Unisensor

Reconstruction of 3D morphology from optical sectioning of biological objects

Description of task for ESGI workshop 17-21 August '09 - formulated by Unisensor

Unisensor have developed a new instrument for detecting and analysing microscopic particles in various types of fluid based on a novel technology involving optical sectioning of the sample. Examples of particles to be measured are blood cells, bacteria, sperm cells, and mammal oocytes and embryos (unfertilised and fertilized eggs, respectively). The aim of the task is to reconstruct the 3D morphology of the sample objects from a series of images through different slices of the sample.

The measurement principle

The detection principle is illustrated in figure 1. It consists of a microscope which can be adjusted automatically in the vertical direction. By moving the camera up and down stepwise and acquiring images for each step, the entire sample can be imaged in optimal focus and the 3D information of the sample is thus available for analysis. The contents of the sample can be analysed in numerous ways depending on the nature of the sample. The more advanced analysis requires information about the 3D morphology of the objects.

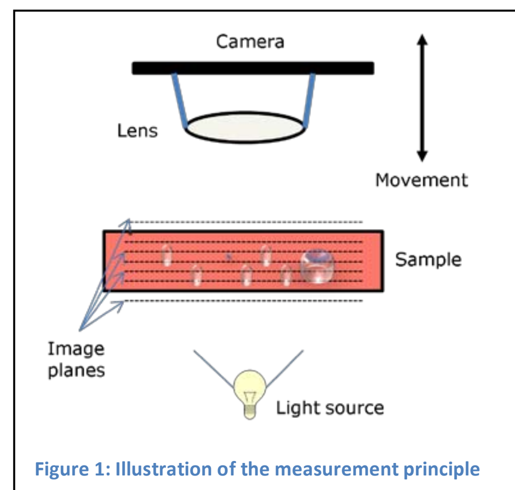

Figure 1: Illustration of the measurement principle

Challenges

The technique of sectioning is well known from other similar applications, such as fetal ultrasound scanning, skin tomography and CT and NMR scanning techniques. The most important specific challenge in this case lies in the fact that the lens has a finite depth of field, i.e. parts of the objects within a certain range of the focus plane will be in focus in the same picture. This has the advantage that fewer images are necessary in order to cover the entire sample, but the disadvantage that each image will include a strong component of the neighbouring images in a blurry (out-of-focus) form. Other challenges involve optical artefacts due to spatial and temporal coherence of the illumination source, increasing scattering of

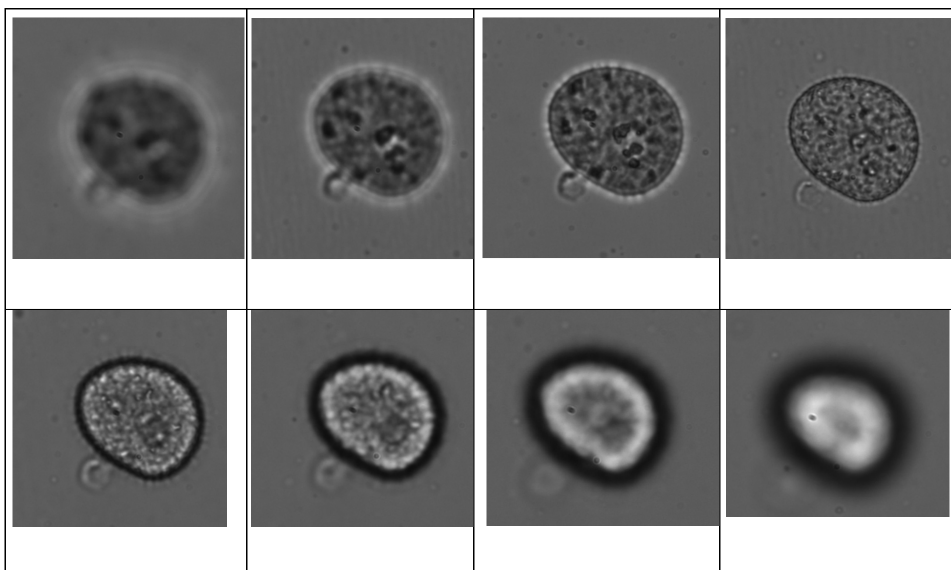

Figure 2: Set of bright field images taken through a mouse Oocyte (). Diameter of Oocyte is 70μm.

the illumination light down through the sample (changing point spread function). These issues could indicate the need for using a complex point spread function, i.e. including both the phase and strength of the field.

