List of Publications

Journals Articles
(Included in the thesis)

1. Use of common path phase sensitive spectral domain optical coherence tomography for measurement of refractive index.

2. Determination of elastic properties of resected human breast tissue samples using optical coherence tomographic elastography.
   A. Srivastava, **Y. Verma**, K. D. Rao and P. K. Gupta
   *Strain* 47, 75–87 (2011).

3. Imaging of human breast tissue using polarisation sensitive optical coherence tomography

   *Skin research and technology* 16, 428 (2010)

5. Real-time *in-vivo* imaging of adult Zebrafish brain using optical coherence tomography.
   K. D. Rao, A. Alex, **Y. Verma**, S. Thampi, P. K. Gupta

6. Optical coherence tomography using a tapered single mode fiber tip.
   *Applied Physics B. 87, 607-610 (2007)*

8. Imaging growth dynamics of tumor spheroids using optical coherence tomography.
   *Biotechnology Letters, 29, 273-278 (2007).*

   K. D. Rao, **Y. Verma**, H. S. Patel and P. K. Gupta,
   *Current Science, 90, 1506, (2006).*

   *(Not included in the thesis)*

10. Single mode fiber based polarization sensitive optical coherence tomography using swept laser source.
    P. Sharma, **Y. Verma**, K. D. Rao, and P. K. Gupta,
    *Journal of Optics 13, 115301-06 (2011).*

    *Journal of Innovative Optical Health Sciences, 4, 59-66 (2011).*

12. Effect of He-Ne laser irradiation on hair follicle growth cycle of swiss albino mice.
    *Skin Pharmacol Physiol, 23, 79–85 (2010).*

**In Edited Volumes:**
1. *In-vivo* imaging of adult Zebrafish using optical coherence tomography.
   **Y. Verma**, K. D. Rao, A. Alex, and P. K. Gupta

2. Effect of He-Ne laser irradiation on hair follicle growth in testosterone treated mice investigated with optical coherence tomography and histology.
   The paper was adjudged by ILA as one of the five best posters presented at NLS-07.

3. Tapered single mode fiber tip for high lateral resolution imaging in Optical Coherence Tomography.
   *The International Biomedical Optics Symposium (BiOS)* San Diego USA, SPIE  p. 64360Y, 2007.

4. Optical Coherence Tomography.
   K. D. Rao, **Y. Verma** and P. K. Gupta

**Conference Presentations / Publications:**

5. Use of common path phase sensitive spectral domain optical coherence tomography setup for refractive index measurements.
   *International conference on opto-electronics, fiber optics and photonics, held at Guwahati, December 2010.*

6. Swept source based fiber optic polarization sensitive optical coherence tomography setup for tissue birefringence.
   P. Sharma, **Y. Verma**, K. D. Rao, and P. K. Gupta
International conference on opto-electronics, fiber optics and photonics, held at Guwahati, December 2010.

7. Combined Raman spectroscopy-optical coherence tomography for analysis of tissue pathology.
   *International conference on opto-electronics, fiber optics and photonics, held at Delhi, 2009.*


9. A new approach for mueller matrix measurement using fiber based optical coherence tomography.
   *International Conference on Optics within Lifesciences OWLS-10, Biophotonics Asia, July 2-4, 2008, Singapore.*

10. Analysis of optical coherence tomography images for binary classification of resected human breast tissues.
    *International Conference on Optics within Lifesciences OWLS-10, Biophotonics Asia, July 2-4, 2008, Singapore.*

11. Polarization Sensitive Optical Coherence Tomography using a single detector and dual reference beams.
    *Saratov Fall Meeting (SFM), September 25-28,2007, Saratov, Russia.*
12. Effect of He-Ne laser irradiation on hair follicle growth in testosterone treated mice investigated with optical coherence tomography and histology.


