Microbiology & staining

By

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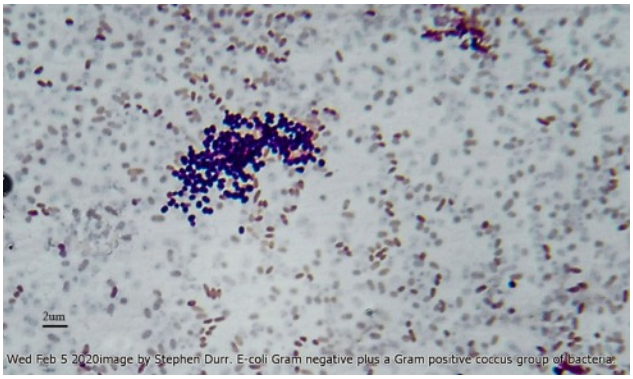
Having acquired some microbiology slides from a friend, I decided to have a go at some of the staining techniques that are frequently used by microbiologists. The one I chose is probably the most famous of all, the Gram stain, which has been in use since the 1880s. Hans Christian Gram wanted to make bacteria more visible to him while looking at lung tissue; he was interested in the aetiology of diseases like pneumonia and went on to publish his results in 1884. Its primary use in medical science today is to differentiate bacteria into two distinct types, Gram-positive and Gram-negative. Gram-positive cells are stained purple due to their cell wall being made up mostly of peptidoglycan.

Crystal violet is poured onto the stain and left for about 60 seconds; this stains the cells purple. The next step is to add some Iodine to the smear; Iodine acts as a mordant and helps bind the crystal violet stain to the cell wall of the Gram-positive bacteria. Alcohol is then washed over the stain until no more crystal violet runs from the stain, making the Gram-negative cells barely visible. The counterstain Safranin is then applied, which turns the Gram-negative cells a pink colour.

Gram-negative bacteria have thinner cell walls with more lipids than Gram-positive cells. The Gram-negative cell wall dissolves when the alcohol wash is applied to the stain; this allows the crystal violet stain to escape.

With the staining completed, the Gram-positive cells are stained a purple colour while the Gram-negative cells are pink.



Very little information can be had from the morphology of most bacteria with many species looking similar. Scientists have had to invent new tests to determine what species you are looking at down the microscope. One such test is the agglutination assay which looks for antibodies in a serology laboratory. The antigen reacts with its reciprocate antibody, and this causes clumping of the bacterial cells. Further confirmation is always needed, and more tests are likely to be carried out to discover the nature of the pathogen. I use an egg incubator for culturing bacteria, which are relatively cheap to buy and are surprisingly good at maintaining a constant temperature. Safe cultures of various bacteria can be purchased from Blades biological Ltd in Kent.

This differential staining technique gives the microbiologist information about the different species of bacteria, which can eventually be used by the doctors, who then prescribe whatever treatment is required by the patient. Instructions on how to carry out the full staining technique are on the internet, all the chemicals that you might need can be purchased from E-Bay. Another critical requirement is the different types of agar, which again can be bought from the internet quite easily. If you wish to see any bacteria clearly under the light microscope once they have been suitably stained, an oil immersion lens is a must, X60 or X 100 are especially useful. To look at living microbial life, then Phase contrast objectives are beneficial. Aseptic techniques must be used at all times and be very careful of what you culture and how you dispose of the unwanted culture dishes. I have included a few examples of some of the smears that I stained over a few weeks.

