Staining

**Staining** is an auxiliary technique used in [microscopy](http://en.wikipedia.org/wiki/Microscopy) to enhance contrast in the [microscopic](http://en.wikipedia.org/wiki/Microscope) image. Stains and [dyes](http://en.wikipedia.org/wiki/Dye) are frequently used in [biology](http://en.wikipedia.org/wiki/Biology)and [medicine](http://en.wikipedia.org/wiki/Medicine) to highlight structures in [biological tissues](http://en.wikipedia.org/wiki/Biological_tissue) for viewing, often with the aid of different [microscopes](http://en.wikipedia.org/wiki/Microscope). Stains may be used to define and examine bulk tissues (highlighting, for example, [muscle fibers](http://en.wikipedia.org/wiki/Muscle_fiber) or [connective tissue](http://en.wikipedia.org/wiki/Connective_tissue)), [cell](http://en.wikipedia.org/wiki/Cell_(biology)) populations (classifying different [blood cells](http://en.wikipedia.org/wiki/Blood_cell), for instance), or[organelles](http://en.wikipedia.org/wiki/Organelle) within individual cells.

Biological staining is also used to mark cells in [flow cytometry](http://en.wikipedia.org/wiki/Flow_cytometry), and to flag [proteins](http://en.wikipedia.org/wiki/Protein) or [nucleic acids](http://en.wikipedia.org/wiki/Nucleic_acid) in [gel electrophoresis](http://en.wikipedia.org/wiki/Gel_electrophoresis).

*In vivo* vs *In vitro*

[*In vivo*](http://en.wikipedia.org/wiki/In_vivo) staining ( Intra Vital Staining ) is the process of dyeing living tissues—*in vivo* means "in life". By causing certain cells or structures to take on contrasting colour(s), their form (morphology) or position within a cell or tissue can be readily seen and studied.

[*In vitro*](http://en.wikipedia.org/wiki/In_vitro) staining involves colouring cells or structures that have been removed from their biological context. For example, [crystal violet](http://en.wikipedia.org/wiki/Crystal_violet) stains only [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) bacteria in [Gram staining](http://en.wikipedia.org/wiki/Gram_staining). A [safranin](http://en.wikipedia.org/wiki/Safranin) counterstain is applied that stains all cells, allowing identification of Gram-negative bacteria.

**In vitro methods**

**Preparation**

The preparatory steps involved depend on the type of analysis planned; some or all of the following procedures may be required.

[**Fixation**](http://en.wikipedia.org/wiki/Fixation_(histology))–which may itself consist of several steps–aims to preserve the shape of the cells or tissue involved as much as possible. Sometimes [heat fixation](http://en.wikipedia.org/wiki/Heat_fixation) is used to kill, adhere, and alter the specimen so it accepts stains. Most chemical fixatives (chemicals causing fixation) generate [chemical bonds](http://en.wikipedia.org/wiki/Chemical_bond) between [proteins](http://en.wikipedia.org/wiki/Protein) and other substances within the sample, increasing their rigidity. Common fixatives include [formaldehyde](http://en.wikipedia.org/wiki/Formaldehyde), [ethanol](http://en.wikipedia.org/wiki/Ethanol), [methanol](http://en.wikipedia.org/wiki/Methanol), and/or [picric acid](http://en.wikipedia.org/wiki/Picric_acid). Pieces of tissue may be embedded in [paraffin wax](http://en.wikipedia.org/wiki/Paraffin_wax) to increase their mechanical strength and stability and to make them easier to cut into thin slices.

**Permeabilization** involves treatment of cells with (usually) a mild [surfactant](http://en.wikipedia.org/wiki/Surfactant). This treatment dissolves [cell membranes](http://en.wikipedia.org/wiki/Cell_membrane), and allows larger dye molecules into the cell's interior.

[**Mounting**](http://en.wikipedia.org/wiki/Microscope_slide#Mounting) usually involves attaching the samples to a glass microscope slide for observation and analysis. In some cases, cells may be grown directly on a slide. For samples of loose cells (as with a blood smear or a [pap smear](http://en.wikipedia.org/wiki/Pap_smear)) the sample can be directly applied to a slide. For larger pieces of tissue, thin sections (slices) are made using a [microtome](http://en.wikipedia.org/wiki/Microtome); these slices can then be mounted and inspected.

**Staining proper**

At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a [mordant](http://en.wikipedia.org/wiki/Mordant): a chemical compound that reacts with the stain to form an insoluble, coloured [precipitate](http://en.wikipedia.org/wiki/Precipitation_(chemistry)). When excess dye solution is washed away, the mordanted stain remains.

