concentration was plotted for each of the EL and LED excitation method used. **(A)** Blue LED illumination (1 s-diamond, 2 s-triangle and 3 s-rectangle) and blue EL illumination (5 min-circle). **(B)** Expansion of the lower concentration range, 0 nM-0.625 nM, of SNAP-25 dose response curve from (A). **(C)** White LED illumination (1 s-X, 2 s-vertical line and 3 s-diamond/dashed line) and the shorter exposure times used for the blue EL (15 s- triangle, 30 s,- circle and 60 s- rectangle). **(D)** Expansion of the lower concentration range for the SNAP-25 dose response plotted in (C). **(E)** White EL illumination (15 s- circle, 30 s-diamond and 60 s- triangle). **(F)** Expansion of the lower concentration range for the SNAP-25 dose response plotted in (E). Standard deviations were determined from three separate readings of each chip.

Figure 5: *In vitro* activity analysis of BoNT-A light chain (LcA) cleavage of FITC/DABCYL-SNAP-25 peptide detected by FRET. Various concentrations of LcA (0 nM-20 nM) were used to cleave FITC/DABCYL-SNAP-25 (5 μM). The cleavage products were excited by LED or EL and fluorescence intensity measured by CCD. **(A)** Correlation between the S/N ratio of the cleavage assay measurements in chip B and chip C. LcA dose response curves plotted as a function of the excitation source for **(B)** chip B and for (C) chip C. For both plots; blue LED exposure of 2 s (black circle), blue LED exposure of 3 s (white circle), white LED exposure of 3 s (black triangles), white LED exposure of 2 s (white rectangle), blue EL exposure of 30 s (black rectangle), blue EL exposure of 60 s (white rectangle), blue EL exposure of 300 s (black diamond). Note that the LcA concentration axis is plotted on the log scale, so the zero (blank) concentration is not present. Standard deviations were determined from three separate readings of each chip.

Supporting Information:

Figure S1: Cleavage activity assay for BoNT (A) Schematic of the LcA FITC/DABCYL-SNAP-25 derived FRET assay. The SNAP-25 peptide substrate for BoNT-A is labeled with the FITC donor/DABCYL acceptor FRET pair. Interaction of the substrate with, in this case, the light chain LcA derivative results in cleavage of the

peptide sequence, disrupting FRET and resulting in increased emission from the FITC donor. Modified from ref [48]. (B) EL-CCD and LED-CCD images of the LcA FITC/DABCYL-SNAP-25 derived FRET assay. A 50% dilution series on the LcA was prepared giving a final concentration range from 0 nM-20 nM and a total of 10 samples, with each sample containing 5 μM FITC/DABCYL-SNAP-25 peptide. The dilution series was loaded in triplicate into each 30-well chip and the fluorescence, measured using a CCD camera was excited using; (I) for blue EL excitation exposure of 300 s and (II) blue LED excitation exposure of 2 s.

Figure S2: Light uniformity analysis of LED illumination source. (A) CCD image showing the light intensity of the white LED. (B) ImageJ 3D analysis of (A) showing the light LED light uniformity.

Figure S3: The layout of the 30 well chip. I. A schematic image of the chip and II. an actual image of a chip with various amounts of unquenched SNAP-25 peptide. The chip contains three replicas (separated by dotted lines) with replica #1 in columns 1 and 2, replica #2 in columns 3 and 4, and replica #3 in columns 5 and 6. In each replicate, nine concentrations of a 50% dilution series ranging from 0.019 nM - 5 nM, plus a blank control (no SNAP-25) were loaded, with the highest concentration in well #1 and the lowest (control) in well #10.