Supplementary information for:

Evaluation of ToF-SIMS imaging for semi-quantitative mapping of BODIPY-labeled fibronectin surface gradients

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Table S 1. Summary of total counts, Cu counts, and normalized values for each condition in BCA-stained fibronectin standards. Data are presented as mean ± standard deviation from four regions of interest (ROIs), each with an approximate scanning size of 100 µm × 100 µm.

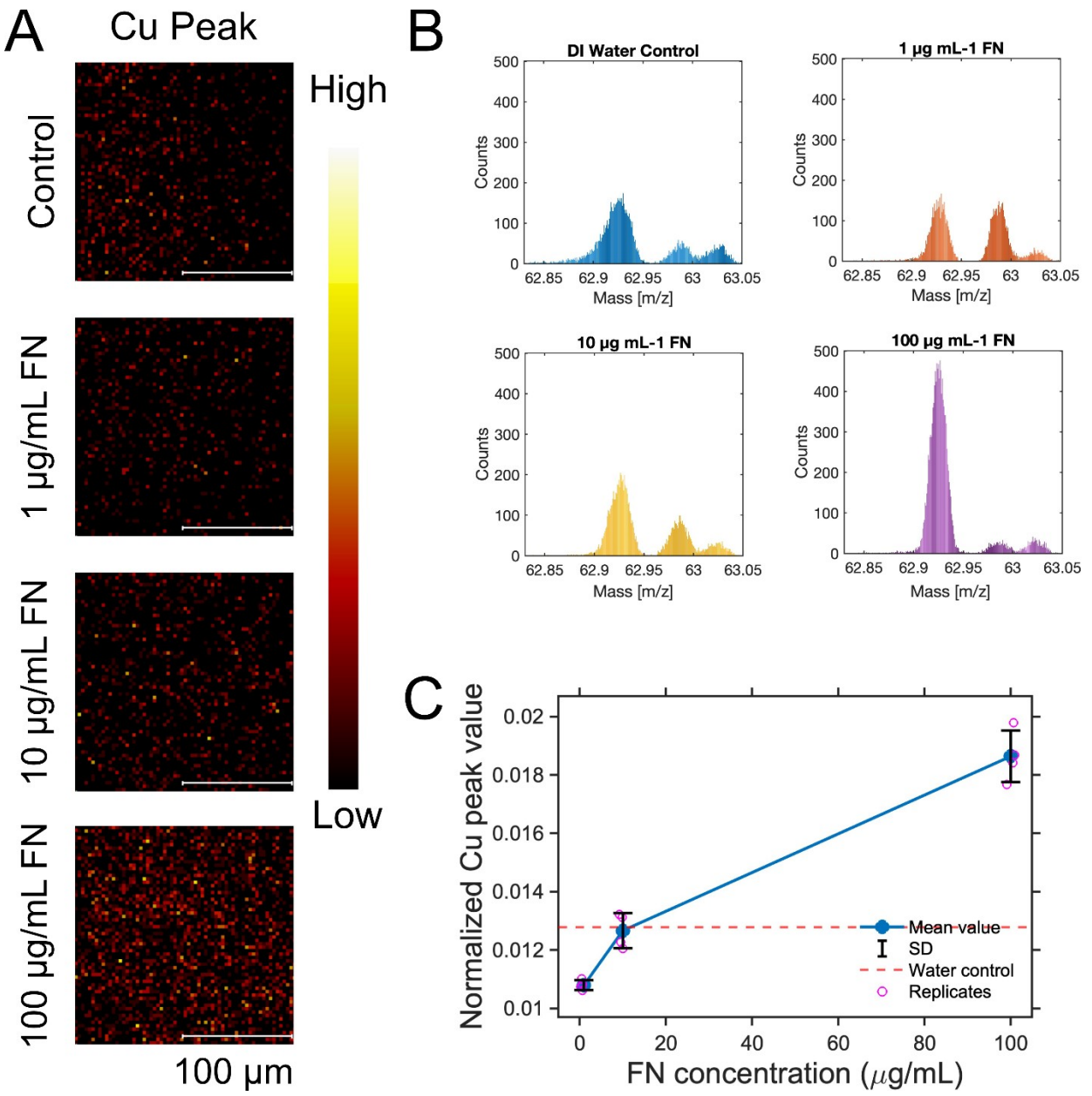
Condition	Total Count (mean ± SD)	Cu Count (mean ± SD)	Normalized Value (mean ± SD)
Control	174,655 ± 63,720	2,141 ± 489	$1.28\times10^{-2} \pm 1.98\times10^{-3}$
1 µg/mL FN	171,636 ± 14,044	1,854 ± 154	$1.08\times10^{-2} \pm 1.71\times10^{-4}$
10 µg/mL FN	168,117 ± 20,139	2,122 ± 176	$1.27\times10^{-2} \pm 5.97\times10^{-4}$
100 µg/mL FN	163,730 ± 12,637	3,047 ± 181	$1.86\times10^{-2} \pm 8.77\times10^{-4}$

