H. Ralph Schumacher, MD, Lan X. Chen, MD, PhD, Gilda Clayburne, MLT

Synovial Fluid Workshop

- **Introduction**
- Gross examination
- Save sterile fluid for cultures or research
- Microscopic examination
  - Wet preparations
    - Regular light
    - Polarized light
  - Stained smears. May not be needed
- Leukocyte count. Not always needed.

No disclosures

REFERENCES
Evidence-base

- Schumacher HR, Reginato AJ: Atlas of Synovial Fluid Analysis and Crystal Identification. Philadelphia, Lea & Febiger, 1991.
- Gatter RA, Schumacher HR: A Practical Handbook of Joint Fluid Analysis, 2nd ed. Philadelphia, Lea & Febiger, 1991.
- Eisenberg JM, Schumacher HR, Davidson PK, Kaufmann L: Usefulness of synovial fluid analysis in the evaluation of joint effusions. Arch hitem Med 144:715, 1984.
- Galvez J, Saiz E, Linares LF, et al: Delayed examination of synovial fluid by ordinary and polarized light microscopy to detect and identify crystals. Ann Rheum Dis 61:444, 2002.
- Schumacher HR, Chen LX, Pessler F: Synovial biopsy in the evaluation of nonrheumatic systemic diseases causing arthritis. Curr Opin Rheum 20:61, 2008.
- Chen LX, Schumacher HR: Current trends in crystal identification. Curr Opin Rheum 18:171, 2006.
- Corominas H, Clayburne G, Diaz-Lopez C, Schumacher HR:Apatite crystal identification in dried smears and synovial fluid pellets with alizarin red staining. Clin Exp Rheum 25:935, 2007.

Normal Synovial Fluid

	Range	Average
Knee joint volume (cc)	0.18 – 3.5	1.1
WBC/mm^3	13 – 180	63
%PMN	0 – 25	6.5
Albumin g/100cc		1.02
Globulin g/100cc		0.05
Glucose	approximately same as plasma	
Electrolytes	approximately same as plasma	

Ropes & Bauer

Table 3.
Number of patients With and Without a Change in the Most Likely Diagnosis Following Synovial Fluid Analysis

Initial Most Likely Diagnosis	Same Final Most Likely Diagnosis	Different Final Most Likely Diagnosis	Changed %
Osteoarthritis	31	6	16
Rheumatoid arthritis	24	5	17
Gout	25	9	26
Infectious arthritis	11	3	21
Pseudogout	9	1	10
Traumatic arthritis	7	2	22

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Normal | Non Inflammatory | Inflammatory | Purulent | Bloody

Joint Fluid Characteristics

	Normal	Group I (Non-Inflammatory)	Group II (Inflammatory)	Group III (Septic)
Volume (knee, in rat)	**<3.5**	**>3.5**	**>3.5**	**>3.5**
Viscosity	**Very high**	**High***	**Low**	**Variable**
Color	**Colorless**	**Straw**	**Straw to opalescent**	**Variable with organism**
Clarity	**Transparent**	**Transparent**	**Translucent, opaque at times**	**Opaque**
WBC/mm^3	**200**	**300-2000^t**	**2000 - 100,000**	**> 50,000^{tt} usually > 100,000**
%PMN	**< 25**	**< 25**	**> 50 often**	**> 75^{tt}**
Culture	**negative**	**negative**	**negative**	**usually positive**

*Rapid accumulation of fluid will lower viscosity

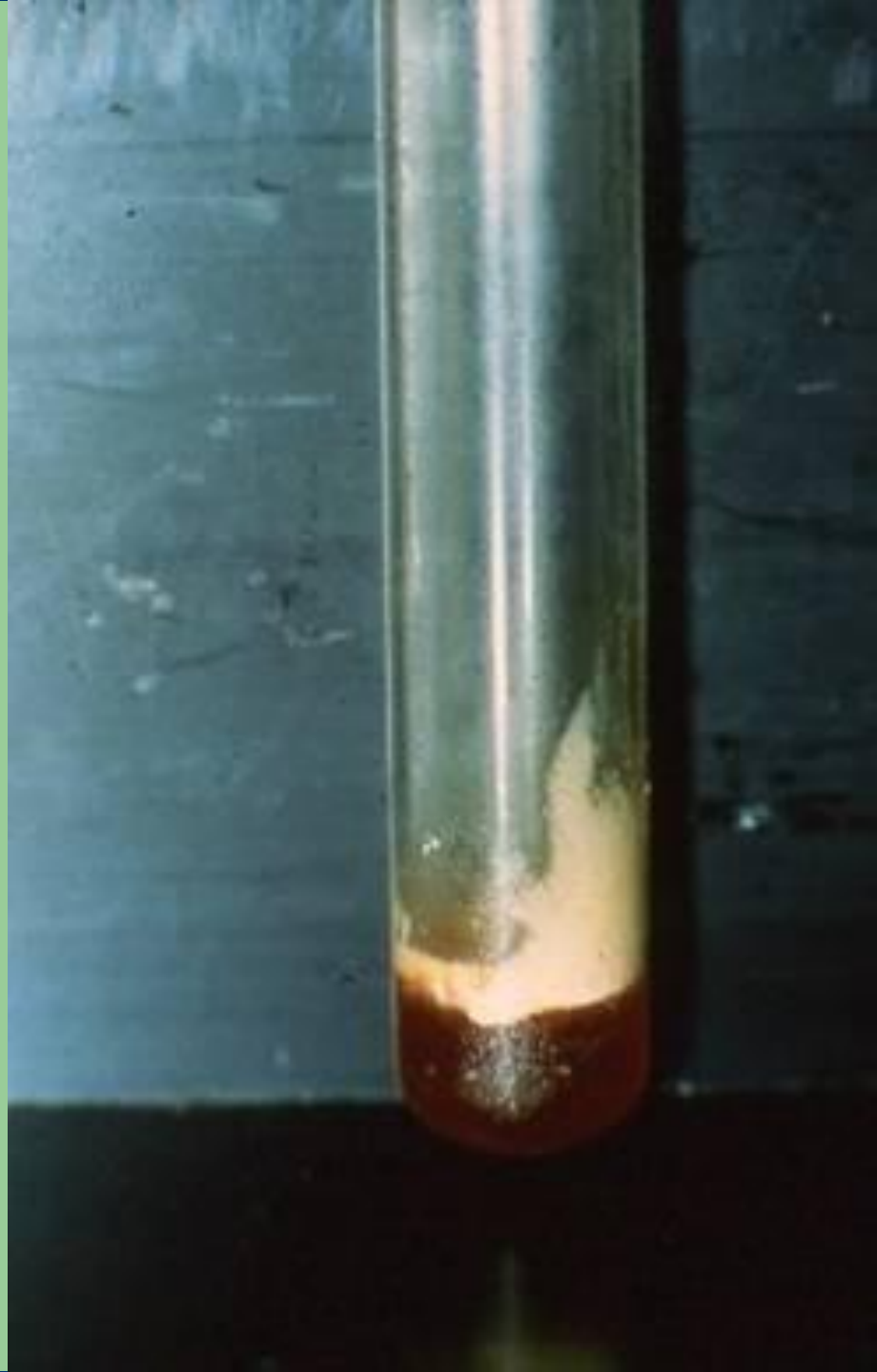
^{t}2000 is an approximation. Usually less than 500

^{tt} may be lower with partially treated or low-virulance organisms

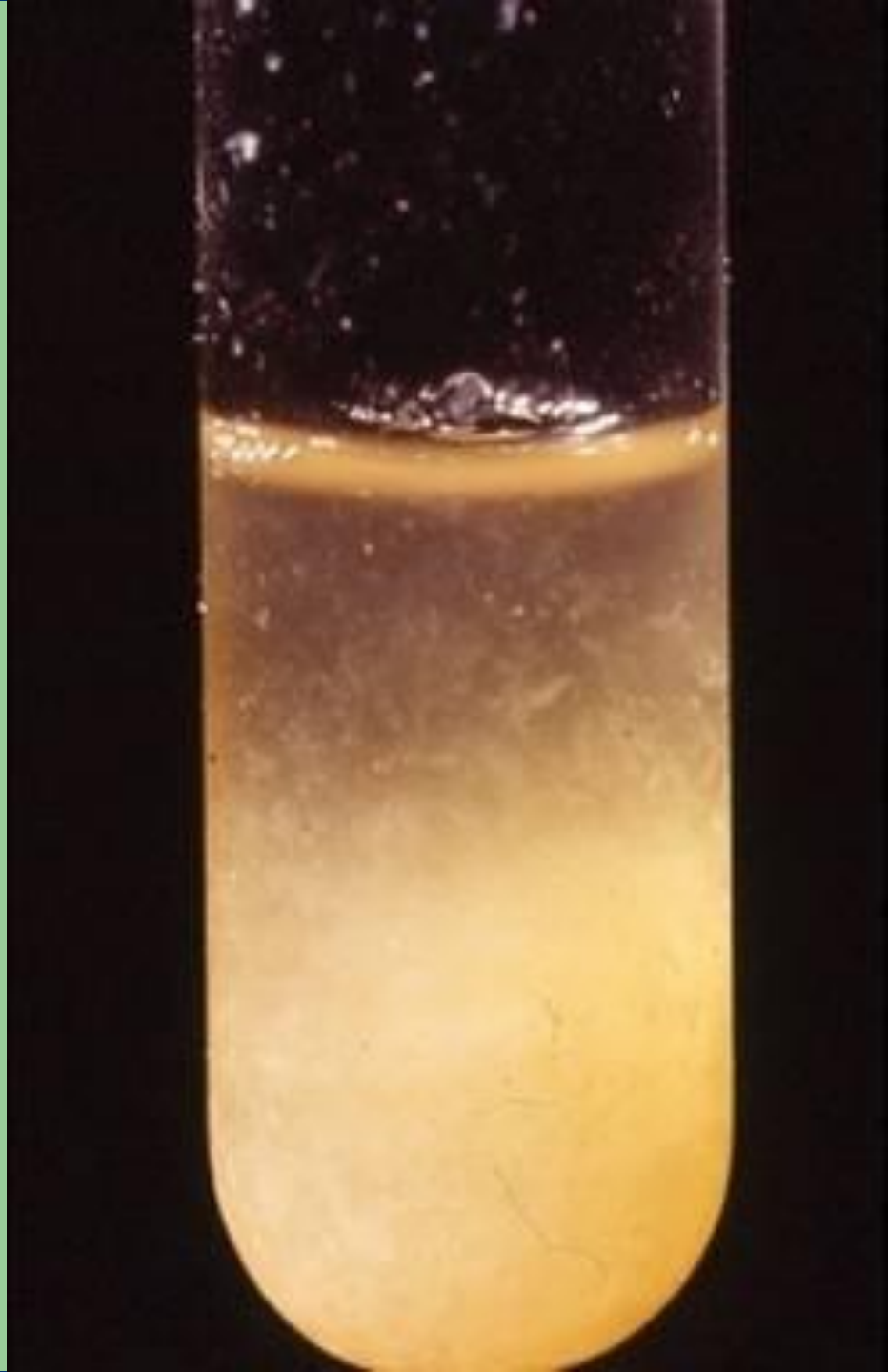
What do you think of this opaque, creamy fluid?

What can cause this “cream of tomato soup” synovial fluid?

Fat on the surface after centrifugation of bloody effusion due to intra-articular fracture



Opaque synovial fluid not due to cells but due to amyloid

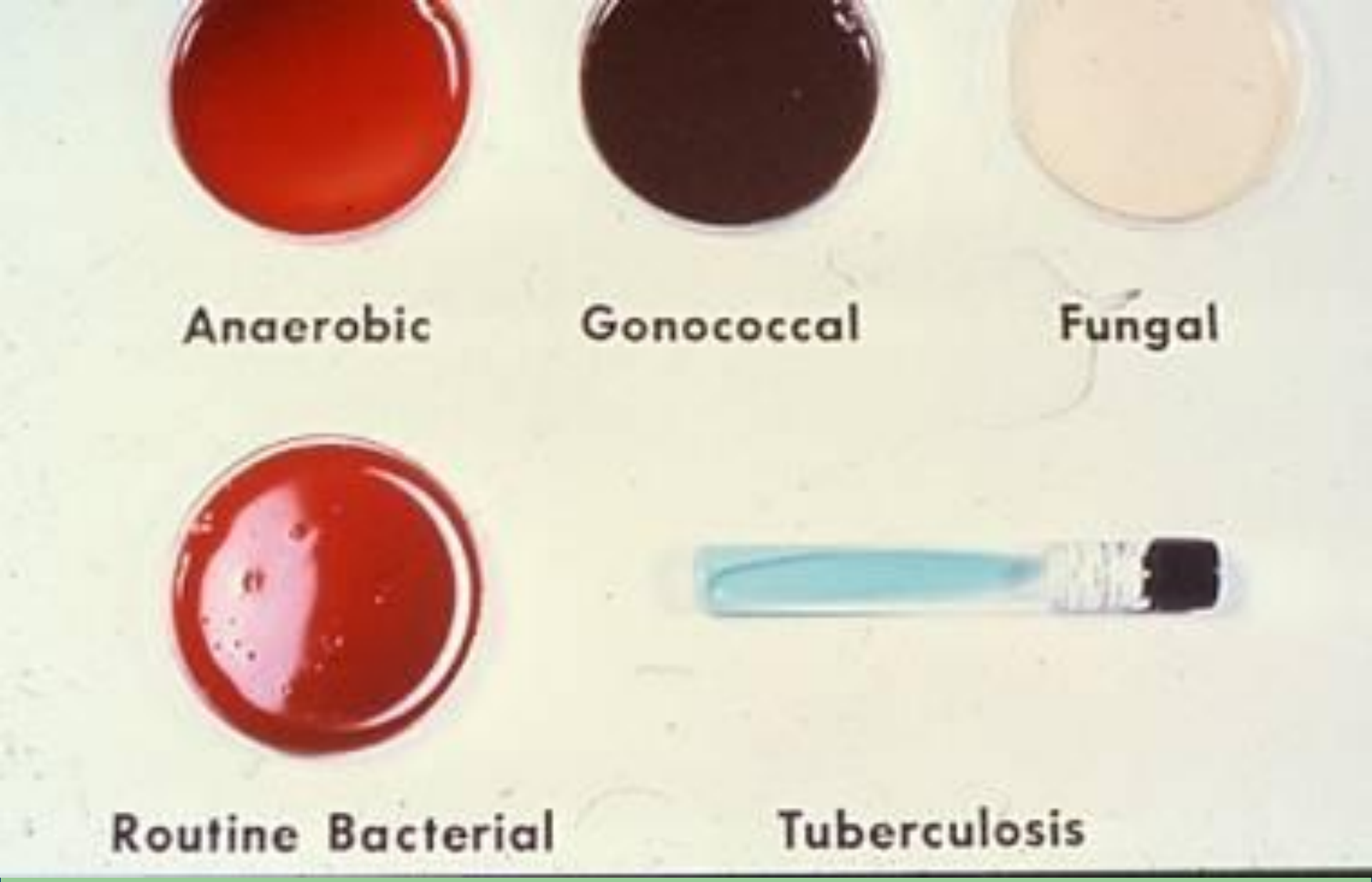


“Gold paint” synovial fluid loaded with cholesterol crystals

Rice Bodies

Very viscous knee synovial fluid due to myxedema.
Can also be seen in ganglia and cysts on Heberden nodes.

Clumps of urate crystals in 1st MTP joint fluid



If infection is being considered send unadulterated fluid to the laboratory with instructions as to which infections are concerns.

Synovial Fluid Workshop

- Introduction
- Gross examination
- Cultures
- **Microscopic examination**
  - **Wet preparations**
    - **Regular light**
    - **Polarized light**
  - Stained smears
- Leukocyte count

If synovial fluid is not obtained, maintain suction on the syringe as you withdraw.

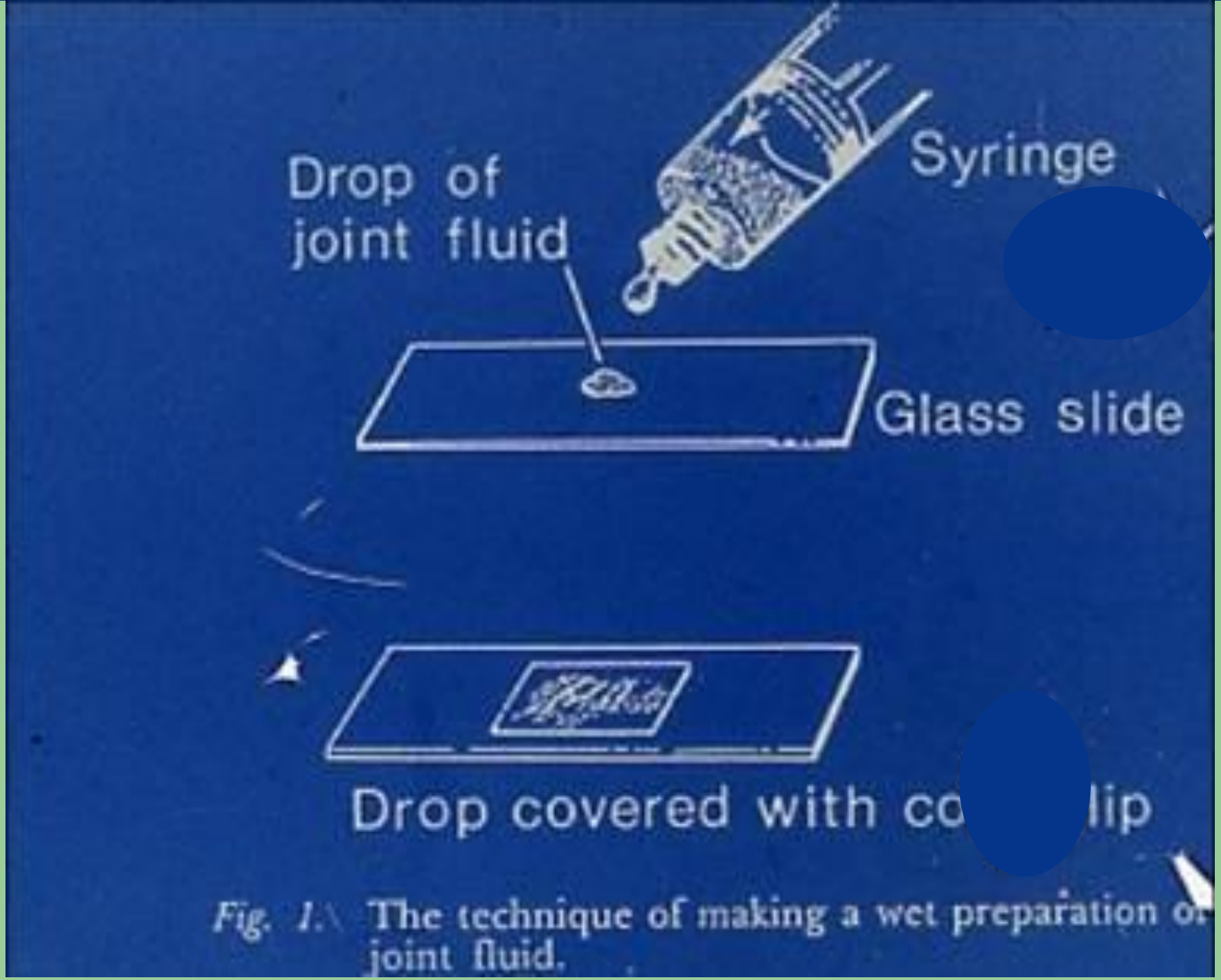
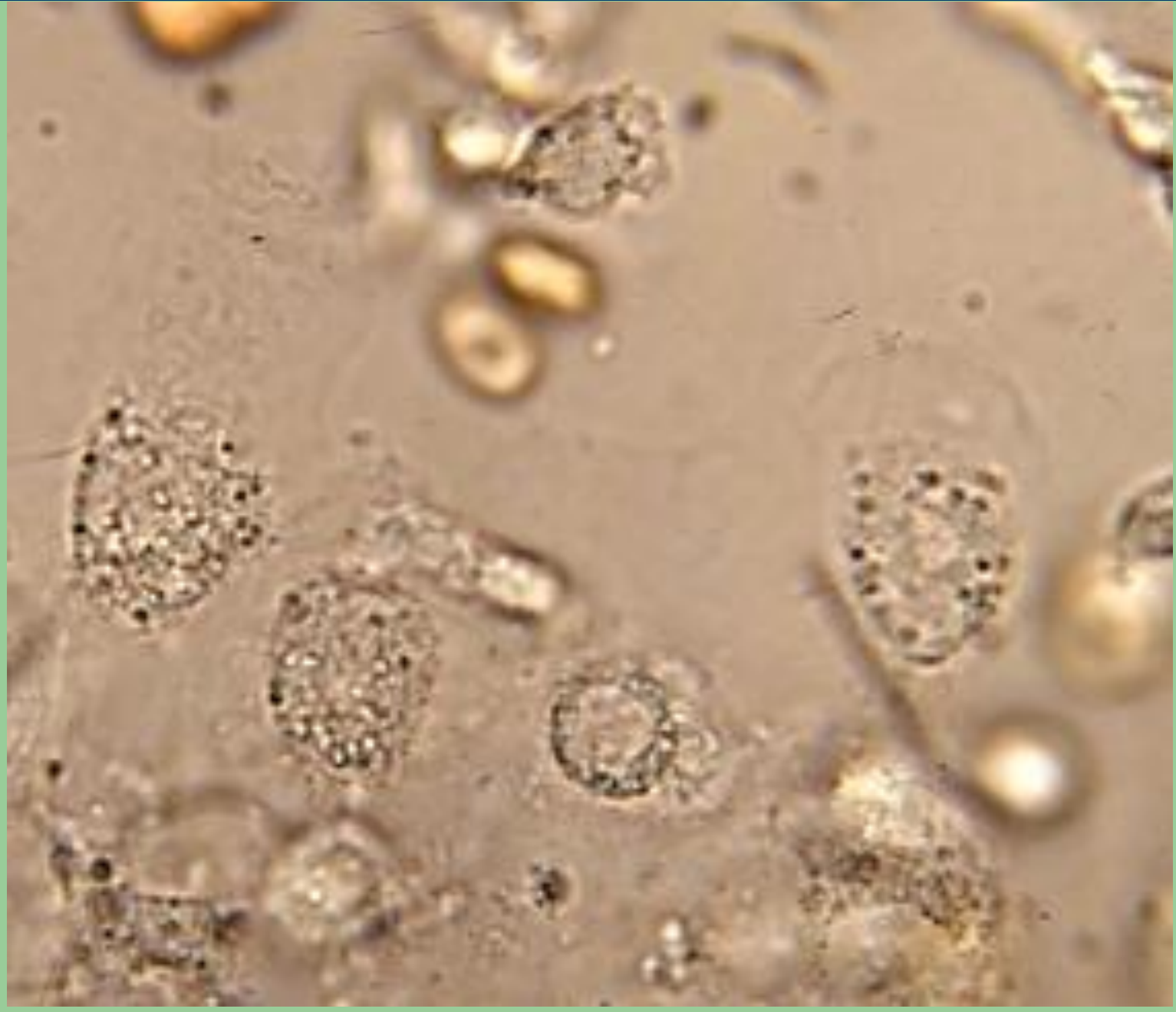


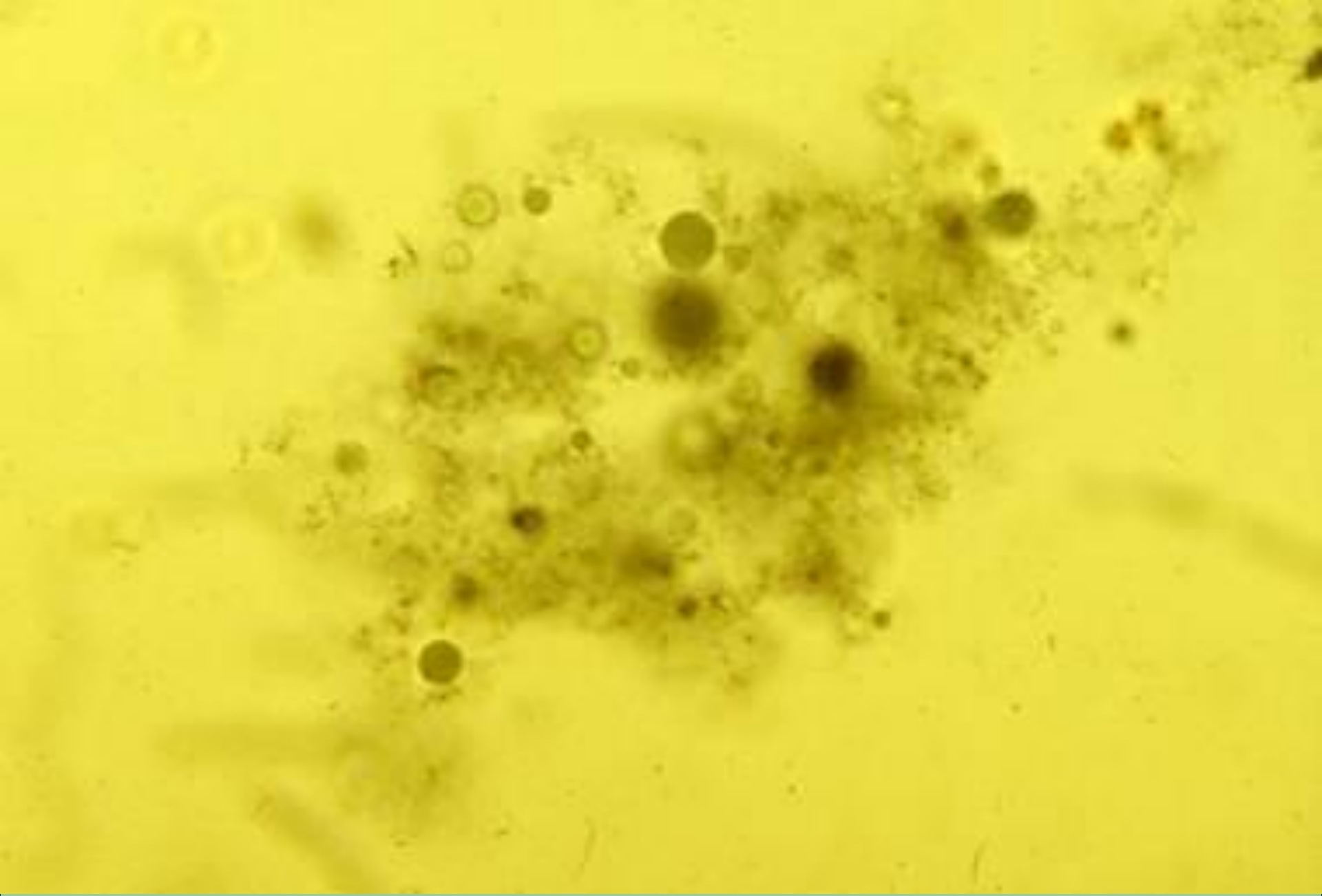
Fig. 1. The technique of making a wet preparation of joint fluid.

