Chapter 6

Phase tomographic by lateral shearing Digital Holographic microscope

6.1 Introduction

Investigation of biological cells or other micro objects in transmission mode with quantitative phase contrast using digital holographic microscope has gained a lot of interest. Previous chapters discussed about digital holographic microscopy and various geometries to implement it and their application for investigating biological specimen. But data captured from a single direction of observation yields the integrated or averaged optical thickness [49, 120] adding the local spatial variations (in the direction of the beam propagation) due to the internal structures and other optical inhomogeneity of the sample. As explained, refractive index value in transparent or semi-transparent specimen may not be constant throughout due to variation in its internal structure, defects or discontinuity in the specimen and thus 3D distribution of refractive index can provide important information about the internal structures of the micro-object/cell under investigation. With digital holographic microscope image of the object can be reconstructed from various distances (numerical focusing), but 3D distribution of refractive index inside the object is difficult to reconstruct.
The 3D distribution of refractive index of the object is important to obtain information concerning the distribution and the optical properties of the intracellular

organelles, cellular structure, and is important in studies of cell and tissue light scattering. Different techniques are adopted for measuring refractive index of different types of cells. But these techniques cannot be generalized for all type of cells or subcellular components. One common method to measure refractive index is by the immersion of cells in liquids of suitable index of refraction and observing them using phase contrast microscopy. But this in turn may alter cell properties. Interference microscopy (quantitative phase contrast microcopy) including digital holography provides a method to measure index of refraction, but it gives an average value of refractive index of the cell and not its 3D spatial variation [121]. In order to overcome this limitation, the integrated phase data, acquired by digital holography from many directions (different angles), can be combined with tomographic reconstruction concepts and quantitative knowledge of the 3D position-dependent refractive index or optical path length change of the sample can be calculated.

Two methods can be employed to obtain multiple projection data of the sample (i) rotating the sample [47, 48, 122, 123, 124] or (ii) rotating the probe itself [50, 125, 126]. For employing rotating sample method, in interference microscopy for phase tomography, source and detector positions are fixed in the microscopic setup and sample is rotated along the axis perpendicular to beam propagation [47, 48, 122, 123, 124]. Rotating the sample makes it possible to cover the entire angular range of the object. But it is difficult to fix the axis of rotation and special arrangement is needed for that. Speed of data acquisition also reduces due to mechanical rotation of the object. Also the rotation may induce small movements and that causes the need of special reconstruction methods to re-center the object on rotation axis. Biological sample to be investigated using this method should be immersed in suitable liquids (to reduce diffraction from the edges) and kept inside micropipette [47, 121]. Sample preparation and need of translation or rotational mechanism for rotating the sample in micropipette makes the technique complicated. So the use of sample rotation method is more suitable for solid non-biological objects such as optical fibers [123, 124, 125]. In rotating the illumination beam method, source beam is rotated such that it illuminates object from various angles [50, 125, 126]. The rotating beam approach does not perturb the sample during data acquisition, and is thus suitable for imaging live cells

in their inherent state [50, 125, 126]. Speed of data acquisition is comparatively faster with the method for study of cell dynamics by the use of galvanometer-mounted or motorized tilting mirror with sample and the detector fixed [50, 126]. But the main drawback of this method is lack of coverage of entire angular range as clear aperture of imaging system is finite [50, 126]. Also since the beam is rotated, fringe density at the detector plane also changes, which requires an adjustment of the reference beam or requires a change in the reconstruction algorithm [50].

Section 5.2 of the previous chapter discussed self-referencing digital holographic microscope using lateral shearing geometry [82]. This geometry has many advantages like ease of implementation, common path nature and automatic adjustment of beam ratios for high contrast fringes. Also the angle between the wavefronts from the front and back surface of the glass plate remains same as it is decided by the amount of shear introduced (thickness of the glass plate). Also this geometry (self-referencing) does not require a separate reference beam, making it possible to implement it without spatial filtering assemblies [126]. So lateral shearing digital holographic microscope (LSDHM) is ideal for phase tomography of transparent samples when coupled with rotating illumination beam technique. This chapter discusses the implementation of phase tomography method using LSDHM.

6.2 Digital holographic tomography using a single laser diode module

Phase tomography with LSDHM is achieved in two ways. In the first of the techniques, a single source is be used to illuminate the object from various angles. Fig.6.1 shows the schematic of the technique. It couples the collimated output from a laser diode module (Thorlabs CPS635F, λ=635nm), into the sample placed on a LSDHM. The source is mounted on a translation (resolution of 0.1mm) and rotation stage (resolution of 0.5°) so that the beam trans-illuminate the object from different angles. This arrangement is necessary to record a set of holograms by projecting source beam from various angles so that tomographic inversion can be achieved. The sample is magnified by a microscope objective lens with 40X magnification and numerical aperture of 0.65. Two laterally sheared versions of this object wavefronts are created by a

fused silica glass plate of 5mm thickness mounted at 45° to the propagation direction of the wavefronts. These beams superpose at the detector plane creating the holograms (interference patterns). In the case of LSDHM, amount of shearing is much larger than the size of the magnified object at the detector plane, leading to images from the back and front surface of the glass plate being spatially separated creating holograms (Fig.5.2). For sparse object distributions this formation of holograms is due to superposition of portion of the wavefront, unmodulated by object information (for example wavefront reflected by the back surface of the glass plate) with the portion of the same wavefront modulated by object information (for example wavefront reflected by the front surface of the glass plate). Holograms are recorded by a CCD array (Thorlabs DCU223M, 8 bit, 4.65μm pixel pitch). It should be noted that the recorded holograms using the lateral shearing method are equivalent to off-axis holograms.

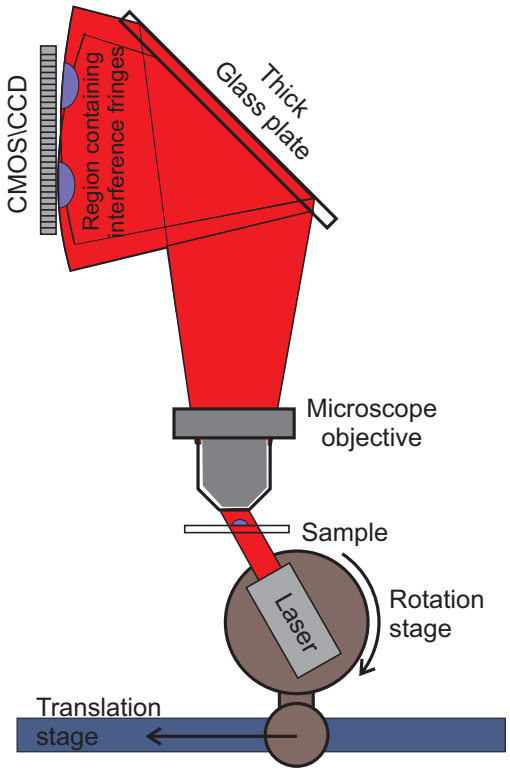

Figure 6.1: LSDHM phase tomography system using single laser source.

