Dual-Enhanced Fluorescent Biosensors Using Metal-Coated Piezoelectric Nanoimprinted Substrates

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Contents

Electrochemical Determination of Substrate Surface Area 2
UV-Vis Absorbance of Substrate Components 3
Temporal Profile of the Piezoelectric Fluorescence Enhancement 4
The Averaging of Fluorescence Spectra 5
Determination of β-phase Content and Reflectance of the Nanoimprinted Substrate 6
Substrate Reusability 7
Piezoelectric Response of Substrates 8
Fluorescence Spectra at Different Concentrations 9

Electrochemical Determination of Substrate Surface Area

Cyclic voltammetry in 1 mM ruthenium(III) hexamine was used to determine the substrate surface area. (Figure S1) The scan-rate-dependent peak heights were used in the Randles-Sevcik equation to determine the substrate surface area:

$$i_p = 0.4463 n^{\frac{3}{2}} F^{\frac{3}{2}} A \frac{D^{\frac{1}{2}} c v^{\frac{1}{2}}}{(RT)^{\frac{1}{2}}}$$

where *n* is the number of electrons transferred, *F* is the Faraday constant, *A* is the electroactive area, *D* is the diffusion coefficient, *c* is the concentration of electroactive species, *v* is the scan rate, *R* is the gas constant, and *T* is the temperature.

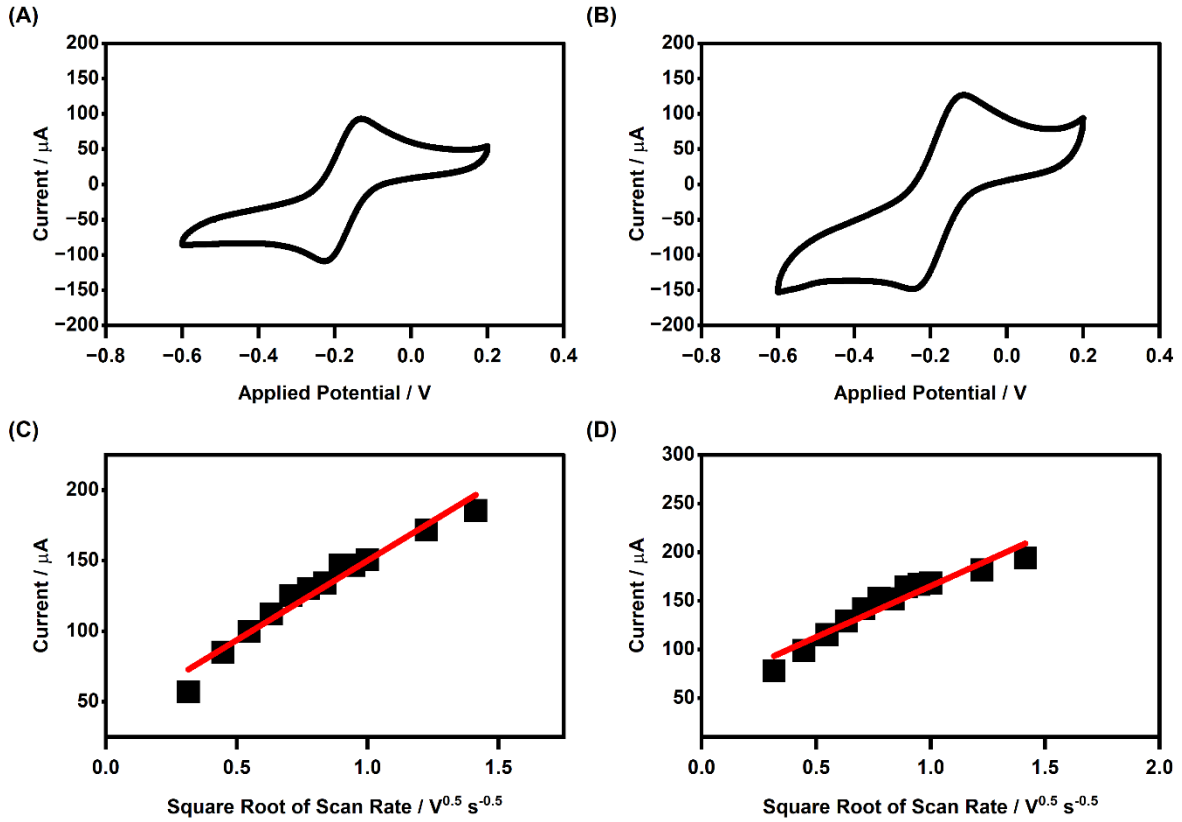

Figure S1 shows sample cyclic voltammograms for the (A) flat and (B) nanoimprinted, and scan rate-dependent peak height for the (C) flat and (D) nanoimprinted substrates.

