below 600nm are found at a reduced amount in cancerous colon. To illustrate the phenomenon, the tissue in the phantom model was stained with proper staining agent so as to make emission below 600nm.

The identification of cancerous growths was illustrated in different speckle interferometric configurations such as in-plane, out-of-plane and shear configurations. Cancerous growths of size greater than or equal to 3mm were easily identified employing in-plane and out-of-plane configurations. Abnormality size less than 3mm was difficult to be identified using the above-mentioned configurations as smaller abnormality size demanded more number of in-plane and out-of-plane fringes upon increased loading for their identification, which eventually resulted the decorrelation of interference fringes. On the other hand, using the shear configuration, the identification of abnormalities of smaller size was possible. Despite the formation of a kink at the abnormality site as in the case in-plane and out-of-plane fringes, the abnormality position was also indicated by a separate fringe system in the shear configuration. Hence the detection of abnormalities of sizes $\approx 1\text{mm}$ was possible with the use of shear configuration. Besides the detection of small abnormalities by looking at the interferometric fringes, the normal colon images taken using the endo-speckle-fluoroscope system for identifying any visible abnormalities at the colon surface were also demonstrated. The interaction of the proposed endo-speckle-fluoroscope with the colon wall required much theoretical aspects to be considered such as speckle fringe formation on curved surfaces, the effect of divergent illumination in speckle interferometric fringes, transmission of speckle patterns through the image fiber, collection of fluorescence emission from the curved colon surface etc. A modified theory for the fringe formation in cavity surfaces for different interferometric configurations was introduced. Experimental verification of the derived theory was done by employing phantom tissue curved surfaces. The theory was again

modified incorporating the effect of illumination beam divergence. An experimental illustration for the effect of illumination beam divergence was also provided in the case of flat as well as curved surfaces. The width of the interference fringes were found to be increasing with the introduction of a divergent beam, thus decreasing the total number of fringes at the interrogated area as compared to that obtained with a collimated beam. Such an analysis was of much help as the illumination provided by the endo-speckle –fluoroscope was divergent in nature. Also, the effect of image fiber in the transmittance of speckle pattern, while using the endo-speckle-fluoroscope in the speckle correlation modality was analyzed. The speckle fringe formation theory for the endo-speckle-fluoroscope in speckle correlation modality incorporating the curved specimen nature and illumination divergence was presented.

The endo-speckle-fluoroscope in fluorescence spectrum analysis modality required necessary theoretical analysis relating different parameters of the probe and the considered colon surface for estimating the amount of fluorescence intensity collected using the probe. In this context, a theoretical analysis dealing with the excitation, fluorescence generation and emission collection aspects was carried out. Numerical plot was drawn relating the total number of fluorescent photons collected using the probe from the colon wall and the distance of the probe from the colon wall. The optimum probe distance from the colon wall at which the maximum collection of photons occurred, was noted. The variation of emitted fluorescence from the different points in the colon surface depending on their distance from the probe axis was also studied. Maximum collection of emission was found to be along the probe axis.

The applicability of the concept of speckle interferometry for curved surfaces was extended to the non-destructive testing of technical cavities as well (Pls. see Appendix C, D, and F). Hence this has potential to find applications in the NDT of pipes and pressure vessels.

In brief, the main conclusions and contributions of this research project are given as mentioned below.

5.2 Main contributions and conclusions:

- A novel diagnostic tool called “Endo-speckle-fluoroscope” based on multiple modalities of imaging such as normal imaging, speckle correlation imaging and fluorescence spectrum analysis was proposed and illustrated, which is capable of early diagnosis of colon cancer.
- A detailed analysis of the incorporated techniques was carried out and the feasibility of application of the techniques in the proposed diagnostic tool was understood.
- The design of the endo-speckle-fluoroscope was done incorporating novel concepts in illumination and imaging necessary for the system to work in multiple modalities simultaneously.
- Experimental investigations on the early diagnosis of colon cancer were carried out using different imaging modalities incorporated in the diagnostic tool.

- Theoretical analyses related to the different aspects of the probe in its different diagnostic modalities were carried out in detail. Some of the main contributions in this category are:
 - a) Extended theoretical formulation of DSPI fringe formation on curved object surfaces.
 - b) Effect of illumination beam divergence in endoscopic DSPI fringe formation.
 - c) Image fiber effect in the speckle pattern transmission in DSPI endoscopy.
 - d) Analysis on relationship between different imaging parameters related to the probe as well as the colon surface in the collection of fluorescent photons.

- An extension of the application of speckle interferometric techniques on technical cavities was done, which can find application in the NDT of pipes and pressure vessels.

CHAPTER 6

FUTURE RESEARCH DIRECTIONS

This last chapter of the thesis discusses the future research directions, the approaches to be adopted for modifying and improving the current achievements. The concept of incorporation of more number of diagnostic modalities in the same endo-speckle-fluoroscope to further increase the accuracy of diagnosis is discussed. The various application areas of the modified endoscope probe are also mentioned.

Currently, the project was concentrating on developing the concepts and methodology for the multi-modal imaging employing phantom models, differing animal models. The future work will be concentrating towards employing the developed probe concepts in animal models. Such investigations require some modifications in the current probe design, which will be discussed below.

6.1 Steering and locomotion aspects of the endo-speckle-fluoroscope

To have a complete inspection of the cavity walls with the developed endo-speckle-fluoroscope, the endoscope distal end is to be deflected at all possible directions (up and down, right and left). A suitable mechanism such as control knob mechanism [120] or incorporation of shape memory alloy (SMA) wires, springs or rods [121] may be adopted in the final endoscope design, so that during inspection, the endoscope distal end can be deflected at any desired angle.

For diagnostic use in the real clinical environment, the developed endoscope should be able to move inside the whole length of the considered body cavities. A common practice adopted in today's medical industry is to move the endoscope inside the colon manually. Different design aspects are to be considered while dealing with the motion of endoscope. The diameter of the endoscope is very important in this aspect. For easy movement, the diameter of the instrument should be as small as possible, but too narrow endoscope makes it difficult for the surgeons to push the instrument into different parts of the cavity, especially in colon. The proposed endo-speckle-fluoroscope is designed to have 12mm diameter including the biocompatible sheath, which will be suitable for compromising the above-mentioned issues.

Besides, the movement of the endoscope inside the cavity done manually, an automation of the whole locomotive scheme is another option. The difficulty that the surgeons face in moving the endoscope through the body cavities have opened up new possibilities for automated endoscopy. With the advancement of technology of MEMS (micro electro mechanical systems) in biomedical engineering, endoscope with micro-driving motors or "inchworm" type locomotion can be designed that provides an automatic movement of the endoscope through the internal cavities [122, 123]. Such approaches can be adopted to the proposed endo-speckle-fluoroscope.

6.2 Addition of auxiliary channel in the endo-speckle-fluoroscope

The current design of the endo-speckle-fluoroscope consists of two illumination fiber channels and a channel for the imaging unit. Auxiliary channel will be allowed in the modified endoscope design for the passage of air. Airflow delivered to the inner colon through the endoscope probe has two functions. This helps in (i) inflating the colon during inspection and (ii) for providing the required deformation of the colon wall, necessary for the speckle fringe formation on colon wall surface.

6.3 Miniaturization of the entire system

The E-S-F system proposed here is making use of two separate detection systems for normal imaging or speckle correlation analysis and fluorescence spectrum analysis. By incorporating CCD arrays with fluorescence spectrum analysis capabilities, the entire system can be made more compact and more suitable for clinical environments. Also, by making the optical component selection unit to work in the automated mode, the speed of diagnosis can be enhanced. Work is going on in this direction.

6.4 Enhancement of diagnostic accuracy

- The current research is focused on the application of the designed endoscope in identifying the presence of cancer from body cavities. However, other techniques such as resolving the phase value contained in the image, may also be employed by incorporating the corresponding detection systems [124] at the proximal end of the probe. The separation of different fluorophores' emissions with very close emission wavelength range collected from the probe proximal end is possible by the incorporation of the phase resolved technique. Sometimes, the fluorescence intensity difference between the normal and cancerous regions may be predominant in these resolved wavelengths, and in such cases the incorporation of phase resolved technique will improve the diagnostic accuracy.
- By incorporating phase shifting techniques to the obtained fringe patterns using the E-S-F system, a more detailed outlook regarding the polyp growth, such as its position and shape is feasible. Such phase shifted images, will provide more information about the growth stage of the polyp within the colon layers and thus will increase the diagnostic accuracy of the E-S-F system.

