

Application of Confocal Laser Scanning Microscope Technique to Visualize Dynamic Sorption Behavior of Perylene in Cellulose Acetate Film

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ABSTRACT: Additives distributed in a polymer film were visualized by a confocal laser scanning microscope (CLSM) in this study. Cellulose acetate (CA) was used as the polymer film material. Perylene as a fluorescence reagent and glycerol triacetate (GTA) as a plasticizer for CA, respectively, were selected and mixed for visualizing the absorption of perylene into CA. Under optimized CLSM conditions of an objective lens (dry, 20 \times) and diode laser (408 nm, 290–330 μ W), a fluorescent penetrant, perylene, in GTA solution was detected in CA casting film within around 9 μ m depth of the film in 60 minutes after starting the sorption study. It was also found that the fluorescence intensity of perylene became lower with the film depth. This finding suggested that CLSM could visualize the sorption behavior of perylene

as a dynamic diffusion process. Analytical conditions, such as a scanning range along the Z axis (± 10 or ± 30 μ m from an air-contact surface of the CA film) and a scan speed (1 or 30 fps) of CLSM, did not affect the fluorescence intensity of the sorbed perylene. It was concluded that a newly developed analytical methodology using CLSM was sufficient for visualizing the perylene penetrant across the cross-sectional distribution of the CA film, being capable of monitoring the sorption behavior of compounds in polymer materials without any destruction of a given absorbed film. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 116: 1552–1557, 2010

Key words: additives; films; diffusion; imaging; fluorescence

INTRODUCTION

The sorption of compounds such as liquid additives and vapor components into polymer materials is one of the most deteriorative phenomena for polymer products. In the field of material engineering, various additives are used in the process of manufacturing polymer products for the purpose of improving product functions. For example, in the manufacturing process of thermoplastics for plastic lapping and rubber materials, plasticizers are added to increase the flexibility, toughness, and/or transparency.^{1,2} In those cases, the diffusion of a plasticizer in polymer materials is associated with the stability of the product quality.^{3–5} Food industries aim to avoid serious deterioration due to the loss of flavors responsible for product quality through sorption into packaging materials.^{6–8}

A number of studies on the sorption behavior of chemicals into polymer materials have been

reported,^{6–14} and theoretical approaches to the dynamic behavior of flavors sorption into polymer films have progressed since the mid-1980s. Namely, aqueous and vaporous penetration methods^{12,13} have been widely used for determining the dynamics, and physical and chemical parameters of chemicals and/or polymers, such as the molar volume, solubility parameters (SPs), free energy, and free volume, have been characterized as contributors to sorption.^{9–11} However, these convenient methods provide no crucial information on the real-time distribution behavior of penetrants in polymers.

Although observational methodology using time-of-flight secondary-ion mass spectrometry has been applied to obtain the cross-sectional distributions of penetrants in polymer films and fibers,¹⁵ a destructive treatment of samples is needed to determine the concentration of the penetrants in each sectional region.

This lack of a nondestructive observational methodology led us to the use of confocal laser scanning microscopy (CLSM) for the real-time monitoring of penetrants in polymers.

CLSM has been widely used for the observation and/or analysis of mass transfer phenomena in the

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fields of physiology and pharmacology with the use of fluorescent labeling techniques or fluorescent reagent mixtures.^{16–22} In physiology, some researches using CLSM have been reported for monitoring the formation of poly(ethylene glycol) microgels and nanogels¹⁶ or surface crosslinked structures of poly(vinyl alcohol).¹⁷ In pharmacology, CLSM has been applied to analyze the transport phenomenon of proteins^{18,19} and the dynamics of glucose transporters in lipocytes.²⁰

Recently, attempts to apply CLSM have been reported in the field of material engineering. For example, as an analytical method for evaluating the hydrophilicity of the surface of nylon fibers, the absorption behavior of glycerin containing a fluorescent dye in fibers has been observed by CLSM.²¹ In addition, to investigate the plasticizing effect of a supercritical fluid treatment for polypropylene, the distribution of a fluorescent probe mixed in a film has been analyzed with CLSM.²²

In this study, cellulose acetate (CA) was used as a polymer material. CA is widely used in various industrial fields for applications such as cigarette filters, clothing, water purification, and medical uses,^{23,24} and a number of studies on the sorption behavior of water and alcohols in CA have been reported.^{25–27} As a fluorescent reagent and a plasticizer, perylene and glycerol triacetate (GTA), respectively, were selected for visualizing the distribution of perylene in a CA casting film.

