구면수차가 보정된 공촛점 현미경의 연구

Study on the spherical aberration compensated confocal microscope

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The research in conventional microscope is mainly concentrated to improve the resolution of 2-D image. Compared with conventional microscope, confocal microscope minimizes the spot size of illumination light inside a sample by using a source pin hole and detects only the light which is re-radiated from that focusing point by using a detecting pin hole. The confocal microscope improves the radial resolution of the system and also has an advantage of acquiring 3-D image by improving the optical sectioning ability. A significant factor lowering the resolution of the confocal image for a bio-sample is spherical aberration. The refractive index mismatch between the objective and a sample causes spherical aberration and it lowers the resolution of the system and signal intensity. Since a bio-sample is mainly composed of water, spherical aberration is introduced at the focusing plane inside a bio-sample if we use the oil-immersion objective or the objective which is used in air.

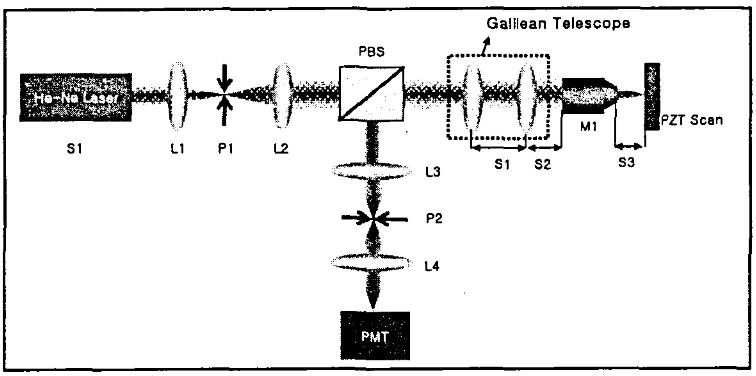

Figure 1. Layout of the Spherical aberration compensated confocal system

The spherical aberration due to the refractive index mismatch between the objective and water is compensated with the Galilean telescope. Figure 1 is the layout of the confocal microscope. Galilean telescope is composed of negative lens and positive lens. Galilean telescope compensates the spherical aberration by adjusting the distance of S1, S2, S3 (Figure 1). When the light travels from air to bio sample(water), negative spherical aberration is introduced in the water and the Galilean telescope introduces positive spherical aberration. Therefore, total spherical aberration is compensated. Figure 2 is the layout of the spherical aberration compensation with the Galilean telescope at the different scan depth of 20μm, 40μm, 60μm and figure 3 is the simulation results of OPD fan in the condition that spherical aberration is compensated. In our experiment, the confocals image of sample bead (silver bead, 170nm, SIGMA -ALDRICH) are acquired and the FWHM (Full-width at half-maximum) of this confocal image is calculated in the both case of without Galilean telescope and with Galilean telescope. It is evident that spherical aberration is compensated by using the Galilean telescope when both results are compared. For this experiment, a sample die which has the form of stairs (Figure 4) is manufactured. A solution of silver bead was dropped and dried at the each step of sample die, and then silver bead was attached to the each step of sample die. After the filling the sample die with water, confocal images of silver bead at the different scan depth are acquired.

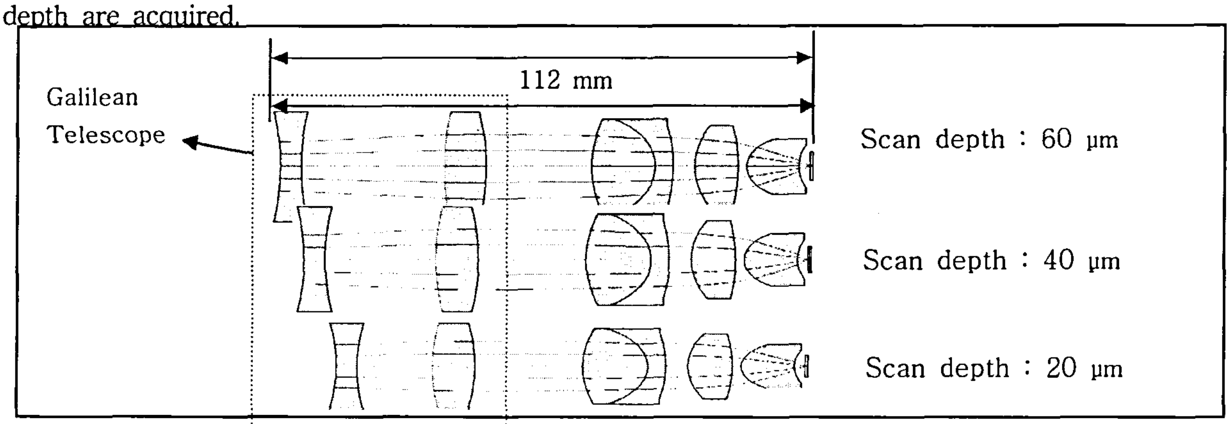

Figure 2. Spherical aberration compensation simulation

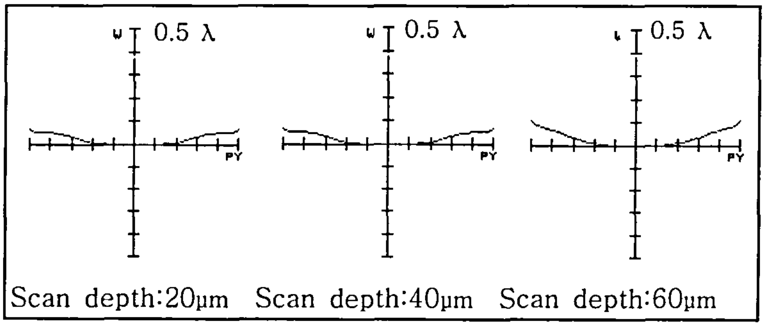

Figure 3. OPD fan at the different scan depth

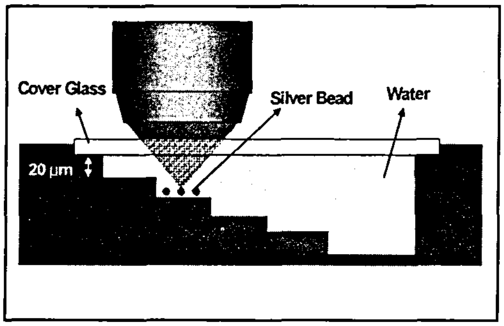

Figure 4. Sample die

Reference

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