Planar Electrochromatography using an Electrospun Polymer Nanofiber Layer

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Supporting Information (SI)

The following information provides a more detailed experimental describing the preparation of the electrospun stationary phases as well as specifics for UTLC development methods. Additional figures and tables are also presented to support the data described within the text.

Materials and Methods

Electrospun Stationary Phase Preparation

Electrospun PAN nanofiber stationary phases were prepared as previously described.[20] The components in the electrospinning apparatus were modified slightly.[26] A stationary metal plate covered served as the collector, and the solution was electrospun for 20 min unless otherwise noted (see Figure S-4 for spot size variation with mat thickness). After electrospinning, the aluminum foil-backed nanofiber mats were cut into UTLC plates for one-dimensional (2.5 x 5.0 cm) or two-dimensional (4.0 x 4.0 cm) separations. UTLC separations were performed directly on the foil-backed nanofiber stationary phases. For PEC separations, the nanofiber stationary phases were peeled off of the foil backing and placed on glass substrates the same size as the stationary phase.

Plate Development

Prior to development, analytes were allowed to dry for 1 h. Spotting was followed by pre-wetting of the stationary phase with mobile phase within 3 mm of the spot using Whatman chromatography paper (Whatman 3MM Chr sheets, Sigma Aldrich). A glass plate covered the stationary phase during separations.

Ultra-Thin Layer Chromatography and Two-Dimensional (2D) Planar Separations

A cylindrical glass jar (volume = 250 mL) topped with a watch glass served as the UTLC development chamber. 5 mL of freshly prepared mobile phase was allowed to equilibrate in the chamber for 15 min before development. Plates were developed vertically to a 25 mm separation distance, and the same visualization technique described in the "Planar Electrochromatography" section in the article was used.

Effect of Nanofiber Alignment

Rather than electrospin randomly-placed nanofibers, alignment of the nanofibers has been shown to be advantageous to the chromatographic performance of the stationary phase.[23] Compared to randomly-placed nanofibers, highly-aligned PAN nanofiber stationary phases have demonstrated increased speed of analysis and efficiency.[23] Consequently, the impact of nanofiber alignment on PEC separation was explored. Both moderately aligned and highly aligned nanofibers were studied and compared to randomly-placed nanofibers. Moderately aligned nanofibers were produced at a drum rotational speed of 500 rpm and highly aligned nanofibers were produced at 1250 rpm; using these conditions, ~45% and ~60% of nanofibers were within 10° of the direction of alignment among moderately aligned and highly aligned nanofibers, respectively.[23] 25:25:50 ACN/2-PrOH/25 mM citrate buffer, pH 5.6 (v/v/v) was used for separations on moderately and highly aligned nanofiber plates.

The effect of nanofiber alignment on analyte migration distance is shown in Table S-2 for 1 min separations using randomly-placed nanofiber stationary phases compared to moderately aligned and highly aligned nanofiber stationary phases. Clearly, migration distances increased with the degree of nanofiber alignment due to an apparent increase in the rate of EOF. Laser dyes migrated 1.6-7.9 times further on highly aligned nanofiber plates compared to randomly-placed nanofiber plates, with the largest differences observed for the negatively charged KR and SR. This trend was attributed to difference in effective pore radius. Larger pore size stationary phases are known to exhibit faster EOF in electrochromatographic separations.[1, 43,44] As pore sizes increase, EOF through the pores is faster as the resistance to flow is diminished.[44] The pore size of highly aligned nanofibers (1200 ± 70 nm) is significantly larger compared to randomly-placed

nanofibers (280 ± 10 nm).[23] Therefore, the four-fold increase in effective pore diameter with nanofiber alignment directly corresponded to increased analyte migration and rate of EOF.