EXPERIMENTAL

Materials

CA flakes with a degree of substitution of 2.5 [weight-average molecular weight (M_w) = 1.0×10^5 g/mol] were a product of Daicel Chemical Industries, Ltd. (Osaka, Japan), and were used without further purification. Perylene (M_w = 252.3 g/mol, excitation wavelength = 411 nm, emission wavelength = 470 nm) and GTA (M_w = 218.2 g/mol) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Sigma-Aldrich (Tokyo, Japan), respectively. Other reagents were commercially available in extrapure grade and were used without further purification. The chemical structures of CA, perylene, and GTA are depicted in Figure 1.

Preparation of the CA film sample

CA flakes were dissolved in acetone to make a 2.0 w/v % solution. An aliquot (200 μ L) of the CA solution was then put into a film-making unit with a penicillin cup and cover glass, as shown in Figure 2. Excess acetone was vaporized at room temperature for 15 h, and the prepared CA film on the surface of

the film-making unit was then dried *in vacuo* over night. The prepared CA film was 95 ± 5 μ m thick.

Preparation of the perylene/GTA solution

A perylene/GTA solution was prepared at room temperature by the dissolution of perylene in GTA to reach a molar ratio ranging from 2.2 to 11×10^{-5} . The molar ratio of perylene (C_{per}) was defined as follows:

$$C_{\text{per}}(-) = \frac{\text{Perylene amount (mol)}}{\text{Perylene amount (mol)} + \text{GTA amount (mol)}}$$

Analysis of the CA, GTA, and perylene/GTA solution by CLSM

The fluorescence spectra of the CA, GTA, and perylene/GTA solution were obtained with a CLSM system (A1 series, Nikon Corp., Tokyo, Japan) equipped with a dry objective lens (CFI Plan Apo VC 20 \times , Nikon). The spectrum of CA was obtained by the direct placement of a film sample on the microscope, whereas the spectra of GTA and perylene/GTA solution were obtained by the pipetting of a 4- μ L solution onto a cover glass placed on the microscope. A diode laser (408 nm, 290–330 μ W) was used as an excitation laser, and the fluorescence excited around the cover glass was detected.

The fluorescence intensity of the perylene/GTA solution was measured under the aforementioned CLSM conditions at room temperature. The intensity of the reflected fluorescence in the wavelength range of 450 ± 25 nm was measured from confocal images around the cover glasses with an optical filter.

Visualization of perylene in the CA film by CLSM

The fluorescence intensity of the distributed perylene in the CA film was measured as follows. Before the perylene measurement by CLSM, the air-contact surface of the CA film was determined from a transmission image with a halogen lamp. Then, CLSM analysis was carried out at 60 min after the placement of a 4- μ L perylene/GTA solution onto the CA film. Confocal images were obtained with the diode laser scanning in the cross-sectional region of the CA film with intervals along the Z axis (Fig. 3); the depth from the air-contact surface of the CA film was ± 10 or ± 30 μ m with intervals of 1 or 3 μ m, respectively. The confocal imaging area was approximately 640×640 μm^2 on the X–Y surface, and both low-speed (scan speed = 1 fps, flame size = 512 pixels \times 512 pixels) and high-speed (scan speed = 30 fps, flame size = 512 pixels \times 512 pixels) modes of

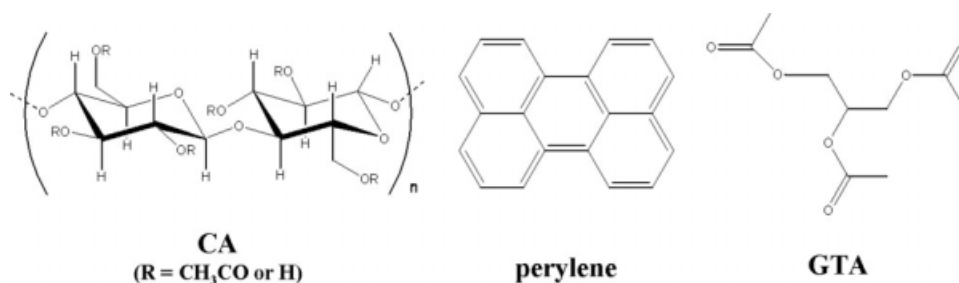


Figure 1 Chemical structure of CA, perylene, and GTA.

scanning were selected for observation. Under each analysis condition, C_{per} was 8.8×10^{-5} .

