Fabrication of Unique Chemical Patterns and Concentration Gradients with Visible Light

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Supporting Information

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SI.1. *General Reagent Information*

All patterning examples were carried out in a nitrogen filled dry-box. The tetrahydrofuran (THF) was purchased from Fischer Scientific and vigorously purged with argon. The solvent was further purified by passing it under argon pressure through a Pure Solv 400-3-MD solvent purification system. The N-methyl pyorlidinone (NMP) was purchased from Aldrich and degassed by three freeze-pump-thaw cycles before it was taken into the glovebox. The *fac*-Ir(ppy)$_3$, acetonitrile, chloroform, mono-*N*-Boc-ethylenediamine, trifluoroacetic acid, triethylamine, iron(II/III)-oxide nanopowder (<50 nm), benzotriazol-1-(yloxy) tripyrrolidinophosphonium hexafluoro-phosphate (PyBOP), pyridine, trichlorosilane, *ω*-undecylenyl alcohol, and Karstedt's catalyst (in xylene, Pt ~2%) were all purchased from Sigma-Aldrich and used as received. The FITC-Avidin was purchased from Invitrogen as a solution of 5 mg of the protein in 2 mL of a 10 mM phosphate-buffer (pH 7.4) containing 40% glycerol, 1% bovine serum albumin, and 0.1% sodium azide. The FITC-Avidin solution was diluted 1:500 with an aqueous solution of 10 mM phosphate buffer (pH = 7.4) and 0.5 M NaCl before use.

SI.2. *General Analytical Information*

Nuclear magnetic resonance spectra were recorded on a Bruker 500 MHz instrument or a Varian 600 MHz Instrument. All ^{1}H NMR experiments are reported in δ units, parts per million (ppm), and were measured relative to the signals for residual chloroform (7.26 ppm) in the deuterated solvent, unless otherwise stated. A HDX Double-Brite Pro Grade 26-Watt Fluorescent Work Light, which was purchased from The Home Depot, was used as the light source. Bulk FT-IR spectra were obtained using a Thermo-Nicholet Avatar-330 IR spectrometer with a single-bounce attenuated total reflection attachment (ATR) (diamond crystal). Fluorescence micrographs were obtained with an Olympus BX51 microscope equipped with a Retiga 2000R camera from Qimaging. Optical micrographs were captured with a Nikon Elipse E600 microscope. Photomasks

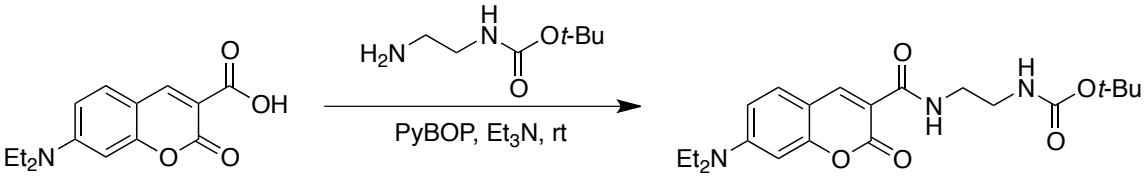
containing transparent rectangles measuring 2.5 mm X 250 mm and 20 mm X 200 mm were purchased from Photronics, Inc. A HEBS5N grayscale lithography calibration mask was obtained from Canyon Materials, Inc. The grayscale mask contained regions of different features including prisms of variable slope, squares of varying optical density, tapered structures, refractive lenslet (positive and negative), refractive microlenses (positive and negative) and linear gradients. A more detailed description of the features can be found at http://www.canyonmaterials.com/descrip_cal_hebs5n.html.

SI.3. *Synthesis of Reagents used in this Study*

Synthesis of the Alkene Functionalized NHS-Ester (1).

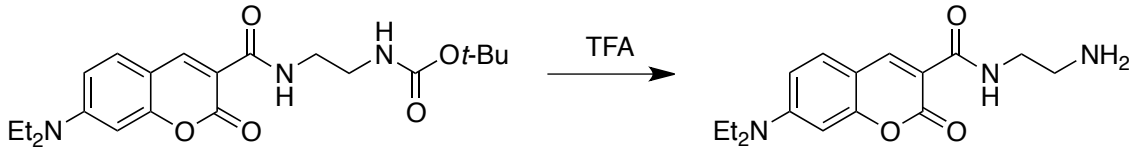
4-Pentenoic acid N-succinimidyl ester was synthesized according to a literature procedure.[1] ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.48 (m, 2H), 2.72 (t, 2H), 2.83 (s, 4H), 5.07-5.14 (m, 2H), 5.84 (m, 1H) ^{13}C NMR (150 MHz, $CDCl_3$), δ 169.06, 168.01, 135.13, 116.59, 30.31, 28.31, 25.56. ATR-IR (cm^{-1}): 3083, 3010, 2979, 2958, 2927, 1815, 1786, 1723, 1643, 1201, 1063.