- Currently, the endoscopic images received from the E-S-F system are two-dimensional. The conversion of endoscopic 2-D images to their 3-D equivalents can definitely provide more detailed informations regarding the disease diagnosis [125]. The incorporation of such techniques and novel concepts in this regard will lead to more accurate diagnostic results.

6.5 Application of the developed system in clinical environments

After incorporating the modifications in the endoscope probe, as discussed in section 6.1 to 6.3 the system is intended to apply in the real clinical environments. In this context, studies will be conducted to analyze the possible issues, which will be associated with the application of the probe in real body cavities, such as the presence of wet tissues and decorrelation of fringe patterns. Although there won't be any change in the experimental configurations, possible variations in the imaging sensitivity resolution etc. have to be looked into. Also, the problems of speckle fringe decorrelation could be avoided by incorporating the double exposure technique for speckle interferometry.

It is to be mentioned that in real colon cavity, the possibility of speckle formation (because of the medium being volatile) due to the spatial phase variations at the illumination wavefront cannot be avoided. In this context, this research aspect of the problem will be looked into during the future studies.

All these aspects will be discussed in the further studies, which will be conducted in the future as part of in-vivo diagnostic procedures using the developed system in real clinical environments.

6.6 Extension of application areas

This research work is intended to develop a diagnostic tool for the early cancer detection. Also, by adding microscopic features to the E-S-F probe, investigations on biological cell characterization can be made possible upon extensive research in this direction.

APPENDICES

APPENDIX A

EDC 2000N COMPUTER CAMERA SPECIFICATIONS:

Camera Model	EDC-2000N
Image Size (Sensing area)	4.84×3.67 mm
Spatial Resolution	652(H)×494(V)
Pixel Well Size	7.4×7.4 μ
Pixel Well Capacity (saturation signal)	30,000 e ⁻
R.M.S. Readout Noise (electrons/pixel)	20 e ⁻
Peak Quantum Efficiency	75% @0.65 μ
Sensor Type¹	TI TC237B monochrome
Exposure Time (S/ controlled)	<1 msec to >5sec
Operating System Compatibility	Windows 95/98/ME/NT/2000, Dos & Linux

SYSTEM REQUIREMENTS:

Electrim EDC-2000 series cameras require a Pentium III or higher CPU and a free PCI slot. Models EDC-2000 and EDC-2000E require DOS or Windows 95/98. The model EDC-2000N requires DOS, Windows 95/98, or Linux. For the model EDC-2000S, Windows NT is required. A video display supporting true color (16 million colors) is strongly recommended, though not required.

DIMENSIONS:

	<u>Width</u>	<u>Height</u>	<u>Depth</u>	<u>Weight (w/o lens)</u>
EDC-2000 size/weight:	1.95 in.	1.95 in.	1.20 in.	4 oz.
EDC-2000N/E/S size/weight:	2.50 in.	2.25 in.	1.35 in.	7 oz.

Lens mount: C or CS (CCTV Standard) **Cable length:** 10 ft. supplied (longer cables can be used)

APPENDIX B

Phantom tissue: mechanical properties

Determination of Young's modulus:(Analysis of data from INSTRON 5566)

Fig. B.1 shows the stress-strain curve obtained when the phantom tissue material was subjected to tensile test under INSTRON 5566.

Fig. B.1 Stress-Strain relationship for the phantom tissue

Calculation of Young's modulus is based on selected linear region of the graph (Tensile Stress value from 0.2 MPa to 0.6MPa)

Average value of tensile stress = 0.39816 MPa

Average value of tensile strain = 1.25938 mm/mm

Young's modulus = Tensile Stress (average) / Tensile strain (average)

= 0.39816 MPa / 125.938 (mm/mm)

= **0.3161MPa**

Determination of Poisson's ratio

Fig. B. 2 shows the relationship between the specimen extension in the linear and lateral directions.

Fig. B.2 Relation between linear (tensile) and lateral (transverse) extensions of the phantom tissue

Average value of tensile extension: 72.499mm

Average value of transverse extension: 35.25mm

For a tissue phantom with equal original dimensions, the value of Poisson's ratio was estimated to be equal to 0.4862

APPENDIX C

DSPI investigations on curved technical specimens

Specimen: Aluminium half-cylinder

Dimensions: 8.5cm length, 8.5cm outer diameter, and 1mm thickness

IN-PLANE CONFIGURATION:

Experiments were carried out with the test specimen in the in-plane DSPI configuration. The test specimen was fixed along bottom and sides, and axial compression was applied on the specimen by the advancement of micrometer attached loading unit from the specimen top.

Fig. C. 1 In-plane displacement contours for the half-cylindrical aluminium specimen (a): fringe pattern for in-plane displacement ($5\ \mu\text{m}$), (b): Fringe pattern for in-plane displacement ($10\ \mu\text{m}$), (c): Numerically simulated in-plane displacement contours

Appendix C

Fig. C. 1(a) shows the in-plane fringes obtained on the application of $5\ \mu m$ displacement in the axial direction. The fringes followed straight-line nature. Figure C. 1(b) shows the increased number of in-plane fringes obtained for an applied displacement of $10\ \mu m$. Figure C.1(c) shows the corresponding simulated contours generated using FEA.

OUT-OF-PLANE CONFIGURATION:

Experiments were carried out with the test specimen in the out-of-plane DSPI configuration. The test specimen was fixed along the edges, and a central out-of-plane loading of micrometer order is given to the specimen by advancing the tip of a screw attached to a translation gauge. Figure C. 2 (a) shows the out-of-plane fringes obtained on the application of $5\ \mu m$ displacement in the out-of-plane direction. The fringes showed a shape of concentric ovals, with increasing thickness from the center. Figure C. 2 (b) shows increased number of out-of-plane fringes obtained for an applied displacement of $10\ \mu m$. Figure C. 1 (c) shows the corresponding simulated contours generated using FEA. As the area of interrogation was the center region of the specimen, the fringes correspond to the center region in the simulated contours.

Fig. C. 2 Out-of-plane displacement contours for the half-cylindrical aluminium specimen (a): fringe pattern for out-of-plane displacement ($5 \mu m$), (b): Fringe pattern for out-of-plane displacement ($10 \mu m$), (c): Numerically simulated out-of-plane displacement contours

SHEAR CONFIGURATION:

Experiments were carried out with the test specimen in the Digital speckle shearography configuration. The test specimen was fixed along the edges, and a central out-of-plane loading of micrometer order is given to the specimen by advancing the tip of a screw attached to a translation gauge. Fig. C. 3 (a) shows the shear fringes obtained on the

application of $5\ \mu\text{m}$ displacement in the out-of-plane direction. The fringes followed the shape of a butterfly. Fig. C. 3 (b) shows increased number of shear fringes obtained for an applied displacement of $10\ \mu\text{m}$. Fig. C. 3 (c) shows the corresponding simulated contours generated using FEA. As the area of interrogation was the center region of the specimen, the fringes correspond to the center region in the simulated contours.

Fig. C. 3 Out-of-plane displacement derivative contours for the half-cylindrical aluminium specimen (a): Shear fringe pattern for out-of-plane displacement ($5\ \mu\text{m}$), (b): Shear fringe pattern for out-of-plane displacement ($10\ \mu\text{m}$), (c): Numerically simulated out-of-plane displacement derivative contours

APPENDIX D

Effect of random loading in DSPI investigations on curved technical specimens:

Specimen: Aluminium half-cylinder

Dimensions: 15cm (length), 10cm (diameter), 1mm (thickness)

For analyzing the effect of random loading on curved specimens, experiments were carried out with a half-cylindrical aluminium specimen in the out-of-plane DSPI configuration. Mechanical loading (by advancing the tip of a screw using an attached translation gauge) of micrometer order was given to the specimen fixed along its sides and bottom for providing necessary out-of-plane deformation). To represent random loading, a combination of in-plane as well as out-of-plane deformation, is given to the specimen. The in-plane loading is given from specimen top using the custom fabricated loading jig, along with the out-of-plane loading from behind. Fig. D.1 - Fig. D.3 shows the out-of-plane displacement contours of the specimen for different in-plane and out-of-plane loads applied to the specimen. Fig. D. 1(a) shows the out-of-plane fringe pattern of the specimen with $5\ \mu\text{m}$ out-of-plane load, and without the application of an in-plane load. Fig. D. 1 (b) shows the corresponding simulated contours generated using FEA.

