Microscopy Techniques

- Cells, casts, lipids, crystals, & microorganisms in the urine can serve as real-time biomarkers of underlying pathology
- Light microscopes illuminate specimens from below to highlight their magnified properties
- **Bright-field (BF), phase-contrast (PC), & polarized light microscopy** are variants of light microscopy
- BF microscopy relies on differences in transparency to visualize a specimen, while PC transforms light path traveling through an object to enhance contours

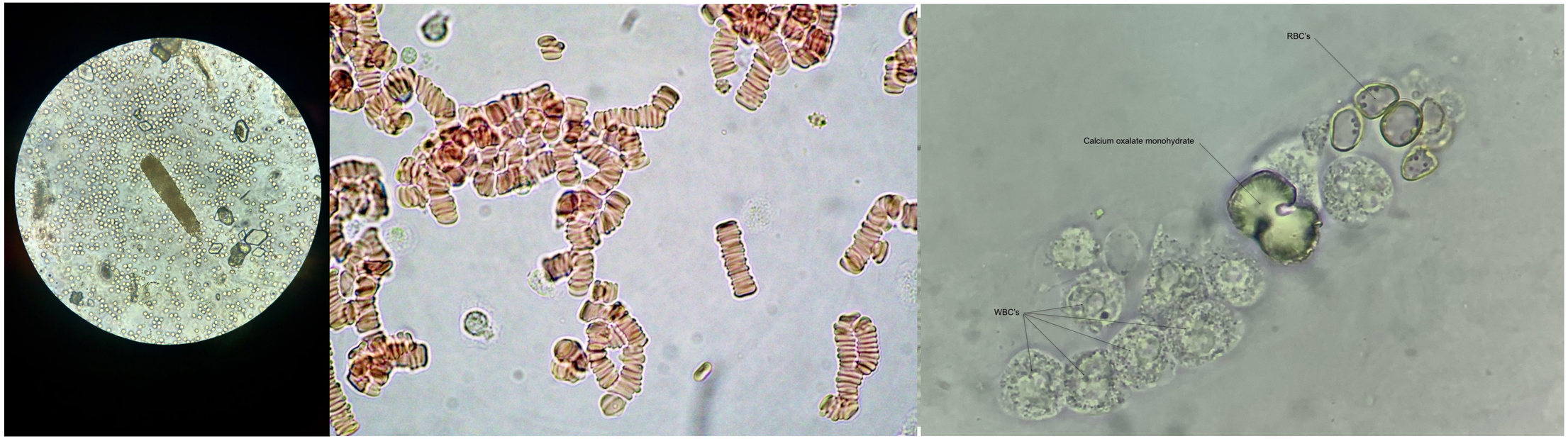

Figure 2. An assortment of unstained bright-field images: muddy brown granular cast (Juan Carlos Velez), red cell rouleaux (José Poloni), mixed cellular cast containing calcium oxalate stone (Jay Seltzer).

Figure 2. Crisp definition of acanthocyte edges, blebs and all, by phase contrast microscopy. Image b Florian Buchkremer.

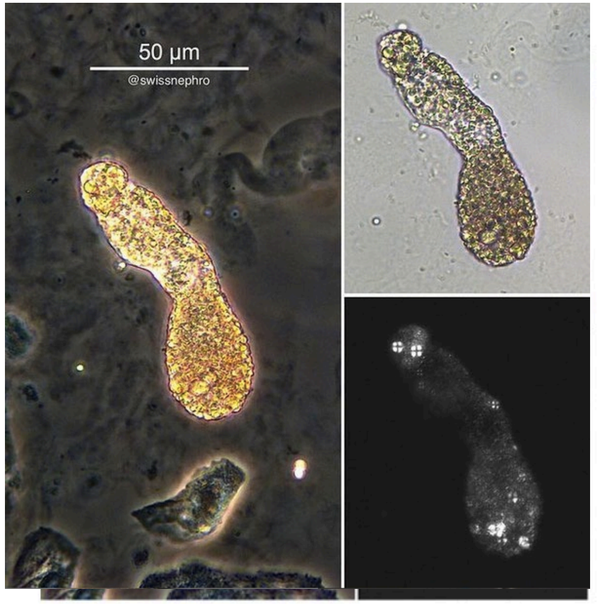

Figure 3. Lipid cast in a patient with nephrotic proteinuria by phase contrast (left panel), bright field (upper right) and polarized light (lower right) microscopy. Image by Florian Buchkremer.

Staining Techniques

- The **Sternheimer-Malbin (SM) stain**, developed in 1948 by Richard Sternheimer and Barney Malbin, is the most widely used for urine sediment analysis.

- The SM stain is composed of crystal violet and safranin, similar to the Gram stain. It is **supravital**, meaning it is applied to live sediment, fresh out of centrifuge!

- Endogenous pigments, including hemoglobin, myoglobin, and bilirubin, can color urinary casts and yield clues to underlying systemic pathology

- The Sudan stain can be used to detect fat globules in urine

Phase-contrast Microscopy

Phase contrast objective lenses

Phase condenser with positions for:
-Brightfield
-Darkfield
-Phase 1
-Phase 2
-Phase 3

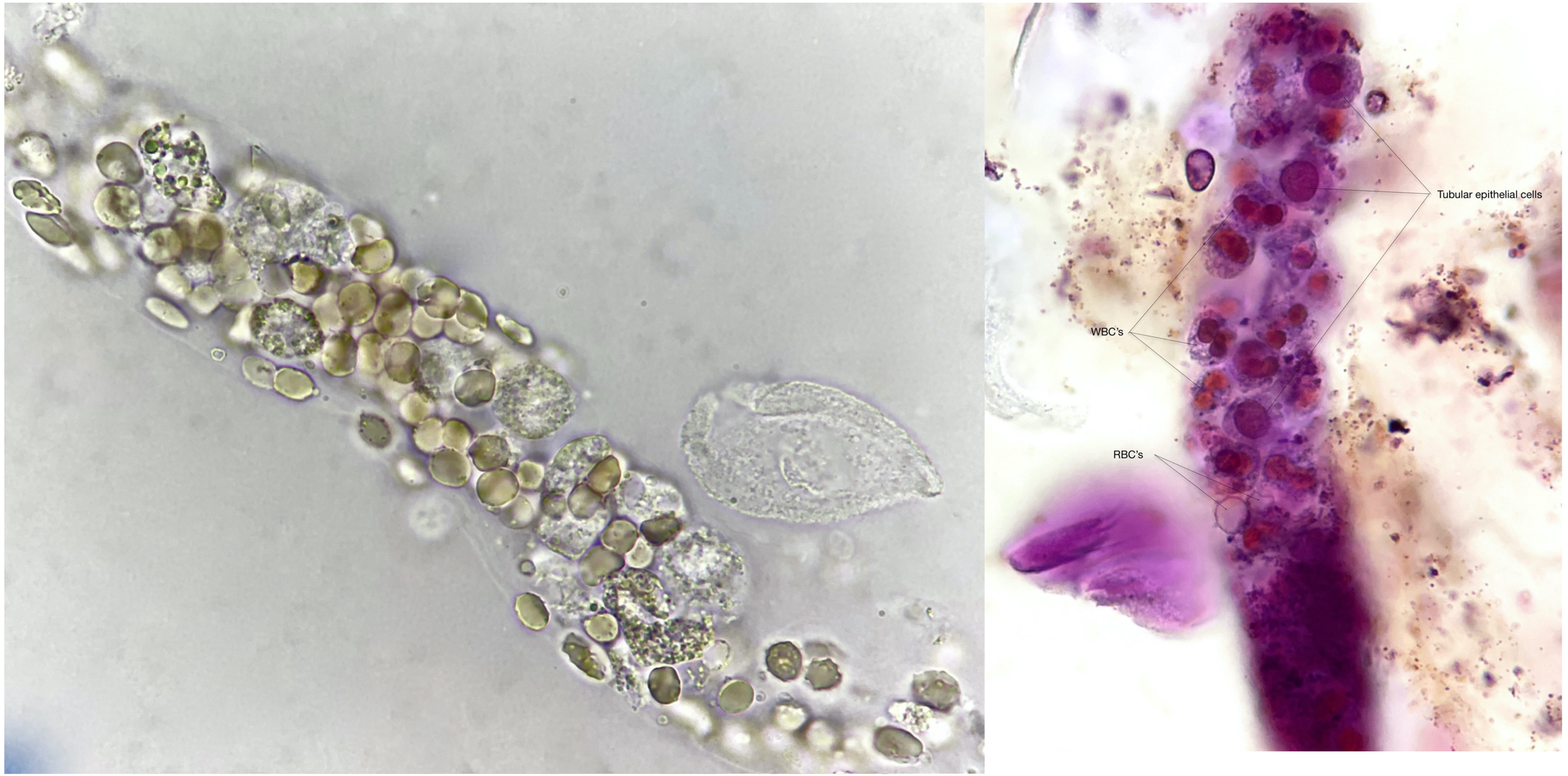

Figure 4. Unstained and stained mixed cellular casts by Jay Seltzer.

