Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Visual and Real-Time Imaging Focusing for Highly Sensitive Laser-Induced Fluorescence Detection for Nanocapillaries at Yoctomoles Level

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Experimental Section

Reagents and materials: Tris(hydroxymethyl)aminomethane (Tris) and ethylenediaminetetraacetic acid disodium salt (Na2EDTA) were products of Sinopharm Chemical Reagent Co., Ltd. (China). Fluorescein disodium salt was obtained from Thermo Fisher Scientific (USA). Hsa-miR-17-5p was purchased from Suzhou GenePharma (China). Fused silica capillaries were specially produced by Polymicro Technologies (USA).

Preparation of eluents and samples: A concentration of 10 mmol/L Tris-EDTA (TE) buffer was composed of 10 mmol/L Tris-HCl and 1 mmol/L Na2EDTA at a pH of 8.0. A stock solution of 1 mmol/L fluorescein disodium salt was prepared with 10 mmol/L TE buffer. The working solutions of fluorescein were prepared by the serial dilution of the 1 mmol/L fluorescein stock solutions with 10 mmol/L TE buffer before they were used. Fluorescein amidite (FAM) labeled Hsa-miR-17-5p was prepared with diethylpyrocarbonate-treated water (DEPC-water). All solutions were prepared with ultrapure water from the GenPure UV/UF system (Thermo Fisher Scientific, USA), filtered through a 0.22 μm filter (Shanghai Derian Instruments, China) and degassed before use.

Fluorescent signal-based focusing and sampling: A detection window was formed based on the removal of a small section of the coating at a location of 5 cm from the end of a nanocapillary. The end of the capillary was fixed on a holder, and was coupled with the three-dimensional translational stage along the x-y-z directions (M562F XYZ, Newport, USA) to allow adjustment of the position of the capillary's detection window. The sampling end of the capillary was inserted through the septum into a 200 μL solution vial inside a small pressure chamber, and a sample or eluent solution was introduced into the capillary in the presence of pressure-regulated nitrogen gas. For fluorescent signal-based focusing, a fluorescein solution (100 nM) was introduced into the capillary in the presence of pressure-regulated nitrogen gas. The position of the detection window was adjusted by the x-y-z three-dimensional translation stage, and the fluorescence signal was monitored. For sampling microRNA, the fluorescein solution was replaced by a sample solution which was introduced into the capillary.

Structure of visual and real-time imaging focusing LIF detection system

The detection system consists of three light paths, namely excitation, fluorescence collection, and image calibration (as shown in Fig. 1).

The excitation light path was a 488 nm OBIS LS laser (Coherent, USA) which was used as an excitation light source. A neutral density filter (NDC-50C-4M, Thorlabs, USA) was adopted for laser power attenuation. The light beam was purified with a laser filter (488 nm/10 nm, Edmund Optics, USA) and shaped through a plane-convex cylindrical lens (LJ1558RM-A, Thorlabs, USA) in one dimension. The light beam was then reflected by a dichroic mirror (Z488bcm, Chroma, USA), was focused by a microscope objective (UPlanSApo100X/1.40OIL, Olympus, Japan), and reached the capillary detection window.

The fluorescence was collected by the same objective along the fluorescence collection light path. After passing through the first dichroic mirror, it was reflected by the second dichroic mirror (AT600dc, Chroma, USA) and was filtered by a bandpass filter (ET535/50 m, Chroma, USA). It was then focused by a lens (AC254-040-A-ML), passed through a slit (200 μm, Thorlabs, USA), and was finally detected by a single-photon counting module (SPCM-AQRH-14, Excelitas Technologies, USA). The single-photon counting module was connected to a data acquisition card (NI USB-6361, National Instruments, USA), and the collected data was stored and displayed using the LabVIEW program written in our laboratory**.**

The image calibration light path installed a light emitting diode (LED) (M625L3, Thorlabs, USA), a filter (ZET 635/20X, Chroma, USA) and an aperture (SM1D12, Thorlabs, USA) above the capillary. The LED was used as the light source to illuminate the detection window of the capillary. Accordingly, the light was filtered, the intensity was adjusted by the aperture, and the signal was then collected by the objective. The light was reflected by a reflector (PFE10-P01, Thorlabs, USA) after it passed through the dichroic mirrors. Subsequently, it was focused by a lens (AC254-075-A, Thorlabs, USA) and then reached the camera (DCC1545M, Thorlabs, USA). Real-time image acquisition and display was performed with the ThorCam software (Thorlabs, USA).

