Supplementary material (ESI) for Journal of Materials Chemistry

Supporting Information

Polymer Nanostructured Material for the Recording of Biometric Features

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Materials. Methyl methacrylate (MMA), n-butyl acrylate (BA), methacrylic acid (MAA), *tert*-dodecyl mercaptan (t-DDM), ethylene glycol dimethacrylate (EGDMA), and ammonium persulfate (APS) were purchased from Aldrich Canada and used as received. 2,2'-azobis(2-methyl-propionitrile) (AIBN) was purchased from Kodak and used as received. Fluorescent monomers, 9-anthryl methacrylate (An-MA), 4-amino-7-nitrobenzo-2-oxa-1,3-diazole-methacrylate (NBD-MA), and 1-hexansulfonic acid, diethyl-[5-(2-methyl-acryloylamino-benzo[a]phenoxazin-9-ylidene)]-ammonium salt (Nile Blue-MA) were synthesized using the procedure described elsewhere.[1,2]

The recipes and the experimental conditions used in each stage of synthesis of multidye multilayer particles are provided in Table 1.

Table 1: Recipe and experimental conditions used in synthesis of multilayer particles

		Stage 1 (An-labeled core)	**Stage 2 (NBD-labeled layer)**		**Stage 3 (Nile Blue-labeled layer)**		**Stage 4 (outmost shell)**
Charged to the reactor (V = 250 mL)	H_2O, g	70.0	55.0		55.0		50.0
	APS, g	0.2	--		--		--
	Latex dispersion, g	--	25.0 (from stage 1)		25 (from stage 2)		20 (from stage 3)
Monomer feeding mixture	MMA, g	20.0	10.0	1.0	10.0	1.0	3.0
	BA, g	--	--	--	--	--	6.5
	MAA, g	--	--	--	--	--	0.5
	EGDMA, g	0.60	0.30	0.03	0.30	0.03	0.1
	t-DDM, g	0.033	0.01	0.01	0.01	0.01	0.02
	AIBN, g	--	--	--	--	--	0.05
	An-MA, g	0.35	--	--	--	--	--
	NBD-MA, g	--	--	0.013	--	--	--
	Nile Blue-MA, g	--	--	--	--	0.06	--
Aqueous feeding solution	H_2O, g	--	10.0		10.0		20.0
	APS, g	--	0.10		0.10		0.10
	$NaHCO_3$, g	--	0.025		0.025		--
Temperature (^oC)	--	80	80		80		80
Feeding time (h)	--	2.5	1.5		1.5		5.0
Latex concentration (%)	--	19.8	13.1		12.0		12.5
Particle size (nm)	--	454	718		1082		1640

Stage 1: Synthesis of An-labeled cores. A combination of batch and semi-batch polymerization processes was used to obtain An-labeled particles. The initiator (APS) and the deionized water were introduced in a three-neck reactor equipped with a condenser, a mechanical stirrer, and a temperature-controlled water bath. The solution was heated to 80 ^{o}C under mechanical agitation (225 rpm) and a gentle flow of nitrogen. A small portion of the monomer mixture (MMA, EGDMA, t-DDM and An-MA) was added into the reactor. After 5 min, the rest of the monomer mixture was fed into the reactor. The feeding time was ca. 2.5 h. The reaction mixture was stirred at 80 ^{o}C for 1 h, cooled to room temperature and stored in the dark place.