In addition, highly aligned nanofiber plates produced lower plate height values (54.2-115 μm) compared to those observed on randomly-placed nanofiber plates (36.0-331 μm) using 1 min separation times. This was attributed to longer migration distances on aligned nanofiber plates (refer to the "Band Broadening" section in the article). To achieve similar migration distances, randomly-placed nanofiber plates required a separation time of 2 min (Figure S-5) and moderately aligned nanofibers required 1.5 min compared to 1 min using highly aligned nanofibers (Table S-3). At these distances, selectivity on randomly-placed nanofiber plates was greater than the selectivity on highly aligned nanofiber plates as noted by a larger range in migration distances (distances ranged 6.93 mm and 4.06 mm, respectively). This was likely due to differences in the amount of exposure of analytes to the stationary phases.[23] Larger migration distances on highly aligned nanofiber stationary phases meant that analytes spent less time interacting with this phase during the 1 min separation which led to lower selectivity. Interestingly, moderately aligned nanofiber plates showed characteristics of both randomly-placed and highly aligned nanofibers in that selectivity remained relatively high similar to randomly-placed nanofibers (distances ranged 9.81 mm) but analytes migrated at fast rates similar to highly aligned nanofibers. *H* values were also compared; in particular *H* was compared between analytes which migrated the furthest, namely R101, R590, and R610, since *H* decreases with migration distance. *H* values on moderately aligned plates run for 1.5 min were lower than those observed on highly aligned plates run for 1 min; moreover, *H* values on randomly-placed plates run for 2 min were typically lower than both aligned nanofiber plates (Figure S-5). This trend correlated directly with developed spot widths on the different plates (Table S-4). In

general, increasing the degree of nanofiber alignment increased spot sizes. Larger spots were attributed to the fast wicking behavior of aligned nanofibers which affected analytes during sample application. The effect was two-fold. First, initial spots took on an elliptical shape, rather than circular shape, in the direction of nanofiber alignment. Because this was also the direction of development, spots generally remained elliptically shaped after development.[26] More importantly, fast wicking led to more diffusion of initial spots between pre-wetting and the start of the separation compared to non-aligned nanofibers. Wicking was noticeably faster for highly aligned nanofibers and therefore spots had more time to diffuse before separation. Overall, randomly-placed nanofiber plates displayed high efficiency and selectivity but provided the lowest migration rates; highly aligned nanofibers plates displayed low efficiency and selectivity but provided the fastest migration rates; and moderately aligned plates offered high selectivity and promoted fast migration rates while maintaining considerable efficiency. These data suggest that moderately aligned stationary phases are ideal for fast and simple separations while randomly-placed stationary phases are ideal for more complex mixtures which may require smaller spots. Therefore, separations in the published manuscript were performed on randomly-placed nanofiber plates.