RESULTS AND DISCUSSION

Fluorescence detection of perylene by CLSM analysis

Figure 4 shows the fluorescence spectra of the CA film, GTA, and perylene/GTA solution when excited by a diode laser at 408 nm. The spectra clearly demonstrate that only the perylene/GTA solution had a significant perylene-induced fluorescence intensity, and this indicated that any interfering fluorescence could be excluded to investigate the distribution dynamics of the penetrant, perylene.

Effect of the concentration of the perylene/GTA solution on the fluorescence intensity

The relationship between C_{per} and the fluorescence intensity of each solution was investigated to optimize the CLSM analysis of the sorbed perylene in the CA film (Fig. 5). Three replicates of the CLSM analysis of perylene (\pm the standard deviation) were performed for this study. As a result, the fluorescence intensity showed good linearity ($r = 0.998$), with C_{per} ranging from 2.2 to 11×10^{-5} .

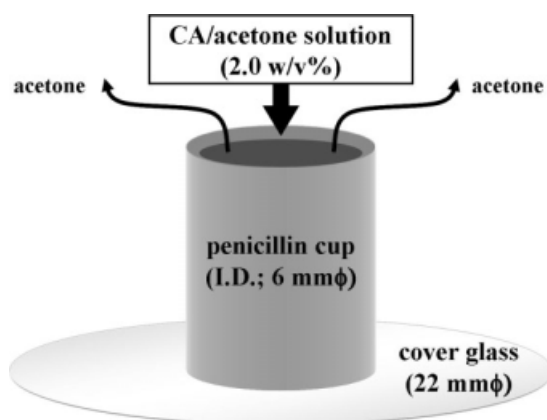


Figure 2 Preparation of the CA film samples on film-making unit consisting of a penicillin cup and a cover glass.

Direct monitoring of the sorbed perylene in the CA film by CLSM analysis

Figure 6 shows chemical images of the X-Y surface at 0-, 2-, 4-, 6-, and 8- μm depths of the CA film treated with the perylene/GTA solution under the following CLMS conditions: a low-speed mode, a $\pm 10\text{-}\mu\text{m}$ range, and 1- μm intervals. Because of the influence of asperity on the air-contact surface of the CA film, an approximately $4.0 \times 10^4 \mu\text{m}^2$ area of the X-Y surface was selected from the observed areas for analysis. The blue color, derived from the fluorescence intensity of the perylene/GTA solution, was detected at 60 min at each depth of the CA film. It was clear that the greater the film depth was, the darker the blue color induced by the sorbed perylene was.

Changes in the fluorescence intensities at each depth of the CA film are shown in Figures 7–9. The intensity was calculated as an average of each pixel in a given analytical area. Three replicates of the CLSM analysis of perylene (\pm the standard deviation) were performed for this study. On the horizontal axis, the 0- μm depth indicates the air-contact surface of the CA film. The results for the sorbed perylene in the low-speed mode in the range of $\pm 10 \mu\text{m}$ with 1- μm intervals are shown in Figure 7, whereas the results for the sorbed perylene in the low-speed mode in the range of $\pm 30 \mu\text{m}$ with 3- μm intervals are shown in Figure 8. Under both CLSM conditions, the perylene-induced intensity was detected to a depth of 9- μm in the CA film and

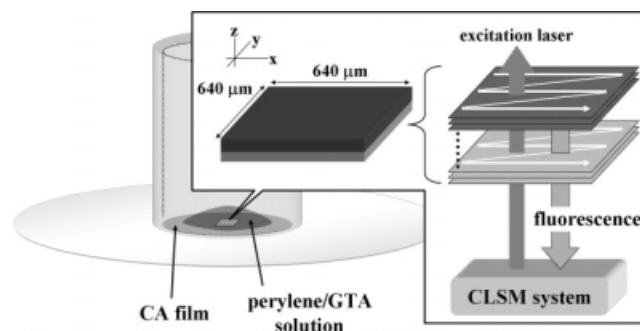


Figure 3 Observation of the perylene/GTA solution in the CA film sample by CLSM.

