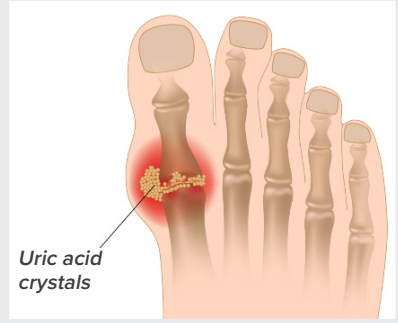
Diagnosing Gout

Raman spectroscopy for decisions at point-of-care

Accurate diagnosis of medical conditions at point-of-care speeds treatment, improves patient outcomes, and reduces healthcare costs. In this application note, we show how Raman spectroscopy is emerging as a tool to accurately diagnose gout and pseudogout for arthritis sufferers. Using a new compact point-of-care Raman spectroscopy (POCRS) instrument powered by a Wasatch Photonics integrated 785 nm Raman system, rapid objective testing of affected joints for painful crystals may be within reach for more clinics than ever before. The instrument has demonstrated comparable performance to a research-grade Raman microscope, and very good agreement with the existing gold standard diagnostic method in a clinical study.

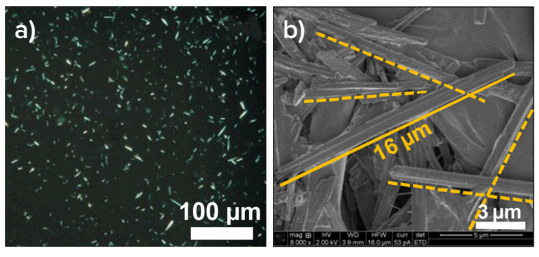
Up to 3% of people suffer from gout, a common form of arthritis that results in intensely painful bouts of joint swelling, warmth, and redness. Gout attacks can last for days or weeks, even causing permanent damage to joints and limiting motion over time if untreated. In a clinical setting, symptoms of gout mimic pseudogout - both are caused by buildup of arthropathic crystals in the joint space, but of different types, necessitating different treatment. Larger hospitals have the resources to distinguish gout from pseudogout and other arthritic conditions, but many smaller clinics or remote facilities must rely on clinical symptoms, resulting in up to 30% of patients being diagnosed incorrectly.



A new compact point-of-care device developed at Case Western University seeks to improve diagnosis by using Raman spectroscopy in combination with a novel, low-cost disposable sample cartridge to isolate and analyze crystals from fluid of the affected joint. Their shoebox-sized instrument can determine the presence, identity, and even concentration of crystals via automated analysis. By supplementing information gleaned from clinical symptoms and existing techniques, this new instrument has the potential to speed accurate diagnosis, improve clinical outcomes, and reduce the number of unwarranted hospital admissions. Its limit of detection has been shown to be more than adequate to observe clinically relevant concentrations of arthropathic crystals, and may even open up new opportunities for early detection of gout and pseudogout, as well as monitoring of treatment.

A THOUSAND TINY NEEDLES

Gout and pseudogout are forms of arthritis caused by a natural inflammatory response to micron-sized crystals deposited in the joint spaces and soft tissues. In gout, those crystals are needle-like MSU (monosodium urate hydrate), caused by excess uric acid in the blood. In pseudogout, the crystals are smaller, block-like CPPD (calcium pyrophosphate dihydrate), the origin of which is not well understood. Though symptoms of many arthritic conditions may be very similar, the short term treatment and long-term management for each condition differs, making accurate diagnosis important.



a) Polarized light microscopy of MSU crystals in digested synovial fluid, and b) SEM image of crystals of MSU on the filter membrane.
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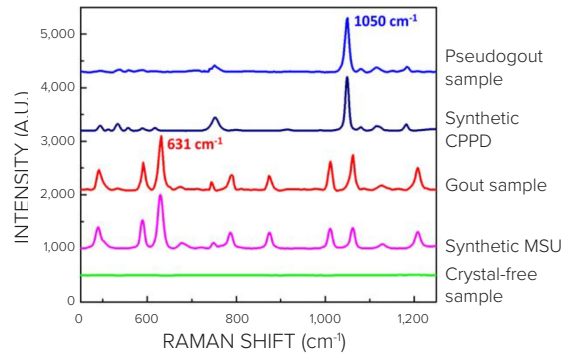


