Canadian Journal of Chemistry**Fluorescence Anisotropy Imaging of a Polydiacetylene Photopolymer Film.**

Journal:	<i>Canadian Journal of Chemistry</i>
Manuscript ID	cjc-2018-0360.R1
Manuscript Type:	Article
Date Submitted by the Author:	02-Nov-2018
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Is the invited manuscript for consideration in a Special Issue?:	R Steer
Keyword:	

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Fluorescence Anisotropy Imaging of a Polydiacetylene Photopolymer Film.

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ABSTRACT

UV-illumination of phase-separated surfactant films prepared from mixtures of photopolymerizable 10, 12-Pentacosadiynoic acid and perfluorotetradecanoic acid results in the formation of fluorescent polydiacetylene fibers and aggregates. In this work, the orientation of polymer strands that comprise the resulting photopolymer structures has been probed using fluorescence anisotropy imaging in combination with defocused single-molecule fluorescence imaging. Imaging experiments indicate the presence of significant fiber-to-fiber heterogeneity, as well as anisotropy within each fiber (or aggregate), with both of these properties changing as a function of film preparation conditions. This anisotropy can be attributed to various alignments of the constituent polymer strands that comprise the larger fibers and aggregates. Intriguingly, when using defocused imaging, fiber images consisted of a series of discrete “doughnut” fluorescence emission patterns, which exhibited intermittent on-off blinking behavior; both of these properties are characteristic of individual emission transition dipoles (single-molecules). Further, all of the individual emission transition dipoles had a uniform orientation with respect to the axis of the fiber, indicating a common orientation of discrete emitters in the larger polymer fiber. The implications of these results for future studies of the electronic properties of conjugated polymers in larger macroscopic systems is noted.

KEYWORDS

fluorescence anisotropy imaging microscopy, 10,12-Pentacosadiynoic acid, polydiacetylene, Langmuir film, single-molecule spectroscopy

1. INTRODUCTION

Polydiacetylenes (PDAs) prepared from photopolymerizable, diacetylene-containing monomers are materials of considerable interest in the scientific literature because of the polymer's potential applications for colorimetric and fluorimetric sensing.¹⁻³ These applications are driven by the strong and rapid optical response of the PDAs, typically a transition from a blue, non-fluorescent form to a red, fluorescent form upon various forms of stimulus. Colour transitions can be driven by a variety of perturbations including optical, thermal and mechanical stress or by the binding of a target molecule of interest. The underlying spectroscopic and molecular-level structure changes that accompany the colour transitions for various PDA systems are well-studied and are reviewed in depth elsewhere.⁴⁻⁵

While PDAs can be prepared using a variety of methods and starting materials, a particularly intriguing method of preparation is through the photopolymerization of monomers that are also surfactant molecules. This provides an opportunity to control and pattern the photopolymer by using surfactant self-assembly processes, for example the formation of micelles, vesicles or Langmuir Blodgett deposition.⁶⁻⁸ One of the more well-studied photopolymerizable diacetylene surfactant monomers is 10, 12- Pentacosadiynoic acid (PCDA), which can undergo UV light-driven topochemical polymerization in vesicles or thin films. Polymerization takes place orthogonal to the PCDA alkyl backbone, resulting in a polymer with conjugation length that lies orthogonal to the underlying substrate upon which the film is deposited (Scheme 1). After initial photopolymerization of the PCDA monomer, further illumination or other forms of perturbation can be used to convert the blue photopolymer into a highly fluorescent, red-emitting photopolymer.

Our research investigating Langmuir Blodgett deposition for patterning PDAs has recently examined the use of phase-separation of immiscible surfactants to achieve this goal.^{6, 9-11} In the phase-separation approach, PCDA is mixed with a perfluorocarbon surfactant (typically perfluorotetradecanoic acid; $\text{CF}_3(\text{CF}_2)_{12}\text{COOH}$; PF) in an organic solvent and the mixture is transferred to the air-water interface in a Langmuir trough. The two surfactants are mutually immiscible and are phase-separated at the air-water interface; the general subject matter of patterning surfactants using this approach has recently been reviewed.¹² Carefully-selected monolayer preparation conditions (mixing ratio, surface pressure) results in the formation of well-defined domains of PCDA dispersed in a matrix of PF. In addition to inducing phase-separation, the PF also serves to mechanically rigidify and stabilize the films at the air-water interface, which

eliminates the need to add additional stabilizing counterions to the underlying subphase. These films can be transferred to a solid substrate and photopolymerized, or alternatively, polymerized directly on the Langmuir trough and then deposited, to yield the highly-fluorescent red PDA photopolymer.