Synthesis of the Amine-Functionalized Coumarin Dye (2)



***tert*-Butyl (2-(7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxamido)ethyl)-carbamate.** Et_2N-Coumarin-COOH (7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid) was synthesized according to the literature method.[2] Et_2N-Coumarin-COOH (1.26 g, 4.82 mmol) and benzotriazol-1-(yloxy)tripyrrolidinophosphonium hexafluorophosphate, PyBOP, (2.51 g, 4.82 mmol) were dissolved in acetonitrile (40 mL) in a 100 mL round bottom flask equipped with a stir bar and septum followed by the addition of triethylamine (1.8 mL, 13 mmol). A solution of mono-*N*-Boc-ethylenediamine (0.834 g, 5.21 mmol)

in chloroform (15 mL) was added and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with brine (50 mL) and extracted with ethyl acetate (2 x 150 mL). The organic portions were combined and washed successively with 10% $NaHSO_4$ (aq) (100 mL), water (100 mL), 10% $NaHCO_3$ (aq) (100 mL), water (100 mL), and finally brine (100 mL). The solution was dried over $MgSO_4$, filtered, and then concentrated under reduced pressure. The resulting solid was purified by column chromatography on silica gel using a gradient of 50 to 70% ethyl acetate in hexanes and dried under vacuum to yield 2.0 g (99%) of a bright yellow powder. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.24 (t, *J* = 7.1 Hz, 6H), 1.44 (s, 9H), 3.36 (q, *J* = 5.7 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 4H), 3.55 (q, *J* = 6.0 Hz, 2H), 5.05 (bs, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 6.65 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.43 (d, *J* = 8.9 Hz, 1H), 8.69 (s, 1H), 8.96 (bs, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.55, 28.53, 39.86, 41.12, 45.21, 79.42, 96.70, 108.47, 110.11, 131.30, 148.33, 152.75, 156.18, 157.81, 162.81, 164.12 ppm. MS (ESI): m/z [M + Na]$^+$ calcd for [$C_{21}H_{29}N_3O_5$ + Na]$^+$, 426.200; found, 426.200.



***N*-(2-Aminoethyl)-7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxamide (2).** Boc-protected *tert*-Butyl (2-(7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxamido)ethyl)- carbamate (91.4 mg, 0.227 mmol) was stirred in a 10 mL round bottom flask with trifluoroacetic acid (2 mL) at 0 °C. After 2 h, excess trifluoroacetic acid was removed *in vacuo* and the free base was isolated by neutralization with 1 M NaOH (aq) followed by extraction with dichloromethane (50 mL). The organic portion was washed with a saturated solution of Na_2CO_3 (aq) (50 mL) then brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield 45.6 mg (66%) of a tacky yellow solid, which was used without further purification. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.23 (t, *J* = 7.2

Hz, 6H), 1.74 (bs, 2H), 2.93 (t, *J* = 6.1 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 4H), 3.51 (q, *J* = 6.0 Hz, 2H), 6.48 (d, *J* = 2.5 Hz, 1H), 6.64 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.42 (d, *J* = 9.0 Hz, 1H), 8.70 (s, 1H), 8.97 (bq, *J* = 6.1 Hz, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.52, 41.86, 42.77, 45.16, 96.65, 108.44, 110.03, 131.20, 148.17, 152.63, 157.72, 162.82, 163.69 ppm. MS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{21}N_3O_3 + H]^+$, 304.166; found, 304.165.

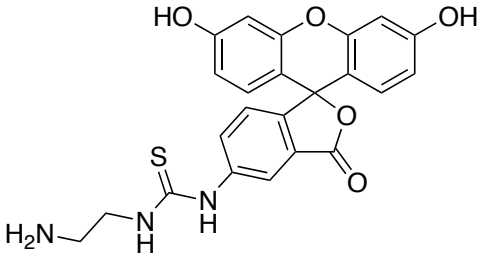
Synthesis of the Alkene Functionalized Biotin (3).[3]

To a solution of (+)-biotin (1.00 g, 4.09 mmol, 1 equiv), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl, 942 mg, 4.91 mmol, 1.2 equiv) and 1-hydroxybenzotriazole (HOBT, 664 mg, 4.91 mmol, 1.2 equiv) in *N,N*-dimethylformamide (DMF, 15 ml) was added 10-Undecen-1-ol (1.24 ml, 6.17 mmol, 1.5 equiv) and triethylamine (5 ml). The reaction was allowed to stir overnight at room temperature. After dilution with dichloromethane, the reaction mixture was washed twice with saturated aqueous ammonium chloride solution. The aqueous fractions were extracted twice with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by flash-column chromatography (dichloromethane:methanol 9:1) giving compound **3** as a white solid (0.87 g, 51%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.76 (m,1H), 4.94 (br d, 1H, *J* = 16.8H), 4.88 (br d, 1H, *J* = 10.2 Hz), 4.46 (dd, 1H, *J* = 6.6 Hz, 6.0 Hz), 4.27 (dd, 1H, *J* = 7.2 Hz, 4.8 Hz), 4.00 (t, 2H, *J* = 7.2 Hz), 3.11 (m, 1H), 2.86 (dd, 1H, *J* = 12.6 Hz, 4.8 Hz), 2.69 (d, 1H, *J* = 12.6), 2.28 (t, 2H, *J* = 7.2 Hz), 1.99 (m, 2H), 1.73-1.52 (m, 6H), 1.47-1.18 (m, 14H). ^{13}C NMR (600 MHz, $CDCl_3$): δ 173.8, 163.7, 139.1, 114.1, 64.5, 61.9, 60.1, 55.4, 40.5, 33.9, 33.8, 29.4, 29.4, 29.2, 29.1, 28.9, 28.6, 28.4, 28.2, 25.9, 24.8. ATR-IR (cm^{-1}): 3250, 2914, 2850, 1735, 1700, 1458, 1189, 1166, 929, 730. HRMS-EIMS (m/z): [M-Na]+ calcd for $C_{21}H_{36}N_2O_3NaS$, 419.2344; found, 419.2335.