Table S 2. Summary of total counts, Br counts, and normalized values for each condition in Eosin-labeled fibronectin standards. Data are presented as mean ± standard deviation from four regions of interest (ROIs), each with an approximate scanning size of 100 µm × 100 µm.

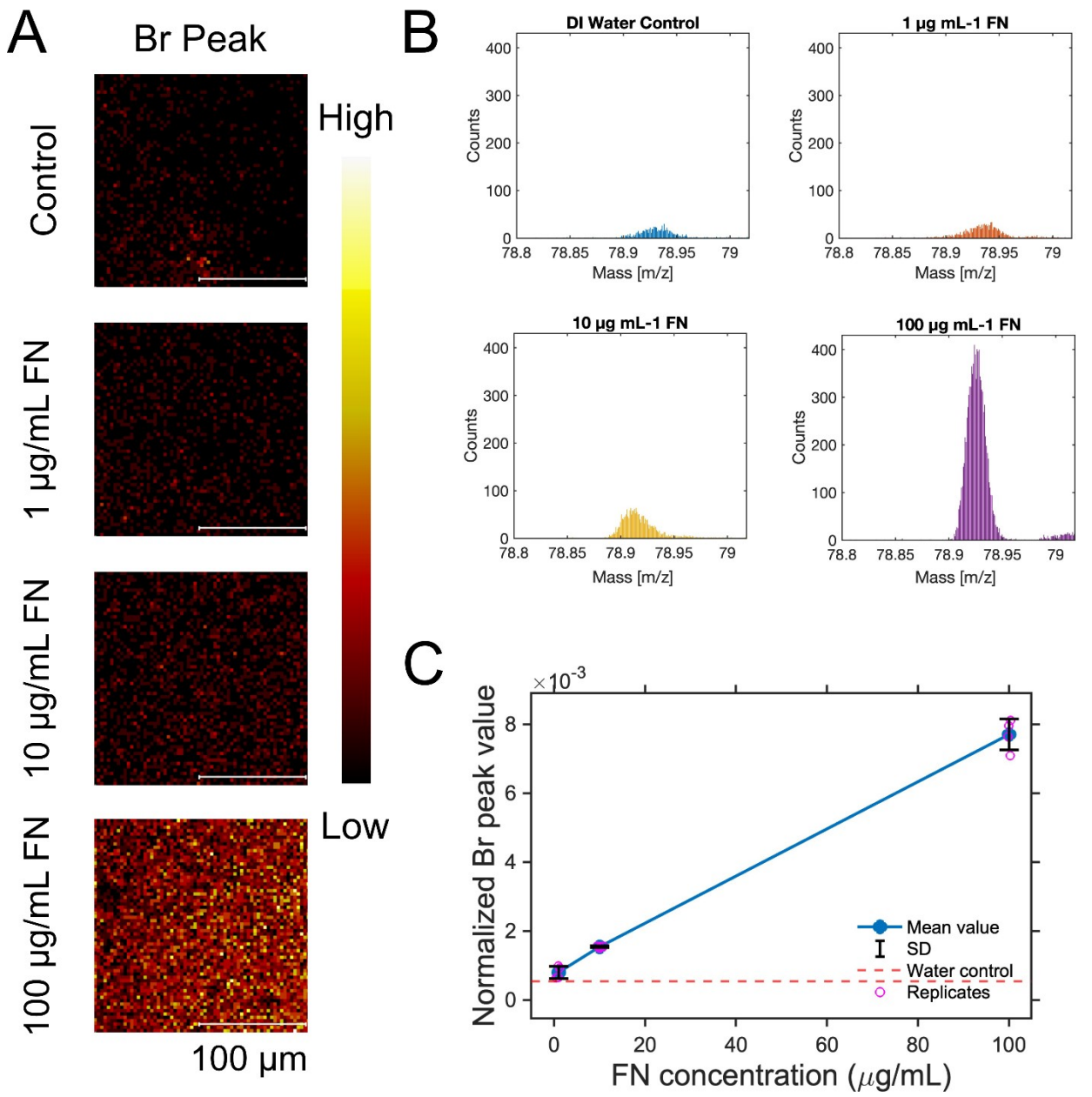
Condition	Total Count (mean ± SD)	Br Count (mean ± SD)	Normalized Value (mean ± SD)
Control	362,472 ± 44,971	205 ± 137	$5.43\times10^{-4} \pm 3.11\times10^{-4}$
1 µg/mL FN	356,600 ± 54,962	287 ± 82	$8.03\times10^{-4} \pm 1.77\times10^{-4}$
10 µg/mL FN	317,606 ± 46,686	491 ± 77	$1.54\times10^{-3} \pm 2.85\times10^{-5}$
100 µg/mL FN	293,649 ± 46,133	2,272 ± 448	$7.70\times10^{-3} \pm 4.48\times10^{-4}$