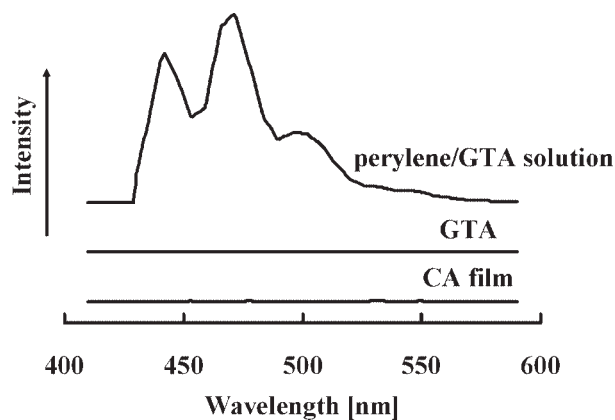


Figure 4 Fluorescence spectra of the CA film, GTA, and perylene/GTA solution excited by a 408-nm diode laser.

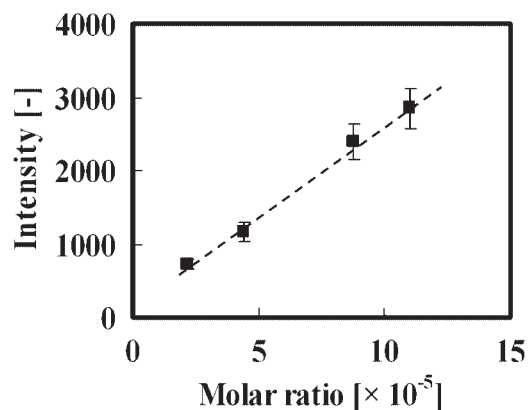


Figure 5 Relationship between the molar ratio of perylene and fluorescence intensity.

became lower with the film depth. The fluorescence phenomena revealed that after the diffusion of the absorbed perylene on the air-contact surface of the CA film started, perylene dynamically sorbed into the inner CA film for 60 min. Because at a film depth greater than 12 μm the perylene-induced intensity was not detected in Figure 8, the scan range of $\pm 10 \mu\text{m}$ along the Z axis was sufficient for monitoring the perylene sorption behavior in this study. In contrast, in a high-speed mode of scanning in the range of $\pm 10 \mu\text{m}$ (Fig. 9), the increase in the intensity of the sorbed perylene was detected to almost the same depth (9 μm) of the film as that in Figure 7. Thus, the scanning speed of a high-speed mode with a wide range along the Z axis in a rapid CLSM analysis in seconds could be useful for dynamic and rapid observations of penetrant distribution in a CA film.

Effect of the optical path length of CLSM on the fluorescence intensity

To evaluate the effect of the optical path length on the fluorescence intensity induced by perylene, a 12- μL perylene/GTA solution was analyzed as mentioned previously with the use of a film-making unit (without a CA film). Figure 10 shows the relationship between the path length of CLSM and the fluorescence intensity of perylene. On the vertical axis, the fluorescence intensity was calculated as an average of each pixel in the observation area at 1 min after the pipetting of the solution onto the film-making unit. On the horizontal axis, the value indicates the distance from the top of the cover glass. As a result, the longer the distance was, the lower the fluorescence intensity was, and this suggested that the power of the excitation laser or the intensity of the fluorescence would decay with an increase in