13. Tapered single mode fiber tip for high lateral resolution imaging in Optical Coherence Tomography.


*BIOS San Diego USA 2007.*


*Eighth international conference on opto-electronics, fiber optics and photonics, held at Hyderabad, December 2006.*

15. Imaging growth dynamics of tumour spheroids using optical coherence tomography.


*Eighth international conference on opto-electronics, fiber optics and photonics, held at Hyderabad, December 2006.*
Figure Captions

Figure 1.1: (a) Schematic of a low coherence interferometry setup. (b) Interference pattern observed for a high coherent light source. Interference is observed for longer distance due to long coherence length. (c) Interference pattern observed for a low coherent source. Interference is observed for short distance due to smaller coherence length. 30

Figure 1.2: Transverse resolution for low and high numerical aperture (NA) focusing lens and the trade-off between transverse resolution vs. depth of field. 35

Figure 1.3: Different scanning configurations used in OCT. (a) A-scan based B-scan which results in z-x plane imaging (z-fast & x-slow scan), (b) T-scan based B-scan which results in x-z plane imaging (x-fast & z-slow scan), (c) C-scan or enface imaging which results in y-x plane imaging (y-fast & x-slow scan). Red arrows (solid) represent fast scan direction while blue arrows (dash) represent slow scan direction. 36

Figure 1.4: SNR as a function of reference-arm reflectivity. Also shown are the signal-to-thermal-noise ratio (SNRth), the signal-to-shot-noise ratio (SNRsh), and the signal-to-excess-intensity noise ratio (SNRex). 39

Figure 2.1: Different modules of an OCT imaging system. 47

Figure 2.2: Schematic of OCT setup. BPF-bandpass filter; C-collimator lens; D-detector; DAQ-data acquisition card; FC-fiber coupler; LA-lockin Amplifier; MTS-motorized translation stage; SLD-superluminescent diode; TIA-transimpedence amplifier. 52
Figure 2.3: Front panel of the TDOCT setup used for setting up the desired imaging parameters and display of OCT images ........................................................................................................54

Figure 2.4: Flow of control in the Labview program (.vi) to acquire and display the OCT image. ........................................................................................................................................55

Figure 2.5: (a) Typical interferogram acquired using the set-up and its demodulated envelope. (b) Zoomed view of interferogram and its envelope (c) OCT signal or A-scan of a microscopic cover slip showing two peaks for front and back interface of the coverslip. .............................................................................................................................................57

Figure 2.6: OCT images of (a) human skin, (b) nail and (c) Zebrafish eye acquired with the developed OCT setup. Image size: a & b: 2 mm x 3 mm; c: 3 mm x 2 mm.............58

Figure 2.7: (a) Schematic of the chemical etching process, (b) Microscopic image of the tapered tip.......................................................................................................................................................59

Figure 2.8: Modified sample arm with the tapered tip.................................................................................................60

Figure 2.9: Beam profiles of the laser beam transmitted through the tapered fiber measured at distances distance of (a) 50 µm , (b) 100 µm and (c) 200 µm from the tip; Calculated spatial mode profile at (d) 50 µm, (e) 100 µm and (f) 200 µm. Images (a-f) are in same magnification. Image size: 25 µm x 25 µm. ........................................................................................................61

Figure 2.10: (a) Beam profiles of the laser beam transmitted through the tapered fiber measured at distances distance of 50 µm, 100 µm and 200 µm. (b) Beam waist as a function of axial distance from tip. .....................................................................................................................62

Figure 2.11: OCT images with the modified set-up with the tapered fiber tip (a-d): (a) Scattering from intralipid placed on coverslip. (b) OCT image of scattering from infrared
viewing card; Image size (a & b): 0.4 mm (depth) x 0.2 mm (lateral). (c) OCT image of plant leaf *Elodea densa* and (d) after applying minimum filter using ImageJ software. Image size (c & d): 0.1 mm (depth) x 0.2 mm (lateral). (e) OCT image of *Elodea densa* taken with conventional OCT set-up with 10 X microscopic objective. Image size: 0.2 mm (depth) x 1.0 mm (lateral). (f) Expanded image of the marked region; size 0.1 mm (depth) x 0.2 mm (lateral).

