Image from http://goo.gl/VOFuor

Buffers and Aldehyde Fixatives

BOTA546B

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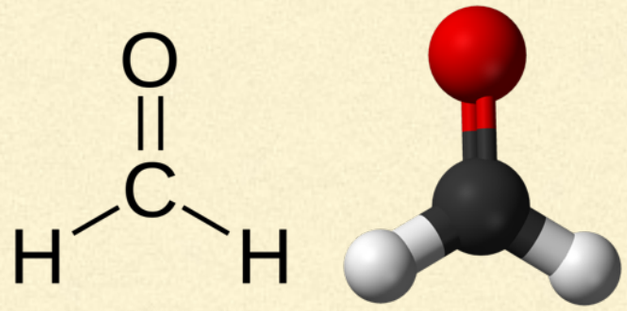
Fixation

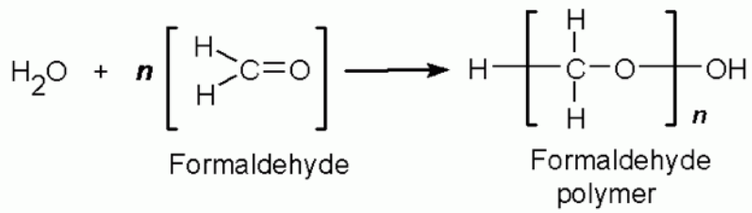
- Fixation: To kill the tissue quickly, and stabilize and preserve its constituents from sample processing and observation procedures
- Aldehyde fixatives are the most commonly used fixatives in microscopy
- Formaldehyde and Gluteraldehyde

Reference: Chandler, D. E., & Roberson, R. W. (2009), Kiernan, J. A. (2000)

Formaldehyde

- Formaldehyde: Small molecule, gas
- hydrated form methylene hydrate (HO-CH2-OH)
- Formalin: 37-40% of formaldehyde (n = 2 to 8) and 60-63% of water (by weight) + 10% Methanol
- Methanol addition: prevents oxidation to formic acid and PFA precipitation
- 10% Formalin =~ 4% Formaldehyde
- Paraformaldehyde: Higher polymers (n up to 100), white powder





Reference: Kiernan, J. A. (2000)

Formaldehyde

- To be useful as a fixative, a solution must contain monomeric formaldehyde as its major solute.
- Dilution with water breaks up the small polymers in formalin
- Takes a couple of days if plain water is used, but almost instantaneous when formalin is diluted with a buffer solution at physiological pH

Reference: Kiernan, J. A. (2000)

Formaldehyde

- Cross-links amines with nearby nitrogens or DNA
- Fixation is reversible by excess water

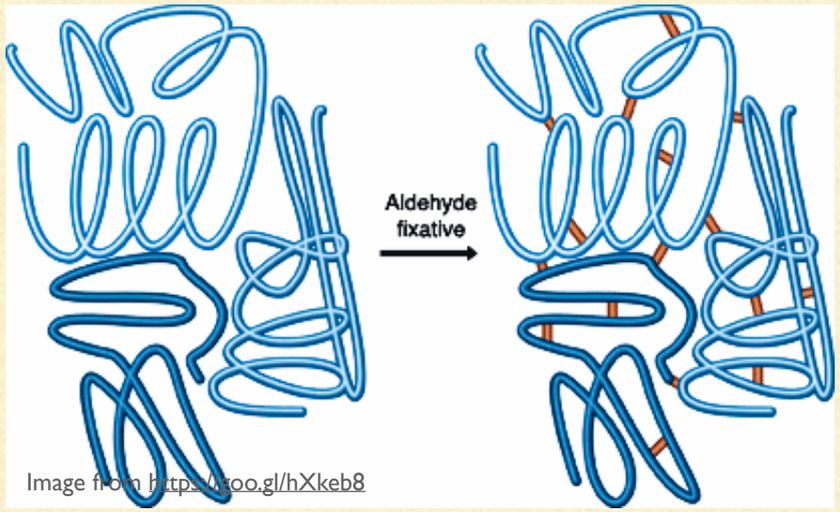


Image from https://goo.gl/hXkeb8

- Initial binding of formaldehyde to protein is largely completed in 24 hours
- Formation of methylene bridges proceeds much more slowly
- Adequate fixation take days