Raman spectra of samples containing MSU & CPPD crystals: clinical, synthetic, and control. (Li, Bolan, Arthritis & Rheumatology 68.7 (2016): 1751-1757. https://onlinelibrary.wiley.com/doi/abs/10.1002/art.39638

GETTING THE RIGHT DIAGNOSIS

The current gold standard in diagnosis of gout and pseudogout uses compensated polarized light microscopy (CPLM) of synovial fluid extracted from the affected joint. In practice, it is only used for ~10% of diagnoses, as it requires a certified operator and a microscope fitted with specialized optics. Even then, CPLM has an average false negative rate of ~30%, with substantial variability between labs and operators. No other technique in use is both sensitive and specific for these conditions. As a result, many diagnoses are made incorrectly based on clinical symptoms, particularly in community health centers and primary care settings, where the majority of arthritic cases present.

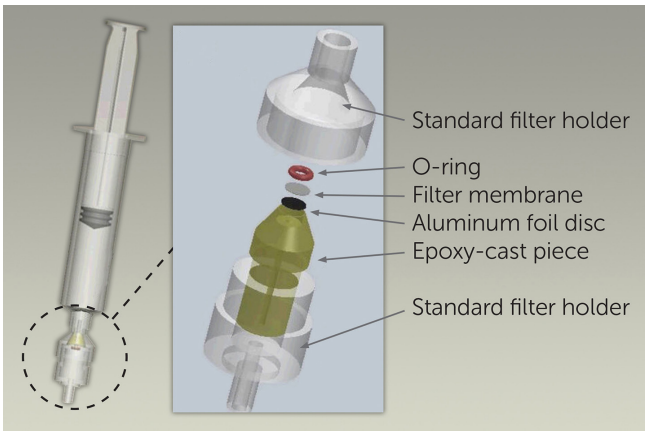
So what is the alternative? Research studies have shown Raman microscopy to be successful in analyzing synovial fluid for the presence of MSU, CPPD and cholesterol crystals. Both MSU and CPPD display strong, distinct Raman fingerprints – MSU with characteristic peaks at 590 and 631 cm^{-1}, and CPPD at 1050 cm^{-1}. In order to translate this technique to the clinic, however, the cost, size, and dependence on the operator needs to be significantly reduced.

The point-of-care Raman spectroscopy (POCRS) system developed at Case Western University achieves each of these objectives by integrating a novel cartridge for the isolation and concentration of arthropathic crystals in synovial fluid with a compact, cost-effective Raman-based detection instrument using a Wasatch Photonics WP 785L integrated Raman system. It opens up the potential for unbiased and unsupervised diagnosis of crystal species at the point-of-care, and within one hour of fluid collection – eliminating the need for storage and shipment to a certified operator for crystal detection via CPLM.

SAMPLE CONCENTRATION VIA MICROFILTRATION

To achieve sufficient signal, Raman spectroscopy requires direct overlap of a small focal spot size (~70 µm) with the crystals of interest (up to 20 µm in length). The team developed a microfilter cartridge to concentrate crystals for detection, mounting it to the sample syringe tip for ease of use. Each sample is first subjected to a 60 minute digestion phase to dislodge crystals from organic debris in the sample for more effective filtering and isolation.

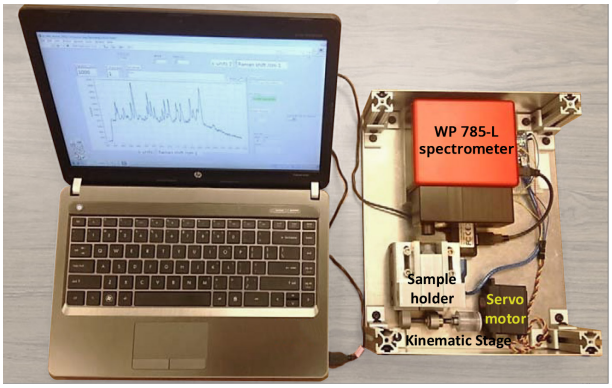
The digested synovial fluid is pushed through the filter cartridge slowly, concentrating the crystals into a ~0.9 mm diameter spot on a polypropylene filter membrane. The filter cartridge is then removed from the syringe and inserted in the POCRS instrument for Raman analysis.