Example

Figure 2 shows an example of the sectioning technique on a mouse Oocyte (an egg cell that is not fertilized), with 8 images taken at different sections through the Oocyte. The inside of the object clearly shows a granular structure, which varies through the egg. The first and the last images are blurred, indicating that the image plane is well outside the object.

Objectives and success criteria

The overall purpose of the task is to develop a method, which can reconstruct the 3D morphology of the object under investigation from the set of images. Focus is especially on identifying sharp changes in contrast, e.g. cell membranes and similar objects. Objects are in general assumed to semi transparent, i.e. weak phase objects.

On the path towards this ultimate goal there are a number of interesting milestones:

- Make computer generated image stack of simple objects (2D slab, sphere, cube, or similar). These images can be used as inspiration for the first version of reconstruction routine.
- Make some observation of the uniqueness of the solutions and consider/discuss potential solutions to improve the 3D reconstruction
- Reconstruct 3D morphology from computer generated images of objects like cubes and spheres etc.)
- Compensate images for complex point spread function. This is mainly important for lenses with high numerical aperture, i.e. small depth of field (and high optical resolution)
- Develop a method to separate focus information from out-of-focus information possibly by comparing two neighbouring images.
- Apply the reconstruction routine on experimental acquired images of
    - well defined 2D patterns
    - Glass sphere clusters
    - White blood cells
    - Mouse egg cell

Available information and data

There is a large amount of background information and actual measurement data available. Among the most important of these should be mentioned:

- Complex point spread function for all involved optical systems
- Measurements on objects with well-known and well-defined 3D morphology
- Measurements on optical systems with low depth of field (makes it easier to separate focus information from out-of –focus information.
- Measurements on optical systems with low depth of field

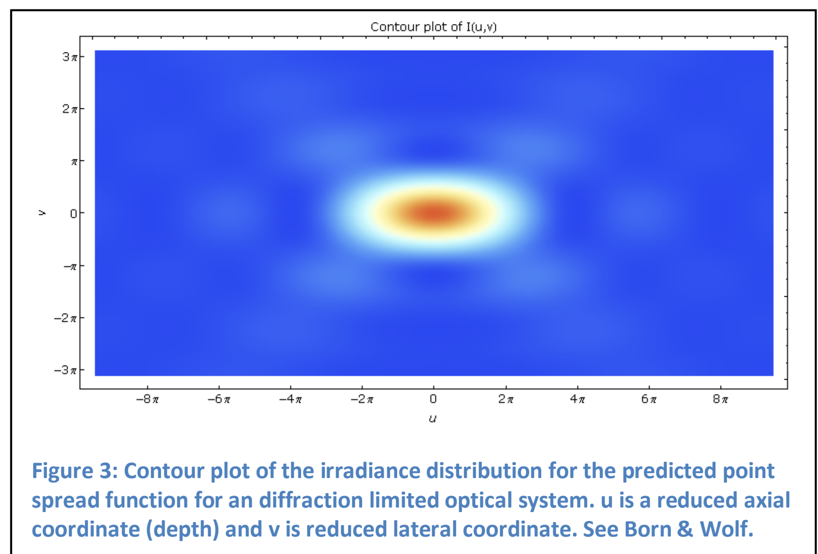

Figure 3: Contour plot of the irradiance distribution for the predicted point spread function for an diffraction limited optical system. u is a reduced axial coordinate (depth) and v is reduced lateral coordinate. See Born & Wolf.