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Performance Estimation of Photothermal Contrast-Applied Microscope

光熱変換コントラストを用いた顕微鏡の性能評価

Midori Manago†, Akira Harata
(Interdisciplinary Grad. School of Engineering Science, Kyushu Univ.)
眞子翠†、原田明 (九大院 総理工)

1. Introduction

A photothermal contrast-applied microscope has a potential for versatility with high sensitivity to optical absorption. Microscopes are used in a variety of scientific fields. An ideal microscope mainly has three specific potentials for chemical species-identification, *In-situ* observation, and high spatial resolution. The fluorescence microscope, the most popular biological microscope, has some disadvantages. The most species to be detected have no native fluoresce: i.e., biologically important DNA bases[1,2,3] need dyeing for fluoresce detection. The fluorescent signal is sometimes suffered from scattering background. The photothermal microscope (PTM) can make up them. Because the PTM has three advantages of being ultrasensitive in measuring optical absorbance, insusceptible to scattering background, and free from sample dyeing.

The thermal lens-based PTM is a two color nonlinear technique, in which a heating (pump) beam is absorbed by the species to be detected, causing heat release and a local change of refractive index. The propagation of the second beam at a different wavelength, the probe, will thus be modified by the produced heat. The intensity of probe beam condensed to a detector is changed because of the change of propagation. These changes produce the observed signal.[4] Because the signal is proportional to the amount of heat generated due to the light absorption, it is expected that real amount of optical absorption is quantitatively determinable with ultrahigh sensitivity.[5] The signal is not sensitivity to weak scattering because the signal only arises from absorbing centers that dissipate heat and the probe only reacts to refractive index changes.

In this report, photothermal images are compared with the optical transmission images for the estimation of the performance as sub-beam-sized resolution imaging.[5]

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m.manago.794@s.kyushu-u.ac.jp

2. Experimental

A sample of a polymer film was marked with a razor blade to modify the surface shape, and was placed on the quartz glass plate.

The experimental setup based on an upright microscope equipped with the objective (20x, numerical aperture 0.40) is sketched in **Figure.1**. The sample stage is scanned in any ranges with arbitrary step widths by the 3-dimensional stage controller.

Both of pump and probe beams are provided by Ti: sapphire laser at 780 nm. One beam is used directly as the probe beam and another is sent to the second harmonic generator (SHG) and converted to 390 nm, as the pump beam. Both of them incidents to an objective along the same axis. These beams are focused by the objective with a little chromatic aberration which makes the difference in the focus positions in the sample. The pump beam is chopped at 1 kHz. The output signal of a photodiode detecting the probe beam passing thorough the optical fiber is demodulated with the reference signal, 1 kHz, by a lock-in amplifier. Then, the PTM signal is obtained.

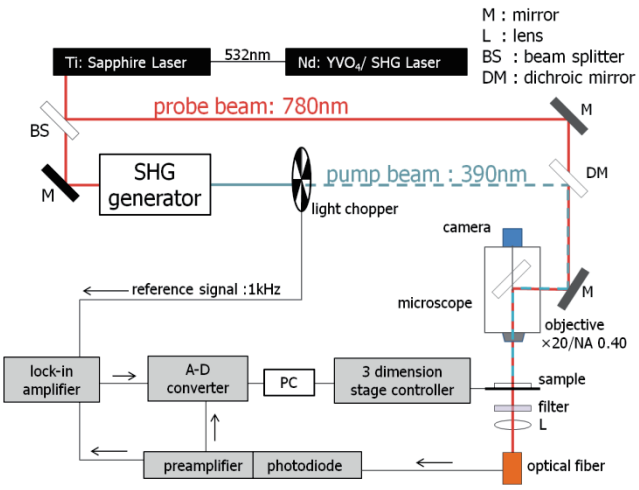

Fig.1 Scheme of the experimental setup

3. Result

Figure.2 shows the result of surface shape images of optical transmission and PTM. They have the area of 100 μm x 100 μm with 1 μm step width. The color bars on the each right hand side of images show the level of signal intensity. The uppers are higher intensity. The transmission image is obtained as the DC component of the diode output. It is demonstrated that the sample surface detail was detected.

