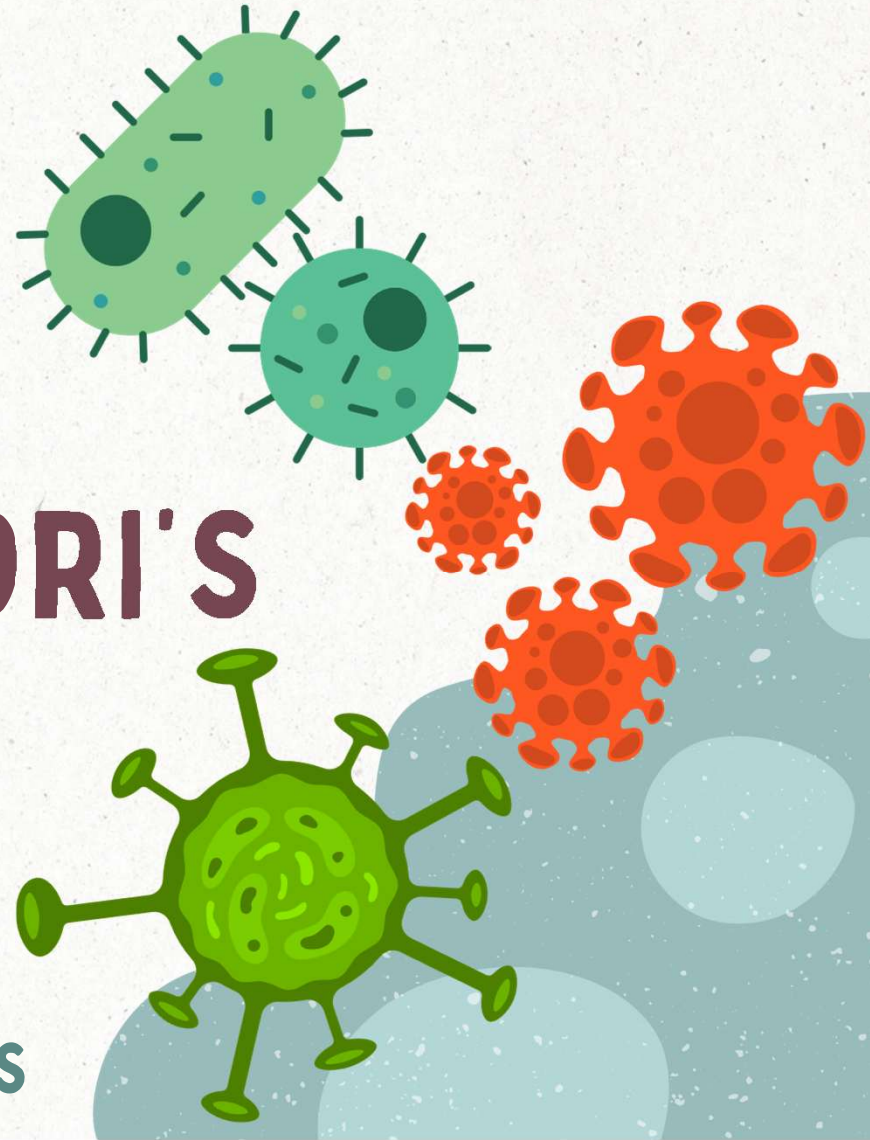


GROCOTT-GOMORI'S METHENAMINE SILVER (GMS)