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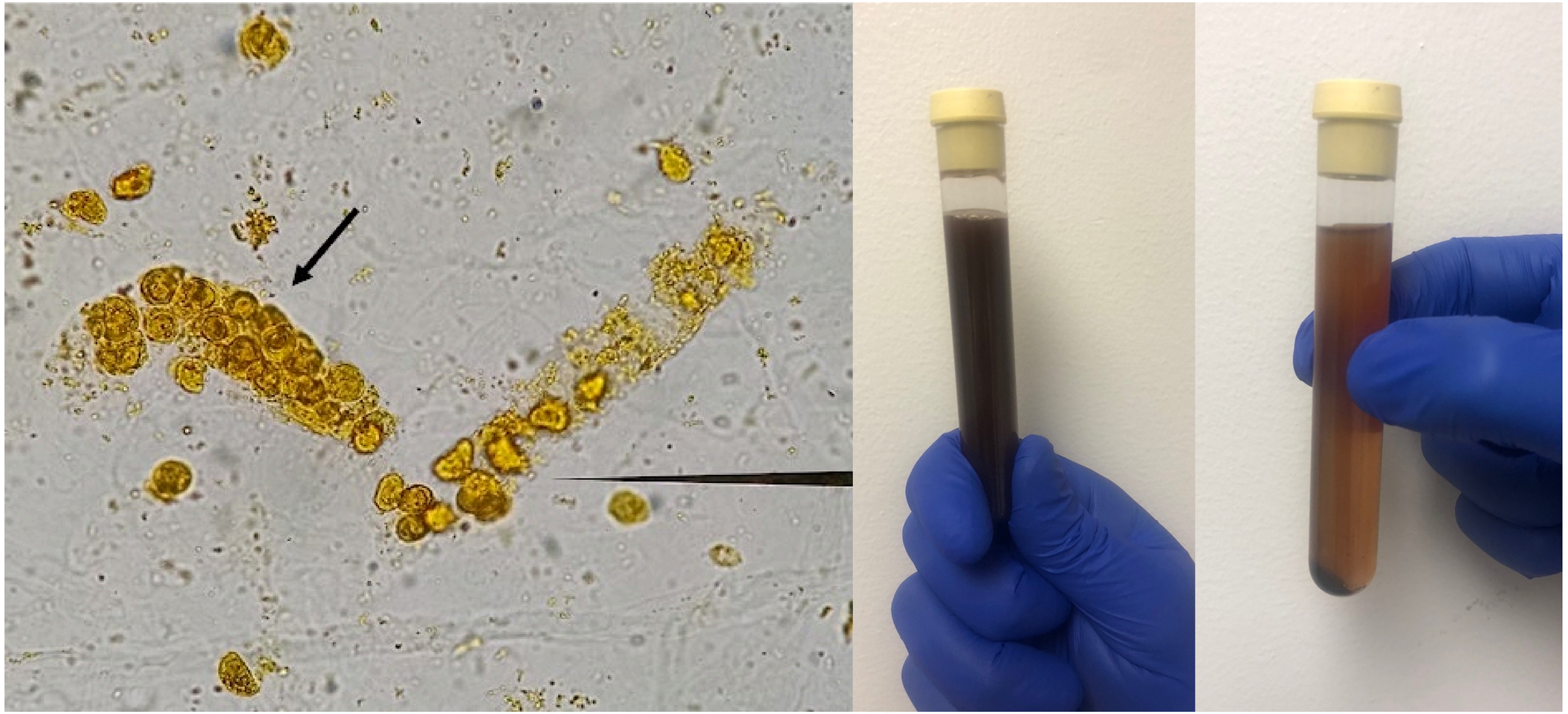

Figure 6. Nature's powerful pigments. The left panel shows bilirubin coloring renal tubular epithelial cells (image from a RFN post by José Poloni). The right panel shows free hemoglobin coloring the supernatant after centrifugation (image courtesy of Samira Farouk).

Urine Microscopy for GN

- Glomerular bleeding with acanthocyturia commonly occurs in various glomerulonephritides, inherited GBM disorders like Alport syndrome, & in non-GN syndromes like membranous & diabetic nephropathy.

- Acanthocytes (a subset of dysmorphic RBCs) are RBCs that develop arm-like projections (or “blebs”) after passing through the GBM

- Acanthocyturia (> 5% urinary RBCs) are fairly specific but poorly sensitive for GN

- RBC casts, formed in the distal nephron, indicate intraluminal bleeding that has occurred somewhere upstream—at the level of the glomerulus or in the tubules.

- RBC casts can also occasionally be found in acute interstitial nephritis

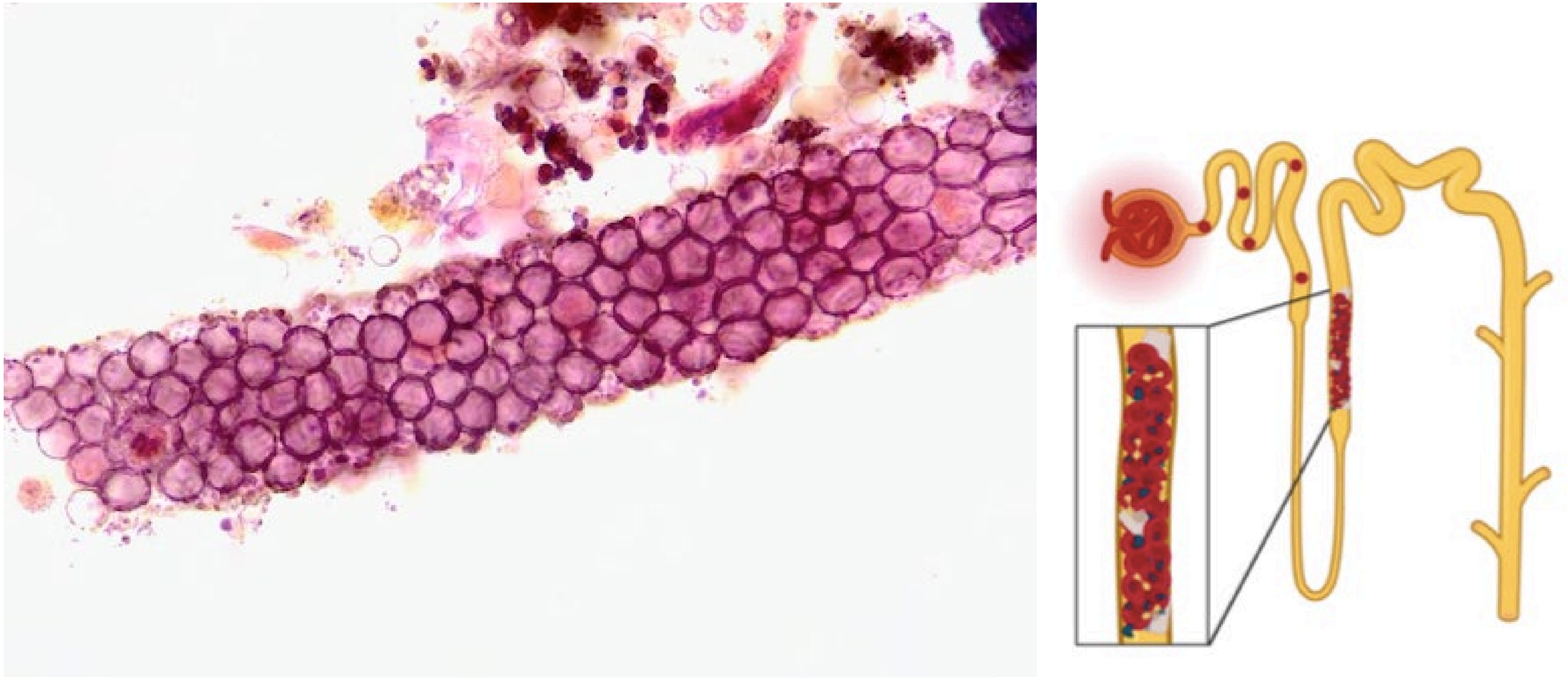

Figure 8. SM-stained RBC cast under bright-field microscopy (Jay Seltzer). The right panel is a schematic showing that all urinary casts are formed in the distal nephron (created with BioRender).

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Urine Microscopy for ATN

- Acute tubular necrosis (ATN) is the most common cause of AKI in hospitalized patients
- ATN can lead to the sloughing of renal tubular epithelial cells (RTECs) & casts in the urine
- Granular casts = cellular debris + plasma proteins trapped in a Tamm-Horsfall protein matrix within the distal nephron. In general, the darker & coarser the cast → more likely that it represents ATN
- A urinary sediment scoring system for AKI predicted the likelihood of a final diagnosis of ATN using the pretest probability and the number of RTECs and granular casts on initial microscopy