Synthesis of Triethylsilane-protected eugenol (4):

The triethylsilane-protected eugenol was synthesized according a literature procedure.[4] ^{1}H NMR (600 MHz, $CDCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.74 (q, 12H), 0.97 (t, 18H), 3.25 (d, 2H), 5.02 (m, 2H), 5.93 (m, 1H), 6.59 (d, 1H), 6.64 (s, 1H), 6.73 (d, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 146.6, 145.0, 137.9, 133.1, 121.3, 121.0, 120.3, 115.3, 39.5, 6.68, 5.15 ppm. TOF-MS (ESI): Calcd: $[M + Na]^+$ ($C_{21}H_{38}O_2Si_2Na$) *m/z* = 401.23; found ESI-MS: $[M + Na]^+$ *m/z* = 401.26. ATR-IR (cm-1): 3079, 2954, 2912, 2877, 1638, 1508.

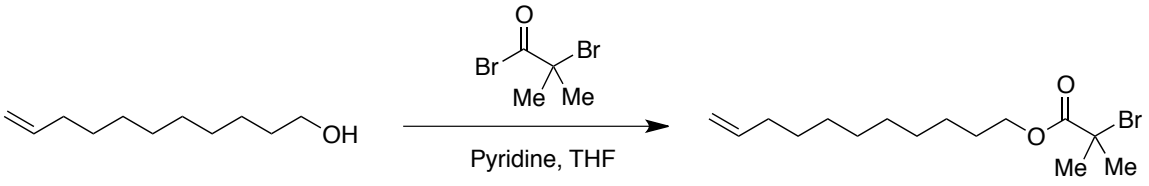
Synthesis of the Amino Functionalized Fluorescein



The amino-functionalized fluorescein dye was synthesized according to a literature procedure.[5] ^{1}H NMR (600 MHz, MeOD): δ 2.90 (t, 2H), 3.33 (t, 2H), 6.56-6.67 (m, 6H), 6.87 (d, 1H), 7.19 (s, 1H).

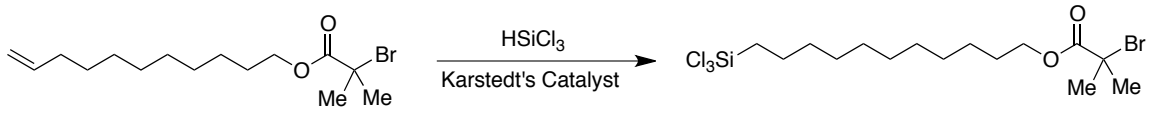
SI.4. *Synthesis of Uniformly Functionalized Alkyl Bromide Silicon Surfaces*[6]

The alkyl bromide functionalized silicon surfaces were prepared in an identical procedure that was described in reference 6. The experimental from reference 6 is below:

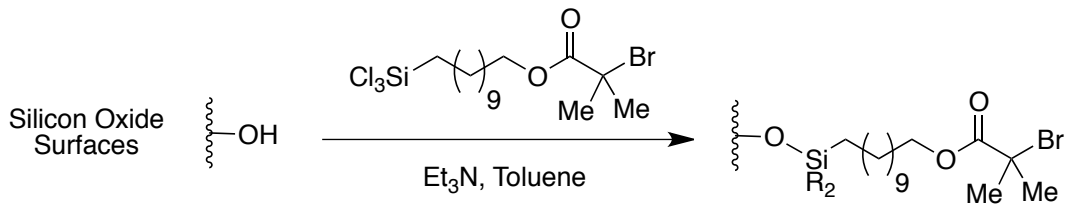


Undec-10-en-1-yl 2-bromo-2-methylpropanoate[7] A 25 mL schlenk flask, which equipped with a magnetic stir bar and a rubber septum, was evacuated and

backfilled with nitrogen and charged with undec-10-enol (1.1 mL, 6.25 mmol), pyridine (525 μL, 6.63 mmol), and tetrahydrofuran (25 mL). 2-Bromoisobutyryl bromide (775 μL, 6.25 mmol) was then added to the reaction mixture in a dropwise fashion over a 10 min period. The solution was stirred overnight at room temperature and then diluted with hexane, washed with 1N HCl, dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified using flash chromatography on silica gel (25 : 1 hexanes : ethyl acetate) to provide the title compound as a colorless oil. ^{1}H NMR spectrum matched literature values.