UV-Vis Absorbance of Substrate Components

Figure S2 shows that PVDF is transparent to visible light, while Cy3 absorbs light with an absorbance peak at around 550 nm, corresponding to the fluorescence excitation laser wavelength of 532 nm.

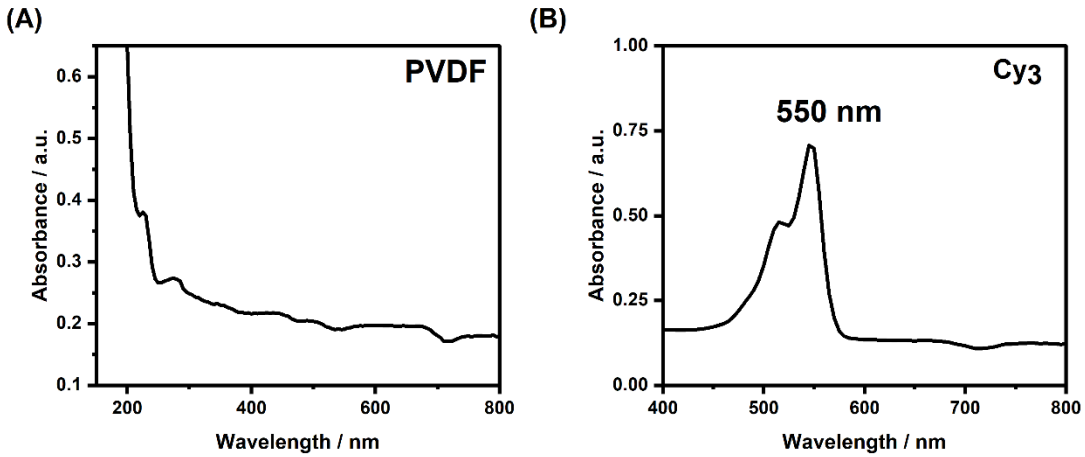

Figure S2 (A) presents the UV–Vis spectrum of a PVDF film, showing its strong absorbance below 300 nm and its transparency to visible light. The (B) UV-vis spectrum of a Cy3 solution shows its characteristic absorption at ~550 nm, which coincides with the fluorescence excitation wavelength of 532 nm used in this manuscript.

Temporal Profile of the Piezoelectric Fluorescence Enhancement

To investigate the temporal evolution of the piezoelectric fluorescence enhancement, time-dependent fluorescence measurements were performed following weight application (Figure S3). Upon applying the 5 g load, the fluorescence intensity increases rapidly, reaching a maximum within ~5 s before gradually decreasing toward the unpressed fluorescence intensity. This shows that the dominant enhancement occurs immediately after loading and persists throughout the fluorescence acquisition window used in this study.

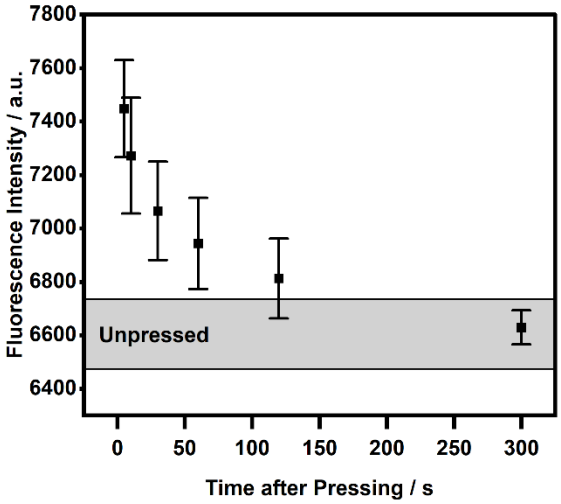

Figure S3 shows the decay of fluorescence enhancement following the application of a 5 g weight at 0 s. The fluorescence intensity observed prior to the application of the weight is shown with the grey band. Error bars are obtained from at least 10 unique spots on each sample.