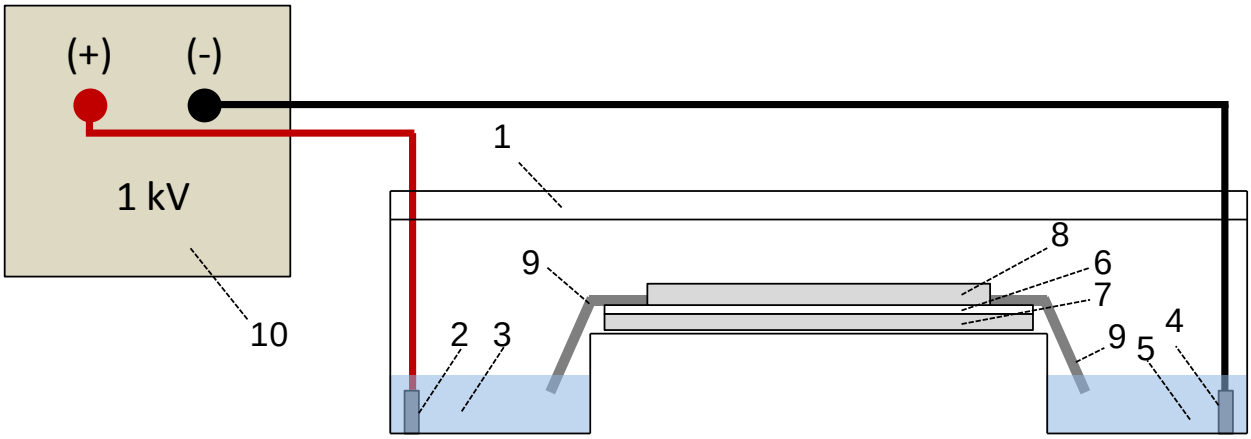


Figure S-1. Apparatus used for PEC. (1) Horizontal chamber with lid, (2) platinum anode, (3) anode reservoir filled with mobile phase, (4) platinum cathode, (5) cathode reservoir filled with mobile phase, (6) electrospun nanofiber stationary phase, (7) glass back plate, (8) glass cover plate, (9) Whatman 3MM wicks, and (10) high voltage power supply.

