A LASER-INTERFERENCE MICROSCOPY FOR SUB-MICRON SCALING

Yuko YAMANISHI,[a] Keiko TAMURA,[a] Kazuo SHIMIZU,[b] Masayuki NAITO,[b] and Tsutomu MASUJIMA*,[a]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan, and Scientific Equipment Division, Olympus Optical Co., Ltd.,[b] 2-3, Kuboyama-cho, Hachioji-shi, Tokyo 192, Japan

Two split laser beams were introduced from the bottom side of the stage of an optical microscope, and combined at the sample position on the stage. The straight stripes which were produced by the interference of the two beams were observed in the focal plane of the objective lens. The distance between two adjacent stripes was 630nm and gave the scale for two-dimensional microscopic image, and the displacement of the stripes gave us information about the thickness and/or concentration of the sample. This optical scale is the measure for dimensional scaling of a microbody in sub-micron resolution.

KEYWORDS laser interference; optical scaling; optical microscope; sub-micron scaling

In order to observe the fine structure of the cell, varieties of optical microscopes have been developed, e.g., the differential-interference contrast microscope, phase-contrast microscope and dark-field microscope. With these microscopes, we can observe the overall image in detail, but it is not so easy to estimate the dimensional scale at the same time. We have developed a laser interference microscope which can analyze the scale of objects by using interference stripes; it was produced by the interference of two introducing laser beams in the observing field. Several interference microscopes have been developed[1-3] and are commercially available; however, they are too complicated in structure, e.g. with two objective optical paths, or have too limited an area for observation and sometimes need scanning systems. We discovered a simple way of scaling by microscopy.

A laser beam from a He-Ne laser (632.8nm, 0.5mW) was introduced from the left side of the

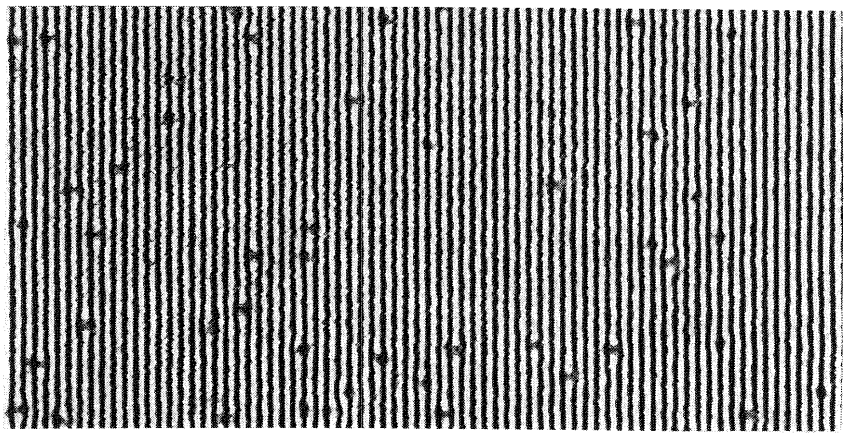

Fig. 1. Laser Interference Stripes Obtained by Crossing Two Laser Beams

Interference stripes interval was 630nm. Black points are latex beads with diameter of 600nm. (Objective lens; x100)

microscope and was divided into two 170° oppositely split beams by a beamsplitter under the stage. Then the two beams were reflected upward to the sample on a slide grass by two flat mirrors which were under the left and right sides of the stage. The interference pattern which was produced by the two laser beams can be seen as many stripes at a focal plane of the objective lens as shown in Fig. 1. Stripes interval was calculated to be 630nm by the following equation (1):

$$d = \lambda / 2\sin\theta \qquad (1)$$

(d; stripes interval, λ; wavelength, θ; half of the crossing angle of two split incident laser beams). The direction of stripes was perpendicular to the plane of two incident crossing beams. Black points in Fig. 1 are particles of latex beads (Sekisui Co. Ltd., Tokyo) with diameter of 600nm, and they are close to the distance between two adjacent stripes (stripes interval). Since the stripes interval depends on the laser wavelength, shorter laser wavelength can be used to get a narrower stripes interval. However, in another sense, it is preferable to use longer wavelength to keep the living cell intact. Using those interference stripes, we can estimate the two-dimensional scale of objects in the microscope field at sub-micron resolution without calibration, which is necessary for using conventional scale plates at each magnification. The displacement of interference stripes also reflects the thickness of the objects.

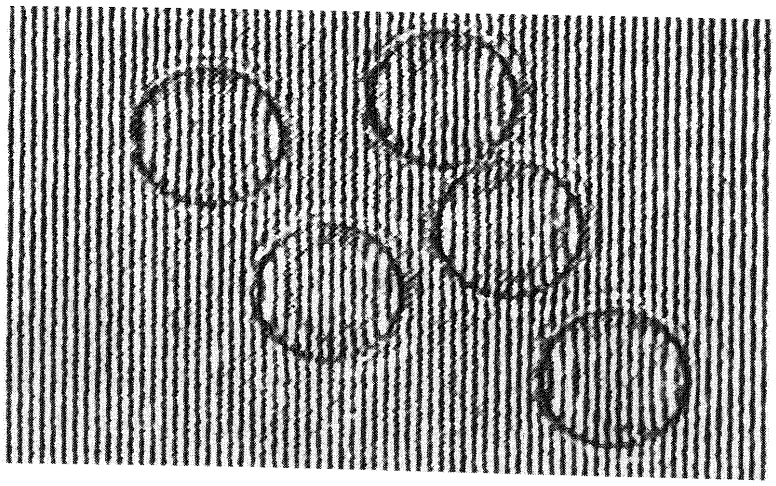

Fig. 2. Standard Microspheres (7.2 μm in Diameter) Observed by Our Laser Interference Microscope
Laser-stripes interval was calculated to be 580nm by equation (1) in this case. (Objective lens; x100)

The image of standard polystyrene microspheres (7.2 μm) is shown in Fig. 2. The diameter can be measured directly from the number of stripes that are connecting with a sphere. If the refractive index of the sample is known, thickness or concentration of substance or density of object can be estimated.

This microscope system has many applications, not only in biology but in various aspects of industrial use. The method of three-dimensional imaging and coupling with the conventional microscope are under investigation.

REFERENCES

1) F. H. Smith, *J. Royal Micro. Soc.*, **87**, 165 (1967).
2) F. H. Smith, *Microscope*, **20**, 153 (1972).
3) D. J. Goldstein, *J. Microscopy*, **102**, 143 (1974).

(Received May 11, 1994; accepted June 1, 1994)