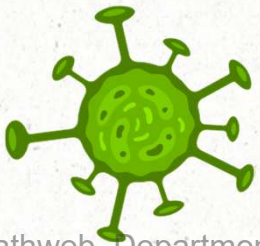
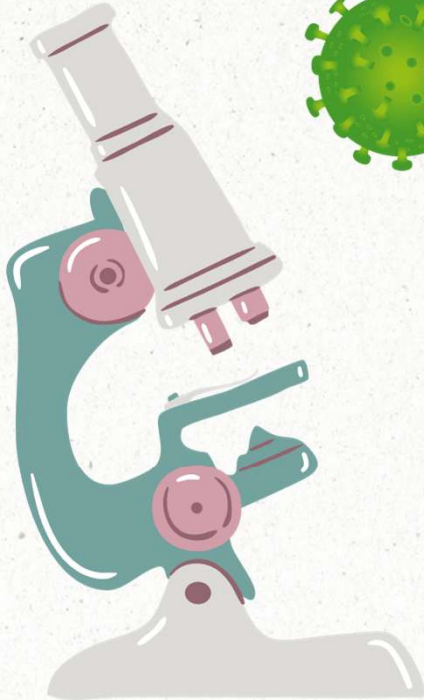
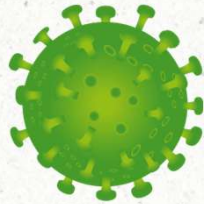
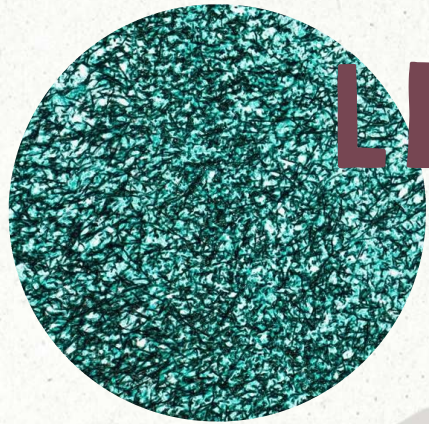
JIA QIAN, MAGHA, AMOS

Pathweb, Department of Pathology, NUHS



LEARNING OBJECTIVES

- Understand silver impregnation for fungal detection
- Identify fungi against background tissue
- Appreciate the importance of oxidation, toning, and washing steps



GMS

Used for the identification of carbohydrates in fungal microorganisms.

Fungal cell walls → **black**

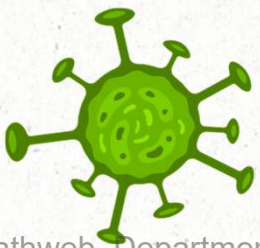
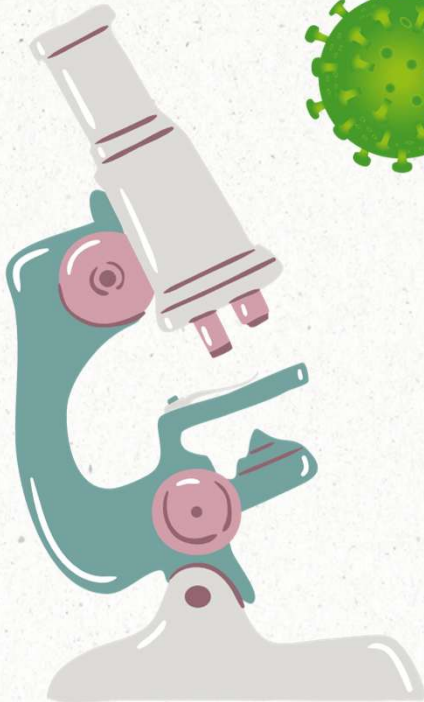
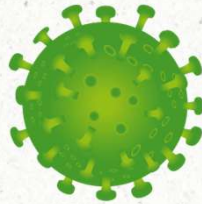
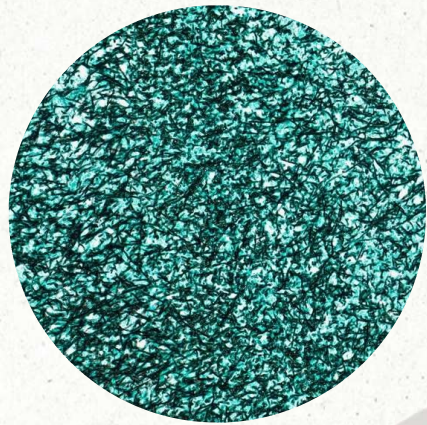
Background tissue → **green or light green**

The stain works based on oxidation and silver reduction:

Fungal cell wall polysaccharides are oxidised to aldehydes, which reduce silver ions to form black deposits.

