

A light micrograph showing numerous long, thin, filamentous bacteria. The filaments are tangled and appear to have small, dark-stained cells or structures along their length. The background is a light, slightly grainy grey. A central text overlay reads "Staining Filamentous Bacteria".

Staining Filamentous Bacteria

Filaments Identifiable by Staining

Gram Positive

Microthrix parvicella

Nocardia

Nostocoida limicola

Type 0041/0675

Type 1851

Type 0914

Neisser Positive

Microthrix parvicella (granules)

Nocardia (granules)

Nostocoida limicola

Type 0092

Tools for Staining



- **Microscope**

- **Bright field (not phase contrast!)**
- **Preferably 1000x oil immersion objective**
- **Minimum of 200x objective**

Tools for Staining

- **Microscope Slides**
- **Clothes pin**
- **Wash bottle**
- **Watch with a second hand**
- **Paper towel**
- **Bon Ami Scouring Powder (optional)**

Tools for Staining



- **Gram Stain Kit**
 - **Gram Crystal Violet Solution**
 - **Gram Iodine Solution**
 - **Gram Decolorizing Solution**
 - **Gram Safranin Solution**

Gram Staining Procedure

1. Gram Crystal Violet Solution
 - Flood slide for 1 minute
 - Rinse with DI water
2. Gram Iodine Solution
 - Flood slide for 1 minute
 - Rinse with DI water
3. Gram Decolorizing Solution
 - Hold slide at 45 degrees and apply dropwise until blue color stops rinsing off
 - Blast with DI water to stop reaction, blot dry with paper towel
4. Gram Safranin Solution
 - Flood slide for 1 minute
 - Rinse with DI water
5. View Slide at 1000x under bright light (not phase contrast)

Tools for Staining

- **Neisser Stain Kit**
 - **Neisser Methyl Blue Solution A**
 - **Neisser Crystal Violet Solution B**
 - **Neisser Bismark Brown Solution**
 - **Transfer pipet**
 - **Container for mixing Solutions A and B**

Neisser Staining Procedure

1. Methyl Blue / Crystal Violet Solution
 - Mix 2 parts Methyl Blue and 1 part Crystal Violet in a small container
 - Flood slide for 30 seconds
 - Rinse with DI water
2. Bismark Brown Solution
 - Flood slide for 1 minute
 - Rinse with DI water and blot dry (do not rub the slide)
3. View Slide at 1000x bright light (not phase contrast)