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Online Photolytic Optical Gating of Caged Fluorophores in Capillary Zone Electrophoresis Utilizing an Ultraviolet-Light Emitting Diode

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Abstract

Photolytic optical gating (POG) facilitates rapid, on-line and highly sensitive analyses, though POG utilizes UV lasers for sample injection. We present a lower-cost, more portable alternative, employing an ultraviolet (UV)-LED array to inject caged fluorescent dyes via photolysis. Utilizing the UV-LED array, labeled amino acids were injected with nanomolar limits of detection (270 ± 30 nM and 250 ± 30 nM for arginine and citrulline, respectively). When normalized for the difference in light intensity, the UV-LED array provides comparable sensitivity to POG utilizing UV lasers. Additionally, the UV-LED array yielded sufficient beam quality and stability to facilitate coupling with Hadamard transformation, resulting in increased sensitivity. This work shows, for the first time, the use of a UV-LED for online POG with comparable sensitivity to conventional laser sources but for lower cost.

Keywords

Capillary zone electrophoresis; Hadamard transformation; Photolytic optical gating; Ultraviolet-light emitting diode

1. Introduction

Physiological function is regulated by neurotransmission where alterations in the storage, release, transport, or reuptake of neurotransmitters leads to various conditions [1]. Quantifying neurotransmitters within an organism is difficult because signaling events are rapid, with cellular and pharmacokinetic responses occurring on second to minute timescales, neurotransmitters are released or present in low concentrations (pM – mM), and sampling from an organism necessitates that only small volumes be removed to avoid disruption of homeostasis [2]. CZE-LIF is often used for neurotransmitter release studies due to the small sample volumes, rapid separations and high sensitivity [3–5].

Direct, online sampling yields the highest temporal resolution of neurotransmitter dynamics [5,6]. Microdialysis probes have been coupled to separation capillaries by flow gating [3,5–8], segmented flow [9–11], and optical gating [12–17]. Of these approaches, optical gating

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Conflict of Interest

The authors claim no conflict of interest associated with this work.

of fluorophore-labeled analytes facilitates the shortest injection lengths, fastest separations and yields the highest separation efficiency [12–17]. For photobleaching optical injections, a continuous laser beam is focused into the capillary to photobleach the sample. Transient blockage of the beam allows plugs of fluorescent material to be injected over the photobleached background [13–16]. In most cases, photobleaching is less than 90 % effective [13], yielding increased noise, and a consequent decrease in sensitivity [13,14]. Photolytic optical gating (POG) circumvents these issues by utilizing caged fluorophores that exhibit markedly decreased fluorescence quantum yields prior to photolysis. Samples are injected upon transient exposure to UV radiation, via photolytic uncaging of the fluorophore to generate highly fluorescent sample plugs [12,17]. POG offers increased sensitivity compared to photobleaching by providing lower background signal and reduced noise [12,17]. Previously, we reported the use of a multi-line laser for online photolytic injection and subsequent LIF detection of amino acids labeled with caged fluorescein succinimidyl ester [12]. LODs for labeled amino acids were approximately 1 nM, showing 2 – 3 orders of magnitude enhanced sensitivity compared to photobleaching injections of fluorescein or NBD utilizing the same optical geometry [13,18]. Though this method is highly sensitive, it is complicated since uncaging the fluorophore necessitates the use of UV radiation and optimum detection utilizes visible radiation [12,17].

Recently, high power LEDs that span the UV and visible spectra have become viable alternative light sources for many spectroscopic methods [19]. LEDs have higher stability when compared to conventional fluorescence lamps [20–22], lower power consumption and longer lifetimes than lasers [20], and are available in a wide spectral range with narrow bandpass [19]. Visible and UV-LEDs have been used for LED-induced fluorescence (LED-IF) detection in CE separations with comparable sensitivity to LIF detection [20,21,23–25]. Additionally, UV-LEDs have been used for photolytic activation of caged dyes within cells to perform imaging studies [26] and for photolytic generation of concentration gradients in ion exchange chromatography [27], though, to our knowledge there are no previous reports of LED-photolytic injection for separation.

Here we demonstrate the first use of an UV-LED array for online photolytic injection of caged fluorophores in CZE. Further, we coupled UV-LED POG to Hadamard transformation capillary electrophoresis (HTCE) [17,28–33] to demonstrate enhanced improvements in sensitivity with minimal instrumental modification.