Synovial fluid cells examined first under regular light.

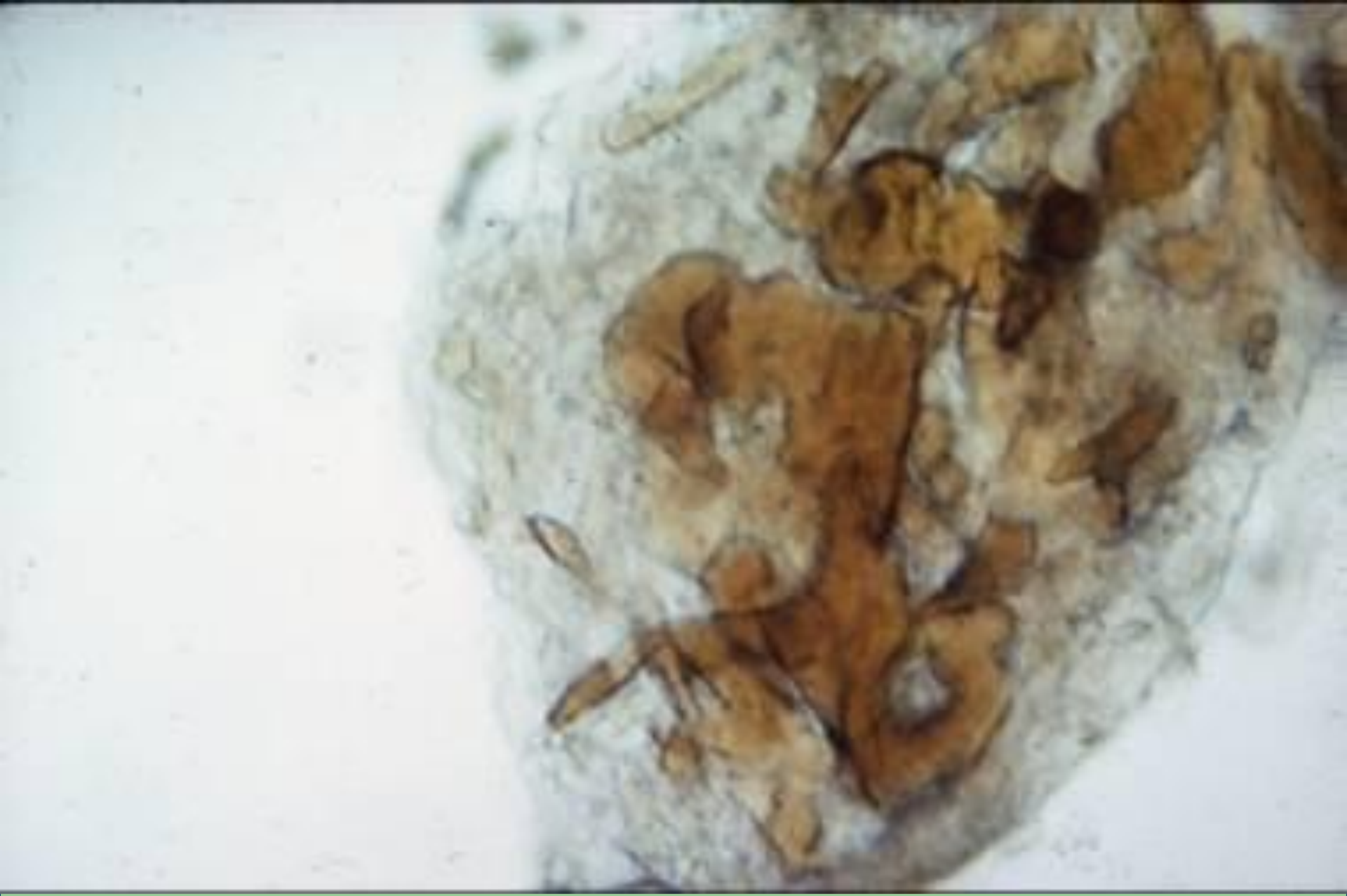


Synovial fluid leukocytes with cytoplasmic inclusions

Synovial fluid neutral fat droplets



Fat droplets stained with Sudan black

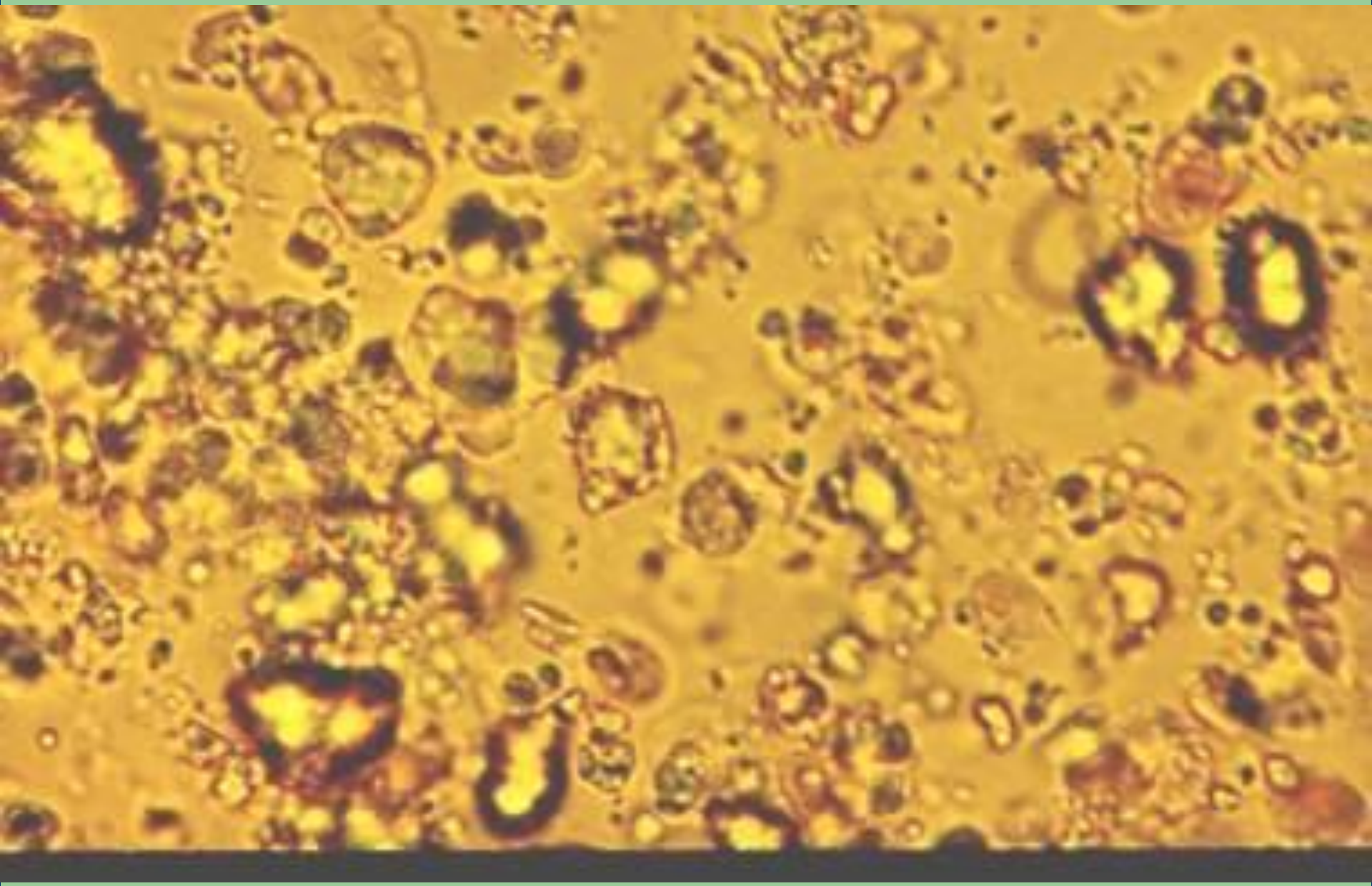


Fragment of synovial villus containing ochronotic shards found floating in synovial fluid.

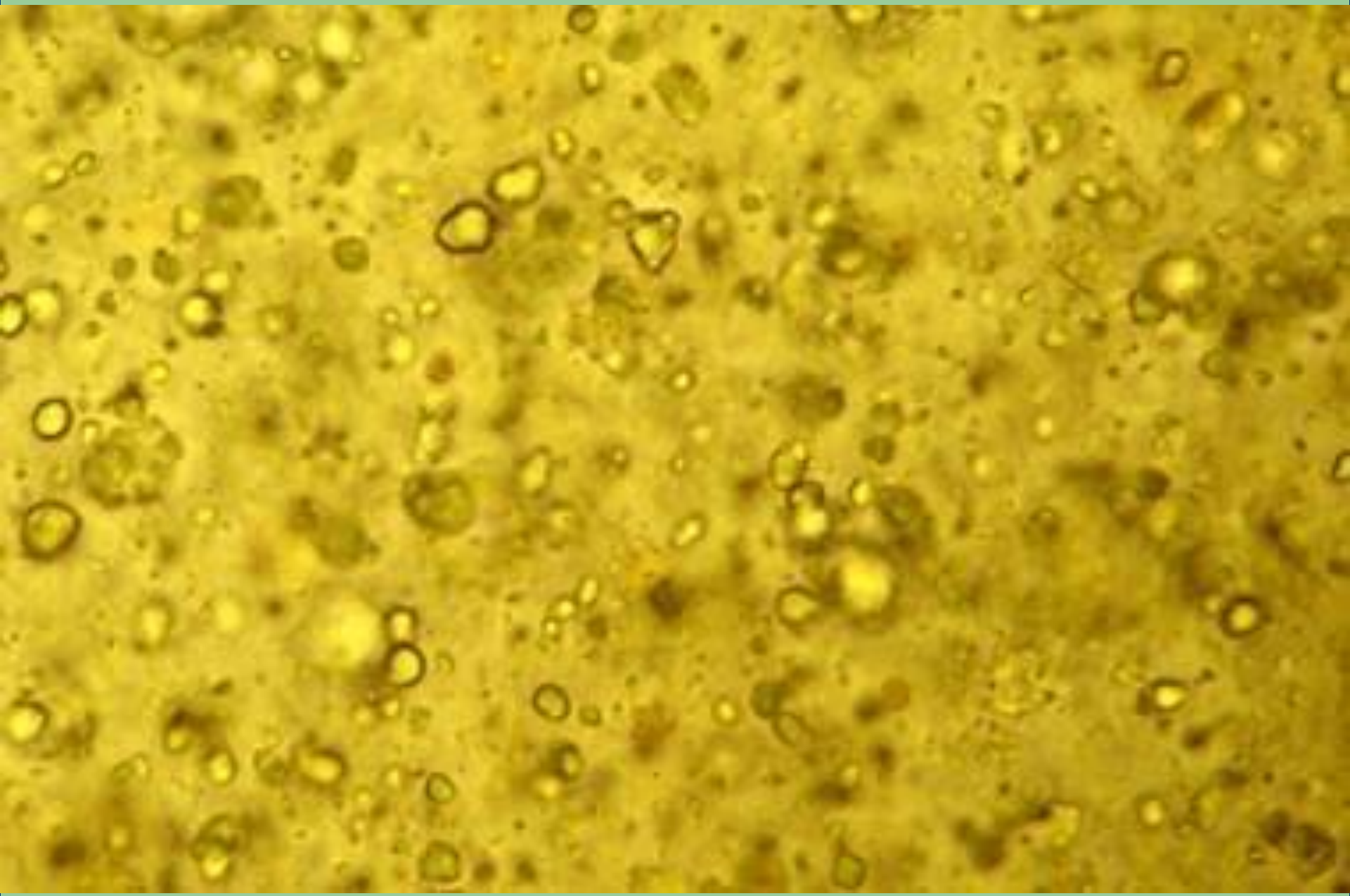
Ochronotic Joint Fluid

Osteoarthritic Joint Fluid

What do you think of this joint fluid?
Regular light microscopy

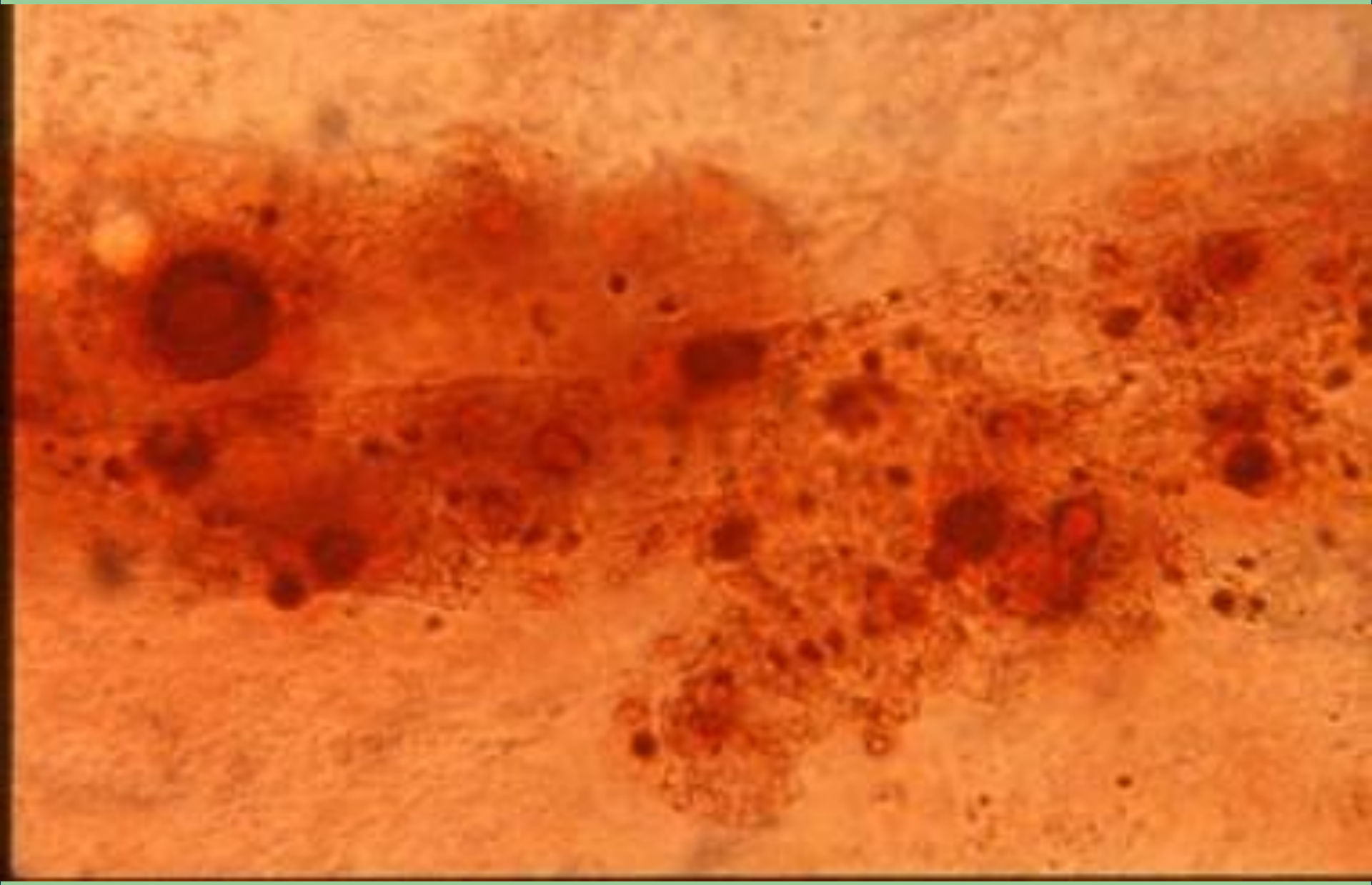


What do you see here?



Apatite crystal clumps by regular light microscopy

Alazarin red S stain for calcium must be passed through a millipore filter

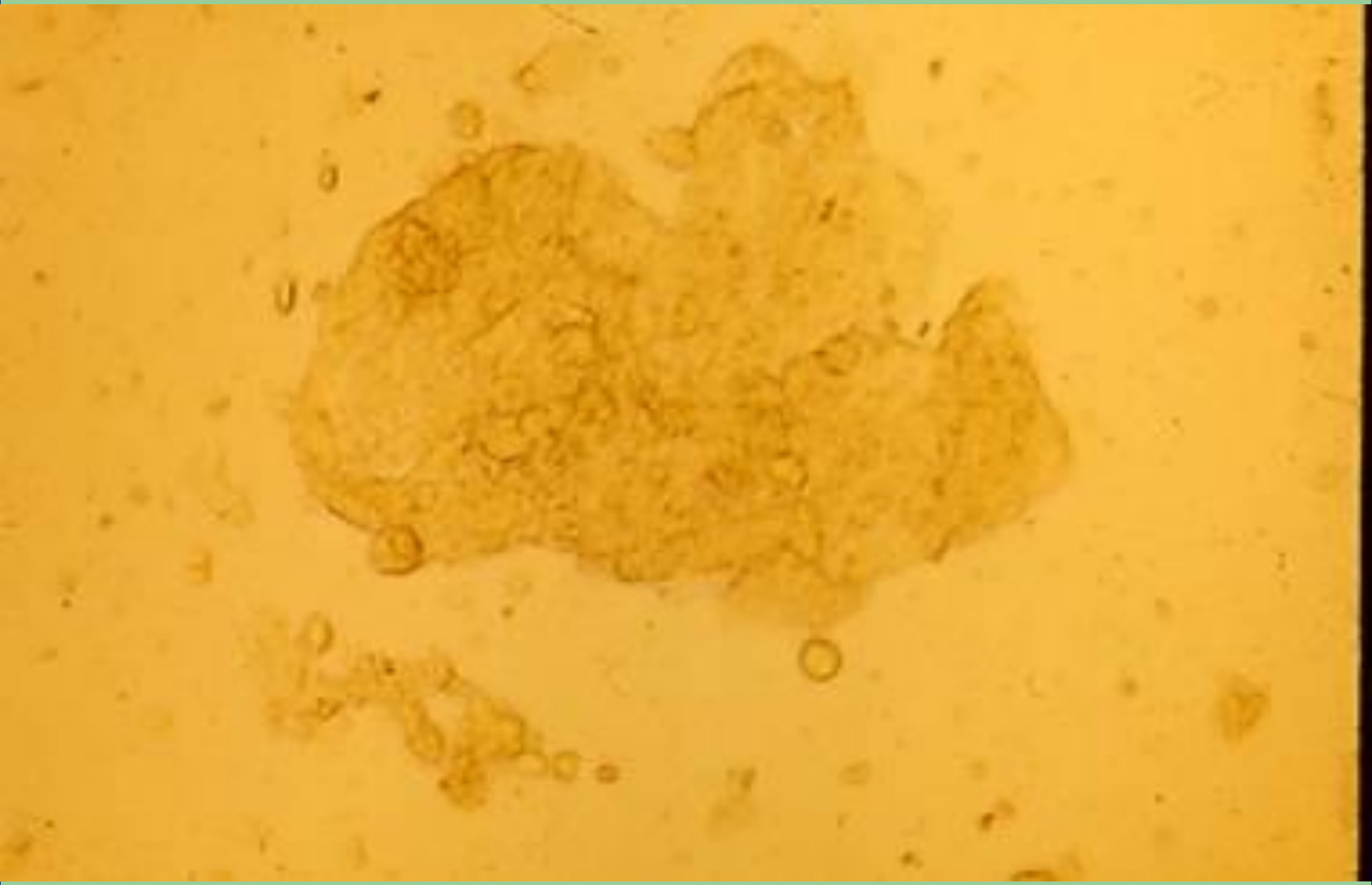


Alazarin red S stained apatite clumps

Individual apatite crystals are seen only by electron microscopy

Calcific periarthritis due to apatite at 2nd MCP joint

Synovial fluid fibrils often seen in osteoarthritis. Regular light

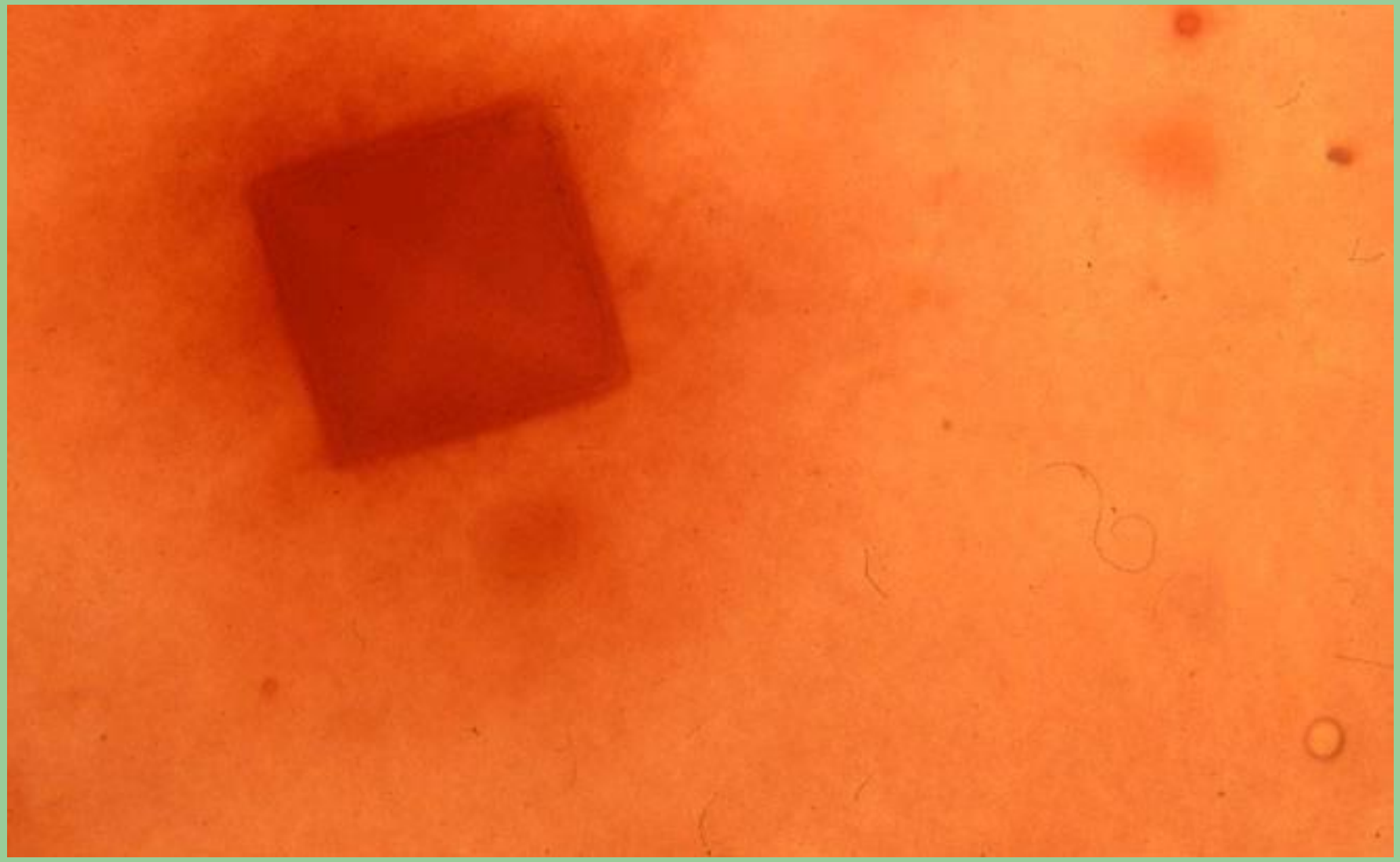


Amorphous clump of synovial fluid amyloid

Congo red positive amyloid

Apple green birefringence of amyloid with plain polarized light

Calcium oxalate crystal



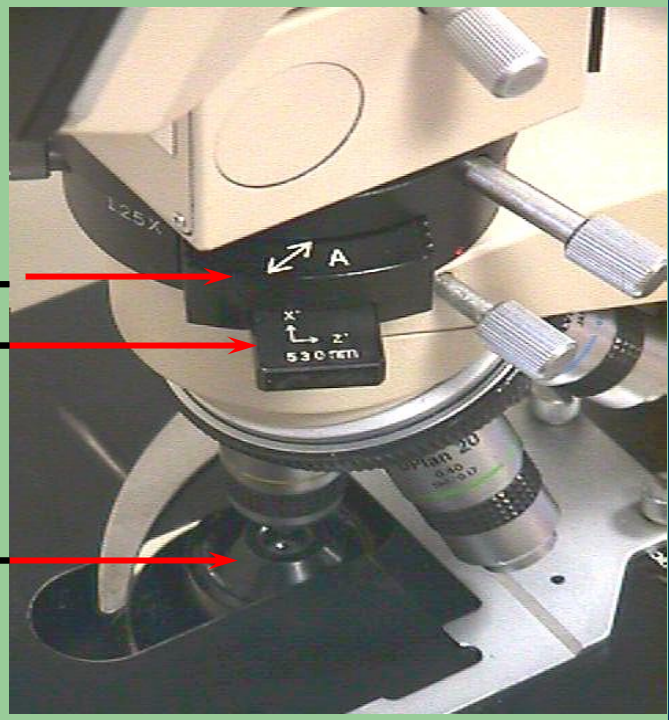
Calcium oxalate crystal stained with Alizarin red S

