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[9] Debonding of OTS molecules may be expected to lead to higher friction in affected monolayer regions, as the enhanced "liquid-like" character of debonded tails would give rise to an enhanced viscous drag opposing the motion of the tip.
[10] Attempts to remove from the surface monolayer molecules (following inscription of patterns in the non-destructive patterning regime) by solvent treatments with sonication and Scotch-tape peeling failed, while exposure to solutions of OTS or NTS invariably resulted in the rapid self-assembly of a stable overlayer in the tip-inscribed areas. Whether the siloxane bonds are or are not affected under such conditions cannot thus be conclusively decided at this stage, the patterned monolayer displaying remarkable stability in all subsequent self-assembly and chemical modification operations.
[11] a) R. Maoz, H. Cohen, J. Sagiv, *Langmuir* **1998**, *14*, 5988. b) C. A. Jones, M. C. Petty, G. G. Roberts, G. Davies, J. Yarwood, N. M. Ratcliffe, J. W. Barton, *Thin Solid Films* **1987**, *155*, 187.
[12] For clarity, the ocatadecylamine contributions to the -CH_2- and -CH_3 bands around 2900 cm^{-1} [11a] were omitted in Figure 3.
[13] Due to the hydrophobic and oleophobic character of the monolayer surface, the sample emerged completely dry and free of traces of bulk liquid contamination from both the acid and ocatadecylamine solutions.
[14] F. Forouzan, A. J. Bard, *J. Phys. Chem. B* **1997**, *101*, 10 876.
[15] H. Shiku, I. Uchida, T. Matsue, *Langmuir* **1997**, *13*, 7239.
[16] Because of the convolution with the tip, the measured lateral dimensions of the particles may be overestimated. AFM images (taken with a sharp tip) of CdS generated by the same process (1 × CdS deposition) on a laterally unrestricted TFSM surface reveal particles with heights between 0.5 and 1.5 nm and lateral dimensions between 3 and 8 nm [6c].

Printing via Photolithography on Micropartitioned Fluid Lipid Membranes**

By *Li A. Kung*, *Jay T. Groves*, *Nick Ulman*, and *Steven G. Boxer**

In a fluid medium there is continuous diffusive mixing among components. In order to retain spatial information such as a concentration gradient or an image in the absence of external forces, the fluid must be partitioned. For ordinary liquids in three dimensions this can be achieved by constructing containers or with small droplets. Lipid bilayer membranes on solid supports are two-dimensional fluids.[1] We have developed methods to partition these membranes into small regions by patterning the properties of the solid support through various methods. These include scratching,[2] blotting, or stamping lipids with poly(dimethylsilane) (PDMS) stamps,[3] stamping proteins via microcontact printing,[4] and using photolithography to create barriers of various compositions.[5,6]

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[**] The authors thank Gordon Kino for encouragement. This work was supported in part by grant N00014-91-J-1050 from the Joint Services Electronics Program (ONR/JSEP), by the NSF Biophysics Program, by the MRSEC Program of the NSF under Award DMR-9808677, and by a grant from SmithKline Beecham. Processing work was carried out at the Stanford Nanofabrication Facility, supported in part by an NSF sponsored Users Grant.

The last method is illustrated schematically in Figure 1; it is the most convenient for patterning small feature sizes over large areas. Although the details of the interface between the barrier and the fluid membrane are not fully understood, the partitions have the operational consequence that membrane-associated components are free to diffuse within each corralled region, but not between regions. This can be exploited to alter the composition of each partitioned corral by irradiation, either destroying[7] or photoactivating[8] a subset of molecules within each partitioned region. This spatially-directed irradiation of the partitioned membrane will be called secondary photolithography.