Common tissue stain with GMS:

Lung, skin, lymph node, gastrointestinal tissue





GMS VS ZIEHL–NEELENSEN (ZN) STAIN



GMS stain is often paired with Ziehl-Neelsen (ZN)

ZN	GMS
Acid-Fast Bacteria (e.g. <i>Mycobacterium</i> <i>Tuberculosis</i>)	Fungi
Bacterial infection	Fungal infection

This is especially useful in lung infections, where symptoms can overlap. Using both stains helps ensure accurate diagnosis and appropriate treatment.

*ACID-WASH ALL GLASSWARE

Don nitrile gloves

→ 3 changes of 5% HCl acid wash

→ 4 changes of DI water rinse

→ Dispose of washing liquids in HCl waste container





PROCEDURES



Dewax GMS
control side

Oxidise section in 10%
Chromic acid (10min)

Bleach in 1% Sodium
Metabisulphite (1min)

Place slides in
pre-heated
**Working
Methenamine
Solution** (5-
7min)



After dewaxing, the section is oxidised using 10% chromic acid to generate aldehydes. This is followed by bleaching to remove excess oxidising agent. Finally, the section is treated with methenamine silver, where fungal elements are stained black.



PROCEDURES



Tone in 0.2%
Gold Chloride ->
sections turn
grey

5% Sodium Thiosulphate
(5min)

Counterstain in 2% light
green (30-40s)

Dehydrate, clear
and mount



After silver staining, 0.2% gold chloride is used to tone the section, improving contrast.

5% sodium thiosulphate removes excess silver to prevent non-specific staining.

A 2% light green counterstain is then applied to provide contrast, followed by dehydration, clear and mounting.

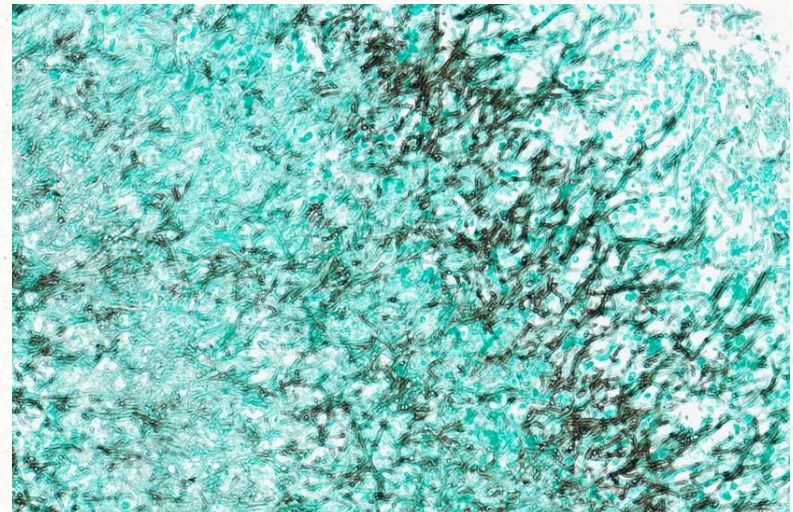
TROUBLESHOOTING: EFFECT OF 10% CHROMIC ACID