Charcot-Leyden crystals in eosinophilic laden synovial fluid

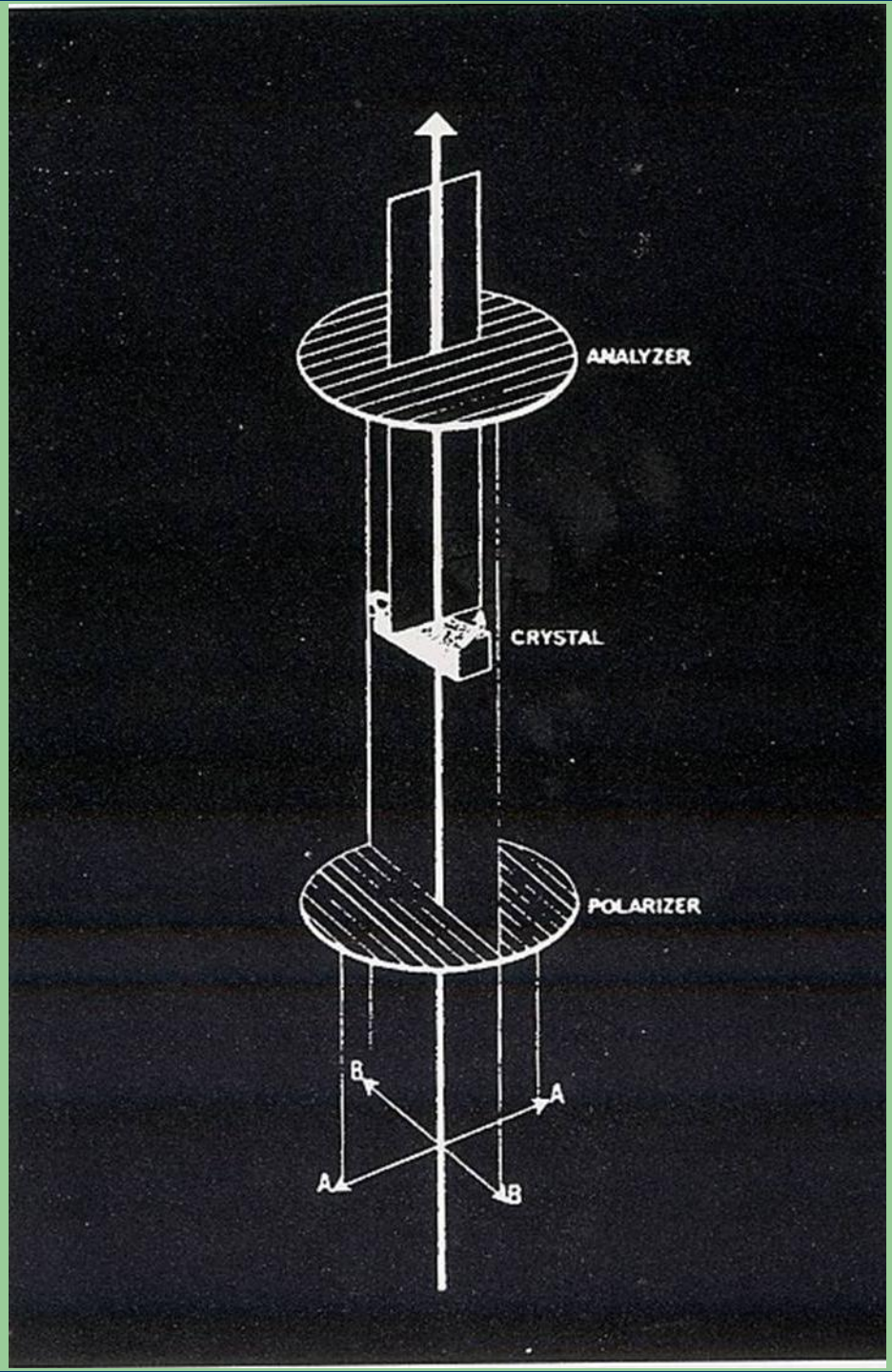
Negatively birefringent MSU crystal

Polarized Light

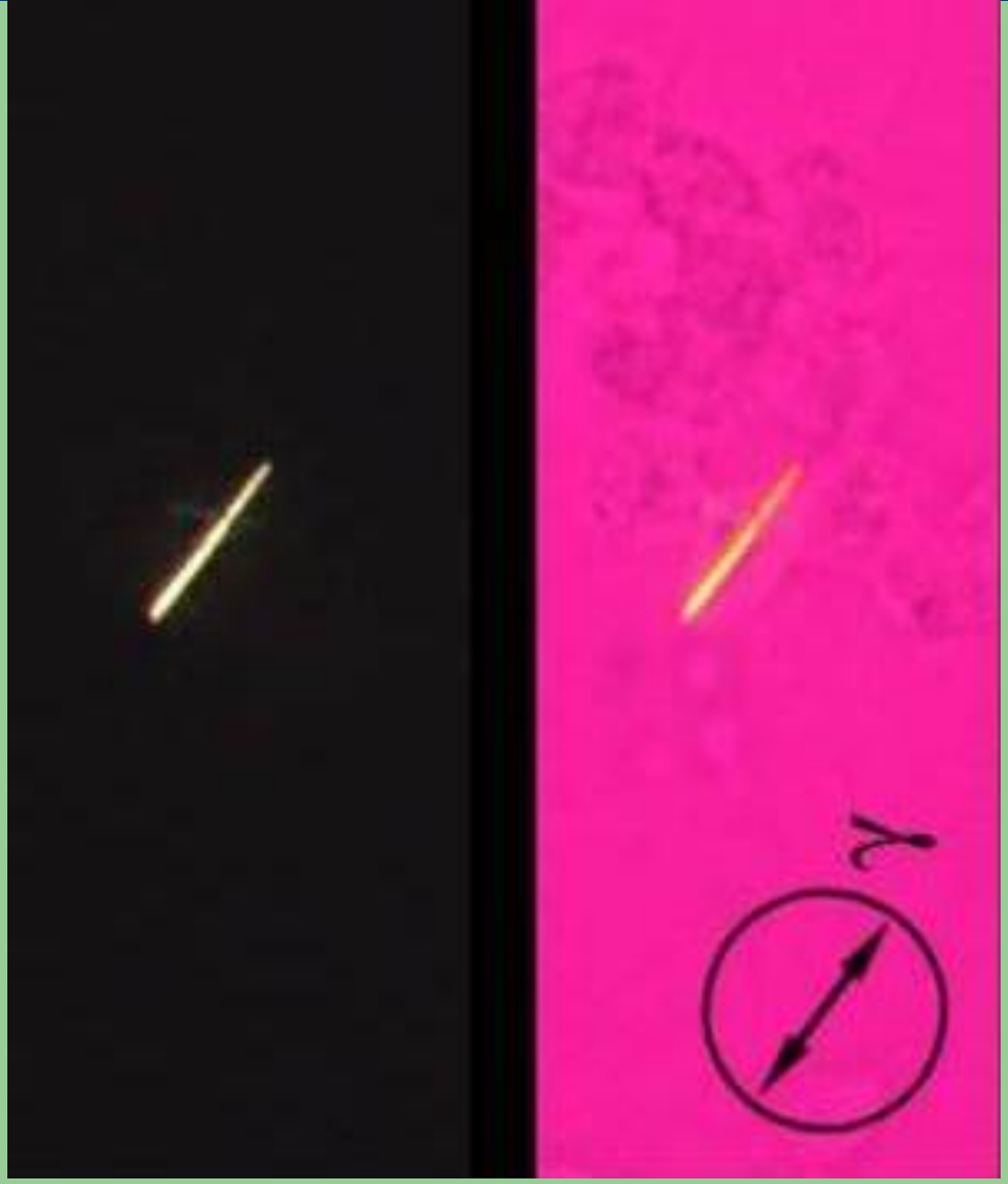
- Polarizing discs
- Rotate until dark field
- Crystal will appear white
- First order red plate
- Background red
- Crystal yellow or blue



A. Ocular
B. Analyzer
C. Compensator
D. Polarizer
E. Condenser


ANALYZER
CRYSTAL
POLARIZER
B
A
A
B

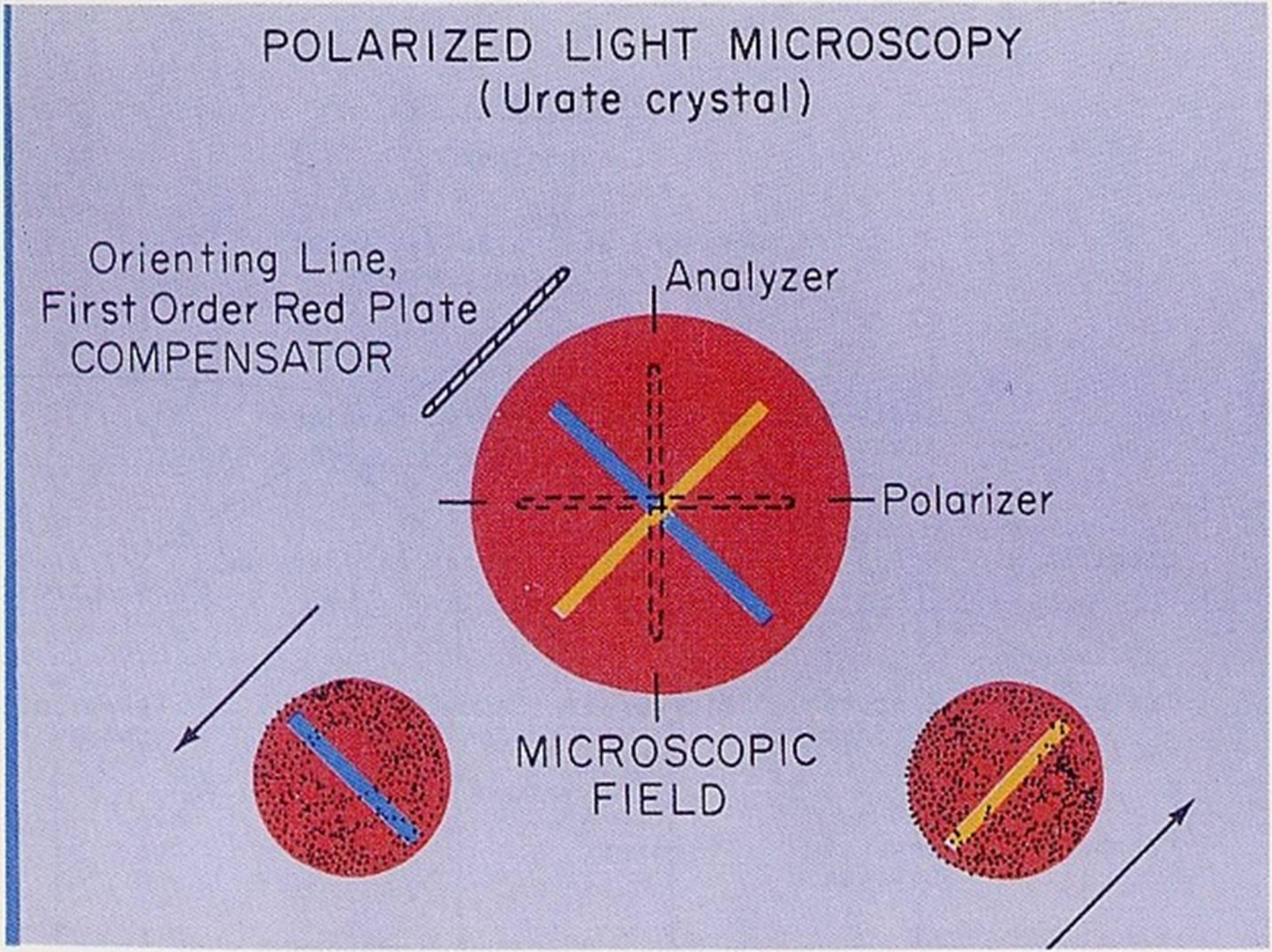
MSU Crystal

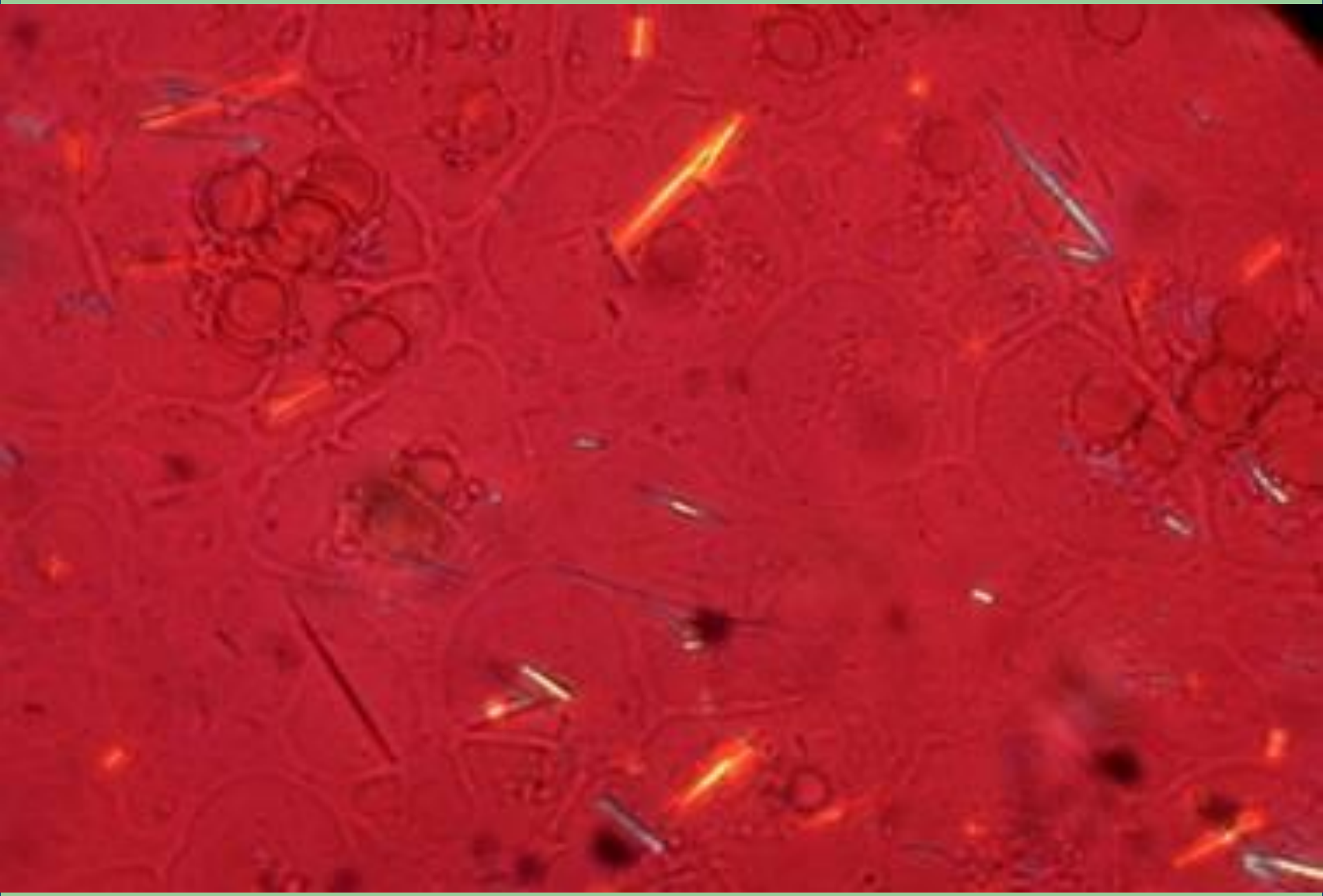


Plain Polarized Light

Compensated Polarized Light

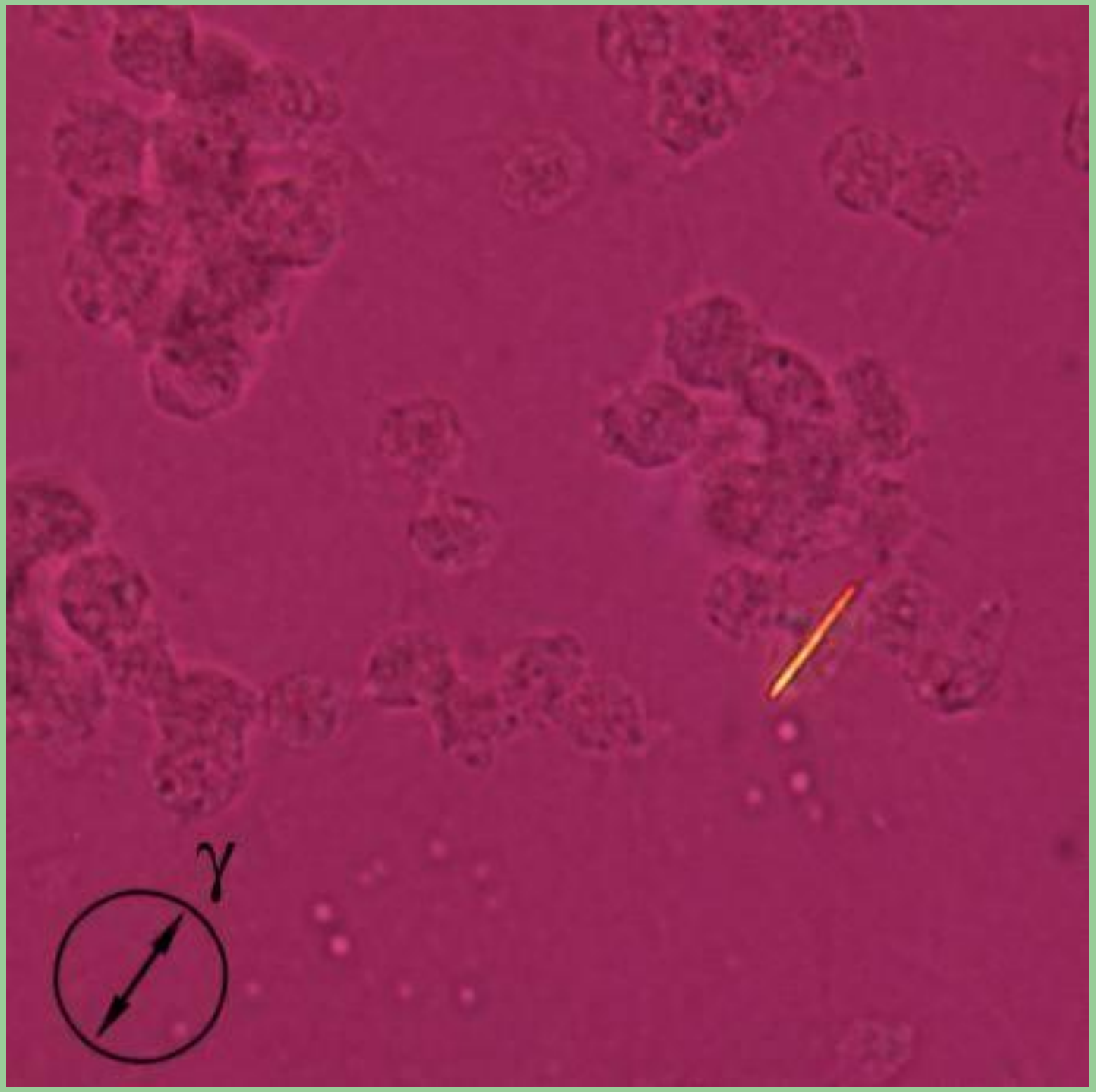
POLARIZED LIGHT MICROSCOPY
(Urate crystal)