Fig. D. 1 Displacement contours for the half-cylindrical aluminium specimen with fixed side and bottom edges (applied displacement- $5\ \mu\text{m}$ (out-of-plane)) (a): Fringe pattern, (b): Simulated contours using FEA

The interrogation area was the center region of the specimen, and hence the obtained fringe pattern corresponds to the center region of the simulated contours.

Figure D. 2 (a) shows the fringe pattern obtained for a combined in-plane ($5\ \mu\text{m}$) and out-of-plane load ($10\ \mu\text{m}$). The corresponding simulated contours are shown in Fig. D. 2 (b). It was found that the application of out-of-plane loading in the presence of an in-plane loading resulted in different type of fringe patterns with half-cylindrical specimens. The number of the concentric oval fringes obtained found to be more centralized with symmetry with the increase in out-of-plane loading ($10\ \mu\text{m}$) along with a fixed in-plane loading of $5\ \mu\text{m}$.

Fig. D. 2 Displacement contours for the half-cylindrical aluminium specimen with fixed side and bottom edges (applied displacement-out-of-plane ($10\ \mu\text{m}$) and in-plane ($5\ \mu\text{m}$)) (a): Fringe pattern, (b): Simulated contours using FEA

When the in-plane loading given was larger compared to the out-of-plane, it resulted in an interesting fringe pattern, both simulated contours and experimental fringes, which was found to be opposite in shape to the fringes obtained for Fig D. 1. This is illustrated in Fig. D. 3. Figure D. 3 (a) represents the fringe pattern obtained for an in-plane load of $10\ \mu\text{m}$ and an out-of-plane load of $5\ \mu\text{m}$. Figure D. 3 (b) shows the corresponding simulated contours.

Fig. D. 3 Displacement contours for the half-cylindrical aluminium specimen with fixed side and bottom edges (applied displacement-Out-of-plane ($5 \mu m$) and in-plane ($10 \mu m$)) (a): Fringe pattern, (b): Simulated contours using FEA

From the analyses carried out, it was inferred that the application of random loading, did affect the nature of DSPI fringes on curved surfaces, and thus the study was found useful while dealing with DSPI fringe formation for curved surfaces, under random loading conditions.

APPENDIX E

Comparison of fringe patterns employing divergent and collimated illumination beams under similar loading conditions

1. Flat object surface

Experiments were carried out with a flat specimen (circular CFRP diaphragm, 6cm diameter) in the out-of-plane DSPI configuration. The specimen was clamped along the edges, and a central out-of-plane loading was given to the specimen by advancing the tip of a screw attached to a translation gauge. The obtained fringe pattern showed concentric circular shape. The specimen was subjected to divergent as well as collimated illumination, keeping all other experimental conditions constant. Fringe patterns were stored with different amount of applied load to the specimen. The obtained results are shown in Fig. E. 1. Fig. E. 1 (a) shows the fringe pattern obtained for an applied load of $5\ \mu m$ with divergent illumination beam. Figure E. 1(b) shows the fringe pattern obtained for a collimated illumination beam for the same applied load ($5\ \mu m$). Similarly, Fig. E. 1 (c) and (d) represent the fringe pattern for $10\ \mu m$ applied load with divergent and collimated beam, respectively. Fig. E.1 (e) and (f) represent the fringe pattern for $15\ \mu m$ applied load with divergent and collimated beam, respectively.

Fig. E. 1 Out-of-plane fringe patterns employing divergent or collimated illumination beams at different applied loads (for flat specimen)

From the above given fringe patterns, it can be seen that a divergent illumination beam causes increase in fringe width compared to a collimated beam. And, this difference is

predominant, while considering small applied loads. A graphical representation of the relationship between the fringe width at the n^{th} fringe and applied load for divergent as well as collimated illumination beams is shown in Fig. E. 2.

Fig. E. 2 Relationship between fringe width and applied load while using divergent or collimated illumination beams (flat surface)

2. Curved object surface

Experiments were carried out with a curved specimen (Aluminium half cylinder, dimensions: 15cm length, 9.6cm diameter and 1 mm thickness) in the out-of-plane DSPI configuration. The specimen was clamped along the top and bottom edges, and a central out-of-plane loading was given to the specimen by advancing the tip of a screw attached to a translation gauge. The obtained fringe pattern showed concentric oval shape. The specimen was subjected to divergent as well as collimated illumination, keeping all other experimental conditions constant. Fringe patterns were stored with different amount of applied load to the specimen. The obtained results are shown in Fig. E. 3. Fig. E. 3 (a) shows the fringe pattern obtained for an applied load of $5\ \mu m$ with divergent illumination beam. Fig. E. 3(b) shows the fringe pattern obtained for a collimated illumination beam for the same applied load ($5\ \mu m$). Similarly, Fig. E. 3 (c) and (d) represent the fringe pattern for $10\ \mu m$ applied load with divergent and collimated beam, respectively. Figure E. 3 (e) and (f) represent the fringe pattern for $15\ \mu m$ applied load with divergent and collimated beam, respectively.

Fig. E. 3 Out-of-plane fringe patterns employing divergent or collimated illumination beams at different applied loads (for curved specimen)

Appendix E

From Fig. E. 3, it can be seen that a divergent illumination beam causes increase in fringe width compared to a collimated beam, in a curved surface. And, similar to a flat surface, in curved surface also, this difference in fringe width caused by divergent and collimated illumination beams is predominant, while considering small applied loads. At larger loads, this difference in fringe width caused by divergent and collimated beams found to be more in the case of curved surface, compared to that of flat surface. A graphical representation of the relationship between the fringe width and applied load for divergent as well as collimated illumination beams is shown in Fig. E. 4.

Fig. E. 4 Relationship between fringe width and applied load while using divergent or collimated illumination beams (curved surface)

APPENDIX F

Non destructive testing of technical cavity surfaces using digital speckle shearography:

1. Identification of surface defects:

Specimen: Aluminium half-cylinder with surface defect

Defect size: 0.5mm

Specimen Dimensions: 15cm length and 10.4cm outer diameter and 1mm thickness

Experiments were carried out with a normal and defective half-cylindrical Aluminium specimen in order to illustrate the identification of surface defects in curved specimen using digital speckle shearography. The normal and defective specimens were tested in the digital speckle shearographic setup, and the obtained results are shown in Fig. F. 1 and Fig. F. 2. Both the specimens were clamped along the top and bottom edges and a central out-of-plane loading of micrometer order was given by advancing the tip of a screw attached to a translation gauge, for providing the necessary deformation. Figure F.1 (a) shows the shear fringes obtained for the normal specimen and the corresponding finite element contours are shown in Fig. F. 1(b).

Fig. F. 1 Displacement derivative contours obtained for a normal aluminium half-cylindrical specimen (a): Fringe pattern, (b): Simulated contours

The same experimental setup and loading conditions was provided for testing the specimen with a surface defect. The defect location was at 5mm distance from the specimen centre as indicated in Fig. F. 2 (a). The shear fringe obtained for the defective specimen is shown in Fig. F. 2 (b). Here, the position of defect is indicated as a kink in the original fringe pattern. The corresponding numerical contours generated using FEA are given in Fig. F. 2 (c). Here, the defect location is identified by the delineation of the contours at the defect site.

