Observation and identification of autofluorescent urine crystals may be linked to a sign of urolithiasis

Syue-Liang Lin¹,² · Chen-Yuan Chung¹ · Zih-Ting Chen¹ · Chih-Chia Huang³ · Yun-Zhen Li¹ · Eric Yi-Hsiu Huang⁴,⁵ · Huihua Kenny Chiang¹,²

Received: 22 March 2022 / Accepted: 15 June 2022 / Published online: 25 July 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Urolithiasis is a common disease of the urinary system. Its recurrence rate is high and may increase medical expenses. Urine stones are composed of urine crystals and other impurities. We discovered the existence of autofluorescence in some of the urine crystals, especially in urolithiasis patients. The fluorescent molecule existed in urine crystals was verified and identified. We have applied micro-Raman and fluorescence microscopy to classify the urine crystals, used confocal laser scanning microscopy (CLSM) to examine the 3D images and spectra of autofluorescence in crystals, used Fourier-transform infrared spectroscopy (FTIR) and mass spectrometry (MS) to identify the type of fluorophore in the autofluorescent urine crystals in urine. Riboflavin was identified as one of the major fluorophores in these autofluorescent urine crystals. The prevalence rates of the autofluorescent crystals in urolithiasis patients and subjects without the history of urolithiasis were to gather statistics. We observed that 80% of urolithiasis patients had autofluorescent crystals. Contrastingly, such crystals existed in only 7% of subjects without the history of urolithiasis. The presence of autofluorescent urine crystals may be linked to a sign of urolithiasis.

Keywords Urine crystal · Urolithiasis · Autofluorescence · Confocal laser scanning microscopy

Introduction
Urolithiasis is one of the most common urologic diseases; it has a high recurrence rate and results in substantial pain and an increased risk of renal failure [1–3]. The prevalence of urolithiasis is approximately 4–15% in North America, Asia, Europe, and Australia [4]. Urinary stones mainly contain of calcium oxalate dehydrate (COD), calcium oxalate monohydrate (COM), uric acid (UA), hydroxyapatite (HAP), dicalcium phosphate dehydrate (DCPD), struvite, and cystine. After identifying the urine stone type of their patients, clinicians could provide adequate medical treatment and dietary advice to prevent the recurrence of kidney stones [5].

The urine stones are composed of urine crystals and other metabolites. The concentration of precursors of stone in the urine of the stone-formers is higher than in normal subject that resulting the greater crystalluria of the stone-formers [6]. The formation of urine stones is a multistep process that includes nucleation (lithogenesis), growth, aggregation, and retention [4, 7, 8]. The process is affected by many factors, both promoters and inhibitors, measured and unmeasured, acting in urine to trigger crystal formation [9]. For urolithiasis patients, the components of urine crystals have a high correlation (90.4%) with the composition of urine stones [10]. Calcium oxalate crystals (COD and COM crystals) were the most common crystals in urolithiasis patients. In a previous study, we found the
existence of urine crystals in the urine of over 80% of the urolithiasis patients [11]. Knowing the types of urine crystals maybe helpful in understanding the cause of urolithiasis and further preventing its recurrence.

Manual microscopic examination of urine sediment is the gold standard in clinics for analyzing crystal types based on crystal morphology [12]. Automatic microscopic instruments have been widely used for examining urine sediment in clinical practice, with high-throughput and high concordance rate [13]. All abnormal results identified through the automatic procedure should be further confirmed by manual microscopic examination [14].

Spectroscopic and imaging techniques have been used for urine crystal analysis. Raman technique has many advantages for analyzing amorphous irregularly shaped urine crystals. Raman spectroscopy is not affected by water in the surrounding environment, yielding a high signal-to-noise ratio peak, high specificity, and selective qualitative information in biological samples [15, 16]. Micro-Raman spectroscopy (MRS) provide a non-destructive identification of specific vibration peaks in the spectra of urine crystals; the types of the crystals can be characterized accurately and rapidly [17]. Overall, analyzing urine crystals by Raman spectroscopy has the following benefits: (i) simple sample treatment, (ii) independence of water in the environment, (iii) no additional reagents required, and (iv) real-time monitoring.