2. Materials and Methods

2.1 Materials and reagents

5-carboxyfluorescein-bis-(5-carboxymethoxy-2-nitrobenzyl) ether, β -alanine-carboxamide, succinimidyl ester (caged fluorescein succinimidyl ester, CFSE) was purchased from Invitrogen (Carlsbad, CA). Boric acid was purchased from Spectrum (Gardena, CA). D,L-arginine (Arg) was purchased from Sigma (St. Louis, MO). L-citrulline (Cit) was purchased from Alfa Aesar (Ward Hill, MA). All other chemicals were obtained from VWR (Radnor, PA), and used as received. All solutions were prepared using deionized water (Barnstead).

2.2 Sample preparation

CFSE was dissolved in DMSO to generate a 2 mM stock solution. Each amine (Cit or Arg) was labeled individually offline at 100 μ M by reaction with CFSE at a mole ratio of 1.00 amine:1.25 CFSE in 20 mM borate buffer (pH 10) and a reaction volume of 100 μ L. Reactions were performed for 3 hours at room temperature in the dark and diluted with 20 mM borate buffer (pH 8.3) before POG and separation.

2.3 Instrumentation and separation

A UV-LED array consisting of four UV-LEDs (total power = 900 mW, $\lambda_{\max} = 365$ nm, $\Delta\lambda_{1/2} = 9$ nm, Model NC4U133(T), Nichia America Corporation, Southfield, MI, USA) in a two by two matrix was mounted on an aluminum cooling plate (Figure SI-2). UV-LED emission was collected using a fused-silica plano-convex lens ($f = 100$ mm, Melles Griot, Irvine, CA) and focused with an additional plano-convex lens ($f = 100$ mm). A LS3 mechanical shutter with VMM-T1 controller (Uniblitz LS3, Vincent Associates, Rochester, NY) was positioned at the focal point of the second lens. Two additional fused-silica lenses, a planoconvex ($f = 75$ mm) and biconvex ($f = 35$ mm) (Melles Griot) were used to focus the light into the capillary. The power of the UV-LED array at the capillary was measured to be 400 μ W using a Model 1815-C power meter (Newport Corporation, Irvine, CA). Optical gating was controlled by the shutter which responded to a TTL signal written into the LabVIEW (National Instruments, Austin, TX) program used for data collection.

For detection of uncaged CFSE ($\lambda_{\max, \text{Abs}} = 494$ nm), the 496 nm line of an Ar⁺ laser (Innova 70C series, Coherent, Santa Clara, CA), was focused into the center of the separation capillary using a biconvex lens ($f = 25$ mm) (Melles Griot). The beam power at the capillary was 28 mW. The fluorescence signal was collected using a microscope objective (20X, 0.4 N.A., Melles Griot) before passing through a spatial filter, a band pass filter (D525/25 M, Chroma Technology, Rockingham, VT), and a 488 ± 12 nm holographic notch filter (HNF-488.0 – 1.0, Kaiser Optical Systems, Ann Arbor, MI.). Fluorescence was detected by a photomultiplier tube (H957, Hamamatsu Photonics, Bridgewater, NJ), then amplified and filtered by a current amplifier (Model 428, Keithley, Cleveland, OH), and collected by an A/D converter (NI USB-6221, National Instruments) using in-house software written in LabVIEW. Electric fields were applied using a 30 kV power supply (CZE-1000, Spellman High Voltage Corporation, Hauppauge, NY). An instrument diagram is presented in supporting information (Figure SI-1).

CFSE was used as a migration time marker in the electropherograms.

2.4 Capillary preparation

Capillaries (25 μ m i.d.; 360 μ m o.d.; Polymicro Technologies, Phoenix, AZ) were cut to 45 cm total length with a separation distance of 24 cm. Before use, capillaries were washed with consecutive rinses of 0.1 M HCl, H₂O, 0.1 M NaOH, H₂O, and 20 mM borate buffer pH 8.3 for 5 min each. Sample constantly flowed through the capillary inlet due to the applied field and EOF within the capillary; thus, sample injections refer to photolytic conversion of sample.