The Averaging of Fluorescence Spectra

Figure S4 shows the variance in fluorescence spectra across different measurement spots in both the flat and nanoimprinted substrate.

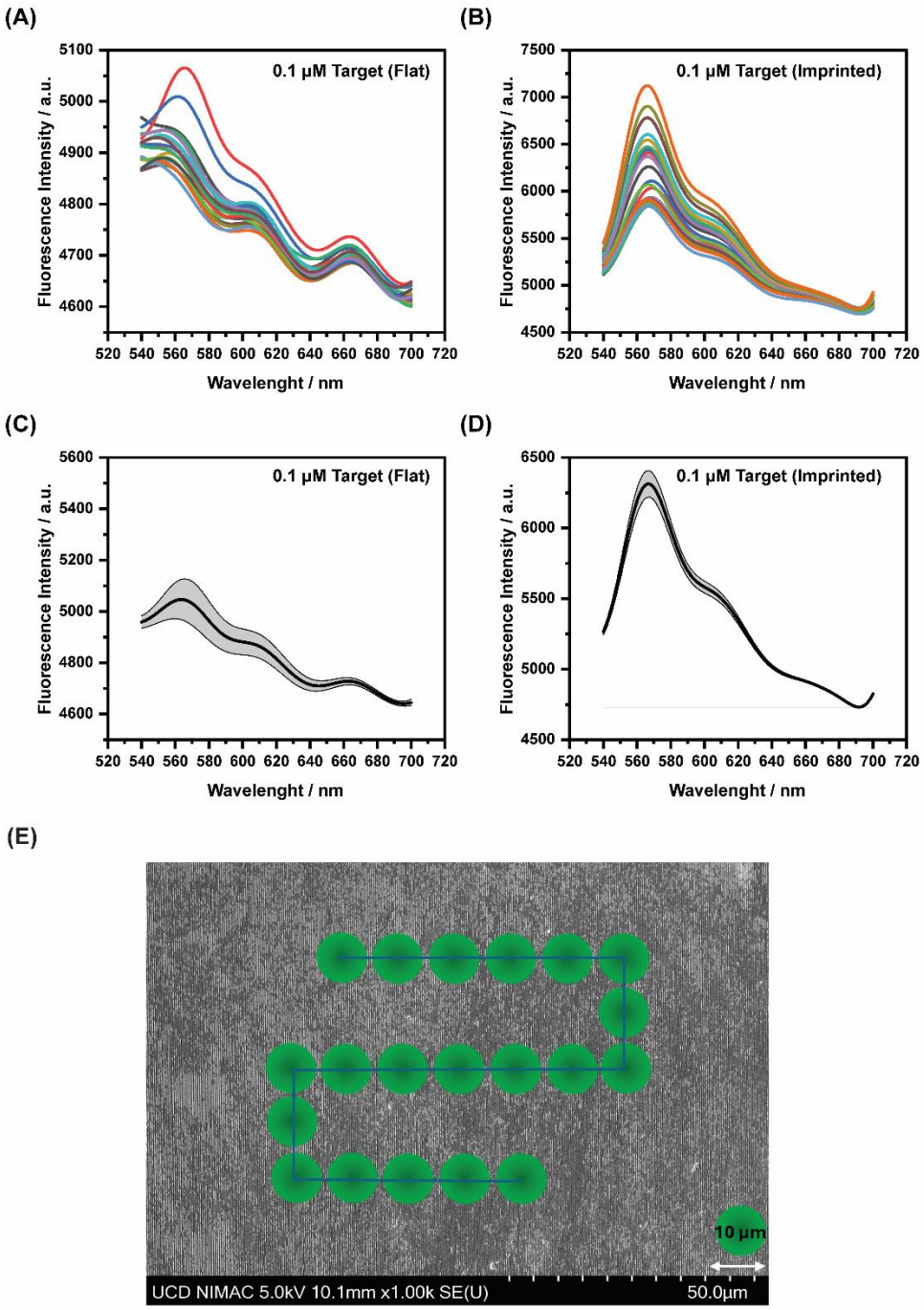

Figure S4 presents the fluorescence spectra from 20 separate measurements on both (A) flat and (B) nanoimprinted Au-PVDF. A target concentration of 0.1 µM is used. The corresponding average fluorescence intensities for both the (C) flat and (D) nanoimprinted films are also shown, where the shaded area represents the standard error from 20 different measurements at different spots on the same substrate. (E) shows an illustration of the 20 separate spots and their scale relative to the nanoimprinted features is also given.