Chiu et al. developed a nanoplatform, based on Fe₃O₄ nanoparticles, to collect crystals from urine and identify the types of crystals through Raman spectroscopy [18].

Since urine crystals are small, amorphous, irregularly shaped, transparent, and colorless, they are difficult to observe under a microscope. Lo et al. further modified the Fe₃O₄ nanomaterial by labeling dyes (crystal violet) to show the position of crystals for faster automatic crystal analysis [11]. Chen et al. analyzed the urine crystals by micro-Raman system and confocal microscopy for studying the correlation between urine crystals formation and gout patients [19].

In our recent studies, we discovered that some urine crystals have autofluorescence, which differs from other literature [20] that including exogenous dye to urine crystals for conditional study. To the best of our knowledge, these findings have never been presented in the literature. Furthermore, we found that the autofluorescent urine crystals appeared in most urolithiasis patients. We think it is interesting to identify the type of fluorophores and investigate the role of the existence of the fluorophores in urine crystals. In this study, we aim to characterize the urine crystals through confocal laser scanning microscopy (CLSM) and Fourier-transform infrared spectroscopy (FTIR) and further identify the type of fluorophores present in the autofluorescent urine crystals through mass spectroscopy (MS).

### Materials and methods

#### Collection, preparation of urines, and crystal extraction

This study was approved by the Institutional Review Board (IRB) of Taipei Veterans General Hospital (TVGH) and National Yang Ming Chiao Tung University (NYCU). All urine samples from subjects without history of urolithiasis (Group A, n = 15) and patients who had definite diagnosis of urolithiasis (Group B, n = 15) were enrolled. The first morning urine samples were collected for further extraction and characterization. The procedures for the synthesis of Fe₃O₄ nano-platforms and treatment of urine were performed according to the methods reported by Chiu et al. [18]. The solution containing Fe₃O₄-bound urine crystals was dropped onto the slide for subsequent crystal analysis. For classifying the crystals, CLSM equipped with the lambda scan equipment for analyzing the autofluorescent images and spectra of the crystals, attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) for identifying the functional groups of organic fluorophores in the crystals, and mass spectrometry (MS) for determining the molecular weight of fluorophore in crystals.

#### Micro-Raman spectroscopy and fluorescence imaging

A customized Raman system with a fluorescent microscope was used in this study, and it combined Raman signal measurement, fluorescence imaging, and polarized light imaging to analyze the urine crystals of extracts. For the Raman signal measurement with 50x/100x microscope objectives, the excitation source was a 70 mW 785 nm laser (LASOS, Germany) and a Raman imaging spectrograph (Acton LS785, Princeton Instrument, NJ, USA). The sample was placed on a wet plate to enhance the signal-to-noise ratio. For fluorescence imaging, the mercury lamp and the camera were mounted with an RGB filter set (λex: 580 nm bandpass filter (R), 475 nm bandpass filter (G), 434 nm bandpass filter (B); λem: 630 nm bandpass filter with bandwidth ~60 nm (R), 535 nm bandpass filter with bandwidth ~38 nm (G), a 470 nm bandpass filter with bandwidth ~38 nm (B)). Raman spectra analysis software (WinSpec/32 Ver.2.6.24.0, Roper Scientific and Origin 9, OriginLab Corporation) and image-processing software (ImageJ, National Institutes of Health, USA) were used for Raman and fluorescence imaging.
3D fluorescent images and spectral analysis by CLSM

A confocal laser scanning microscope (CLSM, ZEISS LSM 880 M equipped with Airyscan and QUASAR detectors) was used to acquire and analyze the 3D fluorescence images and spectra of urine crystals. The fluorescence spectra and distribution information of the fluorophores were measured using the 34-channel QUASAR detectors of the CLSM system. For sample preparation, 1 mL of the preprocessed sample was placed on the slide and covered with a cover glass of 0.17 mm thickness, and the slide was mounted. The data were acquired by LSM 880 M and analyzed using image-processing software (ZEN 2.3 SP1 Ver.14.0.0.201, Carl Zeiss). In this experiment, lasers emitting beams of six different wavelengths (405, 440, 458, 488, 514, and 543 nm) were used as excitation sources. The samples were analyzed along the Z-axis to determine the 3D distribution of autofluorescence in the urine crystals.