With 10% Chromic Acid



- Proper oxidation
- Clear black fungal structures
- Good contrast

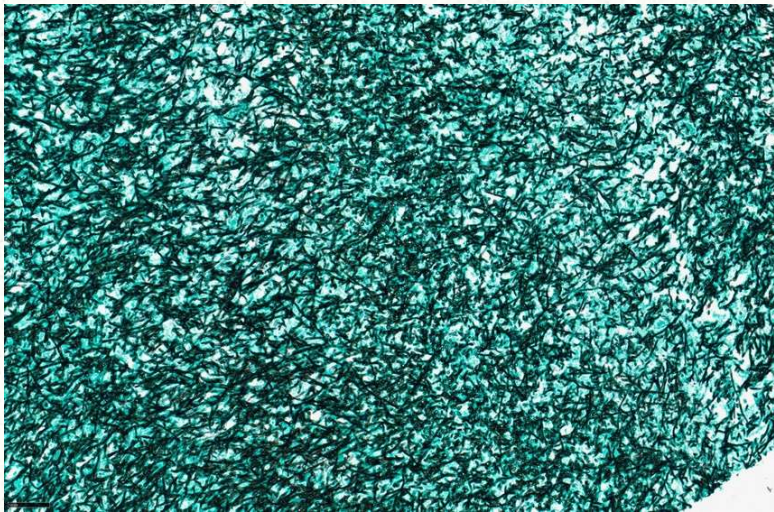
Without 10% Chromic Acid



- Inadequate oxidation
- Weak or absent fungal staining
- Increased background staining

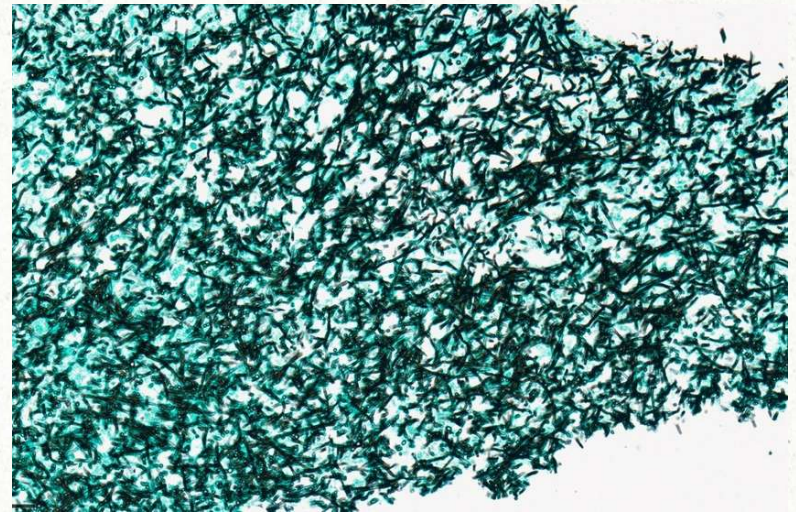
TROUBLESHOOTING: EFFECT OF BLEACH IN 1% SODIUM METABISULPHITE

With 1% Sodium Metabisulphite



- Removes excess chromic acid
- Prevents over-oxidation
- Reduces background staining

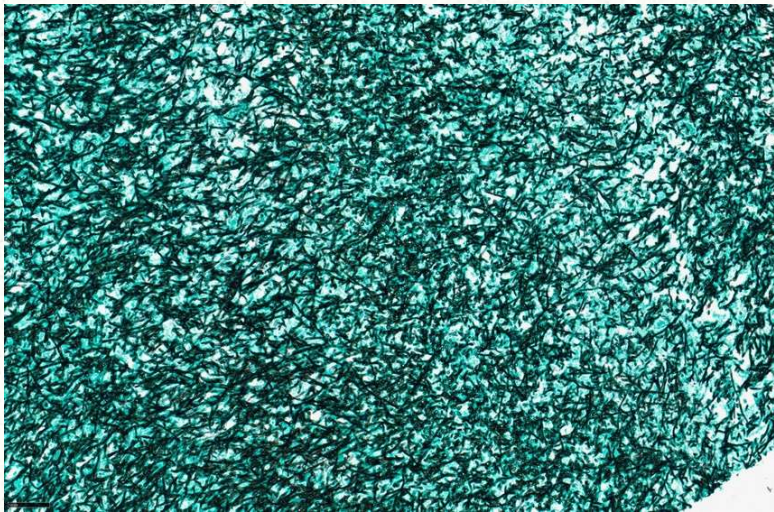
Without 1% Sodium Metabisulphite



- Residual chromic acid remains
- Over-oxidation may occur
- Increased background / non-specific staining