Stage 2: Synthesis of the polymer layer labeled with NBD. The dispersion of An-labeled particles synthesized in Stage 1 and the deionized water were introduced in a three-neck flask equipped with a condenser, a mechanical stirrer and a temperature-controlled water bath. The mixture was heated to 80 ^{o}C under a nitrogen atmosphere under mechanical stirring at 225 rpm. An aqueous solution of APS, $NaHCO_3$ and H_2O, and a mixture of MMA, EGDMA and t-DDM were fed into the reactor using two separate feeding pumps. No dye-labeled comonomer, NBD-MA, was added in this step. In ca. 1.3 h the feeding of the monomers was complete. At this moment, the injection of the aqueous solution was interrupted, and the reaction mixture was stirred for 2 min. Then, a mixture of MMA, EGDMA, t-DDM, and NBD-MA was introduced in the reactor, and feeding of the aqueous solution was renewed. After the injection of the monomer mixture in the reactor was complete, the system was stirred at 80 ^{o}C for 1 h, then it was cooled to room temperature, and stored in the dark place.

Stage 3: Synthesis of the polymer layer labeled with Nile Blue. The procedure of Stage 2 was repeated to synthesize a polymer layer labeled with Nile Blue. Similar to Stage 2, a large portion of the monomer mixture free of the fluorescent comonomer was first fed into the reactor in order to synthesize a thick optically inert 'spacer' layer. Then a fluorescent comonomer, Nile Blue-MA was introduced in the reactor with the rest of the monomer mixture.

Stage 4: Synthesis of optically inert elastomeric shell. The dispersion from *Stage 3* and the deionized water were added to a three-neck flask. The mixture was heated to 80 ^{o}C under the flow of nitrogen and mechanical stirring at 225 rpm. An aqueous solution containing H_2O, APS, and a mixture of MMA, BA, MAA, EGDMA, t-DDM and AIBN were fed into the reactor via two separate pumps for 5 h. The dispersion was stirred at 80 ^{o}C for 1.5 h, then cooled to room temperature and stored in a brown bottle.

Particle Characterization. Particle synthesized in Stages 1-3 were examined using scanning electron microscopy (SEM, Hitachi S-5200 scanning electron microscope) at an accelerating voltage of 1.0 kV and a current of 10 mA. Particles dimensions after the last stage were characterized using photon-correlation spectroscopy (Zeta-sizer 3000, Malvern Instruments).

Film Preparation. Latex dispersion (1 wt %, 10 mL) was treated with 1 mL of 0.1 wt % aqueous solution of NaOH. The dispersion was centrifuged at 3400 rpm for 1 min, the supernatant was removed, and the sediment was redispersed in the deionized water. After the particles settled, the water was allowed to evaporate and the polymer film was formed at room temperature.

Characterization of Film Structure and Recording/Reading Experiments. An upright Zeiss LSM510 confocal microscope equipped with an argon laser (λ= 364 nm, maximum power 80mW), an argon-ion laser (λ=458 and λ=488 nm, maximum power 30 mW), and a helium-neon laser (λ= 633 nm (maximum power 5mW) was used to examine the structure of polymer films, to record patterns in the polymer films, and to read the recorded information. For the low resolution imaging, a dry lens (0.75NA) with a working distance of 0.66 mm was used, while for high resolution imaging we used an oil immersion lens (63x/1.4NA) with a working distance of 0.1 mm. The pinhole of each channel was adjusted for each dye so that the optical cross-section was about 1.0 μm. For anthracene, the writing and the reading processes were carried out using the 364 nm laser line. For the NBD fluorophore the destructive readout was reduced by recording at 458 nm and reading at 488 nm. For Nile Blue, both the writing and the reading processes were conducted using a 633 nm laser line.

Selective Dye Photobleaching. To avoid spectral cross-talk between the dyes and achieve their *selective* photobleaching, we conducted a line test by examining the photostability of each dye incorporated in the polymer material. We exposed the film to consecutive irradiation using 364 nm, 458 nm, and 633 nm laser lines and examined the photobleaching of each dye at different laser powers and a different number of scans. The results shown in Figure 1 were used to select the range of laser intensities in which a particular dye can be photobleached without a notable change in fluorescence of two other dyes.