Functional groups of fluorophores analysis using micro-ATR-FTIR

A Micro-Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (micro-ATR-FTIR, Vertex 80v/Tensor 27, Bruker, Germany, wavenumber range of FTIR: 1100–3750 cm⁻¹) was used to analyze the functional groups in fluorophores non-destructively and conveniently by employing multi-point sampling. The analysis was conducted without further preparation and purification by a 100 µm IR-fiber optic ATR-probe [21]. The ATR technique is a suitable measuring method for analyzing the 10–100 µm urine crystals using total internal reflection and evanescent wave.

Mass spectrometry measurements for identifying the fluorophores

Liquid chromatography–tandem mass spectrometry (HPLC/MS-MS, VARIAN 901-MS, Agilent, United States) was used to identify the molecular weights of fluorophores in urine crystals by determining the mass-to-charge ratio (m/z) of the complete structure or fragments of fluorophores. To determine the presence of fluorophores in urine crystals, autofluorescent and non-fluorescent UA and COD urine crystals were used for the mass spectrometry measurements. The ionized components of the samples were prepared by electrospray ionization (ESI) using a high voltage (4.5 kV) to create an electrospray; this was followed by analysis of these components using mass spectrometry [22].

Results

Fluorescence microscopy and Raman spectroscopy analysis of urine crystals

Image J™ was used to quantify the autofluorescence images of the urine crystals. Figure 1 shows the ambient images of the UA (a), COD (b), and COM (c) urine crystal and the autofluorescence images of crystals excited by a mercury lamp combined with an RGB bandpass filter. The signal-to-background ratios (SBR) of the ROI (region of interest) in autofluorescent urine crystals were 9.85 (UA), 2.81 (COD), and 4.75 (COM).

The crystals were characterized by their corresponding Raman peaks and classified into the appropriate urine crystal categories (UA/COD/COM). The Raman spectra of the common urinary crystals were assigned as follows: bands at 999 ± 5 cm⁻¹ for UA, 911 ± 5 and 1470 ± 5 cm⁻¹ for COD, 897 ± 5, 1461 ± 5, and 1489 ± 5 cm⁻¹ for COM. The baselines of spectra were determined by third-order polynomial fitting using the least-squares method, followed by passing the Raman signal through a Savitzky–Golay filter for curve smoothing. The Raman spectra of the clinical urine samples (on the wet slides) are shown in Fig. 2.

We analyzed urine crystals from Group A (subjects without history of urolithiasis) and B (urolithiasis patients). Figure 3a shows the statistical results of Group A: 66% of the subjects had no crystals, 20% had non-fluorescent COD crystals, 7% had non-fluorescent UA crystals, and only 7% had autofluorescent COM crystals. Figure 3b shows the statistical results of the Group B: 100% of the subjects had urine crystals, 20% of the patients had non-fluorescent crystals (10% COD, 10% HAP), while 80% of the subjects had autofluorescent crystals (27% UA, 27% COD, 20% COM, 6% HAP). We observed that 12/15 of the urolithiasis patients had autofluorescent crystals in their urine, whereas autofluorescent urine crystals appeared only 1/15 of the subjects without history of urolithiasis; thus the occurrence of autofluorescent crystals was much higher in urolithiasis patients. We also observed the existing of urine crystals in 5/15 of the subjects without history of urolithiasis.