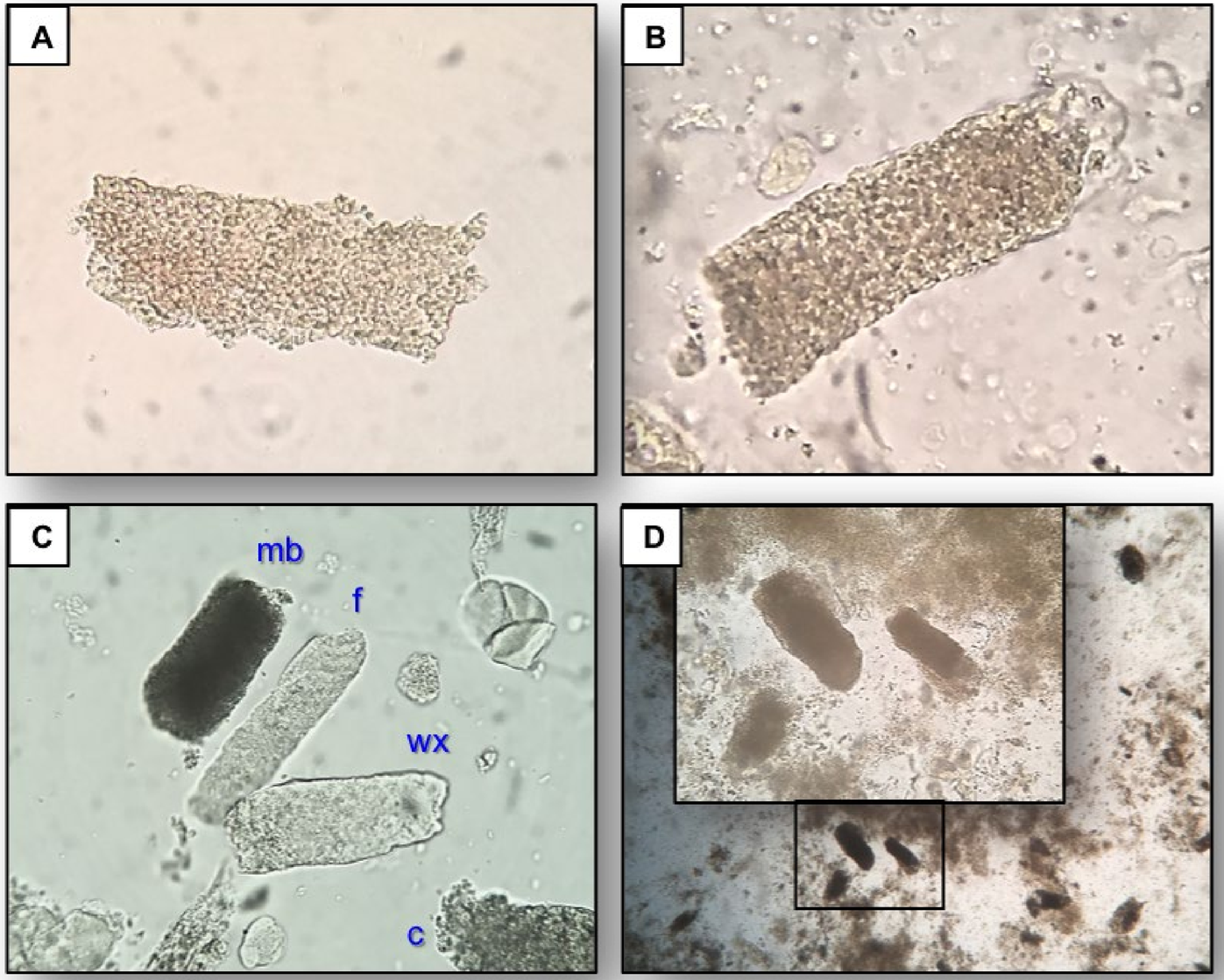

Figure 10. From the RFN post on granular casts by Juan Carlos Velez:
A. Finely granular cast B. slightly coarser granular cast C. "urine poker quads" showing a fine (f), coarse (c) and muddy brown (mb) granular cast accompanied by a waxy (wx) cast D. "Muddiness" of mb granular casts is better appreciated at low power field compared to high power field (inset).

Is urine microscopy associated with severity and worsening of AKI in hospitalized patients?

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Cohort

Prospective study

YALE-NEW HAVEN HOSPITAL

n=249

July 2008-March 2009

Methods

A urinary sediment Scoring based on number of RTE and granular cells was created:

RTE cells/ HPF (points)	Granular casts/ LPF (points)		
	0 (0)	1-5 (1)	≥6 (2)
0 (0)	0	1	2
1-5 (1)	1	2	3
≥6 (2)	2	3	4

RTE: Renal Tubular epithelial cells

Results

n=197 (Prerenal AKI or ATN)

Adjusted relative risk: 7.3; 95% CI (4.5-9.7)
(For AKI worsening with urinary score of ≥3 vs 0)

Urinary Score was **more predictive** for worsening of AKI than AKIN stage

No. of granular casts **(p=0.01)** and RTE (p=0.03) was associated with AKI stage

Conclusion: The urinary sediment's score may be a useful tool to predict worsening of AKI due to either ATN or prerenal AKI during hospitalization.

Perazella Mark A et al. Urine microscopy is associated with severity and worsening of acute kidney injury in hospitalized patients. Clin J Am Soc Nephrol. 2010 Mar;5(3):402-8.

Visual abstract by Priti Meena MD DNB

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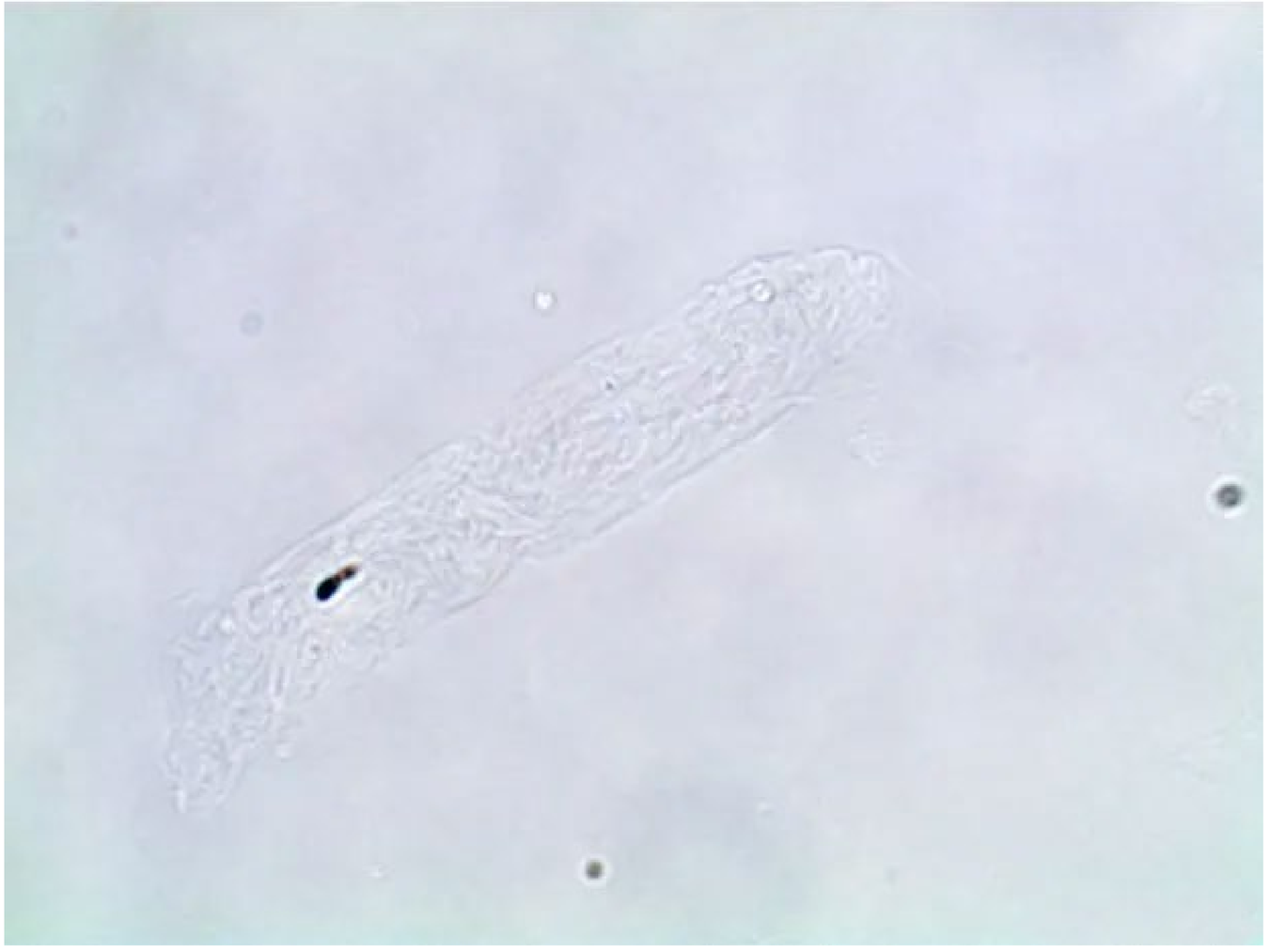