The process of writing was conducted by moving the laser head along the straight line. Figure 1a shows a set of 40 identical straight lines that is organized in four columns

and ten rows. In each column the laser power increased from top to bottom, with an increment between the rows that was determined by the photostability of a particular dye. In each row (from left to right) the number of scans increased from 1 to 8 for the columns marked as 1-8, respectively.

Figure 1b shows a series of lines recorded using irradiation at λ=364 nm and imaged at 364 (I), 488 (II), and 633 nm (III). In Figure 1b (I), in the top three rows of all columns we observed no noticeable photobleaching of anthracene: the irradiance up to 4.8 mW (8 scans at 0.6 mW/scan) was not sufficient to photobleach this dye. The first indication of photobleaching of anthracene occurred in row 4 in column 1 (0.8 mW, 1 scan). In Figure 1b (II) and (III) no photobleaching of NBD and Nile Blue, respectively, occurred when the laser power ranged from 0.8 mW (1 scan) to 6.4 mW (8 scans at 0.8 mW/scan). At higher irradiances, notable dark marks appeared in the films imaged at 488 and 633 nm (Figure 1b (II) and 1b (III), respectively) suggesting non-selective photobleaching of the NBD and Nile Blue at λ=364 nm. In a similar manner, the recording procedure was repeated on a different location of the film using λ=458 nm. Figure 1c shows the lines imaged at 364 nm (I), 488 nm (II) and 633 nm (III). In Image I no traces of recording are notable, an indication that anthracene was not affected by the irradiation at λ=458 nm, regardless of the laser intensity used. As expected, in image Figure 1c(II) recording occurred by photobleaching NBD for laser intensity of 1.2 mW (1 scan). Figure 1c (III), irradiation of the films at λ=458 nm did not significantly affect Nile Blue, until the laser intensity reached 6.0 mW (4 scans) or 9.6 mW (8 scans). Similarly, Figure 1d shows the result of photobleaching by irradiating the polymer film at λ=633 nm. Images I and II show no traces of recording recorded at this wavelength by

photobleaching An and NBD, respectively. Recording by photobleaching of Nile Blue (Figure 1d(III), began at 2.4 mW (1 scan), 1.8 mW (2 scans), 1.2 mW (4 scans), or 0.5 mW (8 scans).