Holograms at different projection angles are recorded by rotating the laser diode module to the desired angle and then translating the laser diode module so that the output laser beam trans-illuminates the sample. In the experiments, a total of 37 holograms for beam angles separated by 2° (-36° to +36° for total span of 72°) are recorded and the phases at these projection angles were reconstructed and used in the tomographic reconstructions.

6.3 Digital holographic tomography using an array of laser diode modules

The use of a translation and rotation stage to create different angles of projection can be speeded up by the use of galvo-mirror scanner placed very close to the sample [50, 126]. Multiple projections can be achieved also by the simultaneous use of multiple laser diode modules. The schematic of the proposed system is shown in Fig.6.2a. An array of laser diode modules (seven laser diode modules) working at 635nm having output power of less than 2mW are made on a 3D printed section of a circle (with its center at the sample plane) as shown in Fig.6.2b.

The projection angles of the beams were separated by 7° and so the range of projection achieved is 42°. Laser diodes are used here because they are comparatively low cost and since they are small in size they make the setup compact. This method eliminates the need of complicated arrangement for translation and rotation. The laser diodes are switched on and off, one by one to acquire projection from all available angles. Here the beam from each diose is allowed to pass through a sample and further through a microscope objective lens with of 40X magnification and 0.65 NA. The same glass plate used in the previous setup creates holograms at the detector plane. The CCD array mentioned in the previous section is used for the recording of holograms.

6.4 Tomographic inversion

To reconstruct a 3D optical path length profile of the sample, from the phase data obtained at different projection angles, procedure based on the back-projection method for tomographic inversion is used [82]. Towards this, two-dimensional projection data

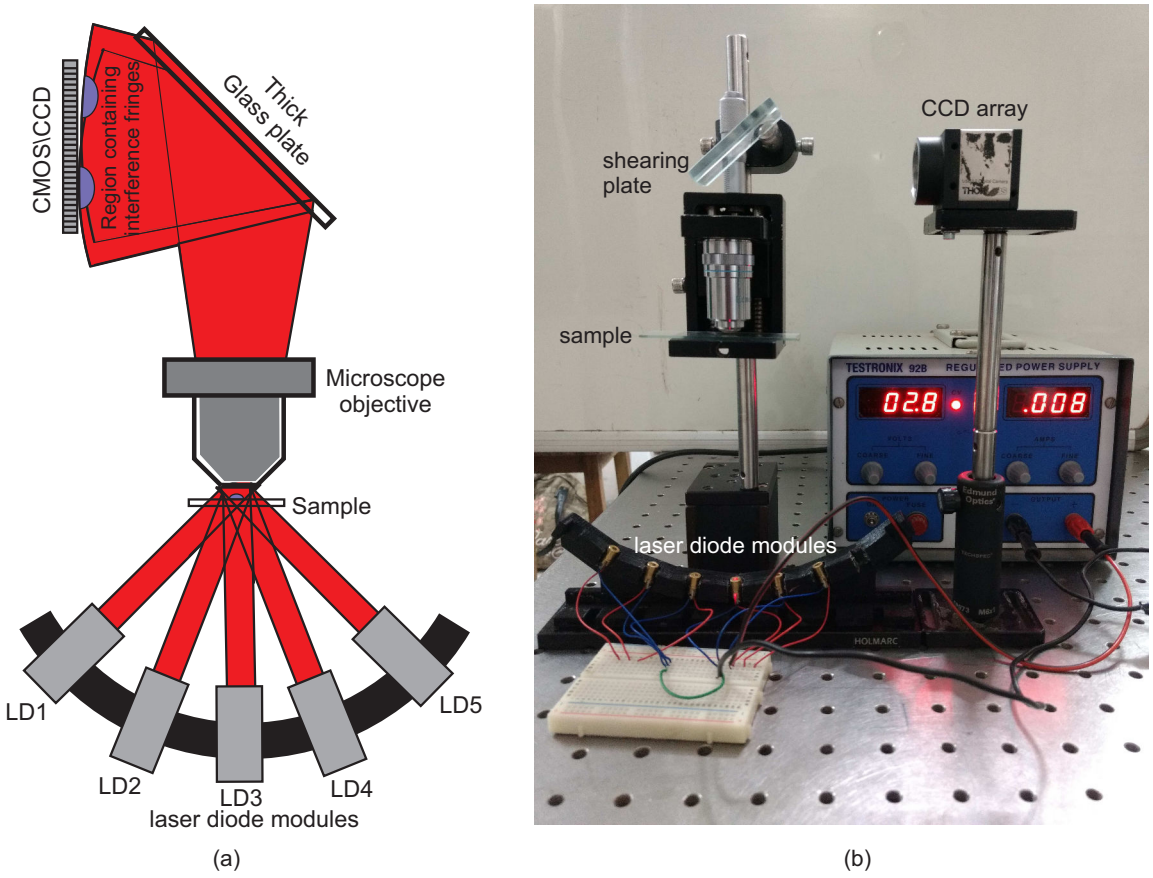

Figure 6.2: LSDHM phase tomography system using multiple diode laser sources. (a) Schematic of the setup. (b) Photograph of the setup.

(Fig.6.3) are acquired from a three-dimensional volume or 3D distribution of physical quantity such as refractive index change or optical path length change in the case of transparent object such as cells, by cutting the measured volume into two dimensional slices. After calculation many of such slices are collected and stacked to build the three-dimensional results. So here two-dimensional distribution of the physical quantity f(z, y) (see Fig.6.3), which is the refractive index distribution, in single plane is considered. The total change in optical path length suffered by a source beam due to change in refractive index inside object as it travels in a straight line through the object, is described in the polar coordinate (ϕ , s) by a line integral

$$P(\phi, s) = \int_l f(z, y) dl \tag{6.4.1}$$

Eqn.(6.4.1) represents the of the object function $f(x, y)$, along the line that is at distance s from the origin and at angle with respect to the z-axis. All points on this line satisfies the equation and using this and delta function Eqn.(6.4.1) can be re-written as

$$P(\phi, s) = \int \int f(z, y)\delta(zsin\phi - ycos\phi - s)dxdy \tag{6.4.2}$$

This is a projection of object function at the detector plane and can be described as collection of parallel line integral as is given by P(ϕ , s) for a constant angle ϕ. This is known as parallel projection. By moving the source beam or detector or rotating the object, collection of these P for many called as the Radon transform or the sinogram of object function f(z, y) is obtained.

Now to reconstruct the 2D slice of the object, from the projections of object taken by combining line integral at various angles (sinogram), a mathematical operation called back-projection (inverse radon transform) is used. It simply propagates measured sinogram back into the image space along the projection path.

$$f(z, y) = \int_0^{\pi} P(zcos\phi - ysin\phi, \phi)d\phi \tag{6.4.3}$$

Thus using Radon transform and back projection method tomographic images are reconstructed.