Fig. F. 2 Shearographic NDT on aluminium half-cylindrical specimen (a): Defect location within the specimen, (b): Shear fringe pattern indicating the position of defect, (c): Corresponding simulated contours indicating the defect position

2. Identification of subsurface defects:

Specimen: Sandwiched half-cylindrical Aluminium-CFRP (carbon fiber reinforced plastic) composite specimens with and without subsurface defects

Specimen dimensions: 15cm length, 9.6cm diameter and 1.25mm thickness

Defect material: Teflon

Defect size: 3.75mm side length and 0.7mm thickness

Experiments were carried out with a normal and defective half-cylindrical Aluminium-CFRP sandwich specimen in order to illustrate the identification of subsurface defects in curved specimen using digital speckle shearography. The normal and defective specimens were tested in the digital speckle shearographic setup, and the obtained results are shown in Fig. F. 3 - Fig. F. 5. Both the specimens were clamped along the top and bottom edges and a central out-of-plane loading of micrometer order was given by advancing the tip of a screw attached to a translation gauge, for providing the necessary deformation. Figure F. 3 (a) shows the shear fringes obtained for the normal specimen and the corresponding finite element contours are shown in Fig. F. 3(b).

Fig. F. 3 Displacement derivative contours obtained for a normal aluminium-CFRP sandwiched half- cylindrical specimen (a): Fringe pattern, (b): Simulated contours

The same experimental setup and loading conditions was provided for testing the specimen with one subsurface defect. The specimen consisted of 3 layers, one bottom layer of Aluminium and two top layers of CFRP. The defect location was on the second layer, and was at 5.3mm distance from the specimen centre as indicated in Fig. F. 4 (a). The shear fringe obtained for the defective specimen is shown in Fig. F. 4 (b). Here, the position of defect is indicated as a kink in the original fringe pattern. The corresponding numerical contours generated using FEA are given in Fig. F. 4 (c). Here, the defect location is identified by the delineation of the contours at the defect site.

Fig. F. 4 Shearographic NDT on aluminium-CFRP sandwiched half-cylindrical specimen with single defect (a): Defect location within the specimen, (b): Shear fringe pattern indicating the position of defect, (c): Corresponding simulated contours indicating the defect position

The experiments were repeated on an Aluminium-CFRP composite specimen with two subsurface defects. Both the defects were on the second layer, and was at 5.3mm distance from the specimen centre as indicated in Fig. F. 5 (a). The shear fringe obtained for the defective specimen with double defect is shown in Fig. F. 5 (b). Here, the position of defects is indicated as kinks in the original fringe pattern. The corresponding numerical contours generated using FEA are given in Fig. F. 5 (c). Here, the defect locations are identified by the delineation of the contours at the defect sites.

Fig. F. 5 Shearographic NDT on aluminium-CFRP sandwiched half-cylindrical specimen with double defect (a): Defect location within the specimen, (b): Shear fringe pattern indicating the position of defects, (c): Corresponding simulated contours indicating the defect positions.

APPENDIX G

Dongwoo Optron DM 150 Monochromator

Main specification and Technical information

Model	DM 150
Optical design	Czerny-Turner
Focal length	150mm
Aperture ratio	f/4.7
Optical path	Side entrance, Side exit
Mechanical scan range	0 to 1600nm with a 1200gr/mm grating
Operating range	185nm to the far infrared with available grating and detectors
Resolution	0.4nm
Dispersion	5nm
Accuracy	$\pm 0.25\text{nm}$
Repeatability	$\pm 0.04\text{nm}$
Drive step size	0.0025nm per one motor step with a 1200gr/mm grating
Detector coverage	125nm
Standard optical coating	High efficiency Al + MgF2 for gratings and mirrors
Scanning mechanism	Direct digital scanning system
Grating Turret	Dual
Grating size (mm)	32 x 32
Slit	5um to 5mm width adjustable
Mirror size (mm)	40 x 40

APPENDIX H**Specifications of PMT (R928)****HAMAMATSU****PHOTOMULTIPLIER TUBES
R928, R955****Extended Red, High Sensitivity, Multialkali Photocathode
28mm (1-1/8 Inch) Diameter, 9-Stage, Side-On****FEATURES**

- **Wide Spectral Response**
 - R928 185 to 900 nm
 - R955 160 to 900 nm
- **High Cathode Sensitivity**
 - Luminous 250 μ A/lm
 - Radiant at 400nm 74mA/W
- **High Anode Sensitivity (at 1000V)**
 - Luminous 2500A/lm
 - Radiant at 400nm 7.4×10^5 A/W
- **Low Drift and Hysteresis**

The R928 and R955 feature extremely high quantum efficiency, high current amplification, good S/N ratio and wide spectral response from UV to near infrared. The R928 employs a UV glass envelope and the R955 has a fused silica envelope for UV sensitivity extension.

The R928 and R955 are well suited for use in broad-band spectrophotometers, atomic absorption spectrophotometers, emission spectrophotometers and other precision photometric instruments.

GENERAL

Parameter	Description/Value	Unit
Spectral Response		
R928	185 to 900	nm
R955	160 to 900	nm
Wavelength of Maximum Response	400	nm
Photocathode Material	Multialkali	—
Minimum Effective Area	8 $\times$ 24	mm
Window Material		
R928	UV glass	—
R955	Fused silica	—
Dynode		
Secondary Emitting Surface	Multialkali	—
Structure	Circular-cage	—
Number of Stages	9	—
Direct Interelectrode Capacitances		
Anode to Last Dynode	Approx. 4	pF
Anode to All Other Electrodes	Approx. 6	pF
Base	11-pin base JEDEC No. B11-88	—
Weight	Approx. 45	g
Suitable Socket	E678-11A (option)	—
Suitable Socket Assembly	E717-21 (option)	—

Figure 1: Typical Spectral Response

APPENDIX I

Photograph of the colon phantom model

Fig. I. 1 The phantom colon model

APPENDIX J

Staining procedures for the phantom tissue

Selection of the fluorescent dye depends on the emission wavelength corresponding to the fluorophore contents of the colon tissue. Here, Rhodamine 6G was selected as the staining agent (fluorochrome) for the phantom tissue, as it has emission of 550nm for a laser excitation of 532nm, which corresponds to the fluorescence emission wavelength of fluorophore contents in the colon. Appropriate procedures were adopted for staining the chosen dye as written below.

For staining, the selection of a proper solvent was important, which could dissolve the fluorochrome and provide minimum reaction to the phantom tissue. For this purpose, 11 organic solvents (5mg fluorochrome in 10ml solvent) were tried out, which are listed below.

The solvents used are:

1. DCM (Dichloromethane)
2. Chloroform
3. Ethyl Acetate
4. Aceto Nitrile
5. THF (Tetra Hydro furan)
6. Acetone
7. Hexane
8. Ethanol
9. Toluene
10. Methanol
11. H₂O

Of the 11 solvents, chloroform, DCM and acetone showed good solubility for the fluorochrome, but the first two solvents were discarded considering their reaction with the phantom tissue. Acetone was found to be having no reaction with the phantom tissue. Also, the fluorochrome was well absorbed by the phantom tissue while dipping the tissue in the fluorochrome-acetone solution.

Two sets of staining solution were prepared with 1mg fluorochrome in 10ml acetone and 1mg fluorochrome in 20ml acetone. The solution was sonicated for maximum solubility and then filtered using a filter paper. The phantom tissue dipped in the first solution represented the normal tissue and the phantom tissue dipped in the second solution represented the abnormal tissue.

APPENDIX K

Photographs of the phantom tissue

Fig. K.1 Normal phantom tissue stained with 1mg Rhodamine 6G in 10ml acetone

Target abnormality

Fig. K.2 Phantom tissue with simulated cancerous growth (size 1mm) stained with 1mg Rhodamine 6G in 20ml acetone

APPENDIX L

Photograph of the E-S-F system experimental setup (Out-of-plane configuration)

Fig. L. 1 Experimental setup for the E-S-F system to operate in the out-of-plane configuration

APPENDIX M

Photograph of the E-S-F system experimental setup (Shear configuration)

Fig. M. 1 Experimental setup for the E-S-F system to operate in the shear configuration

APPENDIX N

Photograph of the E-S-F system experimental setup (Fluorescence spectrum analysis modality)

Fig. N. 1 Experimental setup for the E-S-F system to operate in the fluorescence spectrum analysis modality

LIST OF ORIGINAL PUBLICATIONS

Journals

1. **Sujatha, N.**, Murukeshan, V. M., Ong, L. S., and Seah, L. K. (2003). An all fiber optic system modeling for the gastrointestinal endoscopy: design concepts and fluorescence analysis. Optics Communications, 219, pp. 71-79.
2. Murukeshan, V. M., and **Sujatha, N.** (2004). Digital speckle pattern interferometry for deformation analysis of inner surfaces of cylindrical specimens. Applied Optics, 43 (12), pp. 2400-2408.
3. Murukeshan, V. M., **Sujatha, N.**, and Ganesan, A. R. (2004). Effect of in-plane sensitive component on speckle fringe formation on curved inner surfaces of engineering specimens. Journal of Holography and Speckle, 1 (2), pp. 63-68.
4. **Sujatha, N.**, and Murukeshan, V. M. (2004). Non-destructive inspection of tissue/tissue like phantom curved surfaces using digital speckle shearography. Optical Engineering, 43(12), pp. 3055-3060.
5. **Sujatha, N.**, Murukeshan, V. M., Rajendran, S., Ong, L. S., and Seah, L. K., (2005) Non-destructive inspection of inner surfaces of technical cavities using digital speckle shearography. Nondestructive Testing and Evaluation, 20 (1), pp. 25-34.

