



VON KOSSA

UNDERSTANDING HISTOLOGY FOR BETTER ANALYSIS

WONG XUE YI

LEARNING OBJECTIVES



- Describe the **silver substitution and photochemical reduction** principle
- Identify **pathological calcification** histologically
- Understand **clinical applications** of Von Kossa stain
- Recognise **limitations and sources of false positivity**

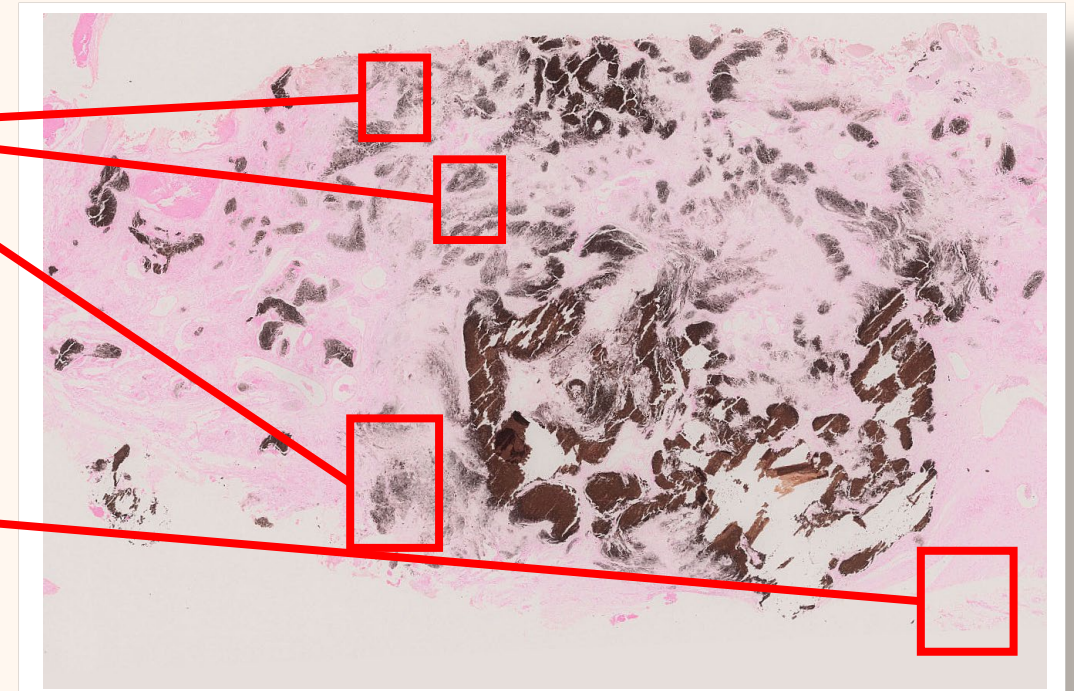
Von Kossa

To illustrate the mineral deposits (calcium) in tissues

- Used in identifying abnormal calcifications (in kidneys, vessels or tumour) and mineralization