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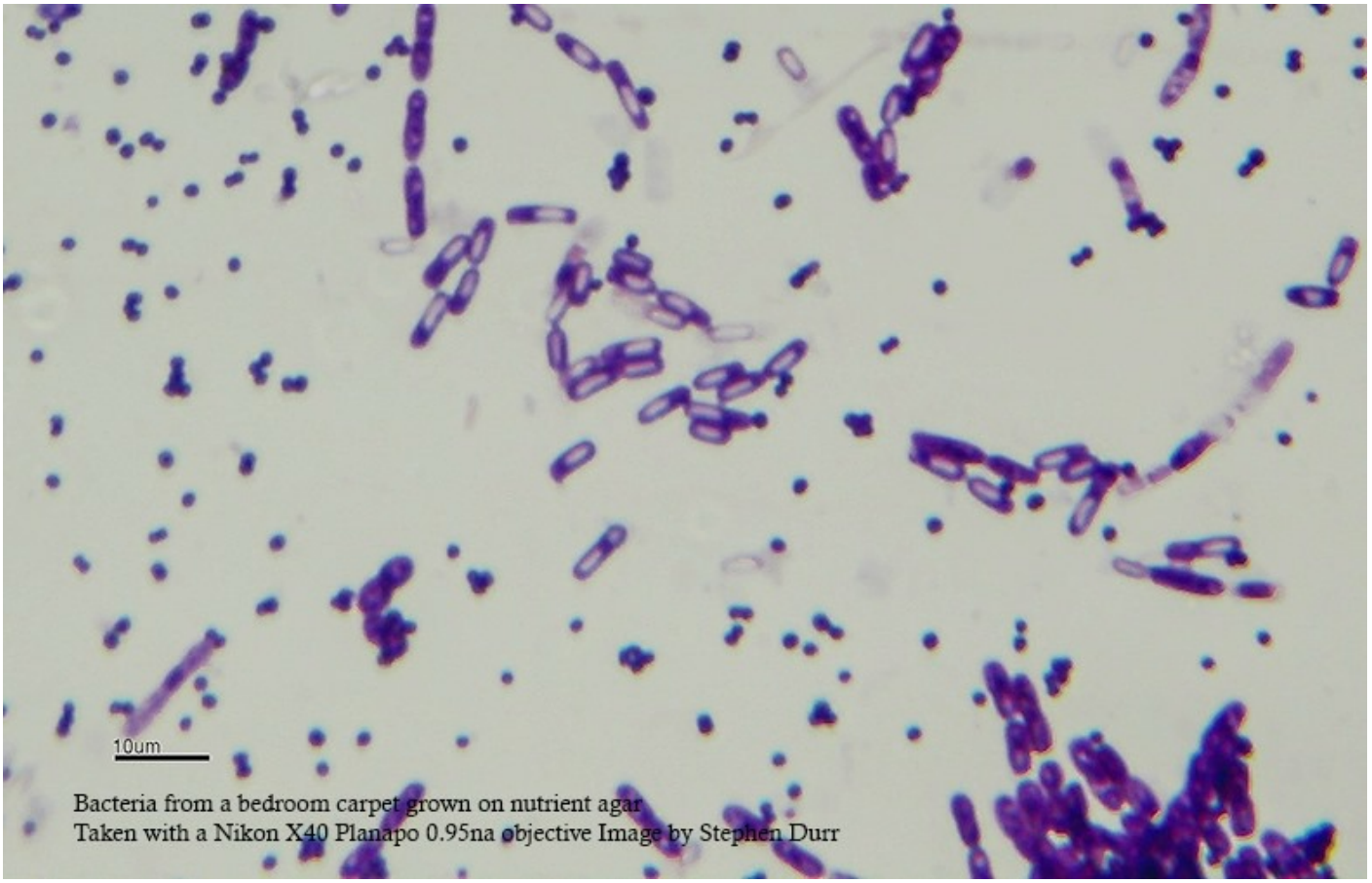
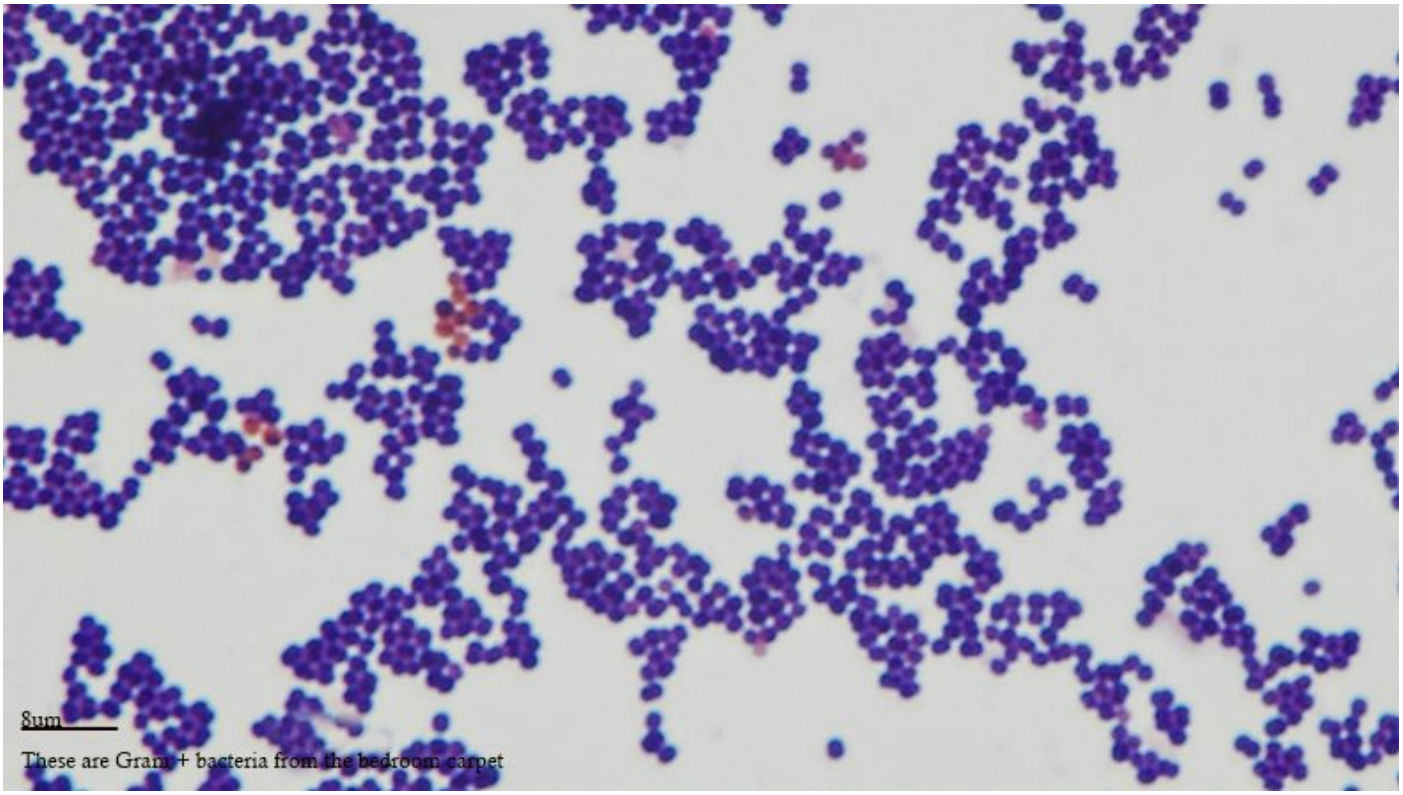