Scheme 1: Illustration of the photopolymerization of 10, 12-Pentacosadiynoic acid monolayer film.

Key observations from our previous studies were that at moderately high fractions of PF (2: 1 mole fraction of PF: PCDA and above), discrete fiber-like domains of PCDA formed and these domains could be converted into red fluorescent photopolymer fibers with UV or visible (532 nm) illumination.⁹⁻¹⁰ The fluorescent fibers in the deposited films had a tendency to align parallel with the long axis of the Langmuir trough barriers, likely because of the mechanical pressure exerted on the phase-separated domains during film compression. Further, polarized fluorescence emission measurements suggested, indirectly, that the fibers consisted of bundles of polymers that adopted various orientations within the fibers. This is in contrast to more crystalline, highly-directional polydiacetylene films that have been reported elsewhere for single-component monolayer films.¹³⁻¹⁴ However, the detailed internal structure of these fibers could not be easily determined during these studies and the precise geometric arrangement of fluorophores in these systems remains unknown.

In the current work, we have re-visited this subject using an alternative fluorescence polarization imaging microscopy approach, Fluorescence Anisotropy Imaging (FAIM), which has enabled a more detailed qualitative investigation of the internal structure of the fibers prepared using the

phase-separation approach.¹⁵ The principle behind these measurements is that the emission transition dipole moment for a PCDA chain can be reasonably treated as lying in the plane of the solid substrate¹⁶⁻¹⁷ (i.e. the X-Y plane in the images below) and thus the intensity and anisotropy of the fluorescence images can be used to extract information about the relative density and orientation of emissive PCDA polymer chains. In the film samples, the PCDA polymer chains are fixed in position, so depolarization from molecular motion will be negligible.¹⁸⁻¹⁹ FAIM measurements show that the film preparation method yields highly heterogeneous PDA fibers, and that within a given film, a number of different fiber morphologies co-exist. Further, the fibers themselves are also highly heterogeneous and exhibit regions of highly-aligned polymer strands as well as regions of lesser alignment, in some cases within the same fiber. The overall structure of the fibers depends strongly upon the PF:PCDA mixing ratio of the film. Intriguingly, defocused fluorescence microscopy imaging experiments, which have previously been used for orientation mapping in single-molecule fluorescence imaging, have revealed that individual (single-molecule) polydiacetylene chains are detectable within the fibers. Results are discussed in context of affiliated systems discussed elsewhere in the literature, including other single-molecule measurements of PCDA reported in crystal matrices.²⁰⁻²¹

2. EXPERIMENTAL SECTION

2.1 Chemicals.

Perfluorotetradecanoic acid and 10,12 pentacosadiynoic acid were purchased from Sigma-Aldrich and were used without additional purification. Hexanes and tetrahydrofuran were received from EMD and Merck EM Science, respectively. Surfactant solutions of the individual components were prepared by dissolving the surfactant of choice in a 9:1 volume ratio mixture of hexanes: THF, and the two surfactant solutions were mixed in appropriate volumes to prepare solutions of the desired composition. Films were deposited onto microscope coverslips (VWR International) which had been cleaned by rinsing with absolute ethanol and subsequent plasma treatment in a commercial plasma cleaner (Harrick Plasma).

2.2 Langmuir-Blodgett film preparation.

Films were prepared using a commercial Langmuir trough system (KSV Instruments) equipped with a dipper for film deposition. Ultrapure water (Millipore, resistivity 18 M Ω ·cm) was used as a subphase, and the water surface was cleaned thoroughly by suction before all deposition experiments. A 100 μ L aliquot of the surfactant solution of interest was spotted onto the subphase surface and the solvent was allowed to evaporate for a minimum of 10 minutes before compression and deposition. Films were compressed at a rate of 10 mm·minute⁻¹ and were deposited onto the pre-cleaned glass coverslips at $\pi = 15$ mN·m⁻¹ using a single up-stroke. Films were allowed to dry at room temperature and were stored in the dark prior to photopolymerization and imaging. For photopolymerization, samples were illuminated with a low-pressure Hg pen lamp (strongest emission line at 254 nm; Edmund Optics) held ca. 10 cm above the coverslips for approximately 10 minutes. Samples treated this way formed fibers (and other aggregates) of strongly fluorescent polymer, as has been reported previously.^{6,9}