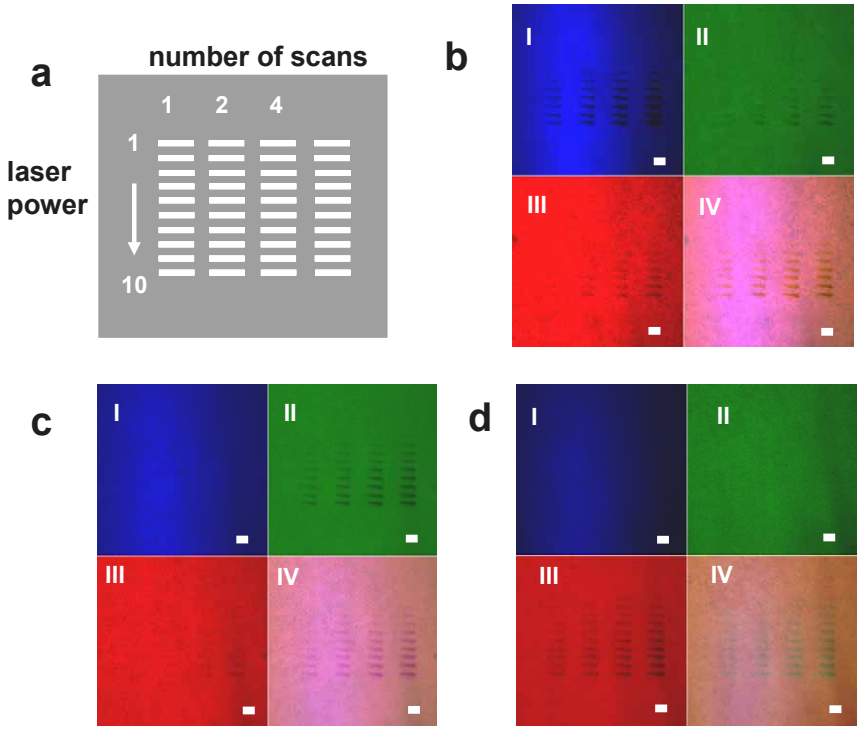

Figure 1. Examination of selective photobleaching of An, NBD and Nile Blue dyes by using a line test. (a) A 'mask' composed of 40 identical lines arranged in 4 columns and 10 rows. The horizontal rows from left to right represent the number of scans used for recording using the 'mask'. The number of scans is specified on the top of each column. In the vertical columns the laser power used for dye photobleaching increases from top to bottom with a predetermined increment characteristic for each dye. (b) A set of lines was recorded λ=364 nm. The images were accessed at 364 nm (1), 488 nm (2), and 633 nm (3). The laser power used to record the lines in column 1 was set at 0.2 mW in the top row, and it increased down the columns with an increment of 0.2 mW. (c) A set of lines was recorded at λ=458 nm and read at λ=364 nm (1), 488 nm (2), and 633 nm (3). The laser power used to record the lines in column 1 was set at 0.3 mW in the top row, and it increased down the column with an increment of 0.3 mW. (d) A set of lines was recorded using λ=633 nm. The images were read at λ=364 nm (1), λ=488 nm (2), and λ=633 nm (3). The laser power used to record the lines in column 1 was set at 0.5 mW in the top row 1 and it increased down the column with an increment of 0.5 mW.

Thus by selecting irradiance for the photobleaching of a particular dye, we avoided a notable change in fluorescence of two other dyes.

Recording of RGB and gray scale images

The recording process included splitting of the original image (Figure 2a) into blue, green and red component images using the "RGB Split" feature of an ImageJ software (National Institute of Health, U.S.A). These binary images (Figure 2b) contained black and white pixels. A white domain in each binary image was specified by the set of coordinates. For the recording, we wrote a macro that directed the laser beam of the confocal microscope to trace out the white pattern.[3] Figure 2c(I-III) shows the patterns recorded by photobleaching anthracene, NBD and Nile Blue in the coordinates specified by the white domains in Figure 2b (I-III), respectively.

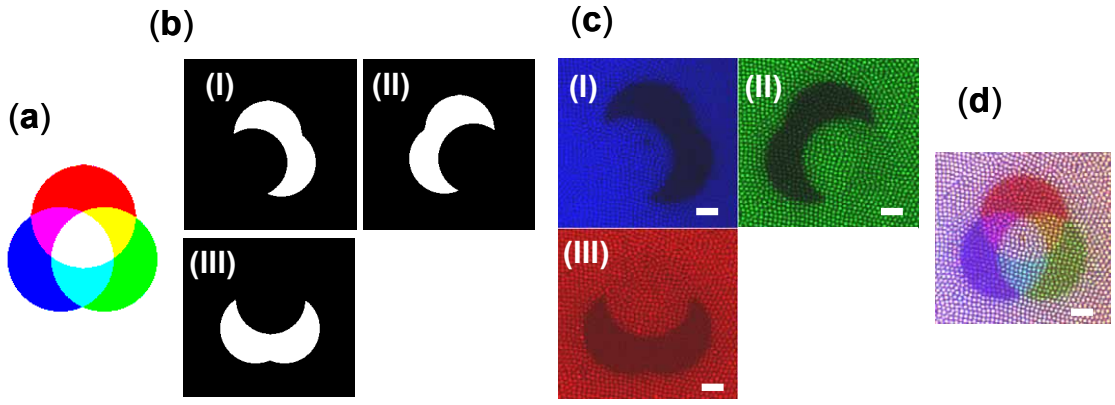

Figure 2. Recording of multicolor images in polymer composite films. (a) Original image; (b) Monochromatic splitting of the original image in three black and white images corresponding to blue (I), green (II) and red (III) colors. (c) Recording the white domains in polymer films at λ=364 nm and laser intensity 1.0 mW (4 scans) (I), at λ=458 nm and laser intensity 1.2 mW (4 scans) (II), and at λ=633 nm and laser intensity 4.0 mW (8 scans) (III); (d) Compiled RGB image. Scale bar is 10 μm.