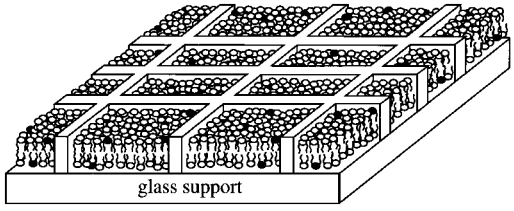

Fig. 1. Schematic illustration of a micropartitioned fluid lipid bilayer membrane. Patterns of differing surface properties on the solid support restrict the supported membrane, creating barriers to lateral diffusion. The solid support is typically glass and many different materials can be used to fabricate the barriers [15]. The lipid bilayer is separated from the glass substrate by a thin (~10 Å) layer of water which serves to lubricate the interaction and allow lateral mobility. The bilayer itself is approximately ~50 Å thick, while the barrier material is typically 10–1000 nm thick and 1–10 μm wide; the drawing is not to scale. This system serves to partition the bilayer so that its components are free to diffuse laterally within each corralled region, but not between them.

In the following, we demonstrate this effect by printing a four-level, grayscale image using a single exposure through a mask consisting of only clear and opaque regions. We take advantage of the fluidity of the substrate membrane to create true grayscale within each pixel on the surface. This contrasts with a dithered image,[9] where alternating white and black points or dots of primary colors are used to approximate gray or color when seen from far away. This approach is a prototype for more complex applications in printing or biotechnology.

Silica surfaces were patterned via standard photolithographic techniques with grids of chrome which were 2 μm wide and 1000 Å high. In the experiments reported here, the grids outline 10 μm squares on the silica surface, but they can be made smaller or larger depending on the application. Supported lipid bilayer membranes were assembled onto the exposed silica regions by vesicle fusion as described in detail elsewhere.[1] Such supported membranes are stable for weeks as long as they are kept under water. Lipid vesicles were prepared with egg phosphatidylcholine (egg PC) doped with a small percentage (0.4–2 %) of fluorescently labeled lipids (Texas Red-DHPE or NBD-PE, Molecular Probes, Eugene, Or.). These dyes are used both for visualization of the membrane by epifluorescence microscopy and for photobleaching by extended irradiation.

For the purpose of this demonstration, spatially-selective photobleaching is the secondary photolithographic process

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used to independently alter the membrane composition within individual corrals and print an image on the fluid membrane. This is achieved by projecting an image of a photomask onto the plane of a micropatterned supported membrane substrate. The photomask is inserted at the field stop of a Nikon E800 fluorescence microscope by using a specially-designed mount. The mask used to create the images is a commercially available photomask such as those commonly used for semiconductor processing applications.[10] It consists of a quartz plate coated with 1000 Å of chrome, patterned by electron beam lithography. The minimum feature size available on a mask of this type is less than 500 nm. A portrait of Abraham Lincoln[11] was cropped and reduced to a 23 pixel by 28 pixel 2-bit image (four levels of grayscale). Each pixel of the portrait was mapped to a pixel on the mask. The mask pixels have differently sized openings or apertures which allow 0 %, 17 %, 35 %, or 50 % of the illumination to pass through, corresponding to each of the four levels of grayscale. The pixel spacing on the mask is 64 μm, which, after a 6.4× reduction in the microscope, is in registry with the substrate partition length of 10 μm. A standard 100 W mercury arc lamp was the illumination source. A Photometrics Sensys cooled charge coupled device (CCD) camera was used to capture the resulting images.

The principle of this printing method is illustrated in Figure 2. Panel 2A is an epifluorescence image of nine 20 × 20 μm corrals; the chrome barriers appear dark (no fluorescence) while the supported bilayer membrane, which is contained within corrals formed by the barriers, fluoresces homogeneously. Panel 2B is an image of the same region taken during a 90 s exposure through a mask. A square region covering 50 % of the middle corral is illuminated. The contrast of this image has been enhanced such that the chrome barriers are made faintly visible through the mask by scattered light or transmission through the mask. Panel 2C is an epifluorescence image of the corralled regions 30 s after irradiation, demonstrating that photobleaching of the dye has occurred exclusively in the middle corral. Panel 2D is an epifluorescence image 10 min after irradiation, following complete mixing of the contents of the irradiated and unirradiated regions within the middle corral. The result is homogeneous fluorescence throughout the middle corral whose intensity reflects the fractional area of the corral that was irradiated in panel 2B and the time of exposure. This process can be executed in parallel on many partitioned regions to create images as described below.