2.3 Fluorescence anisotropy imaging.

The FAIM microscope was based on a modified commercial inverted microscope (Nikon, TE2000) equipped with a high numerical aperture oil immersion microscope objective (Nikon, Apo 60x; NA1.45). The excitation source was a 532 nm CW diode laser that was coupled to the microscope through a polarization maintaining single mode optical fiber (ThorLabs). Fluorescence from the sample was collected with the same objective lens and passed through a polarization splitter (Cairn Research), which enabled simultaneous collection of two orthogonal emission polarization images (parallel, $I_{||}$, and perpendicular, $I_{\perp}$, relative to the excitation polarization) on the same EMCCD camera (Princeton Instruments, Photon MAX camera 1024). In this way, two images, corresponding to the two emission polarization conditions ($I_{||}$ and $I_{\perp}$) could be collected. A schematic illustration of the microscope and the relative orientation of the excitation and imaging polarizations is included in the Supporting Information (Figures S1, S2). For these experiments, excitation polarization was restricted to one orientation (parallel to the image frame) for simplicity. From the simultaneous intensity-based images, fluorescence anisotropy maps, showing the degree of anisotropy, r (Eq. 1), could be generated by image processing at a pixel-by-pixel level using a custom-written Matlab code. In Eq. 1, G is a correction

factor (0.98) to account for any polarization bias of the detection system and B is a background term, which is subtracted from each image using ImageJ.²²⁻²³

$$r = \frac{(I_{\parallel} - B) - G \cdot (I_{\perp} - B)}{(I_{\parallel} - B) + 2 \cdot G \cdot (I_{\perp} - B)} \quad (1)$$

For the defocused single-molecule imaging experiments, a wide field (epifluorescence) microscope was used. The wide field microscope consisted of an inverted optical microscope (IX71, Olympus) equipped with an air-cooled EMCCD camera (Photon Max 512, Princeton Instruments). The excitation source was a 473 nm continuous wave diode laser (Shanghai Laser and Optics Century Co.). The beam's intensity was adjusted using a variable neutral density filter (Thorlabs), before passing it through a half wave plate (Thorlabs) and into a beam expander to give a beam diameter of approximately 2.5 cm. Koehler illumination was achieved by focusing the expanded excitation beam through a lens ($f = 50$ cm) onto the back focal plane of the objective lens (PFO 100x; NA1.3 fluorite oil objective, Olympus). This provided an illumination area of approximately 1 mm in diameter, with an illumination intensity of less than 10 kW/cm². Defocusing was achieved by manually moving the microscope objective away from the sample by ~ 1 micron. The emitted light was collected through the objective, separated from the excitation light by a dichroic mirror (Chroma Technology) and a further long pass filter (Chroma Technology) then passed through a $3.3 \times$ beam expander (Olympus) onto the CCD camera.

3. RESULTS AND DISCUSSION

Mixed monolayer samples of PF:PCDA were prepared as described above, photopolymerized with a UV pen lamp and then imaged using the FAIM microscope. For all films, fluorescence images revealed a heterogeneous collection of fibers dispersed on a dark background (attributed to the non-fluorescent PF) and the fibers emitted a strong red fluorescence upon excitation with green (532 nm) CW laser light, as reported previously.^{6,9} While fibers of a variety of sizes and morphologies could be found in every sample, their length generally decreased as the content of PCDA in the film decreased (*vide infra*). Films with relatively high PCDA content (4PF:1PCDA) contained a significant number of fibers that were > 50 μm in length and spanned the microscope field of view. A typical pair of these fibers are shown in Figure 1; for this image, we have deliberately selected a pair of fibers that are reasonably perpendicular to the polarization axis of

the excitation laser in order to minimize excitation photoselection. In this image, a fixed s excitation polarization was used and two different emission polarizations were collected simultaneously.

Figure 1: Fluorescence anisotropy images collected from phase-separated 4PF:1PCDA photopolymer fibers on glass substrate ($\lambda_{\text{exc}} = 532 \text{ nm}$). Emission polarization ($I_{\perp}$ or $I_{\parallel}$) is indicated in the inset, and different regions of the fiber are labeled (A, B, C) as described in the main body text.

