Highly specific and label-free histological identification of microcrystals in fresh human gout tissues with stimulated Raman scattering

Bohan Zhang,[1] Hanlin Xu,[2] Yinghui Hua[2] and Minbiao Ji[1]

[1]State Key Laboratory of Surface Physics and Department of Physics, Fudan University, Shanghai 200433, China
[2]Department of Sports Medicine, Huashan Hospital, Fudan University

Abstract:
Gout is a common metabolic disease with growing burden, caused by monosodium urate (MSU) microcrystal deposition. In this project, stimulated Raman scattering (SRS) microscopy was utilized to image MSU based on its fingerprint Raman spectra. Our work demonstrated the potential of SRS microscopy for rapid intraoperative diagnosis of gout and may facilitate future fundamental researches of MSU-based diseases.

Schematics of the experimental design

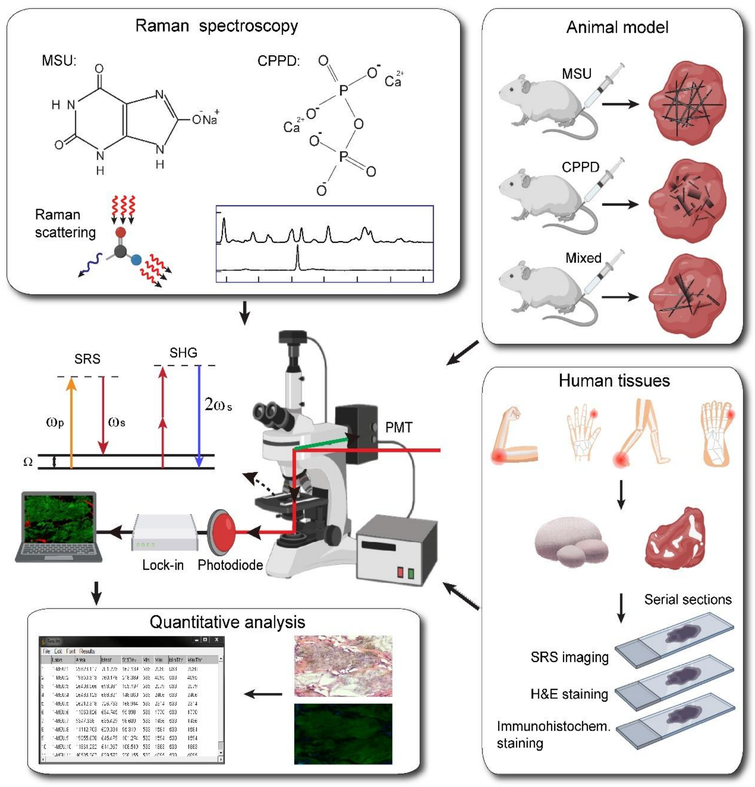


Fig 1. *Left top, spontaneous Raman characterization; Right top, acute GA rat models of MSU, CPDD and comorbidity for testing the capabilities of early diagnosis of gout and differentiation power from pseudogout; Right bottom, human surgical specimens were harvested from different locations; All specimens were imaged with SRS/SHG microscopy to obtain the distributions of MSU and collagen fibers; Left bottom, quantitative SRS analysis*