Reference: Kiernan, J. A. (2000)

Formaldehyde

- Advantage
  - Small molecule
  - rapid penetration
  - preserves native structure
- Good for immunohistochemistry
- Disadvantage:
  - Weak cross-linking
  - Not desirable for EM

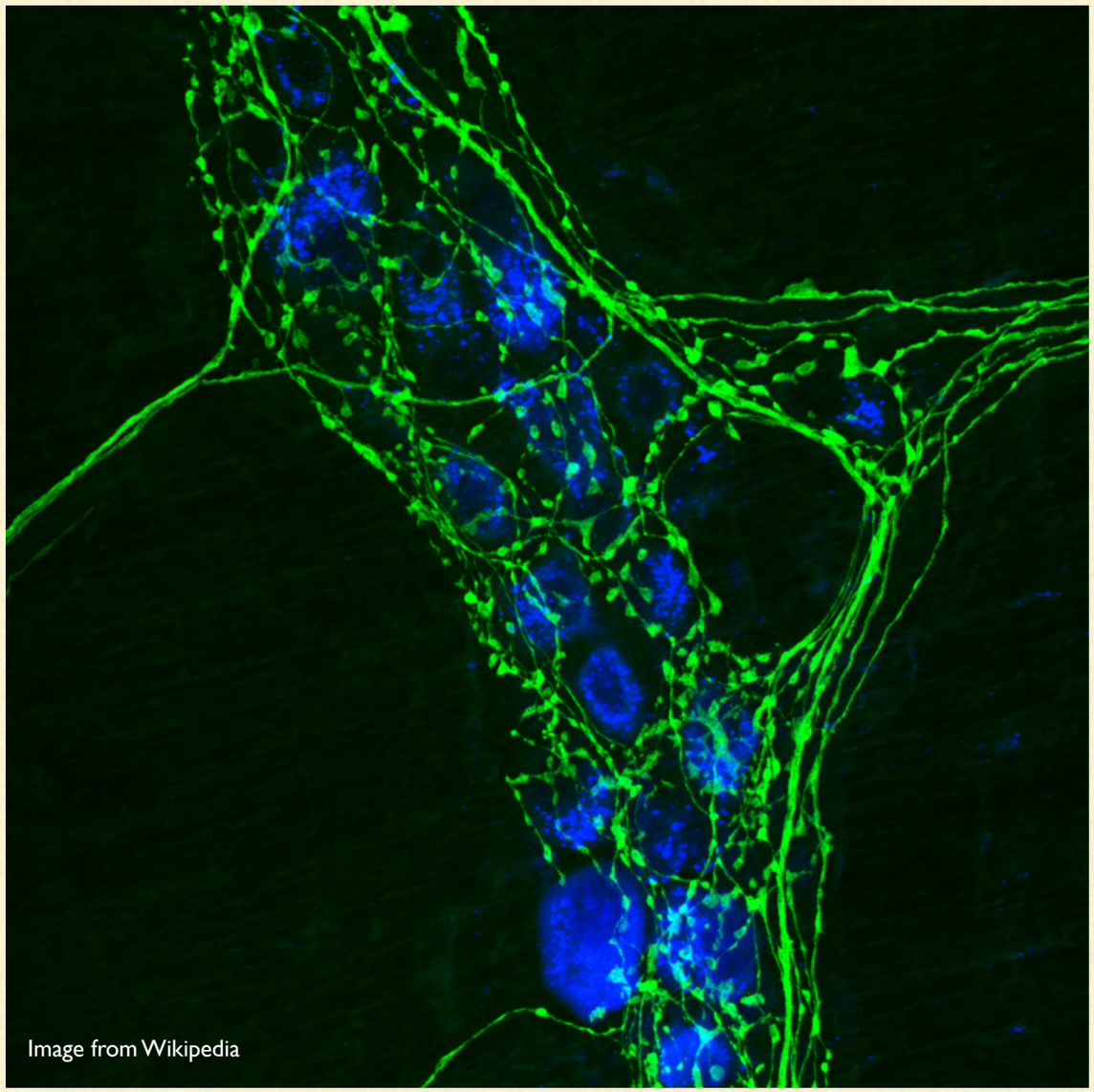
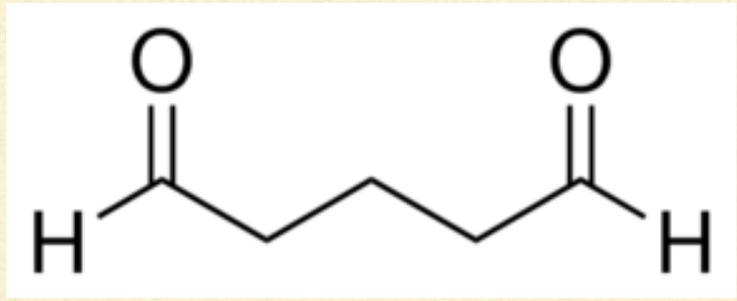