Autofluorescence analysis of the urine crystals by CLSM

CLSM was used to analyze the autofluorescence of urine crystals for measuring the 3D fluorescence images along the Z-axis of the crystal by 594 nm excitation. The 2D cross-sectional fluorescent images of the crystals are shown in Fig. 4. The urine crystals collected from the
urolithiasis patient emitted strong autofluorescence when excited at 594 nm (He–Ne Laser), which may be due to the presence of fluorophores in the crystals. The emission channel was set to 594–720 nm bandpass filter, and a 100x/NA = 1.4, oil DIC microscope objective was used for 3D confocal imaging. The optical sectioning thickness was

![Fig. 1](image)

**Fig. 1** a UA, b COD, and c COM urine crystals under ambient light, and mercury lamp irradiation with the RGB bandpass filter set, respectively

![Fig. 2](image)

**Fig. 2** Micro-Raman spectroscopy results of a UA, b COD, and c COM urine crystals
1.2 μm for analyzing the location of fluorophores. We observed autofluorescence mostly coming from the inside of the crystal.

Figure 5 shows the normalized spectra of the autofluorescent UA, COD, COM urine crystals. The excitation laser wavelength ($\lambda_{\text{ex}}$)/emission peaks ($\lambda_{\text{em}}$) were 405/515 ~ 530, 440/520 ~ 565, 488/560 ~ 580, 514/580 ~ 590, and 543/590 ~ 635 nm, measured by the CLSM. We also calculated the full width at half maximum (FWHM) of the emission spectra of autofluorescent crystals. The FWHM of emission spectra of autofluorescent UA, COD, and COM were 85–115, 135–195, and 150–200 nm, respectively.

**Micro-ATR-FTIR spectra of autofluorescent and non-fluorescent urine crystals**

The results of the micro-ATR-FTIR analysis of the three types of urine crystals are shown in Fig. 6; it shows the IR spectra of the autofluorescent urine crystals (UA/COD/COM), standard materials. In the IR absorption

![Fig. 3](image_url)

**Fig. 3** The percentages of autofluorescent crystals that exist in a subjects without history of urolithiasis ($n$ = 15) and b urolithiasis patients ($n$ = 15) are 7 and 80%, respectively.

![Fig. 4](image_url)

**Fig. 4** CLSM 3D confocal imaging of a single UA crystal with Z-axis scanning with every 1.2 μm step. ($\lambda_{\text{ex}}$: 594 nm; $\lambda_{\text{em}}$: 594–720 nm)

![Fig. 5](image_url)

**Fig. 5** Normalized emission spectra of autofluorescent a UA, b COD, and c COM urine crystal at different excitation wavelengths ($\lambda_{\text{ex}}$: 405, 440, 458, 488, 514, and 543 nm)
spectrum of the UA crystals, we observed the C–N stretching at \( \approx 1066 \text{ cm}^{-1} \), the C = C stretching peak at \( \approx 1577 \text{ cm}^{-1} \), the C = O stretching peak at \( \approx 1641 \text{ cm}^{-1} \), the vibration stretching peaks of the O–H stretching at \( \approx 2700–3350 \text{ cm}^{-1} \), and the N–H stretching peaks at \( \approx 2818 \) and \( \approx 2988 \text{ cm}^{-1} \) in the autofluorescent UA urine crystal.

In the IR absorption spectra of COD and COM crystals, we observed the C–N stretching at \( \approx 1000 \) to \( 1060 \text{ cm}^{-1} \), the C–O stretching peak at \( 1317–1323 \text{ cm}^{-1} \), the intense vibration C = O peak at \( \approx 1614 \) to \( 1615 \text{ cm}^{-1} \) (due to the asymmetric COO⁻ vibration), and the symmetric/asymmetric O–H stretching broad absorption band between \( 2700 \) and \( 3600 \text{ cm}^{-1} \) in the autofluorescent COD/COM urine crystals [23].