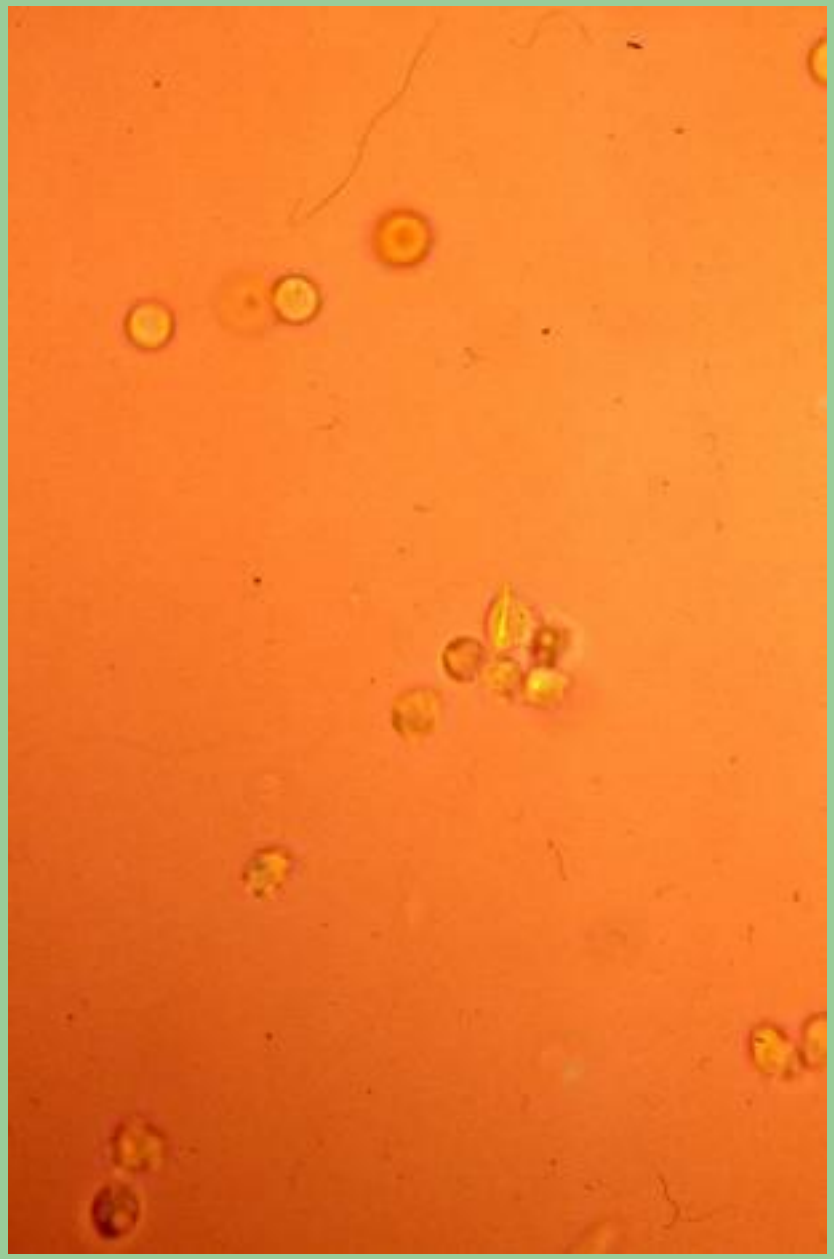


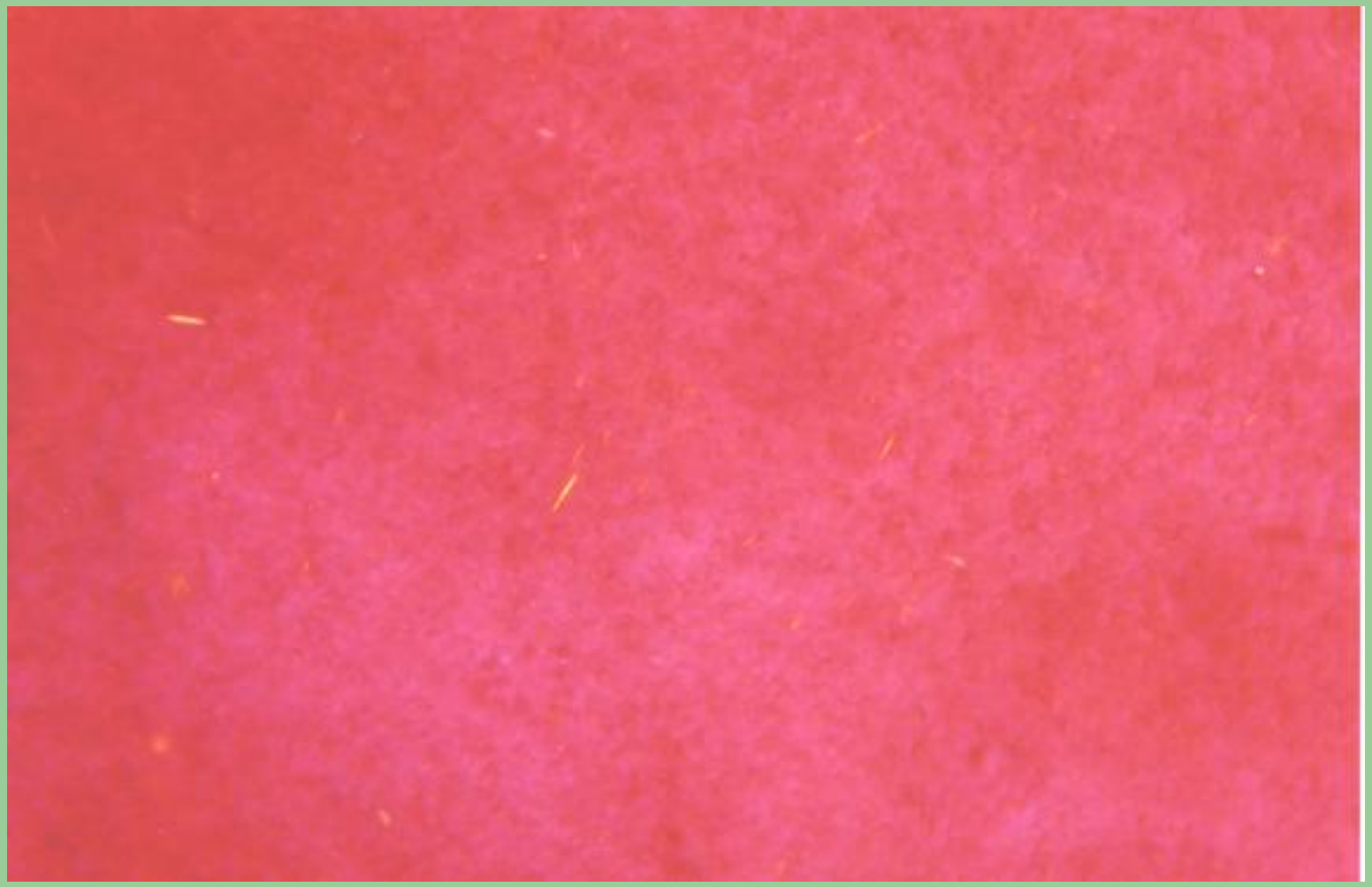
MSU crystals can vary widely in size

MSU Crystal



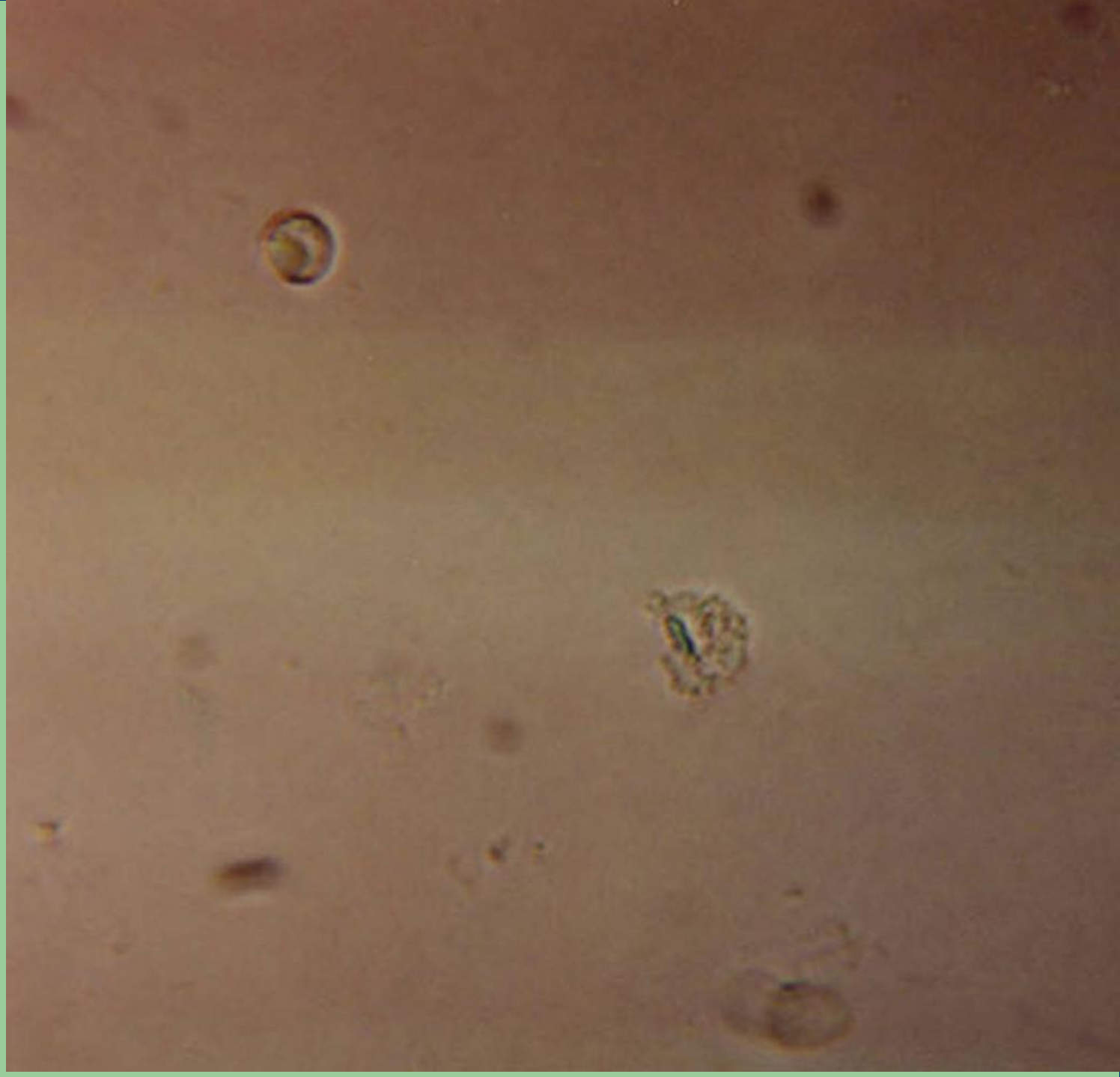
Lower magnification intracellular MSU crystal and unidentified dot-like fragment



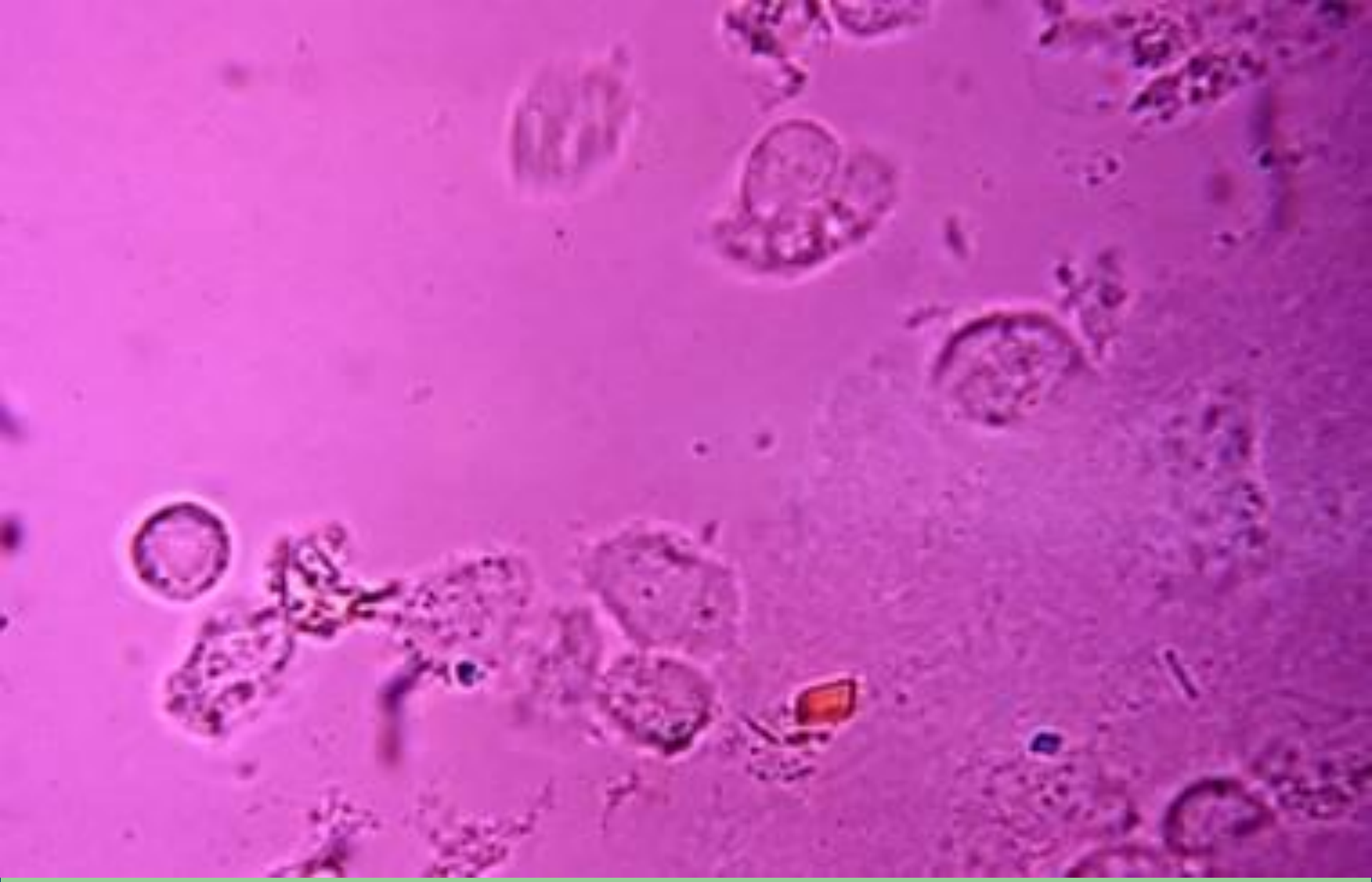


Centrifuged synovial fluid pellet to concentrate MSU crystals

Weakly positively birefringent CPPD crystal in WBC vacuole



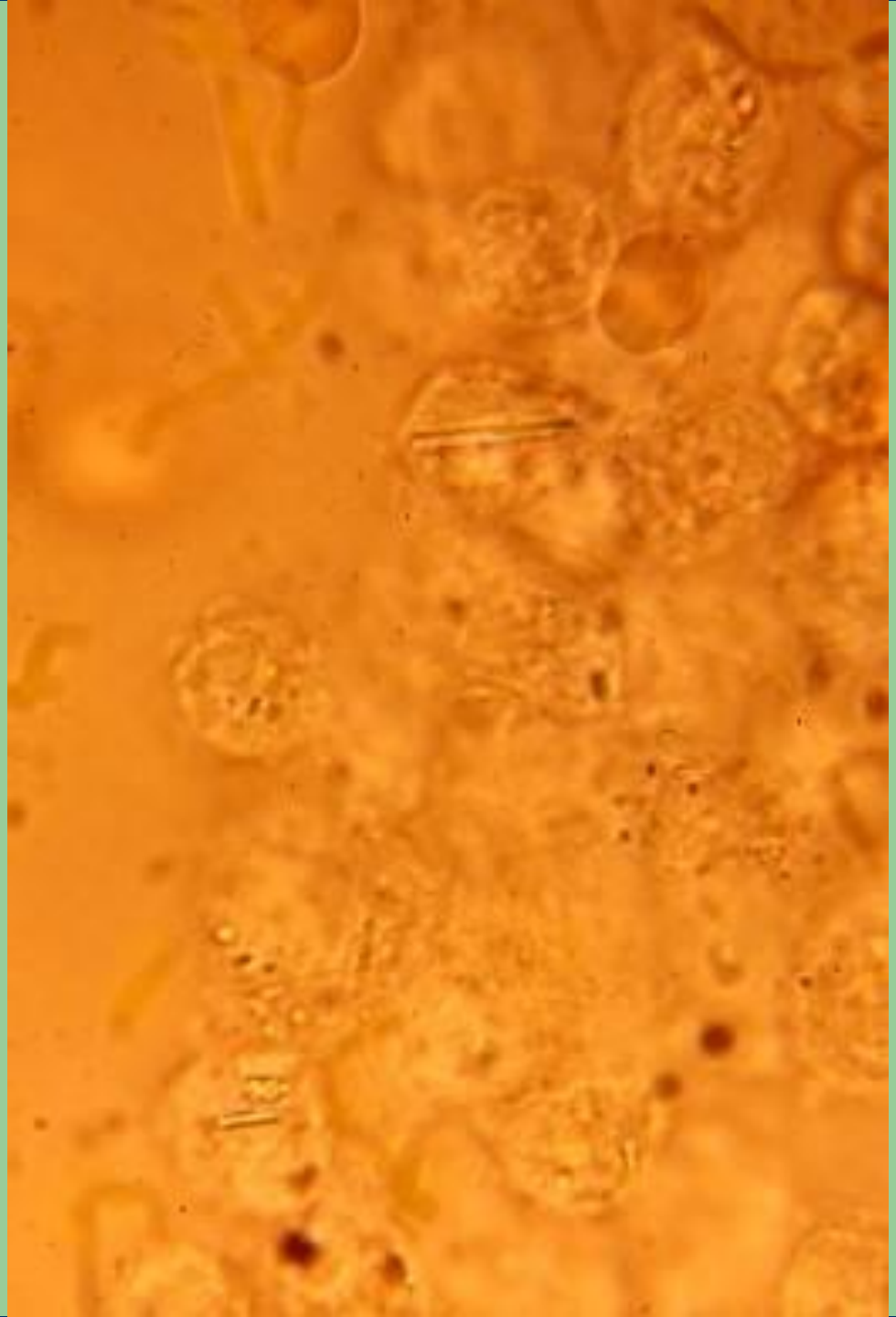
CPPD crystals may be more brightly birefringent



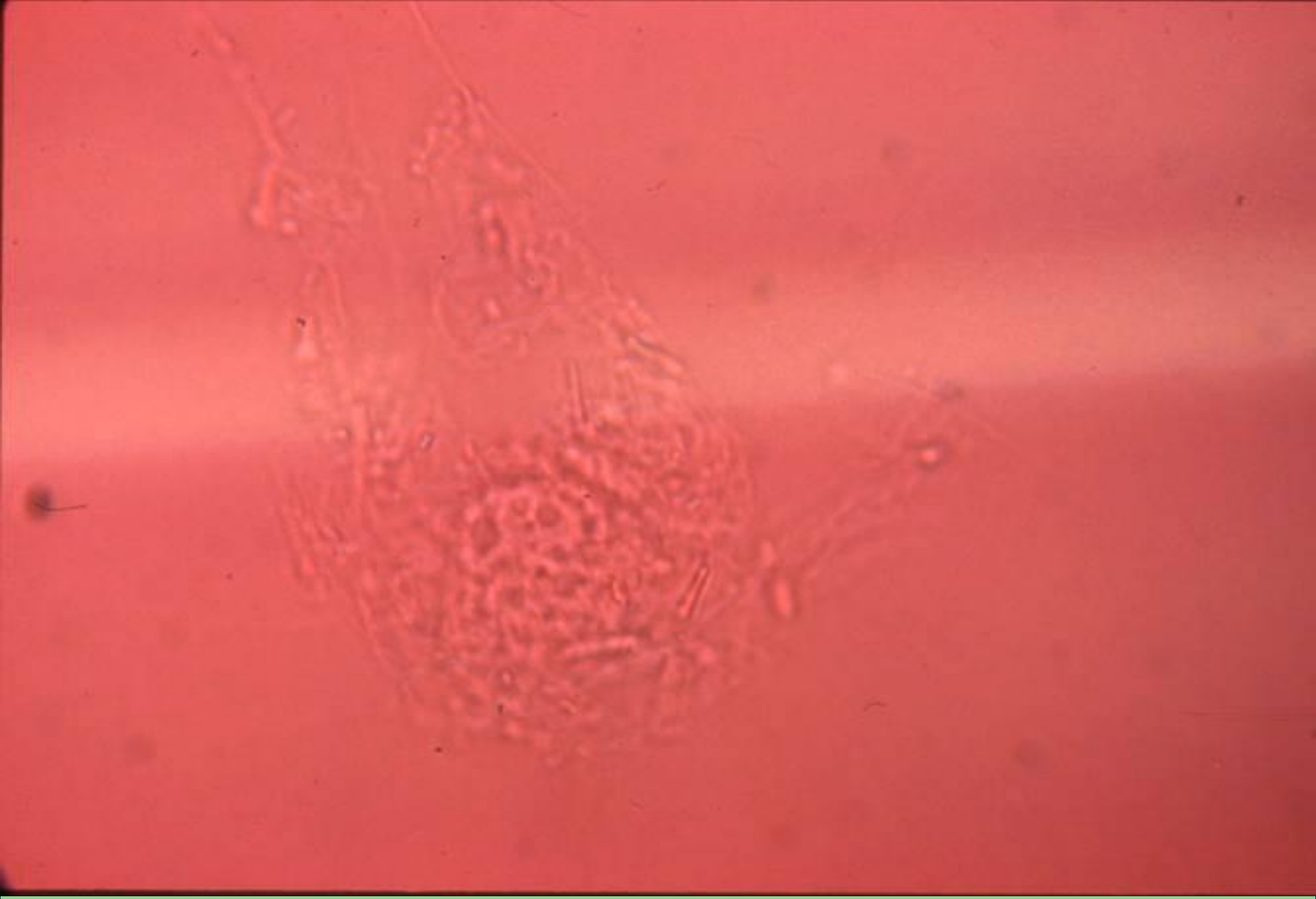
CPPD Crystals Can Be Rhomboid or Rod Shaped

Intracellular CPPD

CPPD may sometimes be seen more easily with regular light.