2.5 Hadamard transformation

Pseudorandom injection sequences based on simplex matrices (S-matrices) [17,28–30,34] were constructed using an in-house Java program implementing the maximal length shift-register method [35]. The algorithm was modified slightly so that each first element was an injection to facilitate proper alignment of the injection sequences. Injection sequences were constructed by duplicating the first row of the S-matrix and removing the last element in the sequence to generate a $2n-1$ element injection sequence, where n represents the number of matrix elements [30]. Matrices of 1023 or 2047 elements with 50 % duty cycles were used for injection. Following injection and separation, the detector monitored summed signal associated with the multiple injections and the different electrophoretic mobility of each compound. The convoluted, raw data was transformed to represent a single injection electropherogram by multiplying the $(n)^{\text{th}}$ to $(2n-1)^{\text{th}}$ data points by the inverse S-matrix [29,30]. HTCE results in a theoretical S/N enhancement proportional to the size of the matrix used for analysis (n) [28–,31,36].

$$S/N \text{ enhancement} = (n+1)/2n^{1/2} \quad (1)$$

Deconvolution of the raw data to acquire an electropherogram with enhanced S/N was performed by multiplying the convoluted, raw data by the inverse S-matrix [30–32]. For matrix multiplication, the matrices must contain the same number of elements in at least one dimension; thus, HTCE experiments are generally performed using the same data collection and injection rates. For the experiments performed in this work, oversampling was used to collect multiple data points during each injection length, which was necessary for synchronizing collection with the first injection. All of the data points collected (500 Hz) within the time of each injection (10 Hz) were binned and then deconvoluted with the inverse S-matrix. All single injection electropherograms were manipulated in the same way for direct comparison.

2.6 Data filtering

Low frequency baseline drifts were subtracted using a LabVIEW program written in-house implementing a median filter with a 600 point window size [37]. A median filter was also used to remove occasional high frequency system interference peaks from the electropherograms using a window size of 3 [37].

2.7 Calculations

LODs ($S/N = 3$) were calculated based on calibration plots ($R^2 > 0.99$) generated using 300 nM, 450 nM, 600 nM, and 900 nM solutions for each amine with 500 ms injection lengths. The background noise was directly proportional to the CFSE concentration within the capillary; therefore, runs were performed with a constant concentration of 1.1 μM CFSE. A small linear dynamic range was used since the lowest concentration approached the LOD of the amines and higher concentrations of amine could not be used while maintaining the same labeling ratio and without increasing the CFSE concentration.

S/N values were calculated by dividing the peak heights by the standard deviation of a 20 s segment of baseline where no peaks were present.

3. Results and Discussion

3.1 Analysis of a UV-LED for online photolytic injection

CZE separations are useful for monitoring dynamic processes since they are rapid and result in high separation efficiencies. Yet, to monitor these dynamic processes, injection into the capillary must be performed rapidly, preventing the use of traditional hydrodynamic and electrokinetic injections. Here we show the use of an online method for rapid injection of analytes utilizing an LED as an injection source. Figure 1A shows CZE-LIF electropherograms resulting from POG injections of 500 nM CFSE in the presence and absence of UV-LED irradiation. Sample peaks were observed only when the UV-LED array was focused into the capillary, demonstrating that the sample was uncaged only in the presence of UV irradiation. Based on these single injection electropherograms, the LOD for CFSE was 15 ± 6 nM, approximately 100 times higher than the LOD from our previous work using the 350–365 nm UV lines of an Ar^+ laser. This difference in sensitivity is due to the approximate 100 fold difference in radiant power between the UV-laser lines and UV-LED. Photolytic injection efficiency is directly proportional to the radiant power of the injection beam, thus the lower power UV-LED array likely yields a smaller sample injection volume and a consequent decrease in the mass of sample injected [17]. Though the sensitivity is lower, this key result clearly demonstrates that a UV-LED array can generate sufficient power to perform POG injections in CZE-LIF. Furthermore, LEDs offer many

advantages over traditional laser sources for POG in that they are smaller, making them more portable and ideal for microchip or minimized instrumentation, consume less power, have longer lifetimes, can be purchased for a variety of wavelengths and are generally cheaper [38].

To explore the precision obtained using an UV-LED array, a series of multiple injections were performed and analyzed within the same separation. Figure 1B shows a representative example of the resulting electropherogram with 20 consecutive POG injections of CFSE using the UV-LED array. The relative standard deviation (RSD) for peak height was 3.4 % (Figure 1C), suggesting that there were small variations in the amount of material that was uncaged during each injection. When the UV laser was used for POG of caged fluorescein, peak height reproducibility was 1.3 % RSD [12]. This variation may be due to differences in intensity as well as the higher power stability and better beam profile of Ar⁺ lasers relative to LEDs. With a higher powered light source, small fluctuations in the output power would have a lesser overall effect on the total power reaching the capillary. Though the UV-LED array resulted in lower reproducibility for multiple consecutive injections, the overall RSD is still under 4 %, suggesting the feasibility for quantitative studies using this injection light source.