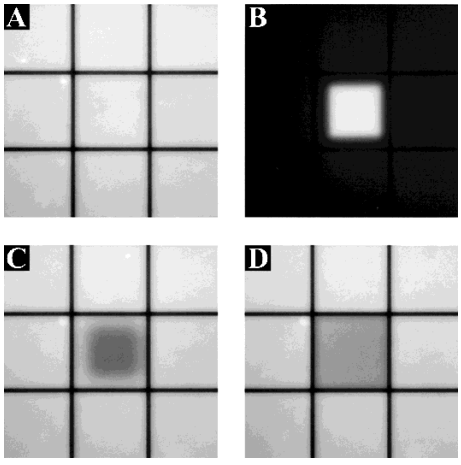

Fig. 2. An example of secondary photolithography on a single corralled region of a supported lipid bilayer. A) Epifluorescence image of nine 20 × 20 μm^2 corrals formed by chrome barriers on glass (seen as dark lines), each containing a fluid bilayer membrane with 2 % NBD labeled lipids (seen as homogeneous light fluorescence). B) Square region of light projected onto the middle corralled membrane. The illuminated box covers 50 % of the corral. C) Epifluorescence image of the corral 30 s after a 90 s exposure using the illumination pattern shown in B). D) The same region after 10 min; photobleached and unphotobleached molecules have fully mixed within the irradiated corral.

The fate of a pattern printed onto an unpartitioned fluid is illustrated in Figure 3. An image of the uniform fluorescence from the membrane prior to exposure is shown in panel 3A. The surface was then exposed for two minutes through a photomask containing the pixelated portrait of Abraham Lincoln. Panel 3B depicts the photobleached pattern on the membrane immediately after the exposure. At this point, some individual pixels from the illumination pattern are still visible, although diffusion within the membrane has already caused some mixing. Typical diffusion coefficients for supported membranes are 4.5 μm^2/s.[12] Images of the patterned membrane at progressively later times (4 min, 30 min, 250 min, and 1100 min) are displayed in panels 3C, 3D, 3E, and 3F. Lateral diffusion in the membrane continues, uninterrupted, until the entire region is thoroughly mixed and the pattern is gone.

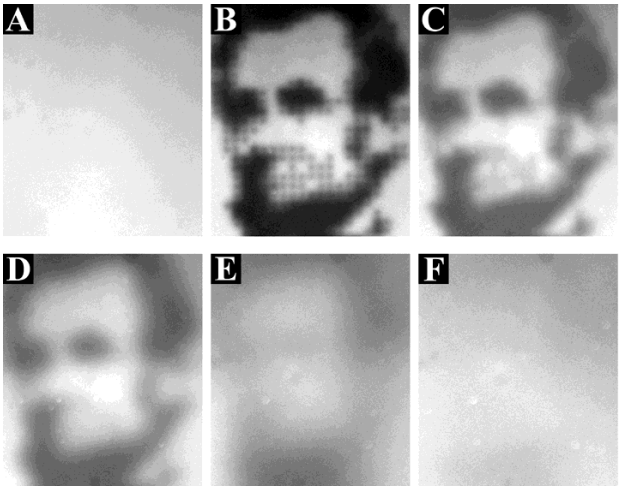

Fig. 3. A series of images demonstrating the dissolution of a pattern by diffusive mixing on a supported membrane that is not partitioned. A) Fluorescence from a region approximately 500 μm across of a supported membrane showing the homogeneity of fluorescence from a Texas Red-lipid probe doped in the membrane at 1 %. B) Image of Lincoln (see Fig. 4) printed onto the supported membrane by photobleaching of the dye fluorescence in small squares. C–F) Images over 4, 30, 250, and 1100 min, respectively, showing the loss of the pattern as diffusive mixing occurs between the photobleached and unphotobleached regions in the membrane.

