CHAPTER 4: UTILIZATION OF TDOCT SETUPS FOR IMAGING ZEBRAFISH

Abstract: In this chapter we describe the results of our studies on the utilization of TDOCT setups for non-invasive, high resolution imaging of the different organs of Zebrafish, a vertebrate model system widely used for studies on ocular development and a variety of human diseases. The ocular images obtained using TDOCT setups could be used to measure corneal and retinal thickness and refractive index profile of the lens. A three dimensional iso-surface model of Zebrafish brain was also reconstructed using high resolution (~ 20 µm) two dimensional OCT images and the major structures of the brain could be clearly seen.

4.1 Introduction

Zebrafish, a popular aquarium fish (Figure 4.1) has life cycle of about 2-3 years. At the age of 3 months it is sexually reproductive and lays 100–200 rapidly developing embryos per week in each clutch [62,63]. The embryos develop externally to the mother and are also completely transparent for the first few days which facilitate easy observation of embryonic development using microscopy. These features of Zebrafish make them a good system for laboratory experiments [64,65]. Zebrafish has also proved to be an important model organism particularly for the developmental research which includes formation of neural connections in the retina and brain, understanding the visual behaviour and the
underlying mechanisms of retinal image formation, etc. Various mutants have been identified in Zebrafish that have relevance to human disease like retinal defects. For studies in these areas, measurement of several ocular parameters of the Zebrafish, like refractive index of the crystalline lens, mean retinal thickness, etc. are required. Most of these measurements have been carried out on extracted eye samples [66]. We have investigated the use of OCT for non-invasive measurements of the ocular parameters and for imaging of other internal organs like brain.

![Figure 4.1: A photograph of adult Zebrafish.](image)

4.2 Non-invasive imaging of intra-ocular structures of adult Zebrafish eye

Zebrafish were procured from local suppliers. For *in-vivo* imaging, the fish was anaesthetized using clove oil following the protocol given by Grush *et al.* [67]. Briefly, clove oil was dissolved in ethanol (1 : 10) and then mixed in 1 liter of water in a glass tank, such that the clove oil concentration was about ~75–100 ppm. The fish lost physical movement within 10 minutes. When the fish showed no response to physical stimulus, it was transferred to a petri dish for imaging. The fish was observed to revive within ~ 1 h after which it was transferred to a freshwater tank. In Figure 4.2a, we show the *in-vivo*
OCT image of the whole eye of the anesthetized Zebrafish. While the cornea, the anterior angle region, and the retina are clearly seen, the lens is not visible in the image due to index matching of the lens surface with the surrounding fluid. During the course of several experiments, it was found that the lens could be imaged with better contrast after dipping the fish in a petri dish containing 10% formalin solution for ~15–30 min. In Figure 4.2b, we show the image of the fish eye after it was dipped in 10% formalin solution. The lens (L) as well as other ocular structures like cornea (C), iris (I), and retina (R) can be clearly seen.

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

Figure 4.3 (a) shows the *in-vivo* image of the cornea of Zebrafish. The image features are in good agreement with the reported images of Zebrafish eye obtained using light microscopy [68]. Corneal thickness in fish is an important parameter that is affected by environmental stress and infection. The maximum corneal thickness was measured to be ~34 µm. Assuming a corneal refractive index of 1.33, the geometrical thickness of
Zebrafish cornea, in the region where the OCT light is perpendicular to the cornea, turned out to be 26 µm (± 11 µm). In their experiments on Zebrafish cornea extracted from dissected eyeballs, Swamynathan et al. [68] estimated its thickness to range from 16 to 20 µm. The difference in the two measurements may be due to the difference in the protocol and may illustrate the usefulness of in-vivo measurements. It should be noted that since the resolution of our set-up is ~ 11 µm, we could not resolve the epithelium layer of the cornea, which has thickness of ~ 8 µm [68]. Use of an ultra-high resolution OCT set-up will be required for quantitative comparison of corneal thickness and microstructures therein. The anterior chamber angle is another important parameter in the structure of the eye. Narrowing of this angle increases the risk of glaucoma. Non-invasive measurement of anterior chamber angle is therefore vital for characterization of structural changes in the anterior region and changes in the intraocular pressure (IOP). In-vivo imaging of the anterior chambers of different mutant variants of Zebrafish may help in better understanding of the genesis of glaucoma. Figure 4.3b shows the in-vivo OCT image of the anterior region of the eye. The anterior chamber angle of the cornea with the iris is clearly visualized. Figure 4.3c shows the expanded OCT scan of the Zebrafish retina with the layered structure. Due to the small retinal thickness and poor axial resolution compared to histology, only broad categorization is possible. The first reflective interface posterior to the lens at the top of the image in Figure 4.3c represents the anterior border of the retina (vitreo–retinal interface) with the retinal nerve fibre layer (RNFL) at the vitreous interface. The outermost band is thought to be that of the retinal pigment epithelium (RPE) choriocapillaris complex showing high scattering. Assuming a retinal refractive index of 1.38 and considering the band above RPE, the retinal thickness can be estimated as 123 ± 11 µm. For comparison a mean retinal thickness of ~ 85 µm for wild-type Zebrafish was
reported by Link et al. [69]. The difference in these values could be due to the fact that the measurements reported by Link et al. were on resected eyes.