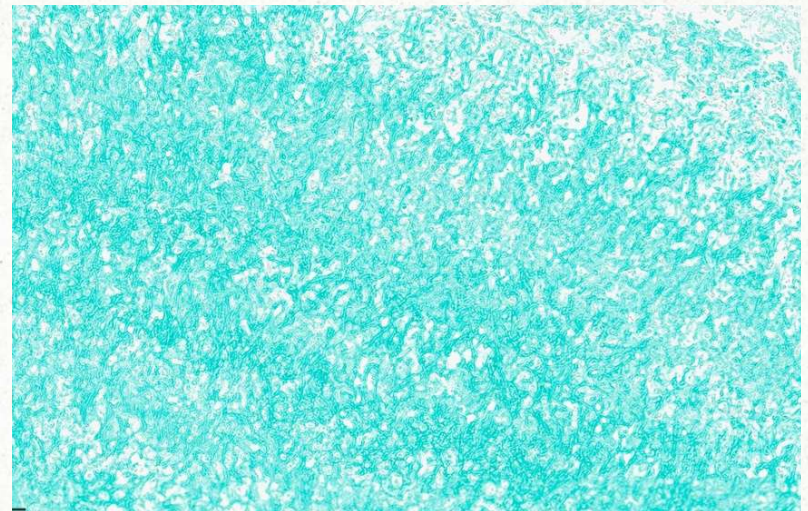
TROUBLESHOOTING: EFFECT OF BORAX IN WORKING METHENAMINE SOLUTION

With borax



- Maintains alkaline pH
- Enables proper silver reduction
- Clear black fungal staining

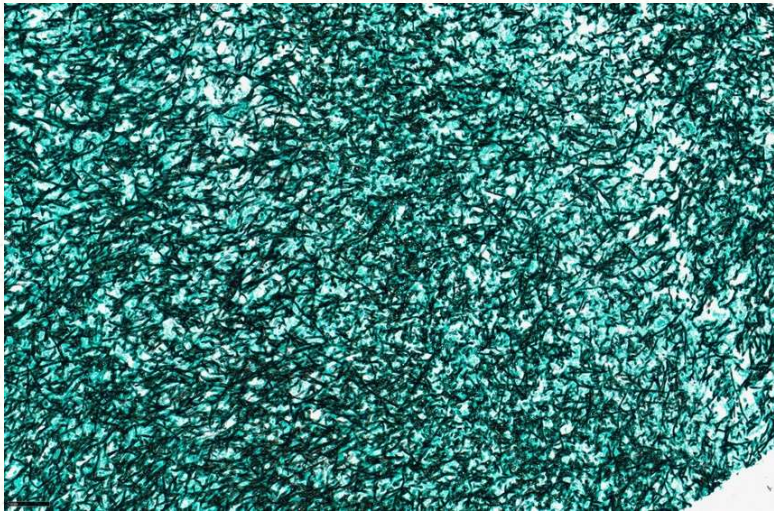
Without borax



- pH not optimal (too acidic)
- Silver reduction impaired
- Weak or absent fungal staining