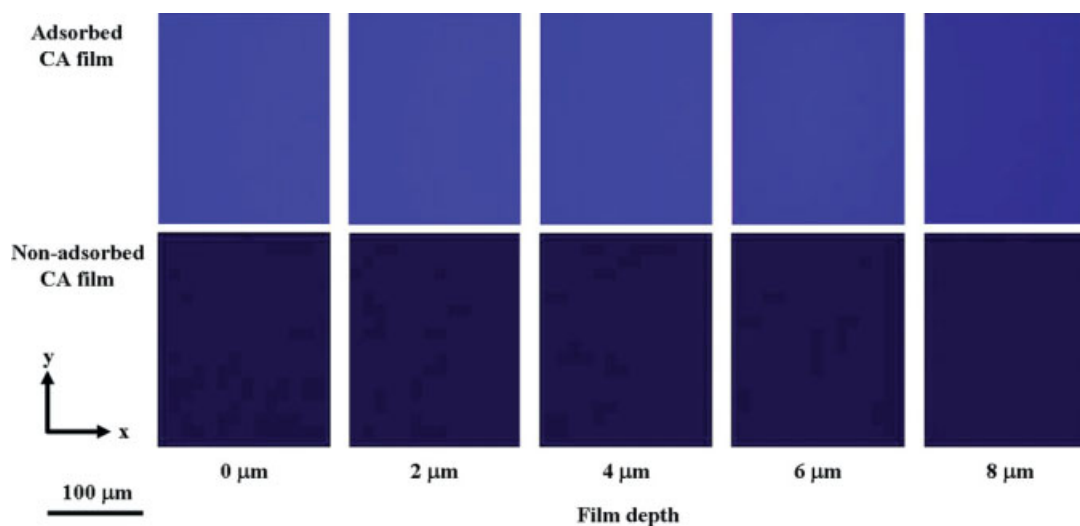


Figure 6 Chemical images of the X-Y surface at several depths of CA films at 60 min after the addition of the perylene/GTA solution obtained under conditions of low-speed mode, $\pm 10\text{-}\mu\text{m}$ range, and 1- μm intervals.

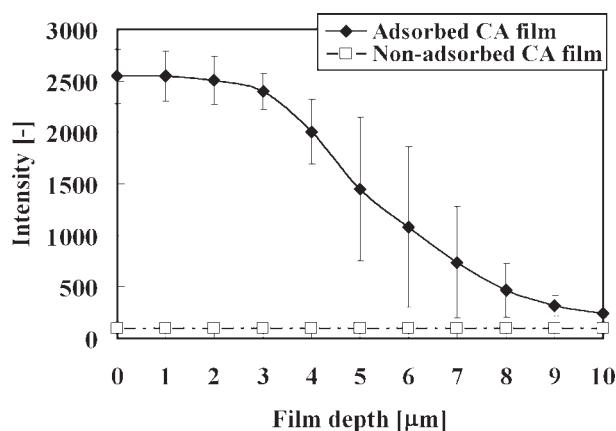


Figure 7 Relationship between the depth of the CA film and the fluorescence intensity derived from the perylene/GTA solution obtained under conditions of low-speed mode, $\pm 10\text{-}\mu\text{m}$ range, and $1\text{-}\mu\text{m}$ intervals.

the optical path length. This result suggested that although the decay behavior of the fluorescence intensity derived from perylene in the CA film was not clarified, the gradient of the amount of perylene had to be sharper than the gradients of the observed fluorescent intensities in Figures 7–9.

As for the sorption of compounds into polymer materials, the physical and chemical parameters of compounds and/or polymers, such as the molar volume, SPs, free energy, and free volume, determine the dynamics.^{9–11} With particular note taken of the SPs, the affinity of a compound for a polymer material can be explained by the two-dimensional distance (δ_c) as follows⁹:

$$\delta_c = [(\delta_{1np} - \delta_{2np})^2 + (\delta_{1p} - \delta_{2p})^2]^{1/2}$$

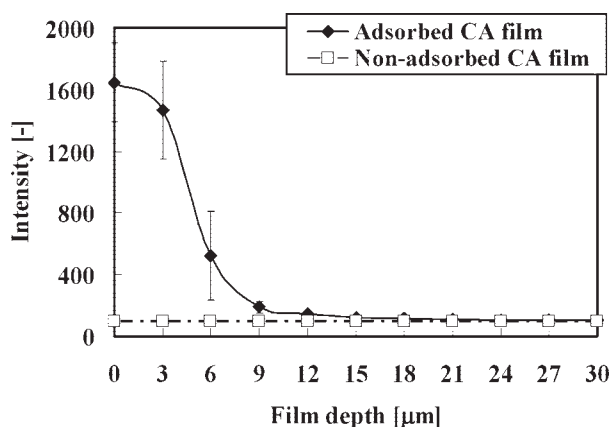


Figure 8 Relationship between the depth of the CA film and the fluorescence intensity derived from the perylene/GTA solution obtained under conditions of low-speed mode, $\pm 30\text{-}\mu\text{m}$ range, and $3\text{-}\mu\text{m}$ intervals.