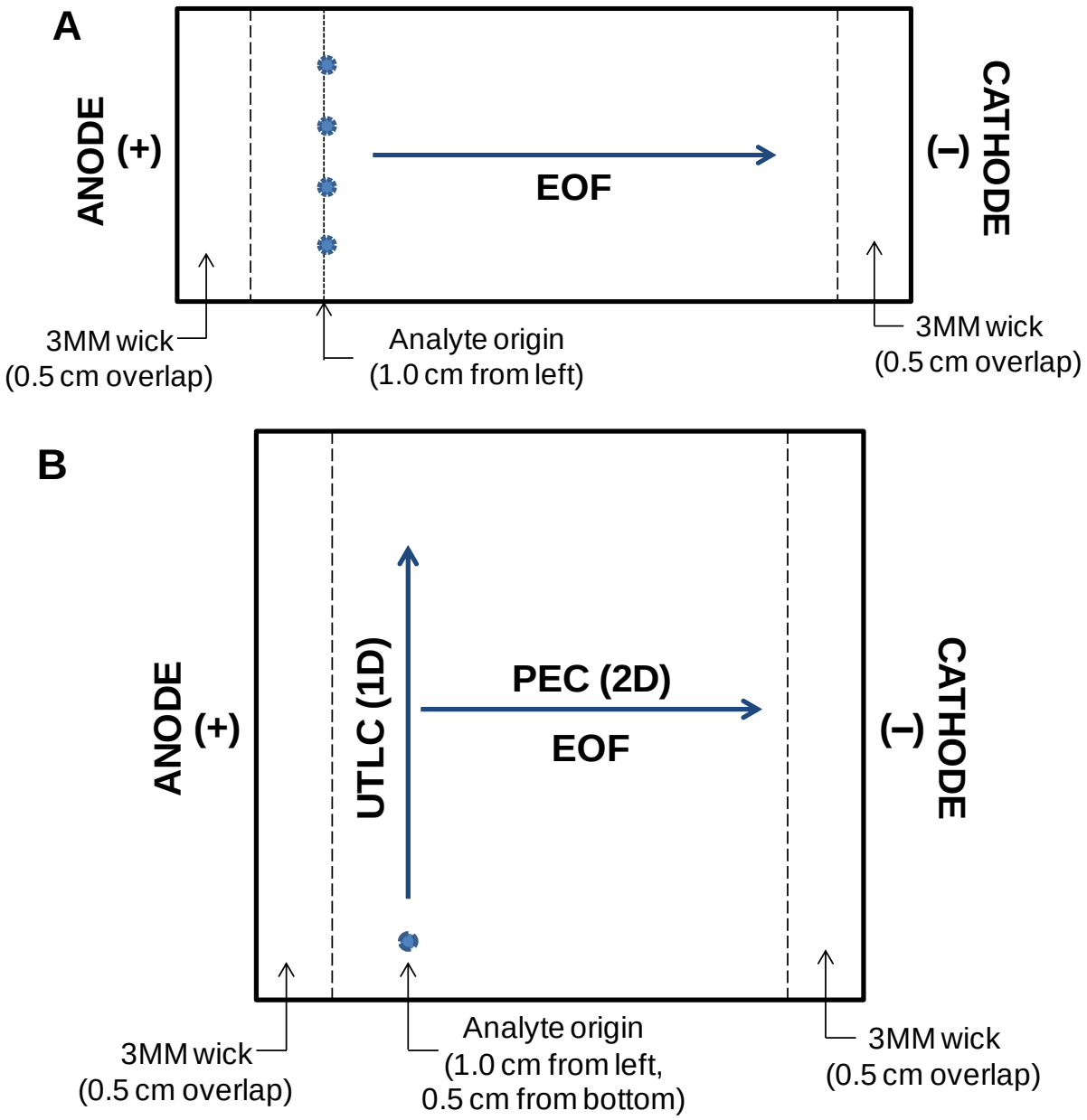
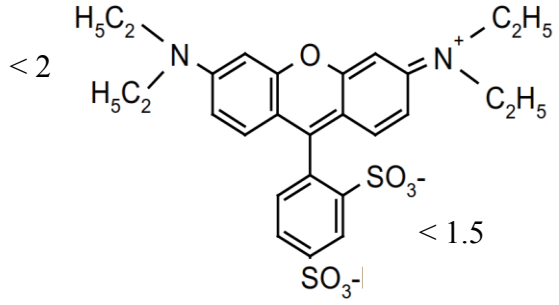
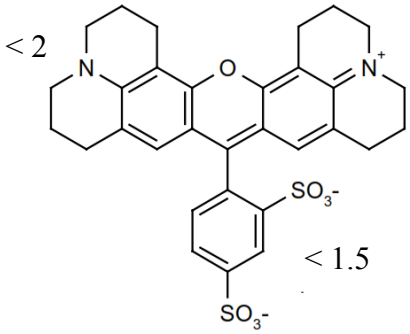
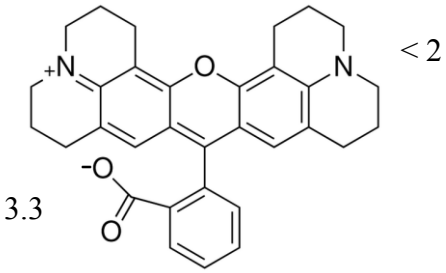
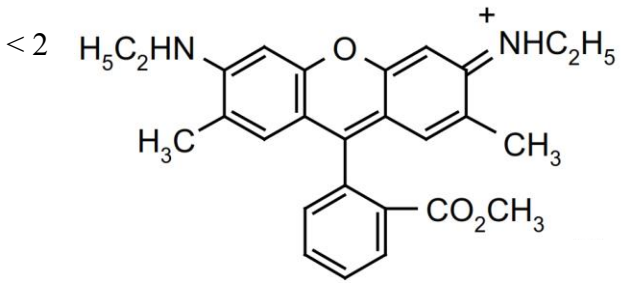


Figure S-2. The location of the analyte origin, wicks, anode, and cathode relative to each other on the 2.5 cm x 5.0 cm electrospun PAN nanofiber plate used for PEC separations (A) and the 4.0 cm x 4.0 cm plate used for 2D UTLC-PEC separations (B). The arrows note the direction of EOF during PEC and the direction of mobile phase flow during UTLC.