Figure 2.12: Schematic of single detector based polarization sensitive optical coherence tomography setup. D- Detector; L- lens; M1,M2: Mirrors; NPBS- Nonpolarizing beam splitter; P-Polarizer; QWP- Quarter wave plate; SLD- Superluminescent Diode.

Figure 2.13: Experimental Set up for measuring Phase retardation of wave plate (WP). SLD: Superluminescent diode; P: Polarizer; A: Analyzer.

Figure 2.14: Measurement of phase retardation of wave plate (design wavelength 665 nm) for various orientations of fast axis.

Figure 2.15: Intensity (a) and cumulative round-trip phase retardation (b) images of chicken breast tissue in-vitro. Image size: 1.2 mm (depth) x 3 mm (lateral).

Figure 3.1: Ray diagram of the rapid scanning FDODL. G-grating; M1-resonant scanning mirror; M2-Double pass mirror [58].

Figure 3.2: Schematic of time domain high speed OCT system. BPF: band pass filter; C1 & C2: circulators; LPF: low pass filter; RSOD: rapid scanning optical delay line; SLD: superluminescent diode.

Figure 3.3: (a) photograph of the high speed OCT system; (b) photograph of hand held probe for *in-vivo* imaging of dermatological samples; (c) an OCT image of finger pad.
acquired with the hand held probe (size: 2.5 mm x 3 mm); and (d) the schematic of the
developed oral probe with an image of oral mucosa (size: 2.5 mm x 3 mm). .................... 80

Figure 3.4: OCT images of (a) threaded screw (size: 2 mm x 2.5 mm), (b) oral mucosa, (c)
finger nail, (d) nail-finger junction, (e) palm and (f) palm with blister. Size: a-f 2 mm
(depth) x 2.5 mm (lateral). .................................................................................................. 81

Figure 3.5: Basic scheme of FDOCT approach ................................................................. 84

Figure 3.6: Typical interference spectrum (a, c) and corresponding A-scan (b, d) after
performing FFT .................................................................................................................. 85

Figure 3.7: Schematic of FDOCT setup. C: collimator lens; G: galvo-scanner; L: imaging
lens; LSC: line scan camera; M: reference mirror; S: sample; SLD: superluminescent
diode; TG: transmission grating ......................................................................................... 87

Figure 3.8: Set of A scans measured for different optical delays between the reference
mirror and object. (a) FFT performed without resampling and (b) after resampling. ....... 89

Figure 3.9: OCT images of biological tissue samples (a) nail fold, (b) skin back side of the
finger, (c and d) finger pad and (e) onion using FDOCT setup. Image size 1.25 mm (depth)
x 3 mm (lateral) .................................................................................................................. 90

Figure 4.1: A photograph of adult Zebrasfish ................................................................. 93

Figure 4.2: OCT images of whole eye of Zebrasfish (a) under anesthesia, and (b) ~ 30
minutes dipped in 10% formaline solution. Image size: 3 mm (depth) and 2 mm (lateral).
Abbreviations: L-lens, C-cornea, I-iris, and R-retina ........................................................ 94

Figure 4.3: (a) In-vivo OCT image of cornea of Zebrasfish. Image size 0.4 mm (depth) x
0.9 mm (lateral); (b) In-vivo OCT image of anterior angle of the cornea with the iris of
Figure 4.4: OCT image of whole eye of Zebrafish. Size of the image is 3 mm in axial and 2.2 mm in lateral direction.

Figure 4.5: OCT images of (a) resected and (b) in-vivo Zebrafish eye lens; (c) ray diagram of light propagation through a graded refractive index spherical lens.

Figure 4.6: (a) Refractive index profile obtained from the mean values of coefficients of Table 4.1. (b) Ray paths for the refractive index profile shown in (a).

Figure 4.7: Cross-sectional images of Zebrafish brain displayed at an interval of 150 µm (The horizontal and vertical bars denote 0.5 mm length).

Figure 4.8: (a) 3-D image of adult Zebra fish brain in axial and segittal plane, (b-c) Orthogonal projection image of brain; (d) crossection of brain in axial plane.