List of publications

6. **Sujatha, N.,** and Murukeshan, V. M. (2004) Combined imaging and fluorescence analysis based endo-fluoroscope system for disease diagnosis in body cavities. (Under review, Surgical Endoscopy)
7. **Sujatha, N.,** and Murukeshan, V. M. Detection of abnormalities in body cavities using endoscopic speckle correlation interferometry. (Under review, Journal of Biomedical Optics)
8. **Sujatha, N.,** Murukeshan, V. M., Ong, L. S., and Seah, L. K. Image fiber effect in speckle pattern transmission and Image fiber guided DSPI endoscopy. (Under review, Optics and Laser Technology)
9. **Sujatha, N.,** and Murukeshan V. M. Effect of illumination beam divergence in DSPI fringe formation. (Accepted, Journal of Holography and Speckle)
10. **Sujatha, N.** and Murukeshan V. M. A multi-modality Endo-Speckle-Fluoroscope system for colon cancer diagnosis. (Pending submission, Gastrointestinal Endoscopy)

International Proceedings / Conferences

1. Murukeshan, V. M., **Sujatha, N.,** Ong, L. S., Seah, L. K. (2003). Imaging considerations in fiber optic endoscopy system for the gastrointestinal endoscopy. Proceedings of SPIE, 5143, pp.170-180.
2. Murukeshan, V. M., **Sujatha, N.,** Ong, L. S., and Seah, L. K. Fiberscope imaging considerations for fluorescence detection in the gastrointestinal path. (SCI 2004, Orlando, Florida, USA)
3. **Sujatha, N.,** Murukeshan, V. M., Ong, L. S., and Seah, L. K. Inspection of human body cavity using speckle correlation and decorrelation methods. (Accepted, ICEM 2004, Singapore)

List of publications

4. **Sujatha, N.**, Murukeshan, V. M., Ong, L. S., and Seah, L. K. Application of Digital speckle shearography for NDT of inner curved surfaces of tissue/tissue-like phantoms, presented at ICO'04, Tokyo (2004))
5. Murukeshan, V. M., **Sujatha, N.**, Seah, L. K., and Ong, L. S. An all fiber optic endoscope probe distal end for disease diagnosis in body cavities, Proceedings of SPIE, 5651, pp. 321-331)
6. **Sujatha, N.**, and Murukeshan, V. M. A dual mode imaging fiberscope system for colon cancer diagnosis, Proc. Photonics 2004, pp. 13.
7. Murukeshan, V. M. and **Sujatha, N.** A flexible endoscope system for intracavity investigations using speckle correlation analysis (Accepted and in press, BIOS 2005, San Jose, California, USA)

LIST OF REFERENCES

1. Rex, D. K., Johnson, D. A., Lieberman, D. A., Burt, R. W., and Sonnenberg, A. (2000). Colorectal Cancer Prevention 2000: Screening Recommendations of the American College of Gastroenterology. The American Journal of Gastroenterology, 95 (4), pp. 868-877.
2. Homepage of colorectal department (2004), Singapore General Hospital, http://www.sghhealth4u.com.sg/health4u/colorectal/hemorrhoids_index.html?colorectal_cancer.htm~right
3. Colon Cancer Fact sheet. (2004). American Cancer Society, pp. 1-5. http://www.cancer.org/colonmd/pdfs/fact_sheet.pdf
4. Rubio, C. A., Jaramillo, E., Lindblom, A., and Fogt, F. (2002). Classification of colorectal polyps: Guidelines for an endoscopist. Endoscopy, 34, pp. 226-236.
5. Shimoda, T., Ikegami, M., Fujisaki, J., Matsui, T., Aizawa, S., and Ishikawa, E. (1989). Early colorectal carcinoma with special reference to its development de novo. Cancer, 64, pp. 1138-1146.
6. Homepage of Oncology channel, colorectal cancer, (2004), <http://www.oncologychannel.com/coloncancer/>
7. D. E. Manshell, Colon polyps and cancer (2004), <http://personalweb.sunset.net/~mansell/polyp.htm>

List of references

8. Rex, D. K., Lehman, G. A., Ulbright, T. M., Smith, J. J., Pound, D. C., Hawes, R. H., Helper, D. J., Wiersema, M. J., Langefeld, C. D., and Li, W. (1993). Colonic neoplasia in asymptomatic persons with negative fecal occult blood tests: Influence of Age, Gender, and Family History. The American Journal of Gastroenterology, 88 (6), pp. 825-831.
9. Sonnenberg, A., Delco, F., and Bauerfeind, P. (1999). Is Virtual Colonoscopy a Cost-Effective Option to Screen for Colorectal Cancer?. The American Journal of Gastroenterology, 94 (8), pp. 2268-2274.
10. Vora, P., and Chapman, A. (2004). Complications from radiographer performed double contrast barium enemas. Clinical Radiology, 59, pp. 364-368.
11. Hara, A. K., Johnson, C. D., Reed, J. E., Ahlquist, D. A., Nelson, H., MacCarty, R. L., Harmsen, W. S., and Ilstrup, D. M. (1997). Detection of colorectal polyps with CT colography: Initial assessment of sensitivity and specificity. Radiology, 205, pp.59-65.
12. Mathur, P., Smith, J. J., Ramsey, C., Owen, M., Thorpe, A., Karim, S., Burke, C., Ramesh, S. and Dawson, P. M. (2003). Comparison of CT and MRI in the preoperative staging of rectal carcinoma and prediction of circumferential resection margin involvement by MRI. Colorectal Disease, 5, pp. 396-401.
13. Tutt, A. N. J., Plunkett, T. A., Barrington, S. F., and Leslie, M. D. (2004). The role of positron emission tomography in the management of colorectal cancer. Colorectal Disease, 6, pp. 2-9.
14. Gregory, B. M., David, J. B. and John, R. M. (2001). The impact of new technology on surgery of colorectal cancer. World Journal of Gastroenterology, 7 (5), pp. 612-621.

15. Scherer, T. D., Obando, J., and Heiss, F. (2003). Endoscopic ultrasound: a primer on today's endoscopes and indications for their use in gastroenterology, Postgraduate Medicine online, http://www.postgradmed.com/issues/2003/04_03/scherer.htm
16. Sergeev, A. M., Gelikonov, V. M., Feldchtein, F. I., Kuranov, R. V., and Gladkova, N. D. (1997). In vivo endoscopic OCT imaging of precancer and cancer states of human mucosa. Optics Express, 1 (13), pp. 432-440.
17. Platell, C. F. E, Philpott, G., and Olynyk, J. K. (2002). Flexible sigmoidoscopy screening for colorectal neoplasia in average risk people: evaluation of a five year rescreening interval. Medical Journal of Australia, 176, pp. 371-373.
18. Lieberman, D. A., and Smith, F. W. (1991). Screening for Colon Malignancy with Colonoscopy, The American Journal of Gastroenterology, 96 (8), pp. 946-951.
19. Boppart, S. A., Deutsch, T. F., and Rattner, D. W. (1999). Optical imaging technology in minimally invasive surgery. Surgical Endoscopy, 13, pp. 718-722.
20. Bahcall, J. K., and Barss, J. T. (2001). Fiberoptic endoscope usage for intracanal visualization. Journal of Endodontics, 27 (2), pp. 128-129.
21. Hemming, A. W., Nagy, A. G., Scudamore, C. H., and Edelmann, K. (1995). Laparoscopic staging of intra abdominal malignancy. Surgical Endoscopy, 9, pp. 325-328.
22. Volkov, V. V., Danilov, A. V., Vassin, L. N., and Frolov, Y. A. (1990). Flexible endoscope for intra ocular surgery. Archives of Ophthalmology, 108, pp. 1037-1038.
23. Webster, J. G. (2001). Minimally invasive medical technology. London: Institute of Physics Publishing, pp. 136-150, ISBN: 0 7503 0733 1.