We next explored the efficiency of the separation as a function of injection time. Band broadening due to injection is a primary source of peak broadening observed in separations employing optical injections [14,15]. The uniformity of the injection beam as well as the beam profile would be expected to contribute to this parameter, factors that vary significantly between collimated lasers and the UV-LED array geometry employed here. The total band broadening can be defined as:

$$\sigma_{\text{Tot}}^2 = \sigma_{\text{Inj}}^2 + \sigma_{\text{Det}}^2 + \sigma_{\text{LD}}^2 + \sigma_{\text{X}}^2 \quad (2)$$

where σ_{Inj}^2 , σ_{Det}^2 , and σ_{LD}^2 define the contributions to the total variance as a function of injection, detection, and linear diffusion, respectively and σ_{X}^2 represents the contribution from all other sources, including Joule heating [14,39,40]. Injection is a substantial source of variance in CZE separations due to the need to precisely inject small volumes of sample onto the low volume capillary. Figure 2 describes the effects of injection on the separation efficiency as well as the overall variance. When the separation efficiency for the CFSE peak was plotted versus injection time (t_{inj}), a linear trend was observed (Figure 2A). Further investigation of this phenomenon showed that the decrease in efficiency was attributed to increased band broadening due to injection (Figure 2B).

Band broadening in optical injections employing lasers was investigated extensively by Moore and Jorgenson [14]. We explored the contributions to band broadening in a similar manner. In this case, a plot of σ_{Tot}^2 versus t_{inj}^2 provides a linear plot where the y-intercept (0.13 s^2) represents all the sources of variance except σ_{Inj}^2 and the slope represents the contribution from σ_{Inj}^2 [14]. The variance due to injection is a function of the injection profile, described by a constant K, and the injection volume according to the following equation [14,39–41]:

$$\sigma_{\text{Inj}}^2 = V_{\text{inj}}^2 / K^2 \quad (3)$$

At fixed flow rates, V_{inj} is directly proportional to t_{inj} , thus when σ_{Tot}^2 is plotted versus t_{inj}^2 the resultant slope describes the injection profile, K (Figure 2B) [14]. Based on Sternberg's theory, the injection profile ranges from exponential ($K = 1$) to plug like ($K = 3.5$) to

Gaussian ($K = 6$) [41]. Prior work with online laser injection (spot size ca. $5\ \mu\text{m}$) has found $K \sim 3.5\text{--}4.0$ [14], whereas the injection observed here was $K \sim 2.5$, approximating plug-like injection with some exponential characteristics. In this work, all injections were controlled by a shutter which opened to illuminate the capillary. Assuming that the opening/closing of the shutter is instantaneous, the injection is expected to be plug-like ($K = 3.5$). Yet, in this system, the UV light can only be focused into a square spot of approximately $1\ \text{mm}$, affecting both beam quality and intensity at the focal point. To reduce the scatter, the capillary is generally positioned at an angle of 20° to the incident light [42]. Thus, approximately $1\ \text{mm}$ of capillary length is illuminated by the LED upon each injection. Furthermore, the focused spot is the combined intensity of four UV-LEDs in a two by two matrix. Thus, the intensity in the vertical and horizontal directions is likely to vary across the focused spot. These variations in illumination likely result in the observed deviations from plug-like injection ($K = 2.5$).

The total variance associated with all other terms was estimated to be $0.13\ \text{s}^2$ (Figure 2B). These values are summarized in Table SI-1. The total variance related to detection from the individual contributions of detection spot size, amplifier electronics and collection rate was estimated to be less than 0.1% of the total non-injection related variance ($\sigma_{\text{Det}}^2 = 1.9 \times 10^{-4}\ \text{s}^2$) [14,15]. Band broadening due to σ_{LD}^2 was estimated to be $0.034\ \text{s}^2$ using previously determined values for the diffusion coefficient of fluorescein [14]. Interestingly, this value accounts for approximately 25% of the total non-injection-related variance. In prior examples of optically-gated CZE, σ_{LD}^2 accounted for ca. 80% of non-injection-related variance. The discrepancy likely results from the capillary i.d., which is limited by the beam profile of the UV-LED. The $25\ \mu\text{m}$ i.d. capillaries used here yield $500\text{--}15000$ fold increase in variance due to Joule heating compared to $5\text{--}10\ \mu\text{m}$ i.d. capillaries used for laser-derived optical injections [14,15].