Determination of β-phase Content and Reflectance of the Nanoimprinted Substrate

Figure S5A shows the infrared absorption spectrum used to determine the β-phase content of the PVDF films. Figure 3SB provides the reflectance spectra of the flat and nanoimprinted substrates to show that nanoimprinting modifies the light-substrate interactions.

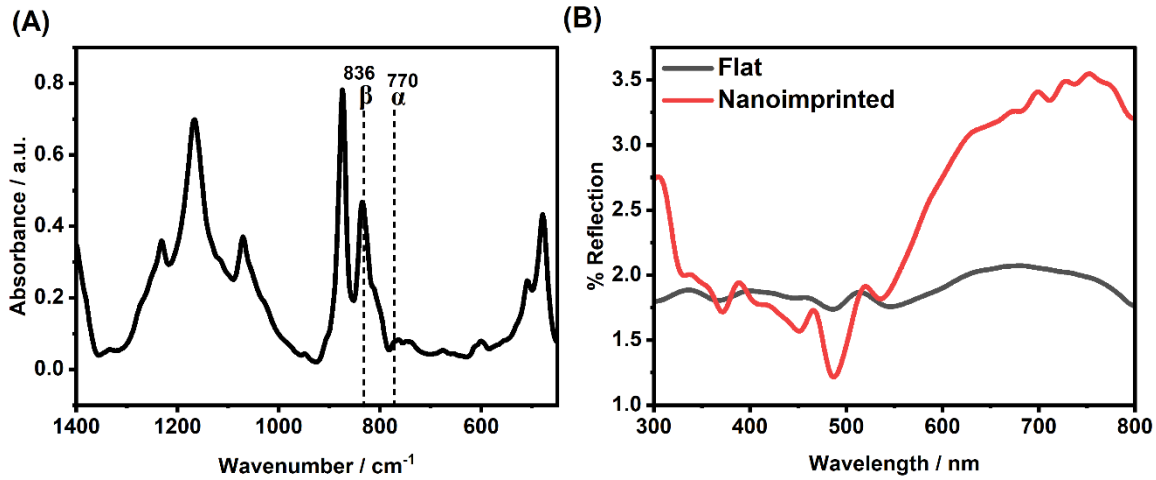

Figure S5 shows the (A) Infrared absorption spectrum (FTIR) of the flat PVDF film with the absorption bands of the α-phase at 770 cm^{-1} and the β-phase at 836 cm^{-1}, and(B) shows the reflectance spectra of flat (black curve) and nanoimprinted (red curve) Au-coated PVDF substrates. The flat gold film exhibits characteristic interband absorption features (450–550 nm), while the nanoimprinted substrate shows strong plasmonic modulation induced by the grating.

Substrate Reusability

Figure S6 shows the fluorescence spectra demonstrating the reusability of the substrate through ozone UV cleaning.