Imaging MSU microcrystals in tophi specimens

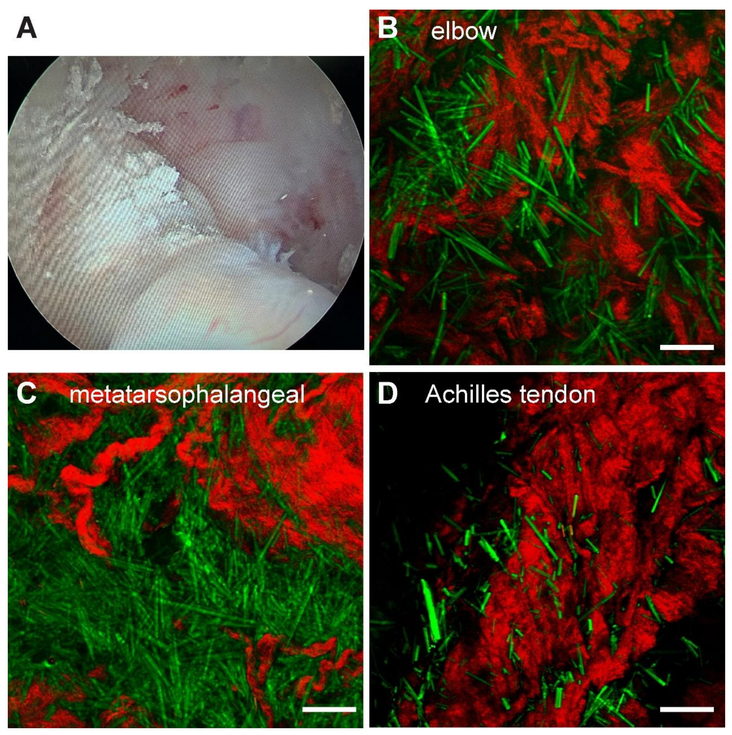


Fig. 4. Rapid diagnosis on fresh human surgical tissues of GA patients. *(A) Chalky tissues were harvested under arthroscopy. SRS images of unprocessed fresh tissues revealed intact MSU (green) depositions from (B) the elbow joint (n = 1), (C) the first metatarsophalangeal joint (n = 1) and (D) Achilles tendon (n = 1). Scale bar:10 μm.*