**Mass spectra of urine crystals**

For further verification of fluorescent objects in the urine crystals, the autofluorescent crystals and non-fluorescent crystals were analyzed by ESI–MS (Fig. 7). Autofluorescent and non-fluorescent crystals were chosen for mass spectrometry and comparison measurement. The data for the UA and COD crystals provided representative results. The signals of m/z were 215.14, 247.17, 279.20, 311.22, 343.25, 375.28, 407.25, 412.28, 445.29, and 478.22 which appeared only in autofluorescent crystals and not in standard materials.

**Discussion**

**Autofluorescence of urine crystals**

In the statistical results of the autofluorescent crystals (Fig. 3), we observed that urine crystals were more prevalent in urolithiasis patients (100 vs. 34%). More COD crystals were found in urine of subjects without history of
uro lithiasis; however, more COM crystals were found in urine of urolithiasis patients, which is consistent with the previous study [24]. The percentage of autofluorescent urine crystals in normal was 7% which is much lower than the 80% identified within urolithiasis patients. In those with urine crystals, autofluorescence were more common in urolithiasis patients than in those without history of urolithiasis (80 vs. 20%). Moreover, the highest percentage of urine crystals in both non-symptomatic subjects and urolithiasis patients were COD crystals. Compared to other types of crystals, the autofluorescent UA, COD, and COM urine crystals accounted for 80% of urolithiasis patients. These results match well with the top three urine crystals of urolithiasis accounted for 80% of urolithiasis patients. These results were COD crystals. Compared to other types of crystals, the autofluorescent UA, COD, and COM urine crystals accounted for 80% of urolithiasis patients.

We further analyzed the fluorescent images and spectra of urine crystals using 3D CLSM to determine the fluorophores distribution. The fluorescence images (Fig. 4) showed that the fluorophores were mainly distributed inside the urine crystals. According to the results, it can be understood that the fluorophores were gradually embedded in the interior structure of the urine crystals during their growth process.

The fluorescence spectra of UA/COD/COM urine crystals were further analyzed using a CLSM equipped with the QUASAR system. Figure 5 shows that the autofluorescent urine crystals had different emission peak locations when excited by different lasers. All autofluorescent urine crystals (UA/COD/COM) have similar different emission peak locations at 515–530, 520–565, 525–565, 560–580, 580–590, and 590–635 nm upon excitation at 405, 440, 458, 488, 514, and 543 nm, respectively. We speculate that multiple fluorophores contributed to the broadband spectra of fluorescent crystals excited by different lasers.

To clarify whether this broadband result is caused by spectroscopic system factors or fluorophores in urine crystals, we measured the spectrum of the pure fluorescent using CLSM. The results, presented in Fig. S1, show the same peak when it was excited by different lasers. This presents the patterns of the emission spectra and indicates that the autofluorescent crystals possibly contained multiple fluorophores. We also further compared the full width at half maximum (FWHM) of the autofluorescent urine crystals and pure fluorescent (riboflavin). The FWHM of the emission spectra of riboflavin was maintained at the same value (85 nm, in Fig. S1) and the FWHM of autofluorescent urine crystals were 85–115, 135–195, 150–200 nm for UA, COD, and COM in Fig. 5, excited by different lasers. In contrast, the FWHM of autofluorescent crystals become narrower with excited with longer wavelengths’ lasers, while the FWHM of the riboflavin did not change. We think that there were multi-fluorophores in these urine crystals. The spectral overlap percentages of riboflavin and autofluorescent crystals were approximately 73% in UA, 55% in COD, and 48% in COM, indicating that the COM and COD might contain more fluorophores than UA.

Previous studies have shown the existence of flavin-based molecules with emission peaks of fluorescence spectra at ~ 530 nm in urine [25]. We speculated that the emission peaks of fluorescent urine crystals at ~ 510–560 nm might originate from riboflavin fluorophores in the crystals. We found that the autofluorescent urine crystals are mainly composed of different types of fluorophores and UA/COD/COM components.