Table S 3. Summary of total counts, F counts, and normalized values for each condition in BODIPY conjugated fibronectin standards. Data are presented as mean ± standard deviation from four regions of interest (ROIs), each with an approximate scanning size of 100 µm × 100 µm.

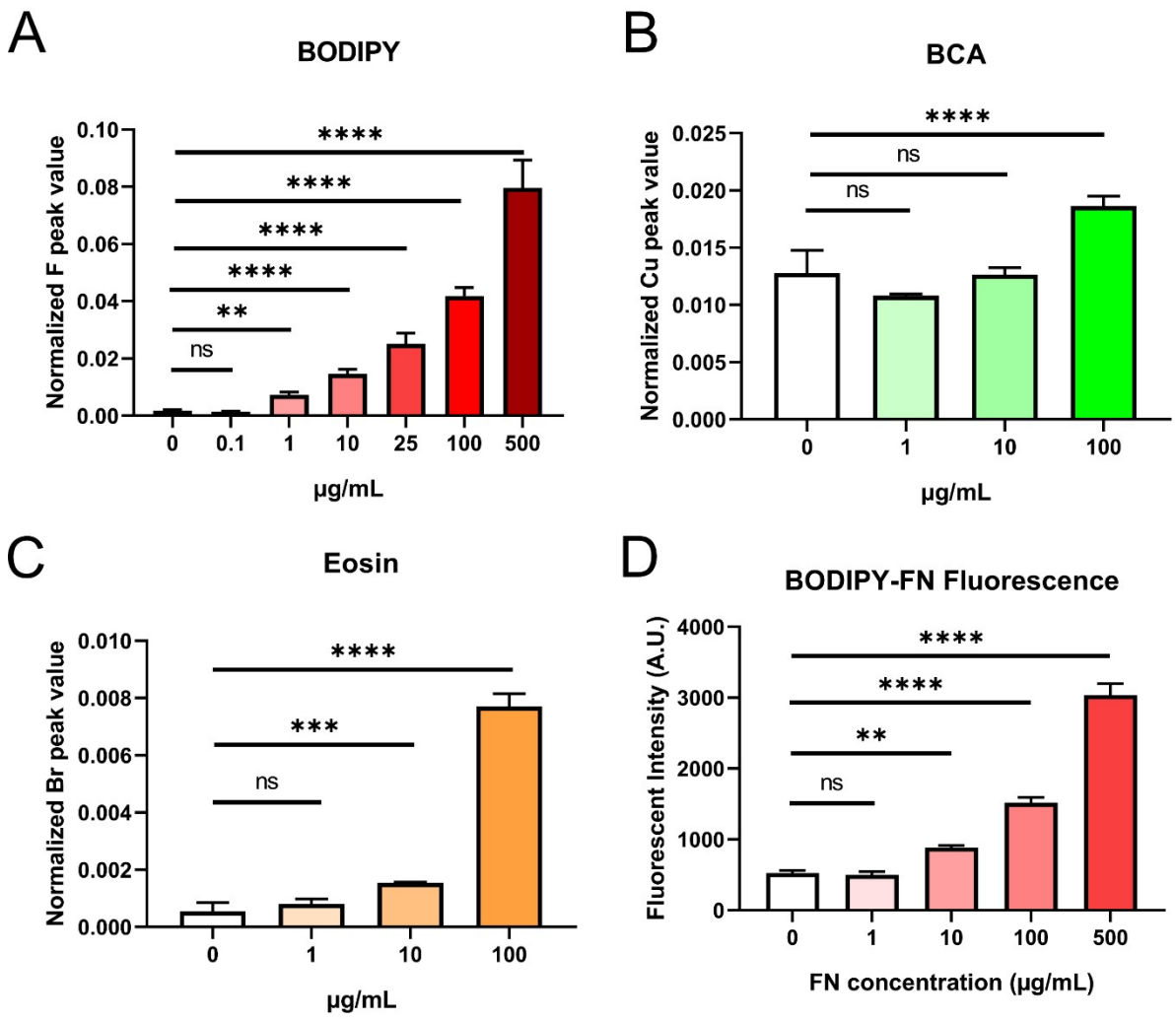
Condition	Total Count (mean ± SD)	F Count (mean ± SD)	Normalized Value (mean ± SD)
Control	360,695 ± 39,051	654 ± 74	$1.83\times10^{-3} \pm 2.94\times10^{-4}$
0.1 µg/mL FN	373,663 ± 80,307	564 ± 172	$1.49\times10^{-3} \pm 1.62\times10^{-4}$
1 µg/mL FN	371,459 ± 23,260	2,270 ± 467	$7.30\times10^{-3} \pm 1.02\times10^{-3}$
10 µg/mL FN	359,560 ± 49,511	5,297 ± 1,112	$1.46\times10^{-2} \pm 1.57\times10^{-3}$
25 µg/mL FN	364,388 ± 37,795	9,156 ± 1,646	$2.51\times10^{-2} \pm 3.77\times10^{-3}$
100 µg/mL FN	363,236 ± 57,319	15,291 ± 3,040	$4.19\times10^{-2} \pm 2.87\times10^{-3}$
500 µg/mL FN	485,247 ± 137,104	38,067 ± 10,209	$7.96\times10^{-2} \pm 9.74\times10^{-3}$



