# LABEL-FREE AUTOFLUORESCENCE-DETECTED MID-IR PHOTOTHERMAL MICROSCOPY

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The instrumentation and methods to perform autofluorescence-detected photothermal mid-IR (AF-PTIR) microscopy are demonstrated experimentally and applied for chemically-selective label-free imaging of an active pharmaceutical ingredient (API) within a mixture with common pharmaceutical excipients. In AF-PTIR, the heat released from mid-IR absorption induces changes in two-photon excited UV-fluorescence (TPE-UVF) intensity. The spectral dependence of the fluorescence modulation locally informs on chemical composition with a spatial resolution dictated by the diffraction limit of visible light. AF-PTIR is shown to provide an additional level of selectivity in nonlinear optical imaging by mid-IR spectroscopy enabling mapping of the API distribution in the presence of TPE-UVF and second harmonic generation active excipients (**Fig. 1**). AF-PTIR provides high selectivity and sensitivity in image contrast for aromatic APIs, complementing broadly applicable commercial methods such as optical photothermal mid-IR (O-PTIR) microscopy.

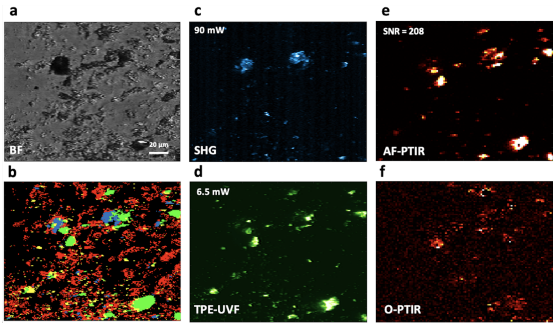


**Figure 1**. (a), (c – f) – Bright field, second harmonic generation (SHG), TPE-UVF, AF-PTIR and O-PTIR images of the field of view respectively. (b) – Segmentation results showing the spatial distribution of individual components (lactose particles are shown in blue, indomethacin in green, $TiO_2$ in yellow and Mg stearate is shown in red).