Low-cost, disposable syringe with custom microfiltration cartridge for processing of joint fluid samples for Raman spectroscopy.

SENSITIVE, CONSISTENT RAMAN ANALYSIS

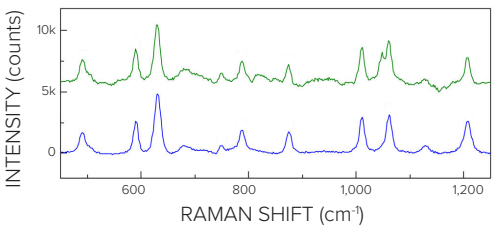
The compact POCRS instrument is comprised of a highly sensitive Wasatch Photonics Raman system adapted for OEM use (WP 785L), a filter cartridge holder mounted on a translation stage, a servo motor to move the translation stage, and a micro-controller to drive the servo motor. The WP 785L integrated Raman system includes a spectrometer with a spectral resolution of 10 cm^{-1} (50 µm



Point-of-care Raman system for the detection of arthropathic crystals in the diagnosis of gout and pseudogout.

slit width) and a 785 nm laser delivering approximately 50 mW of laser power into a ~70 µm spot on the sample via a single lens with 25 mm focal length. The low f-number of this system (f/1.3) maximizes the Raman signal that can be collected from the sample to improve the limit of detection. The instrument collects spectra at ~30 µm steps across the filter cartridge to increase the chance of seeing deposited crystals, even if scarce. Fluorescence background from organic material in the sample is minimized by using a short spectral acquisition time (0.5 seconds, 60 scans averaged per step). This short acquisition time is possible due to the high sensitivity and low noise of the spectrometer design.

Raman spectra for each point are processed in a series of steps: subtraction of the background fluorescence, normalization to the 809 cm^{-1} peak of the polypropylene membrane, and subtraction of the pure polypropylene membrane spectrum. A typical spectrum collected and processed via this method exhibits a signal to noise ratio of ~60. As can be seen by looking at the processed spectra of clinical MSU crystals (green spectrum) vs synthetic MSU crystals (blue spectrum), for highly concentrated samples, most peaks indicative of the analyte (491, 590, 631 cm^{-1}, etc.) can be clearly seen after processing. In this application, the presence of MSU peaks at 590 cm^{-1} and 631 cm^{-1} or the CPPD peak at 1050 cm^{-1} are sufficient to confirm the existence and identity of crystals.

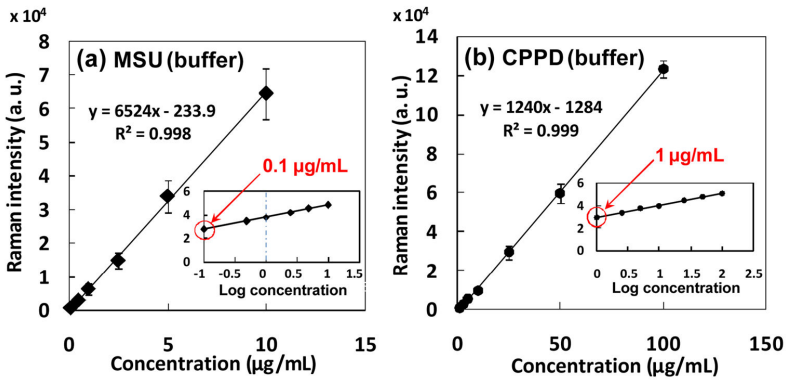


The processed Raman spectrum of a clinical MSU crystal sample shows excellent agreement with synthetic MSU crystals, validating the method.

The sensitivity and selectivity of the POCRS instrument to detect MSU and CPPD crystals was validated through a variety of tests with synthetic and clinical samples to determine limit of detection and ability to measure in synovial fluid. Comparison of results against established techniques such as Raman microscopy, SEM, and CPLM affirmed the ability of the technique to accurately determine the presence and identity of crystals in synovial fluid.