**The functional group of fluorophores in urine crystals with autofluorescence**

The difference in FTIR absorption spectra (Fig. 6) between the autofluorescent and non-fluorescent crystals might indicate the IR absorption by the fluorophores in urine crystals. Compared with the non-fluorescent standard materials of UA + COD + COM, the autofluorescent crystals have the following additional or shifted absorption peaks: C–N (~ 1000 to 1060 cm⁻¹), C–O (~ 1324 cm⁻¹), C = O (~ 1655 cm⁻¹), and O–H (2750–3750 cm⁻¹), stretching might belong to the functional groups of fluorophores in the crystals. The O–H and C–O stretching only appeared in autofluorescent UA crystals and not in UA standard material. The C = O, C–O, and C–N stretching peaks shifted in autofluorescent COD and COM absorption spectra.

A previous report showed that riboflavin, which has O–H, C = O, C–O, and C–N functional groups in the structure, in urines might promote the formation of urinary stones [26]. This was consistent with the additional peaks and shifted peaks in fluorescent crystals. The IR absorption peaks at 1000–1060 cm⁻¹ (C–N stretching) and ~ 1654 cm⁻¹ (C = O stretching) originated from the structure of the isoalloxazine ring [27–29]. These results were consistent with our inference that riboflavin is most likely the main fluorophore in autofluorescent urine crystals.

**The molecular weight of fluorophore in UA/COD/COM urine crystals**

Figure 7 shows the signals of 215.14, 247.17, 279.20, 311.22, 343.25, 375.28 (m/z) belonged to the fragments of riboflavin by NIST database, which only appeared in the ESI–MS data of the autofluorescent crystals and could be identified. The 375.28 (m/z) was assigned to deprotonated riboflavin (376.37 g/mol, 376.37–375.28 = 1.09 ~ the m/z of H⁺, proton). We identified that riboflavin is one of the fluorophores in urine crystals from urolithiasis patients through the FTIR and MS spectra.

The NH₂ groups existed in many fluorescent substances in human urine, such as serotonin, tryptophan, catecholamine, kynurenine, biotin, neopterin, folic acid, uroporphyrin, and kynurenine, biopterin, neopterin, folic acid, uroporphyrin,
and coproporphyrin, etc. The signals of m/z of 407, 412, 445, and 478 in MS data could not match to the melatonin (M.W. 232), tryptophan (M.W. 204), catecholamine (M.W. 151), kynurenine (M.W. 208), bioterin (M.W. 237), neopterin (M.W. 253), folic acid (M.W. 441), uroporphyrin (M.W. 830), coproporphyrin (M.W. 654), etc. in the urine as we know. The concentrations of other possibilities (fluorophores) in urine crystals might be lower than the detection limits of IR and MS. In addition, the m/z of the other possibilities (fluorophores) might be out of the MS measurement range in this study, 200 < m/z < 600. Some crystal matrix protein (CMP), the fragment 1 of human prothrombin, is the predominant protein found in the organic matrix of urine crystals. The CMP or other protein may be the possibilities with weak autofluorescence [30–32].

Implications of autofluorescence urine crystals and urolithiasis

We identified that autofluorescent urine crystals appeared in patients with the definite diagnosis of urolithiasis. The implications were that we discover autofluorescent urine crystals in individuals with ambiguous status of urolithiasis, there are higher chances of diagnosing urolithiasis in such subjects. Our findings may assist in the identifications of urolithiasis in subjects without history of urinary stones. However, a possible artifact is that the urine crystals are soaked in the urine that containing various substrates, so that the autofluorescent molecules in the urine might adsorbed on the surface of urine crystals.

To the best of our knowledge, we presented a novel finding of urine crystals with autofluorescence especially in urolithiasis patients. However, there are some limitations in our study. The samples size of both groups was small. Despite that, the contrasting result of autofluorescent urine crystals in both groups was so obvious that we are confident that our findings are convincing. We will conduct further studies to enroll more individuals for further analysis. The enrolled subjects without history of urolithiasis may have urinary stones. Such individuals may be patients with early, or insignificant urolithiasis. We seek to answer this specific question in the future study.