To evaluate the capabilities of UV-LED POG for analysis of biogenic amines, we explored amino acids in the nitric oxide synthase pathway. Nitric oxide (NO) is a small gaseous molecule that acts as a neurotransmitter in the central nervous system and modulates pain perception, feeding and sleeping behaviors, and thermoregulation [43]. Arg is the biogenic amine substrate for neuronal- and endothelial nitric oxide synthase, enzymes that oxidize the guanidinium group of Arg to form NO and Cit [44,43]. Samples of $450\ \text{nM}$ Arg and Cit were labeled offline with CFSE and analyzed by CZE-LIF with UV-LED POG. Both CFSE-labeled biogenic amines were detected, though the predominant peak ($142\ \text{s}$) in the electropherogram corresponded to excess CFSE (Figure 3A). Investigation of peak height reproducibility, an indicator of injection reproducibility, for 20 consecutive online injections of the amine mixture yielded RSD values of $7.0\ \%$ and $6.5\ \%$ for Arg and Cit, respectively, while the migration times yielded values of $< 0.4\ \%$ for each compound (Figure 3B). These results demonstrate that the UV-LED array can be used for reproducible, online photolytic injection of CFSE-labeled amines, which suggests the feasibility of this approach for analyzing biological samples, including neurochemical analytes.

Using the UV-LED array as a light source, LODs were calculated to be $270 \pm 30\ \text{nM}$ for Arg and $250 \pm 30\ \text{nM}$ for Cit, significantly higher than the ca. $1\ \text{nM}$ LODs reported for CFSE-labeled amino acids using POG with a UV laser [12]. This difference in sensitivity is a result of lower intensity at the focal point for the UV-LED array compared to the laser, and the consequent decrease in injection efficiency. At the capillary, the UV-LED array was measured to be 125 times less intense than the UV-laser lines of the Ar^+ laser. Importantly, the sensitivity of the visible wavelength LIF detector was equivalent to UV-laser POG, thus differences in sensitivity are correlated to the photolytic injection volume. When the LODs for the UV-LED are normalized for the difference in intensity, the sensitivity compared to UV-laser POG is within a factor of 3 , with the slight increase due to increased background

noise resulting from higher CFSE concentrations employed here. Additionally, the current UV-LED array geometry yields four distinct regions of light that were visible at the focal point of the beam (Supporting Information). The net injection efficiency using the UV-LED array may be increased in future iterations by improving the collection geometry of UV-LED emission and the subsequent focal intensity, or by improving the LED beam quality to more closely match that of a collimated laser.

3.2 Utilization of UV-LED POG for HTCE

A variety of multiplexing techniques have been developed for S/N enhancement in CZE [17,29,30,45–48]. HTCE is a mathematical multiplexing technique which decreases the random noise within a system by increasing signal throughput utilizing a pseudorandom injection sequence based on Hadamard matrices [17,28–30]. In HTCE, consecutive injections are performed before the analytes from the previous injection are detected, distributing the random error, or noise associated with the detector, over multiple electropherograms. Following deconvolution of the raw data, the noise is decreased, while the analysis time is only minimally affected [28]. For accurate S/N enhancement in HTCE, the injections must be highly reproducible or the S-matrix will lose fidelity resulting in a diminished S/N enhancement. Figures 1C and 3B showed that peak heights and migration times were reproducible for 20 consecutive injections of CFSE, CFSE-labeled Arg, and CFSE-labeled Cit, suggesting the feasibility of utilizing the UV-LED array for HTCE injections.

Figure 4 demonstrates the S/N enhancement observed using the UV-LED for POG-HTCE. Injection of a single sample plug of 200 nM CFSE yields a S/N ratio of 5.8 (Figure 4A). Figure 4B shows the convoluted electropherogram resulting from consecutive injection of CFSE using an S-matrix encoding 2047 elements. Deconvolution of the electropherogram shown in Figure 4B, yields a CFSE peak with S/N = 90 (Figure 4C).