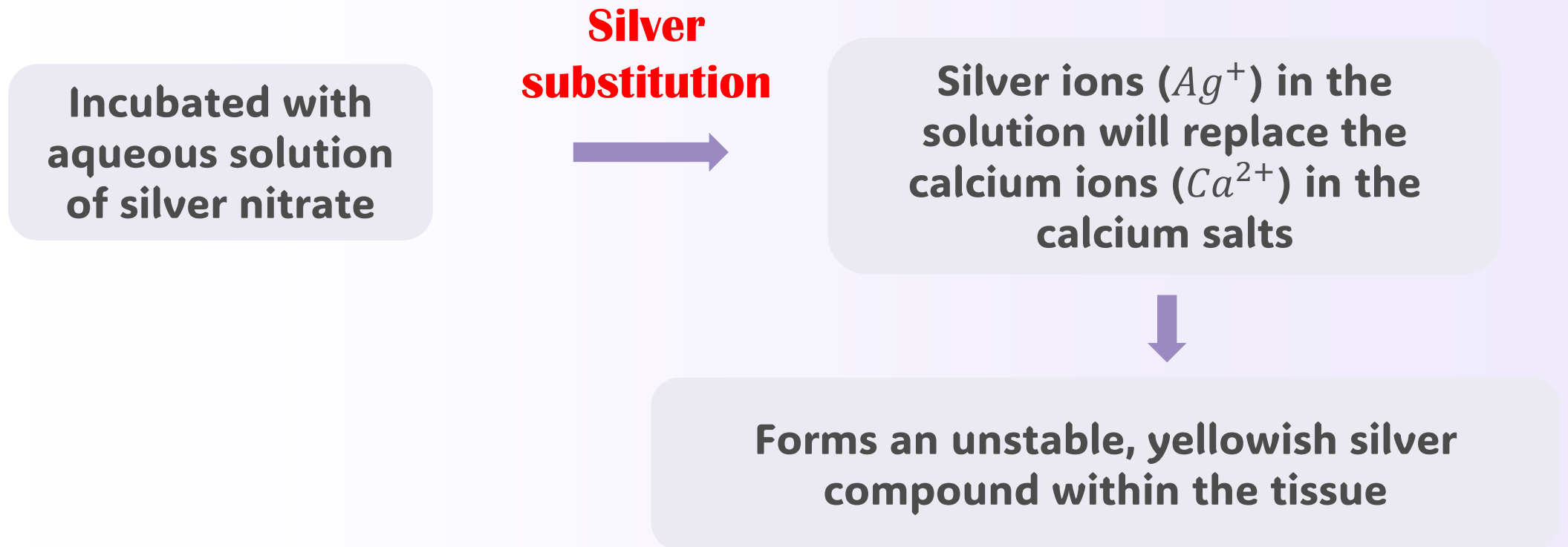
Calcium deposits
Black

Cytoplasm
Pink



Staining Principle

- Formalin fixed tissue, cut at 4 μm
- Two-step process: **silver substitution** and **photochemical reduction**



Exposed the tissue section to strong light
source (1000 W, 1 hour)

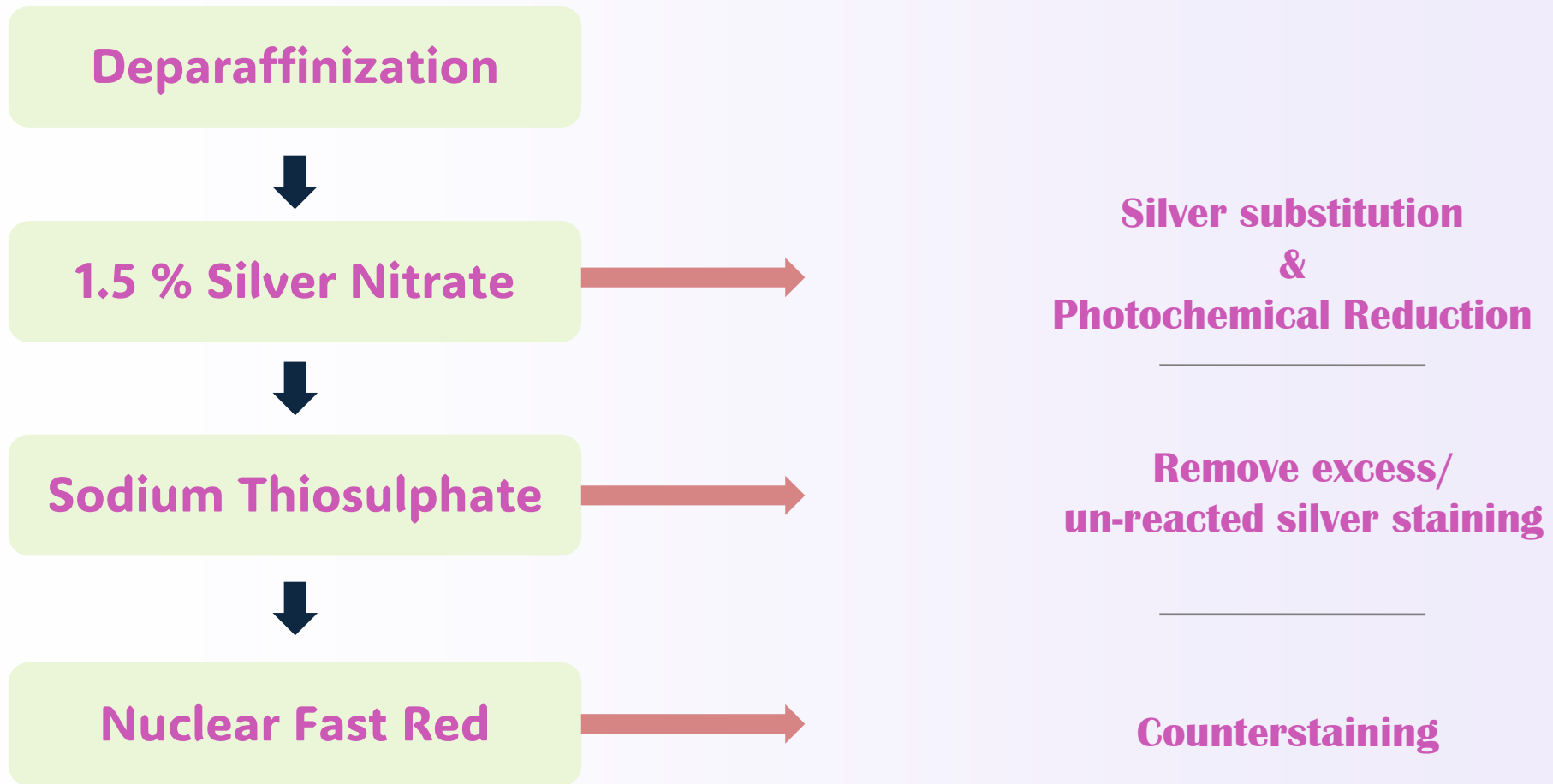
**Yellow silver
compound within
the tissue**



