Supplementary information

On-chip mirror enhanced multiphoton upconversion super-resolution microscopy

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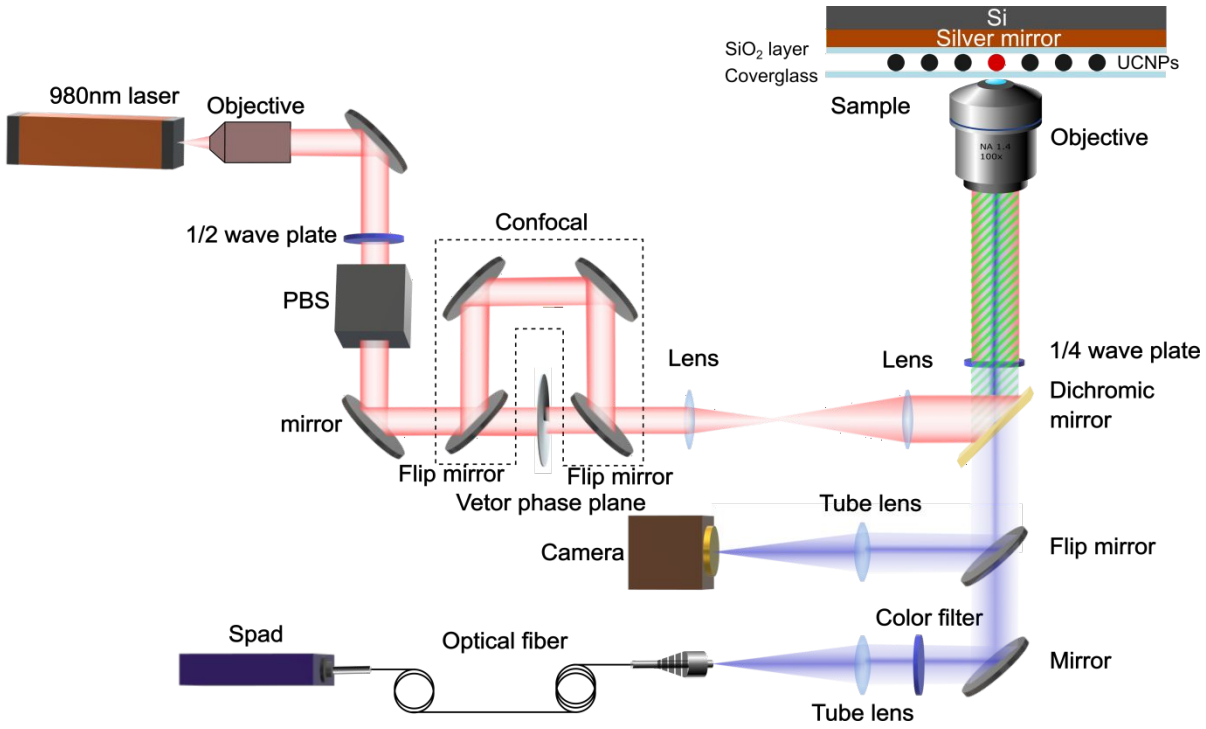
Supplementary Note 1 Experimental setup

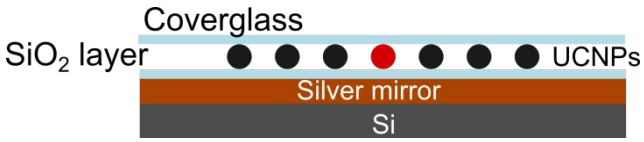


Supplementary Figure 1. Scheme of the purpose-built Mirror enhanced NIRB nanoscopy setup.