RUNNING THE GAUNTLET OF SAMPLES

To correlate crystal concentration to signal and establish limit of detection, synthetic MSU and CPPD crystals in buffer solution were used to generate calibration curves at concentrations spanning 0.1 – 50 µg/mL for MSU, and 0.1 – 100 µg/mL for CPPD. Three samples were measured at each crystal concentration, summing the Raman spectra and retesting at three randomly chosen cross sections of the filter. The coefficient of variation calculated ranged from 2% to 8%, showing good repeatability of the technique.



Calibration curves and limit of detection for synthetic (a) MSU and (b) CPPD crystals in buffer solution. Reproduced from Li, Bolan, Analyst 139.4 (2014): 823-830 with permission from The Royal Society of Chemistry.

The lowest concentration detected using this method was 0.1 µg/mL for MSU, and 1 µg/mL for CPPD. The long, thin shape of the MSU crystals likely increased their detection probability via this cross-section method relative to the smaller, brick-like CPPD crystals. Validation of the instrument using synthetic crystals mixed in synovial fluid with no history of joint disease also showed good correlation of signal with concentration, though signal was lower for MSU crystals in this matrix, possibly due to attenuation by residual organics on the filter. Given that clinical samples are reported anecdotally to be in the range of 10-100 µg/mL, the measured limit of detection of the POCRS system is well below the clinically relevant concentration in both cases. This bodes well for the ability of the technique to detect crystal-related arthritis more sensitively than current techniques, and at an earlier stage.

VALIDATING THE METHOD – SEM & RAMAN MICROSCOPY TO CPLM

Scanning electron microscopy (SEM) and Raman microscopy were performed to validate the presence of crystals and their identity. SEM provided visual confirmation of the presence of crystals consistent with the expected size and shape. Raman microscopy supported this with chemical images of crystal distribution across the membrane based on the intensity of the 631 cm^{-1} peak of MSU.

The final step was a benchmark study against the "gold standard" – CPLM. A total of 174 clinical samples from symptomatic patients were analyzed both by the POCRS instrument and by certified CPLM experts with >20 years of experience. Results were consistent in 89.7% of samples, with kappa coefficients indicating excellent agreement between the two methods for gout and very good agreement for pseudogout. A small number of samples identified by the instrument as being positive for MSU crystals were missed by CPLM, and vice versa. It is interesting to note, but not entirely surprising, that 11 samples identified by POCRS as containing CPPD crystals were missed by CPLM, as CPPD crystals are known to be challenging to detect microscopically due to their weak birefringence. If POCRS is able to detect these crystals more consistently than CPLM, it may offer clinicians additional and valuable information to aid in the diagnosis of pseudogouts.

While the POCRS method identifies the type of crystals, this information alone would not constitute a conclusive diagnosis of gout or pseudogout, as they remain clinical diagnoses. In practice, it is hoped that POCRS will be seen as complementary in the diagnosis of gout and pseudogout, enabling clinics not equipped to perform CPLM to quickly screen for the presence of crystals causing gout and pseudogout versus other arthritic conditions.

CONCLUSION

Raman spectroscopy offers a chemically definitive and highly sensitive method for identification and quantification of the crystals causing gout and pseudogout. By using a low-cost, disposable syringe-filtration technique to isolate crystals from synovial fluid, a sample can be quickly analyzed with a fully integrated and automated Raman analysis instrument incorporating a Wasatch Photonics integrated 785 nm Raman system. This relatively low-cost point-of-care instrument would offer a much greater number of clinics the ability to detect clinically relevant concentrations of arthropathic crystals in synovial fluid using minimally trained staff, thus complementing clinical assessment of symptoms and current methods. By improving the accuracy and availability of correlated diagnosis, this instrument has the potential to reduce the length of urgent care/ER stays, decrease hospital admission rates, and improve patient reported outcomes. As we look to this and other Raman-based techniques for point-of-care diagnostics, time spent in the clinic looks just a little less painful.

For a more detailed version of this app note and links to all references, visit the Knowledge section on our website.

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