List of references

-
24. Holland, P. (2001). The fundamentals of...Flexible Endoscopes, Biomedical Instrumentation and Technology, pp. 343-348.
 25. Tomkinson, T. H., Bentley, J. L., Crawford, M. K., Harkrider, C. J., Moore, D. T., and Rouke, J. L. (1996). Rigid endoscopic relay systems: a comparative study. Applied Optics, 35 (34), pp. 6674-6683
 26. Dario, P., Menciassi, A., Stefanini, C., and Accoto, D. (2002). Miniaturization of biomedical micromachines, 2nd annual international IEEE EMBS special topic conference on microtechnologies in medicine and biology, May 2-4, pp. 291-296.
 27. Classen, M., Knyrim, K., Seidlitz, H. K., and Hagenmuller, F. (1987). Electronic Endoscopy – The latest technology. Endoscopy, 19, pp. 118-123.
 28. Manual of Colonovideoscope model Q160L/I/S (2001), Olympus, Japan.
 29. Rol, P., Jenny, R., Beck, D., Frankhauser, F., and Niederer, P. F. (1995). Optical properties of miniaturized endoscopes for ophthalmic use. Optical Engineering, 34 (7), pp. 2070-2076.
 30. Mason, P. (2004). Biopsy, endoscopy and body imaging. The pharmaceutical journal, 272, pp. 573-575.
 31. Paul, J. (2001). M2A swallowable imaging capsule, ELE282 Biomedical engineering seminar I, Feb 26, http://www.ele.uri.edu/Courses/ele282/S01/Jeson_1_Capsule.pdf
 32. Park, H. J., Nam, H. W., Song, B. S., Choi, J. L., Choi, H. C., Park, J. C., Kim, M. N., Lee, J. T., and Cho, J. H. (2002). Design of bi-directional and multi-channel miniaturized telemetry module for wireless endoscopy. 2nd annual international IEEE EMBS special topic conference on microtechnologies in medicine and biology, May 2-4, pp. 273-276.

List of references

-
33. Ell, C., Remke, S., May, A., Helou, L., Henrich, R., and Mayer, G. (2002). The first prospective controlled trial comparing wireless capsule endoscopy with push enteroscopy in chronic gastrointestinal bleeding. Endoscopy, 34, pp. 685-689.
 34. Jones, R., Wykes, C. (1983). Holographic and Speckle Interferometry, London: Cambridge University Press, Chapter 4, ISBN: 0521232686.
 35. Sirohi, R. S. (1993). Speckle Metrology. New York: Marcel Dekker, Chapter 2 & 3, ISBN: 0 8247 8932 6.
 36. Vest, C. M. (1979). Holographic Interferometry. USA: John Wiley & Sons, Inc., Chapter 7, ISBN: 0471906832
 37. Murukeshan, V. M. (2003). Editorial - Trends in digital speckle pattern interferometry. Optics and lasers in engineering, 39 (4), pp. 409-410.
 38. Jones, R., Wykes, C. (1981). General parameters for the design and optimization of electronic speckle pattern interferometers. Optical Actuators, 28 (7), pp.949-972.
 39. Schnars, U., and Juptner, W. P. O. (1994). Digital recording and reconstruction of holograms in hologram interferometry and shearography. Applied Optics, 33 (20), pp. 4373-4377.
 40. Steinchen, W., Yang, L. X., Kupfer, G., Mackel, P., and Vossing, F. (1998). Strain analysis by means of digital sherography: potential, limitations and demonstration. Journal of Strain Analysis, 33 (2), pp. 171-182.
 41. Peterson, M. O. (1991). Digital speckle pattern shearing interferometry: limitations and prospects. Applied Optics, 30 (19), pp. 2730-2738.

42. Parker, S. C. J., and Salter, P. L. (1999). A novel shearography system for aerospace nondestructive testing. Proceedings of Institution of Mechanical Engineers, 213, pp. 23-33.
43. Sirohi, R. S, Tay, C. J., Shang, H. M., and Boo, W. P. (1999). Nondestructive assessment of thinning of plates using digital shearography. Optical Engineering, 38 (9), pp. 1582-1585.
44. Murukeshan, V. M., Ganesan, A. R., and Sirohi, R. S. (1996). Design and development of a compact fiber optic phase shifting ESPI system for engineering metrology. Proceedings of the 14th World Conference on Non Destructive Testing, pp. 1525-1528.
45. Vikram, C. S. (1993). Novel applications of speckle metrology. Chapter 5 in Speckle Metrology edited by R. S. Sirohi, pp. 225-227, ISBN: 0 8247 8932 6.
46. Sinclair, D. C. (1965). Demonstration of chromatic aberration in the eye using coherent light. Journal of Optical Society of America , 55, pp. 575-578.
47. Fercher, A. F., Peukert, M., and Roth, E. (1986). Visualization and measurement of retinal blood flow by means of laser speckle photography. Optical Engineering, 25, pp. 731-735.
48. Fujii, H., Asakura, T., Nohira, K., Shintomi, Y., and Ohura, T. (1985). Blood flow observed by time varying laser speckle. Optics Letters, 10 (3), pp. 104-106.
49. Fujii, H., Nohira, K., Yamamoto, Y., Ikawa, H., and Ohura, T. (1987). Evaluation of blood flow by laser speckle image sensing. Part 1. Applied Optics, 26 (24), pp. 5321-5325.
50. Tuchin, V. V., Ampilogov, A. V., Bogoroditsky, A. G., Rabinovich, E. M., Ryabukho, V. P., Ul'yanov, S. S., and V'yushkin, M. E. (1991). Laser speckle and optical fiber sensors for micromovement monitoring in biotissues. Proceedings of SPIE, 1429, pp. 62-73.

51. Aizu, Y., and Asakura, T. (1991). Biospeckle phenomena and their applications to blood flow measurements. Proceedings of SPIE, 1431, pp. 239-250.
52. Fukuoka, S., Hotokebuchi, T., Jingushi, S., Fujii, H., Sugioka, Y., and Iwamoto, Y. (1999). Evaluation of blood flow within the subchondral bone of the femoral head: Use of the laser speckle method at surgery for osteonecrosis. Journal of Orthopaedic Research, 17, pp. 80-87.
53. Vogel, A. (1986). Comparison of two sensitive speckle methods applied to the measurement of tooth deformation. Optik, 72, pp. 95-101.
54. Kasprzak, H., Podbielska, H., and Pennig, D. (1991). Speckle photography for investigation of bones supported by different fixing devices. Proceedings of SPIE, 1429, pp. 55-61.
55. Ul'yanov, S. S., Ryabukho, V. P., and Tuchin, V. V. (1994). Speckle interferometry for biotissue vibration measurement. Optical Engineering, 33 (3), pp. 908-914.
56. Kuzmin, Y. S., Ul'yanov, S. S., Tuchin, V. V., and Ryabukho, V. P. (1996). Speckle and speckle interferometric methods in cardiodiagnostics. Proceedings of SPIE, 2732, pp. 82-99.
57. Lokberg, O. J., Hogmoen, K., and Holige, O. M. (1979). Vibration measurement on the human ear drum *in vivo*. Applied Optics, 18 (6), pp. 763-765.
58. Roman, J. F., Moreno, V., Salgueiro, J. R., Suarez, D., Fernandez, P., Gallas, M and Blanchard, A. (1995). Electronic speckle pattern interferometry (ESPI) applied to the study of mechanical behavior of human jaws. Proceedings of SPIE, 2628, pp. 184-195.

