

**Byoungsok Jung,<sup>†</sup> Yonggang Zhu,<sup>‡</sup> and Juan G. Santiago<sup>\*†</sup>**

<sup>†</sup>Department of Mechanical Engineering, Stanford University, Stanford, CA 94305

<sup>‡</sup>CSIRO Manufacturing and Infrastructure Technology, Highett, VIC 3190, Australia

**\* Correspondence:**

Juan G. Santiago

Department of Mechanical Engineering, Stanford University

Building 530

Stanford, CA 94305, USA

Email address: [juan.santiago@stanford.edu](mailto:juan.santiago@stanford.edu)

Fax: (650) 723-7657

**Title of the Primary Article: (ac060949p)**

Detection of 100 attomolar Fluorophores Using a High Sensitivity On-Chip CE System and Transient Isotachophoresis

**Abstract**

This Supporting Information publication contains supplementary materials for the manuscript, “Detection of 100 attomolar Fluorophores Using a High Sensitivity On-Chip CE System and Transient Isotachophoresis.” Provided here are details of the calibration process for quantifying analyte concentration before and after stacking. Further information is also provided that describes the dependence of signal intensity on microscope objective specifications and exposure time.

### **Calibration of Sample Preparation:**

We quantified the relationship between fluorescent signal intensity and sample concentration as a function of exposure time and CCD intensifier gain by direct measurement of signal intensity after each dilution. We measured fluorescent signals of Alexa Fluor 488 (1 pM to 1  $\mu$ M) in a glass microchannel using 60x water immersion objective (NA = 0.9). We varied exposure times between 3 to 300 ms and the micro channel plate (MCP) gain of our ICCD camera from 0-100%. This quantification across variations in gain and exposure time was critical as we varied these parameters in order to accommodate five to six orders of magnitude changes of signal strength in our various experiments. The dynamic range of our CCD is limited to 12 bit (minus the few bits occupied by the noise threshold). The signal intensity of the CCD camera was directly proportional to the exposure time and the second power of MCP gain. The normalized signal intensity of diluted sample solutions was linearly proportional to the sample concentration,  $C_S$ , as shown by the calibration in Figure S-1. For these data, we prepared three independent lines of dilution samples, and each data point represents an average of the three independent sample solutions. These measurements validate the sample dilution process, and validate that normalized signal intensity data can be interpreted as measurements of absolute sample concentration.

### **Calibration for Optimization of Detection System:**

Figure S-2 shows the signal intensity of fluorescent samples as a function of sample concentration,  $C_S$ , exposure time of CCD camera,  $\Delta t$ , numerical aperture (NA) and magnification (M) of objectives using an inverted epifluorescent microscope. For this calibration, we measured fluorescence signals of sample inside square cross-section glass capillaries (inner dimension of 100  $\mu$ m) filled with four concentrations of bodipy ranging from 0.1 to 20  $\mu$ M. We used nine microscope objectives (magnifications of 4x, 10x, 20x, and 40x) with numerical apertures of 0.1, 0.16, 0.2, 0.25, 0.3, 0.4, 0.45, 0.5, and 0.65. We used six exposure times for the CCD camera, ranging from 5 to 100 ms. The result shows that signal intensity ( $I$ ) is proportional to the sample concentration, exposure time of CCD camera, and the fourth power of NA, and is also inversely proportional to the second power of the objective magnification:  $I = AC_S\Delta tNA^4M^{-2}$ , where the constant factor  $A$  takes into account other (fixed) parameters including photophysical properties of fluorophores, characteristics of the microscope (e.g., properties of the tube lens) and filter set, detector efficiency, and excitation source. Illumination intensity and light collection efficiency each depend on the square of NA in epi-illumination mode (leading to the fourth power dependence).