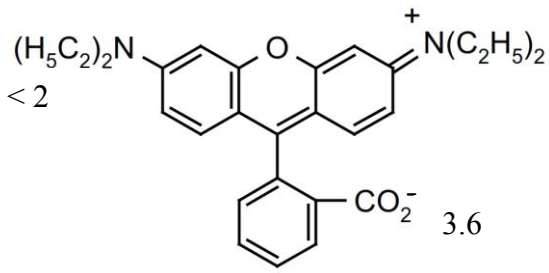
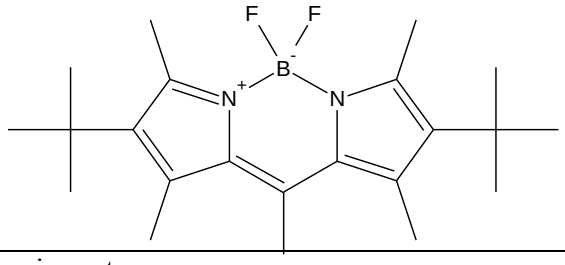
Table S-1 Chemical structures of the investigated laser dyes. pK_a values are listed next to the various functionalities present. The autoprotolysis constants (pK_{ap}) of aqueous solvents used in this system are 15.07 for 2-propanol-water mixtures (50% 2-propanol) and 15.48 for acetonitrile-water mixtures (50% acetonitrile)[49,50,51]

Laser Dye	**Structure and pK_a**[a]
Kiton red 620	
Sulforhodamine 640	
Rhodamine 101	
Rhodamine 590 chloride	

[a]The listed pK_a values are for the analytes in water.

Continued

Table S-1 cont.

Laser Dye	**Structure and pK_a**[a]
Rhodamine 610 chloride	 < 2
Pyrromethene 597	

[a]The listed pK_a values are for the analytes in water.

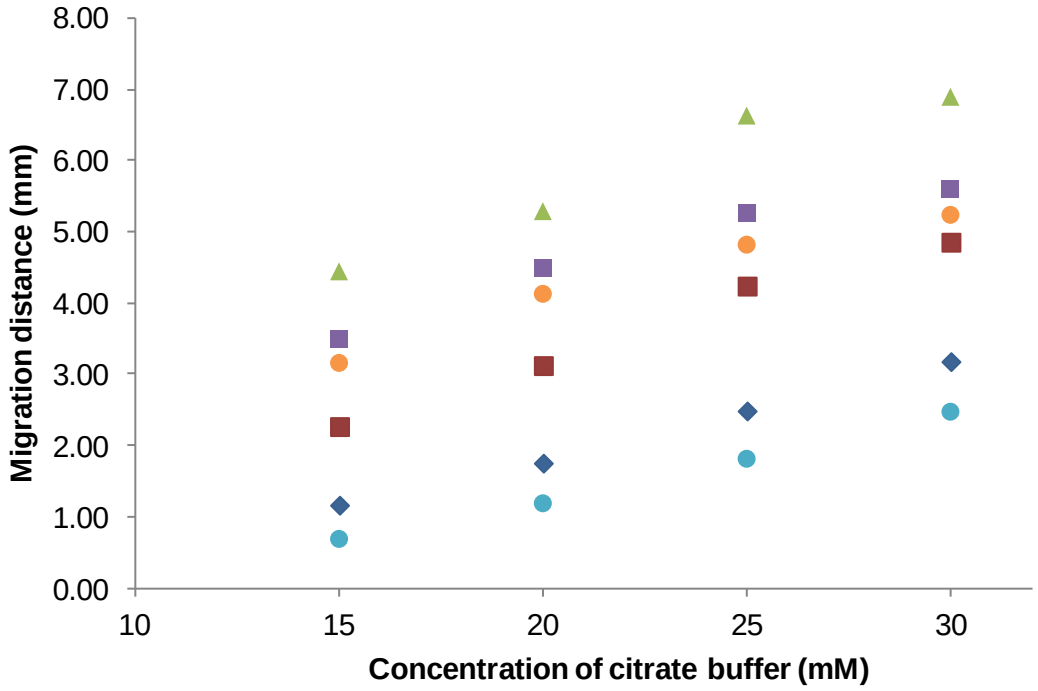


Figure S-3. Migration distance of laser dyes versus citrate buffer concentration in the PEC system. Laser dyes: KR (♦), PM (■), R101 (●), R590 (▲), R610 (■), and SR (●). Experimental conditions: potential applied for 1 min, 25:25:50 ACN/ 2-propanol/ citrate buffer, pH 6.0 (v/v/v) ran at 1 kV.