A very different result is obtained when the illumination pattern from the same photomask is projected onto a parti-

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tioned fluid membrane surface, shown in Figure 4. Panel 4A is an image of the membrane before secondary photolithography, illustrating the original array of identical membrane corrals partitioned by grid patterns on the substrate. The photomask pattern is depicted in panel 4B. The mask is designed to project a precisely defined spot of light into each corral on the substrate. The relative dose of light delivered to an individual corral is determined by the fractional area exposed.

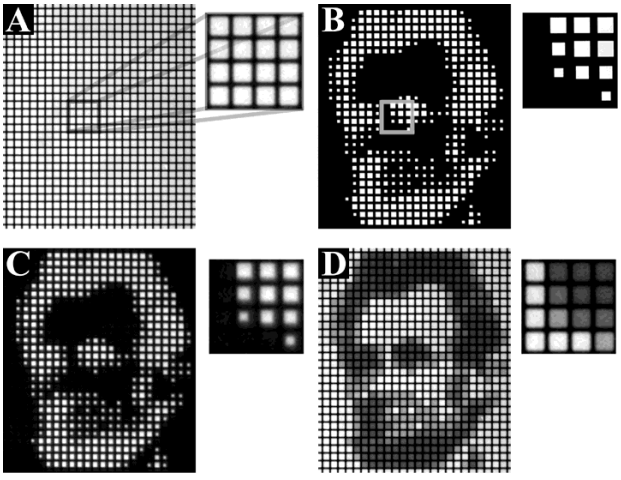

Fig. 4. A series of images depicting the secondary photolithography process. A) A lipid bilayer doped with NBD-labeled lipids before printing. B) A bright field image of the photomask shows four different size openings of 0 %, 17 %, 35 %, and 50 % of the pixel area. C) The photomask is placed in the field stop of the microscope, with its pixels aligned with those of the substrate. The aluminum oxide grids on the sample surface are faintly visible through the mask. The sample is exposed to light for 30 s, and the mask is then removed, creating a grayscale image (D) of Lincoln on the patterned membrane.

The partitioned membrane substrate and pixel pattern on the mask were aligned by manual rotation and translation of the substrate on the microscope stage after the mask has been placed into the microscope. The projected image of the photomask and the size of the substrate partitions agree to within 1 %, causing slight variations in quality across the field of view as the alignment between mask and substrate diverged. This crude procedure could be greatly improved by automation using a mask aligner.

Panel 4C is an image of the light that is projected onto the partitioned substrate through the photomask. The different areas exposed are visible in the enlarged sections shown on the right. The substrate was exposed for 30 s through this mask at a level that is well below saturation in order to retain grayscale contrast in the final printed image. Panel 4D is a fluorescence image from the partitioned membrane after the irradiation has been turned off and the image allowed to relax for 1 min. A true grayscale image is created in a single exposure from a mask that has only two tones, black and white. Diffusive mixing within partially exposed corrals leads to intermediate levels. This image remains indefinitely so long as the supported membrane is under water.

Each pixel on the printed surface is 10 μm on a side, corresponding to a printing resolution of approximately 2400 dots per inch. The grayscale resolution here, however, is much higher than that of a 2400 dpi printer that dithers to achieve a gray-scale effect. Furthermore, a photomask incorporating more gradations of opening sizes can easily be created to print higher bit-depth images. The resolution of the patterns printed in this demonstration was chosen for convenience (for example, 12 000 dpi can be achieved by switching to a standard 100× objective). Optical microscopic imaging systems often have near diffraction-limited resolution (<1 μm). Thus it is reasonable to assume that printing onto micropartitioned fluids could be extended to this resolution using the projection lithography configuration described here.