Bacteria from a bedroom carpet grown on nutrient agar
Taken with a Nikon X40 Planapo 0.95na objective Image by Stephen Durr



These are Gram + bacteria from the bedroom carpet

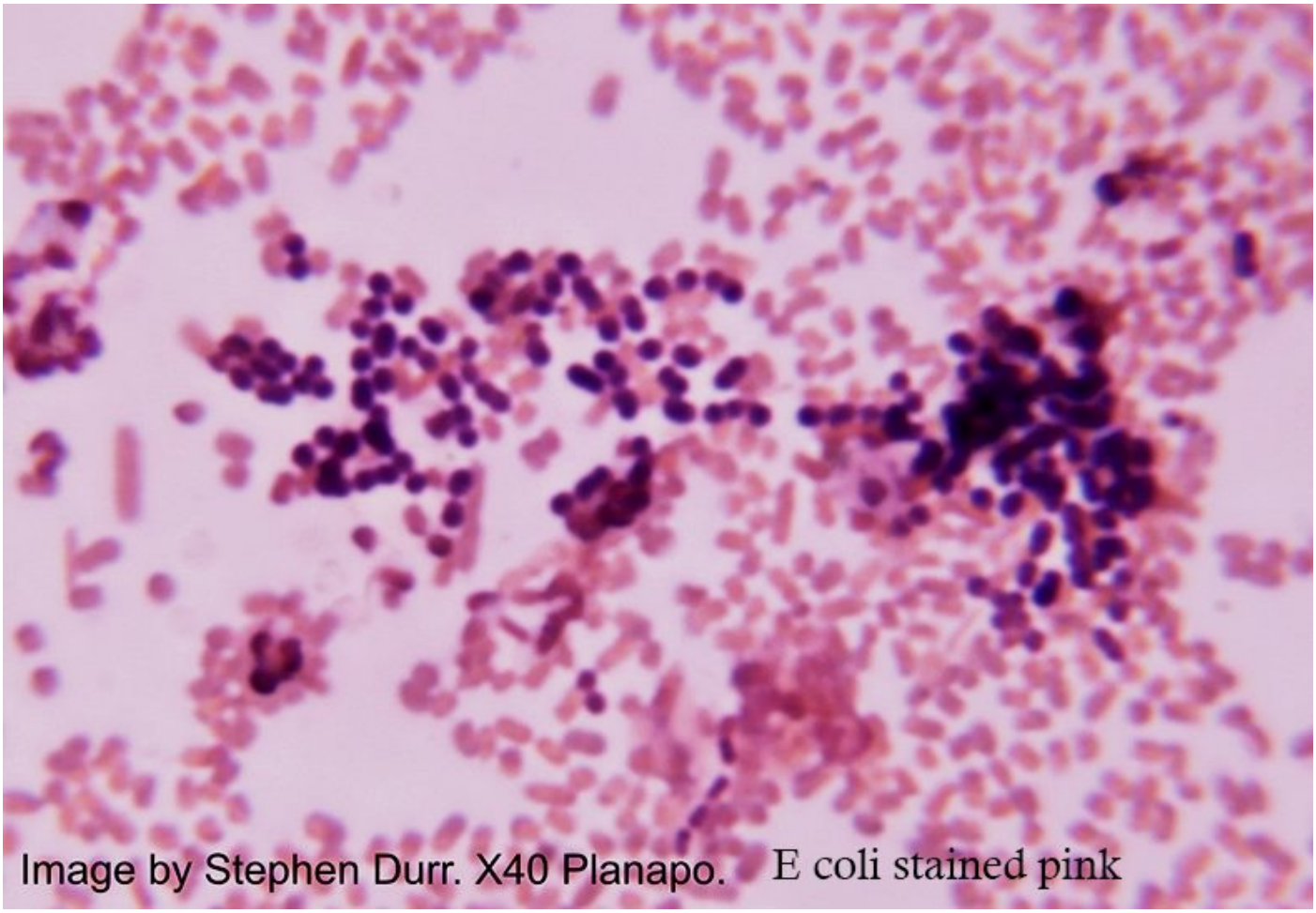


