Supporting information for

## Direct detection of neuron-specific enolase by a spectrometer-free colorimetric plasmonic biosensor

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[Optical setup]

The optical setup is described in our previous paper [1]. Briefly, a white light from a tungsten halogen lamp (HL-2000-HP, Ocean optics) was incident on the sensor surface after passing through an optical fiber and several optical elements shown in the figure. The sensor substrate was fixed on top of the XY stage after attaching a flow cell made of a PDMS gasket. The reflected light from the sensor surface was measured by both a spectrometer (FLAME, Ocean Optics) and a color CMOS camera (acA2440-35ucMED, Basler) using the second beam splitter. For imaging, a C mount lens (C23-5026-2M, Basler) was attached to the CMOS camera, and a color filter (FGT165M, Thorlabs) was inserted to increase the color temperature of the light source.



Figure. S 1 Sketch of the optical setup.



Figure S 2. HSV color space coordinate.

[Image processing]

Figure S3 (a) shows the original reflection image of the sensor surface. Firstly, the RGB values at each pixel were converted to HSV values. Figure S3(c) shows the histogram of Hue, Saturation, and Value respectively. Value is corresponding to brightness. For simplicity, we employed Values as a parameter to define the interest region. As shown in Figure S3(b), the pixels with low values were removed for further image analysis.



Figure S3. (a) Original image, (b) binary image of the sensor surface used for image analysis, (c) histogram of Hue, Saturation, and Value taken from the original image.



Figure S 4. Reflection spectra were taken before (blue solid line) and after (red dashed line) the introduction of the control sample including human IgG. The concentration of human IgG was adjusted to 22 nM.



Figure S 5. Selectivity characterized by spectroscopic method.



Figure S 6. Quantitative plot for the detection of NSE taken by spectroscopic method.  $K_d$  and LOD were determined to be 4.63 nM and 180 pM, respectively.

[References]

[1] M. Toma, Y. Itakura, S. Namihara and K. Kajikawa, Adv. Eng. Mater., 2022, DOI: 10.1002/adem.202200912.