A FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING (FRAP) TECHNIQUE FOR THE MEASUREMENT OF SOLUTE TRANSPORT ACROSS SURFACTANT-LADEN INTERFACES

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ABSTRACT

The technique of Fluorescence Recovery After Photobleaching (FRAP) has been applied to the measurement of interfacial transport in two-phase systems. FRAP exploits the loss of fluorescence exhibited by certain fluorophores when over-stimulated (photobleached), so that a two-phase system, originally at equilibrium, can be perturbed *without disturbing the interface* by strong light from an argon-ion laser and its recovery monitored by a microscope-mounted CCD camera as it relaxes to a new equilibrium. During this relaxation, the concentration profiles of the probe solute are measured on both sides of the interface as a function of time, yielding information about the transport characteristics of the system. To minimize the size of the meniscus between the two phases, a photolithography technique is used to selectively treat the glass walls of the cell in which the phases are contained. This allows concentration measurements to be made very close to the interface and increases the sensitivity of the FRAP technique.

INTRODUCTION

Solute transport across surfactant-laden liquid-liquid interfaces is important in areas as diverse as industrial and analytical separations, tertiary oil recovery, controlled and targeted release drug delivery, and cell membrane transport. However, the mechanism by which this transport takes place is still poorly understood. The process is a complex one, in which electrostatics, steric effects and fluid dynamics all play a role. The disentanglement of these competing influences requires an ability measure unambiguously the resistance to solute transport offered by an interface under a variety of system conditions. Existing techniques, however, suffer from a number of serious limitations. Most of the methods currently employed, such as the stirred cell[1][2] or the rotating diffusion cell[3] involve convection of the fluids adjoining the interface. This requires a detailed knowledge of the hydrodynamic conditions near the interface, which is rarely available. Moreover, convection results in non-uniformities in the interfacial surfactant layer, leading to ambiguity in the interpretation of the results. Limitations of this type can be overcome through the use of purely diffusive systems, in which concentration profiles are recorded as a function of time as the solute moves from one phase to another in response to a perturbation from equilibrium. However, the methods used to detect concentration fields, such as interferometry, frequently require solute concentrations of a magnitude sufficient to perturb the interface itself. In addition, inherent in this kind of technique is the need to observe concentrations very close to the interface, but this is made difficult owing to the presence of the meniscus between the phases which may be up to 200 μm thick. Therefore, what is required in order to measure unambiguously interfacial transport coefficients is a purely diffusive technique with a minimal interphase meniscus and a highly sensitive detection technique.

The technique of Fluorescence Recovery After Photobleaching (FRAP) has been adapted to meet these requirements. FRAP uses a fluorescent solute in a system originally at equilibrium, and perturbs the system using an intense beam of light. This overstimulates the fluorophore and causes it to cease fluorescent emission, a phenomenon known as photobleaching. Over time, the photobleached solute molecules diffuse out of the bleached zone, and the still-fluorescent molecules that were outside the bleached zone diffuse inwards as the system recovers towards equilibrium. This recovery is monitored over a period of time, and from this an interfacial transport coefficient is calculated. For example, if photobleaching takes place close to the interface in the upper phase, transport of *visible* fluorophore will take place from the lower phase to the upper phase as the system recovers. If in this case the system comprised of a lower aqueous phase and upper reversed-micellar phase, such a bleaching pattern would provide information about forward transport from the aqueous phase into the reversed-micellar phase. To reduce the thickness of the meniscus, a photolithography technique[4] is used which results in a meniscus 40 μm deep.

EXPERIMENTAL

The FRAP apparatus is shown in Figure 1. The two phases between which we wish to measure transport are contained in a glass-walled cell *(k)*. The system is allowed to come to equilibrium, with the solute of interest (chiefly derivatives of fluorescein, which have excitation and emission wavelengths of 488 nm and 512 nm respectively) distributed between the upper and lower phase.