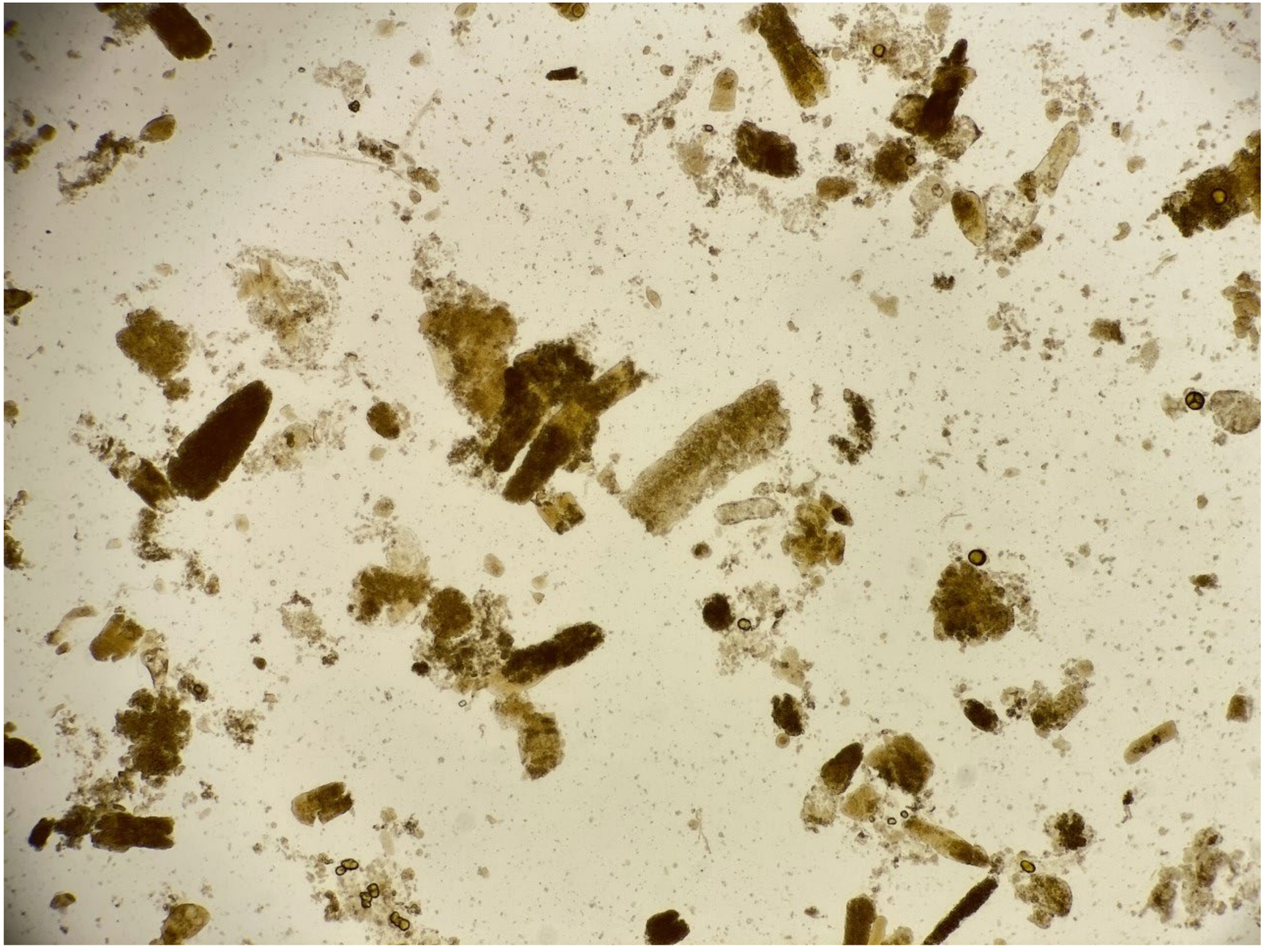

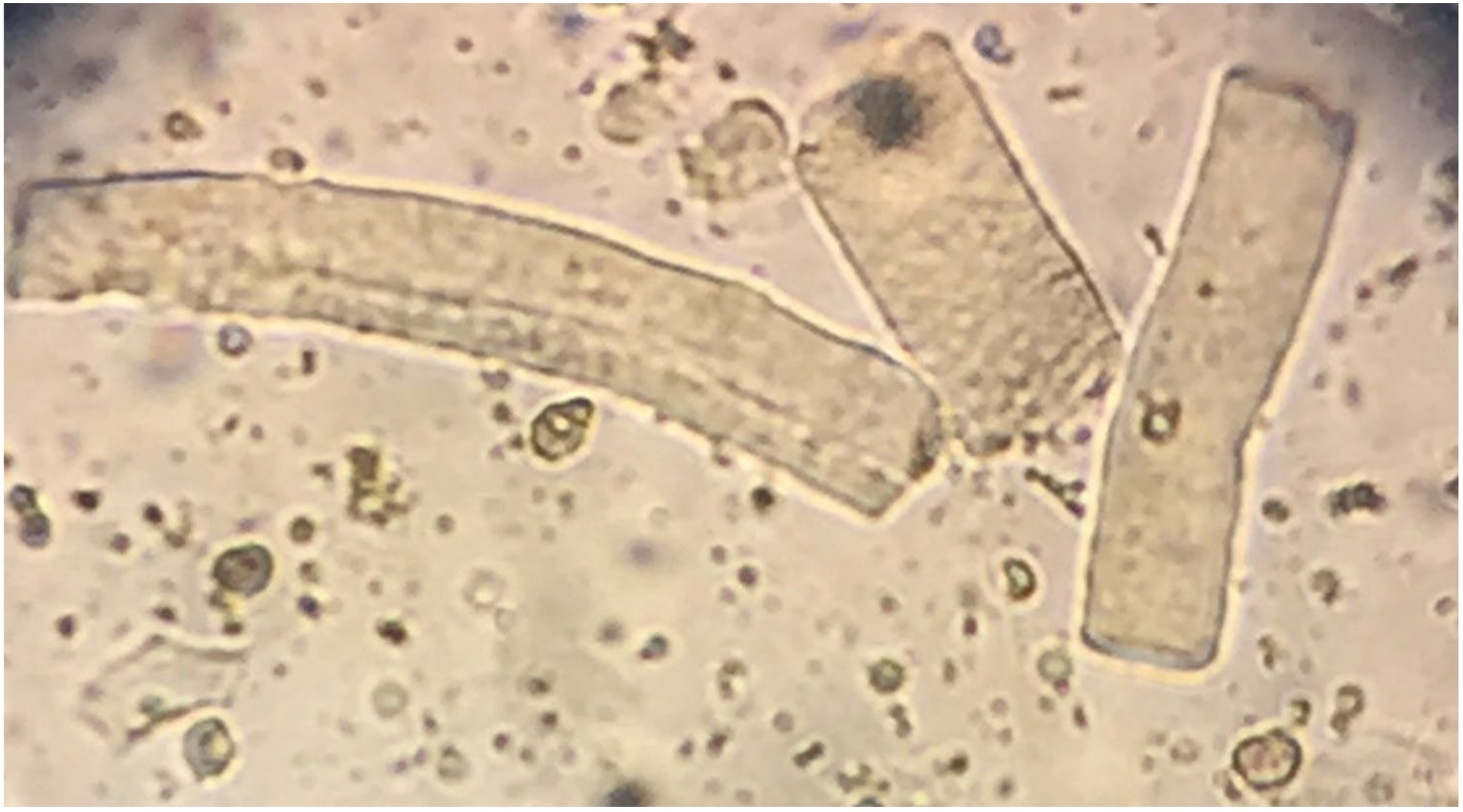

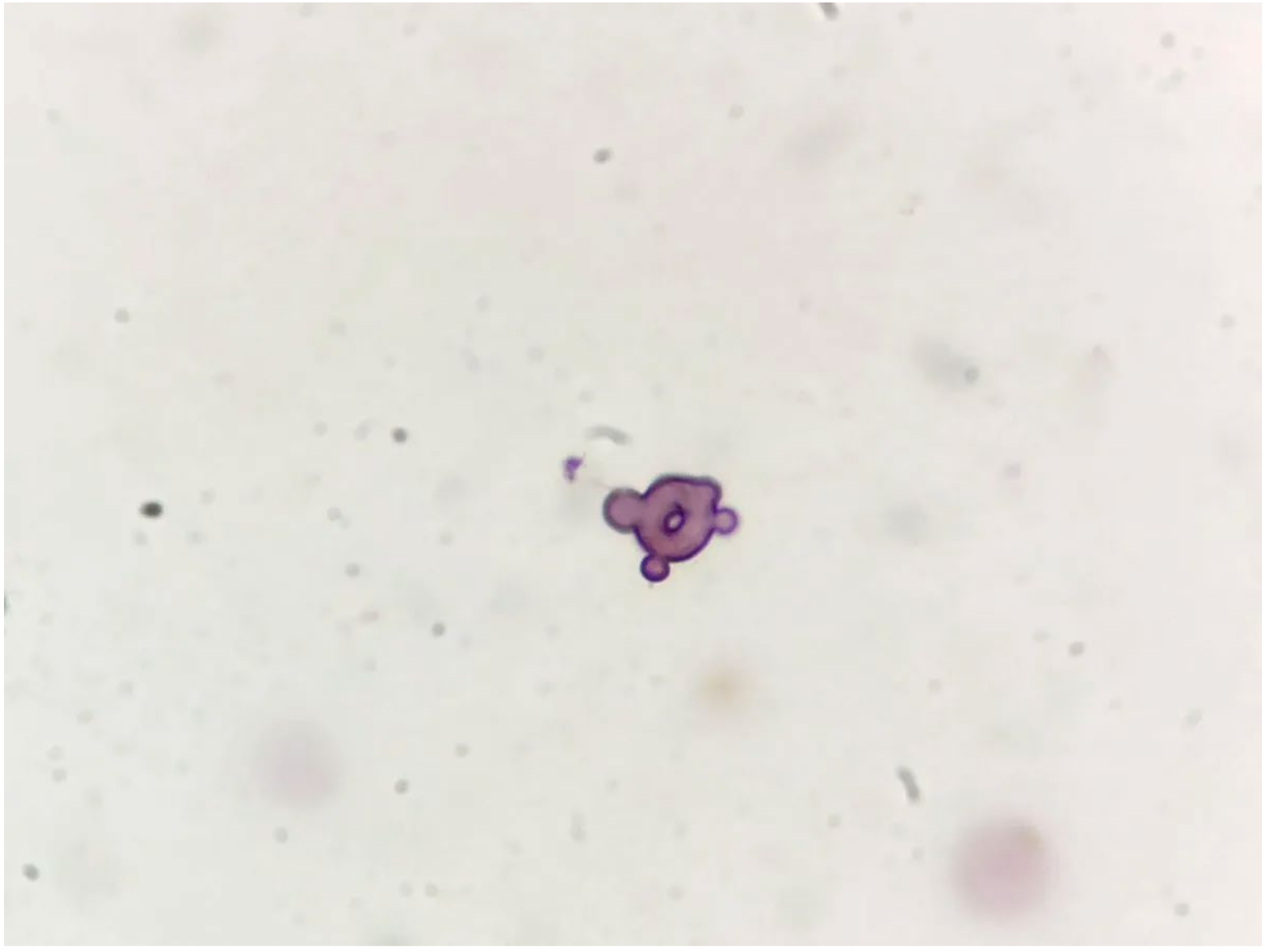

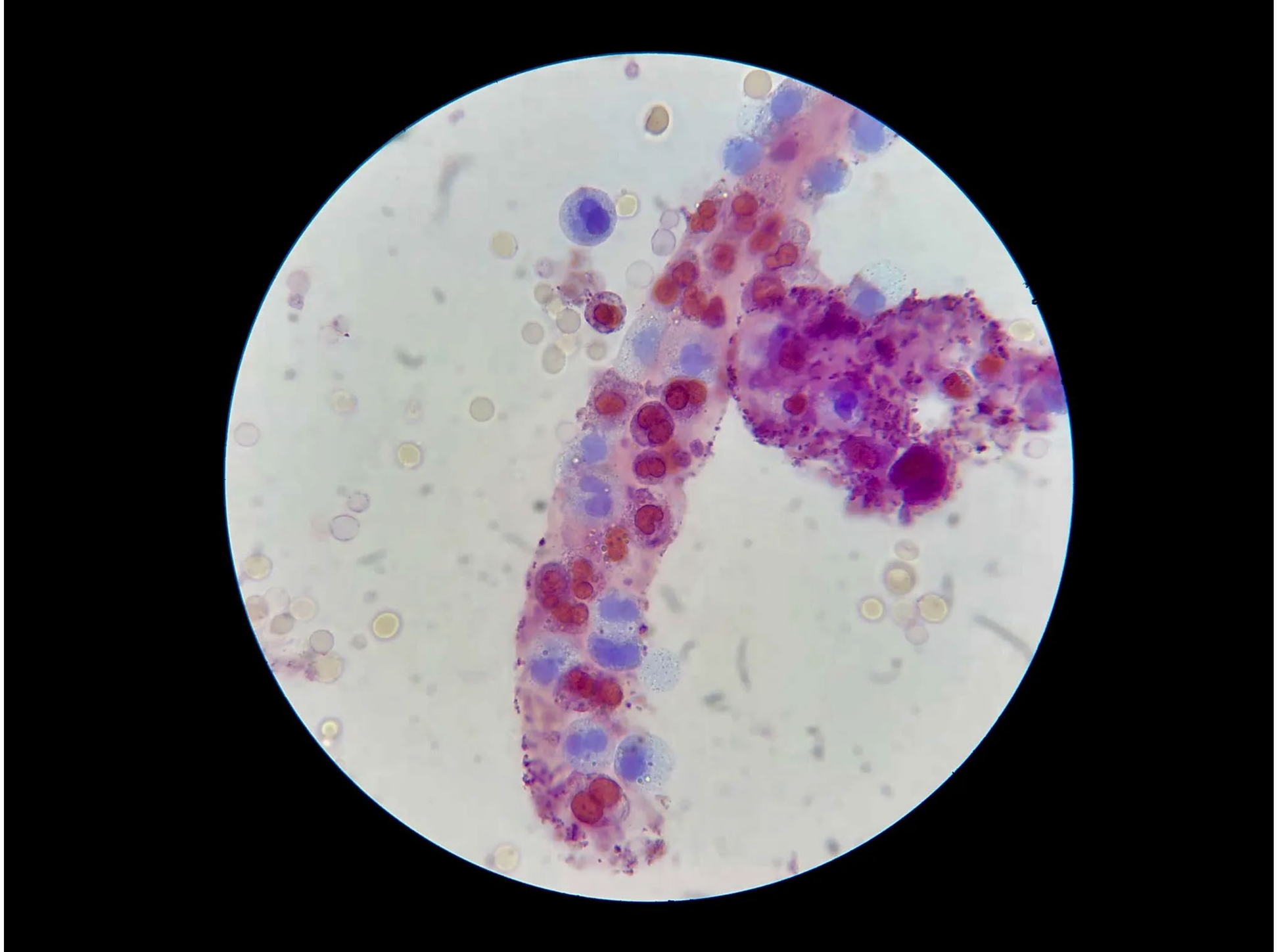