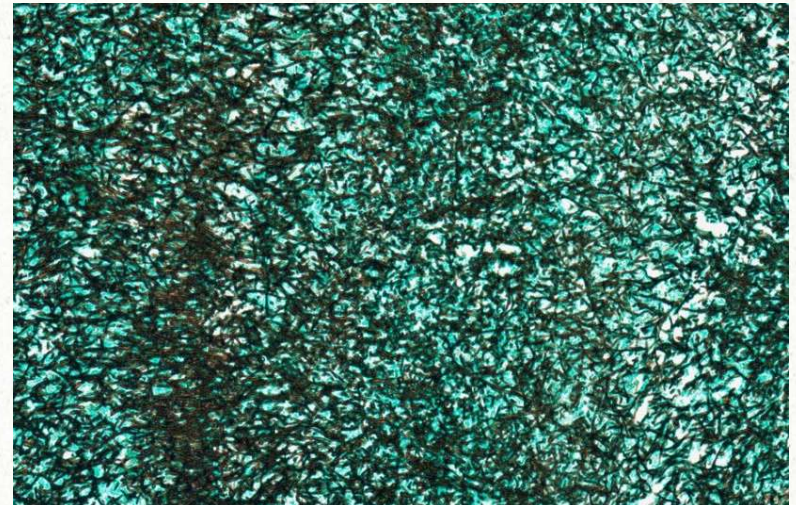
TROUBLESHOOTING: EFFECT OF 0.2% GOLD CHLORIDE

With 0.2% Gold chloride



- Tones silver deposits (enhances contrast)
- Improves sharpness of fungal structures
- Stable black/grey staining

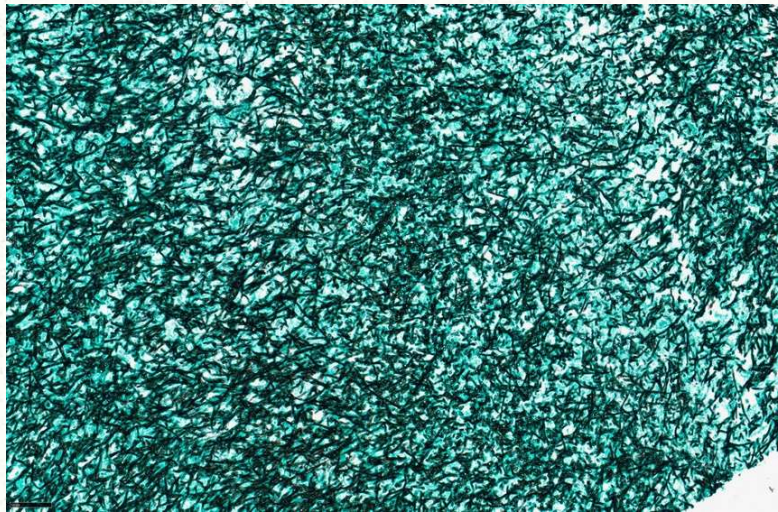
Without 0.2% Gold chloride



- Poor contrast
- Fungal structures appear less distinct
- Staining may appear brown or weak

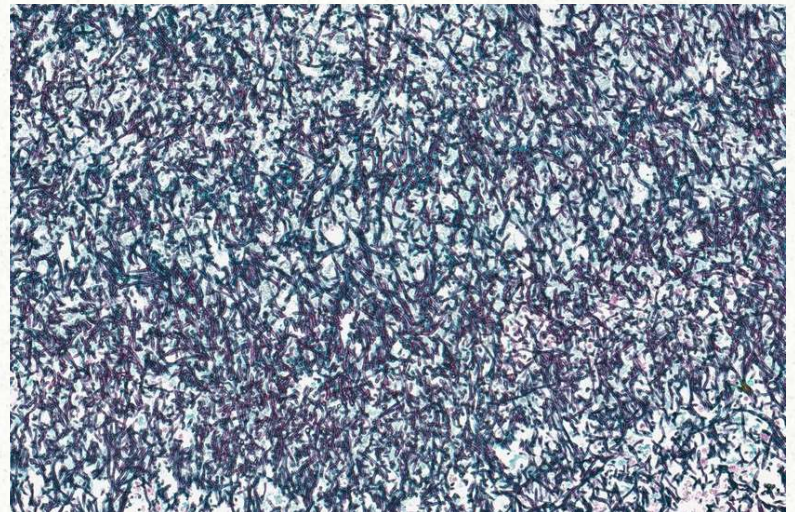
TROUBLESHOOTING: EFFECT OF 5% SODIUM THIOSULPHATE