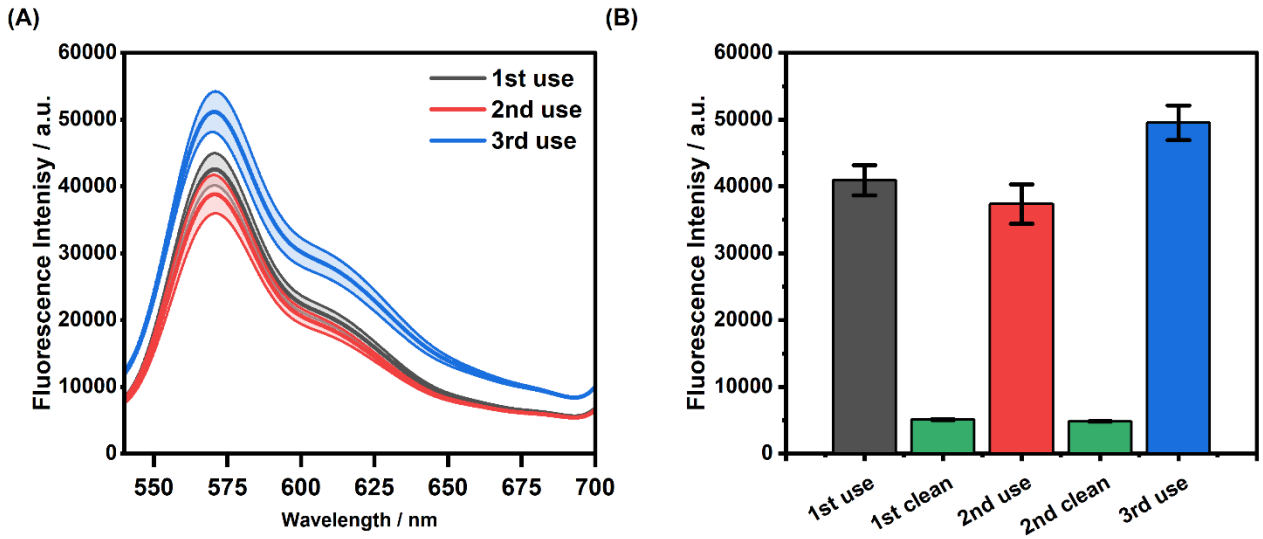

Figure S6 (A) Fluorescence spectra and (B) bar charts of 10 μM target DNA on a fresh substrate (red), after re-immobilization following ozone UV cleaning for 40 min (black), and after another cycle (blue). The shaded area in (A) and the error bars in (B) represent the standard error from 20 different measurements at different spots on the same substrate.

Figure S7 shows the AFM images of the substrate demonstrating that the nanoimprinted features are retained.

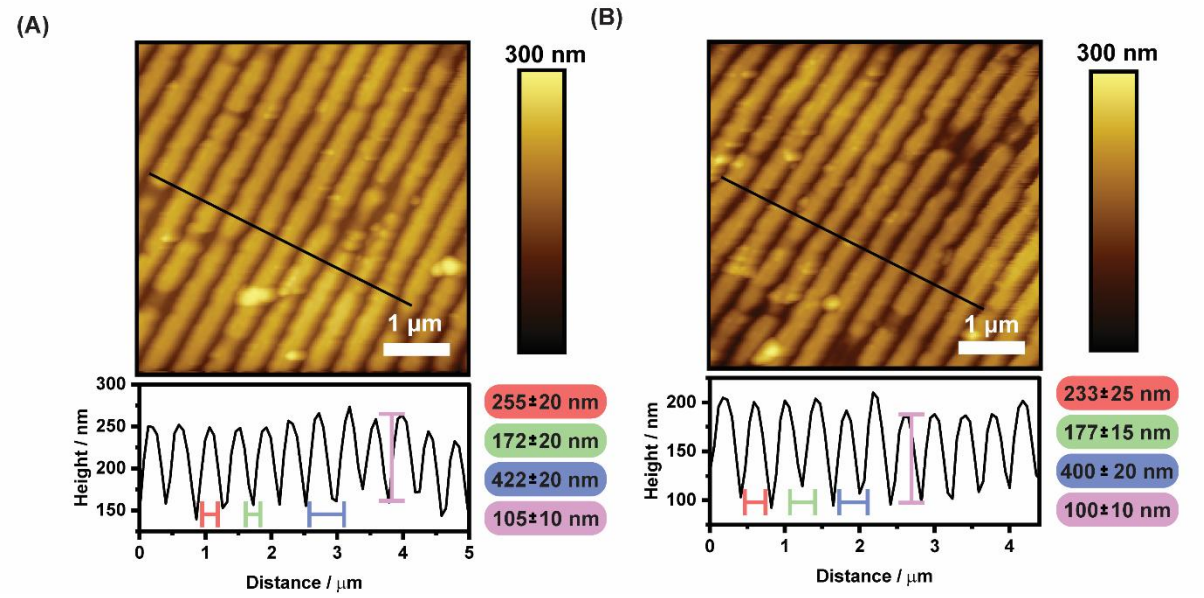

Figure S7 shows the AFM image and profile of the substrate (A) before and (B) after ozone UV cleaning.