The reconstruction algorithm is an important factor in determining the spatial resolution and quantification of the refractive index. The selection of algorithm depends

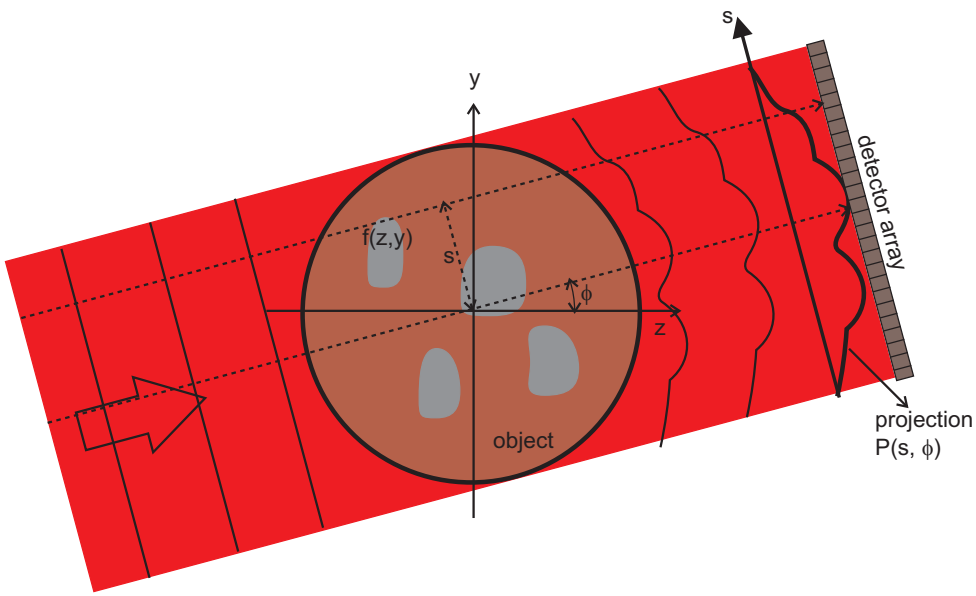

Figure 6.3: Collection of projection data using LSDHM

on the way of interpreting the experimentally measured phase change. Here since the phase of the transmitted field is approximated as a line integral of the refractive index along the direction of propagation, the back-projection algorithm based on the Radon transform is used [50, 82, 126, 127].

6.5 Phase tomography results using single laser diode module

Setup using single laser diode module (Fig.6.1), is tested using 20μm diameter glass microspheres (refractive index=1.56) immersed in microscope immersion oil (refractive index=1.52) as shown in Fig.6.4. Projection data (holograms) are obtained by illuminating the object at different angles by rotating and translating the source. Similarly, a set of reference holograms are also recorded with no sample present in the field of view, but with the same background (immersion oil) present in the field of view. The resulting set of background phase images is subtracted from the sample phase images, to eliminate phase noise due to aberrations and misalignment. Reconstructed Phases at various projection angles are then used for the tomographic

reconstructions using a simple slice-by-slice implementation of the back-projection algorithm. Fig.6.4 shows representative holograms recorded for different beam angles Fig.6.4a to Fig.6.4e). In the experiment, a total of 37 holograms for beam angles separated by 2° (-36° to +36° for total span of 72°) are recorded and the phases at these projection angles are reconstructed and used in the back projection algorithm for tomographic reconstructions. Fig.6.5 shows the sinogram of the slice along the line shown in Fig.6.4h. This sinogram is created by putting together the reconstructed phase values for all the projection angles for the same slice.

Tomographic reconstructions require projections from -90° to +90° (total of 180°). In the present case only a portion of this range is available due to finite size of clear aperture of magnifying lens. The adverse effect of having this limited range of angles is reduced by the use of iterative method for tomographic reconstruction as shown in Fig.6.6 [50]. It starts by assigning zero phase value to all projection angles other than those experimentally available (Fig.6.6a). This is then used in the back projection algorithm (inverse Radon transform) described in theory to get the tomographic data (Fig.6.6b). This tomographic data is then used to generate the projection data (sinogram) for the range -90° to +90° (Fig.6.6c). It should be noted that the generated projection data uses an interval of 2°, which was employed in the experiments. Interpolation can be used to generate data at finer intervals. But this will not provide any additional information in the tomographic reconstructions. In the generated projection map (sinogram), experimentally recorded projection data (phase reconstructed from holograms) replaces the generated data for the angles (green rectangle in Fig.6.6c) for which experimental data is available, generating the new sinogram for tomographic inversion (Fig.6.6d). The whole process is repeated till a convergent solution is obtained. It takes about 4 to 6 iterations of the algorithm to arrive at the convergent solution. This reconstruction algorithm is used for the method which employs an array of laser diode modules also.

Once the reconstruction algorithm provides a unique solution, it can be used to gain information about the refractive index profile of the object under investigation. Fig.6.7 shows the reconstructed refractive index profile in the case of the 20μm diameter glass micro-sphere in the y-z plane (z is the direction of beam propagation for