To perturb the system, a high intensity beam of light at 488 nm from the argon ion laser *(a)* is used to photobleach a given area of the cell. For example, if photobleaching takes place close to the interface in the upper phase, transport of *visible* fluorophore will take place from the lower phase to the upper phase as the system recovers. The light from the laser reflects off mirror *(b)*, and falls on a half silvered mirror (c), splitting the beam into the bleaching beam, *cdfghi*, and the observation beam, *clmi*. When bleaching is required, the shutter *(e)* is opened, allowing the full power of the bleaching beam to fall on the cell. A computer controlled translation stage *(g)* is included in the path to scan the beam rapidly back and forth across the cell parallel to the interface. This eliminates the complicating factor of concentration gradients *along* the interface, provided that the scanning is rapid relative to diffusional timescales in the system. A long focal length converging lens *(r)* controls the width of the beam. When the 488 nm beam reaches the dichroic mirror *(j)*, the properties of the mirror are such that the light is reflected into the cell and photobleaching occurs.

The task of the observation beam, *clmi*, is to illuminate the cell when a concentration measurement is required. When the observation beam falls on the dichroic mirror, it is reflected towards the cell where it stimulates fluorescence emission. The emitted light at 512 nm can pass through the dichroic mirror and enter the microscope *(u)*, where the image falls on a CCD camera*(v)* and is captured by a frame-grabber board (Data Translation, Inc.) installed in an IBM PC. The width of the cell along the beam is sufficiently small (2 mm) that it lies within the depth of field of the microscope. The image produced by the CCD camera is therefore a two dimensional projection of the contents of the cell. A rotating filter wheel *(n)* controls the level of illumination, and allows two different illumination levels to be used in systems where the solute partition coefficient differs significantly from unity. To prevent photobleaching by the observation beam due to long exposure times, a shutter *(p)* ensures that the observation beam falls on the cell only when an image is required. A beam expander *(w)* ensures a more uniform illumination field, and an optical flat *(o)* bleeds off a small fraction of the beam towards a second CCD camera *(t)* to allow monitoring of any variation in the laser beam power with time.

The operation and timing of the shutters, the translation stage, the filter wheel and the image acquisition from both cameras is controlled by the PC-based virtual instrumentation package Labview (National Instruments, Inc., TX).

The presence of a meniscus between the phases would ordinarily prevent sensitive measurement of interfacial resistance by optical techniques because the meniscus produces a blind zone up to 300 μm thick around the interface. To minimize the meniscus between the two phases so that sensitive measurements can be made, the surface of the glass cell walls is treated by a photolithography technique so that the upper part of the cell, corresponding to the organic phase, is hydrophobic, and the lower part, corresponding to the aqueous phase, is hydrophilic. The edge of the meniscus adheres with a 90° contact angle to the line where the surface properties change, and judicious filling of the cell results in a meniscus that is as little as 40 μm deep.

Early results indicated that natural convection was a major problem when conducting ground-based FRAP experiments. The local heating that occurred on bleaching resulted in gross movements of fluid, as shown in Figure 2. Refinements to the imaging and control apparatus has enabled the use of fluorophore concentrations as low as 50 nM, at which level the bleaching-induced heating is insufficient to cause the onset of convection.

DATA ANALYSIS

The emission intensity at any point in the cell is proportional both to the observation beam intensity and to the concentration of the fluorophore at that point. In order to obtain the two-dimensional concentration field in the cell, it is necessary to take a pre-bleach image (when the system is at equilibrium and all

concentrations are known) so that all subsequent, post-bleach images may be calibrated and the dependency on observation beam profile removed.

If (i) the time required for the bleaching beam to scan across the cell is very small relative to the diffusional timescales of the system, and (ii) the width of the scan is very large compared to the observation area, the diffusional recovery is essentially one-dimensional. This allows us to use an average concentration across the observation area (parallel to the interface), and produce a one-dimensional concentration profile. The interfacial resistance in the system is taken as that value which provides the best fit of the one-dimensional unsteady state diffusion equation to all the concentration profiles *simultaneously* . (The diffusion coefficient in either phase can be measured independently through a FRAP experiment in which only one phase is present.)