As seen in Figure 1, fluorescence emission from the fibers was both highly heterogeneous and anisotropic. One of the fibers showed negligible overall fluorescence in the p -polarized emission channel whereas the other fiber showed a strong overall fluorescence emission in the p -polarized channel. This indicates a large fiber-to-fiber variation in alignment of the constituent polymer emission dipole moments. At the level of the individual fiber, distinct heterogeneities are observed in emission, and we now describe the emission from the left-most fiber in Figure 1 in more depth, noting that other fibers shared many of these features. The fiber of interest exhibited linear segments of reasonably uniform fluorescence intensity (label “A”; most clear in the $I_{\parallel}$ image),

alongside circular spots (label “B”) of strong fluorescence intensity which were spaced at seemingly random intervals along the length of the fiber, and finally segments of very weak fluorescence (label “C”). Segments of uniform intensity can be assigned to lengths of the fiber where there is uniform alignment of polymer transition dipoles running perpendicular to the long axis of the fiber. The highly-fluorescent spots were typically larger in diameter than the straight segments of the fiber, which suggest that these regions were bulges or kinks in the fiber where the local concentration of fluorescent polymer was comparatively large. While the fiber segments labeled “C” may appear to be non-fluorescent, careful inspection of the anisotropy map reveals that there is still significant fluorescence from these regions. Intriguingly, the angle that these segments of the fibers makes to the incident polarization is not significantly different from the rest of the fiber. This result suggests that the polymer transition dipoles in these regions have a much different orientation than other parts of the fiber indicating that there are very different alignments of the polymer transition dipole moments in different regions of the fibers. We note that the values for emission anisotropy (r) were ~ 0.12 for the linear segments (“C”) and ~ 0.20 for the spots, indicating a relatively modest preferred orientation of emission dipoles in the systems.

In some fibers, we observed segments where there was effectively no fluorescence intensity in either emission channel and hence no appreciable signal detected in the anisotropy map. Figure 2 shows a fiber where this effect can be seen. For this fiber, the segment between labels B and C showed effectively no fluorescence signal. While it is possible that this is the result of excitation photoselection, there is emission observed from different segments of the fiber with the same spatial alignment relative to the excitation beam, so this is exceedingly unlikely. We have previously reported that the conversion of the PDA into its red fluorescent form in these films is only partial, and that under the treatment conditions used here there is typically $\sim 20\%$ of the total photopolymer remaining in its blue, non-fluorescent form¹¹; these results are consistent with those reported by Spagnoli et al.²⁴ for affiliated systems. It is likely that in these films, the non-fluorescent fiber segments are simply just regions of the fiber that are comprised of non-fluorescent PDA.

Figure 2: Fluorescence anisotropy images collected from phase-separated 4PF:1PCDA photopolymer fibers on glass substrate ($\lambda_{\text{exc}} = 532 \text{ nm}$). Emission polarization is indicated in the inset ($I_{\perp}$ or $I_{\parallel}$), and different regions of the fiber are labeled as described in the main body text.

The overall morphology and fluorescence anisotropy of the fibers changed as a function of the composition of the mixed films. As the fraction of PCDA in the films decreased, extended fibers became increasingly rare and shorter (tens of microns) fibers were typically observed, as well as less well-structured aggregates of fluorescent material. A representative image of a films with 5PF:1PCDA composition is shown in Figure 3. Like their longer counterparts, the short fibers and aggregates were also heterogeneous in terms of their shape, size and fluorescence anisotropy. There was a strong tendency for the short fibers to also be wider and more irregular in shape than the long, linear fibers. While there were still fibers that had r values similar to those described above ($r \sim 0.12$), there were numerous short, thick fibers and aggregates that had anisotropies that were markedly larger ($r \sim 0.35$ and above), indicating a stronger tendency to form aligned polymer transition dipoles in the shorter aggregates. We note that the peak anisotropy values for the shorter

fibers and aggregates are similar to those we have reported previously using a different measurement and polymerization approach⁹; for fibers prepared using laser-based polymerization, we reported an average fiber anisotropy of $r = 0.32 \pm 0.03$ (reported as a polarization value P ; values related through $r = 2P / (3-P)$). This result indicates there is a much greater tendency for the polymer transition dipoles to align in the shorter fibers and aggregates in comparison with the extended fibers. At present, the underlying explanation for this effect remains unclear. Several groups have observed that the photopolymerization process can induce film buckling in these systems and affiliated photopolymerizable film systems (see ²⁵⁻²⁶ for example). In buckling, internal mechanical stress within a 2D film causes the rigid polymer backbone to mechanically lift off from the underlying substrate surface to relieve this stress. We posit that that internal stress in the short, wide fibers and aggregates is substantially greater than the long, linear fibers, which leads to buckling and in turn, the mechanical stress of buckling forces stretch the PDA polymer fibers into alignment. However, this remains conjecture and more detailed experiments, possibly time-resolved monitoring of film structures as a function of illumination time, might prove to be useful here.

