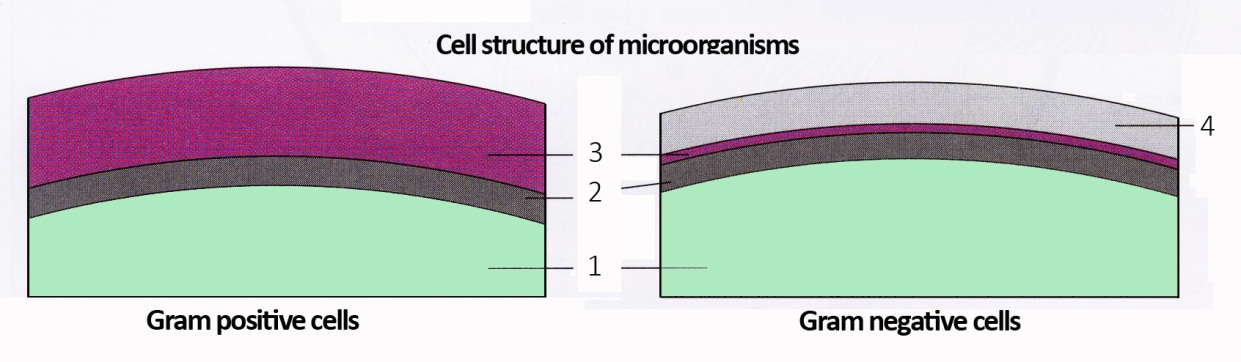
**Appendix: Information about Gram staining**

Hans Christian Gram, a Danish scientist, showed in 1884 that some microbes can be stained with crystal violet dye and become purple (Gram positive species), while others cannot retain this dye in their cell walls (Gram negative species). This is due to important differences in cell wall chemistry.

Indeed Gram positive bacteria have a thick outer cell wall of Peptidoglycan (giant molecules composed of amino acids and sugars) whereas in Gram negative bacteria the peptidoglycan layer is rather thin with an outer membrane of lipid and polysaccharide.

**Cell structure of microorganisms**



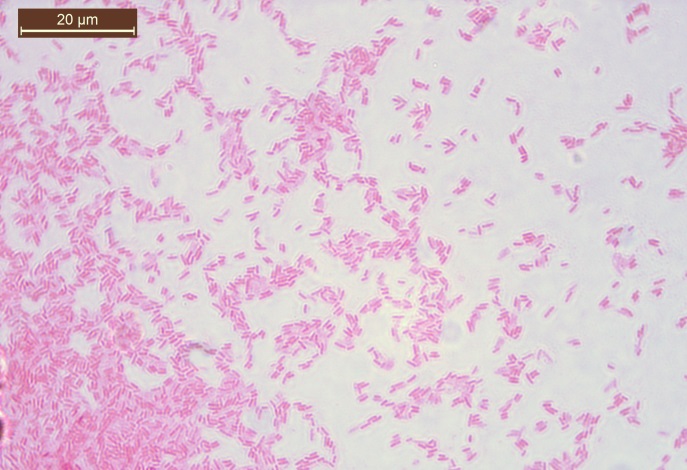
1 Cytoplasm; 2 Plasma membrane; 3 Peptidoglycan (murein) layer; 4 Outer membrane of lipid and polysaccharide.

**Gram negative cells**

**Gram positive cells**

During the staining procedure, in Gram positive microorganisms the crystal violet-iodine complex cannot subsequently be dissolved from the cells with decolorizing agents such as alcohol or acetone. The cells remain blue-violet. In Gram negative microorganisms the crystal violet-iodine complex is dissolved by alcohol or acetone and the cells turn pink to red as a result of the coloring by Safranin solution.

Single culture Gram negative staining



Single culture Gram positive staining