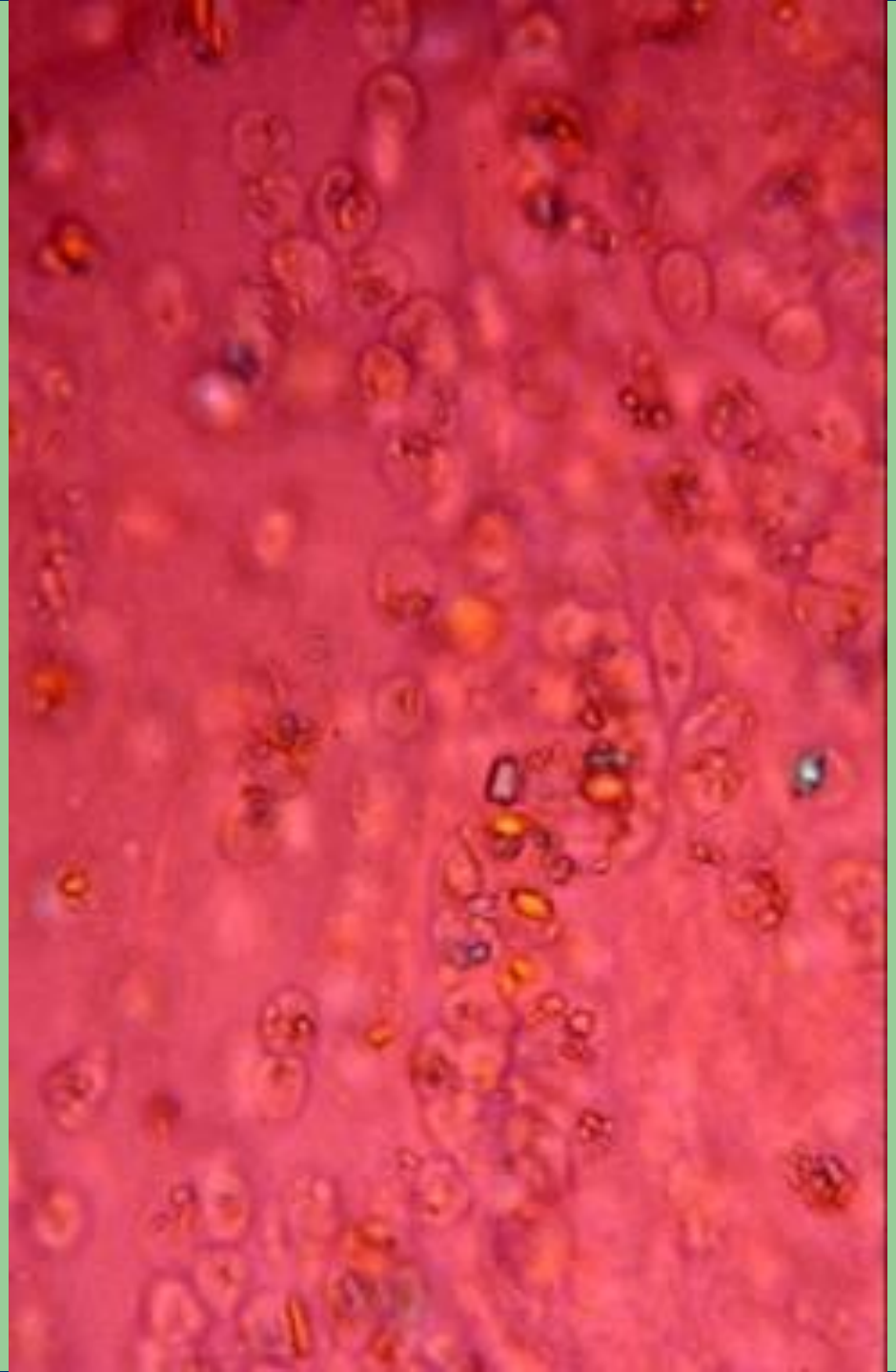


Faintly positively birefringent CPPD can be very small

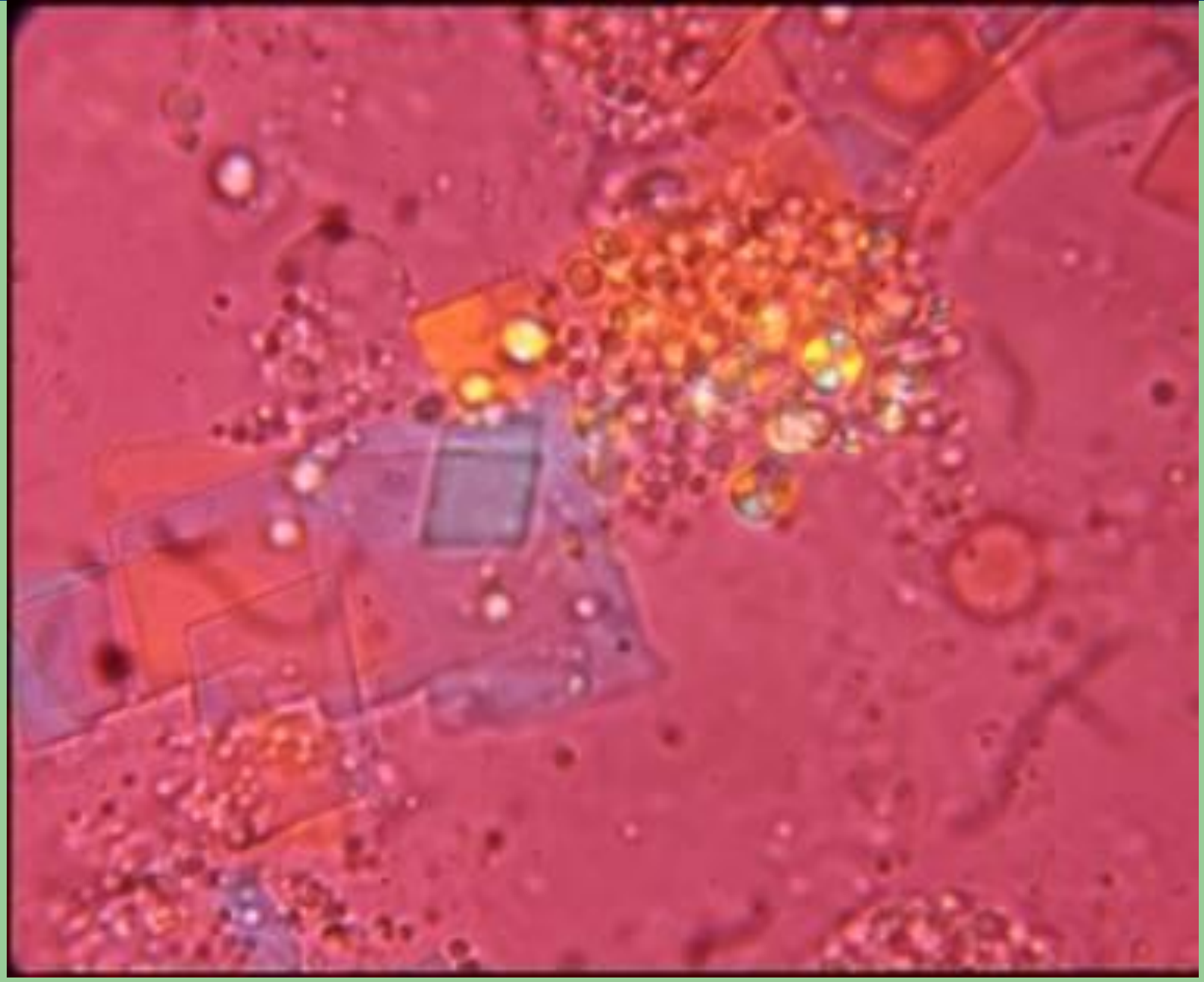


CPPD May Be Non-birefringent

CPPD concentrated in a cartilage fragment



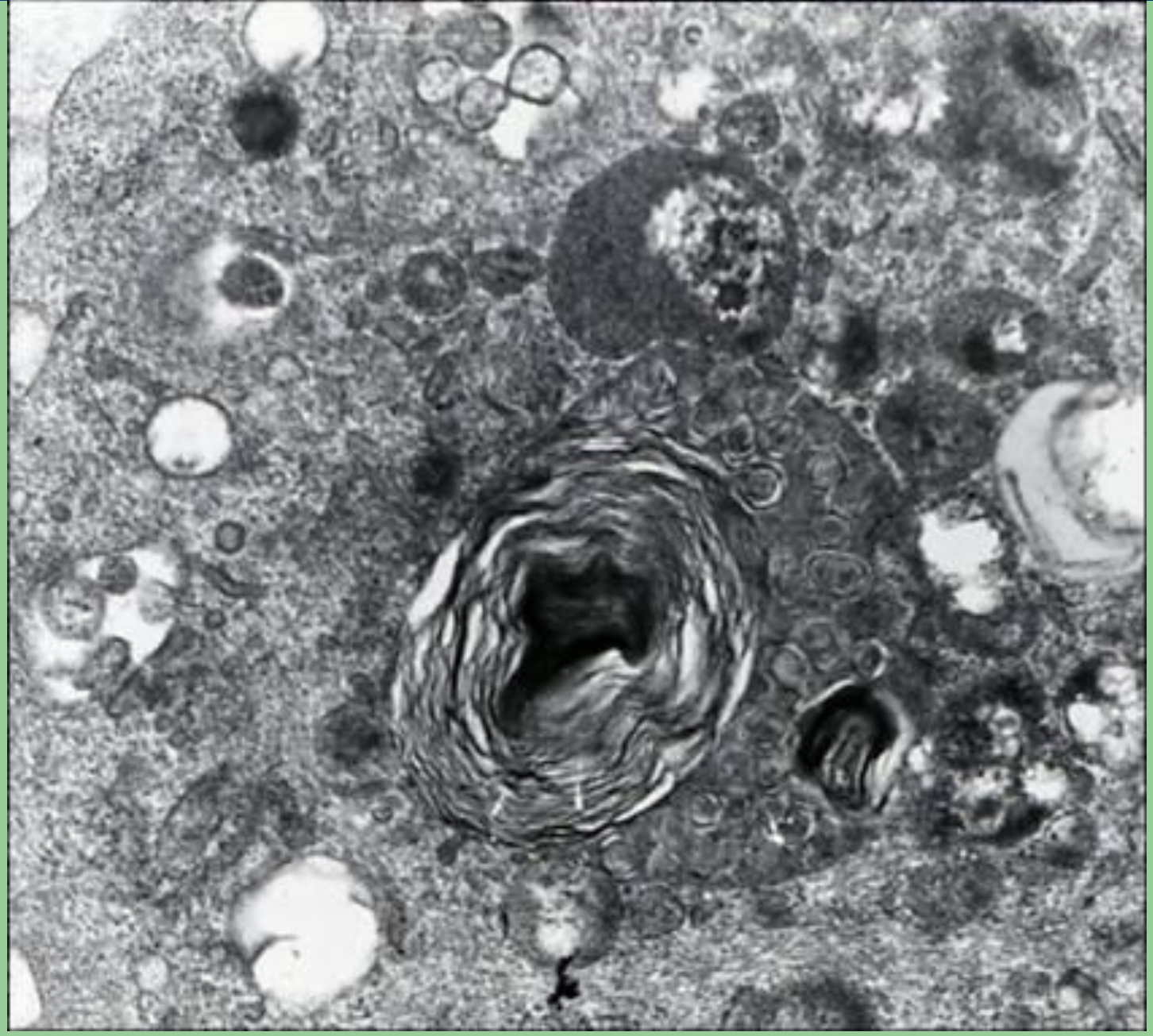
Single cell containing MSU and CPPD crystals



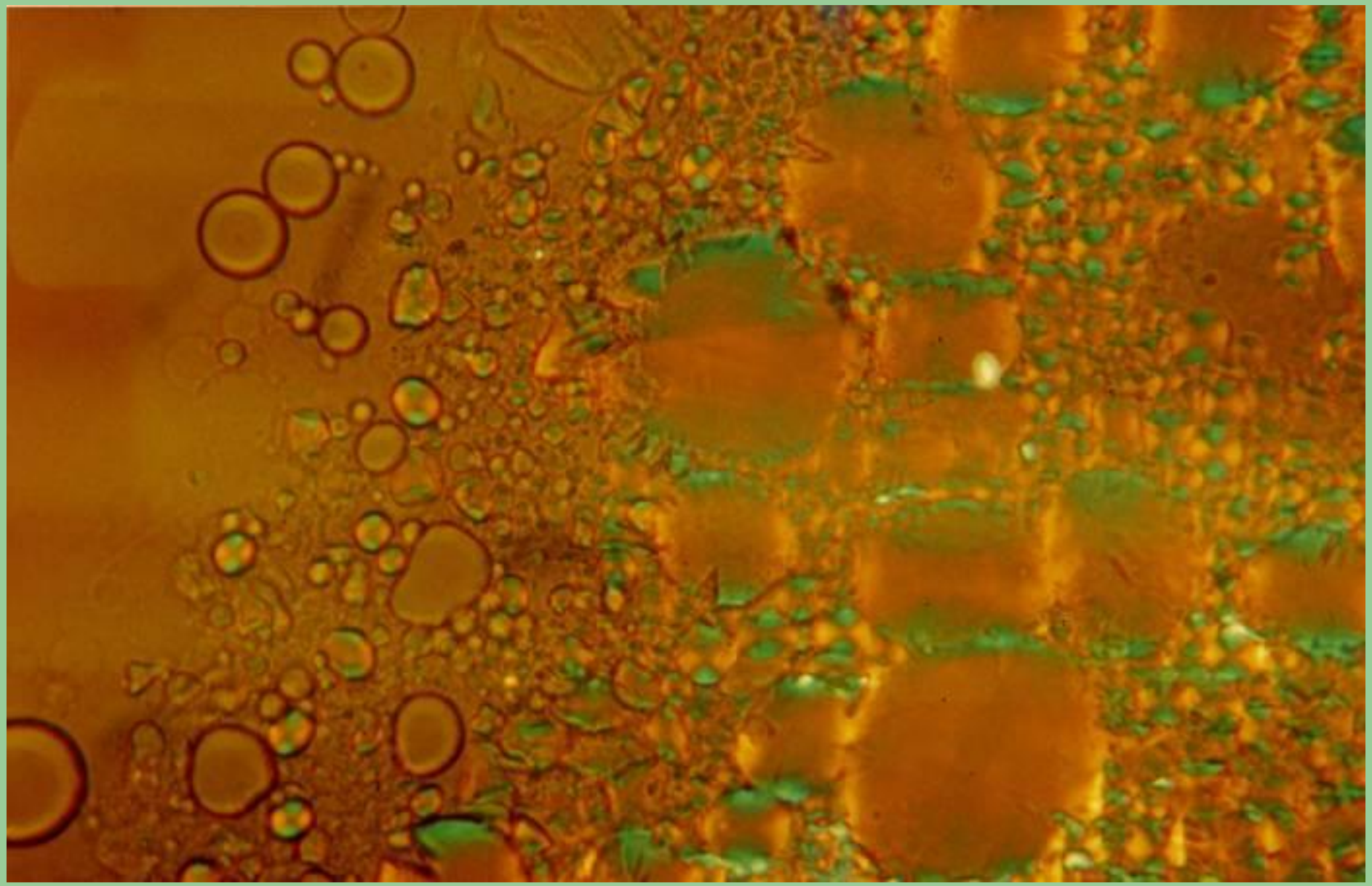
Cholesterol and lipid liquid crystals

Lipid Liquid Crystals

- Appear as maltese crosses
- Positively birefringent
- Associated with some acute otherwise unexplained arthritis
- Can be phagocytized
- Seen as membranous arrays by EM
- Possibly derived from RBC or other cell membranes.
- Don’t confuse with urate microspherules (negatively birefringent) or talc.

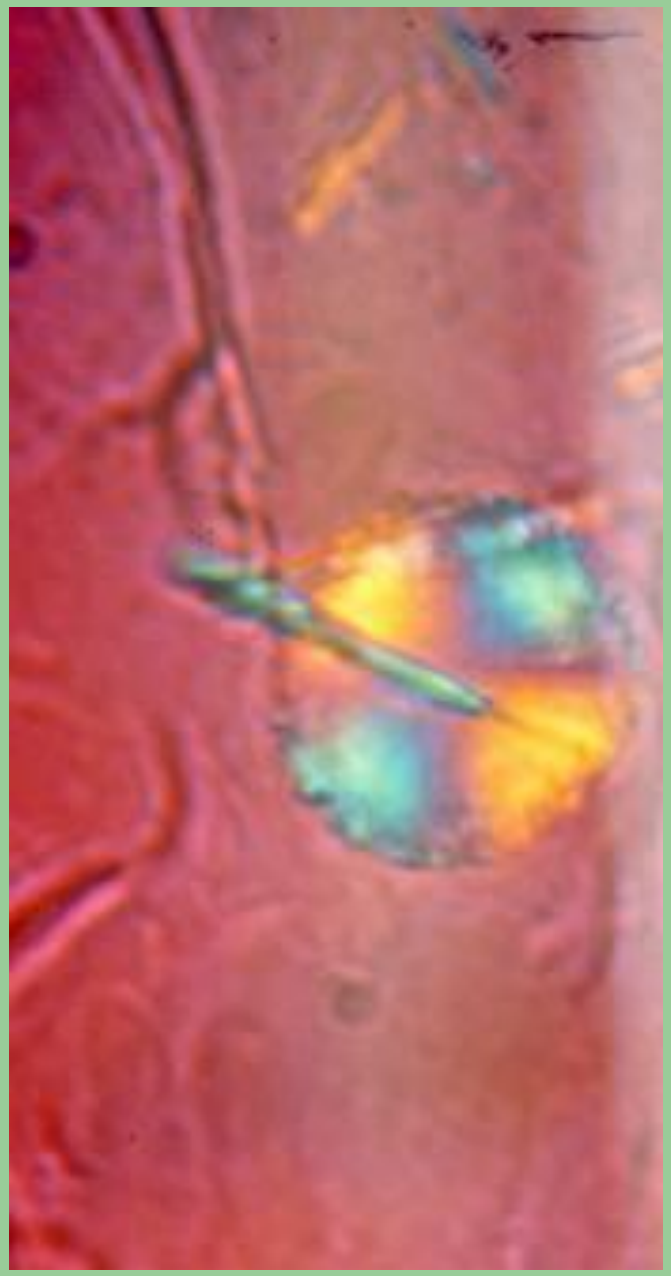


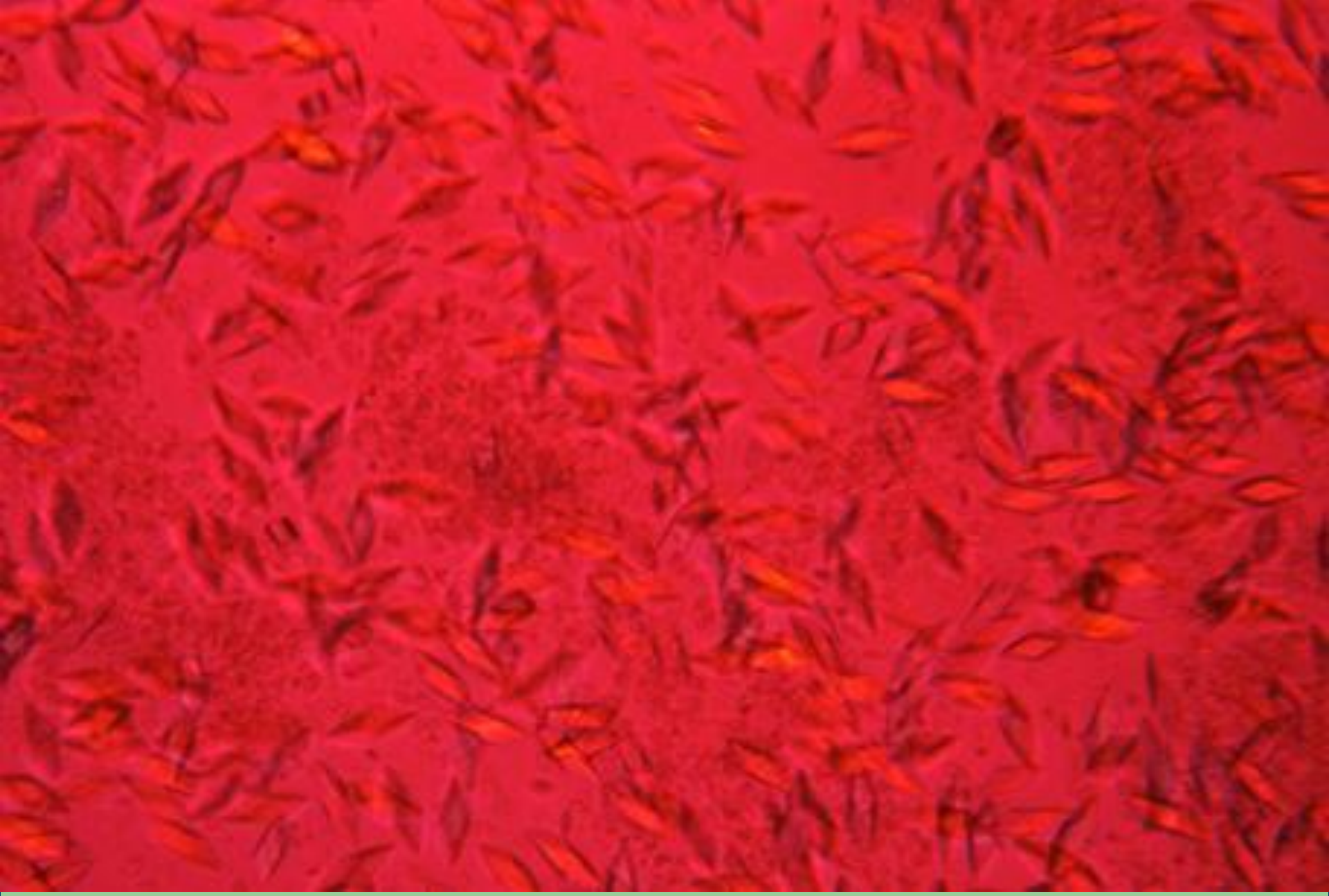
Membranous arrays of phospholipid in lipid liquid crystals by EM



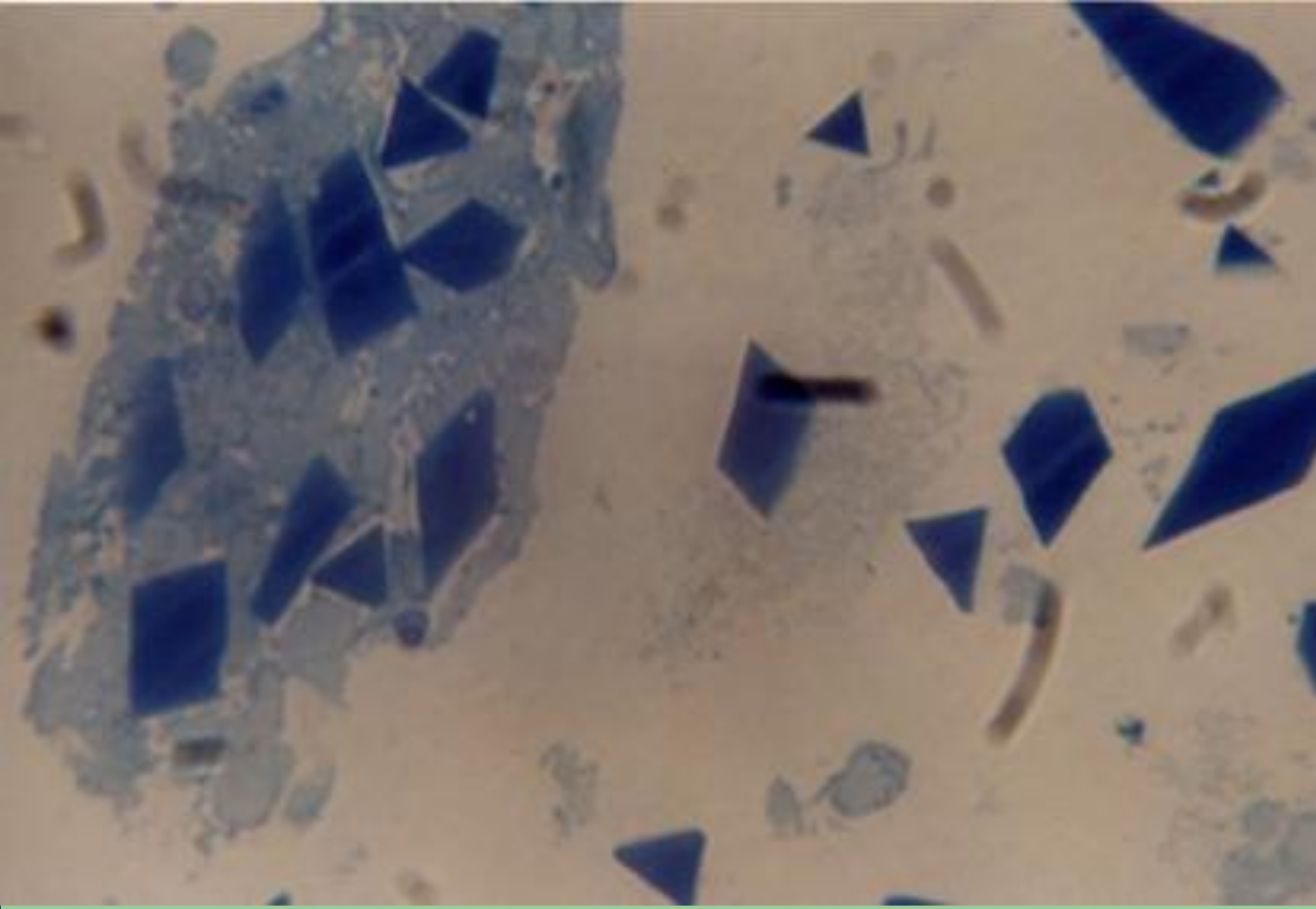
Massive positively birefringent lipid liquid crystals

Negatively birefringent MSU crystal overlying positively birefringent Maltese cross lipid liquid crystal

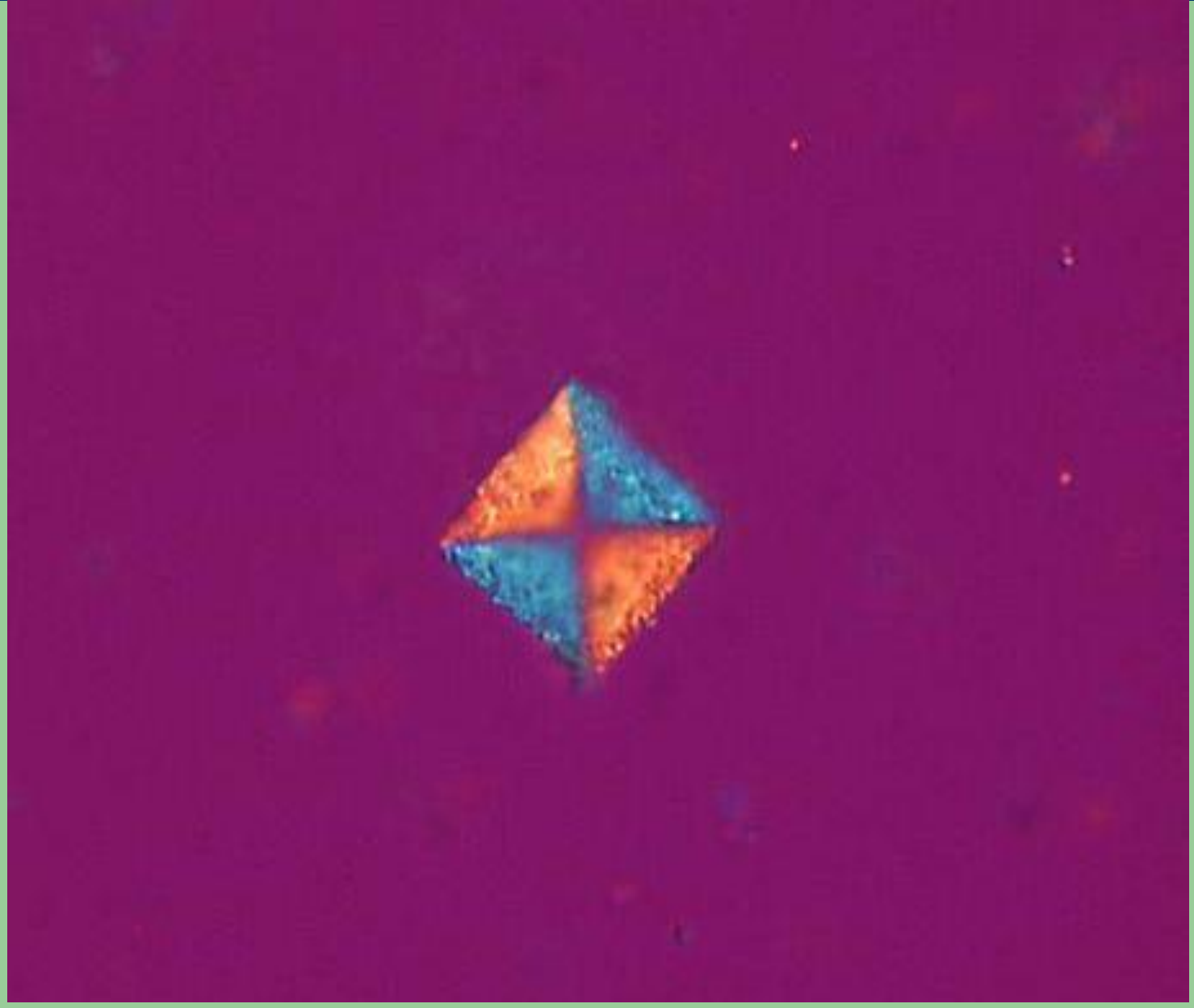




What do you see here?