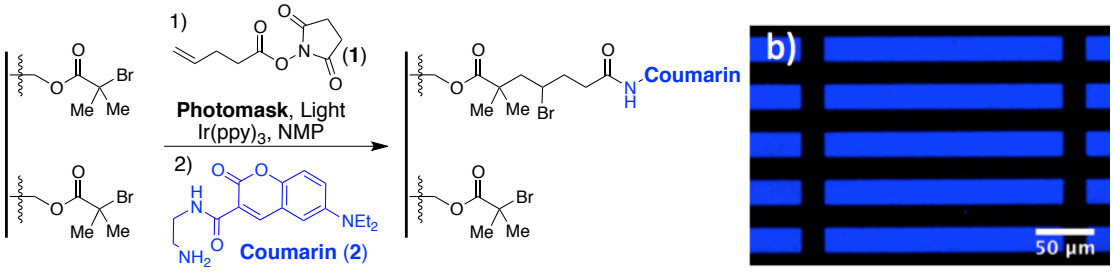


11-(Trichlorosilyl)undecyl 2-bromo-2-methylproanoate[7] A 10 mL schlenk flask, which equipped with a magnetic stir bar and a rubber septum, was evacuated and backfilled with nitrogen and charged with undec-10-en-1-yl 2-bromo-2-methylpropanoate (1.35 g, 4.2 mmol), trichlorosilane (4.2 mL, 42.6 mmol) and a solution of Karstedt's catalyst in xylene (5 μL, 2 wt% Pt in xylene). The solution was stirred at room temperature. Conversion was determined by ^{1}H NMR, and the reaction was found to go to completion in 2 hours. The reaction mixture was then concentrated under reduced pressure to give the title compound as a clear oil. The compound was used without further purification. ^{1}H NMR spectrum matched literature values.



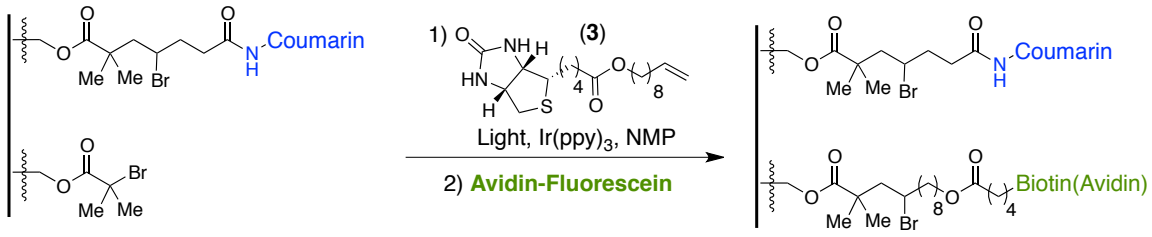
Alkyl bromide functionalized Surface. Silicon substrates with 100 nm of oxide were purchased from Silicon Quest International. The substrates were diced into 1.5 cm X 1.5 cm squares and cleaned by sonication in acetone, followed by isopropyl alcohol, and finally deionized water. The wafers were then immersed in a piranha solution at 90 °C for 20 minutes (**CAUTION:** Piranha solutions are extremely energetic and may result in explosion or skin burns if not handled with extreme caution)**.** The substrates were then washed with copious amounts of deionized water, dried under a stream of nitrogen gas, and stored in a vacuum oven for 1 hour. After drying, the wafers were placed in petri dishes and covered with 30 ml of a solution containing 125 µl of 11-(trichlorosilyl)undecyl 2-bromo-2-methylproanoate in 250 ml of dry toluene (0.05% v/v) and 50 ml of dry triethylamine. The petri dishes were covered under a blanket of nitrogen and allowed to react overnight. Upon completion, the functionalized substrates were rinsed thoroughly with toluene followed by ethanol to remove the triethlyamine salts which precipitated during the reaction. The wafers were dried under vacuum and stored under ambient conditions.

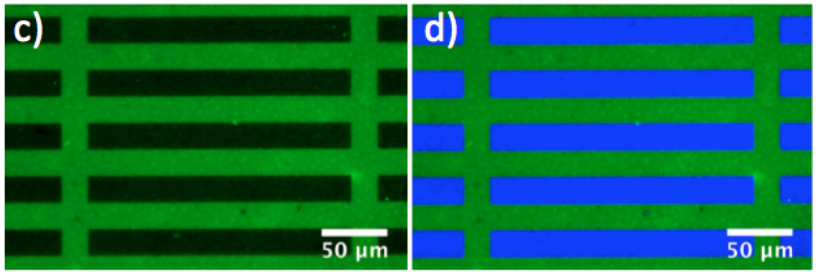
SI.5. *Patterning Chemically Differentiated Regions of Coumarin and Biotin (Experimental for Figure 2)*



20 x 200 µm coumarin-containing rectangles. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with 2,5-dioxopyrrolidin-1-yl pent-4-enoate (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was

pipetted onto a silicon substrate, which had been uniformly functionalized with alkyl bromide initiators, until it was completely covered. A binary photomask containing 20 x 200 μm clear rectangles was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 30 minutes. After irradiation, the substrate was washed 3 times with NMP and then placed in a solution containing the coumarin dye **1** (1.0 mg), Et_3N (50 μL) and NMP (5 mL). After 1 hour the silicon substrate was removed from the dye solution and washed 5 times with NMP. The resultant pattern was visualized with fluorescence microscopy.