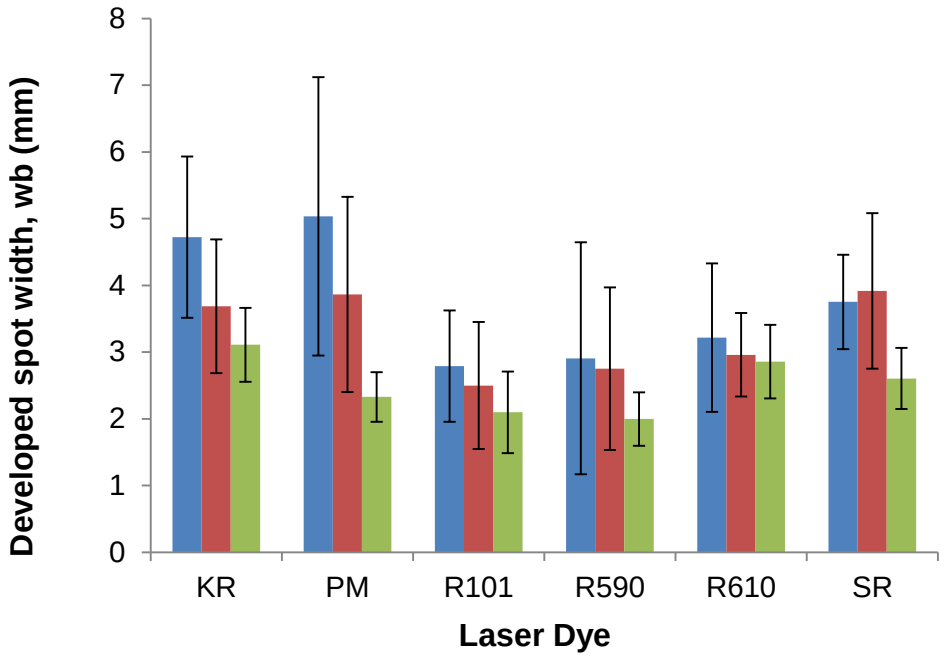


Figure S-4. Developed spot widths, w_b, of laser dyes separated for 1 min on nanofiber plates generated at different electrospinning times: 5 min, 12 µm thick (■); 10 min, 25 µm thick (■); or 20 min, 27 µm thick (■). Experimental conditions: 25:25:50 ACN/ 2-propanol/ 25 mM citrate buffer, pH 5.6 (v/v/v) mobile phase ran at 1 kV.