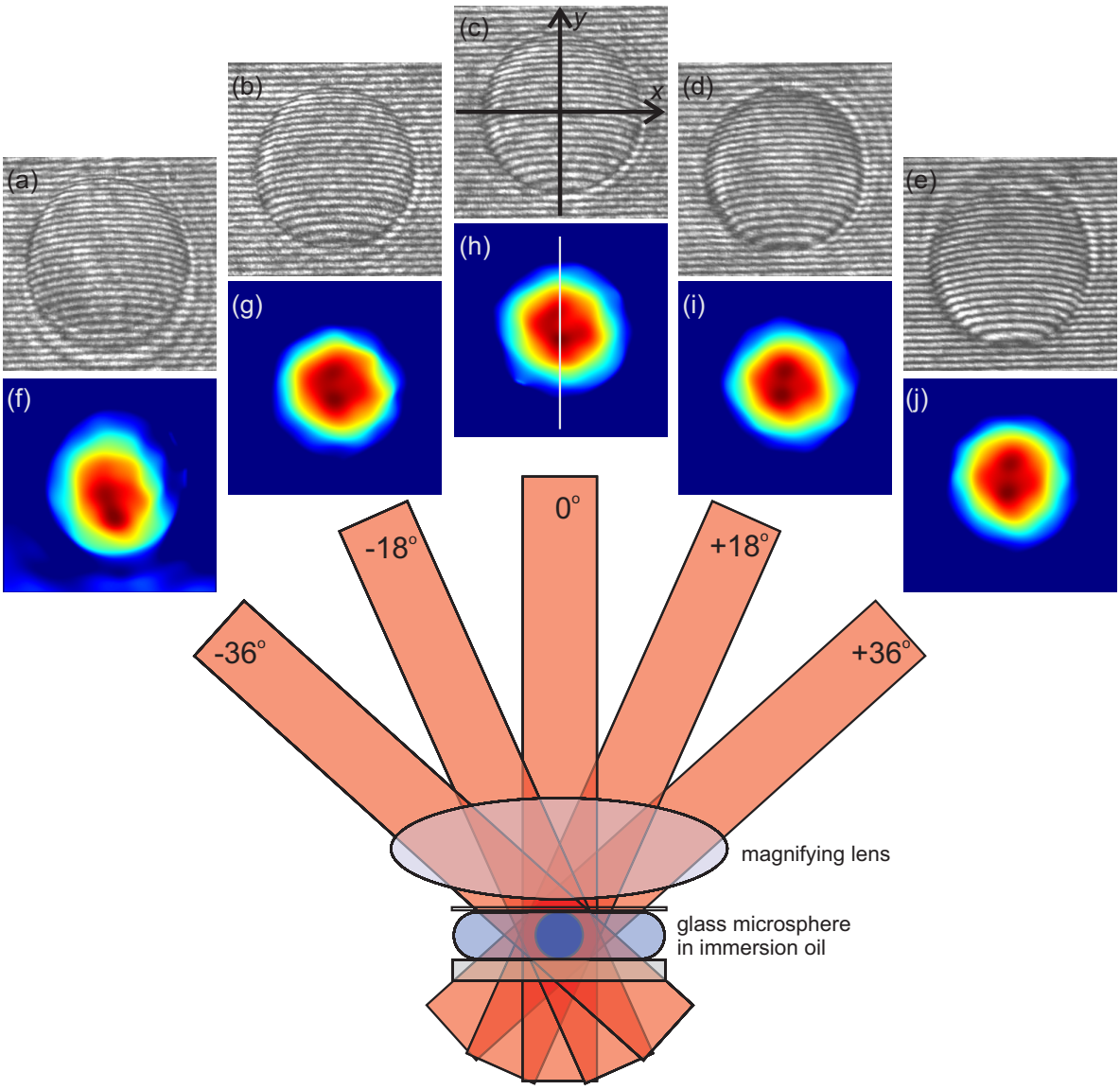

Figure 6.4: Data acquisition and phase reconstruction. (a) - (e) Holograms of 20μm diameter glass microsphere for beam angles of -36°, -18°, 0°, 18° and 36° respectively. (f) - (j) reconstructed object phase at these projection angles.

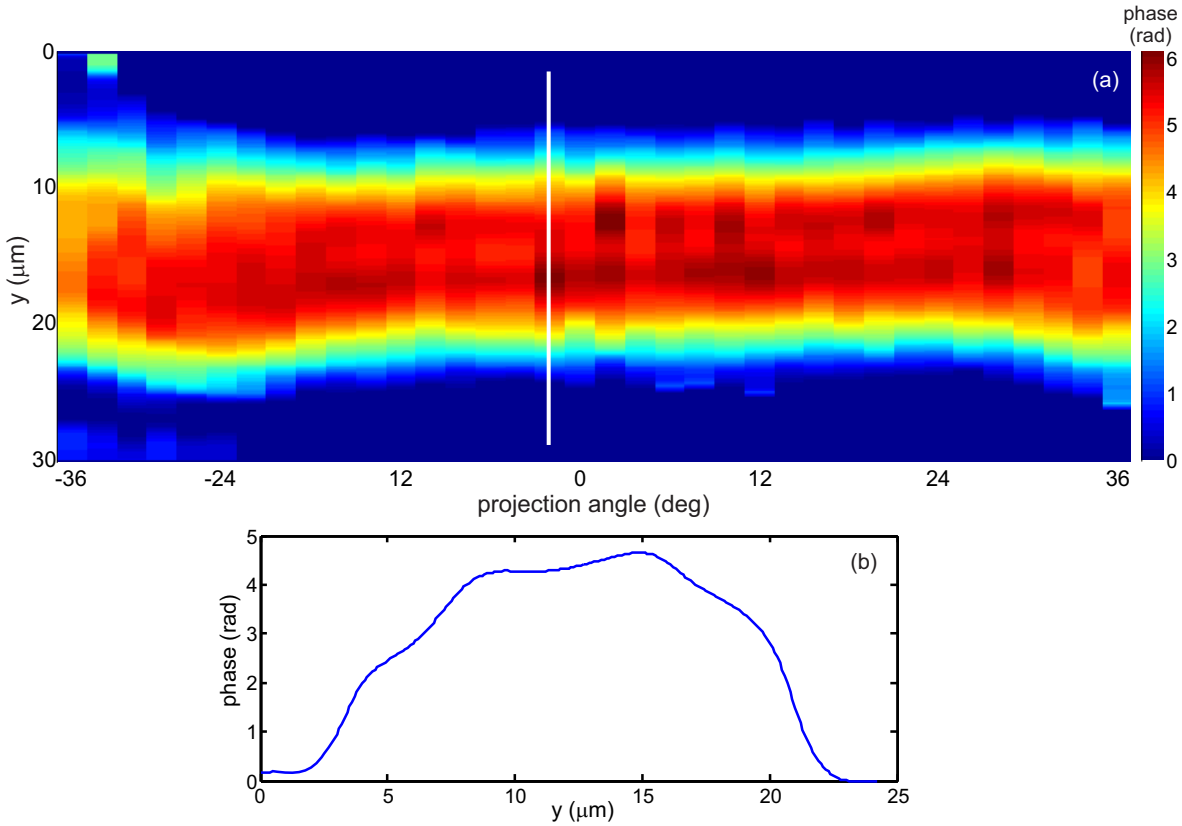

Figure 6.5: (a) Sinogram for 36 projections spanning total angle of 72° obtained for 20μm glass microsphere immersed in microscope oil. (b) Phase profile for the projection shown by the line in Fig.6.5a

$\phi = 0°$) at several x positions. The Refractive index of the glass microsphere is 1.56 and is immersed in immersion oil of refractive index 1.52. The sections seen in Fig.6.7 can be understood by looking at Fig.6.8, which shows the co-ordinate system adapted in the reconstructions. Fig.6.9 shows the refractive index difference ($n_G - n_O$, n_G is refractive index of the microsphere and n_O is the refractive index of the immersion oil) profile along the line shown in X=10μm plane.

It is expected that the refractive index should be constant inside the microsphere and from the nature (flat top profile) of Fig.6.9, it can be deduced that the refractive index is indeed a constant. The slight variations might be due to the density variations inside the microsphere.

6.6 Phase tomography results using an array of laser diode modules

In the technique using a single laser diode module, one has to move and rotate the laser source to change the direction of projection beam, and for that special and accurate translation arrangement is needed and mechanical vibrations may also disturb the system. So the setup was modified using an array of laser diode modules as shown in Fig.6.2. An array comprising of 7 laser diode modules (λ =635nm, power<2mW) is placed below the platform holding the sample. The projections angles of the beams were separated by 7° and so the range of projection is 42° (-21° to +21°). This setup is tested using used glass microspheres of diameter 10μm and then Red Blood Cells (RBC). Glass microsphere sample is prepared as mentioned in the previous section. And for studying RBC a thin smear of blood is made on microscope slide and then it is covered by a cover glass slide. Holograms are recorded on an 8-bit CCD array of 4.65μm pixel pitch, by serially turning on the laser diodes which leads to acquisition of projection data from various angles and the reconstructed phase at each projection angle is used in tomographic reconstruction process.