The largest area that can be patterned using our current microscope projection system corresponds to an image with approximately 10 000 10 μm^2 pixels. Figure 5 is a larger view of the substrate illustrating an array of patterned Lincolns, all produced in a single exposure. The variations among the Lincoln images arise from the slight mismatch in the alignment of the substrate and the photomask mentioned previously. With a dedicated mask alignment system, these irregularities can be greatly reduced, and the area to be printed can be increased to several square centimeters or more.

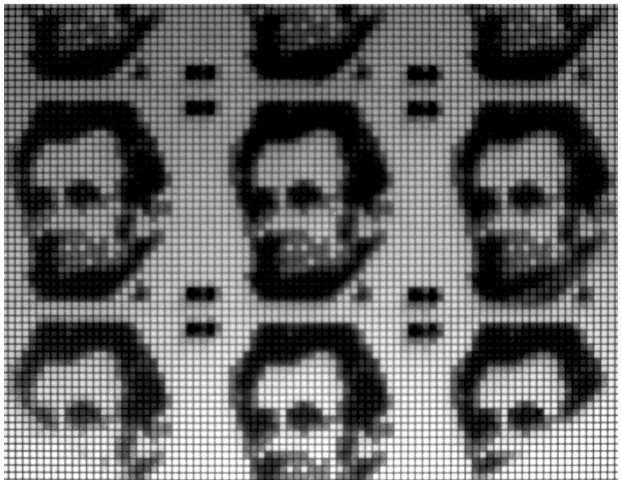

Fig. 5. A larger view of the substrate through a 10× objective. This image is approximately one half of the field of view through the objective (908 μm by 714 μm) and shows pieces of nine Lincoln faces.

The results depicted in Figures 2 and 4 demonstrate that it is possible to alter the composition of many regions of a partitioned membrane in parallel using secondary photolithography. The primary photolithography creates a patterned surface which allows diffusive mixing within each region for true grayscale, but retains the overall spatial information delivered by the secondary process. Both the probe (fluorescence) and image (Lincoln) were chosen for convenience; however, any spatially-directed photoactivation[8] or destruction and any useful pattern could be used. A mask is a readily-accessible method for encoding the image, and can be used with a microscope or a mask aligner. Alternatively, a spatial-light modulator could be used.[13]

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Applications of this technology include making arrays of cell surface mimics which can be used in parallel screening assays. Grayscale printing coupled with appropriate photochemistry makes it possible to vary the concentration of a molecular species embedded in, or attached to, the membrane. For example, photoactivatable lipid headgroups can be activated at variable levels, which may lead to attachment of varying quantities peptides, carbohydrates or nucleic acids to create different concentrations of these biologically-active molecules across the surface. Such arrays could be used to perform many concentration-dependence experiments in parallel. Membranes are the natural interface to cells, so parallel experiments on cell arrays may also be possible. The method is not limited to a single exposure, that is, an initial fraction of photoactivatable groups within a membrane corral could be activated and react, then another fraction of photoactivatable groups within the same membrane corral could exposed, using the same or a different mask, and allowed to react with a different molecule. Because diffusive mixing occurs within each fluid membrane pixel, this offers a method for preparing defined mixtures within each corralled region of the membrane. The advantages of photochemistry might also be realized via temporal modulation of light to the surface. Proteins or other biological molecules already residing or placed on the surface can be induced by photo-activating variable amounts of caged ligands or reactants in flash experiments.

We use a supported lipid bilayer membrane as the print medium both to demonstrate the concept and because it is an area of current research interest. Taking a broader perspective, a different substrate system, such as encapsulated electronic ink,[14] could be used in a fluid printing system to make high-resolution images on paper or other substrates. Color scale can be achieved by sequential exposures at different wavelengths that selectively activate or destroy colored pigments in the substrate.