RESULTS

Figure 3 shows one-dimensional concentration profiles obtained for a single phase fluorescein/ PEG/dextran/water system. Fluorescein concentration is 100 nM. An 800 μm bleaching beam has been scanned across the middle of the image. The recovery from the bleaching with time is clearly visible. The curves in Figure 3 give the best fit of the one-dimensional unsteady state diffusion equation to these data, using a diffusion coefficient of 0.4×10^{-5} cm^2s^{-1} (this is for a single-phase system, with no interface present). The initial condition used in solving the diffusion equation is derived from the experimental concentration profile obtained immediately after bleaching. In this way, diffusion coefficients for our chosen fluorophores can be measured directly, leaving the interfacial resistance as the only unknown parameter in two-phase systems.

Figure 4 shows results obtained from an experiment with a fluorescein-containing water-NaCl / AOT-heptane reversed micellar system. These results were obtained before the addition of the rotating filter wheel to the FRAP system. The aqueous phase has been bleached, resulting in 'back' transport of unbleached fluorophore preferentially from the reversed-micellar phase to the aqueous phase. The fit of the diffusion equation given by the curves in Figure 4 is for an interfacial resistance of 5000 s cm^{-1}. The solute partition coefficient is taken into account by the image processing algorithm used to extract the concentration data from the intensity images in such a way that a discontinuity of concentration across the interface, as seen in Figure 4, is attributable to interfacial resistance and not to the effect of a non-unity partition coefficient. Although the fit is not as good as in the single-phase (Figure 3) or two-phase aqueous polymer systems (not shown), and the data for the organic phase is very noisy, an interfacial effect is clearly visible in these preliminary experiments. Further refinement of the technique is necessary to reduce the noise present in the concentration profiles and increase the sensitivity of the technique. Preliminary results obtained on addition of the rotating filter wheel (which allows the use of two different levels of illumination by the observation beam) have been encouraging. In particular, detailed, low-noise images are available in the reversed-micellar phase for the first time (Figure 5). Previously, it had not been possible to observe any bleaching effects in the reversed-micellar phase, which would be necessary for observing 'forward' transport from the aqueous phase into the reversed-micellar phase.

CONCLUSIONS

Results to date show that interfacial transport effects can be observed using the FRAP technique in a variety of systems, and that it will provide a sensitive means to determine interfacial resistance free from the ambiguity inherent in convective methods of measurement.

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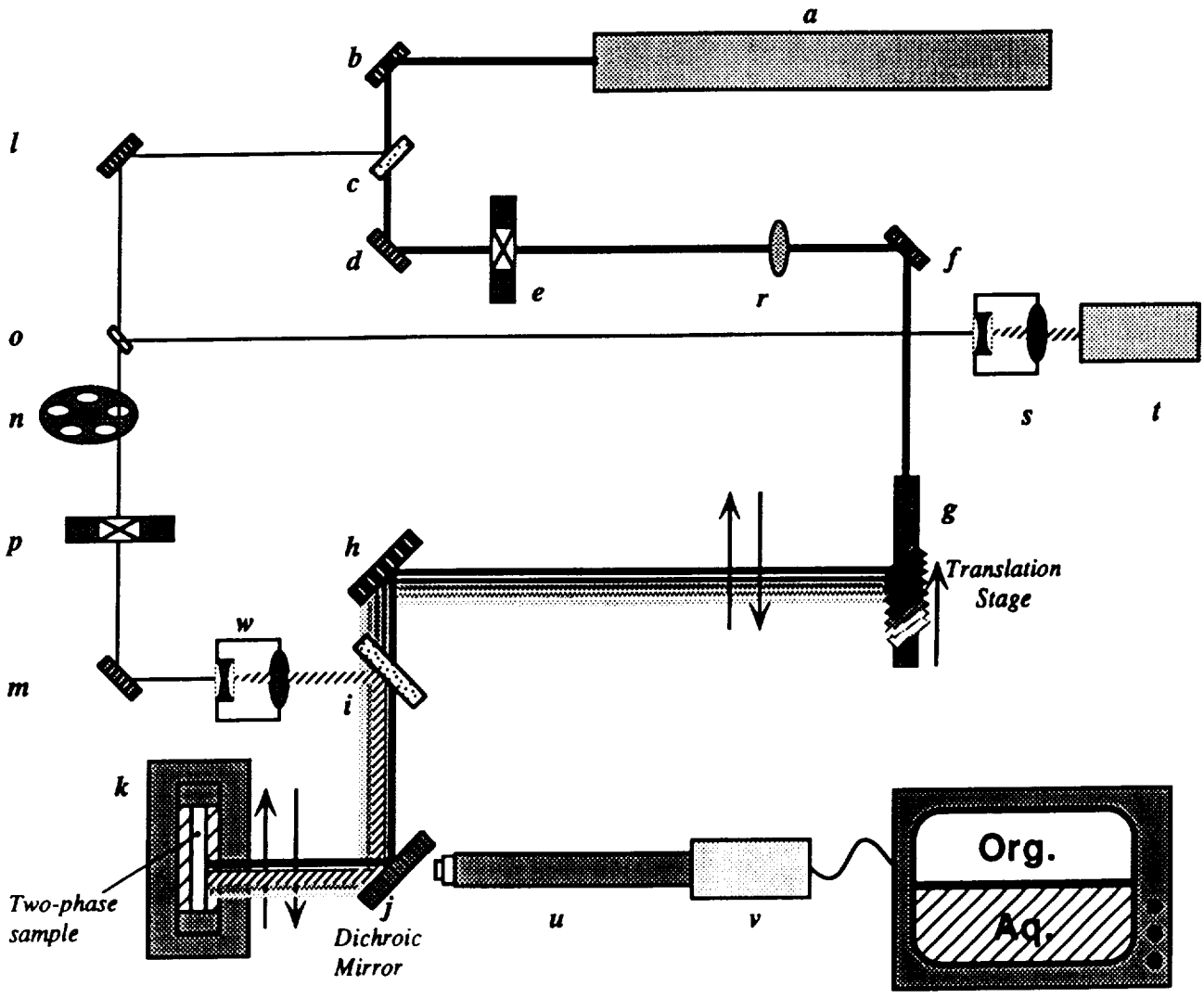