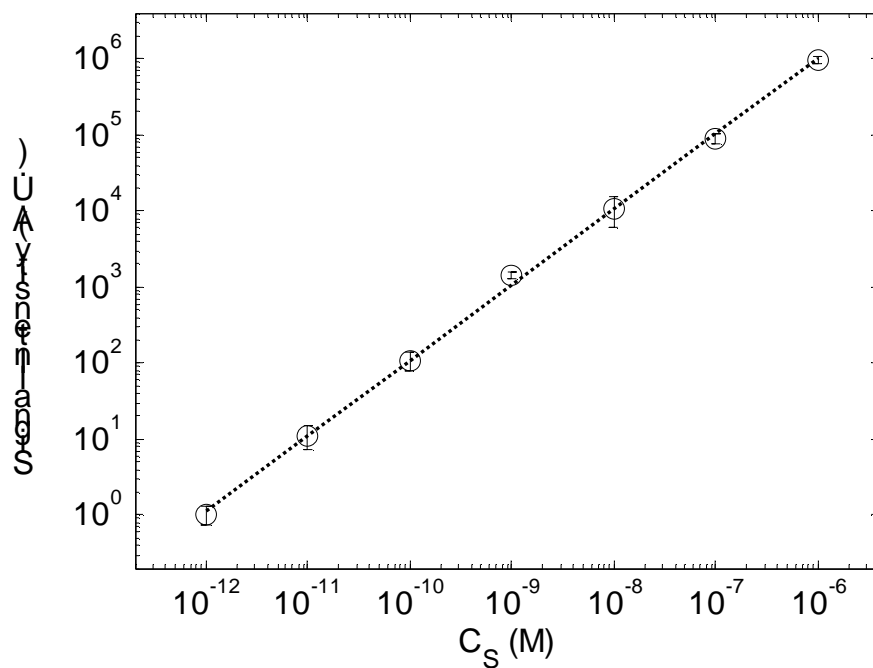


Figure S-1.  
 Calibration of the high sensitivity CE system for various sample concentrations. Concentrations of Alexa Fluor 488 were 1 pM to 1  $\mu$ M. All data shown here were obtained using a 60x objective (NA = 0.9) and an ICCD camera. The error bars reflect 95% confidence intervals as determined from three independently prepared sample solutions.

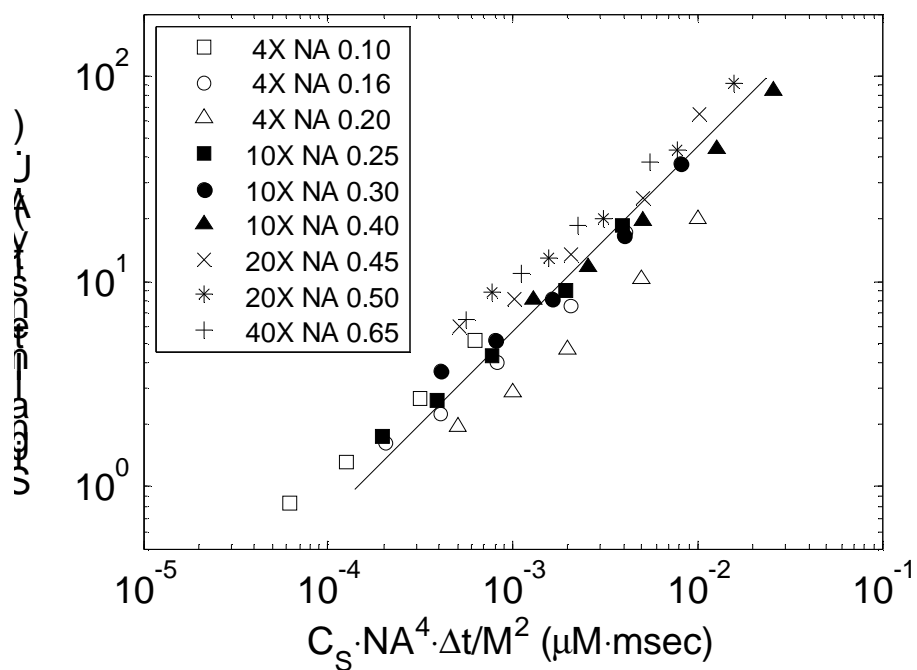


Figure S-2. Signal intensity of fluorophore as a function of sample concentration, numerical aperture and magnification of objective, and exposure time of CCD camera. Each data point represents average of five realizations. The line shown has the form  $I = A C_S \Delta t NA^4 M^{-2}$ , where  $A$  is a constant.