Figure 4.3: (a) In-vivo OCT image of cornea of Zebrafish. 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 Zebrafish. Image size 1.3 mm (depth) x 0.7 mm (lateral); (c) In-vivo OCT image of retina of Zebrafish. Image size 1 mm (depth) x 0.7 mm (lateral).

In Figure 4.4 we show the OCT image of the whole eye of a Zebrafish, dipped in 10% formalin solution. Along the apex of the lens (when the OCT beam was exactly perpendicular to the cornea) some scattering inside the lens was also seen, indicating that the crystalline lens has gradually varying refractive index as a function of depth. It is pertinent to note that although the lens of the fish eye is known to be spherical in shape, the diameters of the lens as measured by OCT were different along the axial and lateral directions. This is because, while in axial direction the measured diameter is the optical length of the lens (geometrical path x effective refractive index), in the lateral direction it
is the true geometrical diameter. The difference in the ratio of the measured diameters can be used to estimate the effective index of refraction of the lens medium. From the image shown in Figure 4.2b, effective refractive index of ~ 1.43 was estimated for the Zebrafish crystalline lens at the centre wavelength of the source (840 nm). The crystalline lens of the fish is usually assumed to be spherical with a gradient refractive index that varies from approximately 1.35–1.38 at the surface to 1.55–1.57 at the core of the lens [70,71].

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.

The refractive index of the fish lens was also measured using focus-tracking method described by Wang et al. [72]. The sample arm light was focused using a high numerical aperture microscopic objective (20X) with the focal plane set initially at the top surface of the lens. This was achieved by monitoring the intensity of the A-scan peak, and maximizing the corresponding peak of the lens surface. Once the focus was set at the top of the lens surface, the objective was moved a distance $\Delta x$ such that the bottom surface of the lens came in focus. The corresponding change in the reference mirror position ($\Delta y$) to
get the A-scan peak intensity was noted. The effective refractive index was determined using the expression [72]

\[
\frac{n^2}{2} = \left[ \frac{\Delta \gamma}{\Delta x} - \frac{\Delta x}{2} \right]^2 + 4 \left( n_0^2 - n^2 \right) + \frac{\Delta x}{2} \left( \frac{\Delta y}{\Delta x} \right)^2 \tag{4.1}
\]

where \( n_0 \) is the refractive index of the surrounding medium of the tissue. Before applying this technique to fish lens, we used it to measure the refractive index of quartz window. The measured value of 1.445 @ 840 nm was found to be in good agreement with the reported value [72].

From the measurements, we estimate the effective (integrated) refractive index of the fish lens as \( \sim 1.43 \) @ 840 nm within 3% rms deviations. The effective refractive index can also be computed assuming the refractive index variation along the beam direction to be of parabolic form [73] given by

\[
n(z) = n_0 \sqrt{1 - \frac{z^2}{a^2}} \tag{4.2}
\]

where \( n_0 \) is the maximum refractive index at the centre of the lens and \( a \) is a constant that indicates the steepness of the gradient in the refractive index. The effective refractive index of the lens \( (n_{\text{eff}}) \) as seen by the OCT beam is then given by