Figure 1. FRAP System

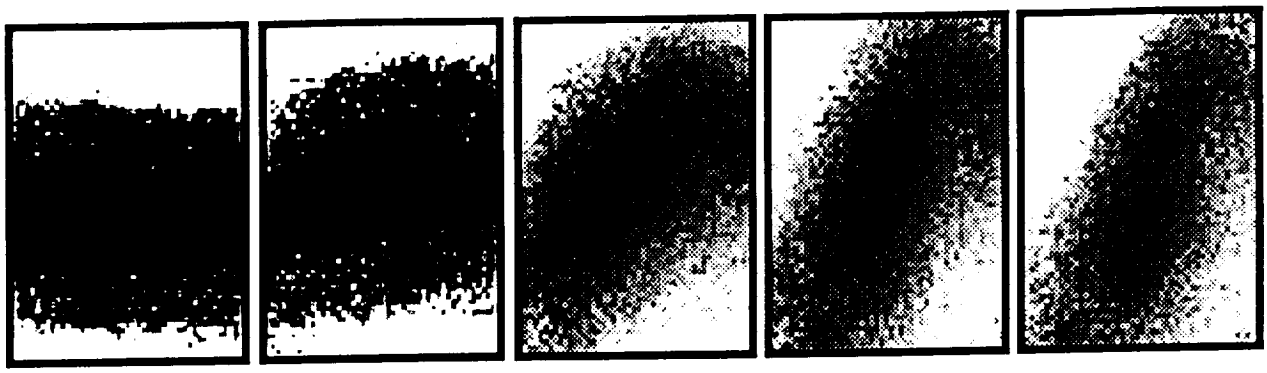

Figure 2. Photobleaching-induced Convection