Figure 3: Fluorescence anisotropy images collected from phase-separated 5PF:1PCDA photopolymer films on glass substrate ($\lambda_{\text{exc}} = 532$ nm). Emission polarization is indicated in the inset ($I_{\perp}$ or $I_{\parallel}$).

Films with the lowest mole fractions of PCDA contained amorphous fluorescent polymer aggregates of various sizes in addition to the fibrous structures. Aggregates of a variety of sizes and shapes were dispersed throughout the film but a fraction of the aggregates had sizes that approached the diffraction-limited resolution of the optical microscope. These discrete photopolymer aggregates were measured in more detail using defocused imaging, a procedure in which the fluorescent objects of interest are moved slightly out of focus and, in the case of discrete emission transition dipoles (single molecules) the resulting images can be used to determine the emitter's three-dimensional spatial orientation.²⁷⁻²⁸ The operating principle of these measurements is that out-of-focus molecules with their emission transition dipole moment aligned with the optical axis of the microscope (z-oriented; out of plane) generate a characteristic "doughnut" shaped emission pattern, and any deviations from this emission pattern can be used to back-calculate the original spatial orientation of the emission transition dipole. This approach has been successfully applied in a number of single-molecule fluorescence imaging experiments, including measuring spatial orientation of chromophores in dendrimers, observation of rotational dynamics in polymer matrices, energy migration in multichromophoric systems and others.²⁸⁻³² As an aside, we note that the simultaneous use of fluorescence anisotropy imaging in conjunction with defocused imaging is somewhat unusual in the literature. Moerner et al.³³⁻³⁴ have demonstrated some variations of this approach for single-molecule imaging, using optical Fourier processing (referred to as 4f optical processing), and the subject is reviewed in the affiliated references. Splitting the fluorescence emission into two parallel channels in a defocused imaging experiment adds considerable complexity to both the mathematical modeling and fitting of the resulting emission pattern; this is beyond the scope of the present work and thus we will restrict discussion to a qualitative analysis of the data only.

Figure 4: Defocused fluorescence anisotropy intensity images collected from phase-separated 5PF:1PCDA photopolymer films on glass substrate ($\lambda_{\text{exc}} = 532 \text{ nm}$). Emission polarization is indicated in the inset ($I_{\perp}$ or $I_{\parallel}$).

A typical image of a 5PF:1PCDA film captured using the defocused imaging approach is shown in Figure 4. Several different types of emission patterns for aggregates were observed in the defocused experiments, with the most common emission patterns highlighted in Figure 4 A-C. The emission patterns of the discrete aggregates were complex but, intriguingly, exhibited patterns that are consistent with expectations for individual emission transition dipoles that have been split into dual channels (e.g. quadrupoles in Figure 4 labelled A, B) and have an assortment of orientations with respect to the microscope focal plane. Nonetheless, these measurements suggest that individual molecules of red PDA might be directly visualized in these films. We note that there is precedence for this in the literature; fluorescence from single red chains of PDA have been documented by Schott et al.²⁰⁻²¹, who reported detection of single chains diluted in single crystals of their monomer measured at temperatures ranging from cryogenic to room temperature. Those authors reported that red chains had high fluorescence quantum yields (~ 0.30 at 15 K) in the zero-

phonon line. More recently, the spatial coherence of excitons has been investigated in isolated PDA chains at cryogenic temperatures using a novel interference pattern approach.³⁵

Figure 5: Zoom-in of defocused fluorescence anisotropy emission patterns observed for fluorescent structures measured in phase-separated 5PF:1PCDA photopolymer films on glass substrate ($\lambda_{\text{exc}} = 532 \text{ nm}$).

To verify that we achieved single-molecule detection in this system, we further explored the phase-separated film samples using defocused fluorescence imaging without a polarizing beam splitter. Surprisingly, we have observed a number of short fluorescent aggregates of PDA that retain their fiber-like structure but have emission properties that are consistent with a series of closely-packed single polymer molecules. Representative defocused images are shown in Figure 6, along with a fluorescence intensity time trajectory (integrated fluorescence intensity of a single spot as a function of time) for an in-focus spot. A time-lapse “movie” of the structures’ emission, for both focused and defocused samples, is included as supplementary information.

