# Photothermal nanoimaging using two-photon excitation

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Detecting photothermal expansion forces by atomic force microscopy (AFM) has attracted much attention as a promising nanoscale molecular imaging modality. For example, AFM-based infrared spectroscopy (AFM-IR) [1] identifies molecular species without labeling by probing their vibrational fingerprints. Moreover, extending AFM-IR excitation to the near-infrared and visible regions facilitates the investigation of spatial correlations between vibrational and electronic absorption signals at the nanometer scale [2, 3]. So far, most photothermal AFM implementations rely on one-photon excitation, which can suffer from signal interference from other linear optical processes, including scattering and absorption of the photothermal excitation beam by the cantilever. These signal overlaps compromise the specificity and the sensitivity of the acquired photothermal AFM images.

In the present work, we demonstrate two-photon-excited photothermal AFM (TPP-AFM) to mitigate the above-mentioned signal interferences. The working principle of the developed TPP-AFM is shown in Fig. 1. A femtosecond pulse train modulated at $f_\mathrm{O}$ was irradiated to the sample underneath the AFM tip oscillating at $f_\mathrm{C}$. The two-photon photothermal expansion was probed by demodulating the cantilever sidebands at $f_\mathrm{C} \pm 2f_\mathrm{O}$, where the contributions from unwanted linear responses are minimized. We performed a proof-of-concept measurement with microcrystals of fluorescent Coumarin 314. A topography image (Fig. 2a) and a TPP-AFM image (Fig. 2b) were simultaneously acquired under illumination with 1040-nm, 20-mW optical pulses. While the morphological features exhibit a clear correspondence between the two images, small objects (several hundred nanometers) clustered around the crystals appear with higher signal in the photothermal image. This implies that they possess a high absorption cross-section or a high thermal expansion coefficient due to the interaction with the substrate. A confocal fluorescence image of the same crystals (Fig. 2c) show that the fluorescent nature of the fibrous structures and non-fluorescence from the clusters. These results show the utility of TPP-AFM as a nanoscopic imaging modality that cannot be addressed by existing methods. By broadening the excitation wavelength, high-performance molecular nano-imaging based on photothermal contrast will become feasible in the near future.

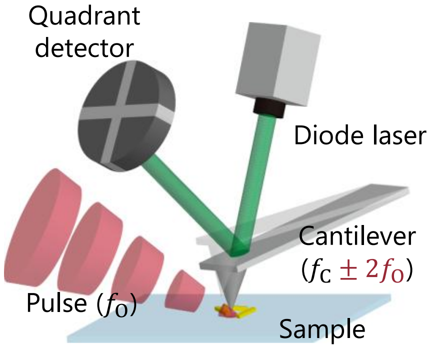

**Figure 1.** Simple schematic of our two-photon-excited photothermal AFM.

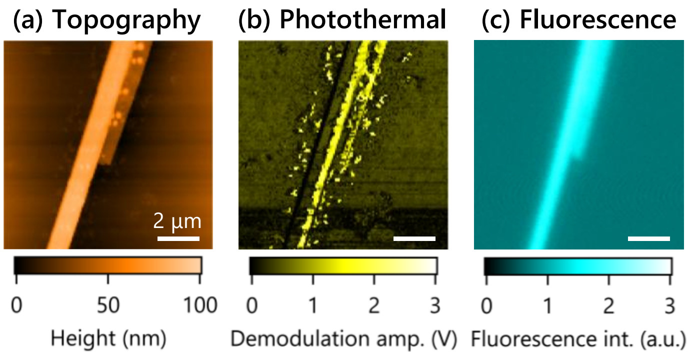

**Figure 2.** Coumarin 314 crystals observed by (a) AFM topography, (b) two-photon-excited photothermal AFM, and (c) confocal fluorescence microscopy.

**Reference:** [1] A. Dazzi, et al. *Chem. Rev.* **117**, 5146–5173 (2017). [2] A. M. Katzenmeyer, et al. *Anal. Chem.* **87**, 3154– 3159 (2015). [3] D. S. Jakob, et al. *Anal. Chem.* **94**, 15564–15569 (2022).