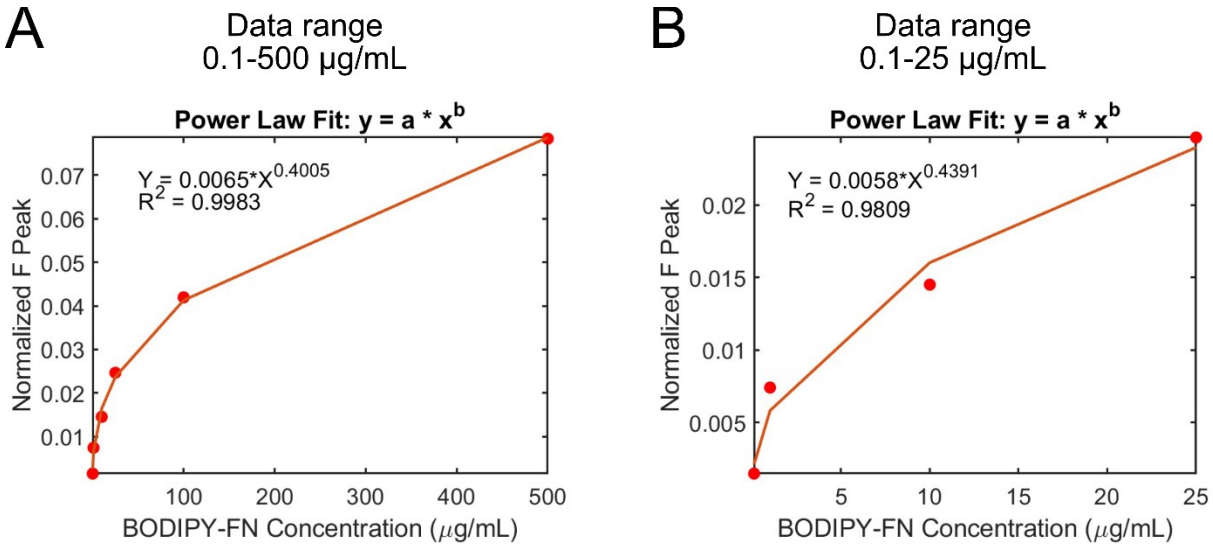
Supplementary Figure 1. ToF-SIMS analysis of varying concentrations of fibronectin (FN) coated onto plastic slide surfaces, using BCA staining as a surrogate marker. **(A)** Representative ToF-SIMS images illustrating copper (Cu) ion signals (m/z 62.83–63.03) across fibronectin (FN) coatings ranging from 1 to 100 µg mL-1, with water as a control. **(B)** Cu ion peak intensities at m/z 62.93 for different FN concentrations, displayed using a uniform y-axis scale for comparison. **(C)** Normalized Cu ion counts, calculated by dividing Cu ion counts by total ion counts for each ROI, presented as pink scatter points in a line plot. The water control is indicated by a red dotted line, mean values are shown as blue dots, standard deviations are shown in black, and pink dots represent 4 ROIs. Measurements were acquired in bunched mode.