**Photochemical
Reduction**

**Deposited silver ions reduced
to black, metallic silver**

Procedure



Troubleshooting

Photochemical reaction



X lamp

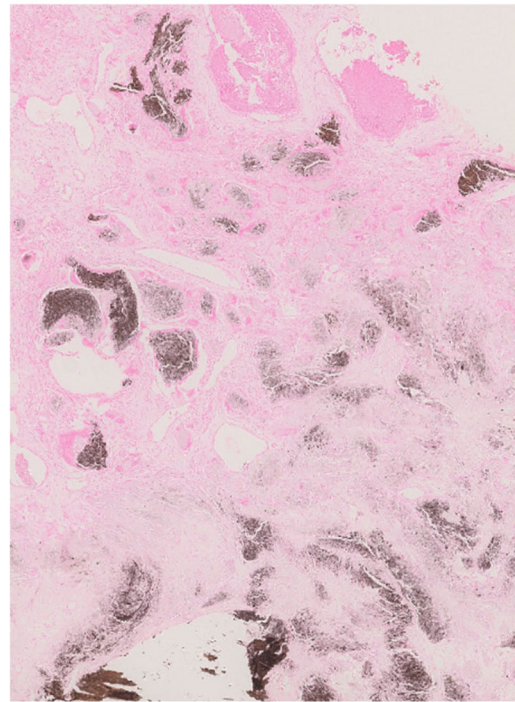
30 mins

Control (1 hr)