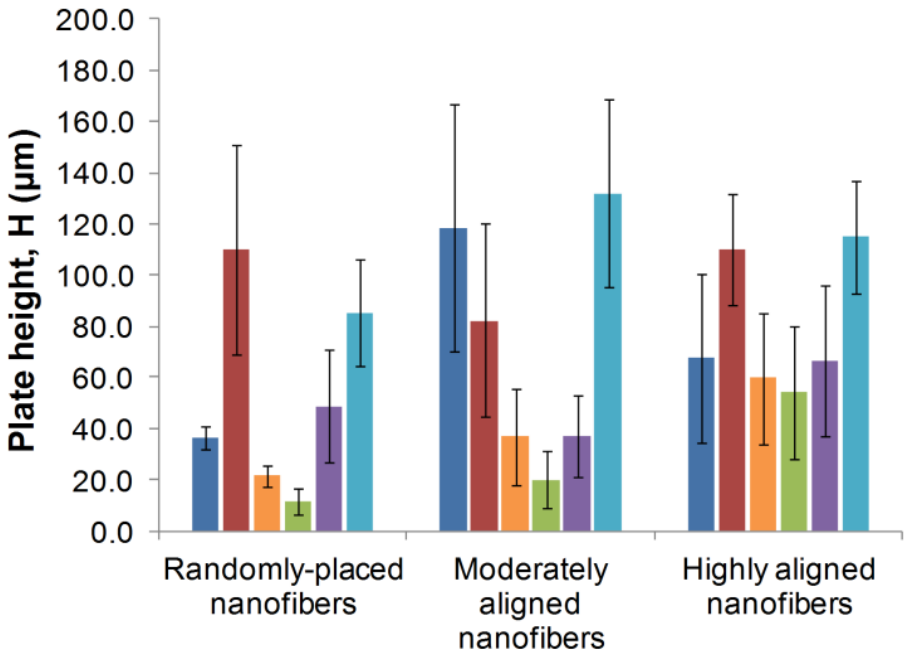


Figure S-5. Plate height values of laser dyes on randomly-placed nanofiber plates ran for 2 min compared to moderately aligned and highly aligned nanofiber plates ran for 1.5 and 1.0 min, respectively. Different times correlate to similar analyte migration distances. Analytes: KR (■), PM (■), R101 (■), R590 (■), R610 (■), and SR (■).

Table S-2. Migration distances (%RSD) and developed spot widths, w_b, of laser dyes on nanofiber plates generated at different electrospinning times: 5 min or 20 min. PEC was performed for 1 min on the 5 min plate and 1.5 min on the 20 min plate to achieve similar migration distances. Experimental conditions: 25:25:50 ACN/ 2-propanol/ 25 mM citrate buffer, pH 5.6 (v/v/v) mobile phase ran at 1 kV.

Laser Dye	5 min, 12 µm thickness		20 min, 27 µm thickness	
	Migration Distance (mm)	w_b (mm)	Migration Distance (mm)	w_b (mm)
KR	4.18 (29)	4.72 (26)	3.92 (24)	2.39 (28)
PM	7.06 (30)	5.03 (21)	6.19 (24)	4.40 (33)
R101	7.92 (11)	2.79 (29)	8.88 (8)	2.11 (32)
R590	10.1 (17)	2.91 (30)	9.64 (5)	1.57 (28)
R610	7.40 (15)	3.42 (35)	8.00 (13)	3.03 (25)
SR	3.59 (20)	3.75 (19)	3.27 (28)	3.02 (24)

Table S-3. Migration distance (%RSD) of laser dyes in UTLC and PEC mode using the same mobile phase. UTLC was performed over a 15 mm separation distance (>8 min) and PEC was performed with a 2 min separation time. Experimental conditions: 25:25:50 ACN/ 2-propanol/ 25 mM citrate buffer, pH 5.6 (v/v/v) mobile phase on PAN nanofiber stationary phases fabricated with a 20 min electrospinning time.

Laser Dye	Migration Distance (mm)	
	UTLC	PEC
KR	13.90 (8)	10.00 (5)
PM	12.64 (18)	13.01 (5)
R101	14.20 (6)	14.79 (11)
R590	10.32 (23)	15.69 (24)
R610	14.27 (7)	14.42 (22)
SR	14.50 (8)	8.76 (10)

Table S-4. Spot widths, w_b (%RSD) of laser dyes on randomly-placed nanofiber plates ran for 2 min compared to moderately aligned and highly aligned nanofiber plates ran for 1.5 and 1.0 min, respectively. Different run times correlate to similar migration distances of analytes. Moderately aligned nanofibers were produced at 500 rpm and highly aligned nanofibers were produced at 1250 rpm.