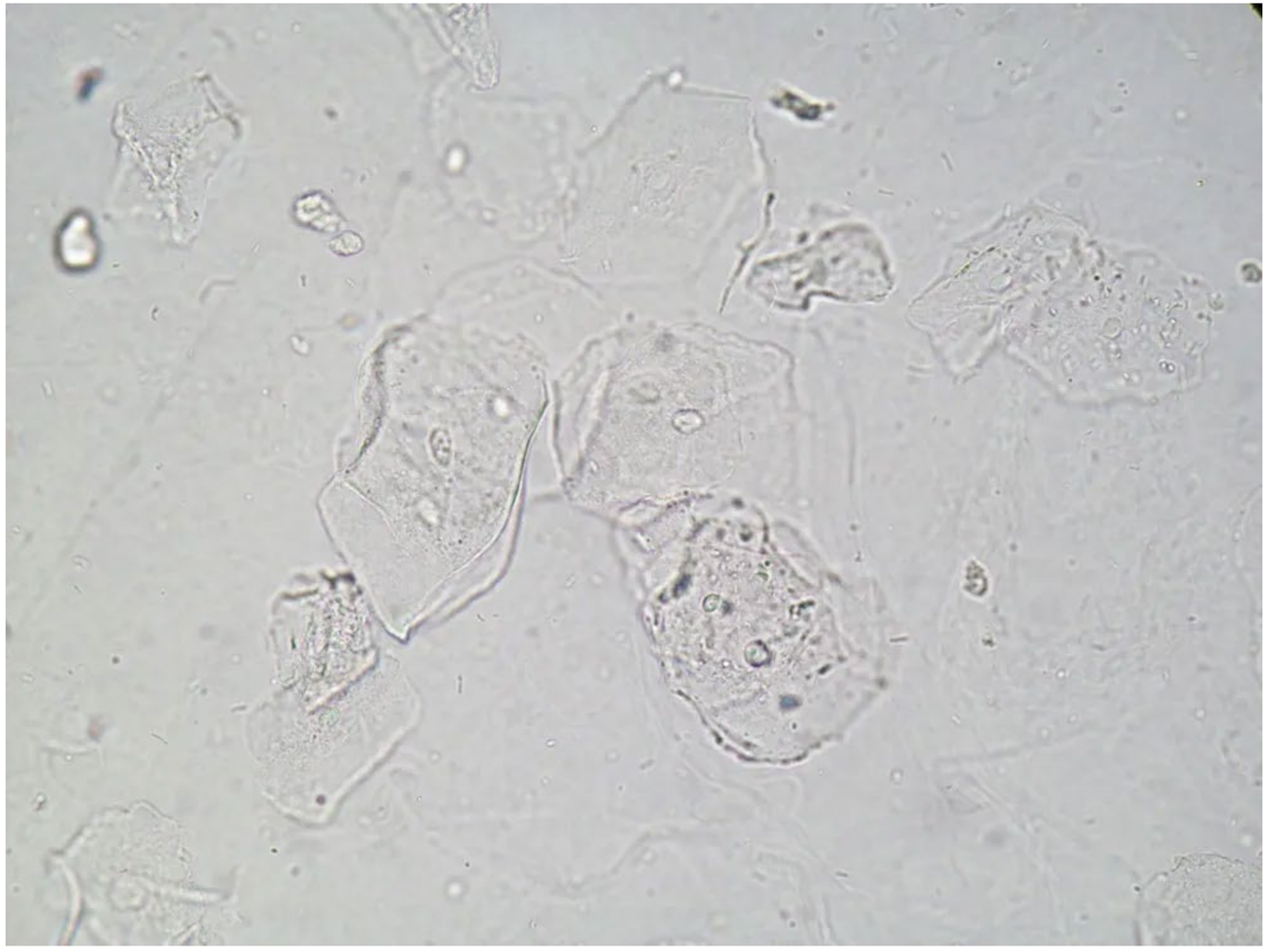

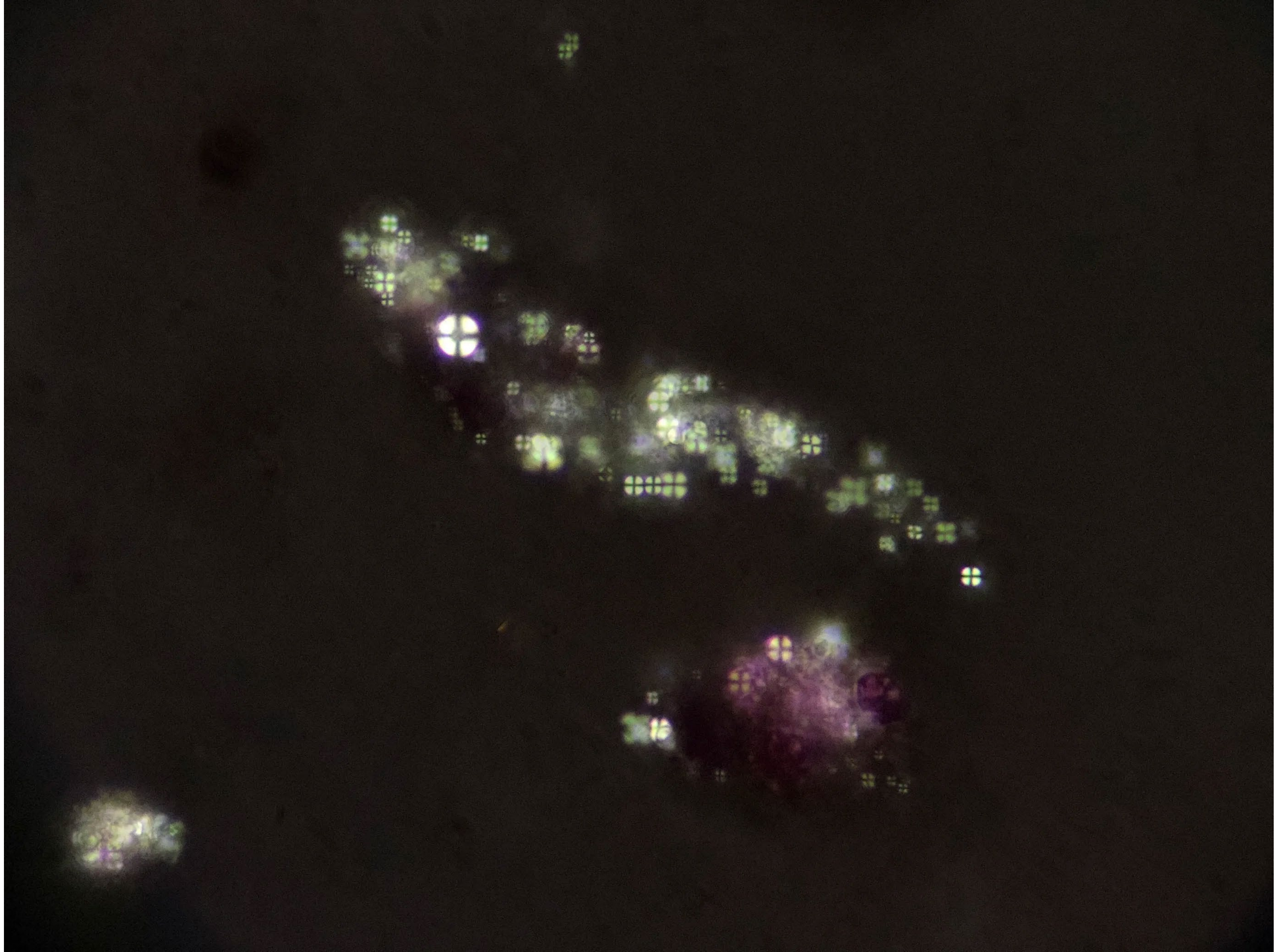

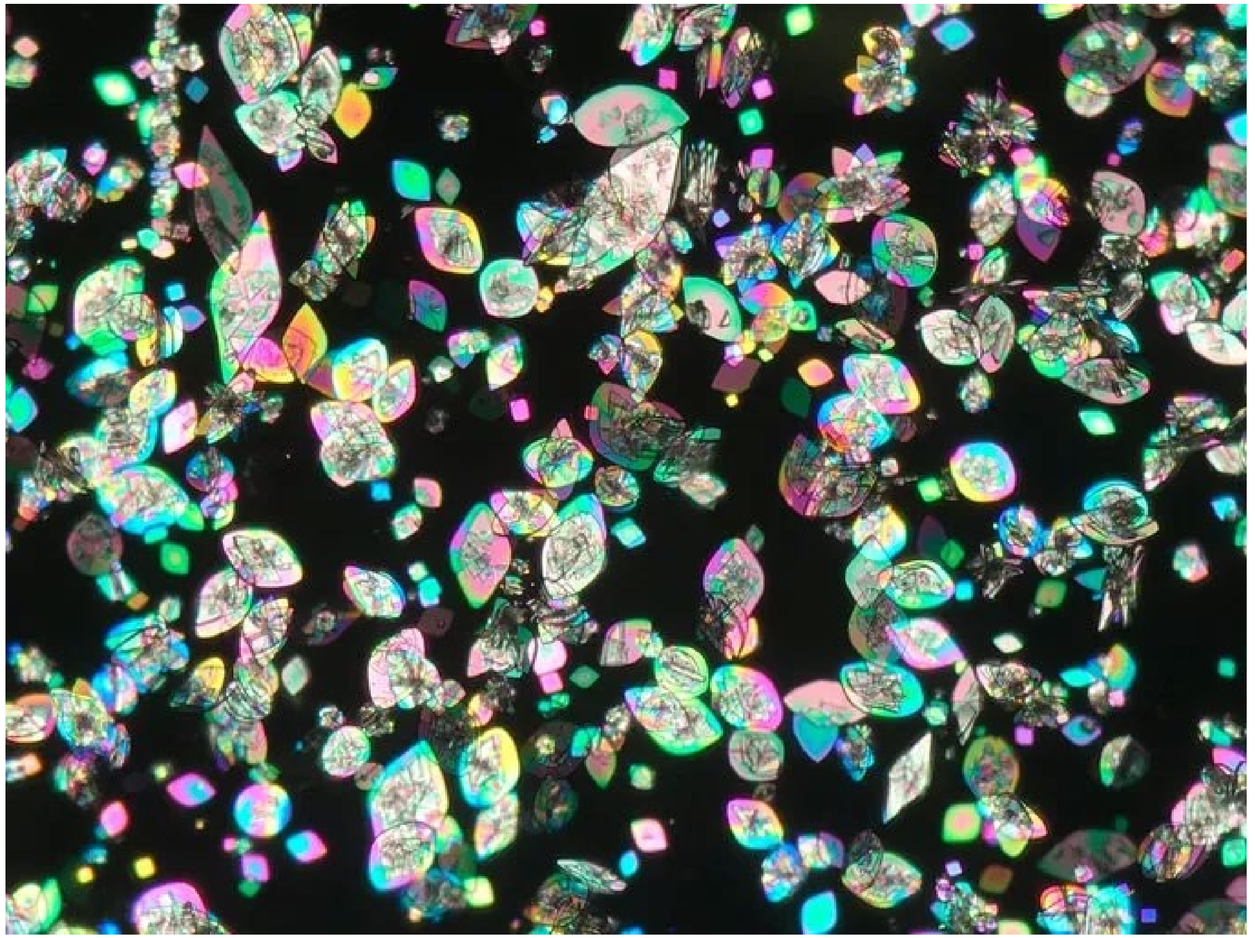