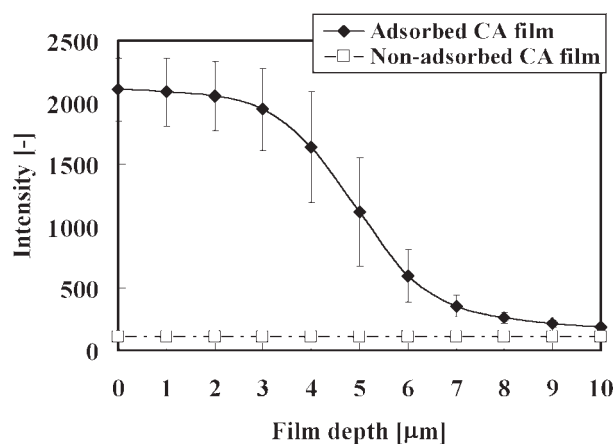


Figure 9 Relationship between the depth of the CA film and the fluorescence intensity derived from the perylene/GTA solution obtained under conditions of high-speed mode, $\pm 10\text{-}\mu\text{m}$ range, and $1\text{-}\mu\text{m}$ intervals.

where δ_t is the total SP value and δ_{np} and δ_p are the nonpolar and polar components of δ_t , respectively. The smaller δ_c is, the higher the affinity is. Table I shows the SP values for CA, perylene, and GTA and the δ_c values of perylene and GTA from CA. The SP values of CA were as previously reported,²⁸ and those of perylene and GTA were calculated with Molecular Modeling Pro computational software (version 6.0.1, ChemSW, Inc., Fairfield, CA). The δ_c value of GTA was smaller than that of perylene, and it was concluded that the affinity of GTA for CA was higher than that of perylene. As a plasticizer for CA, GTA should accelerate the sorption of other compounds into the polymer material, and it was thought that GTA had some effect on the sorption behavior of perylene in the CA film obtained in this study.²⁹ In other words, it could be possible to explain the sorption behavior of liquid additives

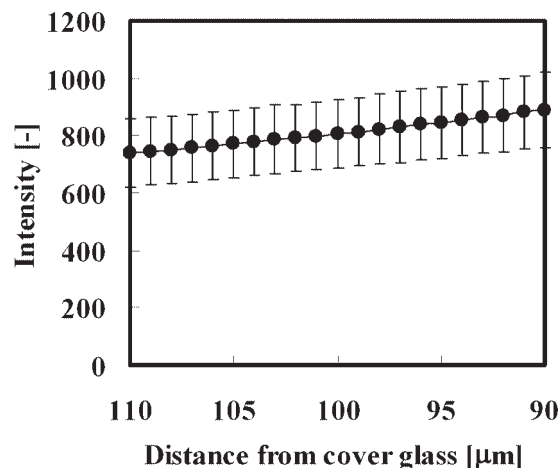


Figure 10 Relationship between the optical path length and the fluorescence intensity derived from the perylene/GTA solution.

TABLE I
SP and δ_c Values of the Compounds

Compound	SP (MPa ^{1/2})			δ_c (MPa ^{1/2}) ^a
	δ_t	δ_{np}	δ_p	
CA	25.1	21.6	12.7	–
Perylene	26.0	26.0	1.3	12.2
GTA	27.2	26.3	6.8	7.6

^a Distance to CA.

such as GTA with a fluorescent reagent such as perylene by this CLSM methodology.

CONCLUSIONS

In this study, we visualized the dynamic distribution of perylene in a CA film by applying a CLSM system for the first time. The CLSM analytical conditions, such as the scanning range along the Z axis and the scan speed, did not affect the results. This methodology has some advantages for evaluating the sorption behavior of a penetrant in a polymer: (1) the proposed nondestructive confocal optics system enables a direct cross-sectional analysis to be performed without any destruction of the sorbed film, and (2) the direct casting of CA onto a cover glass also improves the CLSM monitoring of the sorbed penetrant in the CA film by reducing the reflected lasers caused by random and nonuniform air spaces between the cover glass and film. As for further experiments, a comparison study of the sorption dynamics with different fluorescent reagents and liquid additives and a kinetic investigation of penetrants in CA films are now in progress with this CLSM system.

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