This phase data is used for tomographic reconstructions. Sinogram of the 7 projections along the line in Fig.6.10k is shown in Fig.6.11. Co-ordinate system remains the same as in Fig.6.8. This projection data is used in the iterative reconstruction algorithm (Fig.6.6). Fig.6.12 shows the reconstructed refractive index profile in the

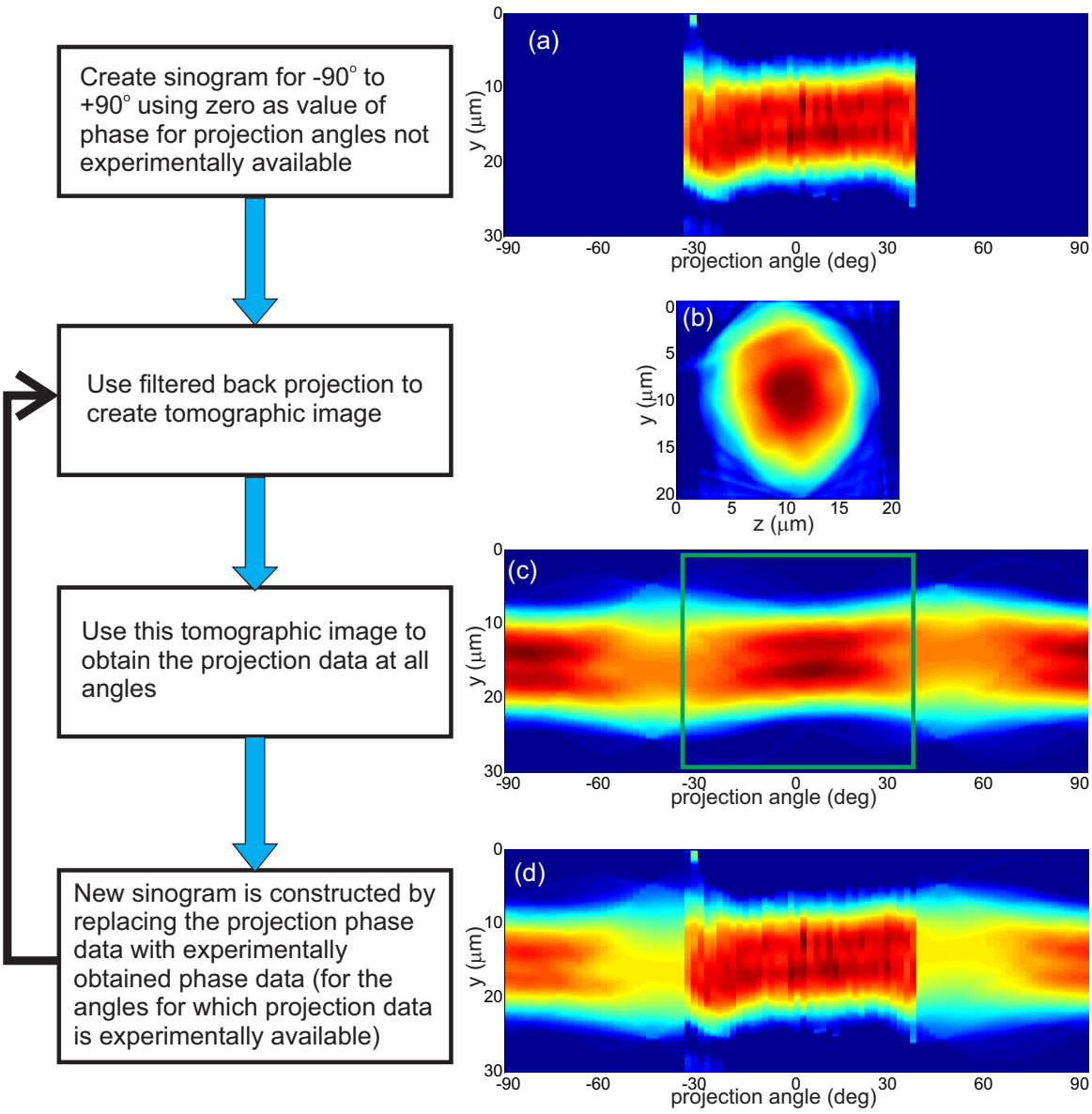

Figure 6.6: Iterative algorithm for tomographic reconstruction

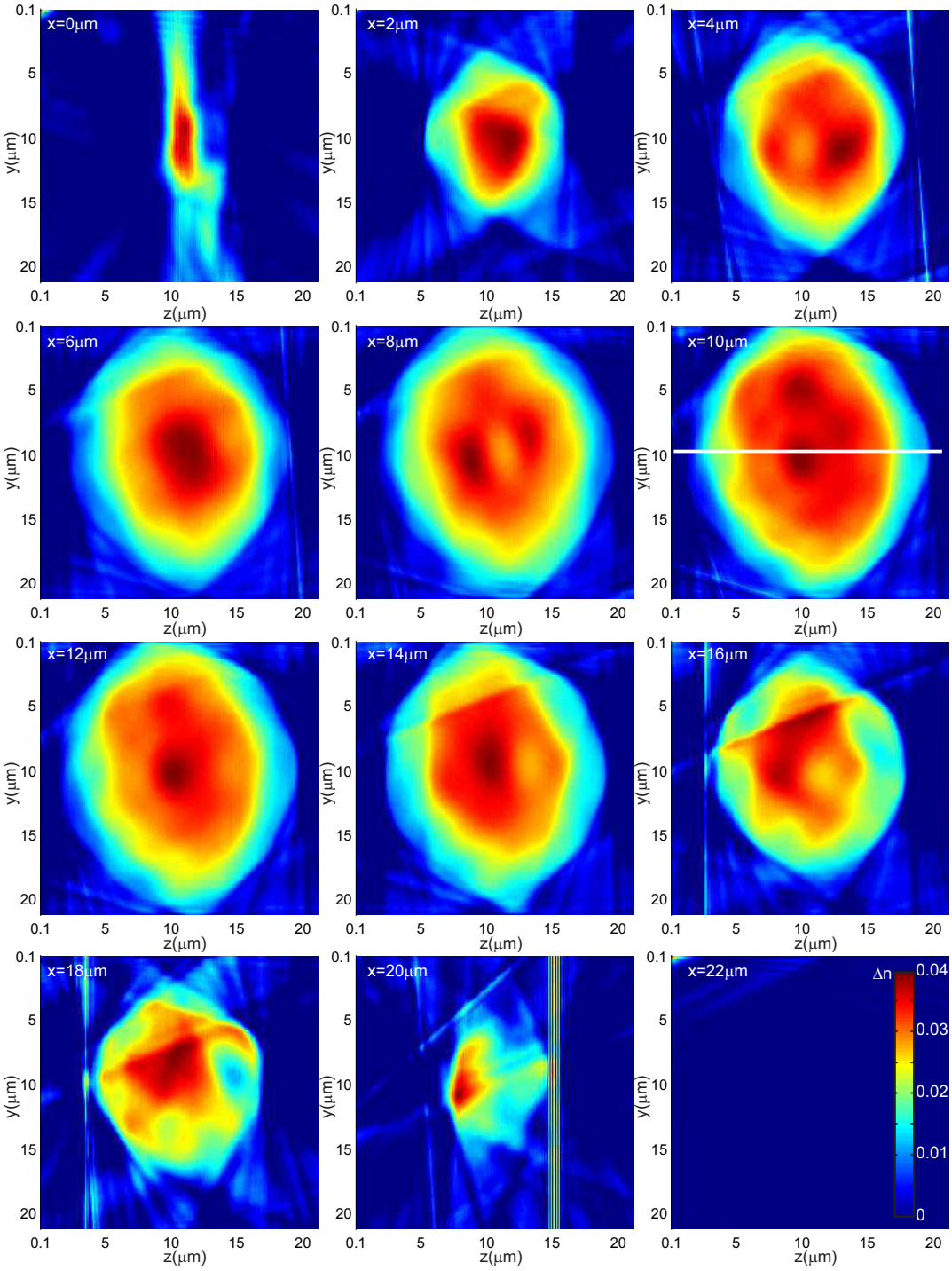

