SUPPORTING INFORMATION

Nanoparticles as Non-Fluorescent Analogs of Fluorophores for Optical Nanoscopy

Simon Hennig[1†], Viola Mönkemöller[1†], Carolin Böger[2], Marcel Müller[1], and Thomas Huser[1,3]*

[1] Biomolecular Photonics, Department of Physics, University of Bielefeld, Universitätsstr. 25, 33615 Bielefeld, Germany

[2] Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany

[3] Department of Internal Medicine, and NSF Center for Biophotonics, 2700 Stockton Blvd., Ste. 1400, University of California, Davis, Sacramento, CA 95817, USA

To determine the photostability of SERS nanotags, we investigated the particles by wide-field "fluorescence" microscopy using a range of different illumination powers and measure the overall intensity for at least 5 min under continuous illumination as well as potential blinking effects on short timescales. The results of the experiment are shown in Figure S1.

SERS nanotags were prepared in H_2O, an aliquot (10 - 20 μl) spread on a cover glass surface and allowed to dry. Using normal wide-field illumination intensities typically used for fluorescence microscopy (up to ~100 W/cm^2), SERS nanotags exhibited high and stable signal intensites without any noticeable blinking or bleaching effects (see Fig. S1b). These measurements were carried out with a signal integration time of 1 s/frame. At laser power densities above 500 W/cm^2 we noticed an initial decrease in overall brightness of the SERS nanotags as shown in Fig. S1a (i-iii). The brightness decreased to ~60% of the initial brightness within 160-180 s and then stabilizes at this value. By ramping up the illumination power density up to ~2.6 kW/cm^2 still no significant signal fluctuations (blinking) were detected - even with reduced integration times of 3 ms/frame. Only when we exceeded a power density of 2.6 kW/cm^2 did signal fluctuations become apparent (see Fig. S1d).

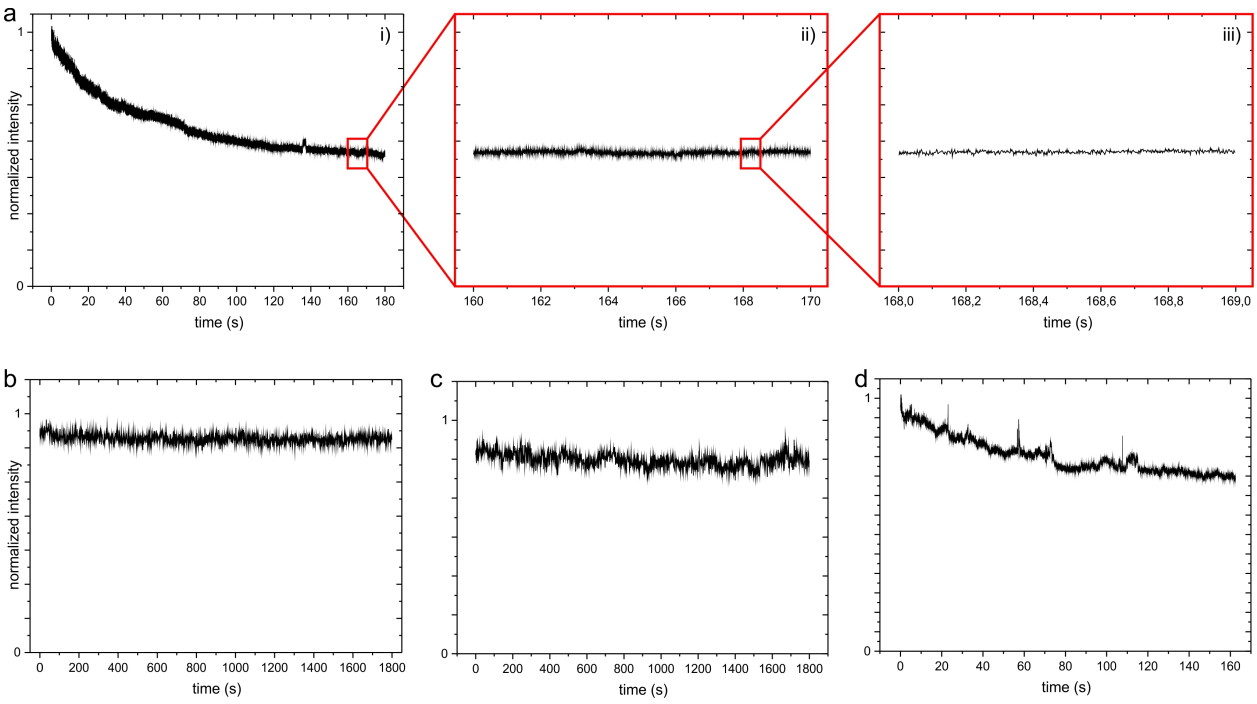

Figure S1. Photostability of single SERS tags under widefield illumination conditions. Excitation of SERS tags using 2 different laser powers indicates excellent longterm photostability after initial signal decline. Short term signal fluctuations can also be observed. **(a)** i: A single SERS nanotag excited at 647 nm 0.5 kW/cm^2 for 180 s. The SERS intensity of the particle exhibits an initial decrease of the intensity, which stabilizes at ~ ½ of the initially intensity. Further expansion to smaller timescales **(ii-iii)** shows no blinking of the single particle. Binning: 3 ms. **(b)** Illumination with normal wide-field conditions of 20 W/cm^2. Here no decrease of the Raman signal can be observed over 30 min. Binning: 1 s. **(c)** Illumination under 0.1 kW/cm^2 shows slightly fluctuations in the detected signal over 30 min Binning: 1s. **(d)** At extreme conditions of 2.6 kW/cm^2 minor fluctuations as well as a previously described decrease of the Raman signal can be observed. Binning: 6.5 ms.