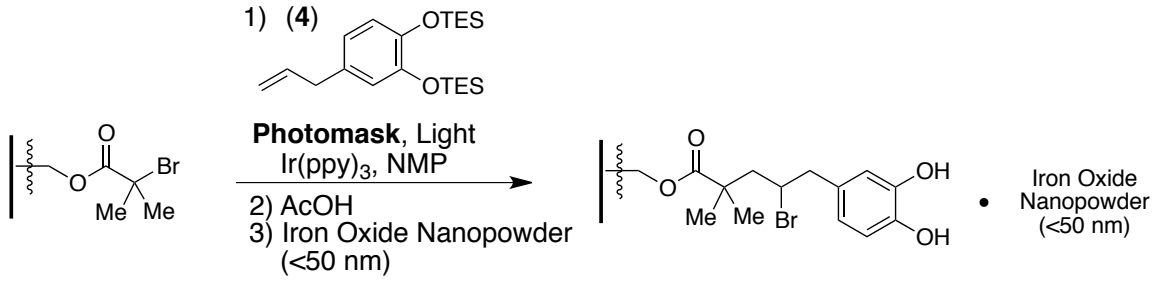


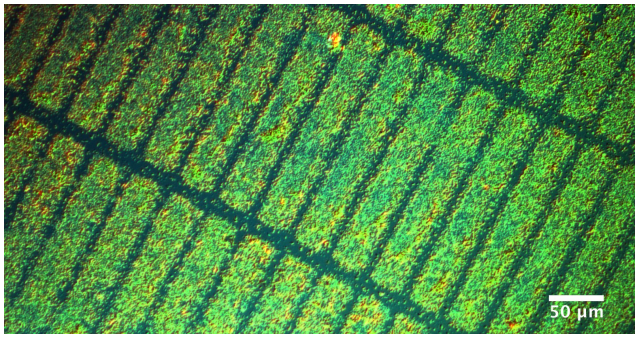


Functionalization of the grid region around the 20 x 200 µm coumarin-containing rectangles with biotin-avidin. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with the alkene functionalized biotin **3** (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto the silicon substrate that contained the 20 x 200 μm rectangles of coumarin until it was completely covered. A glass cover slip was placed on top of the solution to form a

thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 20 minutes. After irradiation, the substrate was washed 3 times with NMP, removed from the glovebox and then placed in a solution of the fluorescein labeled avidin. After 1 hour the silicon substrate was removed from the avidin solution and washed 5 times with water. The substrate was then placed in a solution of water and sonicated for 20 minutes (this process was repeated a total of 3 times). The resultant pattern was then visualized with fluorescence microscopy.

SI.6. *Templated Assembly of Iron-Oxide Nanoparticles Facilitated by the Patterning of Catechol Functional Groups (Experimental for Figure 3)*

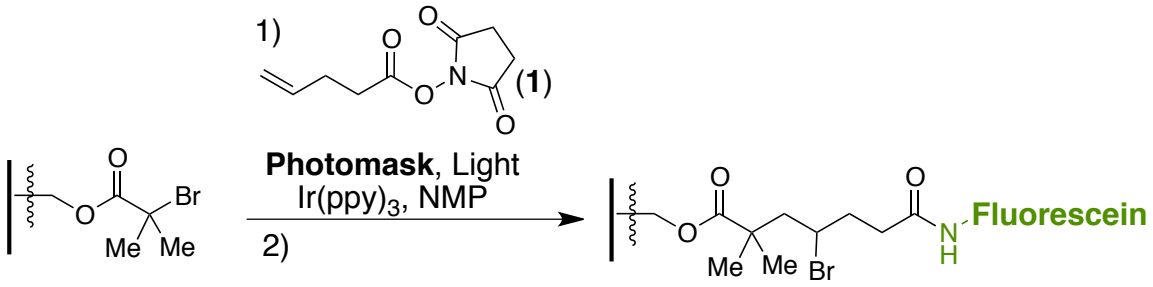




20 x 200 µm iron-oxide nanopowder containing rectangles. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with the protected catechol **4** (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto a silicon substrate, which had been uniformly functionalized with alkyl bromide initiators, until it was completely covered. A binary photomask containing

20 x 200 μm clear rectangles was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 30 minutes. After irradiation, the substrate was washed 3 times with NMP and removed from the glovebox. Under argon atmosphere the substrate was treated with an aqueous solution of acetic acid (0.1 M) for 1 hour. The silicon substrate was then washed with water and placed in a suspension of iron oxide nanopowder in ethanol, which was prepared by sonication of 20 mg of the nanopowder in 5 mL of EtOH for 1 hour. After 24 hours the substrate was removed from the suspension, washed 5 times with ethanol and visualized with optical microscopy.