Laser Dye	Developed Spot Width, w_b (mm)		
	Randomly-placed nanofiber plate (2 min)	**Moderately aligned nanofiber plate (1.5 min)**	**Highly aligned nanofiber plate (1 min)**
KR	2.41 (18)	3.01(22)	3.15 (31)
PM	4.78 (34)	3.39(20)	4.47 (11)
R101	2.25 (11)	2.93(23)	3.47 (40)
R590	1.69 (29)	2.21(30)	3.13 (44)
R610	3.34 (33)	2.77(21)	3.75 (41)
SR	3.46 (20)	4.09(15)	4.39 (11)

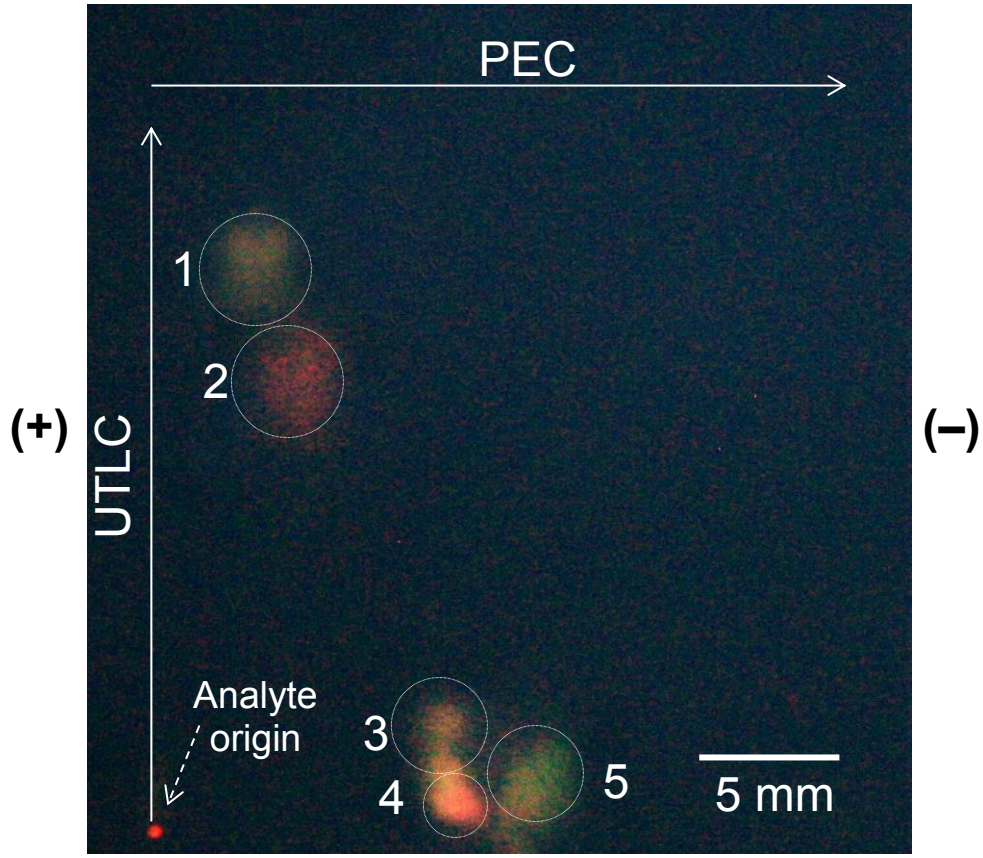


Figure S-6. Digital image of the electrospun stationary phase after a 2D UTLC-PEC separation of five laser dyes. Contrast has been enhanced for clear recognition of analyte colors (red, orange, or yellow). The analyte origin was at the bottom corner. UTLC was performed first using 90:10 2-propanol/ methanol (v/v) over 25 mm. PEC was performed second (perpendicular to UTLC) for 1 min using 25:25:50 ACN/ 2-propanol/ 25 mM citrate buffer, pH 5.6 (v/v/v) at 1 kV. Analytes: (1) SR, (2) KR, (3) R610, (4) R101, and (5) R590.

References (only used in SI)

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(50) Roses, M.; Rafols, C.; Bosch, E. *Anal. Chem.* **1993**, *65*, 2294.

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