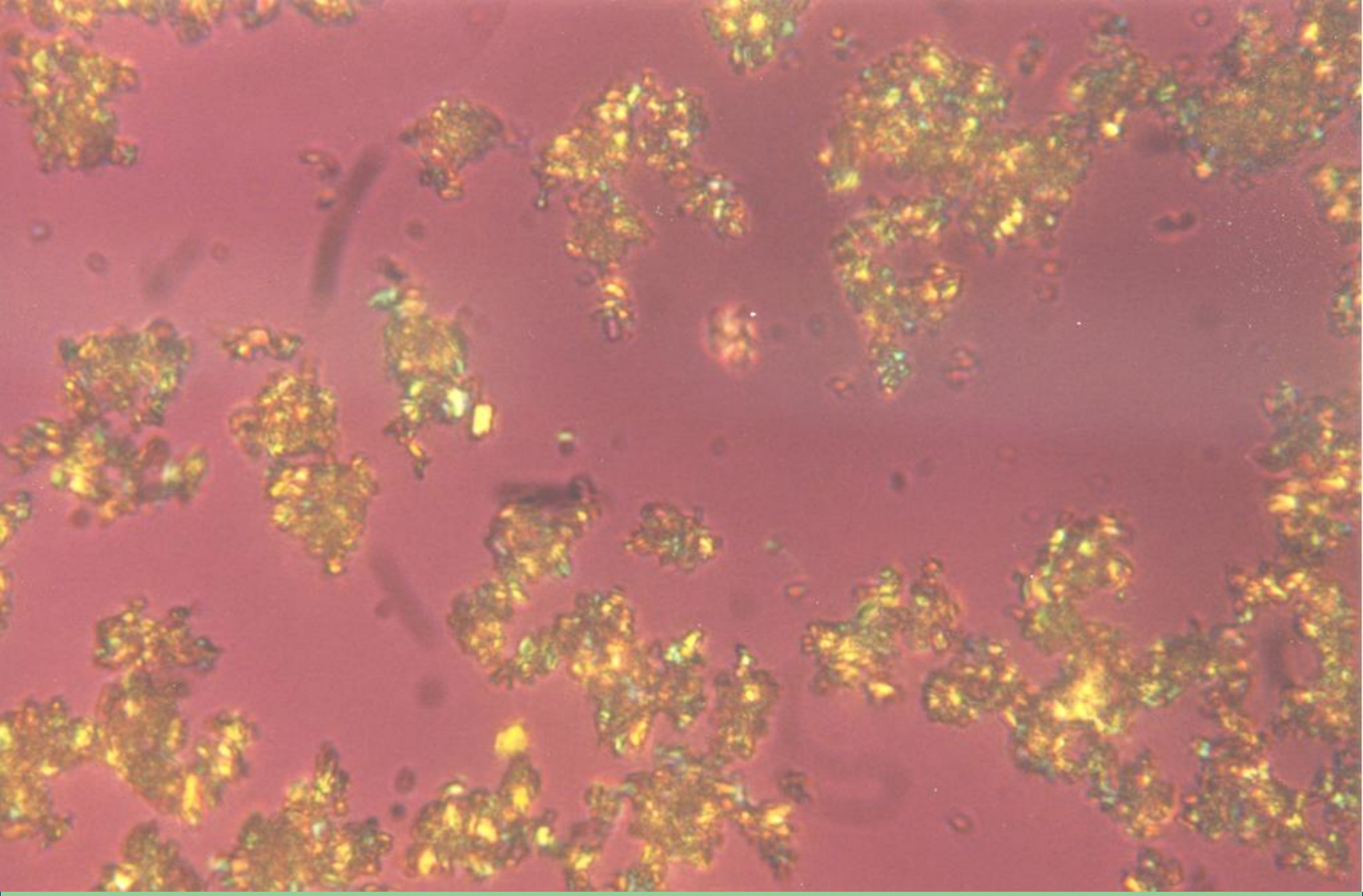
Cryoglobulin and other protein crystals stain with toluidine blue



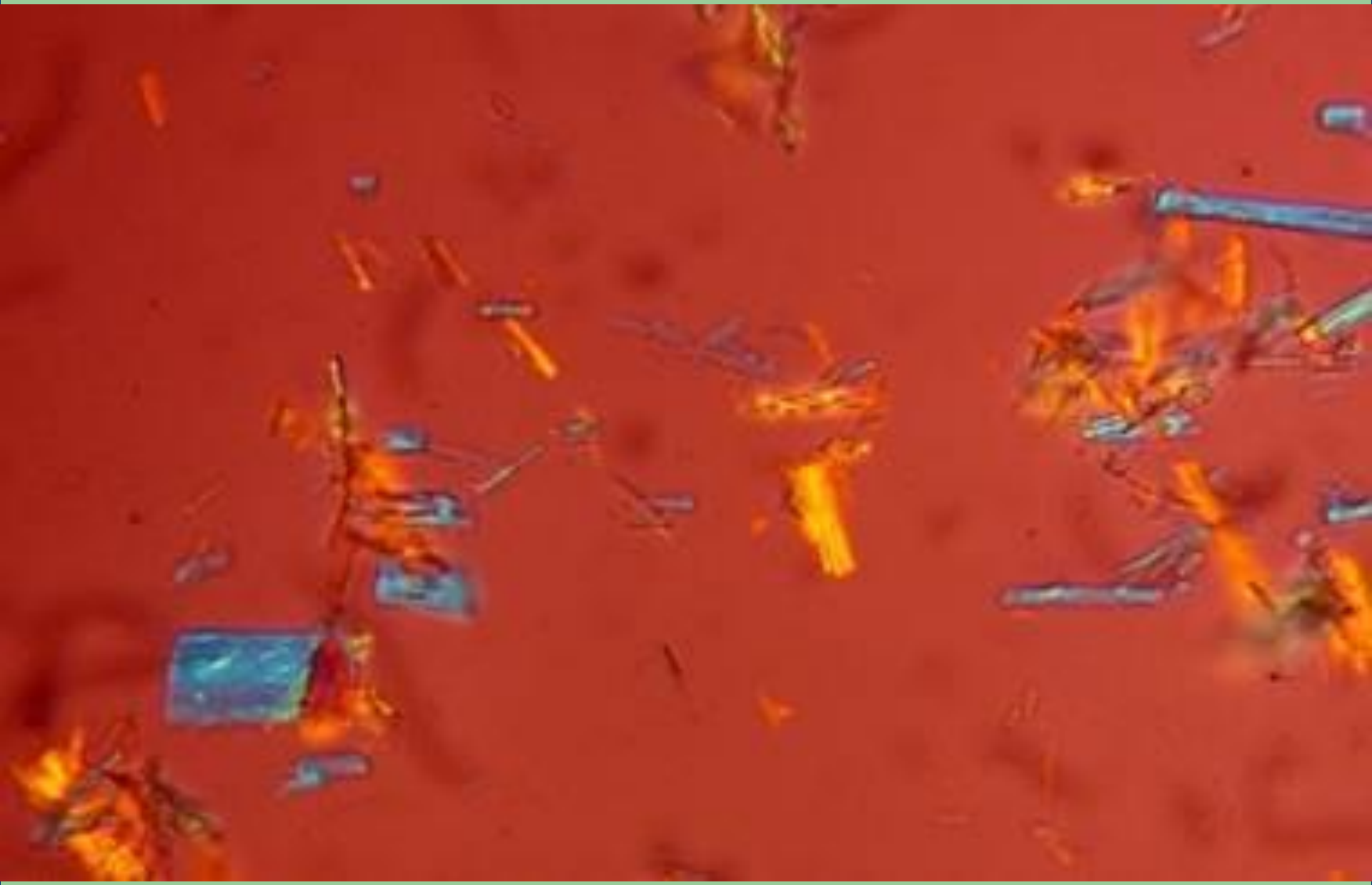
Pyramidal aspect of oxalate crystals are accentuated by polarized light

Artefacts that May Be Seen on Polarized Light Examination of Joint Fluid

- Depot corticosteroids
- Anticoagulant crystals
  - Oxalate
  - EDTA
- Drying artefact
- Glass fragments
- Fibrils from lens paper
- Corn starch from sterile gloves
- Lipids from degenerated cells
- Birefringent nail polish used to seal coverslips

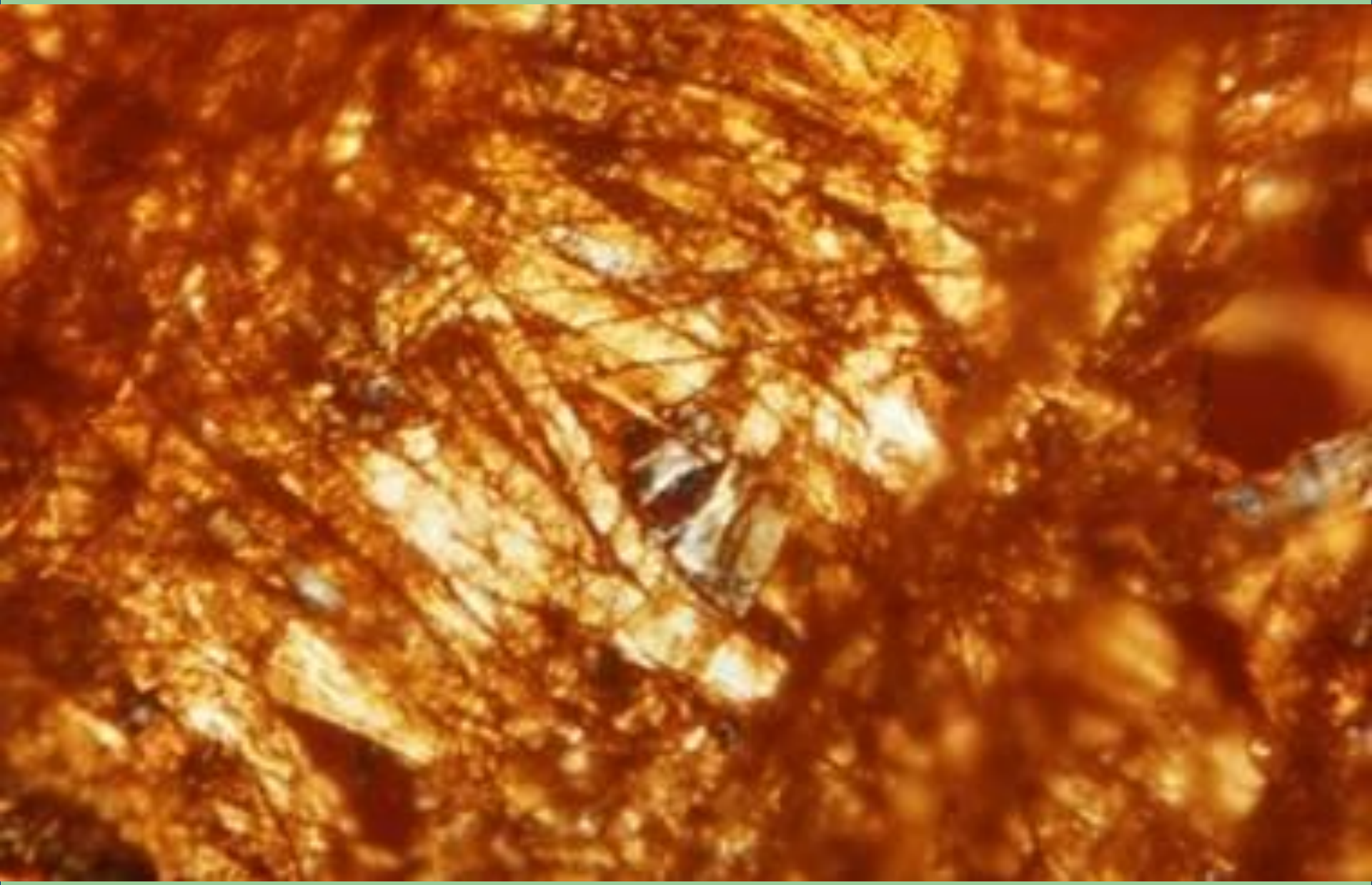


Depot medrol is very bright and irregular

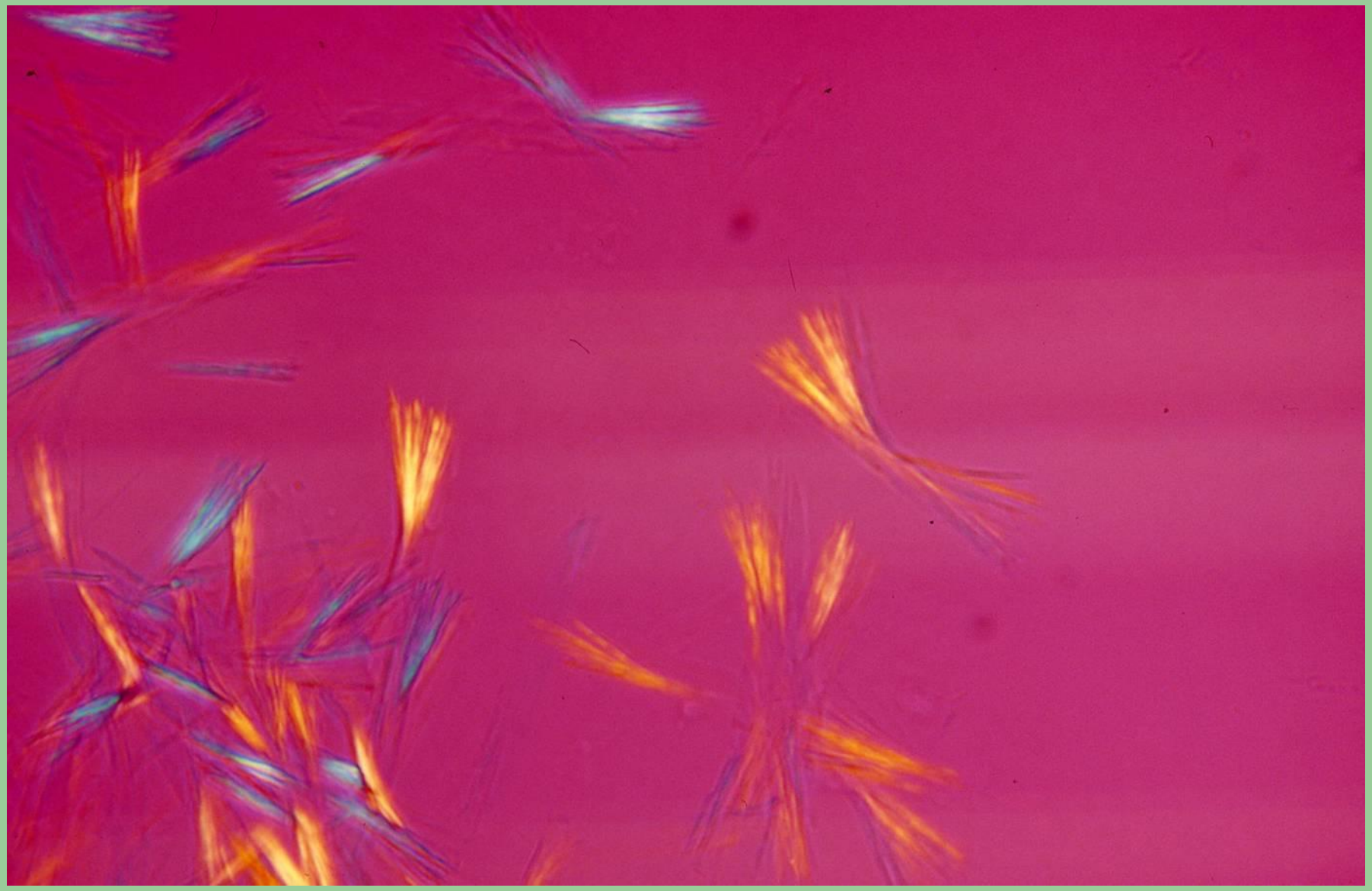


Celestone soluspan can mimic CPPD or cholesterol

Glass fragments from broken coverslips can mimic MSU crystals



Lens paper is brightly birefringent

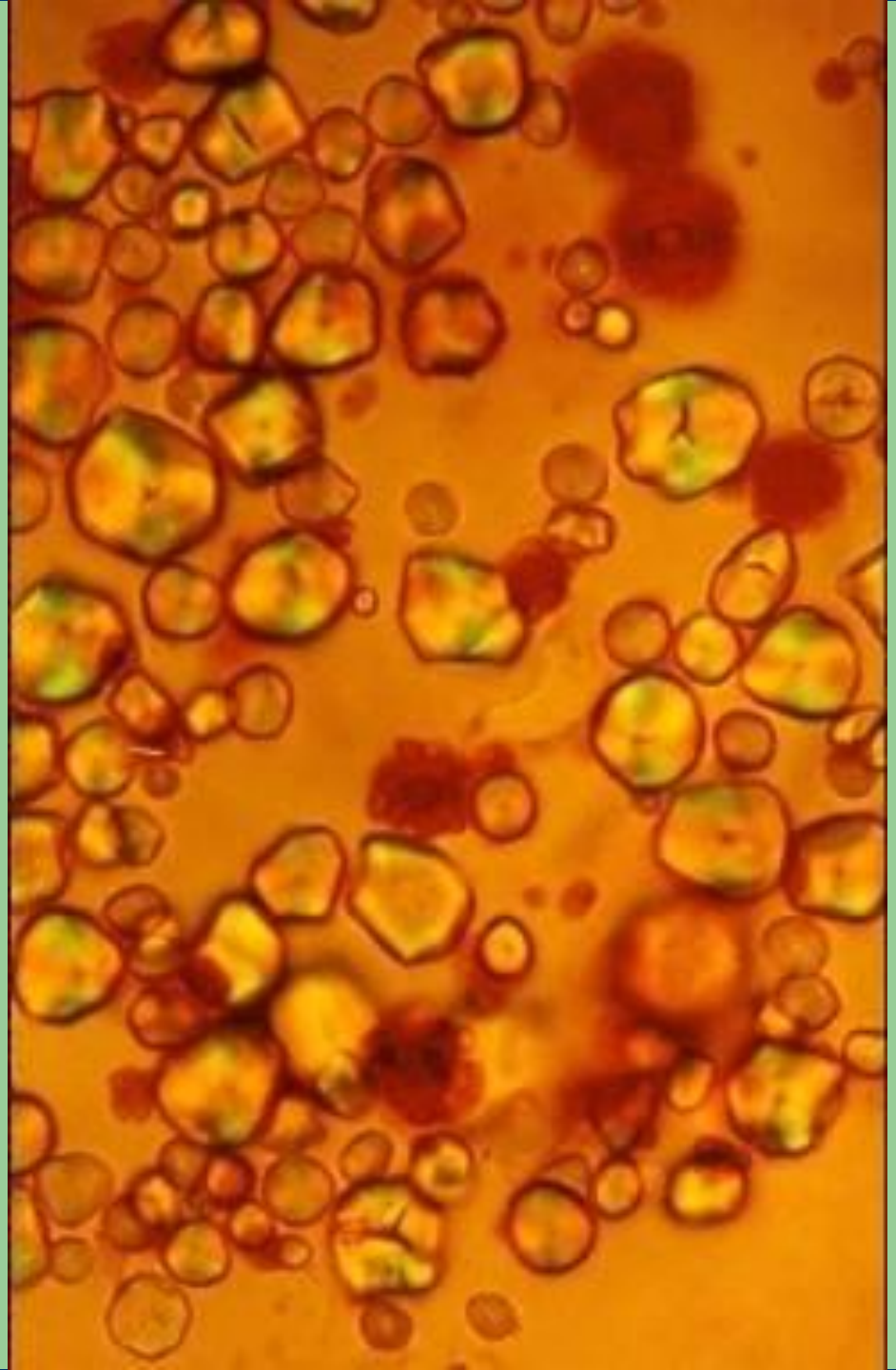


What do you see here?

These negatively birefringent lipid crystals can form in neutral lipid droplets in specimens left over night

Lipid crystals forming in neutral fat droplet

Corn starch from gloves



Green fragment from tube stopper found in synovial fluid. Nail polish used to seal coverslip can seep into specimen.

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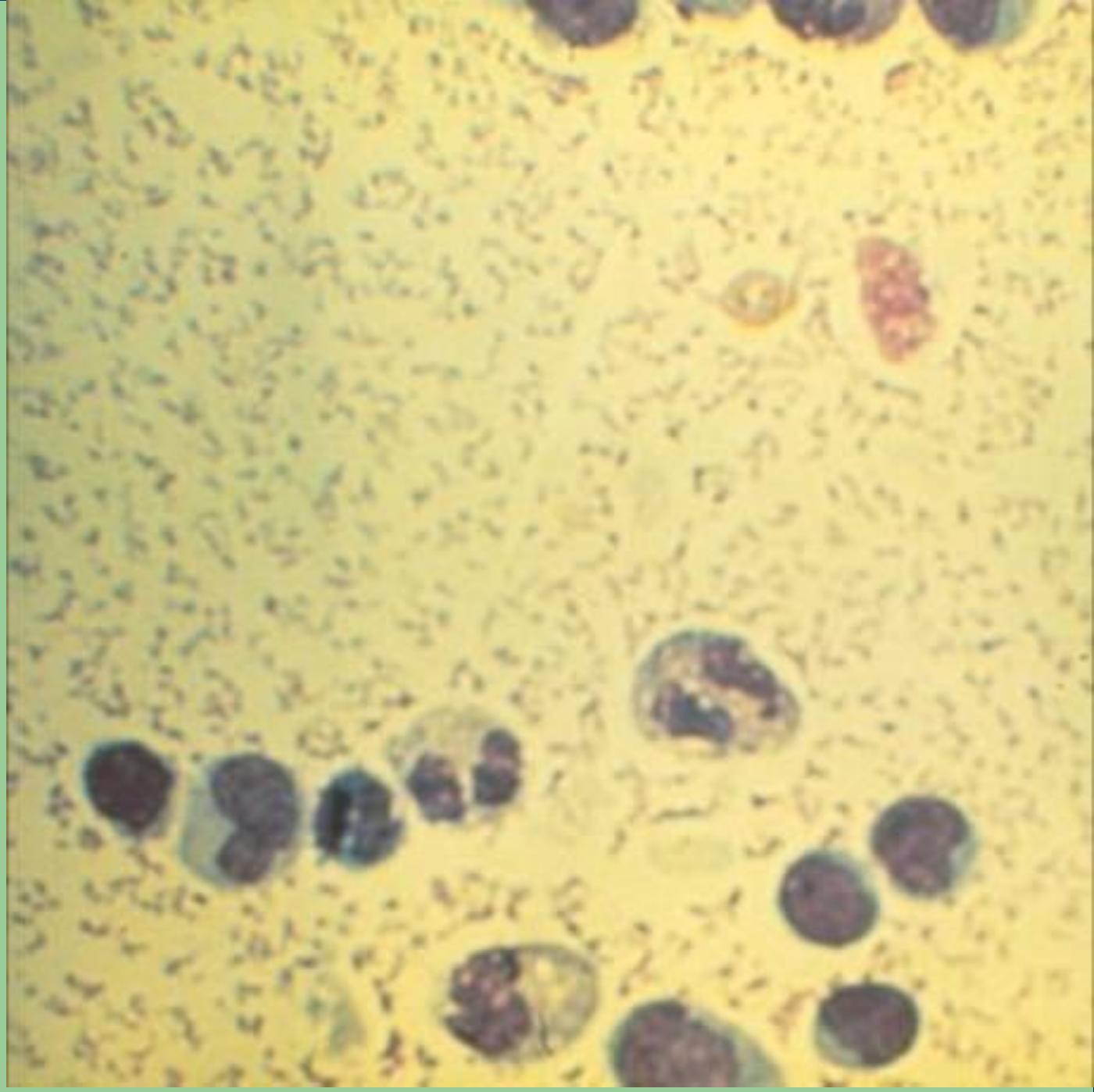
  - **Stained smears**
- Leukocyte count

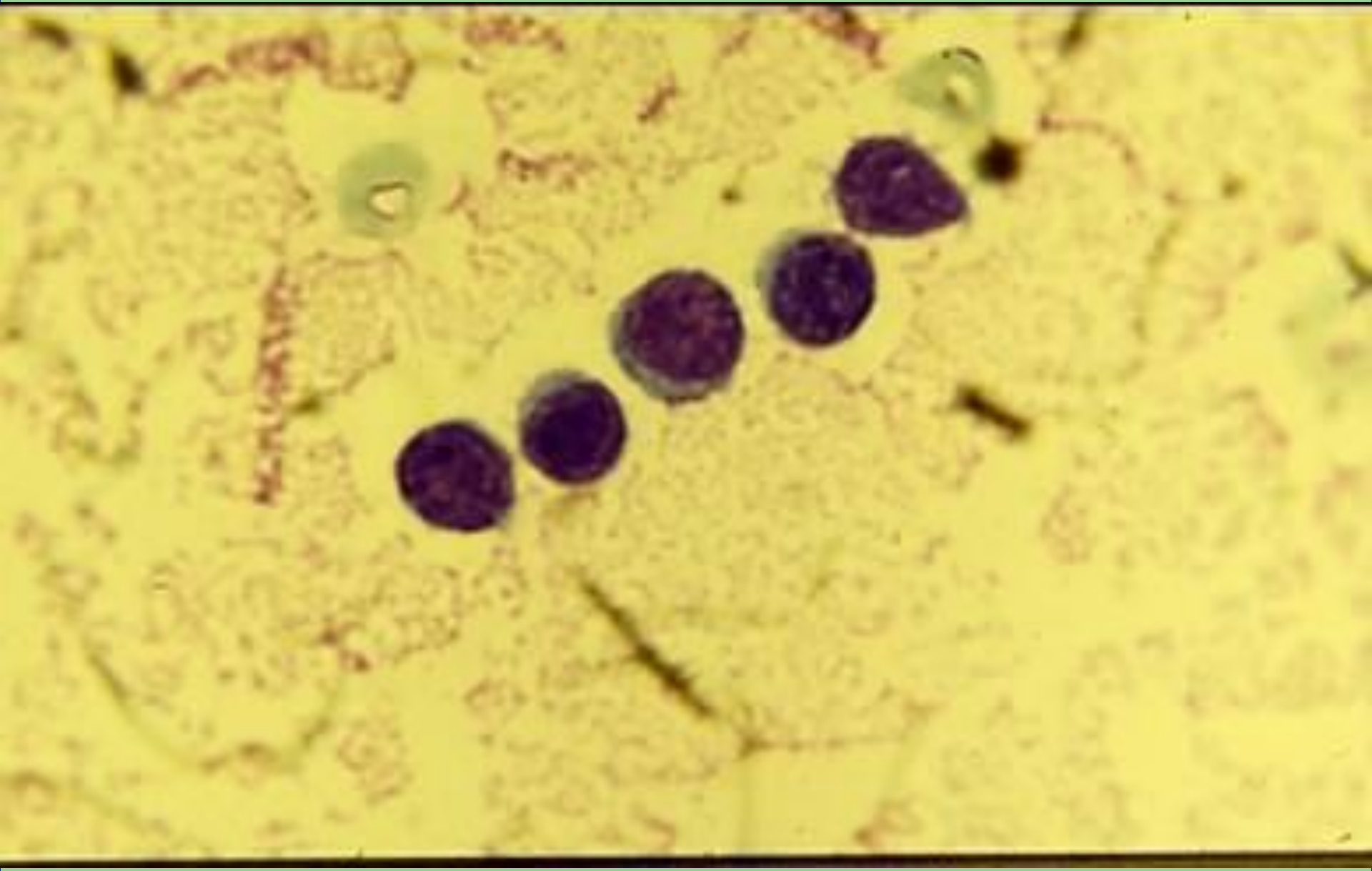
If leucocyte differential or gram stain may be needed make several thin smears for later staining

