Supporting Information

Single-Cell Infrared Vibrational Analysis by Optical Trapping Mid-Infrared Photothermal Microscopy

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After the PS bead was trapped, the MIP intensity of the PS bead at 1494 cm^{-1} was continuously monitored for 6 minutes every 200 ms as shown in Figure S1. The time-dependent MIP signal intensity for the PS bead did not significantly deteriorate for 6 min while pulsed IR laser was ON; however, it decreased when the pulsed IR laser was OFF. When the IR laser was turned ON again, the MIP signal intensity recovered. This result indicates that the MIP signal originates from the IR absorption of the PS bead upon irradiation with pulsed IR light. It was also confirmed that the pulsed IR light did not induce sufficient perturbation of the optical forces that contributed to the optical trapping of the bead. The average normalized MIP signal was 53.4 counts with a standard

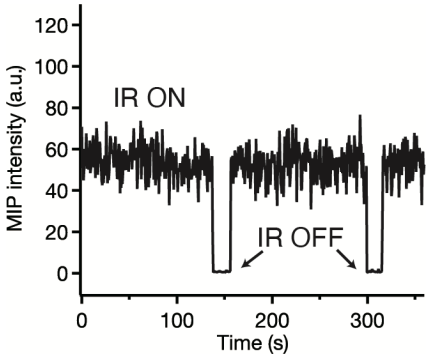

Figure S1. Normalized time-dependent MIP signal intensity of an optically trapped single PS bead. The MIP signal at 1494 cm^{-1} was monitored for 6 min.

deviation of 6.1 counts. The minor fluctuation in the MIP signal intensity was ascribed to the variation in the PS bead position within the tightly focused laser spot and was dependent on situations. However, the MIP signal intensity was sufficiently constant to analyze the individual samples.

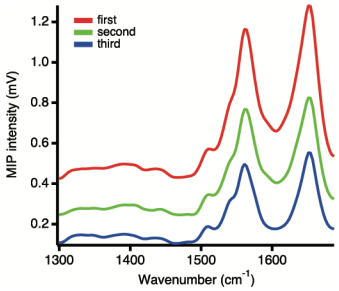

Figure S2. MIP spectra of an optically trapped single RBC continuously recorded 3 times. No notable spectral variation was observed in these spectra, which indicates that visible and IR beams does not induce photodamage to the sample.

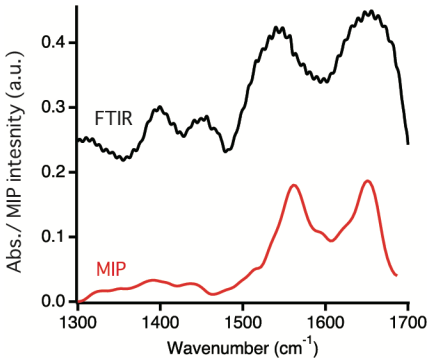

Figure S3. Comparison of the FTIR spectrum of ensemble of RBCs and the MIP spectrum of single RBCs. In both spectra, characteristic IR absorption bands were seen while the slight difference of the relative peak intensity was also seen due to the S/N ratio of measurements. It is noteworthy that absorption bands in FTIR are broader than those in MIP spectrum because the FTIR spectrum represents chemical signatures of ensembled RBCs, which include various factors, such as orientation of RBCs, chemical constitutes and molecular states in many RBCs. On the other hand, the MIP spectrum shows characteristic of only single RBCs, thereby it showed narrow IR absorption bands.