Most of the dyes commonly used in microscopy are available as **certified stains**. This means that samples of the manufacturer's batch have been tested by an independent body, the [Biological Stain Commission](http://en.wikipedia.org/wiki/Biological_Stain_Commission), and found to meet or exceed certain standards of purity, dye content and performance in staining techniques.

**Specific techniques**

**Gram staining**

[Gram staining](http://en.wikipedia.org/wiki/Gram_staining) is used to determine gram status to classify bacteria broadly. It is based on the composition of their [cell wall](http://en.wikipedia.org/wiki/Cell_wall). Gram staining uses [crystal violet](http://en.wikipedia.org/wiki/Gentian_violet) to stain cell walls, [iodine](http://en.wikipedia.org/wiki/Iodine) as a mordant, and a [fuchsin](http://en.wikipedia.org/wiki/Fuchsin) or [safranin](http://en.wikipedia.org/wiki/Safranin) counterstain to mark all bacteria. Gram status is important in medicine; the presence or absence of a cell wall changes the bacterium's susceptibility to some[antibiotics](http://en.wikipedia.org/wiki/Antibiotic).

[Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) bacteria stain dark blue or violet. Their [cell wall](http://en.wikipedia.org/wiki/Cell_wall) is typically rich with [peptidoglycan](http://en.wikipedia.org/wiki/Peptidoglycan) and lacks the secondary membrane and [lipopolysaccharide](http://en.wikipedia.org/wiki/Lipopolysaccharide) layer found in Gram-negative bacteria.

On most Gram-stained preparations, [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative) organisms appear red or pink because they are counterstained. Due to presence of higher lipid content, after alcohol-treatment, the porosity of the cell wall increases, hence the CVI complex (Crystal violet -Iodine) can pass through. Thus, the primary stain is not retained. Also, in contrast to most Gram-positive bacteria, Gram-negative bacteria have only a few layers of peptidoglycan and a secondary cell membrane made primarily of lipopolysaccharide.

**Ziehl-Neelsen stain**

[Ziehl-Neelsen staining](http://en.wikipedia.org/wiki/Ziehl-Neelsen_stain) is used to stain species of [*Mycobacterium tuberculosis*](http://en.wikipedia.org/wiki/Mycobacterium_tuberculosis) that do not stain with the standard laboratory staining procedures like Gram staining.

The stains used are the red coloured [Carbol fuchsin](http://en.wikipedia.org/wiki/Carbol_fuchsin) that stains the bacteria and a counter stain like [Methylene blue](http://en.wikipedia.org/wiki/Methylene_blue)

**Haematoxylin and eosin (H&E) staining**

[Haematoxylin and eosin staining](http://en.wikipedia.org/wiki/Haematoxylin_and_eosin_stain) protocol is used frequently in [histology](http://en.wikipedia.org/wiki/Histology) to examine thin sections of tissue. [Haematoxylin](http://en.wikipedia.org/wiki/Haematoxylin) stains cell nuclei blue, while [eosin](http://en.wikipedia.org/wiki/Eosin) stains cytoplasm, connective tissue and other extracellular substances pink or red. Eosin is strongly absorbed by [red blood cells](http://en.wikipedia.org/wiki/Red_blood_cell), colouring them bright red. Hematoxylin stains the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of hyaline cartilage) blue. In contrast, eosin stains the cytoplasm and collagen pink.

**PAS staining**

[Periodic acid-Schiff](http://en.wikipedia.org/wiki/Periodic_acid-Schiff) staining is used to mark [carbohydrates](http://en.wikipedia.org/wiki/Carbohydrate) ([glycogen](http://en.wikipedia.org/wiki/Glycogen), [glycoprotein](http://en.wikipedia.org/wiki/Glycoprotein), [proteoglycans](http://en.wikipedia.org/wiki/Proteoglycan)). It is used to distinguish different types of glycogen storage diseases.

**Masson's trichrome**

[Masson's trichrome](http://en.wikipedia.org/wiki/Masson%27s_trichrome) is (as the name implies) a three-colour staining protocol. The recipe has evolved from Masson's original technique for different specific applications, but all are well-suited to distinguish cells from surrounding [connective tissue](http://en.wikipedia.org/wiki/Connective_tissue). Most recipes produce red [keratin](http://en.wikipedia.org/wiki/Keratin) and muscle fibers, blue or green staining of [collagen](http://en.wikipedia.org/wiki/Collagen) and [bone](http://en.wikipedia.org/wiki/Bone), light red or pink staining of [cytoplasm](http://en.wikipedia.org/wiki/Cytoplasm), and black [cell nuclei](http://en.wikipedia.org/wiki/Cell_nucleus).