\[
n_{\text{eff}} = \frac{\int r n(z) dz}{\sqrt{r}} \tag{4.3}
\]

is the total optical path along the apex of the lens as seen by the OCT beam and \( r \) is the radius of the spherical lens. Using OCT, \( r \) was measured to be 0.46 mm. The index of the lens surface is reported to be in the range 1.35–1.38 at 632.8 nm [70,71]. As the OCT reflection (@ 840 nm) from the lens surface was feeble, we assume the index at the lens surface to be close to water. We calculated the effective refractive index from equation 4.3 using different values of core index \( n_0 \) in the range 1.54–1.59. Using \( n_0 = 1.54 \) yields
effective refractive index $n_{eff} \sim 1.47$, closer to the experimentally measured value. The relative error in the estimation of effective refractive index of the crystalline lens using the OCT method can be estimated by

$$\frac{\delta n_{eff}}{n_{eff}} = \frac{\delta D_z}{D_z} - \frac{\delta D_x}{D_x}$$

(4.4)

where $D_z$ is the diameter of the lens along the axial direction, $D_x$ is the diameter of the lens along the lateral direction, and $dD_z$ and $dD_x$ are the uncertainties in measurement of the diameters along the axial and lateral directions respectively. Using the values $dD_z \sim 11 \, \mu m$ (axial resolution), and $dD_x \sim 27 \, \mu m$ (lateral resolution with 5× objective), the relative error $|dn_{eff}/n_{eff}|$ is estimated to be $\sim 2\%$. Taking the measured value of $n_{eff} \sim 1.43$, the absolute error in the effective refractive index estimate is $dn_{eff} \sim 0.03$. To the best of our knowledge, there are no experimental measurements of refractive index of intact Zebrafish lens. Even though the fish could not be revived after these measurements, it did not require separation of the lens from the fish eye unlike the previous studies by Garner et al. [70], where MRI was used for determination of graded index of fish lens after physical separation.

4.3 Measurement of gradient refractive index profile of Zebrafish crystalline lens

It is known that the spherical lens of Zebrafish has a gradient refractive index profile for correcting the large spherical aberrations to be expected from a spherical lens. There exists considerable interest in determining the refractive index profile of crystalline lenses. Several techniques are employed for this purpose such as the Abbe refractometer, interference technique, and laser ray tracing methods [70,74,75]. These require either...
sectioning or resection of the lens. Magnetic resonance imaging (MRI) can be used for non-invasive measurement of the refractive index profile of the fisheye lens. However, it is not suitable for measuring the refractive index in the core region of the lens due to the absence of free water [70]. Recently a tomographic method reported for the measurement of the gradient refractive index of a spherically symmetric lens which later has been extended to rotationally symmetric lenses [71,76]. Here, we present the use of OCT for non-invasive measurement of the gradient refractive index profile. In OCT, the backscattered signal is detected only when the optical path length of the light in the reference and the sample arm matches within the coherence length of the source [10,14]. Since, the path length in the reference arm is known, the optical path length in the sample arm and thus the position of the reflecting structure can be calculated. OCT can thus detect the backscattered or reflected light at front and back surfaces of the lens, respectively, and provides a direct measurement of the optical path length of the ray passing through the lens. The optical path of the ray inside the lens can be computed using ray tracing, assuming a polynomial form refractive index profile. Thus by fitting the measured optical path length with the optical path calculated using a ray tracing algorithm, coefficients of the index profile can be extracted. The approach has been employed for determining the index profile of a fisheye lens under both resected and intact (in-vivo) conditions. The slow speed TDOCT setup which uses a motorized translation based reference arm scanning for depth ranging was used to image Zebrafish eye. The details of the setup are discussed in chapter 2 of the thesis. In the sample arm was collimated focused on to the sample with a 5× microscopic objective (NA ~ 0.1). It should be noted that to achieve depth of focus ~ 1mm as required for this study, we purposely under filled the objective to reduce its effective NA. While the clear aperture of the objective was 5 mm, we kept the width of the collimated beam incident on it ~ 1 mm, so that NA reduces to ~0.02. This
resulted in a focal spot size ($\Delta x$) \( \sim 27 \mu m \) and the corresponding depth of focus was \( \sim 1.3 \) mm. Due to the large depth of focus, the beam size can be assumed to be constant throughout the lens.