SI.7. *Patterning Chemical Concentration Gradients of Fluorescein (Experimental for Figures 4 and 5)*

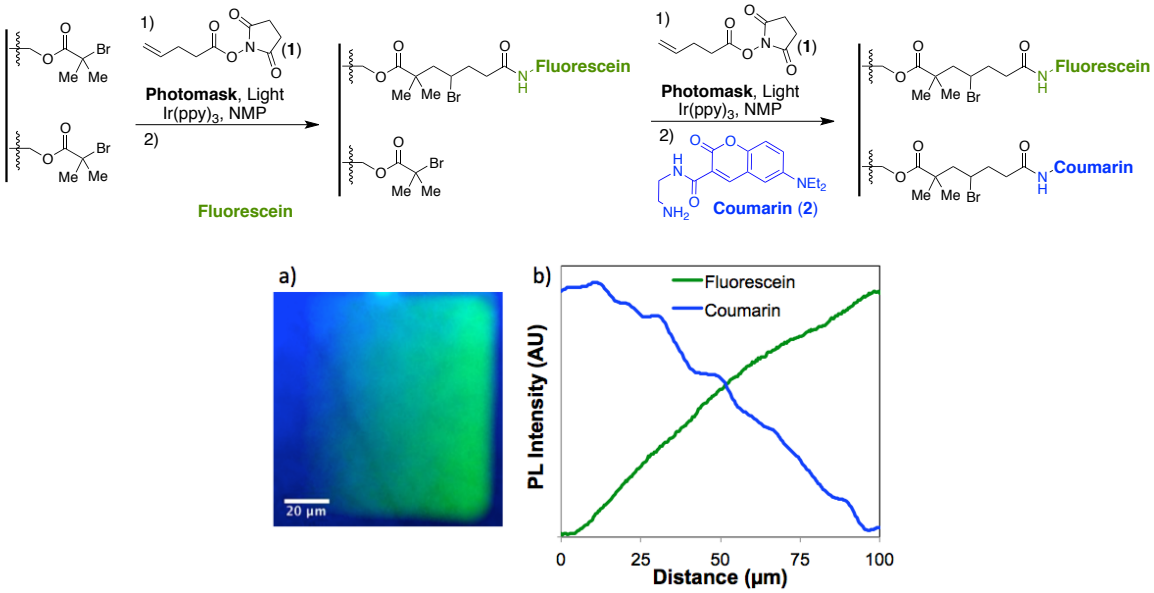


Fluorescein

100 x 100 µm Squares varying linearly in the areal chemical concentration of fluorescein. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with 2,5-dioxopyrrolidin-1-yl pent-4-enoate (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto a silicon substrate, which had been uniformly functionalized with alkyl bromide initiators, until it was completely covered. A grayscale photomask containing an array of 100 x 100 μm squares that varied linearly in transmittance was placed on top of the solution to form a

thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 10 minutes. After irradiation, the substrate was washed 3 times with NMP and then placed in a solution containing the fluorescein dye (1.5 mg), Et_3N (50 μL) and NMP (5 mL). After 1 hour the silicon substrate was removed from the dye solution and washed 5 times with NMP. Images were taken with a fluorescence microscope and the data was analyzed with FIJI. The software FIJI was downloaded from the internet at http://fiji.sc/Fiji.

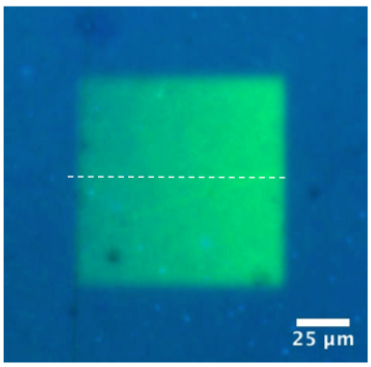
SI.8. *Fabrication of Dual Chemical Concentration Gradients of Fluorescein and Coumarin (Experimental for Figures 6)*

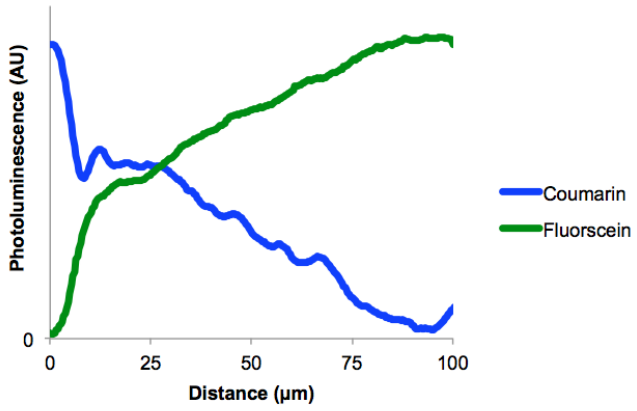


Dual chemical concentration gradients of fluorescein and coumarin. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[$Ir(ppy)_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with 2,5-dioxopyrrolidin-1-yl pent-4-enoate (15 mg), NMP (900 μL) and the $Ir(ppy)_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto a silicon substrate, which had been uniformly functionalized with alkyl bromide initiators, until it was completely covered. A grayscale photomask containing an array of 100 x 100 μm squares that varied