SRS/SHG imaging of crystalline and amorphous MSU

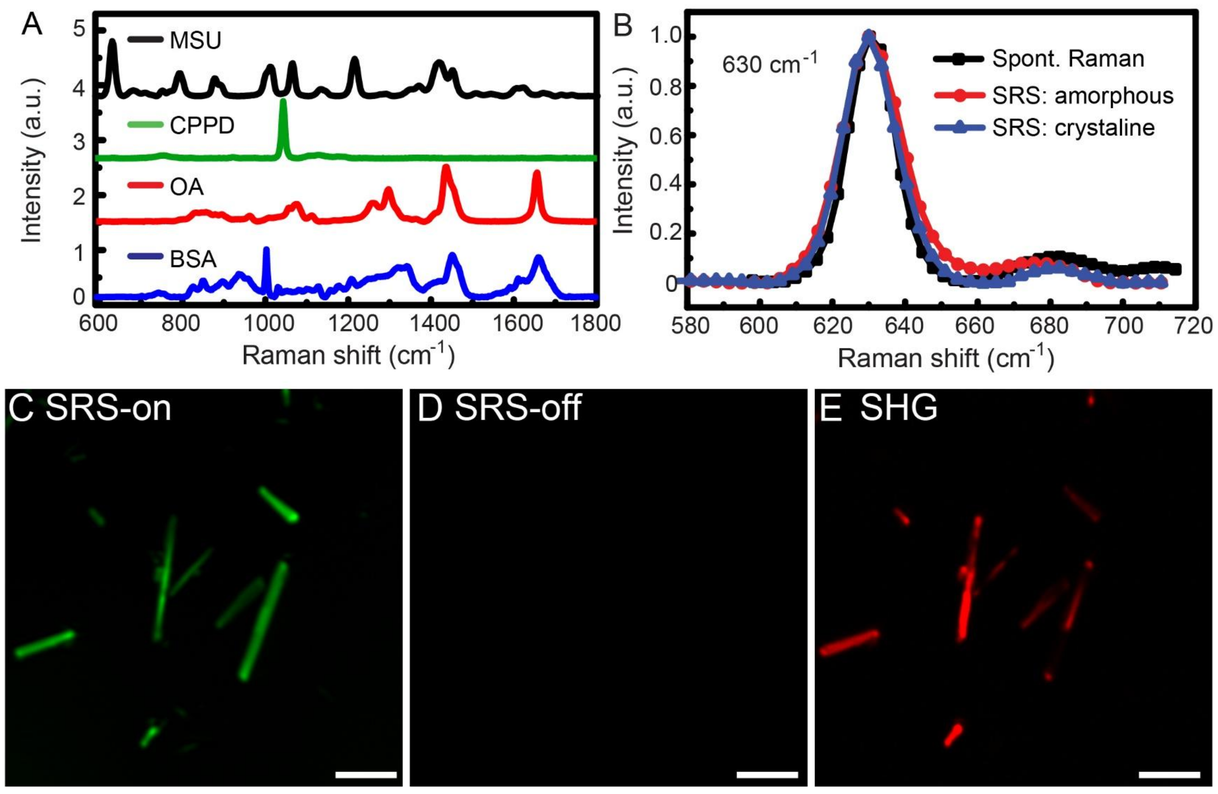


Fig 2. Spontaneous and stimulated Raman spectra of standard chemicals. *(A) Spontaneous Raman spectra of MSU, CPPD, lipid (OA) and protein (BSA). (B) Raman and SRS spectra of crystalline and amorphous MSU samples.(C) on-resonance SRS image of MSU crystals at 630 cm^{-1}; (D) off-resonance SRS image takend at 700 cm^{-1}; (E) SHG image of the same crystals;*

Imaging thin frozen sections of human tissues

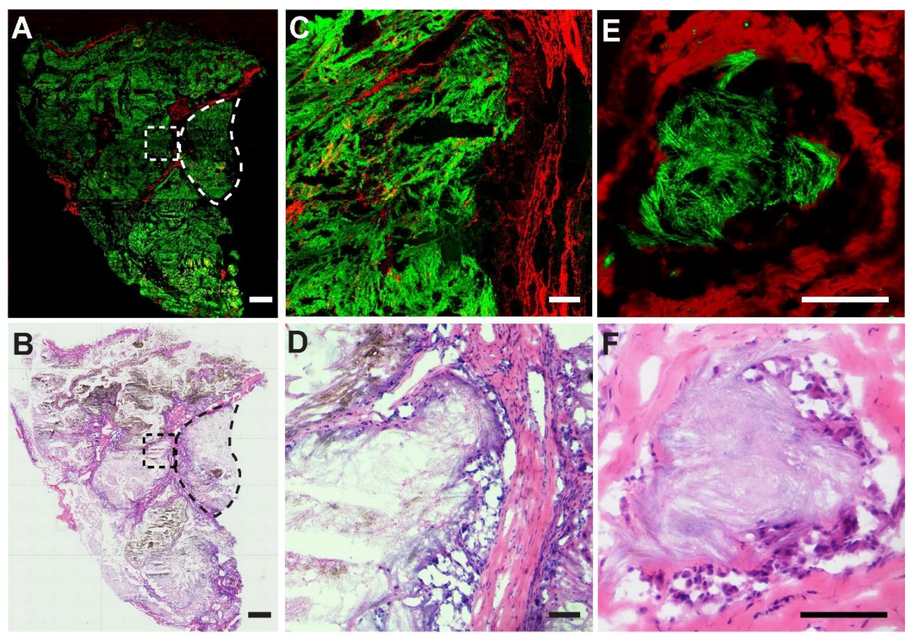


Fig 5. *(A) Representative stiched large-scale SRS/SHG image and (B) H&E image of adjacent tissue sections, showing the distributions of MSU (green) and collagen fibers (red), with a typical granuloma-like structure (dashed circle). (C-D) Enlarged images of the dashed square area in (A-B). (E-F) A typical tophi structure containing an enveloped crystal core. Scale bar: 500 μm (A-B), 100 μm (C-F).*