Received: November 23, 1999
Final version: February 28, 2000

[1] A. A. Brian, H. M. McConnell, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 6159.
[2] P. S. Cremer, J. T. Groves, L. A. Kung, S. G. Boxer, *Langmuir* **1999**, *15*, 3893.
[3] J. S. Hovis, S. G. Boxer, *Langmuir* **2000**, *16*, 894.
[4] L. A. Kung, L. Kam, J. S. Hovis, S. G. Boxer, unpublished.
[5] J. T. Groves, N. Ulman, S. G. Boxer, *Science* **1997**, *275*, 651.
[6] N. Ulman, J. T. Groves, S. G. Boxer, *Adv. Mater.* **1997**, *9*, 1121.
[7] D. Axelrod, D. E. Koppel, J. Schlessinger, E. Elson, W. W. Webb, *Biophys. J.* **1976**, *16*, 1055.
[8] S. P. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991**, *251*, 767.
[9] R. W. Floyd, L. Steinberg, *Proc. SID* **1976**, *17*, 75.
[10] For example, from Align-Rite Corporation, Burbank, CA.
[11] Abraham Lincoln, three-quarter length portrait, standing, facing left; American Memory edition timeline No. 0873a, 1864, Library of Congress.
[12] J. T. Groves, S. G. Boxer, *Biophys. J.* **1995**, *69*, 1972.
[13] H. I. Fujikake, K. Takizawa, H. Kikuchi, T. Fujii, M. Kawakita, M. Yokozawa, *Opt. Rev.* **1996**, *3*, 89.
[14] J. Jacobson, B. Comiskey, C. Turner, J. Albert, P. Tsao, *IBM Syst. J.* **1997**, *36*, 457.
[15] J. T. Groves, N. Ulman, P. S. Cremer, S. G. Boxer, *Langmuir* **1998**, *14*, 3347.

Chemical Control over Ceramic Porosity Using Carboxylate–Alumoxane Nanoparticles**

By *Rhonda L. Callender* and *Andrew R. Barron**

A ceramic can be defined as a non-polymeric, non-metallic, inorganic solid.[1] The useful applications of a ceramic are determined by its composition, chemical and physical properties, lattice defects, particle morphology, and surface structure.[2,3] Control of these variables has traditionally been obtained through physical processing, i.e., variation of mechanical and/or thermal treatments.[4] One notable exception has been the synthesis of complex ceramic systems such as zeolites and meso-porous materials, where achieving the desired porosity has been accomplished almost exclusively through chemical control over the size and structure of the pores. In contrast, relatively little effort has been aimed at demonstrating chemical control over porosity for a simple bulk ceramic material such as aluminum oxide.

In general, the amount and type of porosity desired in a bulk ceramic depends on its intended application.[5] Porosity is a consideration in engineering ceramic applications[6] where the strength and toughness of ceramic matrix composites (CMCs) are largely affected by the porosity as well as the individual pore size, structure, volume, and location. In addition, the flexibility of porosities in combination with excellent mechanical strength and tolerance to solvents, pH, oxidants, and temperature extremes has led to ceramic-based membrane technologies taking an increasingly important role in pollution prevention, resource recovery, and waste treatment activities.[7] It would be desirable, therefore, to develop a chemical route (independent of physical processing) for controlling the porosity of bulk engineering ceramics. In this regard, we have investigated the chemical control over porosity of simple bulk ceramics by exploiting the flexibility of the organic periphery of a class of preceramic nanoparticles: carboxylate-alumoxanes.

Carboxylate-alumoxanes, $[Al(O)_x(OH)_y(O_2CR)_z]_n$, consist of an alumina core surrounded by covalently bound carboxylate ligands. Previous research in this laboratory has demonstrated that carboxylate-alumoxanes are readily prepared by the reaction of the mineral boehmite, $[Al(O)(OH)]_n$, with a carboxylic acid (HO_2CR), whereby the carboxylate anion "unzips" the boehmite structure, abstracts, and stabilizes a nanoparticulate fragment by replacing oxide and hydroxide groups with acid functionalities.[8,9] We have also demonstrated that metal-doped carboxylate-

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[**] This research was supported by the National Aeronautics and Space Administration (NASA) and the Environmental Protection Agency (EPA).

  0935-9648/00/1005-0734 $ 17.50+.50/0