Figure 6.7: Sectional images (y-z sections) of the 20μm diameter glass microsphere. Different sections can be understood from Fig.6.8. The colour bar shown in the last figure (for x=22μm) is common for all

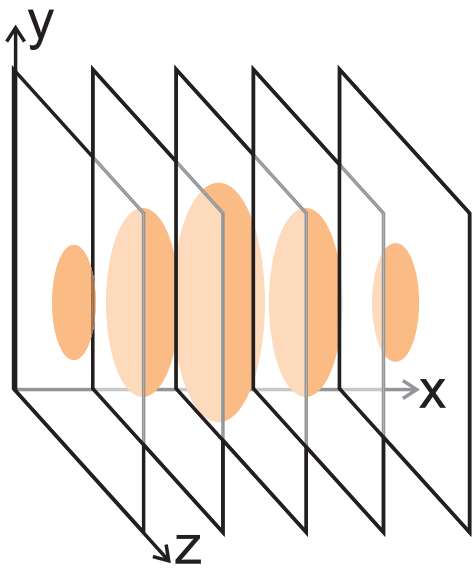

Figure 6.8: Co-ordinate system used in the representation of back projection data

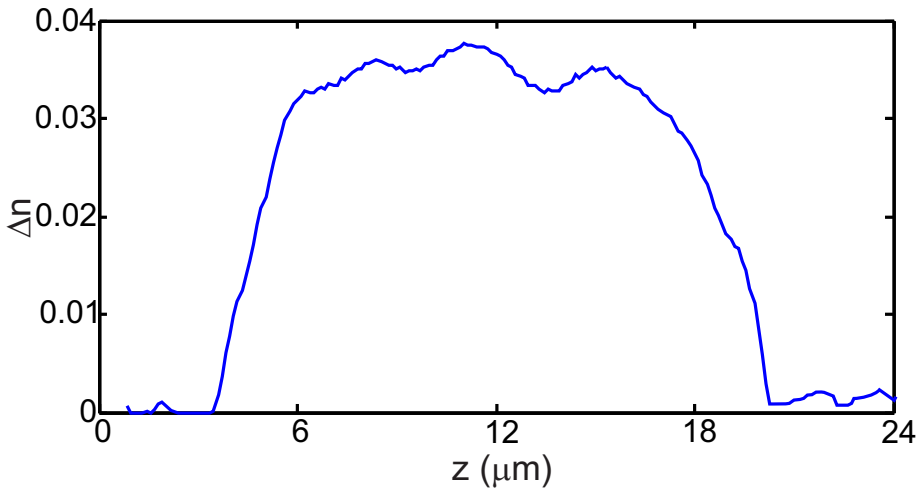

Figure 6.9: Refractive index profile along the direction of beam propagation for x=10μm plane