Conclusion

In this study, we discovered (1) the existence of autofluorescent urine crystals, and (2) these autofluorescent urine crystals were mostly found in urolithiasis patients. We analyzed the types, images, spectra, functional groups, and fluorophores of urine crystals using the MRS, CLSM, FTIR, and ESI–MS systems, and identified riboflavin as one of the major fluorophores in the autofluorescent crystals.

Autofluorescent urine crystals were found in 80% of the urolithiasis patients, which was much higher than their occurrence in non-symptomatic patients (7%) in the study. The appearance of autofluorescent crystals in urine might be a potential risk indicator and may be linked to an early sign of urolithiasis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00240-022-01343-0.

Acknowledgements This research was supported by the Ministry of Science and Technology, Taiwan (NSC 102-2221-E-010-001-MY3, MOST 109-2221-E-010-001-MY3, MOST 109-2218-E-010-002).

Author contributions SLL wrote the main manuscript text; CYC, ZTC, and YZL conducted the experiments and analyzed all of the results in this manuscript. CCH provided some advices about this research. SLL, EYHH, and HHKC revised the manuscript. All authors reviewed the manuscript.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no conflict of interest.

References

1. Bihl G, Meyers A (2001) Recurrent renal stone disease—advances in pathogenesis and clinical management. Lancet 358(9282):651–656
2. Coe FL, Parks JH, Asplin JR (1992) The pathogenesis and treatment of kidney stones. N Engl J Med 327(16):1141–1152
3. Worcester EM, Coe FL (2010) Calcium kidney stones. N Engl J Med 363(10):954–963
4. Basavaraj DR, Biyani CS, Browning AJ, Cartledge JJ (2007) The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAU-EBU Update Series 5(3):126–136
5. Han H, Segal AM, Seifert JL, Dwyer JT (2015) Nutritional management of kidney stones (nephrolithiasis). Clin Nutr Res 4(3):137–152
6. Robertson WG, Peacock M, Nordin BEC (1971) Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. Clin Sci 40(5):365–374
7. Werness PG, Bergert JH, Smith LH (1981) Crystalluria. J Cryst Growth 53(1):166–181
8. Ratkalkar VN, Kleinman JG (2011) Mechanisms of stone formation. Clin Rev Bone Miner Metab 9(3):187–197. https://doi.org/10.1007/s12018-011-9104-8
9. Daudon M, Hennequin C, Boujelben G, Lacour B, Jungers P (2005) Serial crystalluria determination and the risk of recurrence in calcium stone formers. Kidney Int 67(5):1934–1943
10. Lu HS-H, Chen K-K, Lin AT, Chang Y-H, Wu HH, Hsu TH, Chang LS (1994) Urinary crystals in patients with and without urolithiasis. J Urol Assoc ROC 5(3):163–168
11. Lo P-A, Huang Y-H, Chiu Y-C, Huang L-C, Bai J-L, Wu S-H, Huang C-C, Chiang HK (2019) Automatic Raman spectroscopic urine crystal identification system using fluorescent image-guided
2D scanning platform with Fe3O4 crystal violet nanoclusters. J Raman Spectrosc 50(1):34–40
12. Kesson A, Talbott J, Gyory A (1978) Microscopic examination of urine. Lancet 312(8094):809–812
13. Fontanella CG, Carniel EL (2021) Computational tools for the investigation of the male lower urinary tract functionality in health and disease. J Med Biol Eng 41(2):203–215
14. Chien T-I, Kao J-T, Liu H-L, Lin P-C, Hong J-S, Hsieh H-P, Chien M-J (2007) Urine sediment examination: a comparison of automated urinalysis systems and manual microscopy. Clin Chim Acta 384(1):28–34