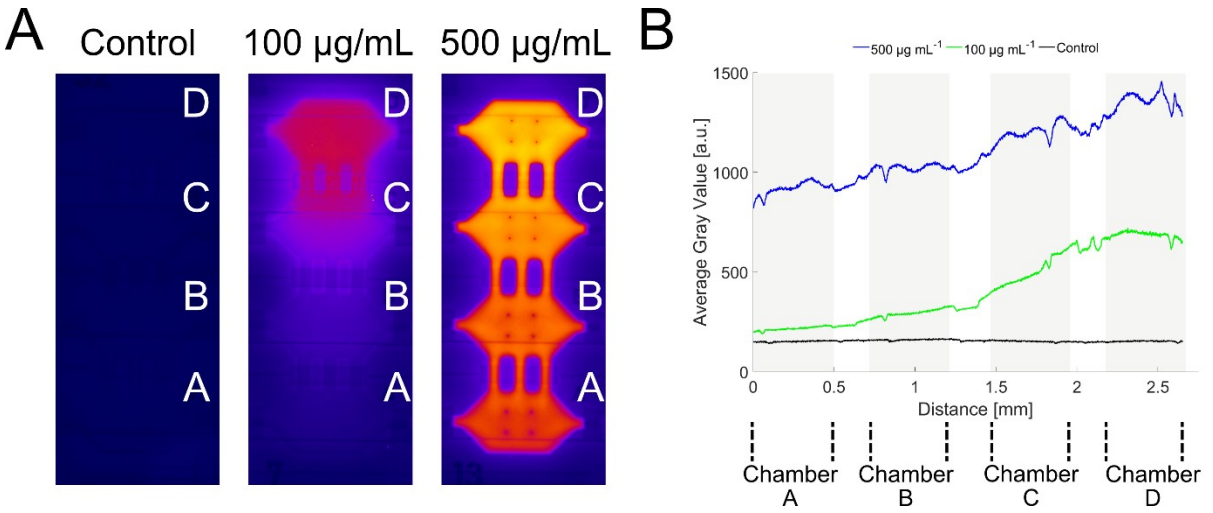
Supplementary Figure 2. ToF-SIMS analysis of varying concentrations of fibronectin (FN) coated onto plastic slide surfaces, using Eosin staining as a surrogate marker. **(A)** Representative ToF-SIMS images illustrating bromine (Br) ion signals (m/z:78.818-79.018) across FN coatings ranging from 1 to 100 µg mL-1, with water as a control. **(B)** Br ion peak at m/z 78.92 for different FN concentrations displayed using a uniform y-axis scale. **(C)** Normalized Br ion counts, calculated by dividing Br ion counts by total ion counts for each ROI, presented as scatter points in a line plot. The water control is indicated by a red dotted line, mean values are shown as blue dots, standard deviations are shown in black, and pink dots represent 4 ROIs. Measurements were acquired in bunched mode.