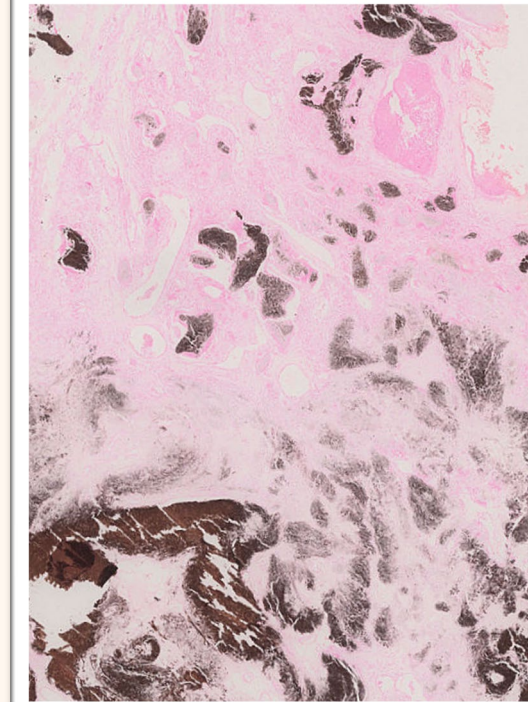
3 hours



X reaction



**pale staining
X contrast**

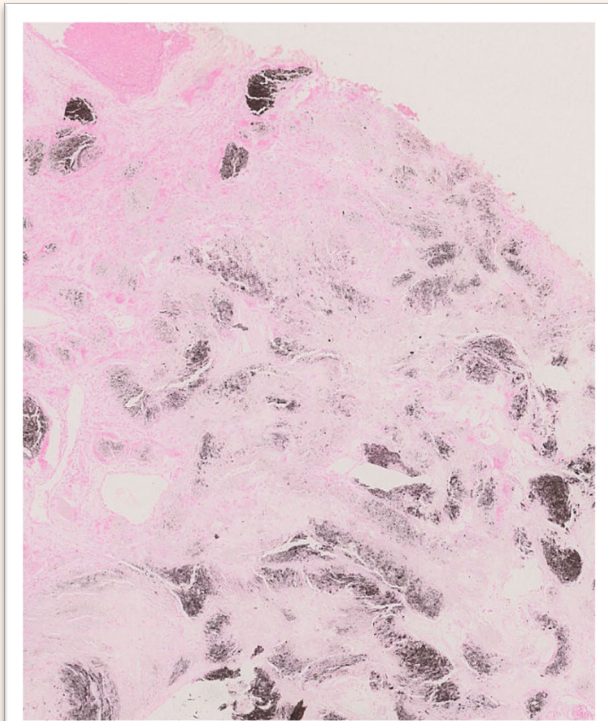


**↑ Incubation time,
may lead to false positive**

Troubleshooting

Concentration of Silver Nitrate

Con. Of Silver Nitrate (0.5%)

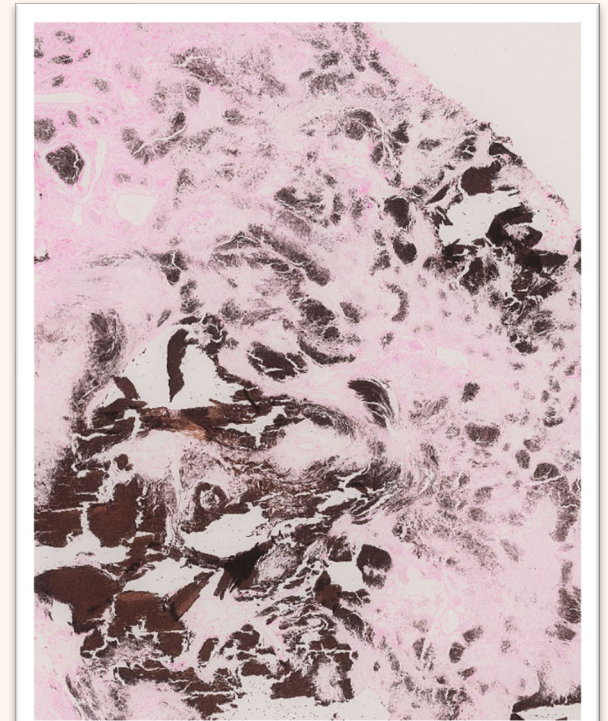


Few Ag^+ to react

Control (1.5%)



Con. Of Silver Nitrate (10.0%)