With 5% Sodium Thiosulphate



- Removes unreacted silver
- Prevents non-specific background staining
- Produces clean and stable staining

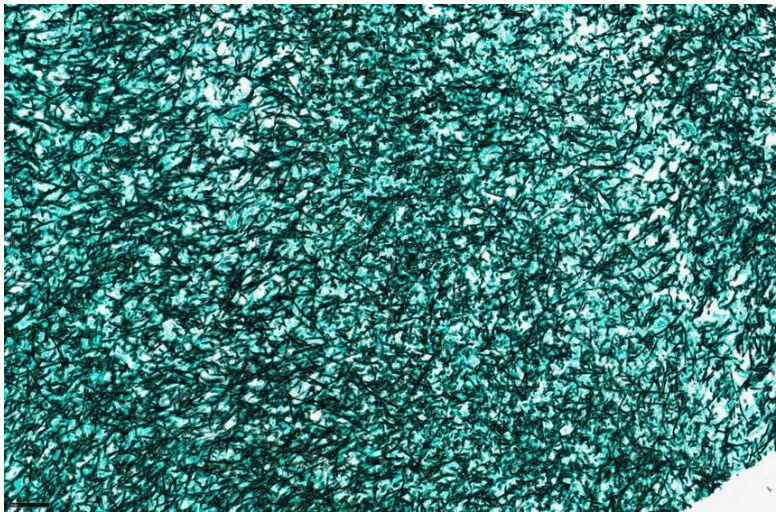
Without 5% Sodium Thiosulphate



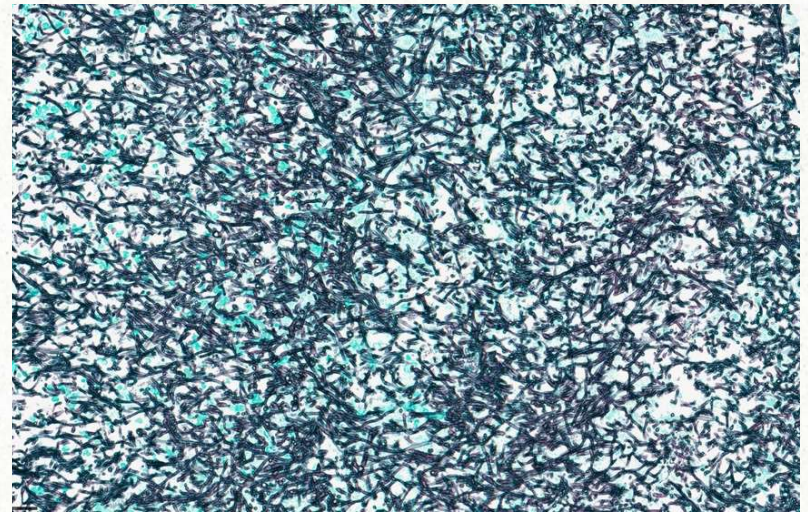
- Residual silver remains
- Increased background / non-specific staining
- Stain may darken over time

BSS MACHINE AND MANUAL STAIN

Manual Stain



BSS Machine (Autostainer)



Manual staining is faster, especially when handling a small number of slides, and allows flexibility in timing. However, it is operator-dependent and may lead to variability. In contrast, the BSS machine provides more consistent and reproducible results, although it may take longer due to batch processing

TAKE HOME MESSAGES

- Fungal cell walls stain black via silver reduction
- Proper oxidation, bleaching, and toning ensure specificity and contrast
- GMS complements ZN, especially in pulmonary infections



REFERENCES



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