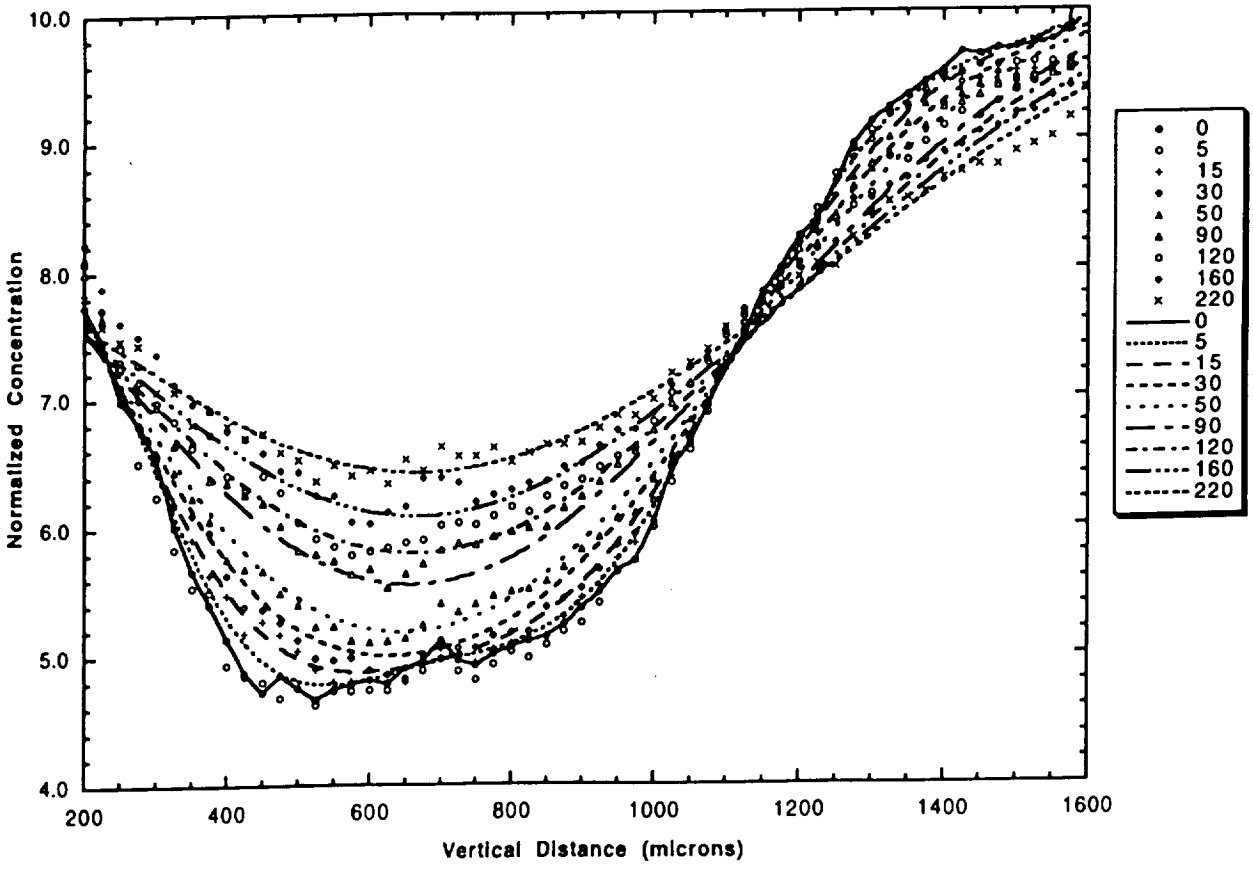

Figure 3. Fluorescein In PEG/Dextran/Water System

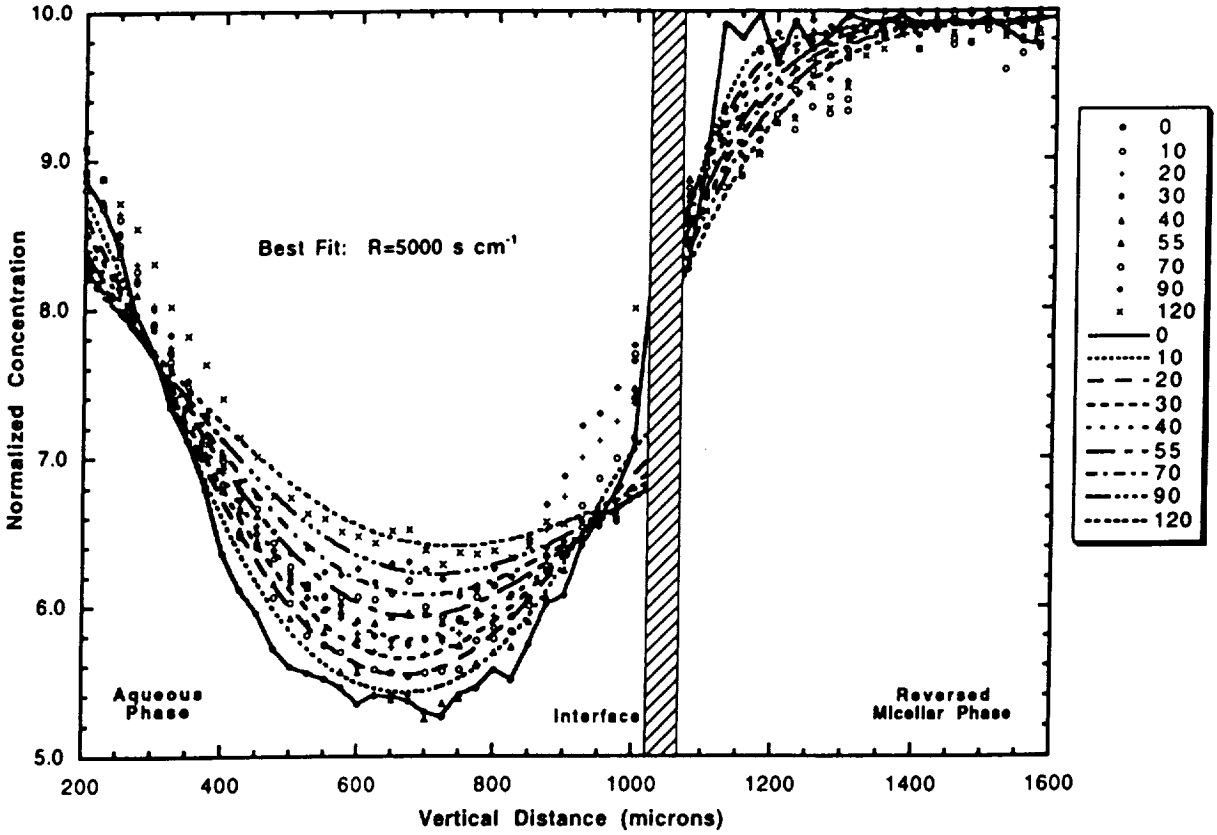