**Too much Ag^+ to react,
may lead to false positive**

Troubleshooting

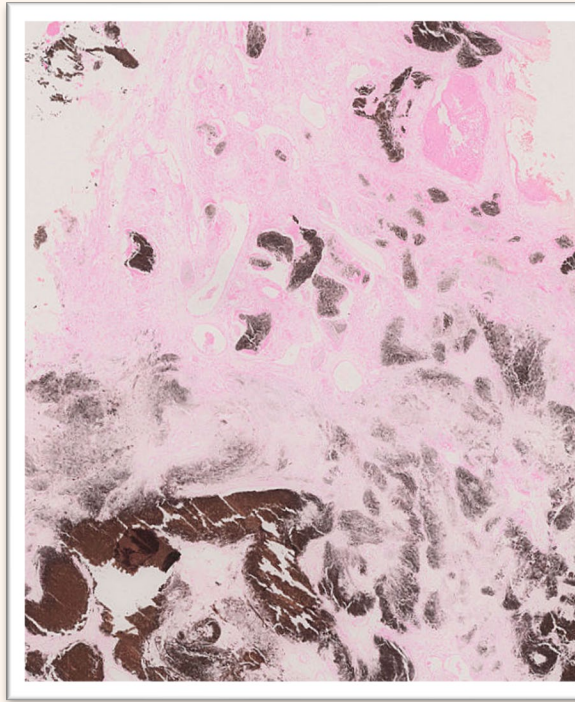
Steps

X nuclear fast red



x background staining

Control



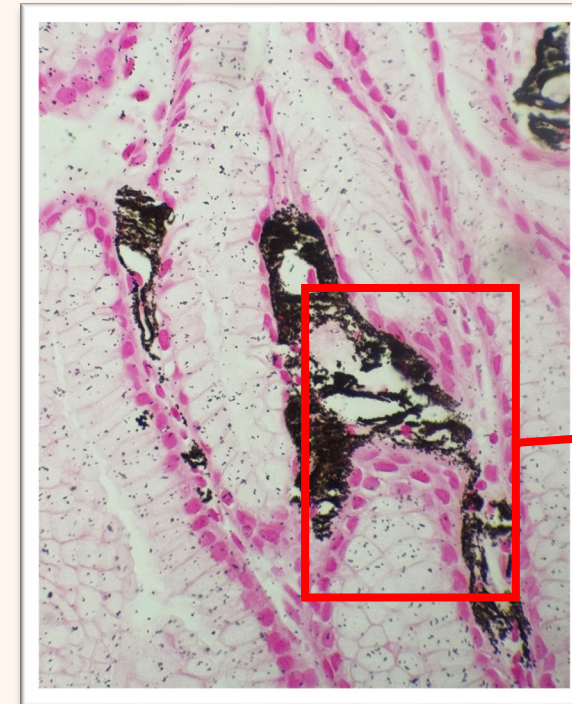
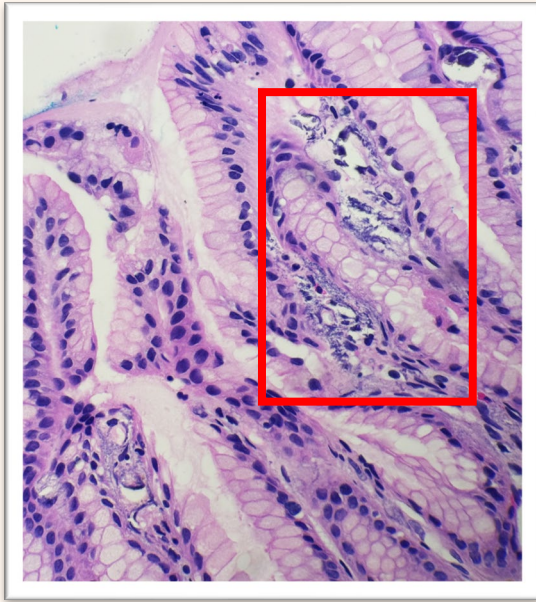
X sodium thiosulphate



**Nonspecific staining,
dirty background**

Case Study

Eg: Patient (male, 69 years old) reported with of mucosal calcinosis in stomach cardiac biopsy.
(positive to von Kossa staining)



Calcium deposits
Black

➤ Mucosal calcinosis:

Rare pathological condition characterized by the deposition of calcium salts in the mucosal tissues, often associated with various underlying conditions:

- Hypercalcemia
- Chronic kidney disease

Case Study

➤ Abnormal Blood test results:

- Serum urea: 20.3 mmol/L
- Serum creatinine: 836 umol/L

➤ Findings:

- Patient with diabetic nephropathy, hypertension (HTN) and hyperlipidemia (HLD)
- Metabolic disturbance –Calcium enters the cells through passive diffusion, and accumulation occurs when the cell's ability to extrude calcium is overwhelmed