List of references

-
59. Kishen, A., Murukeshan, V. M., Krishnakumar, V., Lim, C. S., and Asundi, A. (2003). Digital Speckle pattern Interferometric (DSPI) and thermo-graphic investigations on the thermal responds in human teeth. Optics and Lasers in Engineering, 39 (4), pp. 489-500.
60. Kemper, B., Dirksen, D., Avenhaus, W., Merker, A., and Bally, G. V. (2000). Endoscopic double-pulse electronic-speckle-pattern interferometer for technical and medical intracavity inspection. Applied Optics, 39 (22), pp. 3899-3905.
61. Lakowicz, J. R. (1999). Principles of Fluorescence Spectroscopy, New York: Kluwer academic/ Plenum Publishers, Chapter 1, ISBN: 0 306 46093 9.
62. Ramanujam, N. (2000). Fluorescence spectroscopy in vivo. Encyclopedia of Analytical Chemistry, Chichester: John Wiley & sons Ltd, pp. 20-56.
63. Cerussi, A. E. (1999). Quantitative frequency domain fluorescence spectroscopy in tissues and tissue like media. Ph.D. Thesis, University of Illinois at Urbana-Champaign.
64. Marcon, N. E., and Wilson, B. C. (1998). The value of fluorescence techniques in gastrointestinal endoscopy – better than an endoscopist's eye? II: The north American experience. Endoscopy, 30, pp. 419-421.
65. Shiozaki, H., Tahara, T., and Kobayashi, K. (1990). Endoscopic screening of early esophageal cancer with lugol dye method in patients with head and neck cancers. Cancer, 66, pp. 2068-2071.
66. Messman, H., Kullmann, F., Wild, T., Knuchel-Clarke, R., Ruschoff, J., Gross, V., Scholmerich, J., and Holstege, A. (1998). Detection of dysplastic lesions by fluorescence in a model of colitis in rats after previous photosensitization with 5-aminolevulinic acid. Endoscopy, 30, pp. 333-338.
-

List of references

-
67. Stepp, H., Baumgartner, R., Beyer, W., Knuchel, R., Rick, K., Steinbach, P., Stepp, H. G., and Kriegmair, M. (1995). Bladder tissue diagnosis utilizing Protoporphyrin IX fluorescence detection. Proceedings of SPIE, 2324, pp. 20-31.
68. Frimberger, D., Zaak, D., Stepp, H., Baumgartner, R., Schneede, P., Schmeller, N., and Hoestetter, A. (2001). Autofluorescence imaging to optimize 5-ALA induced fluorescence endoscopy of bladder carcinoma. Urology, 58, pp. 372-375
69. Leunig, A., Rick, K., Stepp, H., Gutmann, R., Alwin, G., Baumgartner, R., and Feyh, J. (1996). Fluorescence imaging and spectroscopy of 5-Aminolevulinic acid induced protoporphyrin IX for the detection of neoplastic lesions in the oral cavity. American Journal of Surgery, 172, pp. 674-677.
70. Mayinger, B., Neidhardt, S., Reh, H., Martus, P., and Hahn, E. G. (2001). Fluorescence induced with 5-aminolevulinic acid for the endoscopic detection and follow-up of esophageal lesions. Gastrointestinal Endoscopy, 54 (5), pp. 572-578.
71. Zonios, G. I., Cothren, R. M., Arendt, J. T., Wu, J., Dam, J. V., Crawford, J. M., Manoharan, R., and Feld, M. S. (1996). Morphological model of human colon tissue fluorescence. IEEE Transactions on Biomedical Engineering, 43 (2), pp. 113-122.
72. Romer, T. J., Fitzmaurice, M., Cothren, R. M., Richards-Kortum, R., Petras, R., Sivak, M. V., and Kramer, J. R. (1995). Laser-induced fluorescence microscopy of normal colon and dysplasia in colonic adenomas: Implications for spectroscopic diagnosis. The American Journal of Gastroenterology, 90 (1), pp. 81- 87.
73. Kapadia, C. R., Cutruzzola, F. W., O'Brien, K. M., Stetz, M. L., Enriquez, R., and Deckelbaum, L. I. (1990). Laser – induced fluorescence spectroscopy of human colonic mucosa – Detection of adenomatous transformation. Gastroenterology, 99, pp. 150-157.
-

List of references

-
74. Cothren, R. M., Richards-Kortum, R., Sivak, M. V., Fitzmaurice, M., Rava, R. P., Boyce, G. A., Doxtader, M., Blackman, R., Ivanc, T. B., Hayes, G. B., Feld, M. S., and Petras, R. E. (1990). Gastrointestinal tissue diagnosis by laser induced fluorescence spectroscopy at endoscopy. Gastrointestinal Endoscopy, 36 (2), pp. 105-111.
75. Cothren, R. M., Sivak, M. V., Dam, J. V., Petras, R. E., Fitzmaurice, M., Crawford, J. M., Wu, J., Brennan, J. F., Rava, R. P., Manoharan, R., and Feld, M. S. (1996). Detection of dysplasia at colonoscopy using laser-induced fluorescence: a blinded study. Gastrointestinal Endoscopy, 44 (2), pp. 168-176.
76. Wang, T. D., Dam, J. V., Crawford, J. M., Preisinger, E. A., Wang, Y., and Feld, M. S. (1996). Fluorescence endoscopic imaging of human colonic adenomas. Gastroenterology, 111, pp. 1182-1191.
77. Zonios, G. I., Cothren, R. M., Arendt, J. Wu, J., Crawford, J. M., Dam, J. V., Manoharan, R., and Feld, M. S. (1995). Fluorescence spectroscopy for colon cancer diagnosis. Proceedings of SPIE , 2324, pp. 9-13.
78. Rogers, B. H. G. (1990). Colonoscopy with fluoroscopy. Gastrointestinal Endoscopy, 36 (1), pp. 71-72.
79. Marchesini, R., Fumagalli, S., Pignoli, E., Sichirillo, A. E., Tomatis, S., Palma, S. D., Fante, M. D., Spinelli, P., Croce, A. C., and Bottiroli, G. (1995). Light induced fluorescence of human colon tissue: dependence on histological and histochemical properties studied by means of a simplified model for simulation. Proceedings of SPIE, 2324, pp. 76- 83.
80. Bohorfoush, A. G. (1996). Tissue Spectroscopy for gastrointestinal diseases. Endoscopy, 28, pp. 372-380.

List of references

-
81. Lam, S., MacAulay, C., LeRiche, J., Ikeda, N., and Palcic, B. (1995). Fluorescence imaging of early lung cancer. Proceedings of SPIE, 2324, pp. 2-8.
 82. Lam, S., MacAulay, C., Hung, J., LeRiche, J., Profio, A. E., and Palcic, B. (1993). Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscope. Journal of Thoracic and Cardiovascular Surgery, 105, pp. 1035-1040.
 83. Palcic, B., Lam, S., Hung, J., and MacAulay, C. (1991). Detection and localization of early lung cancer by imaging techniques. Chest, 99, pp. 742-743.
 84. DuVall, A., Wilson, B. C., and Marcon, N. E. (1997). Tissue Autofluorescence. Annals of Gastrointestinal Endoscopy, 10, pp. 25-30.
 85. DaCosta, R. S., Linge, L., and Kost, J. (1997), Confocal fluorescence microscopy / macroscopy and microspectrofluorimetry analysis of human colorectal tissue, Journal of Analytical Morphology, 4, pp. 192-194.
 86. Panjehpour, M., Overholt, B. F., and Vo-Dinh, T, Haggitt, R. C., Edwards, D. H., and Buckley, F. P. (1996). Endoscopic fluorescence detection of high grade dysplasia in Barrett's esophagus. Gastroenterology, 111, pp. 93-101.
 87. Mayinger, B., Horner, P., Jordan, M., Gerlach, C., Horbach, T., Hohenberger, W., and Hahn, E. G. (2001). Endoscopic fluorescence spectroscopy in the upper GI Tract for the detection of GI Cancer: Initial Experience. The American Journal of Gastroenterology, 96 (9), pp. 2616- 2621.
 88. Laifer, L. I., O'Brien, K. M., Stetz, M. L., Gindi, G. R., Garrand, T. J., and Deckelbaum, L. I. (1989). Biochemical basis for the difference between normal and atherosclerotic arterial fluorescence. Circulation, 80, 1893-1901.