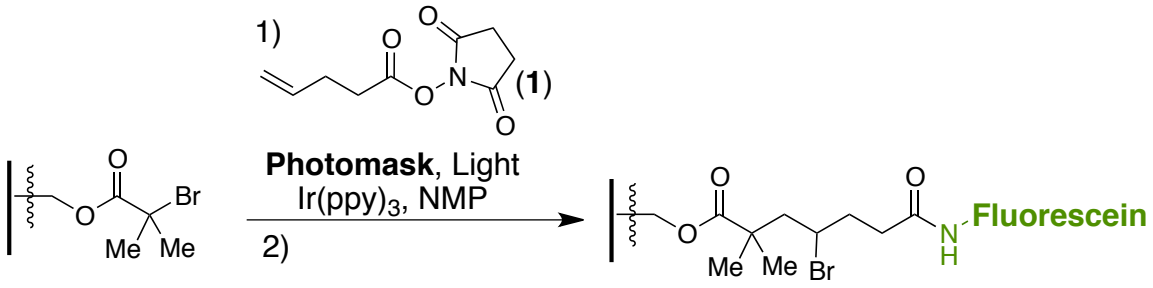
linearly in optical density was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 10 minutes. After irradiation, the substrate was washed 3 times with NMP and then placed in a solution containing the fluorescein dye (1.5 mg), Et_3N (50 μL) and NMP (5 mL). After 1 hour the silicon substrate was removed from the dye solution and washed 5 times with NMP. For a second time, the reaction solution was pipetted on the substrate, which now contained a chemical concentration gradient of fluorescein. A coverslip was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 15 minutes. After irradiation, the substrate was washed 3 times with NMP and then placed in a solution containing the coumarin dye (1.0 mg), Et_3N (50 μL) and NMP (5 mL). After 1 hour the silicon substrate was removed from the dye solution and washed 5 times with NMP. Images of the dual gradient were taken with a fluorescence microscope and the data was analyzed with FIJI. The software FIJI was downloaded from the internet at http://fiji.sc/Fiji.

Additional example of a dual gradient of fluorescein and coumarin. Using the general procedure above an additional example of a dual fluorescein and coumarin gradient was fabricated. See data below:





SI.9. *Synthesis of Complex Non-Linear Chemical Concentration Gradients of Fluorescein (Experimental for Figure 7)*