Considerations

1. Specificity

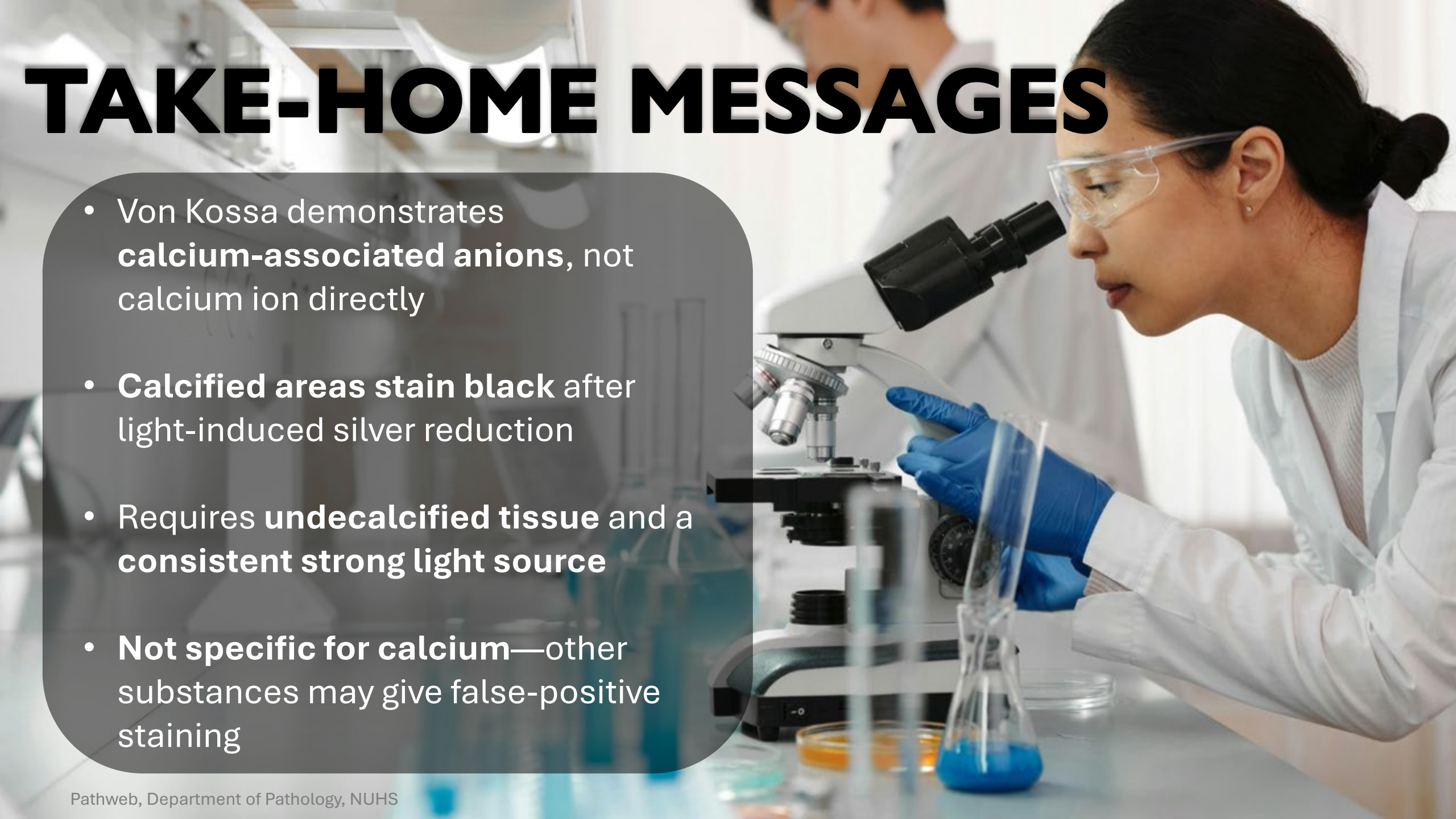
- Not specific for the calcium ion but also demonstrate the presence of associated anions like phosphate and carbonate; and other substances (urate and iron)
- Leading to potential false positives

2. Decalcification

- Required undecalcified sections
- Strong acid decalcifiers will remove the calcium deposits

3. Light source consistency

TAKE-HOME MESSAGES



- Von Kossa demonstrates **calcium-associated anions**, not calcium ion directly
- **Calcified areas stain black** after light-induced silver reduction
- Requires **undecalcified tissue** and a **consistent strong light source**
- **Not specific for calcium**—other substances may give false-positive staining