The optical setup was a free space design system which built on a commercial microscope Olympus IX73) equipped with a 3-axis closed loop piezo stage (Nano Nano-LP Series, Mad city labs). A single-mode fibre-coupled 980 nm diode laser (BL976-PAG900, controller CLD1015, Thorlabs; maximum output power 900 mW) was used as the excitation source. After collimation, the excitation beam was transmitted through the first half wave plate (HWP, WPH05M-980, Thorlabs) and polarized beam splitter (PBS, CCM1-PBS252/M, Thorlabs). This allowed precisely adjusting the excitation power by rotating HWP electronically. And then, the vortex phase plate was used to generate a doughnut-shaped point spread function (PSF)

at the focal plane. A 4f optical system (L5 and L6) was used to transfer the image plane into the back aperture of the objective lens. Then reflected by a short-pass dichroic mirror (T875spxrxt, Chroma), and focused through a high numerical aperture objective (UPlanSApo, 100×/1.40 oil, Olympus) to the sample slide. The quarter-wave plate (QWP, WPQ05M-980/808, Thorlabs) was adopted to transform the excitation beam from linear polarization to circular polarization to obtain optical super-resolution images. Photo luminescence signal from the sample was collected by the same objective, then directed to the detection unit by the dichroic mirror. Before being coupled into a multi-mode fiber (MMF, M24L02, Thorlabs) for detection, the emission signals went through either a bandpass (BPF, ET805/20M, Chroma) or a short pass filter(SPF,FF01-842/SP-25, Semrock)to completely remove the excitation light. A single-photon counting avalanche photodiode (APD, SPCM-AQRH-14-FC, Excelitas) was used for detection, controlled by a Labview program. The MMF could also be connected to a spectrometer equipped with an EMCCD detector (iXon Ultra, Andor).

Supplementary Note 2: Schematic of materials, and sample fabrication



Supplementary Figure 2. Schematic structure of the mirror sample

The on-chip mirror enhanced sample had been fabricated similarly to our previously reported method[1]. The mirror used in our experiment is a custom-made (ANFF, Australia), first-surface mirror, using a sliced silicon wafer as a base. A silver film about 200 nm is sputtered on a silicon wafer, and then the SiO_2 layer is deposited as a spacer by thermal evaporation. In this experiment, a series of spacer thicknesses was prepared (50 nm, 100 nm, 150 nm, 200 nm, 250 nm, 500 nm). The AFM characterized values are 63 nm, 118 nm, 164nm, 205 nm, 247 nm, and 510nm. In the main text and the simulation, the distance between the particle and mirror is the spacer thickness plus the effective radius of the UCNP (22.3 nm). Hence in this work, the characterized spacings between the particle's centre and mirror surface are 85.3 nm, 140.3 nm, 186.3 nm, 227.3 nm, 269.3 nm, and 532.3 nm.

During the experiment, the UCNPs are dispersed on the top of mirror samples by the dip-casting method, which provides a better distribution of individual nanoparticles as compared with the drop-casting method. In this dip-casting method, we first dilute the concentration of UCNPs to 0.01 mg/ml. Then, we took 2 ml from the solution and placed them into a shallow dish. Finally, we used a tweezer to hold the mirror, then, we dipped it into the dish, took it out, and left it for drying. A schematic of the sample is shown in Supplementary Figure 2. To avoid the refractive mismatch, we use objective oil to fill the gap between SiO_2 and the top cover glass. The sample of the UCNP in glycerol solution was prepared in a similar way. UCNPs coated with home-made polymer were purified three times and then diluted into 0.01 mg/ml. Then, the solution was added to 99% Glycerol. The Glycerol with the UCNPs was diluted into a concentration of 93% with water, then transferred into a home-made micro-chamber. As the last step, the micro-chamber was closed with a cover glass.

Reference

(1) Liu, Y.; Zhou, Z.; Wang, F.; Kewes, G.; Wen, S.; Burger, S.; Ebrahimi Wakiani, M.;

Xi, P.; Yang, J.; Yang, X.; Benson, O.; Jin, D. Axial Localization and Tracking of Self-Interference Nanoparticles by Lateral Point Spread Functions. *Nat. Commun.* **2021**. https://doi.org/10.1038/s41467-021-22283-0.