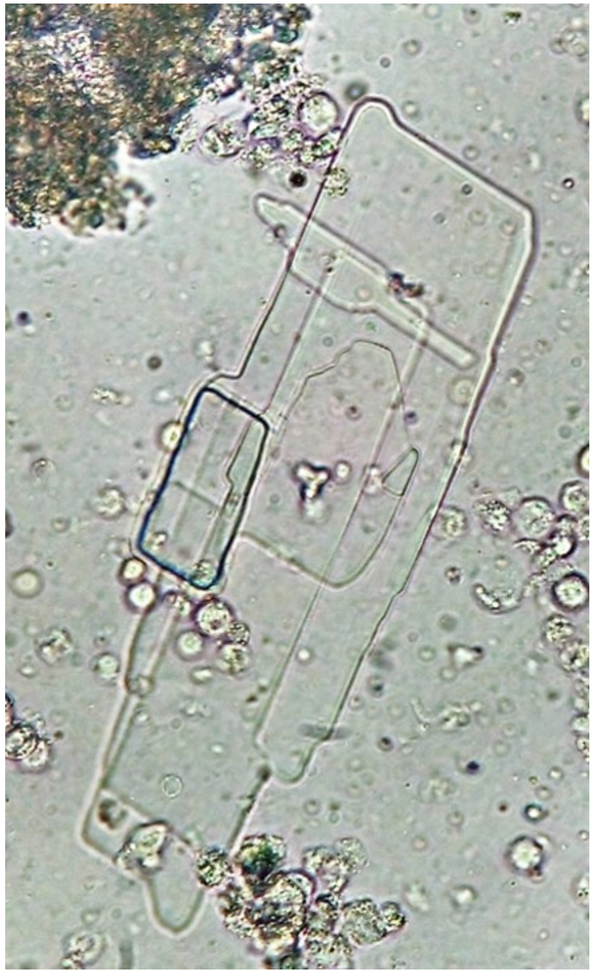

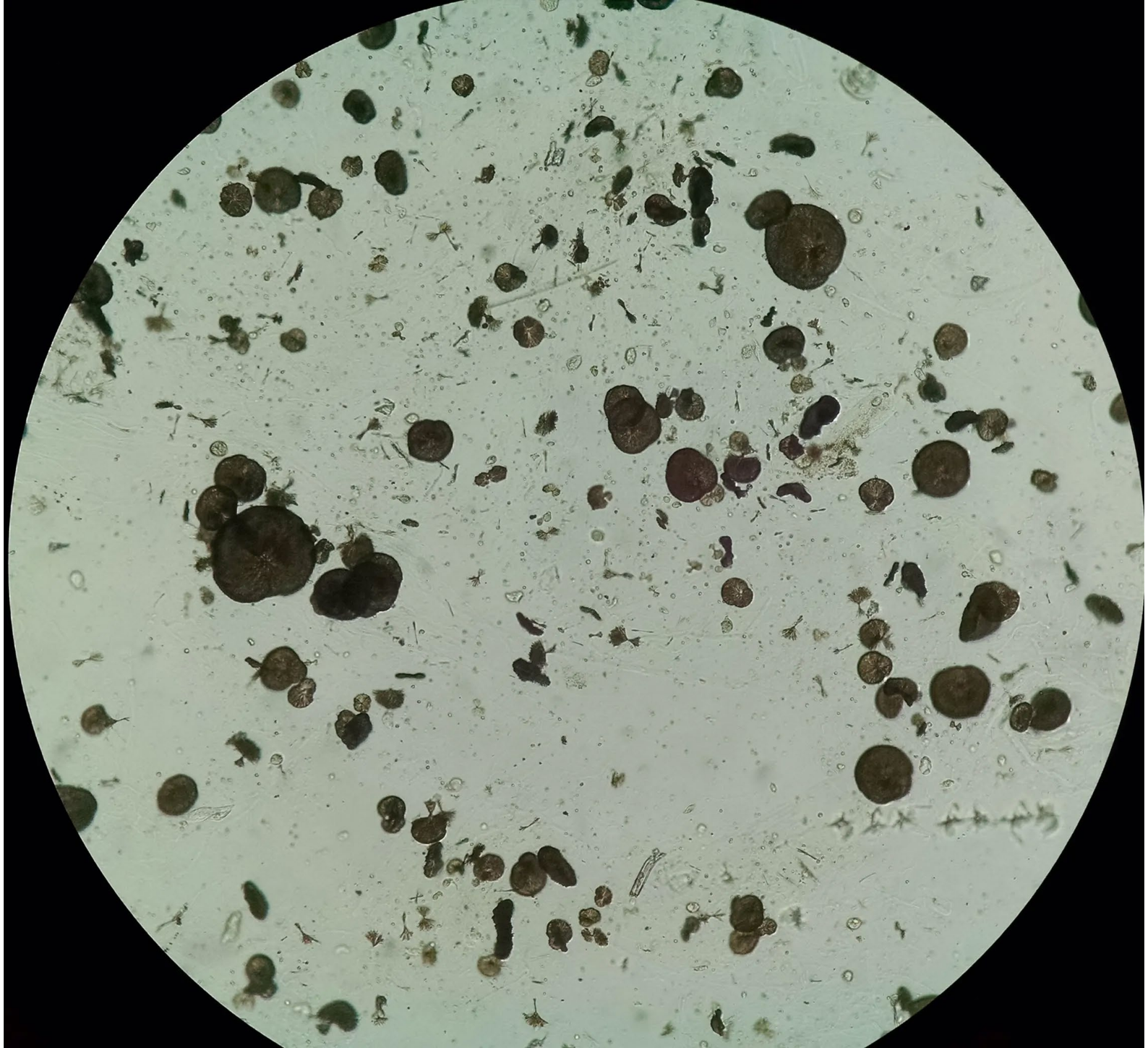

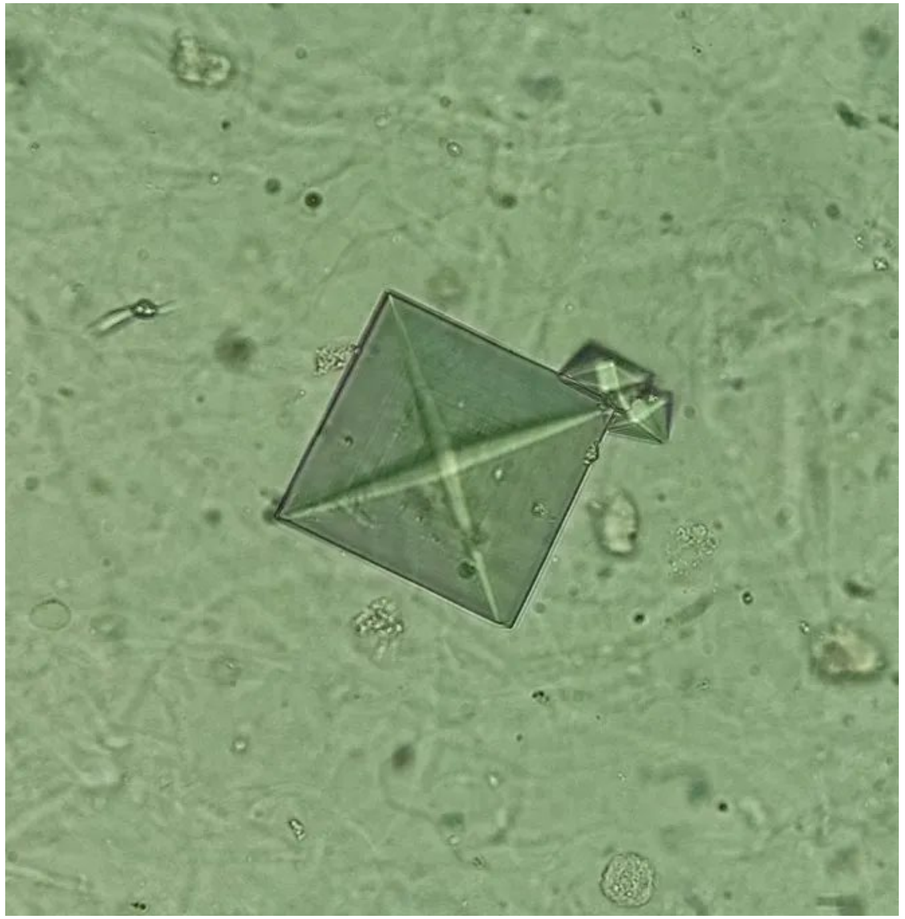

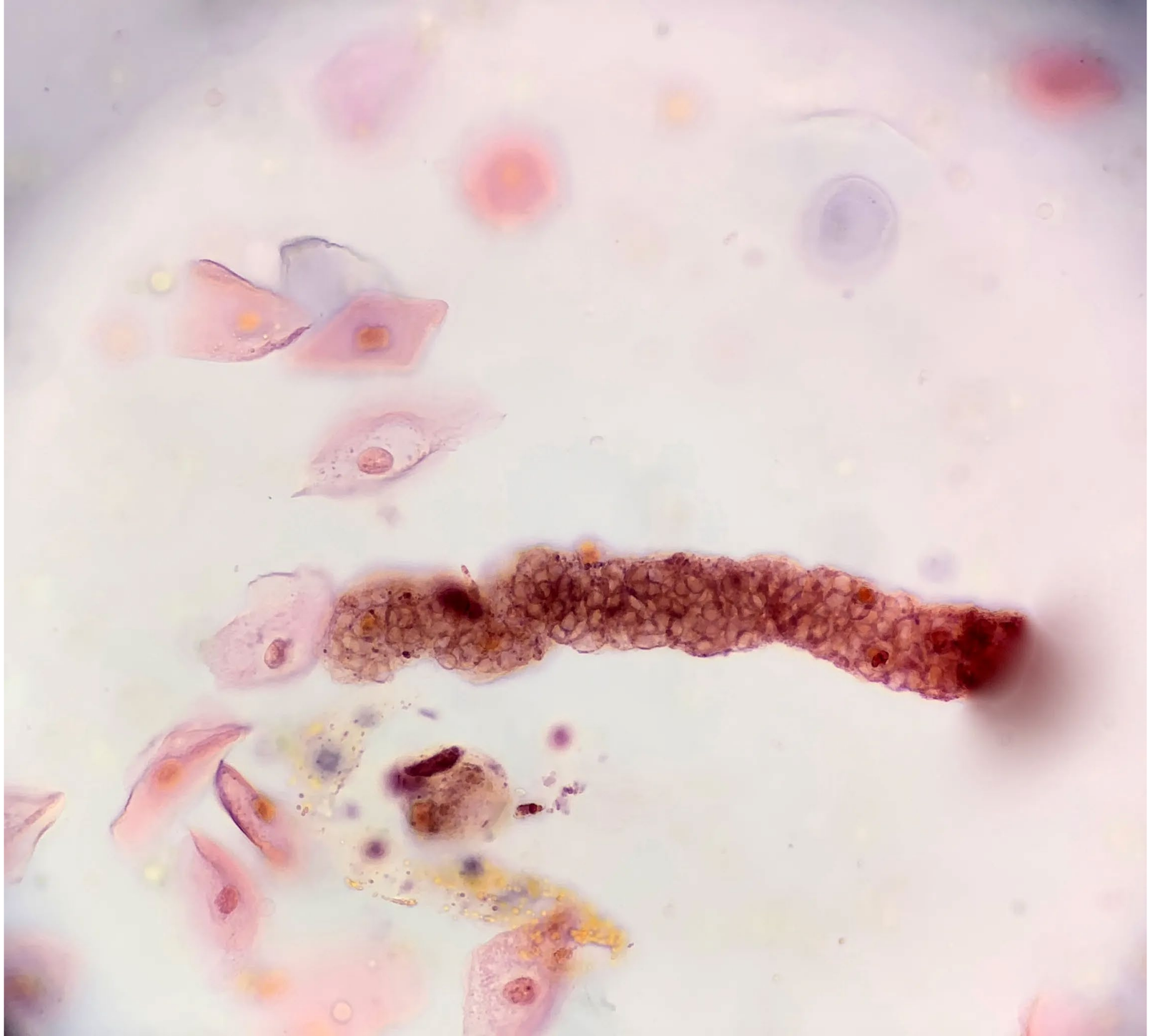