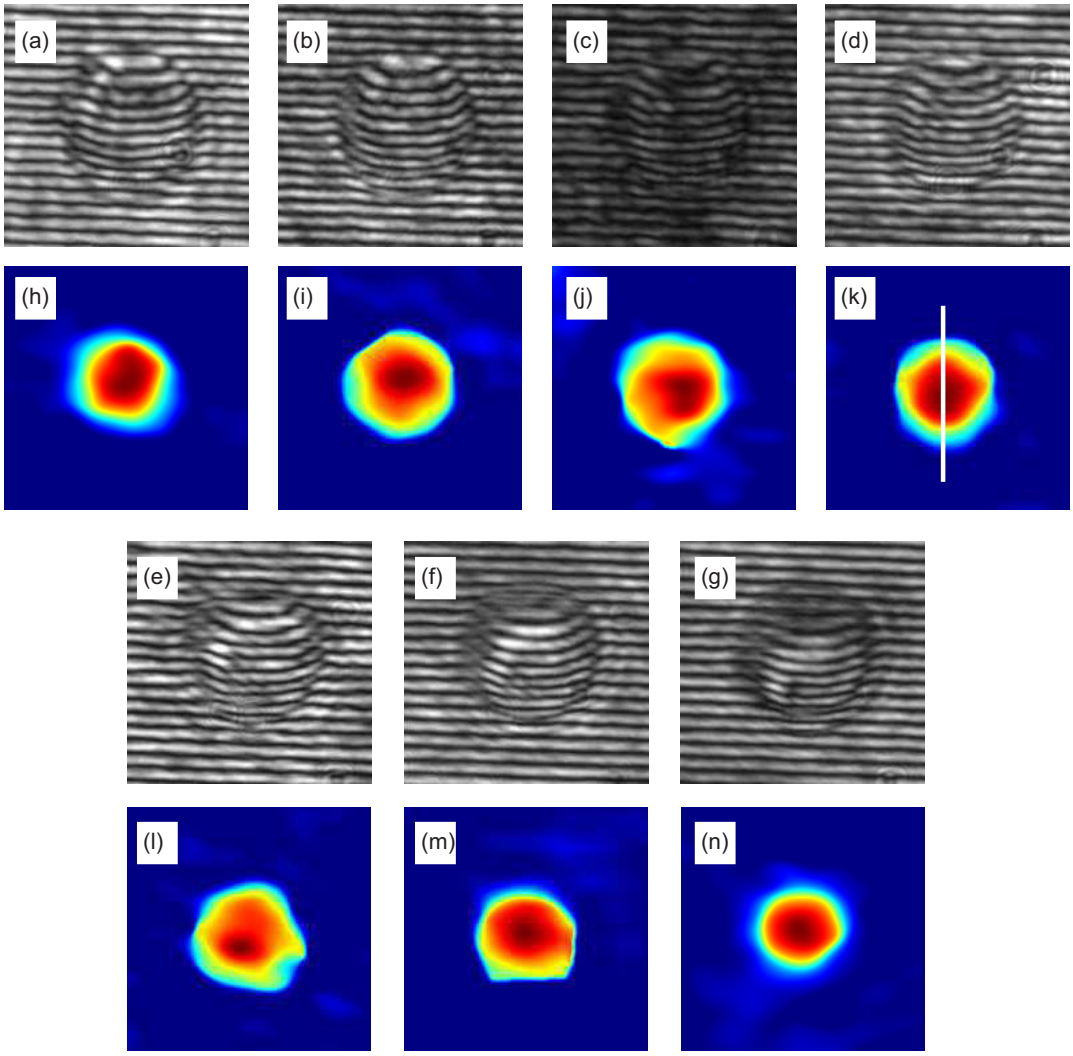

Figure 6.10: Holograms and reconstructed phases for setup employing array of laser diode modules. (a) - (g) Holograms of 10μm diameter glass microsphere for beam angles of -21°, -14°, -7°, 0°, 7°, 14° and 21° respectively. (h) - (n) reconstructed object phase at these projection angles.

y-z section at several x positions.

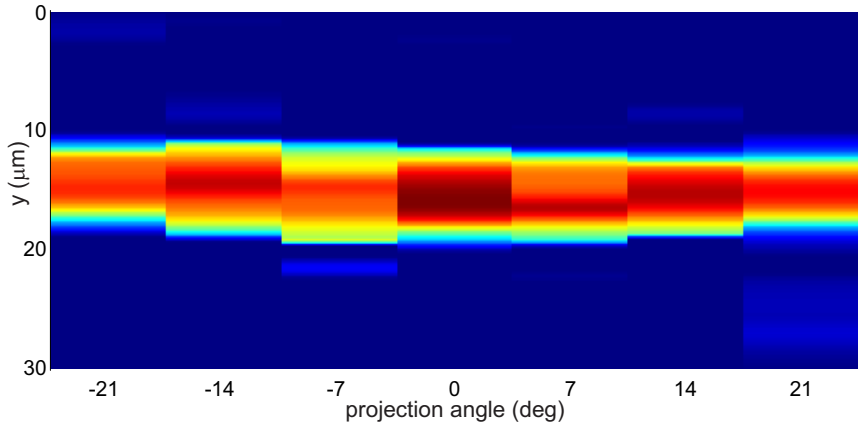

Figure 6.11: Sinogram of the 10μm glass microsphere obtained from 7 projections in the case of laser diode array.

Fig.6.13 shows the refractive index profile along the direction of beam propagation obtained using the laser diode array. It also shows a flat top profile and the obtained refractive index values are close to the expected values. From Fig.6.13, it can be seen that since the number of projections in this case is less, the reconstruction does not achieve the quality in the case shown in Fig.6.9.

Experiments are also conducted on red blood cells using the same setup. Fig.6.14 shows the recorded holograms as well as phase maps for three projection angles in the case of red blood cells.

Tomographic reconstructions are carried out using the same algorithm described in the previous section. Fig.6.15 shows the reconstructed images in the x-y plane at different z positions. From the figures, the features (like its donut cross-section profile) at different depths of the blood cell can be seen.

The main motive behind the development of phase tomographic imaging system using lateral shearing digital holographic microscope is to demonstrate the potential of this simple geometry in reconstructing the local phase profiles of micro-objects. Especially the setup using an array of laser diode modules has the potential to measure local refractive index profiles of micro-objects, without the need for a mechanical scanning device to generate projection data. Refractive index difference of glass

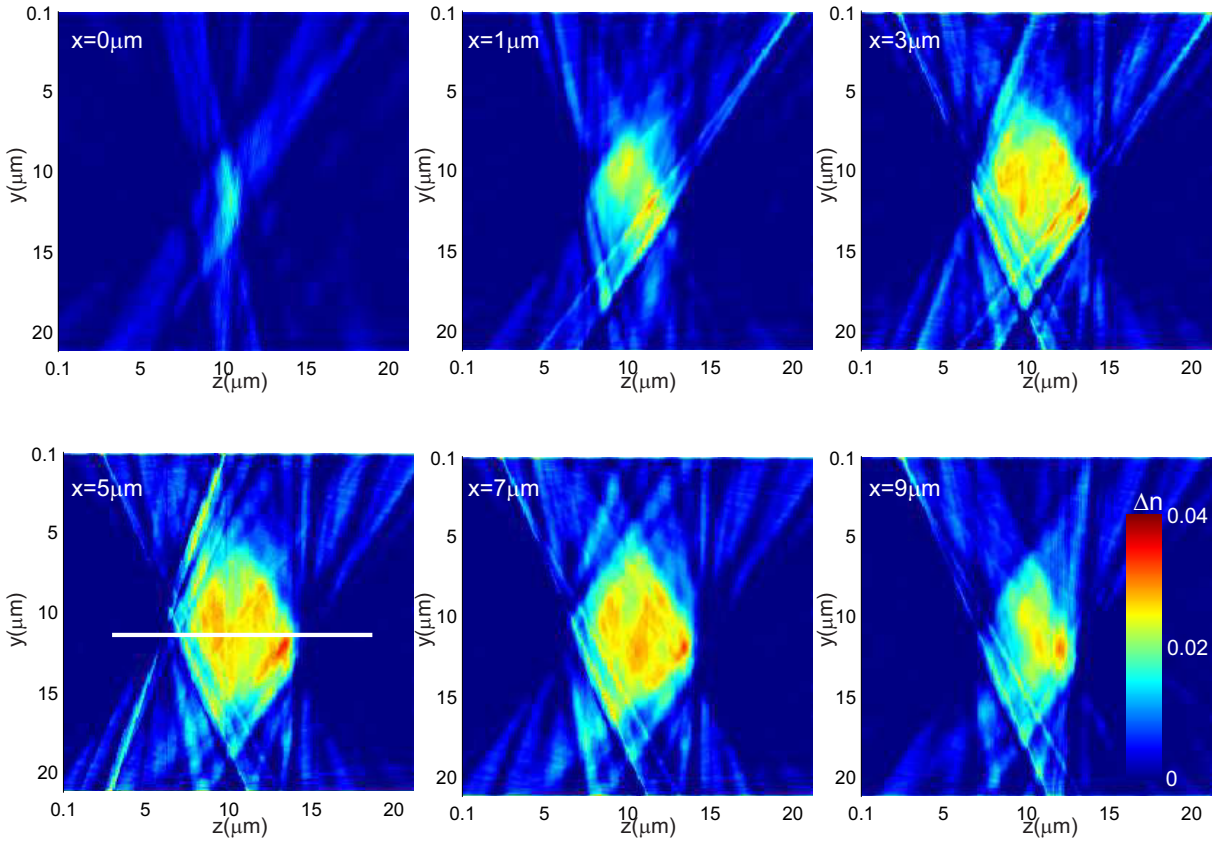

Figure 6.12: Sectional images (y-z sections) of the 10μm diameter glass microsphere imaged with laser diode array. The colour bar shown in the last figure (for x=9μm) is common for all.