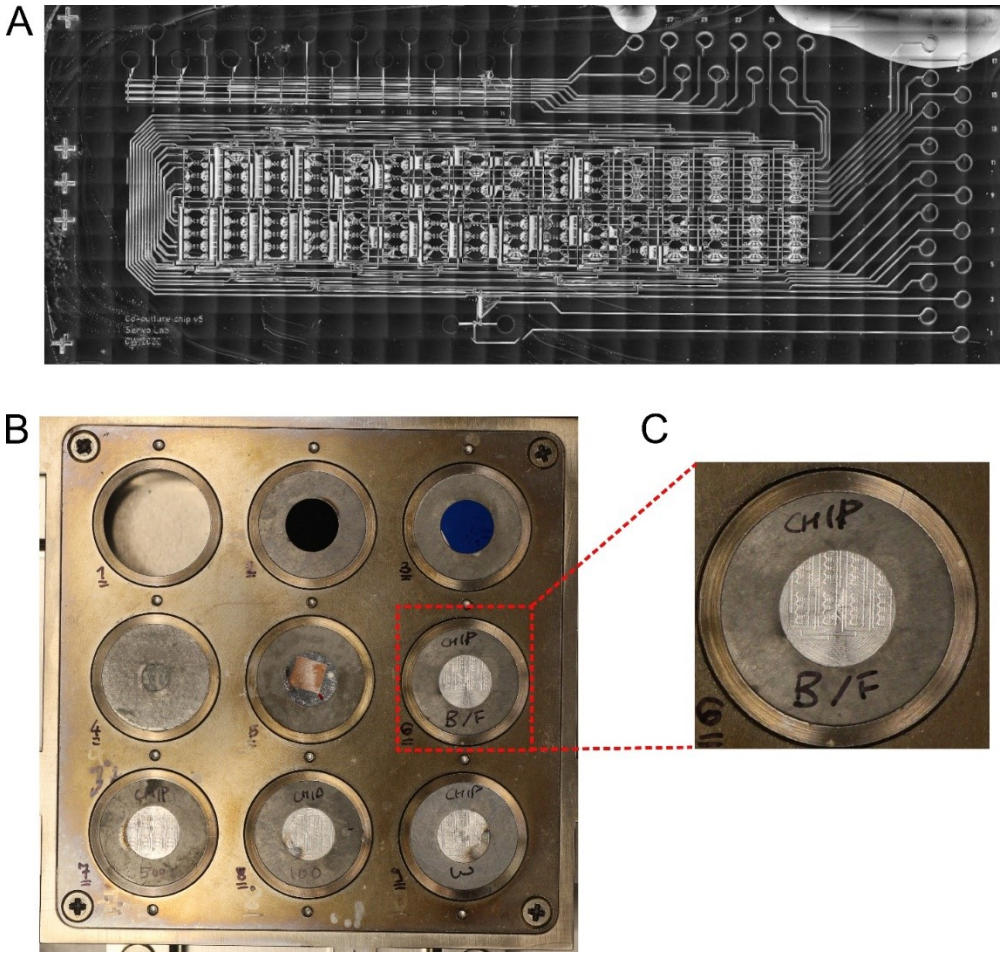
Supplementary Figure 3. ToF-SIMS analysis of varying concentrations of BODIPY–FN **(A)**, fibronectin labeled with surrogate BCA **(B)**, fibronectin labeled with Eosin **(C)**, and fluorescence measurements of BODIPY–FN at corresponding concentrations **(D)**. Statistical comparisons were made against the water control (0 µg mL^{-1}) using one-way ANOVA with a post hoc Dunnett test. ns: not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.



Supplementary Figure 4. Power law fits were applied to the BODIPY-FN standard concentrations for **(A)** data in the range of 0.1–500 µg mL^{-1} and **(B)** data in the range of 0.1–25 µg mL^{-1}. The fitting equations and R-squared values are provided on the graphs.



Supplementary Figure 5. BODIPY-FN gradient formation in the microfluidic chip visualized by fluorescence imaging. (A) Fluorescence images of BODIPY-FN gradients formed by 24-hour diffusion from source chambers containing 100 µg mL^{-1} or 500 µg mL^{-1} BODIPY-FN, compared to the water control. (B) Averaged line profiles of fluorescence intensity across chambers A–D, demonstrating gradient formation.



Supplementary Figure 6. Images of the microfluidic chip used to generate BODIPY-FN gradients and prepare samples for ToF-SIMS analysis. **(A)** Overview of the microfluidic chip after removal of the PDMS chamber, revealing the underlying plastic surface. **(B)** Sample plate showing the microfluidic slide cut into smaller pieces for ToF-SIMS measurement. **(C)** Close-up image of the microfluidic chambered slide used for ToF-SIMS analysis.