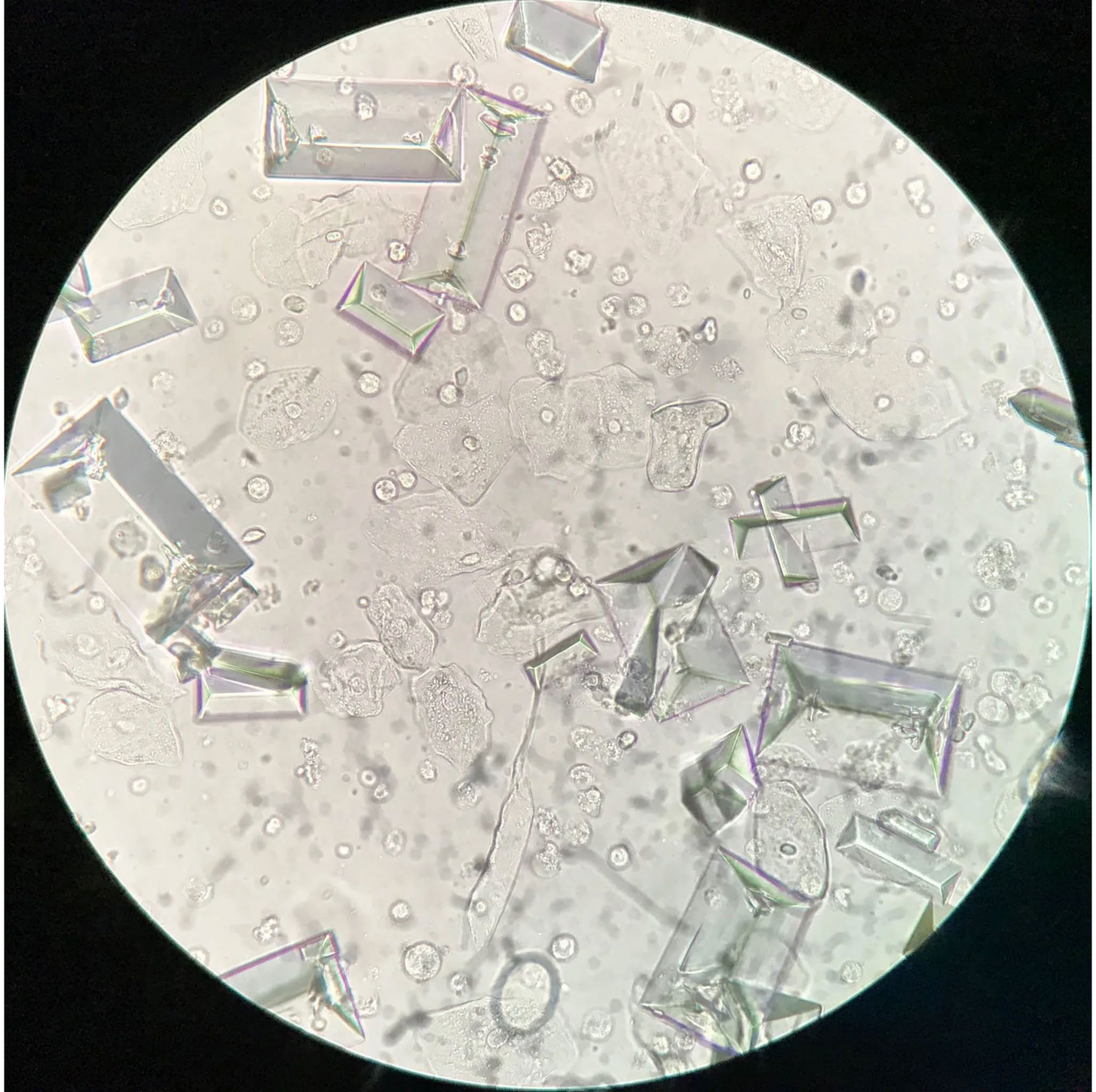

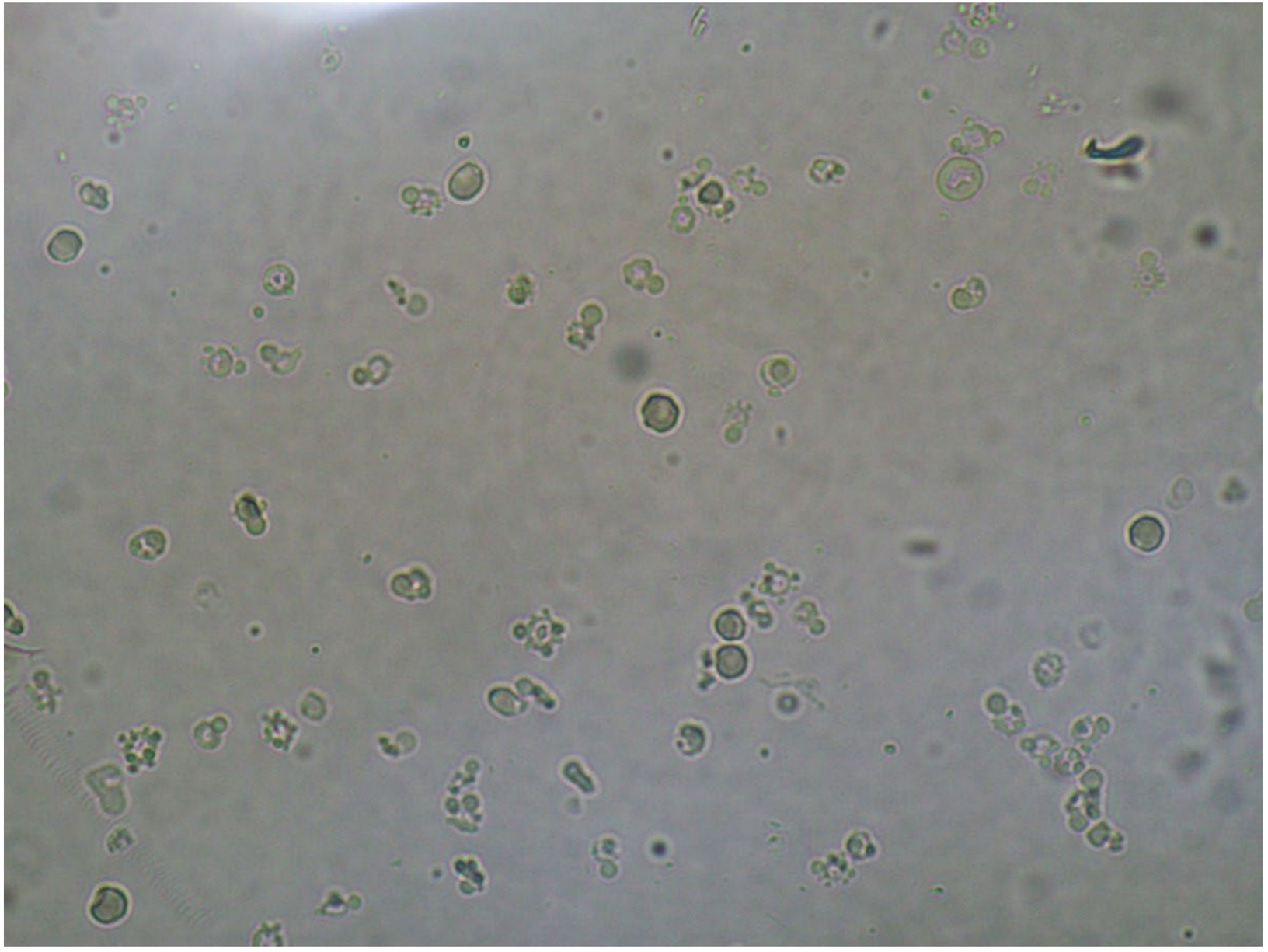

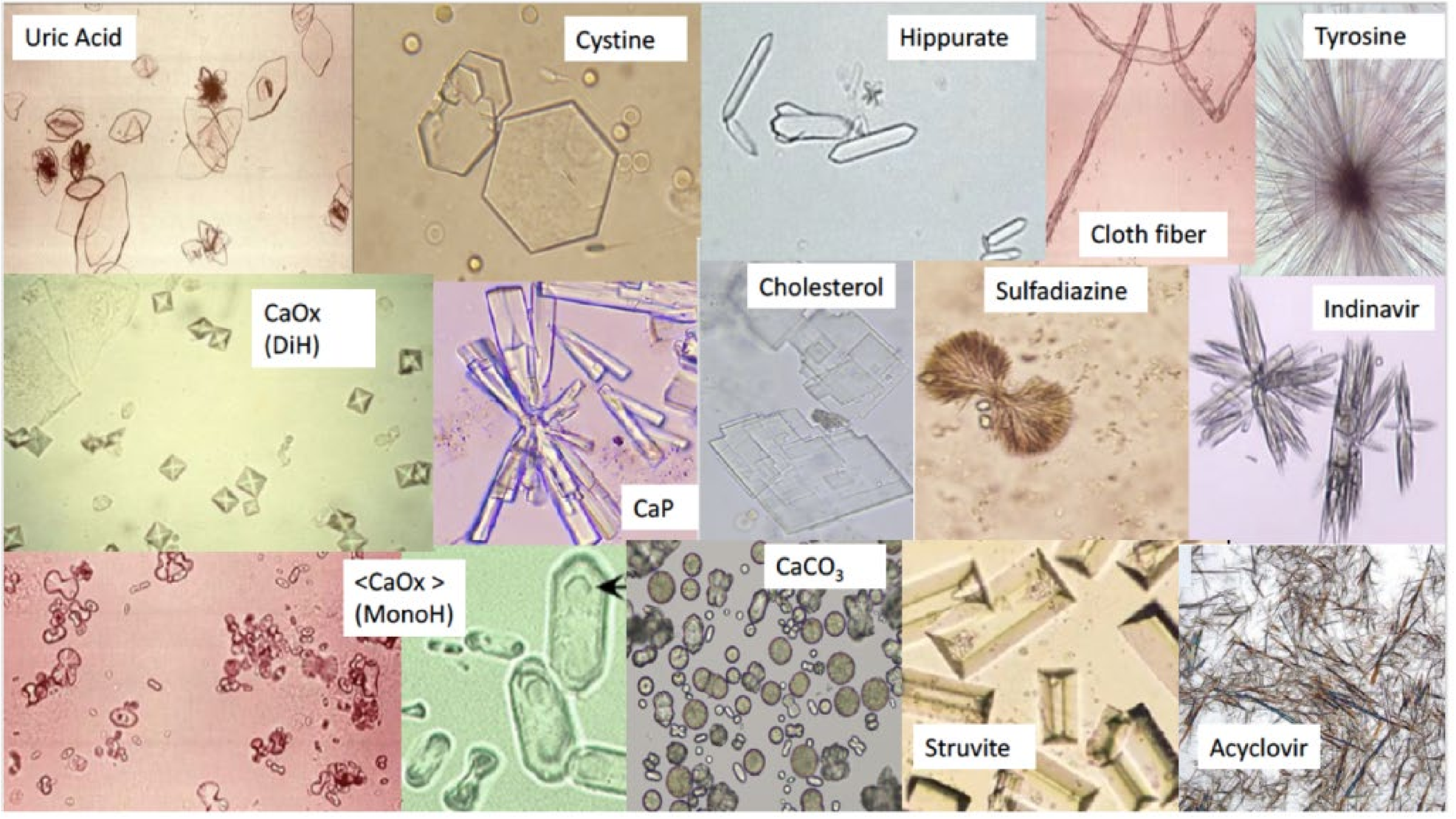

CaP: Calcium Phosphate
CaOx: Calcium oxalate
MonoH: Monohydrate
DiH: Dihyrdate
$CaCO_3$: Calcium carbonate

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