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.

In order to acquire a two-dimensional image of the lens, the fish/resected eye lens was put on a linear translation stage that was controlled by a stepper motor. Unlike angular scanning of the beam for two-dimensional retinal imaging used in OCT, a parallel beam of light was translated across the pupil for imaging of the lens. For in-vivo imaging, the fish
was anaesthetized using clove oil as discussed in the previous section. Following the loss of physical movement (within 10 minutes.) and response to physical stimulus, it was transferred to a petri dish for imaging. After imaging, the fish was transferred back to a fresh water tank for its revival. A typical OCT image of a resected fisheye lens is shown in Figure 4.5 a. Figure 4.5 b shows the image of a fisheye structure in the meridional plane acquired in-vivo. The images consist of a number of A-scans (depth scans) at different transverse locations of the sample. From these images, the optical path length of the incident light rays that travelled through the lens was measured. These measured data was then used as the experimental input data for fitting with the path calculated from the assumed polynomial form refractive index profile [77]. To retrieve the gradient refractive index profile, we consider a spherical lens with radius \( R \). In a radial gradient medium, the gradient refractive index profile is normally represented by a polynomial with only even powers of \( r \) [75, 78]:

\[
 n(r) = \sum_{j=0}^{3} a_j r^{2j} = a_0 + a_1 r^2 + a_2 r^4 + a_3 r^6 \tag{4.5}
\]

and

\[
 r = \frac{[(x^2 + z^2)^{1/2}]}{R}
\]

where \( r \) is the normalized radial distance from center to edge. This is attributed to the fact that if a linear term in \( r \) was present, the paraxial theory would no longer apply and no useful imaging can be obtained and in presence of the other odd terms the effect on aberrations can be serious [79].

Consider a collimated beam of light passing through the lens as shown schematically in Figure 4.5 c. The ray A experiences refraction at point B on the surface of the lens. Afterwards, due to the refractive index gradient of the crystalline lens, the ray follows a curved path. It emerges from point C at the back surface of the lens and intersects the optic
axis at point D. The optical path inside the lens can be obtained evaluating \( \int_C^{n(r)} ds \) where
\( ds \) is arc length along ray path [80]. For retrieval of the refractive index, optical path lengths measured from the OCT images i.e. \( BC_{\text{exp}} \) needs to be fitted with the calculated theoretically optical paths i.e. \( BC_{\text{theo}} \) for a given gradient refractive index profile. The theoretical optical paths i.e. \( BC_{\text{theo}} \) were calculated using the ray tracing method reported by Sharma et al. [80] in the following manner:

In gradient refractive index medium the propagation of the ray is governed by the following expression [80]

\[
\frac{d}{ds} \left[ n(r) \frac{dr}{ds} \right] = \nabla n(r) \tag{4.6}
\]

where \( n(r) \) is the gradient index medium, \( r \) is the position vector and \( ds \) is the length along the ray. Equation 4.6 can be modified to transform it in a numerically solvable form:

\[
\frac{d^2 R}{dt^2} = \frac{1}{2} \nabla n^2 \approx n \nabla n \tag{4.7}
\]

Where \( t = \int \frac{ds}{n} \) or \( dt = \frac{ds}{n} \);

The modified variables \( R \) and \( D \) are defined such that the above equation 4.6 transform in to a second order differential equation as below:

\[
\frac{d^2 R}{dt^2} = D(R) \tag{4.8}
\]

Where \( R = (x, z) \); and \( D = n(\frac{dn}{dx}, \frac{dn}{dz}) \). An optical ray vector \( T = n(\frac{dx}{ds}, \frac{dz}{ds}) \) can be defined as \( T \equiv dr / dt \equiv \frac{dr}{ds} \equiv \hat{i}n \cos \alpha + \hat{k}n \cos \gamma \), where \( \alpha \) and \( \gamma \) are angles ray direction makes with \( x \) and \( z \) axes respectively. The above matrix Equation 4.8 can be
solved to generate successively \((R_1, T_1), (R_2, T_2), \ldots, (R_n, T_n)\) points for a given initial condition using \((R_0, T_0)\) using Runge-Kutta algorithm as below [80]:

\[
R_{n+1} = R_n + \Delta t [T_n + \frac{1}{6}(A + 2B)],
\]

\[
T_{n+1} = T_n + \frac{1}{6}(A + 4B + C),
\] (4.9)

where the matrices \(A, B, \) and \(C\) are defined as

\[
A = \Delta t D(R_n);
\]

\[
B = \Delta t D \left(R_n + \frac{\Delta t}{2} T_n + \frac{1}{8} \Delta t A\right);
\] (4.10)

\[
C = \Delta t D \left(R_n + \Delta t T_n + \frac{1}{2} \Delta t B\right)
\]

where \(\Delta t\) is the step size for the computation for desired accuracy. Optical path \(BC_{\text{theo}}\) can be calculated by measuring the arc length for every step along the ray (inside the lens only) and multiplying it with refractive index calculated for that particular point in the lens. The coefficients \(a_j\) were retrieved with the use of the nonlinear least squares curve-fitting algorithm that minimizes the difference between theoretically calculated optical path \((BC_{\text{theo}})\) and the optical path length measured experimentally \((BC_{\text{expt}})\) from the acquired OCT images i.e.

\[
\sum_{m=1}^{M} \left[BC_{\text{theo}}^m - BC_{\text{expt}}^m\right]^2
\] (4.11)

where \(M\) is the total number of rays measured for different lateral shifts and

\[
BC_{\text{theo}} = \sum \left(\sum a_j \left|\frac{R_{n+1} + R_n}{2}\right|^{2j}\right) \times |R_{n+1} - R_{n-1}|
\] (4.12)

where \(R_n\) is the position vector of a grid point and \(n\) is chosen such that \(R_n\) is within the lens. For quantitative estimation of the errors, random noise corresponding to
measurement inaccuracy of 11 \( \mu m \) was added to the optical path length data. This leads to uncertainties in the coefficients of the refractive index profile (i.e., \( a_i \) \( \sim 10^{-3} \)). The maximum error in refractive index due to these uncertainties was estimated to be \( \sim 0.013 \). The size of the beam also affects the uncertainties in the refractive index estimation. For a beam width of \( \sim 27 \mu m \) at the sample, the maximum error in refractive index was estimated to be \( \sim 0.012 \). The increase in beam width reduces the number of data points and therefore increases the uncertainties in estimating the coefficients, and hence the refractive index. For example, for a spot size \( \sim 100 \mu m \) the error in the refractive index was estimated to be \( \sim 0.016 \). We have also used the images for the measurement of the index profile in the \( Y-Z \) plane. As expected, the refractive index profiles in the two orthogonal planes were found to be the same.

Table 4.1: Optimized coefficients for refractive index profile

<table>
<thead>
<tr>
<th>Samples</th>
<th>Radius (mm)</th>
<th>( a_0 )</th>
<th>( a_1 )</th>
<th>( a_2 )</th>
<th>( a_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-vitro</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.46</td>
<td>1.545</td>
<td>-0.117</td>
<td>-0.034</td>
<td>-0.034</td>
</tr>
<tr>
<td>2*</td>
<td>0.46</td>
<td>1.540</td>
<td>-0.109</td>
<td>-0.032</td>
<td>-0.037</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>1.545</td>
<td>-0.119</td>
<td>-0.033</td>
<td>-0.033</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
<td>1.543</td>
<td>-0.113</td>
<td>-0.033</td>
<td>-0.032</td>
</tr>
<tr>
<td>In-vivo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
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<td>1.544</td>
<td>-0.116</td>
<td>-0.035</td>
<td>-0.036</td>
</tr>
<tr>
<td>6</td>
<td>0.48</td>
<td>1.545</td>
<td>-0.125</td>
<td>-0.031</td>
<td>-0.029</td>
</tr>
</tbody>
</table>