**Silver staining**

[Silver staining](http://en.wikipedia.org/wiki/Silver_stain) is the use of [silver](http://en.wikipedia.org/wiki/Silver) to stain [histologic sections](http://en.wikipedia.org/wiki/Histologic_section). This kind of staining is important especially to **show**[**proteins**](http://en.wikipedia.org/wiki/Protein) (for example type III [collagen](http://en.wikipedia.org/wiki/Collagen)) and [DNA](http://en.wikipedia.org/wiki/DNA). It is used to show both substances inside and outside [cells](http://en.wikipedia.org/wiki/Cell_(biology)). **Silver staining is also used in**[**temperature gradient gel electrophoresis**](http://en.wikipedia.org/wiki/Temperature_gradient_gel_electrophoresis)**.**

**Sudan staining**

[Sudan staining](http://en.wikipedia.org/wiki/Sudan_stain) is the use of Sudan dyes to stain sudano-philic substances, usually [lipids](http://en.wikipedia.org/wiki/Lipid). [Sudan III](http://en.wikipedia.org/wiki/Sudan_III), [Sudan IV](http://en.wikipedia.org/wiki/Sudan_IV), [Oil Red O](http://en.wikipedia.org/wiki/Oil_Red_O), [Osmium tetroxide](http://en.wikipedia.org/wiki/Osmium_tetroxide), and [Sudan Black B](http://en.wikipedia.org/wiki/Sudan_Black_B) are often used. Sudan staining is often used to determine the level of [fecal fat](http://en.wikipedia.org/wiki/Fecal_fat) to diagnose [steatorrhea](http://en.wikipedia.org/wiki/Steatorrhea).

Common biological stains

Different stains react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas. Some of the most common biological stains are listed below. Unless otherwise marked, all of these dyes may be used with fixed cells and tissues; vital dyes (suitable for use with living organisms) are noted.

**Acridine orange**

[Acridine orange](http://en.wikipedia.org/wiki/Acridine_orange) (AO) is a nucleic acid selective **fluorescent cationic dye** useful for **cell cycle determination**. It is cell-permeable, and interacts with DNA and RNA by intercalation or electrostatic attractions. When bound to DNA, it is very similar spectrally to fluorescein. .

**Carmine**

[Carmine](http://en.wikipedia.org/wiki/Carmine) is an intensely **red dye used to stain**[**glycogen**](http://en.wikipedia.org/wiki/Glycogen), while Carmine alum is a nuclear stain. Carmine stains require the use of a mordant, usually [aluminum](http://en.wikipedia.org/wiki/Aluminum).

**Cresyl violet**

[Cresyl violet](http://en.wikipedia.org/wiki/Cresyl_violet_stain) stains the acidic components of the **neuronal cytoplasm** a violet colour, **specifically**[**nissl**](http://en.wikipedia.org/wiki/Nissl_body)**bodies**. Often used in brain research.

**Crystal violet**

[Crystal violet](http://en.wikipedia.org/wiki/Methyl_violet), when combined with a suitable mordant, stains [cell walls](http://en.wikipedia.org/wiki/Cell_wall) purple. Crystal violet is an important component in Gram staining.

**Eosin**

[Eosin](http://en.wikipedia.org/wiki/Eosin) is most often used as a counterstain to haematoxylin, imparting a pink or red colour to [cytoplasmic](http://en.wikipedia.org/wiki/Cytoplasm) material, [cell membranes](http://en.wikipedia.org/wiki/Cell_membrane), and some extracellular structures. It also imparts a strong red colour to [red blood cells](http://en.wikipedia.org/wiki/Red_blood_cell). Eosin may also be used as a counterstain in some variants of Gram staining, and in many other protocols. There are actually two very closely related compounds commonly referred to as eosin. Most often used is eosin Y (also known as eosin Y ws or eosin yellowish); it has a very slightly yellowish cast. The other eosin compound is eosin B (eosin bluish or imperial red); it has a very faint bluish cast. The two dyes are interchangeable, and the use of one or the other is more a matter of preference and tradition.