15. Kraft C, Steiner G, Beleites C, Salzer R (2009) Disease recognition by infrared and Raman spectroscopy. J biophotonics 2(1–2):13–28
16. Butler HJ, Ashton L, Bird B, Cinque G, Curtis K, Dorney J, Esmonde-White K, Fullwood NJ, Gardner B, Martin-Hirsch PL (2016) Using Raman spectroscopy to characterize biological materials. Nat Protoc 11(4):664–687
17. Chiu YC, Yang HY, Lu SH, Chiang HK (2010) Micro-Raman spectroscopy identification of urinary stone composition from ureteroscopic lithotripsy urine powder. J Raman Spectrosc 41(2):136–141
18. Chiu Y-C, Chen P-A, Chang P-Y, Hsu C-Y, Tao C-W, Huang C-C, Chiang HK (2015) Enhanced Raman sensitivity and magnetic separation for urolithiasis detection using phosphonic acid-terminated Fe3O4 nanoclusters. J Mater Chem B 3(20):4282–4290
19. Chen Z-T, Wang C-H, Chiang HK (2020) Characterization of auto-fluorescence urine crystals from gout patients using confocal microscopy and micro-Raman system for urolithiasis prediction. Proc of SPIE 11359:113591P
20. Sours RE, Fink DA, Cox KA, Swift JA (2005) Uric acid dye inclusion crystals. Mol Cryst Liq Cryst 440(1):187–193
21. Elmer P (2005) Spectroscopy attenuated total reflectance (ATR). Technical Note 27(11):1
22. Ho CS, Lam C, Chan M, Cheung R, Law L, Lit L, Ng K, Suen M, Tai H (2003) Electrospray ionisation mass spectrometry: principles and clinical applications. Clin Biochem Rev 24(1):3
23. Sekkouni K, Cheriti A, Taleb S, Belboukhari N (2016) FTIR spectroscopic study of human urinary stones from El Bayadh district (Algeria). Arab J Chem 9(3):330–334
24. He JY, Deng SP, Ouyang JM (2010) Morphology, particle size distribution, aggregation, and crystal phase of nanocrystallites in the urine of healthy persons and lithogenic patients. IEEE Trans NanoBioscience 9(2):156–163
25. Zhang Y, Wang Y, Cao W-W, Ma K-T, Ji W, Han Z-W, Si J-Q, Li L (2018) Spectral characteristics of autofluorescence in renal tissue and methods for reducing fluorescence background in confocal laser scanning microscopy. J Fluoresc 28(2):561–572
26. Trinchieri A, Lizzano R, Marchesotti F, Zanetti G (2006) Effect of potential renal acid load of foods on urinary citrate excretion in calcium renal stone formers. Urol Res 34(1):1–7
27. Zheng Y, Dong J, Palfrey BA, Carey PR (1999) Using Raman spectroscopy to monitor the solvent-exposed and “buried” forms of flavin in p-hydroxybenzoate hydroxylase. Biochemistry 38(51):16727–16732
28. Unno M, Sano R, Masuda S, Ono T-a, Yamauchi S (2005) Light-induced structural changes in the active site of the BLUF domain in AppA by Raman spectroscopy. J Phys Chem B 109(25):12620–12626
29. Pearle MS, Nakada SY (2014) Practical Controversies in medical management of stone disease, vol 20. Springer, New York
30. Stapleton A, Simpson R, Ryall RL (1993) Crystal matrix protein is related to human prothrombin. Biochem Biophys Res Commun 195(3):1199–1203
31. Stapleton A, Ryall RL (1995) Blood coagulation proteins and urolithiasis are linked: crystal matrix protein is the Fl activation peptide of human prothrombin. Br J Urol 75(6):712–719
32. Cook AF, Grover PK, Ryall RL (2009) Face-specific binding of prothrombin fragment 1 and human serum albumin to inorganic and urinary calcium oxalate monohydrate crystals. BJU Int 103(6):826–835

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.