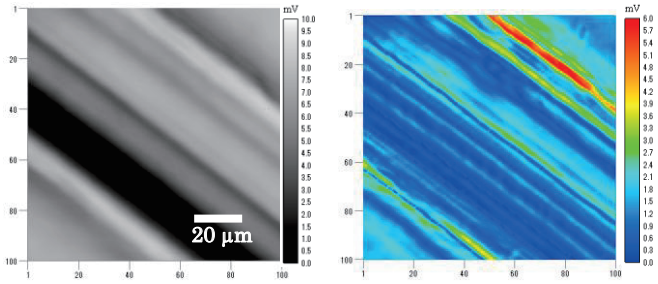

Fig.2 Result of the sample surface shape images. Optical transmission image (left), PTM image (right).

Figure.3 shows the one dimensional signal intensities of optical transmission and PTM. They are detected 30 μm length on the sample with four different step widths, 0.2 and 1 μm respectively. The highest and lowest points of each signal rising lengths are chosen. From their heights among 10-90 % points, the widths are evaluated as the resolution. **Table. 1** summarizes the resolutions for each step width (0.2, 0.3, 0.5 and 1 μm). The PTM resolutions are higher than optical transmission's: sub-beam-sized resolution is demonstrated. Because the heat distribution is changed by the sample's surface shape, the difference of the refractive index change was induced efficiently.

The PTM has a resolution in the depth direction. **Figure.4** shows the different of depth resolving images. These images were obtained on the same area with 100 μm x 100 μm of the area size with 1 μm step width. The depth direction level is changes, an arbitrary level (0 μm), lower 10 and 20μm of the sample, respectively. These images have the different intensity contributes by the measurement level.

4. Conclusion

The performances of PTM were examined for the ability of inhomogeneous responsiveness as a sub-beam-sized resolution, and depth-resolved imaging. The result demonstrated the potential of PTM to a versatile microscope.

Reference

1 A. Gaiduk, M. Yorulmaz, P. V. Ruijgrok, M. Orrit: Sci., 330 (2010) 353
2 T. Larsen, S. Schmid, L. G. Villanueva and A. Boisen: ACS Nano, 7 (2013) 6188
3 D. A. Nadosekin, E. I. Galanzha, E. Dervishi, A. S. Biris and V. P. Zharov: small, 1 (2014) 135
4 A. Gaiduk, P. V. Ruijgrok, M. Yorulmaz and M. Orrit: Chem. Sci., 1 (2010) 343
5 A. Harata, T. Kitamori and T. Sawada: J. Jpn. Soc. Colour Materi, 68 (1995) 606

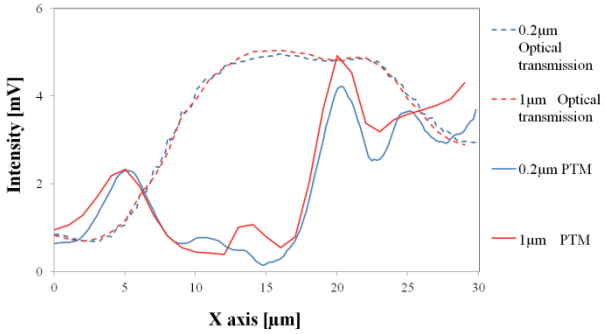

Fig.3 The one dimension intensities of optical transmission with PTM for two step widths (0.2, 1 μm). The dashed line shows the optical transmission signal and the solid line shows the PTM signal.

Table. 1 Comparison of Resolution* for Step Widths

Step width [μm]	Optical transmission [μm]	PTM [μm]
0.2	6.8	3.0
0.3	6.5	3.2
0.5	7.1	2.1
1	7.1	2.5

*Resolution: Estimated as the distance between 10-90% signal rising length

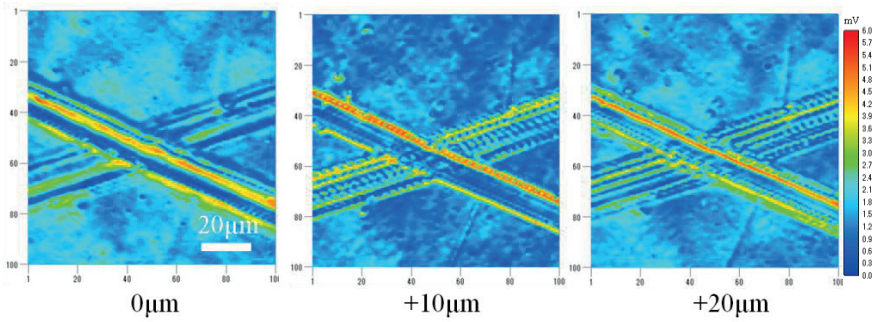

Fig.4 The comparison of depth resolving images.
0 μm means an arbitrary level.
+ mark shows the measuring position of the sample, the larger figure of the mark's right is the lower position of the sample.