Supplementary figures

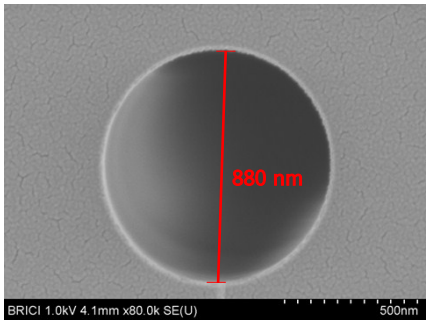

Fig. S1 SEM image of the cross-section of an 880 nm inner diameter capillary.

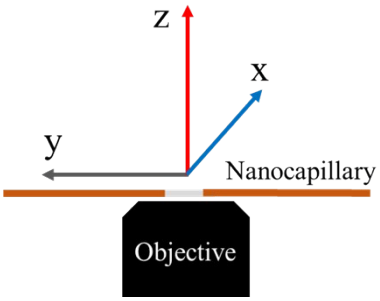

Fig. S2 Definition of the x-, y-, and z-axes (x: the axial direction of the capillary, y: the radial direction of the capillary; z: the distance between the objective and the capillary).

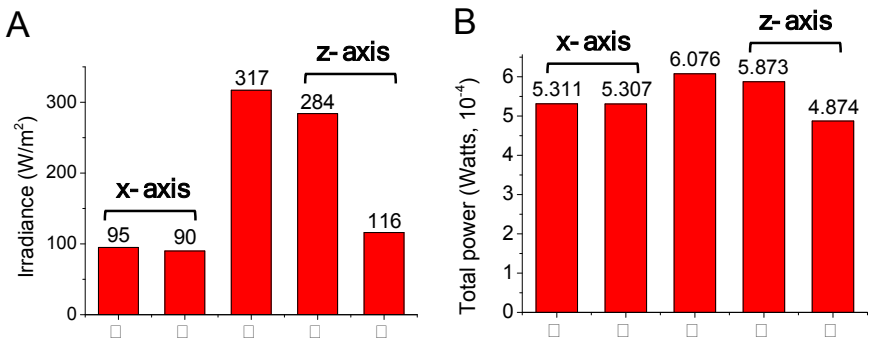

Fig. S3 The irradiance and the total power of simulation by the optical software Zemax. (A)The irradiance of the simulation. (B)The total power of the simulation.

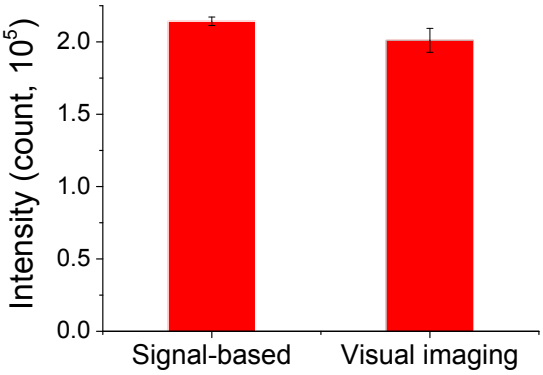

Fig. S4 The signal evoked owing to fluorescence signal-based focusing and visual and real-time imaging focusing.

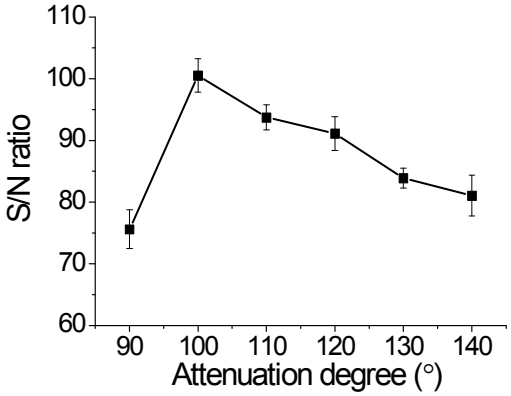

Fig. S5 Effect of degree of attenuation of neutral density filter on the S/N ratio. The capillary had a radius of 440 nm and a total length of 35 cm (30 cm effective). A concentration of 10 nmol/L fluorescein sodium solution was flushed into the capillary at 800 psi.

Performance characterization of LIF system

The fluorescein sodium solutions with concentrations from 10 pmol/L to 10 nmol/L were flushed into the capillary. The detected fluorescence intensities are shown in Figure S6A. The inset presents the expanded range of 0 - 100 pmol/L. A highly linear relationship (correlation coefficient of 0.9993) was observed between the fluorescence intensity and the

concentration of fluorescein sodium solution (Fig. S6B). The linear range of the present system was greater than three orders of magnitude.