Figure 5.1: (A): Intensity images (i) normal, (ii) malignant, and (iii) benign of resected breast tissue samples. (B): Retardation images (i) normal, (ii) malignant, and (iii) benign of resected breast tissue samples. Image size: 1 mm (depth) × 2mm (lateral).

Figure 5.2: Phase retardation depth profile of malignant (circle) and benign (square) breast tissue. The linear fitting of the depth profile is shown by solid (benign) and dashed (malignant) lines. The arrow shows tissue top surface.

Figure 5.3: (a) Intensity and (b) retardation images of breast tissue. Arrows show the tumor margin. Image size: 1 mm (depth) × 2 mm (lateral).
Figure 5.4: (a) Schematic of arrangement for compressive loading of tissue samples; (b) plot of the force versus average displacement of the top surface of normal and malignant breast tissue samples. ................................................................. 120

Figure 5.5: Original (a) and post-compressed (b-f) OCT images of gelatin phantoms. Axial load (in steps of ~ 245 N/m$^2$) is applied along z-direction to compress the phantoms. For better clarity, OCT images corresponding to alternate load values have been shown above. The displacement of the top layer of the phantom (phantom-cover slip interface) in successive images shown above is about 40 µm............................................................... 124

Figure 5.6: Displacement vector maps for a series of axial compressive loads as obtained for gelatin phantoms by cross correlation technique with three different kernel sizes (31×31, 41×41 and 51×51 pixels). The maps correspond to the OCT images shown in Figure 5.5. (Vertical dimension of each plot (from top to bottom): 1.38, 1.34, 1.30, 1.26 and 1.22 mm. Horizontal dimension: 2.0 mm. Numbers below each row indicate the compressive stress applied in N/m$^2$.) ........................................................................................................ 125

Figure 5.7: Original (a) and post-compressed (b) OCT images of normal, benign and malignant breast tissue samples. The compressive stress is applied along z-direction (axial). Displacement in successive images shown above is about 40 µm...................... 127

Figure 5.8: Displacement vector maps for a series of axial compressive loads as obtained for normal, benign and malignant breast tissue samples by cross correlation technique. (Kernel size= 41×41 pixels). (Vertical dimension of each plot (from top to bottom): 1.66, 1.63, 1.59, 1.54 and 1.50 mm. Horizontal dimension: 2.0 mm). The number below each image indicates the compressive stress applied in units of N/m$^2$.) ................................................. 128
Figure 5.9: Stress-strain curves for normal, benign and malignant breast tissue samples.

Figure 5.10: Images of spheroids grown for different durations (a) 0, (b) 4, (c) 5, (d) 6 and (e) 7 days; A) OCT and B) Phase contrast images. Zero day represents time when cell aggregate is transferred from hanging drop to agar coated petridishes. Scale bar: 500 µm

Figure 5.11: Comparison of dimensions of spheroids measured by microscopy and OCT.

Figure 5.12: (a) Volume of spheroids determined by microscopy and OCT. Inset shows volume of spheroids determined by OCT in expanded scale. (b) Total number of cells in spheroids grown for different days. Cell count was determined using Hemocytometer.

Figure 5.13: OCT cross sectional images of the spheroid grown by liquid over-lay method (a-b), a & b are OCT images of same spheroid acquired twice to show the reproducibility of high scattering regions, fluorescence image of a typical spheroid grown for 10 days time stained with propidium iodide. Scale bar: 50 µm (c). Spheroids were stained with propidium iodide (10 µg/ml) for 15 min. The fluorescence was viewed under microscope using 540 nm band pass excitation and 590 nm long pass emission filters respectively. Scale bar: 40 µm.

Figure 6.1: Post-infection time-dependent bacterial counts obtained from wounded skin tissue of mice. The error bar represents the standard deviation around the mean calculated from three different experiments. Data represented at each infection time point are obtained from three mice.
Figure 6.2: PSOCT image of the normal mice skin. Back scattered (a; image size: 1.5 mm × 3 mm), optical retardation (b; image size: 1.5 mm × 3 mm) OCT images of resected mice skin and the histology image (c) of the corresponding tissue. Scale bar: 200 µm...