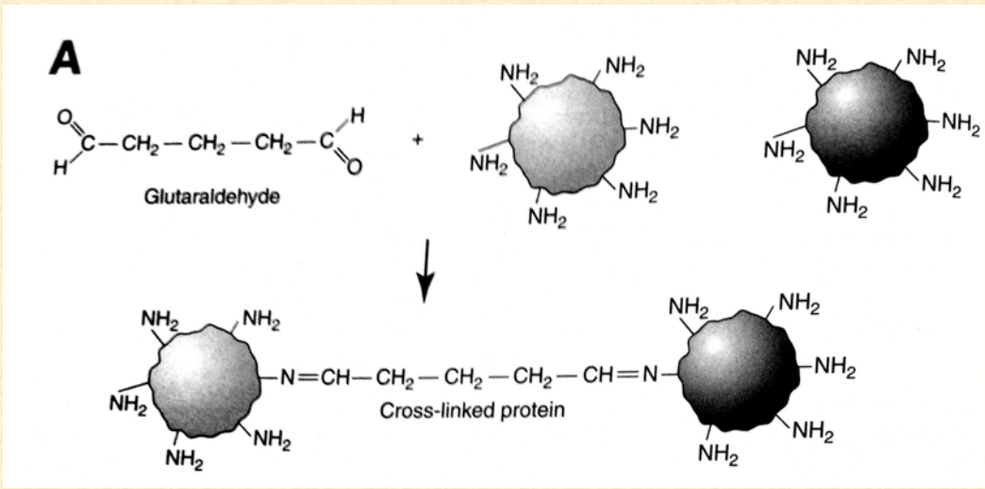
Image from Wikipedia

Reference: Chandler, D. E., & Roberson, R. W. (2009)

Gluteraldehyde

- Gluteraldehyde: Two aldehyde groups linked by a three carbon chain
- strong cross-linking of amine groups
- irreversible
- longer molecule, two aldehyde groups
- slowly decomposes to glutaric acid and polymerise to form cyclic compounds





Reference: Kiernan, J. A. (2000), Chandler, D. E., & Roberson, R. W. (2009)

Gluteraldehyde

- Advantage:
  - Strong cross-linking
  - good preservation at ultra-structure levels
- Disadvantage
  - Relatively large molecule, slow penetration.
  - Slow fixation, artifacts due to physiological and autolytic activity of cells (4C fixation)
  - Not good for immunostaining