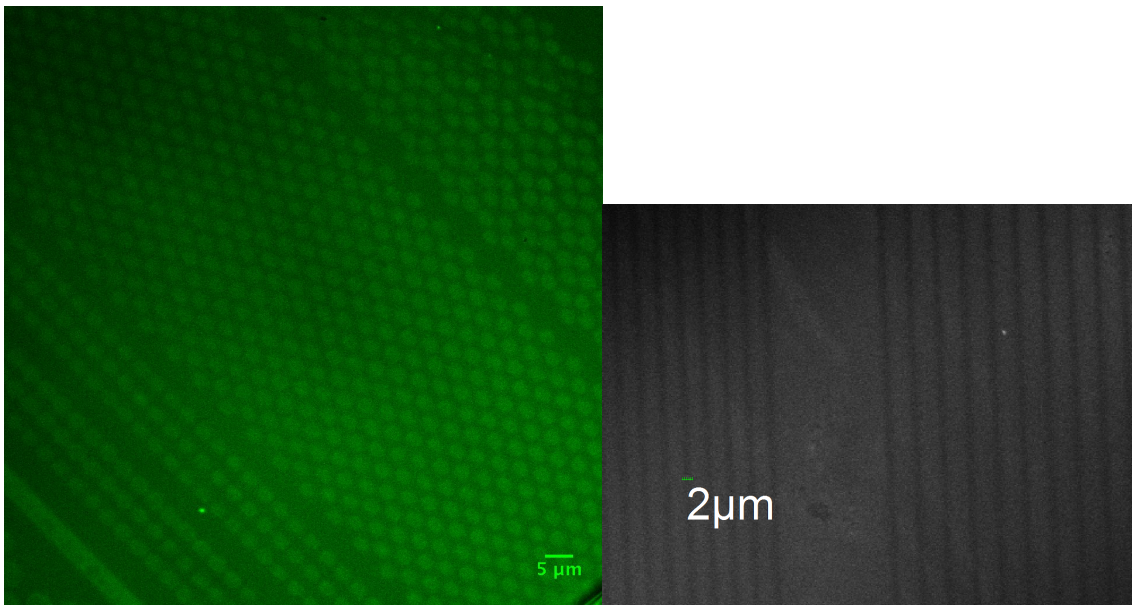


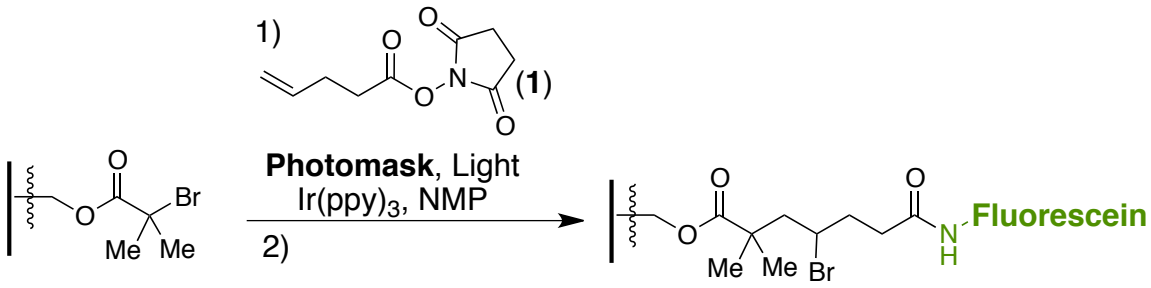
Fluorescein

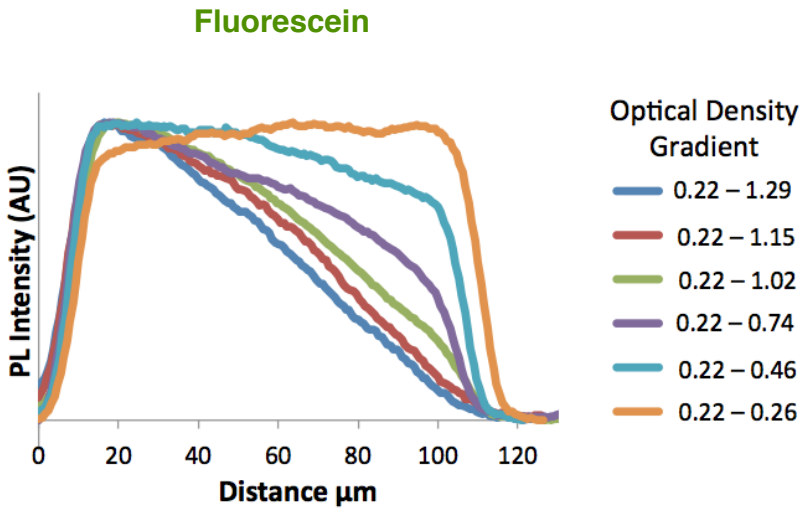
Chemical gradients of fluorescein that increase or decrease radially in concentration from the center of each feature. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with 2,5-dioxopyrrolidin-1-yl pent-4-enoate (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto a silicon substrate, which had been uniformly functionalized with alkyl bromide initiators, until it was completely covered. A grayscale photomask containing an array of 50 x 50 μm positive and negative lenses that increased or decreased in optical density from the center of each feature was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 10 minutes. After irradiation, the substrate was washed 3 times with NMP and then placed in a solution containing the fluorescein dye (1.5 mg), Et_3N (50 μL) and NMP (5 mL). After 1 hour the silicon substrate was removed from the dye solution and washed 5 times with NMP. Images were taken with a fluorescence microscope and the data was analyzed with FIJI. The software FIJI was downloaded from the internet at http://fiji.sc/Fiji.

SI.10. *Additional Patterning Example*

Functionalization of biotin on a surface: feature sizes down to ~1 μm. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 μmol) in 1 ml degassed NMP was prepared. A separate vial was charged with the alkene functionalized biotin **3** (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto an alkyl bromide functionalized silicon substrate until it was completely covered. A binary photomask was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 20 minutes. After irradiation, the substrate was washed 3 times with NMP, removed from the glovebox and then placed in a solution of the fluorescein labeled avidin. After 1 hour the silicon substrate was removed from the avidin solution and washed 5 times with water. The substrate was then placed in a solution of water and sonicated for 20 minutes (this process was repeated a total of 3 times). The resultant pattern was then visualized with fluorescence microscopy (see image below).







100 x 100 µm Squares varying linearly in the areal chemical concentration of fluorescein using a grayscale mask that varied linearly in optical density. In a nitrogen atmosphere glove box, a stock solution containing 1.2 mg of *fac*[Ir(ppy)$_3$] (1.8 µmol) in 1 ml degassed NMP was prepared. A separate vial was charged with 2,5-dioxopyrrolidin-1-yl pent-4-enoate (15 mg), NMP (900 μL) and the Ir(ppy)$_3$/NMP stock solution (100 μL) to form the reaction solution. The reaction solution was pipetted onto a silicon substrate, which had been uniformly functionalized with alkyl bromide initiators, until it was completely covered. A grayscale photomask containing an array of 100 x 100 μm squares that varied linearly in optical density was placed on top of the solution to form a thin layer in contact with the substrate. The wafer was placed ca. 7 cm. below the light source and was irradiated with a 26-watt fluorescent lamp for 10 minutes. After irradiation, the substrate was washed 3 times with NMP and then placed in a

solution containing the fluorescein dye (1.5 mg), Et_3N (50 μL) and NMP (5 mL). After 1 hour the silicon substrate was removed from the dye solution and washed 5 times with NMP. Images were taken with a fluorescence microscope and the data was analyzed with FIJI (see data above). The software FIJI was downloaded from the internet at http://fiji.sc/Fiji.

SI.11. *References.*

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