**Iodine**

[Iodine](http://en.wikipedia.org/wiki/Iodine) is used in [chemistry](http://en.wikipedia.org/wiki/Chemistry) as an indicator for [starch](http://en.wikipedia.org/wiki/Starch). When starch is mixed with iodine in solution, an intensely dark blue colour develops, representing a starch/iodine complex. Starch is a substance common to most plant cells and so a weak iodine solution will stain starch present in the cells. Iodine is one component in the staining technique known as [Gram staining](http://en.wikipedia.org/wiki/Gram_staining), used in[microbiology](http://en.wikipedia.org/wiki/Microbiology)

**Rhodamine**

[Rhodamine](http://en.wikipedia.org/wiki/Rhodamine) is a protein specific fluorescent stain commonly used in fluorescence microscopy.

**Safranin**

[Safranin](http://en.wikipedia.org/wiki/Safranin) (or Safranin O) is a nuclear stain. It produces red nuclei, and is used primarily as a counterstain. Safranin may also be used to give a yellow colour to collagen.

Electron microscopy

As in light microscopy, stains can be used to enhance contrast in [transmission electron microscopy](http://en.wikipedia.org/wiki/Transmission_electron_microscopy). Electron-dense compounds of heavy metals are typically used.

**Phosphotungstic acid**

[Phosphotungstic acid](http://en.wikipedia.org/wiki/Phosphotungstic_acid) is a common [negative stain](http://en.wikipedia.org/wiki/Negative_stain) for [viruses](http://en.wikipedia.org/wiki/Virus), [nerves](http://en.wikipedia.org/wiki/Nerve), [polysaccharides](http://en.wikipedia.org/wiki/Polysaccharide), and other biological tissue materials.

**Osmium tetroxide**

[Osmium tetroxide](http://en.wikipedia.org/wiki/Osmium_tetroxide) is used in optical microscopy to stain [lipids](http://en.wikipedia.org/wiki/Lipid). It dissolves in fats, and is reduced by organic materials to elemental osmium, an easily visible black substance. Because it is a heavy metal that absorbs electrons, it is perhaps the most common stain used for morphology in biological electron microscopy. It is also used for the staining of various polymers for the study of their morphology by TEM. OsO4 is very volatile and extremely toxic. It is a strong oxidizing agent as the osmium has an oxidation number of +8. It aggressively oxidizes many materials, leaving behind a deposit of non-volatile osmium in a lower oxidation state.

**Ruthenium tetroxide**

[Ruthenium tetroxide](http://en.wikipedia.org/wiki/Ruthenium_tetroxide) is equally volatile and even more aggressive than osmium tetraoxide and able to stain even materials that resist the osmium stain, e.g. polyethylene.

Other chemicals used in electron microscopy staining include: [ammonium molybdate](http://en.wikipedia.org/wiki/Ammonium_molybdate), [cadmium iodide](http://en.wikipedia.org/wiki/Cadmium_iodide), [carbohydrazide](http://en.wikipedia.org/wiki/Carbohydrazide), [ferric chloride](http://en.wikipedia.org/wiki/Ferric_chloride), [hexamine](http://en.wikipedia.org/wiki/Hexamine), [indium trichloride](http://en.wikipedia.org/wiki/Indium_trichloride), [lanthanum nitrate](http://en.wikipedia.org/w/index.php?title=Lanthanum_nitrate&action=edit&redlink=1), [lead acetate](http://en.wikipedia.org/wiki/Lead_acetate), [lead citrate](http://en.wikipedia.org/w/index.php?title=Lead_citrate&action=edit&redlink=1), [lead(II) nitrate](http://en.wikipedia.org/wiki/Lead(II)_nitrate), [periodic acid](http://en.wikipedia.org/wiki/Periodic_acid), [phosphomolybdic acid](http://en.wikipedia.org/wiki/Phosphomolybdic_acid), [potassium ferricyanide](http://en.wikipedia.org/wiki/Potassium_ferricyanide), [potassium ferrocyanide](http://en.wikipedia.org/wiki/Potassium_ferrocyanide), [ruthenium red](http://en.wikipedia.org/wiki/Ruthenium_red), [silver nitrate](http://en.wikipedia.org/wiki/Silver_nitrate), [silver proteinate](http://en.wikipedia.org/wiki/Silver_proteinate), [sodium chloroaurate](http://en.wikipedia.org/w/index.php?title=Sodium_chloroaurate&action=edit&redlink=1),[thallium nitrate](http://en.wikipedia.org/w/index.php?title=Thallium_nitrate&action=edit&redlink=1), [thiosemicarbazide](http://en.wikipedia.org/wiki/Thiosemicarbazide), [uranyl acetate](http://en.wikipedia.org/wiki/Uranyl_acetate), [uranyl nitrate](http://en.wikipedia.org/wiki/Uranyl_nitrate), and [vanadyl sulfate](http://en.wikipedia.org/wiki/Vanadyl_sulfate).