Cells seen in synovial fluid

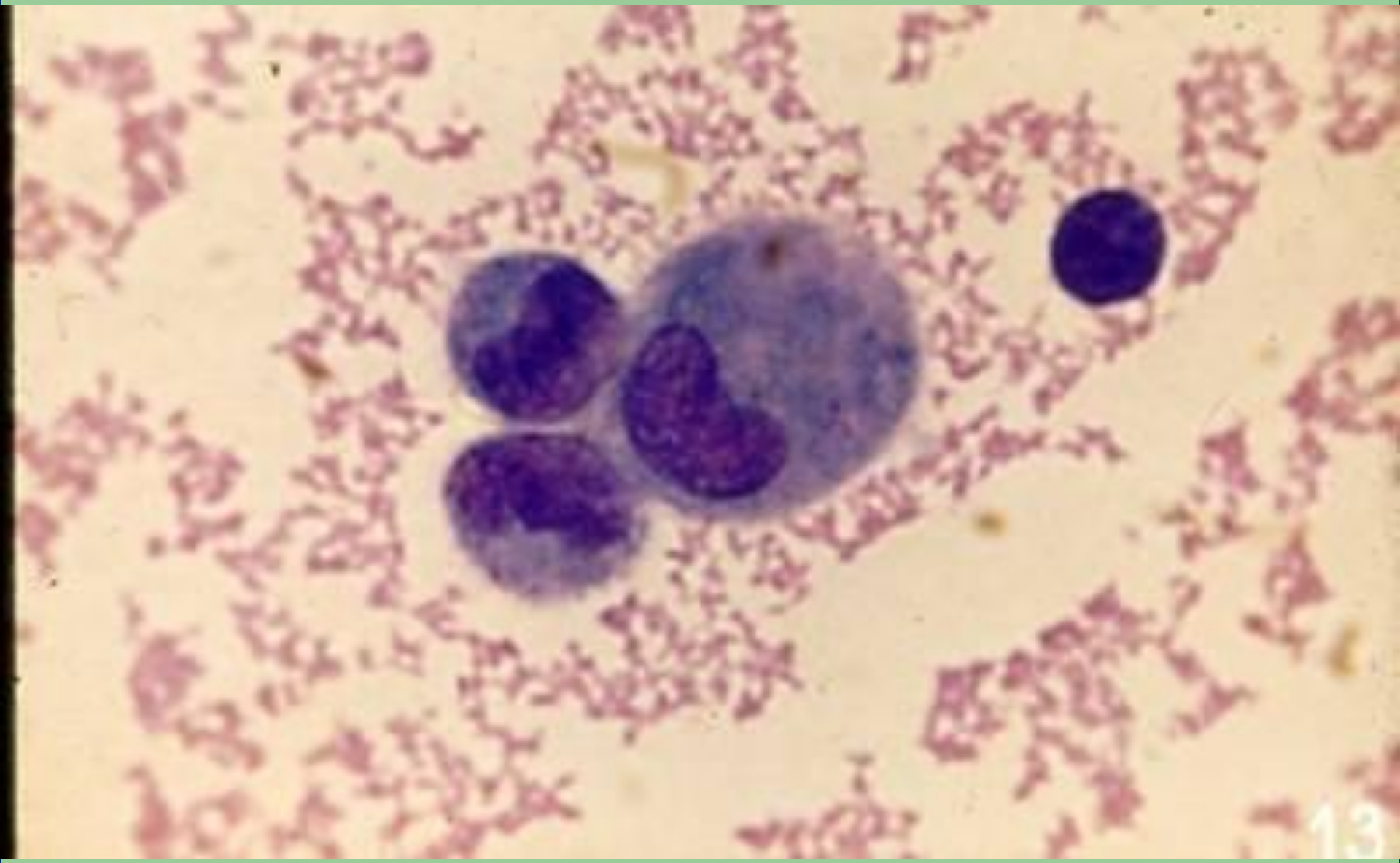
- PMN
- Small lymphocytes
- Activated lymphocytes
- Large granular lymphocytes
- Monocytes
- Large mononuclears
- Synovial lining cells (synthetic type)
- Eosinophils
- Plasma cells
- Mast cells
- Others

Wright stain of synovial fluid showing lymphocytes, monocytes and PMN as often seen in RA

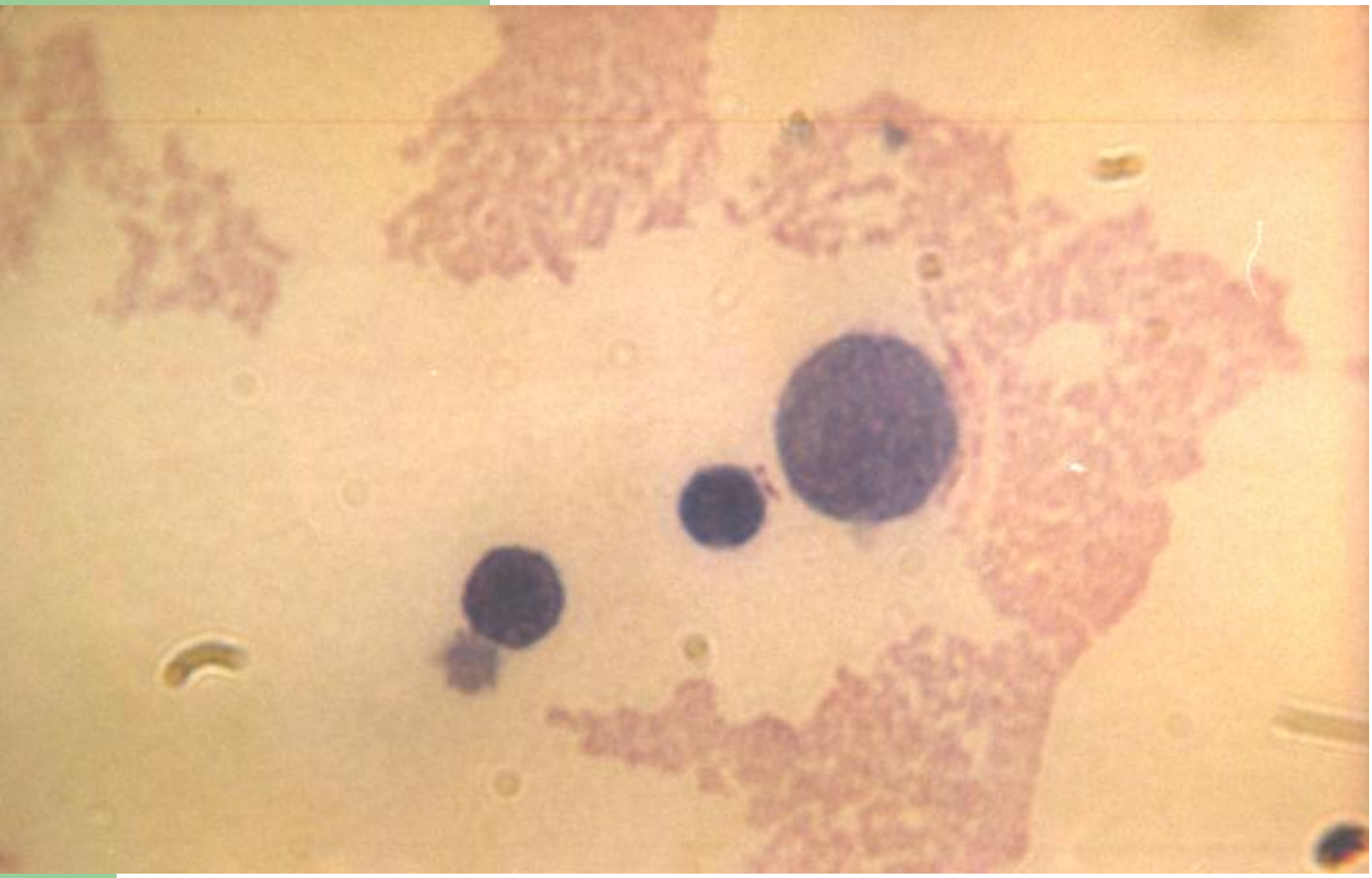




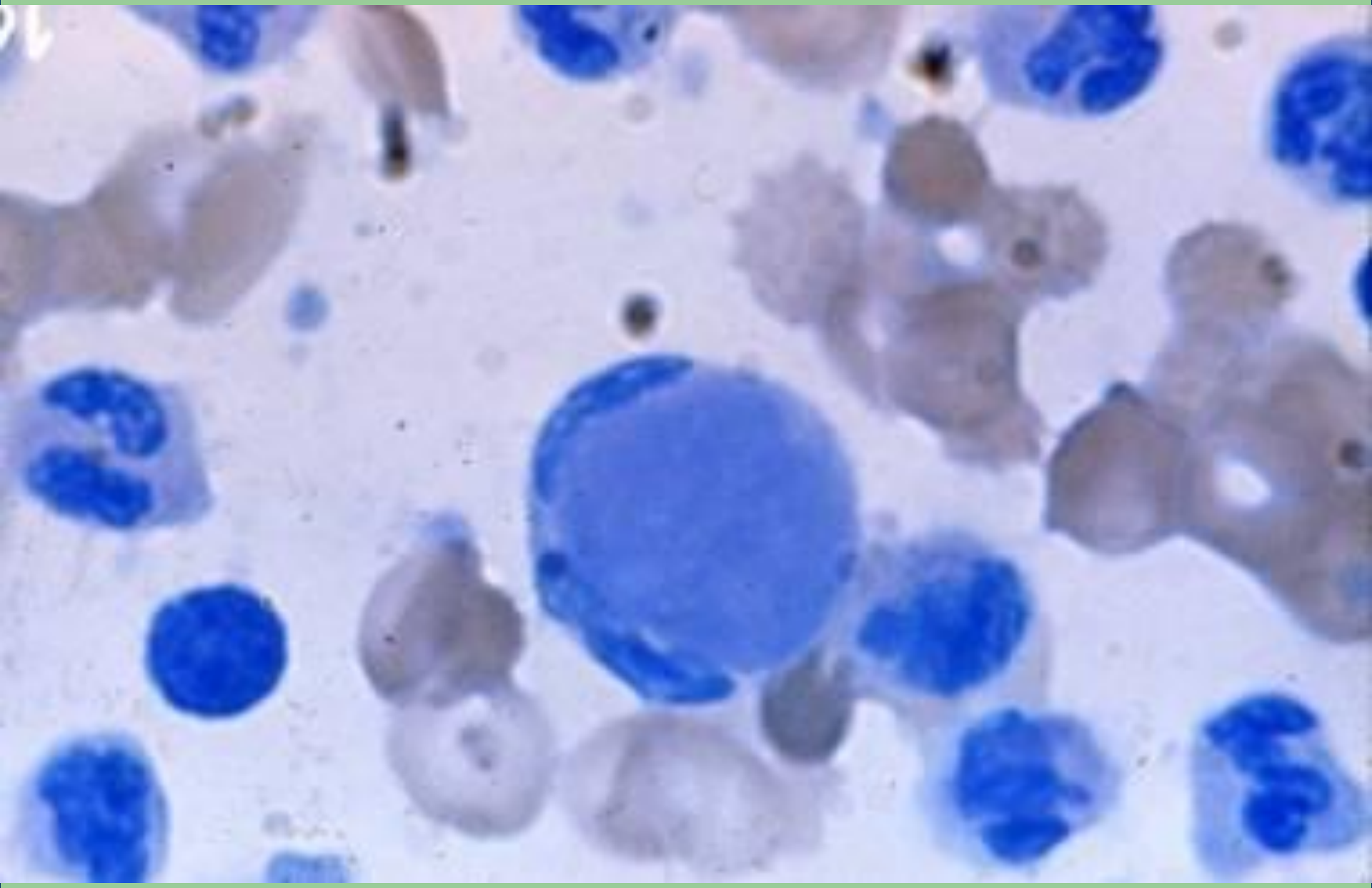
Occasional synovial fluids may have predominantly lymphocytes



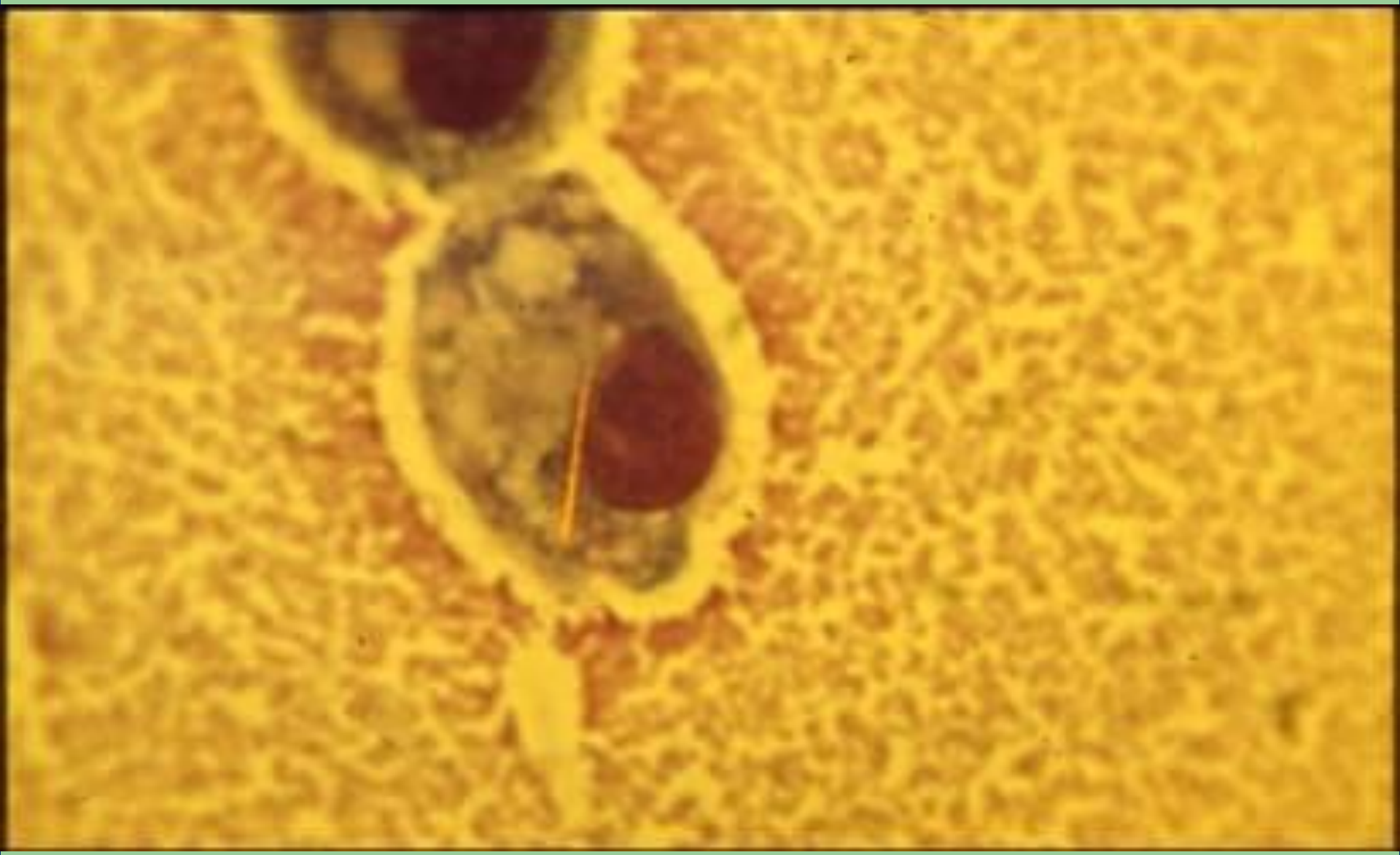
One lymphocyte, two monocytes and the large cell is a synovial lining cell



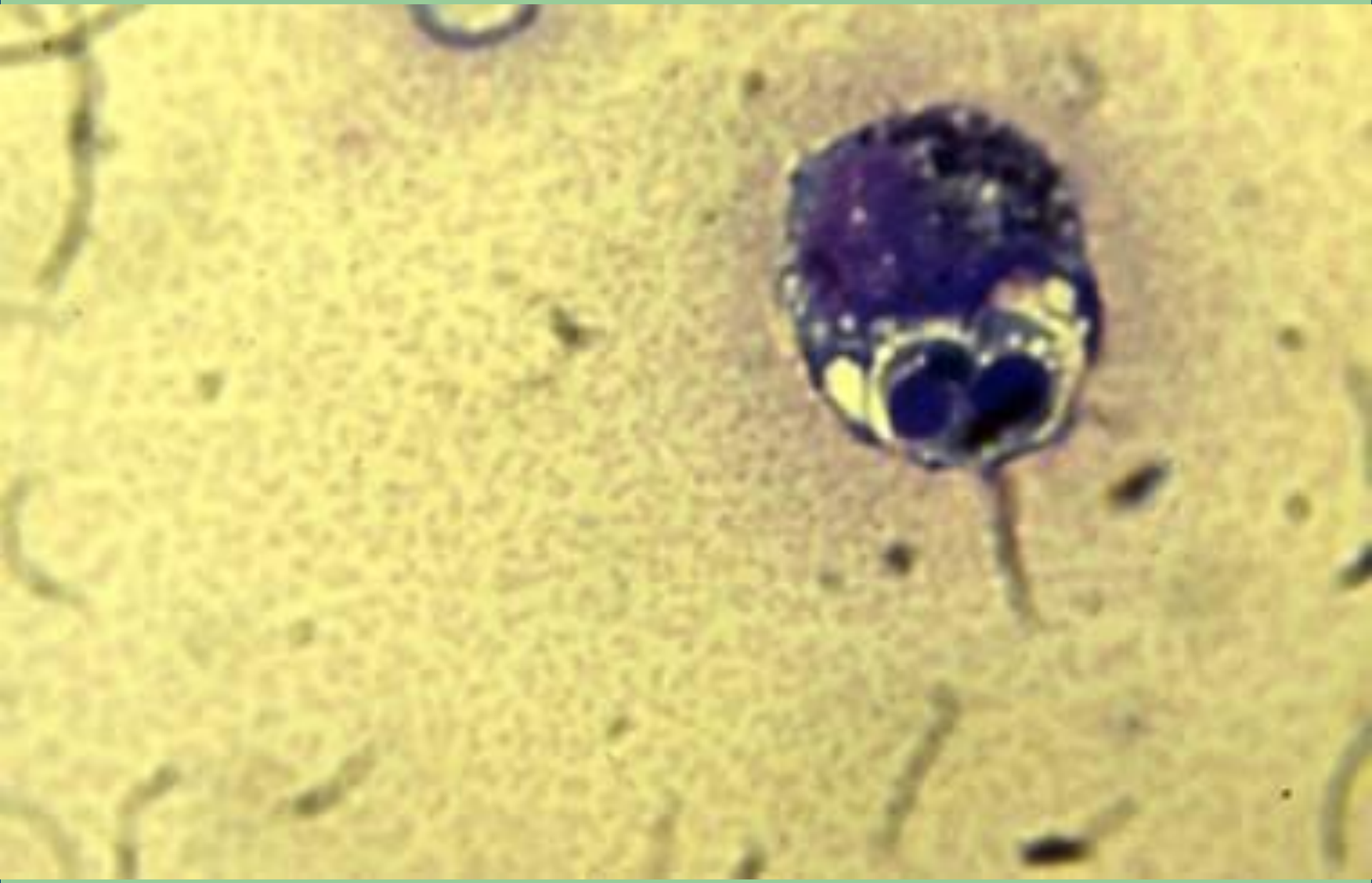
A large cell with a nucleus filling most of the cytoplasm is an activated lymphocyte as may be seen in RA or SLE



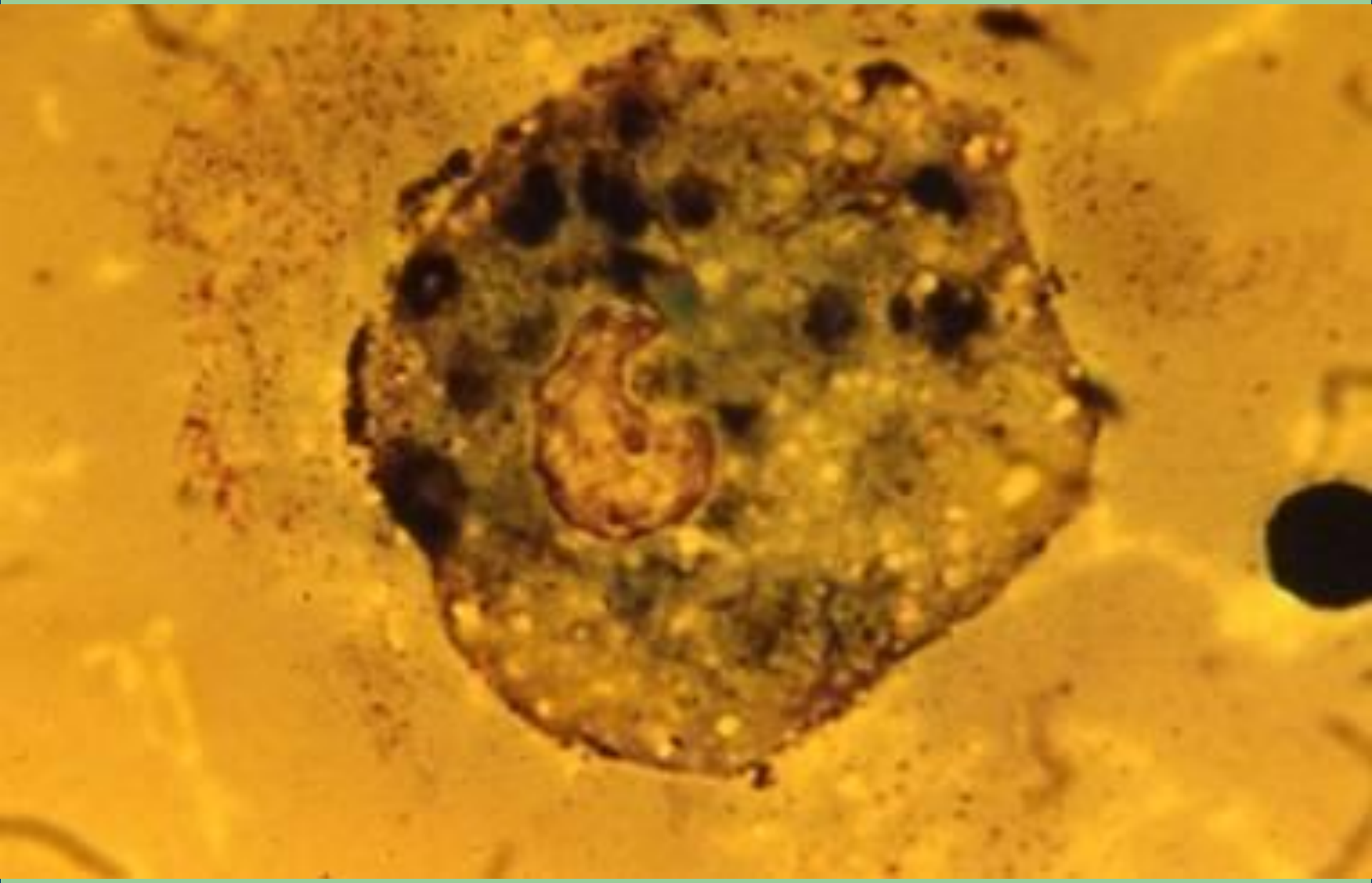
This large cell is an LE cell



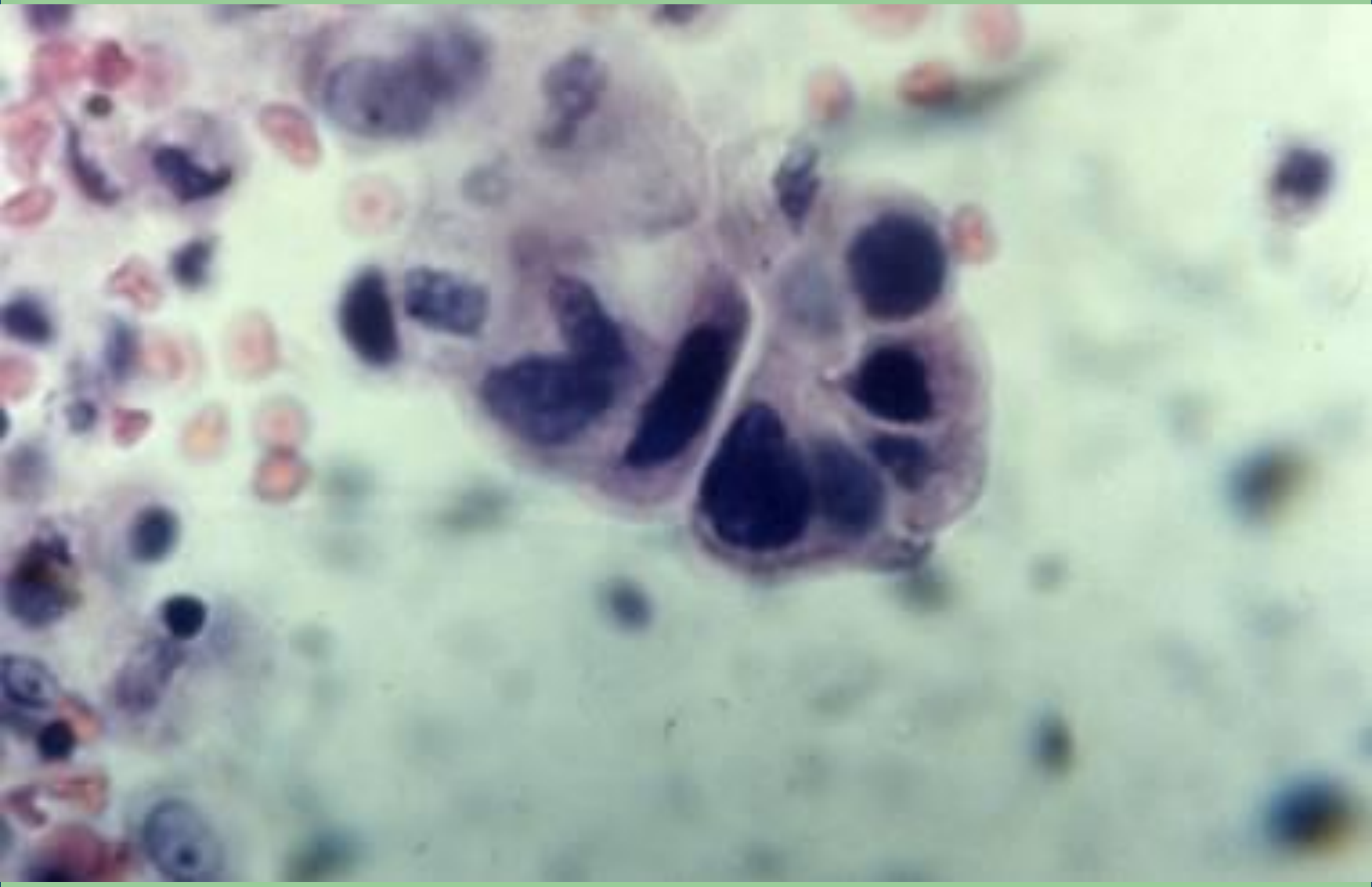
Synovial lining cell with phagocytized MSU crystal