Figure 6.3: Time-dependent structural changes in uninfected wound skin of mice. Left (a, d, g), middle (b, e, h) and right (c, f, i) panels represent backscattered intensity OCT images, PSOCT images and histological images, respectively. Top (a–c), middle (d–f) and lowermost (g–i) rows represent images of resected wounded skin sample imaged on days 2, 4 and 10 of wounding, respectively. OCT images; image size: 1.5 mm × 3 mm. Histology images; scale bar: 100 µm...

Figure 6.4: Postinfection time-dependent structural changes in infected wound skin of mice. Left (a, d, g), middle (b, e, h) and right (c, f, i) panels represent backscattered intensity OCT images, PS-OCT images and histological images, respectively. Top (a–c), middle (d–f) and lowermost (g–i) rows represent images of resected infected skin sample imaged on days 2, 4 and 10 of wounding, respectively. Image size: 1.5 mm × 3 mm....

Figure 6.5: Measured mean phase retardation of normal (N), wound skin without (UI) and with infection (I) measures along the skin depth on day 10 of wound creation. Individual columns represent the mean value per sample measured from six different images taken from two different experiments. The scale bars represent standard deviation around mean. The statistical significance was determined using oneway ANOVA (*Po 0.05).....

Figure 6.6: Kinetics of healing of uninfected and infected wound imaged using real-time OCT. Top panel (a): image of normal skin. Image size: 1.5 mm × 3 mm. The images show, compared with uninfected infected wounds, a delay in different phases of wound healing.
Figure 7.1: Schematic of the common path spectral domain interferometer. SLD, FC, C, L, TG, LSC are the abbreviations for superluminescent diode, fiber-optic coupler, collimating lens, lens, transmission grating and line scan camera respectively. Inset picture shows the sample chamber along with the paths of interfering (reference and sample) beams. ......

Figure 7.2: (A) The spectral interference fringes are shown for empty (solid line) and water filled (dash-dot line) sample chamber, (B) the unwrapped phase and wave number curve shows change in slope for the empty (black line) and water filled sample chamber (dash-dot line). Inset picture shows the stability of the optical path length (OPL) calculated over 100 measurements for a coverslip of thickness 115 µm. .......................... 157

Figure 7.3: Fluctuations in measurements of OPL using (A) separate reference and sample arm and (B) with common path interferometer................................................................. 158

Figure 7.4: FFT of the acquired spectrum before (solid line) and after (dotted line) remove coverslip from the sample chamber. The peak marked by letter A corresponds to the OPL of the coverslip while B and B’ are the peaks corresponding to the thickness of the sample chamber before and after removing the coverslip respectively. ............................... 159

Figure 7.5: The RI data obtained for different concentrations are shown for (A) water (B) 0.5% intralipid (square), and 1% intralipid (diamond). The linear fit to data obtained in water is shown in solid line and that of 0.5% intralipid and 1% intralipid are shown in dotted and dashed lines respectively. ................................................................. 161

Figure 7.6: (A) The schematic diagram of single cell refractive index measurement setup. (B) The measured change in OPL when light passes through the cell and outside the cell. ................................................................. 162
Table captions

Table 1-1: A comparison of biomedical imaging techniques [1,11].................................40

Table 2.1: Various delay lines used in OCT systems and their important parameters. ......50

Table 2.2: Comparison of phase retardation measured with polarimetry and PSOCT......69

Table 4.1: Optimized coefficients for refractive index profile .........................................105

Table 5.1: Percentage error between calculated mean axial displacement and measured
axial displacement for six different kernel sizes..............................................................124

Table 5.2: Means and standard deviations of the estimated modulus of elasticity for the
three classes of breast tissue samples identified as normal, benign (fibroadenoma), and
malignant (invasive ductal carcinoma). ............................................................................131

Table 7.1: Linear fitting parameters for glucose sensing in water and in intralipid
solutions. ...........................................................................................................................161