Image by Stephen Durr. X40 Planapo.
E coli stained pink

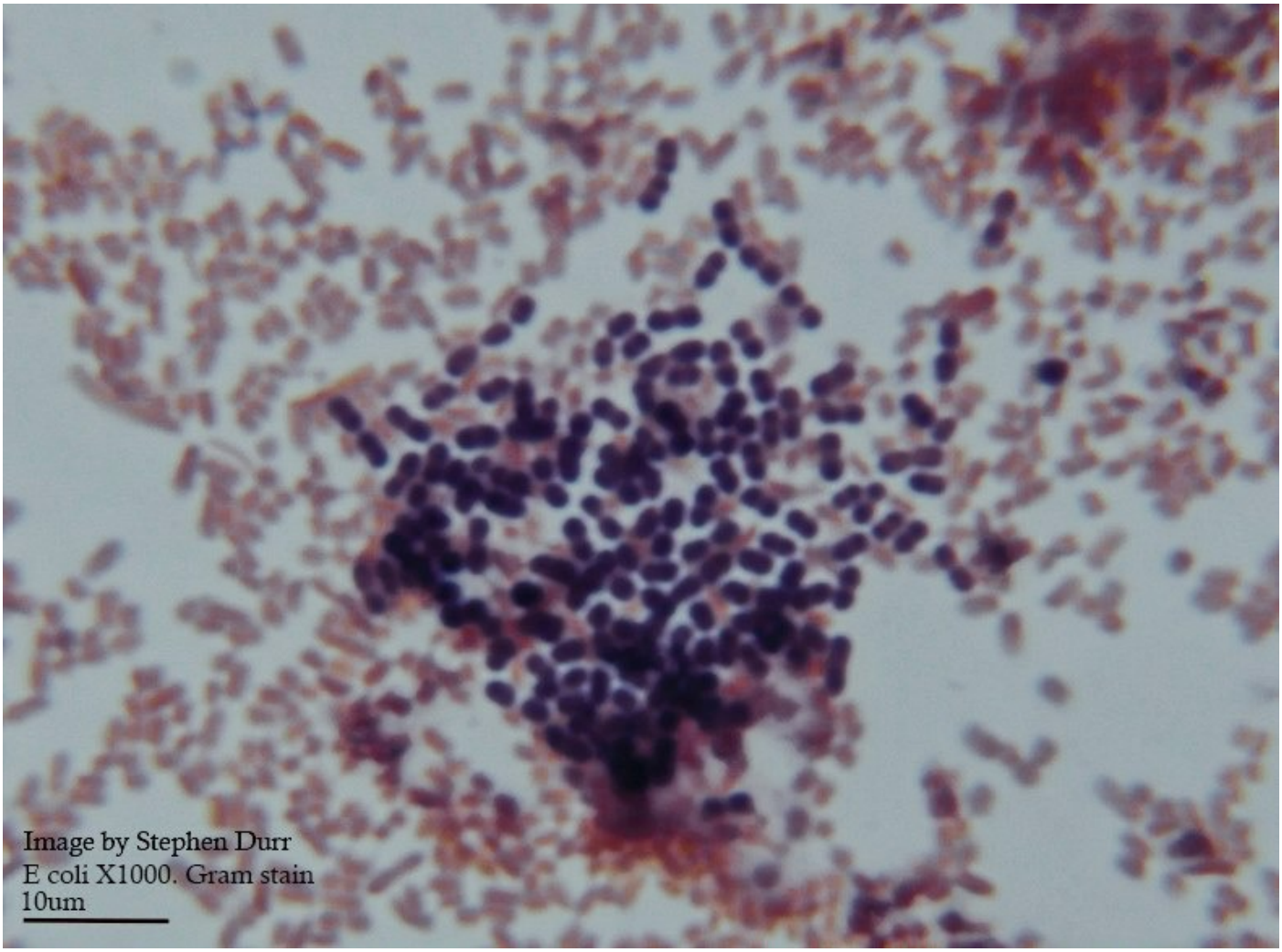


Image by Stephen Durr
E coli X1000. Gram stain
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