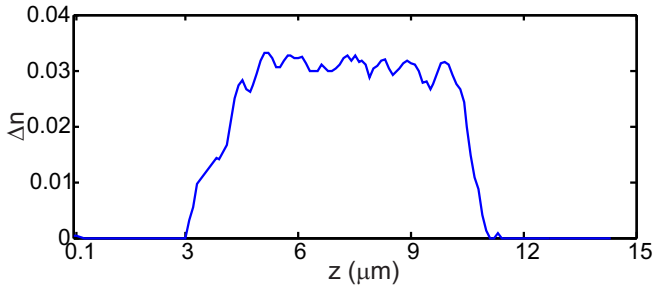

Figure 6.13: Refractive index profile using the setup employing the laser diode array. The profile is in the direction of beam propagation for x=5μm plane

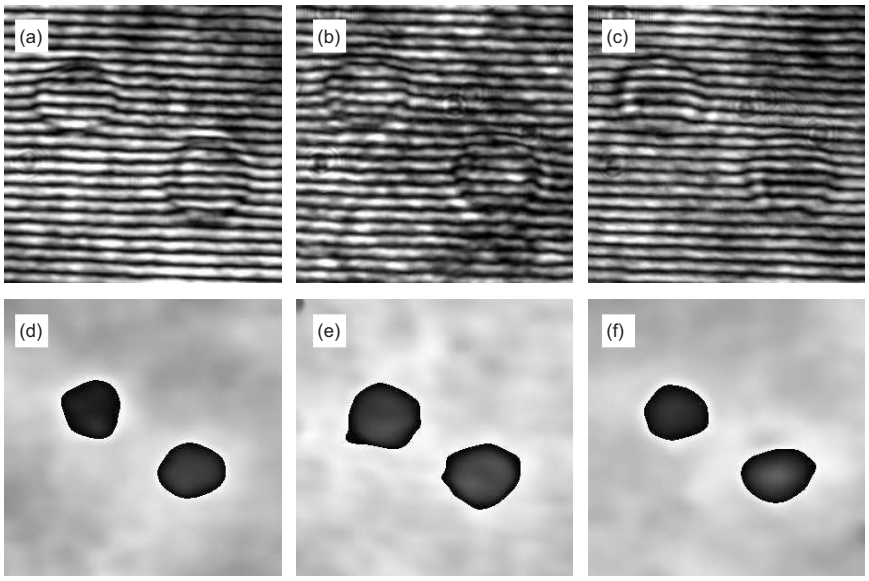

Figure 6.14: (a) - (c) Holograms of red blood cells for beam angles of -21°, 0° and 21° respectively. (d) - (f) reconstructed object phase at these projection angles.

microsphere and its surrounding medium is obtained as expected in both the cases. Next step is to use more number of laser diode modules, which in turn will increase the angle as well as number of projection for better quality reconstructed images.

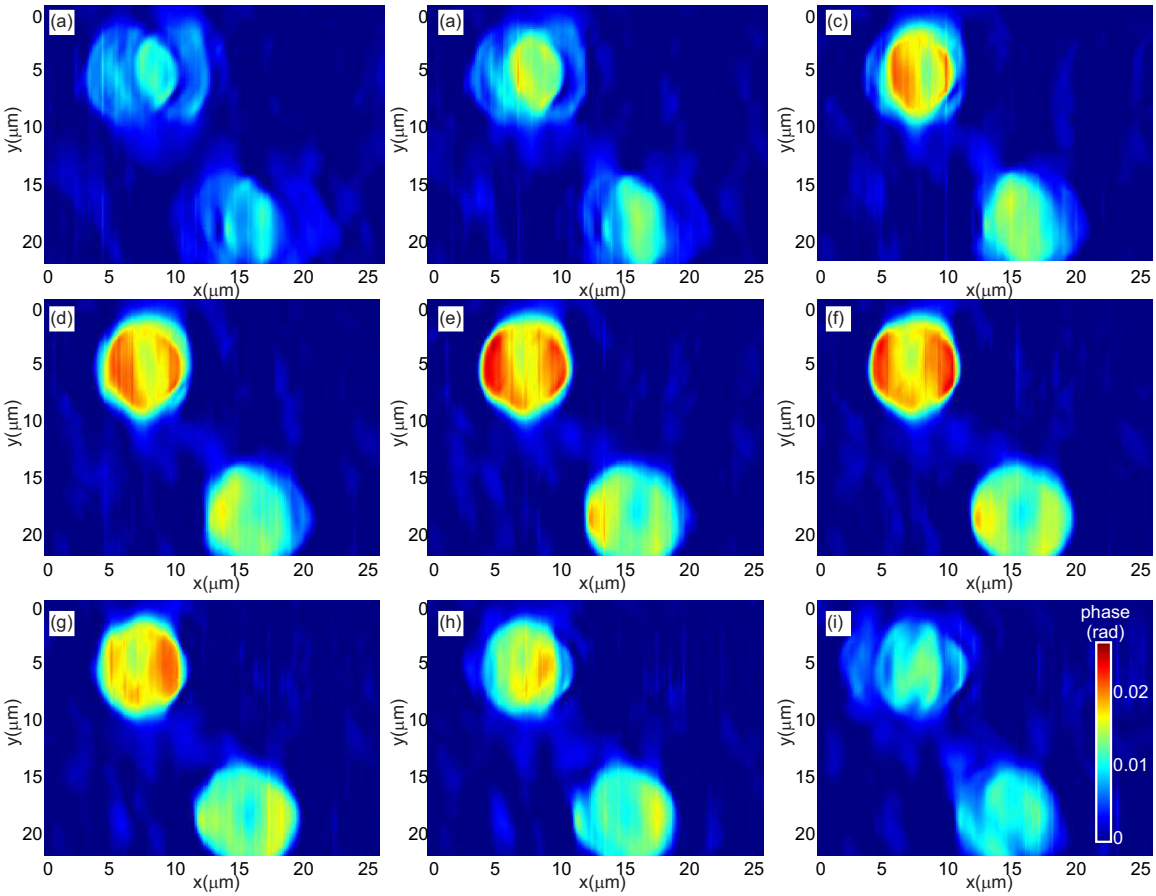

Figure 6.15: Sectional images (x-y sections) of human red blood cells for different z planes separated by 0.2μm.