Figure 4. Water/AOT Reversed Micellar System

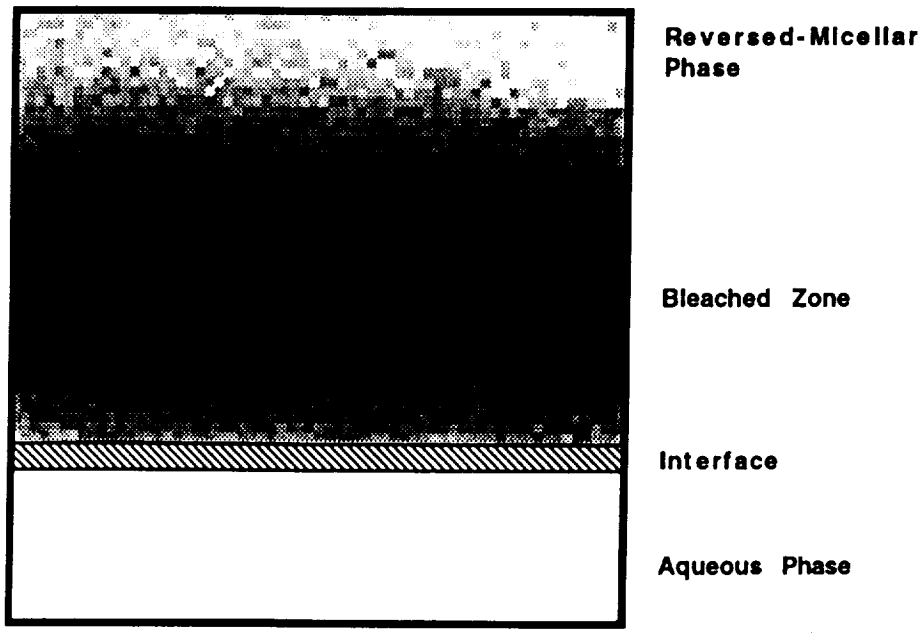

Figure 5. Reversed-Micellar Phase Photobleaching