List of references

-
89. Richards-Kortum, R., Rava, R. P., Fitzmaurice, M., Kramer, J. R., and Feld, M. S. (1991). 476nm Excited laser-induced fluorescence spectroscopy of human coronary arteries: Applications in cardiology. American Heart Journal, pp. 1141-1150.
90. Abe, S., Izuishi, K., Tajiri, H., Kinoshita, T., and Matsuoka, T. (2000). Correlation of in vitro autofluorescence endoscopy images with histopathologic findings in stomach cancer. Endoscopy, 32 (4), pp. 281-286.
91. Domingues, J. P., Correia, C. M., and Cunha-Vaz, J. G. (1995). Light sensors in ocular fluorescence imaging: assessment of parameters. Proceedings of SPIE, 2324, pp. 55-61.
92. Mayinger, B., Horner, P., Jordan, M., Gerlach, C., Horbach, T., Hohenberger, W., and Hahn, E. G. (2001). Light induced autofluorescence spectroscopy for the endoscopic detection of esophageal cancer. Gastrointestinal Endoscopy, 54 (2), pp. 195-201.
93. Wang, K. K., Gutta, K., Laukka, M., and Densmore, J. (1995). Laser induced fluorescence in the detection of esophageal carcinoma. Proceedings of SPIE, 2324, pp. 14-18.
94. Sterenborg, H. J. C. M., Motamedi, M., Wagner, R. F., Thomsen, S., and Jacques, S. L. (1995). In vivo fluorescence spectroscopy for the diagnosis of skin diseases. Proceedings of SPIE, 2324, pp. 32-38.
95. Schomacker, K. T., Frisoli, J. K., and Compton, C. C. (1992). Ultraviolet laser induced fluorescence of colonic tissue: Basic biology and diagnostic potential. Lasers in Surgery and Medicine, 12, pp. 63-78.
96. Mayinger, B., Jordan, M., Horner, P., Gerlach, C., Muehldorfer, S., Bittorf, B. R., Matzel, K. E., Hohenberger, W., Hahn, E. G., and Guenther, K. (2003). Endoscopic light-induced autofluorescence spectroscopy for the diagnosis of colorectal cancer and adenoma. Journal of Photochemistry and Photobiology B: Biology, 70, pp. 13-20.
-

97. Yuanlong, Y., Yanming, Y., Fuming, L., Yufen, L., and Paozong, M. (1997). Characteristic autofluorescence for cancer diagnosis and its origin. Lasers in Surgery and Medicine, 7, 528-532.
98. Marchesini, R., Pignoli, E., and Tomatis, S. (1994). Ex vivo optical properties of human colon tissue. Lasers in Surgery and Medicine, 15, pp. 351-357.
99. Fiarman, G. S., Nathanson, M. H., West, A. B., Deckelbaum, L. I., Kelly, L., and Kapadia, C. R. (1995). Difference in laser induced autofluorescence between adenomatous and hyperplastic polyps and normal colonic mucosa by confocal microscopy. Digestive Diseases and Sciences, 40, pp. 1261-1268.
100. Zuluga, A. F. et.al. (1999). Fluorescent Excitation Emission Matrices of human tissue: A system for *in vivo* measurement and method of data analysis. Applied Spectroscopy, 53 (3), pp. 302-311.
101. Gregory, P. M., Crystal, M. L., Kristin, V. M., and Ramanujam, N. (2002). Optimal methods for fluorescence and diffuse reflectance measurements of tissue biopsy samples. Lasers in Surgery and Medicine, 30 (3), pp. 191-200.
102. Kin, C. C., and Chiang, F. P. (1983). Objective laser speckle method for three dimensional displacement measurement on curved surfaces. Optical Engineering, 22 (1), pp. 153-155.
103. Ratnam, M. M., Evans, W. T., and Tyrer, J. R. (1992). Measurement of thermal expansion of a piston using holographic and electronic speckle pattern interferometry. Optical Engineering, 31(1), pp. 61-69.

List of references

-
104. Rao, M. V., Samuel, R., and Ananthan, A. (2003). Applications of electronic speckle interferometry (ESI) techniques for spacecraft structural components. Optics and Lasers in Engineering, 40, pp. 563-571.
 105. Kim, K. S., Kang, K. S., Kang, Y. J., and Cheong, S. K. (2003). Analysis of an internal crack of pressure pipeline using ESPI and shearography. Optics and Laser Technology 35, pp. 639-643.
 106. Li, X., Liu, X., and Wang, K., (2002). Quantitative detection of the defects in thin-walled pressure vessels with holography and shearing speckle interferometry. Journal of Nondestructive Evaluation, 21 (3), pp. 85-94.
 107. Groves, R. M., James, S. W., and Tatum, R. P. (2003). Pipe weld investigation using shearography. Strain, 39, pp. 101-105.
 108. John, B. F. (1990). Calculus with analytical geometry, USA: Addison Wesley publishing company, Chapter 11, pp. 549-601, ISBN: 0201503638.
 109. Murukeshan, V. M. (1997). Fiber-optic electronic speckle pattern interferometry and its applications. Ph. D Thesis, IIT Madras.
 110. Niederer, P., Hafliger, J., Blessing, P., Lehareinger, Y., Doswald, D., and Felber, N. (2001). Image quality of endoscopes. Proceedings of SPIE, 4158, pp. 1-9.
 111. Tsumanuma, T., Toriya, T., Tanaka, T., Shamoto, N., Seto, K., and Sanada, K. (1991). New image diagnosis system with ultrathin endoscope and clinical results. Proceedings of SPIE, 1420, pp. 193-198.
 112. Home page of Buy-A-Mag corporation (2004), www.buyamag.com
 113. Home page of Simulab corporation (2004), www.simulab.com.

List of references

-
114. Huh, C. H., Bhutani, M. S., Farfan, E. B., and Bolch, W. E. (2003). Individual variations in mucosa and total wall thickness in the stomach and rectum assessed via endoscopic ultrasound. Physiological measurement, 24, pp. 15-22.
 115. Holder, L., Okamoto, T., and Asakura, T. (1993). A digital speckle correlation interferometer using an image fiber. Measurement science and Technology, 4, pp. 746-753.
 116. Takai, N., Kimura, S., and Asakura, T. (1989). Dynamic properties of laser speckles on images transmitted through an image fiber bundle. Journal of modern optics, 36, pp. 181-193.
 117. Lokberg, O., and Krakhella, K. (1981). Electronic speckle pattern interferometry using optical fibers. Optics communications, 38 (3), pp. 155-158.
 118. Born, M., and Wolf, E. (1980), Principles of Optics: Electromagnetic Theory of Propagation, Interference and Diffraction of Light, Pergamon, New York, Chapter 4, ISBN: 0080264816.
 119. Kennedy, T. P. (2002), Understanding ball lenses,
<http://www.edmundoptics.com/techSupport/DisplayArticle.cfm?articleid=245>
 120. Ouchi, T. (2002), Control wire driving mechanism for use in endoscope, United States Patent, Patent number: 6,440,062.
 121. Phee, S. J., Ng, W. S., Chen, I. M., Seow-Choen, F. and Davis, B. L. (1997). Locomotion and steering aspects in automation of colonoscopy. IEEE Engineering in Medicine and Biology, pp. 85-96.

List of references

-
122. Gao, L. M., Chen, Y., Lin, L. M., and Yan, G. Z. (1998). Micromotor based a new type of endoscope. Proceedings of the IEEE Engineering in Medicine and Biology society, 20 (4), pp. 1822-1825.
 123. Reynaerts, D., Peirs, J., and Brussel, H. V. (1996). Design of a shape memory actuated gastrointestinal intervention system. Euroensors X, Leuven, Belgium, 8-11 September, pp. 1181-1184.
 124. Dinish, U.S., Chao, Z. X., Singh, A., Seah, L. K., and Murukeshan, V.M. (2004). Formulation and implementation of phase-resolved optical technique for latent fingerprint imaging: Theoretical and experimental Analysis. Applied Optics, [in press].
 125. Podbielska, H., and Jaroński, J. (1994). Computer aided analysis of the endoscopic fringe pattern images. Proceedings of SPIE, 2340, pp. 180-187.