To record full color photographs with different shades of grey, a 24-bit digital photograph was split into its red, green and blue component images. Each of the

component images had 256 grayscale levels, where the gray levels "0" and "255" represented completely black and completely white shades, respectively. By repeatedly using the threshold function, each component image was further split into 8 binary images, later in the text referred to as 'masks'. Each 'mask' represented a range of grey values from a component image. Figure 3a-c shows three exemplary 'masks' derived from the green component image by choosing gray level thresholds from 0 to 31, from 32 to 61 and from 62 to 91, respectively. By using a macro software[3] that directed the laser beam to trace out the pattern specified by the non-zero coordinates in the 'masks' we recorded a series of images recorded by photobleaching the NBD dye (Figure 3d-f). Figure 3d shows the image recorded by photobleaching the NBD dye in the areas of the material defined by the 'masks' shown in Figure 3a. The photobleaching process was repeated in the same location of the material with the other six binary 'masks' using progressively lower laser powers of 40%, 35%, 30%, 25%, 20%, and 10%, respectively.

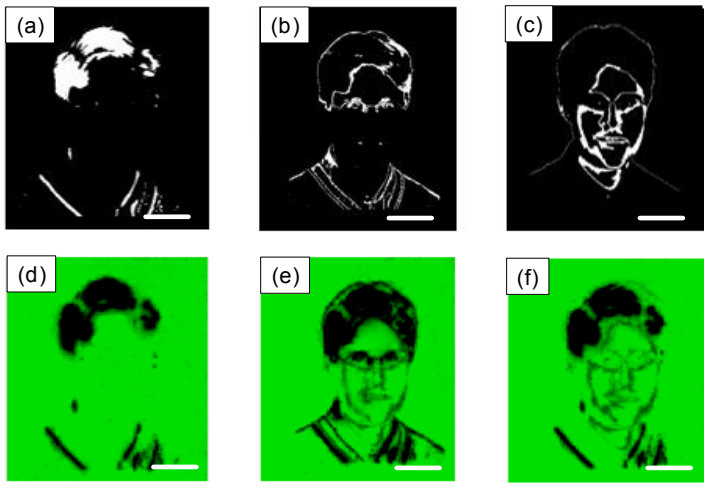

Figure 3. Exemplary binary images ('masks') obtained from the green component image by selecting gray scale thresholds from 0 to 31 (a); from 32 to 61 (b), and from 62 to 91 (c). (d-f): representative images recorded by using 'masks' (a-c), respectively. Photobleaching of NBD was conducted at λ=458 nm and accessed at λ=488 nm and a 500 to 550 nm bandpass filter. We used a 20x/0.75NA objective lens and four scans at an output Ar laser power 2.63 mW (d), 0.75 mW (e) and 1.875 mW (f). Scale bar is 50 μm.

The brightest pixels in the original green component image were not bleached. Figure 2e,f shows accumulated results of recording by using the 'masks' in Figure 2b,c, respectively.

References

1. Pham, H. H.; Gourevich, I.; Oh, J. K.; Jonkman, J. E. N.; Kumacheva, E. *Adv. Mater.* 2004, **16,** 516.
2. Gourevich, I.; Pham, H. H.; Jonkman, J. E. N.; Kumacheva, E. *Chem. Mater.* 2004, **16**, 1472.
3. We used the built-in Visual Basic for Applications (VBA) macro programming feature in the software provided for the Zeiss confocal laser-scanning microscope.