Piezoelectric Response of Substrates

The piezoelectric activities of the flat and nanoimprinted substrates were compared by measuring the generated open-circuit voltage upon application of the load. (Figure S8) The nanoimprinted substrate shows a slightly higher piezoelectric response, likely due to higher stresses generated on the nanostructure, where the load is not as evenly distributed as on the flat substrate.

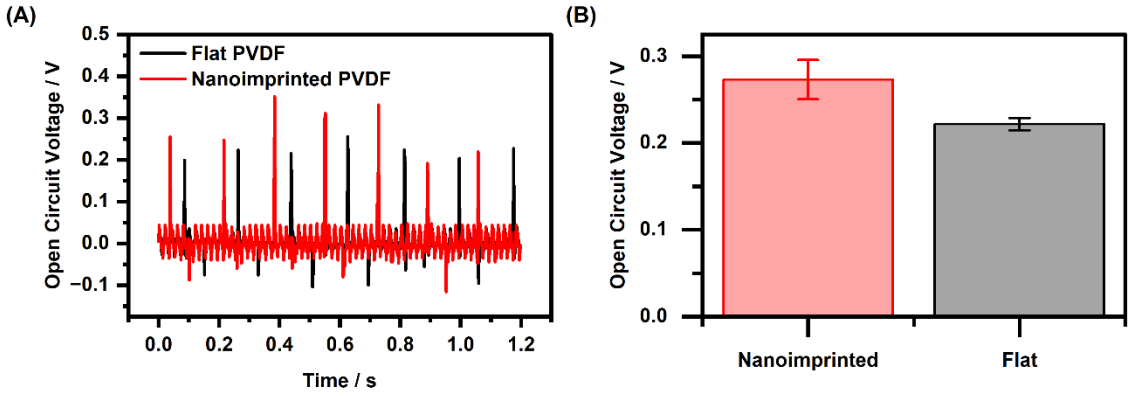

Figure S8 (A) shows the generated open circuit voltage upon pressing each sample, while (B) shows the average voltage spike generated by each sample.

Fluorescence Spectra at Different Concentrations

Figure S9 shows the fluorescence spectra obtained for each target DNA concentration.

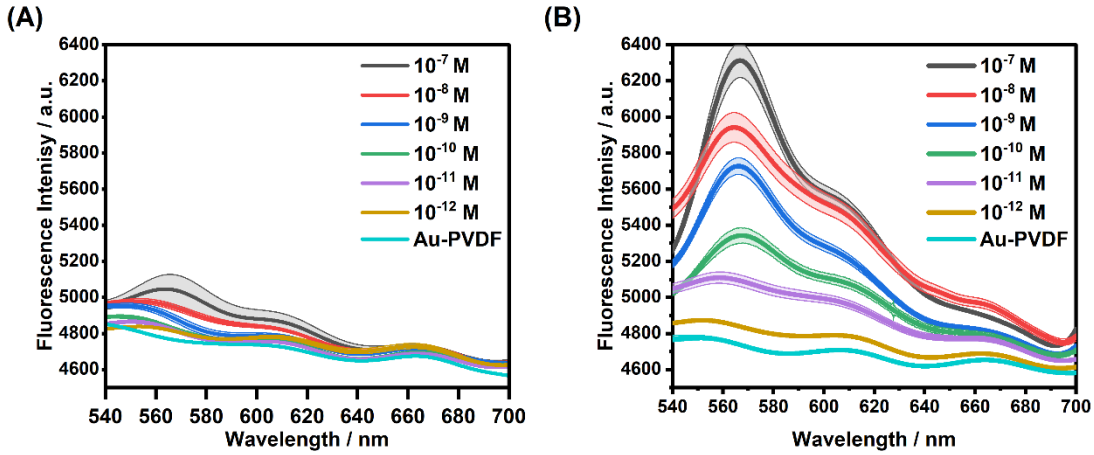

Figure S9 Fluorescence emission spectra of the (A) flat and (B) nanoimprinted Au-PVDF substrates corresponding to different concentrations of target DNA. The excitation wavelength is 532 nm. The shaded area in (A) and the error bars in (B) represent the standard error from 20 different measurements at different spots on the same substrate.