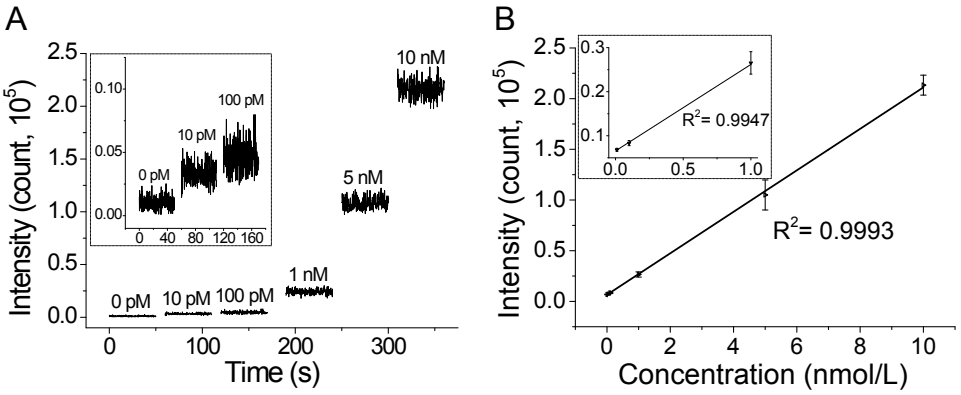

Fig. S6 (A) Fluorescence intensities obtained with various concentrations of fluorescein sodium solutions. (B) Linear fit of the plot of the fluorescence intensity as a function of the concentration of fluorescein solution. The capillary had a radius of 440 nm and a total length of 35 cm (30 cm effective). Fluorescein solutions were flushed into the capillary at 800 psi.

The limit of detection (LOD) of the LIF detection system was evaluated following the injection of fluorescein sodium solutions at different concentrations into the capillary. Each fluorescein sodium solution was repeatedly injected three times, and the results are shown in Fig. S7A. A linear relationship was established between the fluorescence intensity and the fluorescein sodium solution concentration with a correlation coefficient of 0.9887 (Fig. S7B).

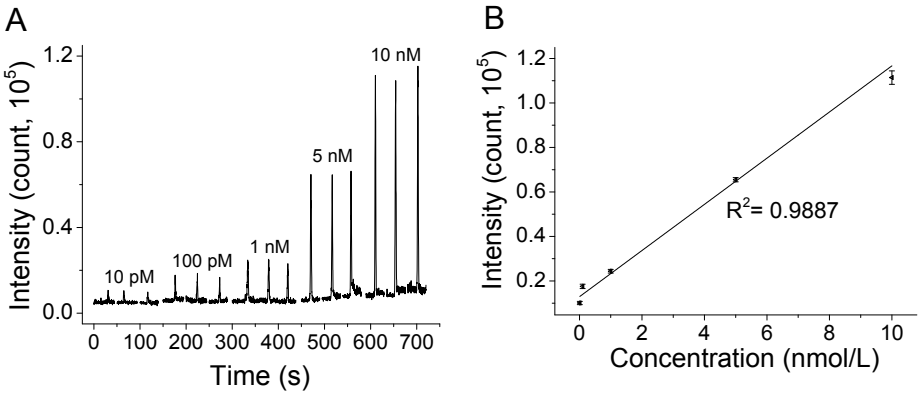

Fig. S7 (A) Fluorescence intensity of fluorescein sodium solutions. (B) Linearly fitted plot of fluorescence intensities and concentrations of fluorescein solutions. The sample was injected at 80 psi for 10 s at three different times, and the separation was carried out at 800 psi.

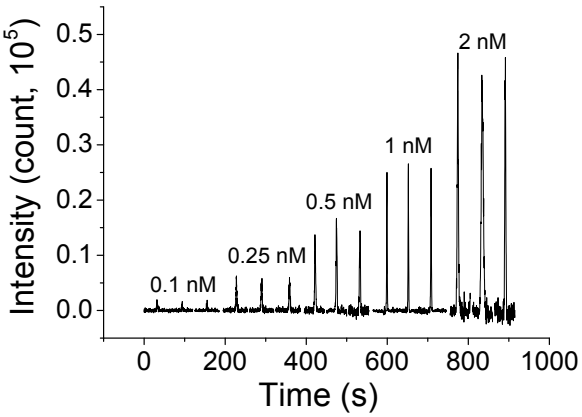

Fig. S8 Fluorescence intensity of FAM-labeled Hsa-miR-17-5p with concentrations of 0.1, 0.25, 0.5, 1, and 2 nmol/L, respectively. The capillary had a radius of 440 nm and a total length of 32 cm (effective radius of 28 cm). The samples were injected at 100 psi for 10 s at three separate times. A TE concentration of 10 mmol/L was used for the eluent with an elution pressure of 800 psi.