*Measurement for orthogonal plane of sample 1
The optimized coefficients of index profile obtained from the resected lens and the \textit{in-vivo} measurements are shown in Table 4.1. The polynomial form refractive index profile obtained from the mean values of coefficients (Table 4.1) is shown in Figure 4.6 a. A gradual decrease in refractive index from $\sim 1.54$ to $1.36$, from the core to the edge of the lens, can be seen in the figure. The difference between the group and phase index values for the case of the crystalline lens of the fisheye, was estimated to be $\sim 0.05$ using the reported ocular dispersion values [81]. The ray paths for a polynomial form refractive index profile obtained from the mean values of coefficients are shown in Figure 4.6 b. The
equivalent focal length (distance from center of lens to focal point) of the lens is seen to be 1.06 mm and the corresponding Matthiessen’s ratio, defined as the ratio of the focal length of the lens to its radius, was estimated to be~ 2.30. These values are in very good agreement with the values reported in the literature [78]. It is important to note here that the optimized coefficients of the gradient refractive index profile indeed lead to small spherical aberrations (longitudinal and transverse). The marginal rays focus closer to the lens than the paraxial rays. For an aperture of 0.85R, the calculated values of longitudinal spherical aberration (LSA) defined as the distance between the focal point of the marginal rays and the focal point of the paraxial rays is ~ 55 µm which significantly smaller for a lens of size ~ 0.9 mm.

4.4 \textit{In-vivo imaging of Zebrafish brain}

The real time OCT setup was used to acquire two-dimensional cross sectional images (XZ plane, as shown in Figure 4.7) of the brain of anesthetized Zebrafish and a 3D model of Zebrafish brain was reconstructed. About 90 cross-sectional images of the brain were acquired by moving the sample in the Y direction. The acquisition time for each 2D cross-sectional image was 0.125 s. Around 90 images were acquired in the XZ plane by manually moving (50 µm) a micrometer controlled stage along Y-axis. The major lobes of the brain such as bulbus olfactorius, telencephalon, tectum opticum, cerebellum, frontal bone and eminentia granularis were clearly distinguishable in these images [82]. The raw images were thresholded for minimizing the speckle noise. Using these images, a three-dimensional model of the Zebrafish brain was constructed (Figure 4.8) with AMIRA software. The iso-surface brain model so developed was found to be in resemblance to the model reported previously [83]. We also compare the OCT images with the Zebrafish brain images posted on the VCCRI fishnet website [84] database. The images shown on
fishnet website are of 17 mm length (adult) fish taken with optical projection tomography [85]. The fishnet database images do not clearly show the structure of bulbus olfactorius and Eminentia granularis. The other structures such as telencephalon, frontal bone, tectum opticum and cerebellum are resolved better in OCT images than the reported images on fishnet database. The cavity inside tectum opticum is clearly distinguishable in our images compared to the fishnet database. Due to the presence of a highly scattering layer in the hind portion of the Zebrafish brain the structures beyond the cerebellum such as crista cerebellaris, medulla spinalis, parasphenoid, palatoquadrate, etc. were not clearly distinguishable. It is pertinent to note that the adult Zebrafish used in this study was ~35 mm long. In a fish of smaller size (few days old) the internal structures of brain could be better visualized with good contrast. Further, using OCT set up with higher sensitivity (~110 dB), one can distinguish the anatomical structures more effectively. The 3D imaging of the internal structures of brain helps rendering of data for better visualization and understanding of the features. *In-vivo* 3D imaging of an adult Zebrafish brain demonstrates the capability of OCT to monitor the developmental changes even for adult Zebrafish when it becomes turbid and unable to visualize under microscopy techniques.
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.
4.5 Summary

We have utilized the OCT setups developed by us to image the various ocular structures of Zebrafish eye such as cornea, iris, eye lens and retina with an axial resolution of ~10 µm and measured various ocular parameters such as corneal thickness, retinal thickness and equivalent refractive index of Zebrafish eye lens. We also successfully demonstrated the use of OCT for non-invasive measurement of the refractive index profile of the eye lens. For the latter we exploited the fact that since OCT provides direct measurement of the optical path length, the gradient refractive index profile can be retrieved by iterative fitting of optical path calculated by ray tracing method with that experimentally measured using OCT at various lateral locations of the sample. The 3D optical imaging of brain at speed (@ 8 fps) and with resolution significantly better than obtained by other techniques like MRI and computed tomography was also demonstrated. The three dimensional images of Zebrafish brain reconstructed using two dimensional OCT images clearly show the major structures of the brain such as bulbus olfactorius, telencephalon, tectum opticum, cerebellum, frontal bone and eminentia granularis.