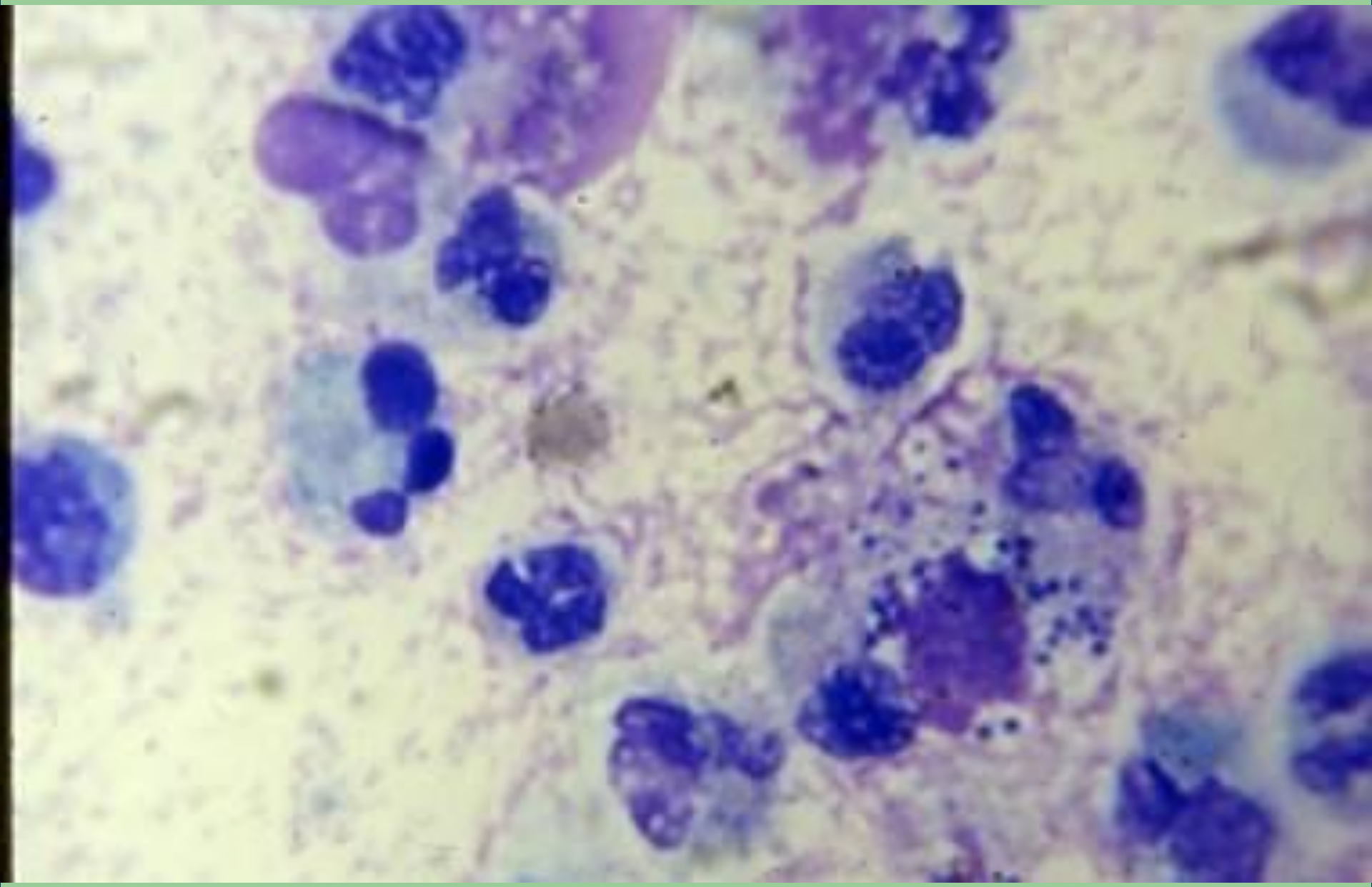
“Reiter cell” typical of spondyloarthritis



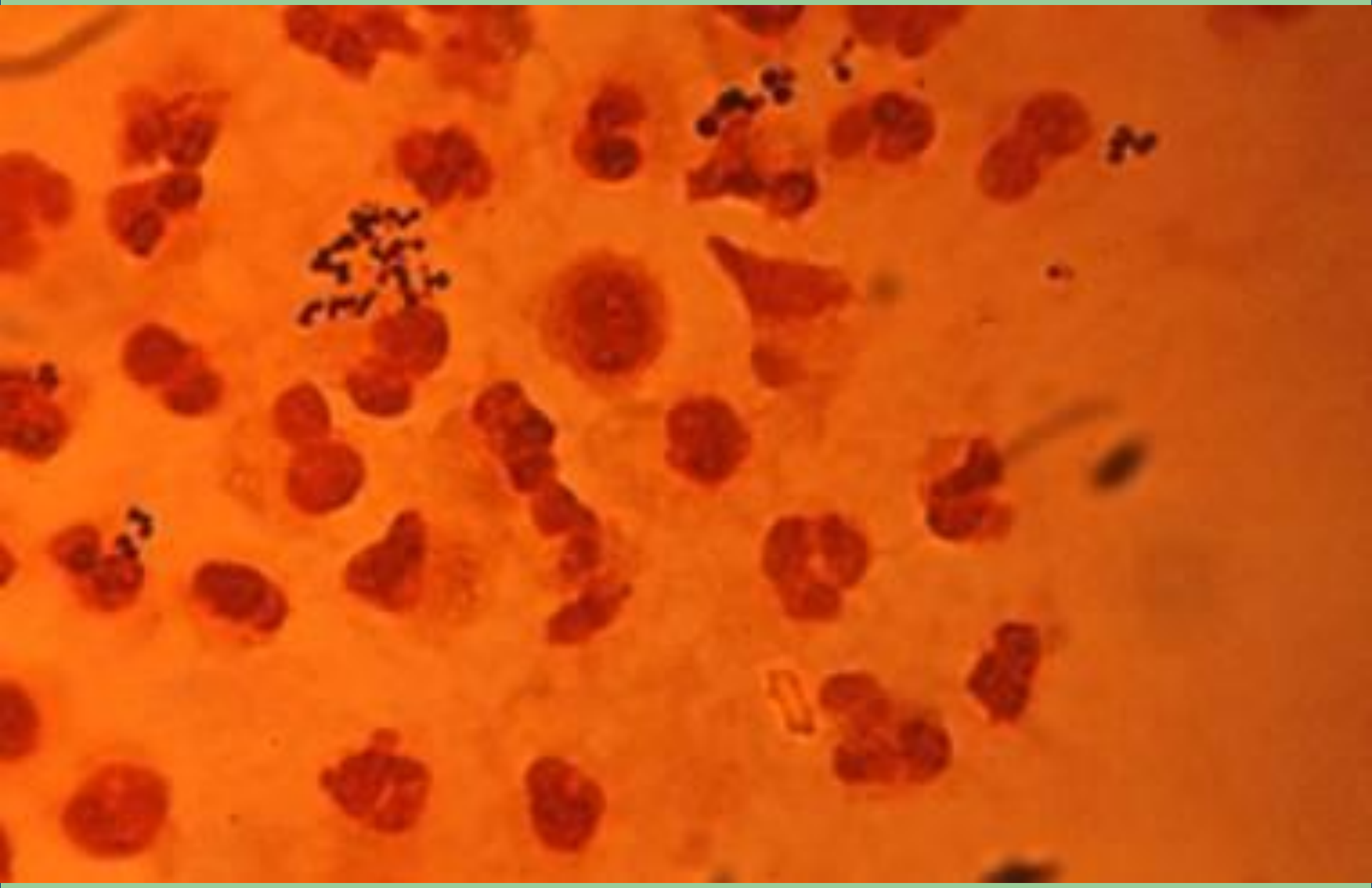
World champion “Reiter cell”



Metastatic adenocarcinoma cells



Bacteria can be suspected on Wright stain



Gram stain showing gram positive cocci

Synovial Fluid Workshop

- Introduction
- Gross examination
- Cultures
- Microscopic examination
  - Wet preparations
    - Regular light
    - Polarized light
  - Stained smears
- **Leukocyte count**

Leukocyte Counts on Joint Fluids

- Use heparin or EDTA tubes
- Leukocyte counts fall with time so test best done promptly
- Use 0.3N saline as diluent to lyse red blood cells.
- Automated counters may become clogged and may count material other than cells so should be avoided
- With clear fluids estimated counts can be made.

  0-2 WBC/HPF means that actual counts will virtually always be less than 2000/mm^3

Abnormally high leukocyte count reported on an automated counter

04/03/97 9:44

SSN: SEX: M AGE: 63 LOC: MICU

Provider :

Specimen: SYNOVIAL FLUID. SPHEM 0401 163

04/01/97 16:25

Test name	Result units Ref. range
FLUID COLOR	YELLOW/CLOUDY
FLUID WBC	257,950
FLUID RBC	400
FLUID SEG%	78
FLUID LYMPH%	2
FLUID OTHER%	BANDS-5, MACROPHAGE-12, SYNOVIAL LINING CELLS-

Comment: ALL BODY FLUID SLIDES ARE REVIEWED BY THE PATHOLOGIST

KEY: "L"=Abnormal low, "H"=Abnormal high, "*"=Critical value

SYNOVIAL FLUID ANALYSIS

Patient name ___ Location MICU

Patient number ___ Physician ___

Diagnosis ___ Attending ___

Indications met Y N Complications N Y

Spec. date ___ Date rec'd ___ Time in ___ Time out ___

Joint ___ Duration ___

Color ___ Clarity ___ Volume ___

WBC ___ Viscosity ___

RBC estimate ___

Differential

Polys

Lymphs

Transformed lymphs

Monocytes

Large mononuclears

Synovial lining cells

Other findings on stained smear

Crystals

MSU

CPPD

Other

"RA cells"

Other findings on wet prep

Alizarin red S

Cultures sent Y N Type

List any other test being done on fluid (chemistries, etc) & special handling for research

Interpretation:

Gout

Joint Fluid Characteristics

	Normal	Group I (Non-Inflammatory)	Group II (Inflammatory)	Group III (Septic)
Volume (knee, in rat)	<3.5	>3.5	>3.5	>3.5
Viscosity	Very high	High*	Low	Variable
Color	Colorless	Straw	Straw to opalescent	Variable with organism
Clarity	Transparent	Transparent	Translucent, opaque at times	Opaque
WBC/mm^3	200	300-2000^t	2000 - 100,000	> 50,000^{tt} usually > 100,000
%PMN	< 25	< 25	> 50 often	> 75^{tt}
Culture	negative	negative	negative	usually positive

*Rapid accumulation of fluid will lower viscosity

^{t}2000 is an approximation. Usually less than 500

^{tt} may be lower with partially treated or low-virulance organisms

Sequential changes in synovial fluid leukocyte counts over a 6 hour time period at room temperature

Synovial fluids	Immediate exam	1 hour	2 hour	3 hour	6 hour
Borderline inflammation #1	4,700	4,200	3,750	3,550	1,850
#2	6,200	6,000	4,800	3,500	1,950
#3	4,850	3,660	2,450	2,200	1,800
#4	3,150	2,250	1,950	1,600	1,300
Marked inflammation #1	17,550	16,400	13,350	12,800	10,500
#2	45,000	42,880	38,650	35,600	30,440
#3	16,600	15,550	12,600	8,700	7,950

Borderline inflammatory SFs >2,000 WBC/mm^3 had decreased into a non-inflammatory range <2,000 WBC/mm^3 after 6 hours

Table 1 Comparison of Leukocyte Estimates per hpf with Counts per mm^3

Estimated WBC/hpf	ACTUAL WBC (mm^3) 0-1000	1050-2000	2050-10,000	10,050-20,000	20,000-50,000	>50,000
0-2	25	1	---	---	---	---
3-4	4	2	3	---	---	---
5-10	2	11	3	---	---	---
10-25	---	4	8	12	2	---
26-50	—	---	2	4	10	1
>50	---	---	---	---	2	4

CAUSES OF NON-INFLAMMATORY JOINT FLUIDS

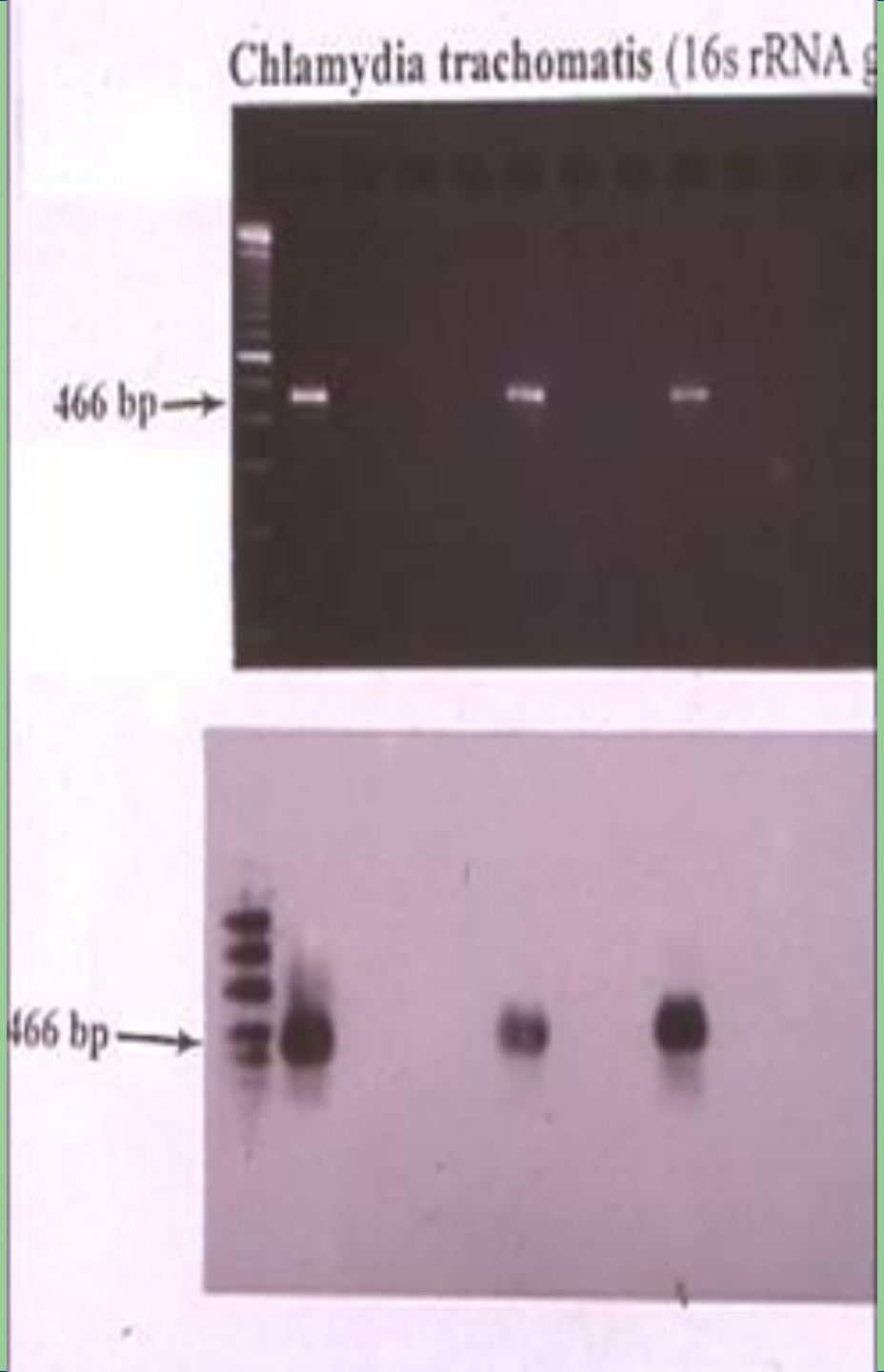
Osteoarthritis
Traumatic arthritis
Acromegaly
Gaucher's disease
Hemochromatosis
Hyperparathyroidism
Ochronosis
Paget's disease
Jaccoud's arthritis
Hemarthrosis, hemophilia
Mechanicaal derangement
Fractures
Osteochondritis dessecans
Epiphyseal dysplasias

Primary tumors
Metatastic tumors
Pigmented villonodular synovitis
Aseptic necrosis
Ehlers-Danlos syndrome
Sickle cell disease
Amyloidosis
Hypertrophic pulmonary osteoarthropathy
Pancreatitis
Charcot joints
Wilson's disease

Other Tests are Rarely Useful

- Rheumatoid factor is not needed and can mislead
- Cytokines, cell surface markers, enzymes, etc. are still mostly for research
- PCR may be an important test in the near future for difficult to identify infections
- Consider synovial biopsies if synovial fluid is not diagnostic. Decide if your question can be better answered by examining tissue.

PCR demonstration of chlamydial nucleic acid in reactive arthritis synovial fluid

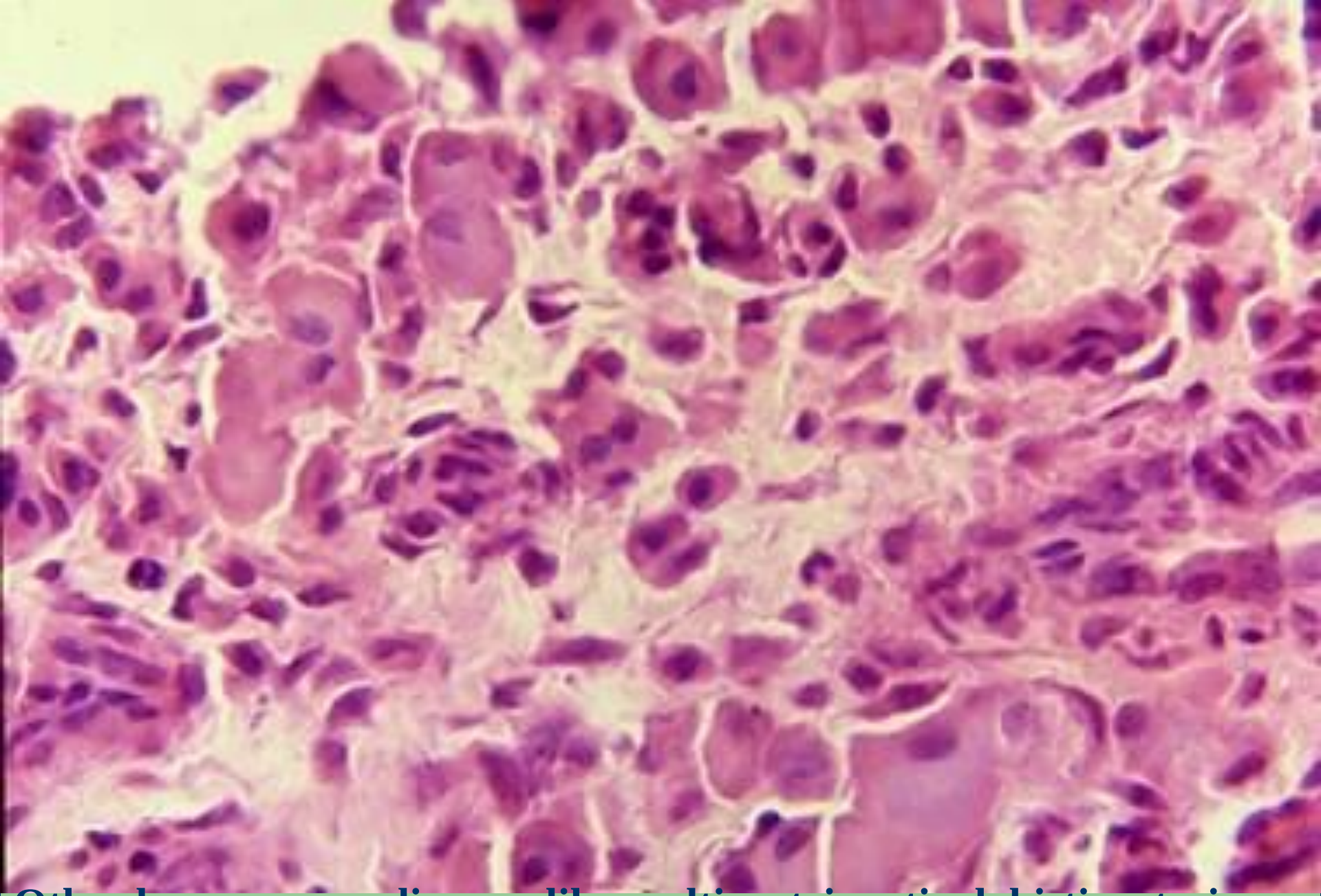


Chlamydia Identification by PCR is More Often Positive in Synovium than Synovial Fluid

- **Total of Patients: 37**

• (+) Synovium	24	(64.8%)
• (+) Synovial fluid	13	(35.1%)
• (+) On both	14	(37.9%)
• (–) On both	11	(29.7%)
• (+) Syn (–) Sf	10	(27.0%)
• (–) Sf (+) Syn	2	(5.4%)

TB granuloma detected in synovium despite negative synovial fluid culture



Other less common diseases like multicentric reticulohistiocytosis may also be detected by synovial biopsy


BD 10ml Syringe
APLICARE
ONE
POVIDONE-IODINE
SWABSTICK
20ml Luer-Lok Tip

Conclusions

- Examining synovial fluid may be the only way to determine the process involving a given joint
- Gross appearance and wet drop examination are most helpful
- Your examination is important and worth documenting on a SF report form


SYNOVIAL FLUID ANALYSIS

Patient name
Patient number
Diagnosis
Location MICU
Physician
Attending
Indications met Y N Complications Y N
Spec. date Date rec'd Time in Time out
Reason if specimen is unsatisfactory
Joint Duration
Color Yellow Clarity Clear Volume
WBC Viscosity poor
RBC estimate

Differential
Polys 71
Lymphs
Transformed lymphs
Monocytes 14
Large mononuclears
Synovial lining cells 3
Other findings on stained smear 1 Reiter

Crystals
MSU whole 0 Pellet 0
CPPD 2 2
Other

"RA cells"
Other findings on wet prep
Alizarin red S neg
Cultures sent Y N Type
List any other test being done on fluid (chemistries, etc) & special handling for research

Interpretation
Gout + CPPD crystals. mild inflammation

UR Sellwall
Faculty signature
Person performing analysis