Detection of intracellular monosodium urate crystals in gout synovial fluid using optical diffraction tomography

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Supplementary information

Supplementary Movie S1. 3D morphological structure of monosodium urate (MSU) crystals.

Supplementary Movie S2. Time-lapse monitoring of macrophagic THP-1 cells with MSU crystals (10 μg/mL). The MSU crystals with RI ≥ 1.385 are indicated in purple.

Supplementary Movie S3. 3D holotomographic image of synovial leukocytes. The MSU crystals with RI ≥ 1.385 are indicated in purple.

Supplementary Movie S4. 3D morphological structure of RAW264.7 cells with MSU crystals (200 μg/mL). The MSU crystals with RI ≥ 1.385 are indicated in purple.

Supplementary Movie S5. Time-lapse monitoring of RAW264.7 cells with MSU crystals (10 μg/mL). The MSU crystals with RI ≥ 1.385 are indicated in purple.

Supplementary Figure S1. Cellular volume of THP-1 cells with or without phorbol 12-myristate 13-acetate (PMA). Data are shown as the mean ± SEM.

Supplementary Figure S2. Three-dimensional (3D) optical diffraction tomography (ODT) of murine RAW 264.7 cells with or without MSU crystals. (**a**) 3D RI distribution of RAW 264.7 cells. (**b**) RAW 264.7 cells treated with 50 μg/mL MSU crystals. (**c**) RAW 264.7 cells treated with 200 μg/mL MSU crystals. Each square shows the x-y, y-z, or x-z plane. Additionally, refer to Supplementary Movies S4–S5. n: refractive index.

Supplementary methods

Culture of RAW 264.7 macrophage cell line

The murine RAW 264.7 macrophage cell line (TIB-71, ATCC, Manassas, VA, USA) was used. RAW 264.7 cells were cultured in Dulbecco's Modified Eagle's Medium (Welgene, Gyeongsan, Korea) supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C in a humidified 5% CO_2 incubator. Thereafter, 1×10^5 RAW 264.7 cells were sub-cultured in a specialized

culture dish (TomoDish, Tomocube, Daejeon, Korea) and incubated in a stage-top humidified incubator (TomoChamber, Tomocube) at 37 °C in a humidified 5% CO_2 incubator. The incubated RAW 264.7 cells were monitored before (**Supplementary Figure S2a**) and after treatment with 50 μg/mL (**Supplementary Figure S2b**) or 100 μg/mL (**Supplementary Figure S2c**) of synthetic MSU crystals (InvivoGen) using ODT (HT-2H, Tomocube) (**Supplementary Movie S4** and **S5**).