Figure 6: (A), (B) Defocused images of short fibers measured in phase-separated 5PF:1PCDA films on glass substrate ($\lambda_{\text{exc}} = 473 \text{ nm}$). (C) Fluorescence time trajectory of an in-focus spot isolated from one of the short fibers.

Fluorescence images of the fiber-like structures consisted of a series of characteristic “doughnut”-like emission patterns that were in close proximity to each other and ran along the length of the structure. Further, the individual spots exhibited intermittent, on-off fluorescence (“blinking”) on the time scale of seconds, which provides strong evidence that emission is from individual polymer transition dipoles. To more clearly illustrate the on-off blinking effect, which is challenging to do for the defocused images because of difficulty in selecting spatially resolved spots in the fibers, we show a fluorescence time trajectory for a single spot taken when the microscope was focused (Figure 6 C). In addition to the on-off blinking, time trajectories also showed a slowly-decreasing background, indicating that a weakly fluorescent background emission (possibly from residual blue polymer) was slowly photobleaching during these experiments.

The “doughnut” emission pattern observed for the defocused individual emitters most closely resemble those for molecules with a z-oriented emission dipole²⁸, but the pattern was not precisely the same; the emission patterns measured here are missing a dark spot in the middle of the bright central maximum. At present, we cannot explain the source of this difference and also cannot directly definitively determine the fluorophore orientation from the emission pattern. We speculate that these are z-oriented dipoles and that the dark central spot might simply be too difficult to detect against the bright central maximum, particularly given the high background

fluorescence of the sample. Further, optical imaging aberrations caused by the finite thickness of the polymer aggregate might also contribute to distortions in the measured emission pattern. These are effects which will be explored in greater detail in future studies.

Inspection of the time-lapse videos for both the focused and defocused images (supplementary information) revealed no apparent synchronization in blinking events for the different dipoles that ran along the length of the fiber, suggesting that there was no excitonic energy coupling of dipoles within these aggregates. Indeed, the dipoles appeared, for all intents and purposes, to behave like discrete individual emitters despite being incorporated into the same photopolymer aggregate. Intriguingly, for all of the fiber-like structures that exhibited the single-molecule behavior, all of the defocused patterns had the same characteristic spatial distribution of intensity. This indicates that all of the polymer transition dipoles had exactly the same orientation along the length of the polymer. Different orientations, for example the transition dipoles lying in-plane, give different (typically bi-lobed) emission patterns. This suggests that these regions of the fibers are highly-ordered at a molecular level, despite the tendency of these shorter aggregates to appear less uniform than the extended polymer fibers. While many of these observations are entirely qualitative and remain open to interpretation, it is clear that the system holds enormous potential for carrying out single-molecule investigations of electronic properties of conjugated polymer molecule systems in complex, multi-molecular aggregates. This is a topic of enormous interest in the current literature and details of the system, such as polymer structure-energy transfer dynamics, will be explored in depth in future single-molecule spectroscopy investigations.

4. CONCLUSIONS

A qualitative investigation of the orientation of PDA photopolymers prepared in phase-separated films using a wide field FAIM microscope has been performed. The general observation was that photopolymers fibers and aggregates formed in these samples were highly heterogeneous in shape and size, with these properties depending strongly upon the composition of the surfactant mixture used to prepare the films. There was also significant heterogeneity of overall emission dipole orientation within each fiber or aggregate, with shorter aggregates tending to show a higher degree of anisotropy in comparison with long, extended fibers. Even with the longer fibers, however, there was a strong degree of heterogeneity consistent with regions of the fibers that contained highly-

aligned PDA chains and other regions with less-ordered alignment. We have also detected individual PDA polymer molecules in these systems using defocused fluorescence imaging. In addition to discrete PDA molecules, we observed a number of shorter fibers in which emission consisted of a series of discrete individual molecules (emission transition dipoles) that ran along the length of the fiber, all with the same spatial orientation. This preliminary result indicates the enormous potential of this system for the investigation of exciton dynamics and other energy transfer processes in conjugated molecule systems using of single-molecule fluorescence methods.