Imaging protein misfolding-Alzheimer's Disease

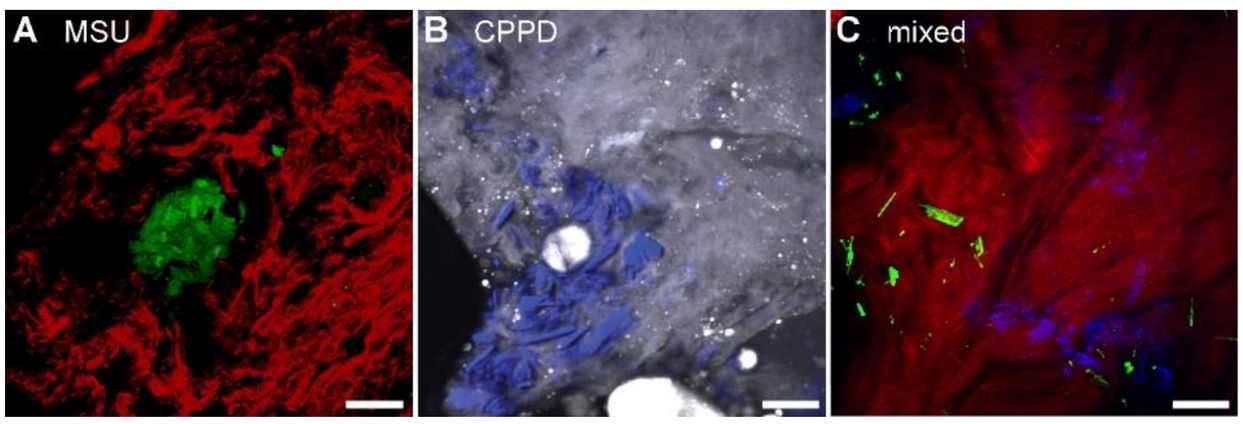
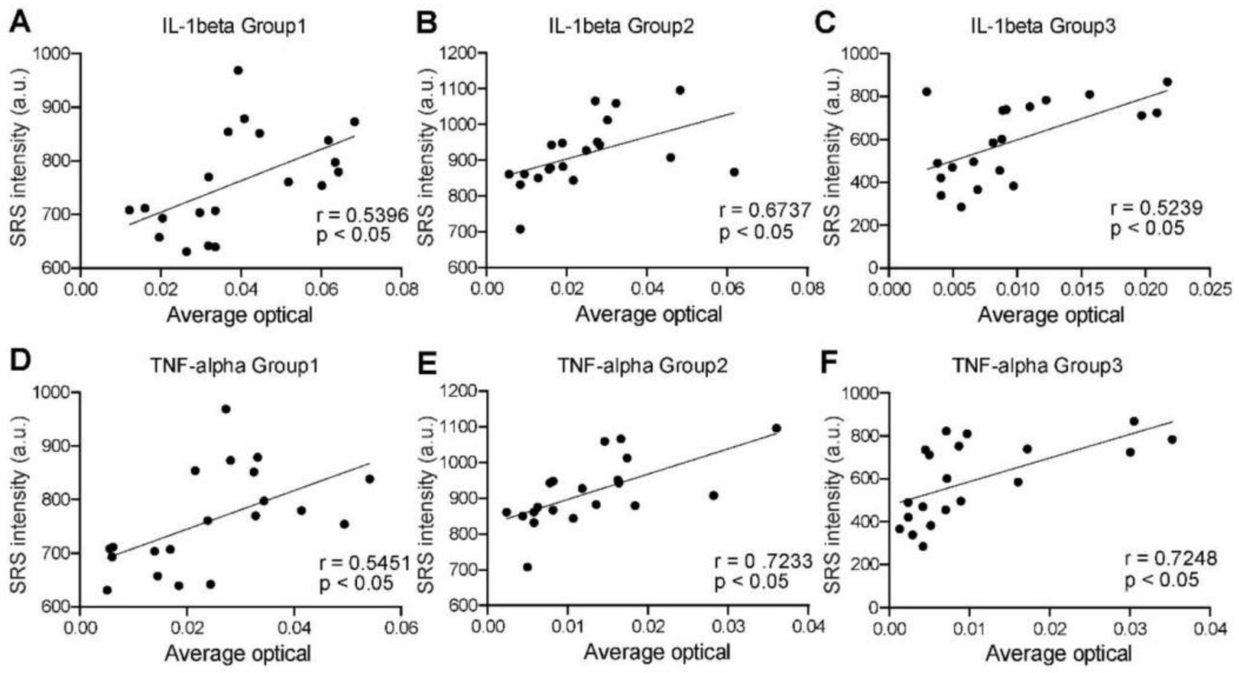


Fig 3. Differentiation of pseudogout and early detection of microcrystals in fresh tissues of rat models with multicolor SRS.

Correlation between SRS microscopy and immunofluorescence



Science Advances, 2018, 4, eaat7715Science Advances, 2018, 4, eaat7715
Theranostics , 2019, 9(9), 2541-2554.

Minbiao Ji: minbiaoj@fudan.edu.cn