S/N enhancement in HTCE is related to the S-matrix length by equation 1 [17,31,28]. Longer matrices facilitate greater signal throughput thereby distributing the noise over a larger number of samples. For CFSE, S/N enhancements of 12.0 ± 1.3 and 17.0 ± 1.4 were observed with matrices of 1023 and 2047 elements, respectively (Table 1), facilitating analysis of labeled analytes at 15 nM. With longer matrices, the S/N enhancement would be expected to increase further, unfortunately as the matrix length is increased, longer analysis times and larger sample volumes are required to perform HTCE [31], introducing a practical upper limit on the matrix size, particularly in dynamic studies where analyte concentrations vary over time.

The S/N enhancements presented here are consistent with those calculated for HTCE using UV-laser lines for POG injection. Previously, S/N enhancements of 12.7 and 17.3 were reported for matrices with 1023 and 2047 elements, respectively [49]. These results provide additional evidence that the UV-LED array is suitable for POG injections since variations in the light source would have resulted in altered injection volumes and a reduced S/N enhancement would have been observed. Interestingly, HTCE performed using both UV-laser lines and the UV-LED array resulted in S/N enhancements of 75 % of the theoretical values. The noise reduction in HTCE is based on the assumptions that the noise is random and independent of previous measurements. When utilizing LIF detection, Shot noise is not completely random, but is dependent on the sample concentration since the noise is proportional to the number of independent events and the number of events at the PMT increases with increasing concentration. Thus, the 75 % enhancement observed for HTCE may arise from reduction of the random portion of the noise, suggesting that HTCE is Shot noise limited. [49].

4. Concluding Remarks

A UV-LED array was demonstrated for POG injection of caged fluorescent samples. When corrected for the differences in light intensity, the UV-LED array provided comparable sensitivity to UV laser lines. Peak width variance was found to model similarly to previous examples of optical gating CZE, though the injection plug shape was pseudo-plug like, likely due to the beam profile and power stability of the UV-LED array. When combined with HTCE, the sensitivity of the analysis was increased by as much as 17 fold, with equivalent enhancements to POG performed using UV laser lines, suggesting that neurotransmitters with physiological concentrations as low as 20 nM could be monitored. This injection scheme, in combination with an LED for detection, could provide a low-cost, high sensitivity, portable device for a range of dynamic analyses, including neuro-chemical analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Arg	arginine
CFSE	caged fluorescein succinimidyl ester
Cit	citrulline
HTCE	Hadamard transformation capillary electrophoresis
LED-IF	LED – induced fluorescence
NO	nitric oxide
POG	photolytic optical gating
UV	ultraviolet

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Figure 1.

Analysis of 500 nM CFSE by POG with a UV-LED array. (A) Single UV-LED POG injection of CFSE with shutter open (top) and closed (bottom). Electropherograms are offset for clarity. (B) Twenty consecutive UV-LED POG injections of CFSE. (C) Peak height (solid squares) and migration time (open squares) were plotted for each POG injection in part B. The dashed lines represent the mean $\pm$ standard deviation of the peak height for all 20 injections. Data points represent parameters for the peak observed at 142 s. Injection time = 500 ms.

Figure 2.

Evaluation of peak efficiency and variance. (A) Peak efficiency as a function of injection length. Data points represent the average efficiency for multiple injections of a CFSE sample using different POG injection lengths ($n = 3$). (B) Peak variance as a function of the squared injection length. Circles represent the experimentally measured σ^2_{Tot} for multiple injections of a CFSE sample using different POG injection lengths ($n = 3$). Dashed line represents σ^2_{Tot} excluding σ^2_{Inj} .

Figure 3. Separation and reproducibility of CFSE-labeled amino acids. (A) Representative electropherogram of single POG injection of 450 nM Arg and Cit labeled with CFSE. Inset identifies labeled amino acids. (B) The peak height (solid squares and circles for Arg and Cit, respectively) and migration time (open squares and circles for Arg and Cit, respectively) were plotted for each of 20 consecutive POG injections in a single run. Injection time = 750 ms.

Figure 4. HTCE analysis of 200 nM CFSE using UV-LED array POG. (A) Single injection of 200 nM CFSE. (B) Convolved raw electropherogram collected using a 2047 element S-matrix. (C) Deconvoluted electropherogram collected using a 2047 element S-matrix. Injection time = 100 ms.

Table 1

S/N Enhancement for CFSE with HTCE

Matrix Size	S/N Enhancement with UV-LED	S/N Enhancement with UV Laser [49]	Theoretical S/N Enhancement
1023	12.0 ± 1.3	12.7 ± 0.6	16.0
2047	17.0 ± 1.4	17.3 ± 0.9	22.6