SUPPORTING INFORMATION AVAILABLE:

Schematic illustration of the FAIM microscope; Illustration of the polarization directions used in these experiments; Focused and defocused single-molecule fluorescence “movies” of aggregates in phase-separated 5PF:1PCDA films on glass substrate ($\lambda_{\text{exc}} = 532 \text{ nm}$).

ACKNOWLEDGMENTS

Financial support for this work has been provided by the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Innovation and the University of Saskatchewan. Support from the Australian Research Council Centre of Excellence in Exciton Science (CE170100026) is gratefully acknowledged. All of the authors would like to thank Ronald P. Steer for many years of excellent science, fruitful collaborations and, of course, friendship. Best wishes for a happy (and research active) retirement, and we hope to share our scientific adventures with you for many years to come.

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CAPTIONS

Scheme 1: Illustration of the photopolymerization of 10, 12- Pentacosadiynoic acid monolayer film.

Figure 1: Fluorescence anisotropy images collected from phase-separated 4PF:1PCDA photopolymer fibers on glass substrate ($\lambda_{\text{exc}} = 532$ nm). Emission polarization ($I_{\perp}$ or $I_{\parallel}$) is indicated in the inset, and different regions of the fiber are labeled (A, B, C) as described in the main body text.

Figure 2: Fluorescence anisotropy images collected from phase-separated 4PF:1PCDA photopolymer fibers on glass substrate ($\lambda_{\text{exc}} = 532$ nm). Emission polarization is indicated in the inset ($I_{\perp}$ or $I_{\parallel}$), and different regions of the fiber are labeled as described in the main body text.

Figure 3: Fluorescence anisotropy images collected from phase-separated 5PF:1PCDA photopolymer films on glass substrate ($\lambda_{\text{exc}} = 532$ nm). Emission polarization is indicated in the inset ($I_{\perp}$ or $I_{\parallel}$).

Figure 4: Defocused fluorescence anisotropy intensity images collected from phase-separated 5PF:1PCDA photopolymer films on glass substrate ($\lambda_{\text{exc}} = 532$ nm). Emission polarization is indicated in the inset ($I_{\perp}$ or $I_{\parallel}$).

Figure 5: Zoom-in of defocused fluorescence anisotropy emission patterns observed for fluorescent structures measured in phase-separated 5PF:1PCDA photopolymer films on glass substrate ($\lambda_{\text{exc}} = 532$ nm).

Figure 6: (A), (B) Defocused images of short fibers measured in phase-separated 5PF:1PCDA films on glass substrate ($\lambda_{\text{exc}} = 473$ nm). (C) Fluorescence time trajectory of an in-focus spot isolated from one of the short fibers.

Figure S1: Schematic illustration of the FAIM microscope.

Figure S2: Schematic illustration of relative orientation of excitation and emission polarization in these measurements. All samples are excited with horizontal (s) polarized light relative to the image frame. In terms of emission, $I_{\parallel}$ is the fluorescence emission parallel to the incident excitation polarization and $I_{\perp}$ is the fluorescence emission perpendicular to the incident excitation.

FIGURES

Scheme 1: Illustration of the photopolymerization of 10, 12-Pentacosadiynoic acid monolayer film.

Figure 1: Fluorescence anisotropy images collected from phase-separated 4PF:1PCDA photopolymer fibers on glass substrate ($\lambda_{\text{exc}} = 532 \text{ nm}$). Emission polarization ($I_{\perp}$ or $I_{\parallel}$) is indicated in the inset, and different regions of the fiber are labeled (A, B, C) as described in the main body text.

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SUPPORTING INFORMATION

Figure S1: Schematic illustration of the FAIM microscope.

Figure S2: Schematic illustration of relative orientation of excitation and emission polarization in these measurements. All samples are excited with horizontal (s) polarized light relative to the image frame. In terms of emission, $I_{||}$ is the fluorescence emission parallel to the incident excitation polarization and $I_{\perp}$ is the fluorescence emission perpendicular to the incident excitation.

The figure displays a chemical structure of a polyunsaturated fatty acid derivative on the left and its corresponding fluorescence microscopy image on the right. The chemical structure shows a long, zigzag hydrocarbon chain with four conjugated double bonds (indicated by three vertical lines) and four terminal carboxylic acid groups (HO-C(=O)-). A red arrow points from the chemical structure to the fluorescence image. The fluorescence image shows a bright, elongated, and slightly curved signal against a dark background, representing the emission from the molecule.