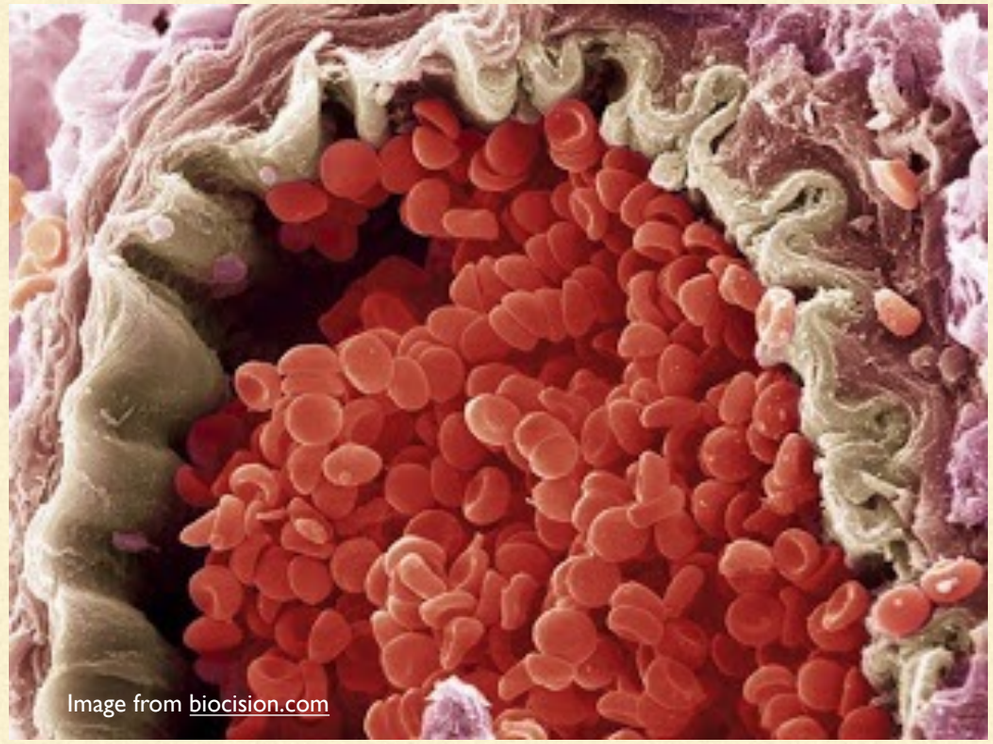


Image from biocision.com

Reference: Chandler, D. E., & Roberson, R. W. (2009)

Buffers

- Provide balanced salt solution as well as pH stability needed to preserve cells and tissues
- Protect the aldehyde solution against pH changes caused by aldehyde breakdown
- Speed up certain changes (i.e. formaldehyde)
- Good's Buffers (Neutral pH, solubility, membrane permeability, biological inertness, optical absorbance, ease of preparation)
- PIPES and MES (Good's)

Reference: Kiernan, J. A. (2000)

Buffers

Table 3.2 Common Buffers for Fixation

Buffer	pK/pH Range	Comments
Phosphate (Na_2HPO_4/NaH_2PO_4)	5.7 to 8.0	Mixture of Na_2HPO_4 and NaH_2PO_4 as determined by pH desired
Sodium cacodylate ($Na(CH_3)_2\ AsO_2 \cdot 3H_2O$)	5.0 to 7.4	Poisonous; contains arsenic; still commonly used but HEPES and PIPES are safer and are replacing cacodylate
S-Collidine (2,4,6-trimethylpyridine)	6.0 to 8.0	Toxic; mild smell is disliked by some; used infrequently
HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid)	7.35	Reacts slowly with glutaraldehyde; can be used between 5 and 50 mM
PIPES (Piperazine-1,4-bis-2-ethanesulfonic acid)	6.8	Reacts slowly with glutaraldehyde; can be used between 5 and 50 mM
Tris-Maleate (Tris (hydroxymethyl) aminomethane maleate)	5.2 to 8.6	Reacts slowly with glutaraldehyde; can be used between 5 and 50 mM

Reference: Chandler, D. E., & Roberson, R. W. (2009)

Buffers

- Bicarbonate buffers not used due to the need for CO_2 control
- phosphate may precipitate from buffer if alcohol > 70%
- HEPES is specially good for low temperatures but photo-toxic
- PIPES+gluteraldehyde minimizes lipid loss
- Sodium cacodylate
- Agent Blue in Vietnam War s

Reference: Kiernan, J. A. (2000)

Quiz!

- What is the difference between formaldehyde, formalin and paraformaldehyde?

References

- Kiernan, J. A. (2000). Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Microscopy Today, 1(5).
- Chandler, D. E., & Roberson, R. W. (2009). Bioimaging: current concepts in light and electron microscopy. Jones & Bartlett Publishers
- Rolls, G., (2015) Fixation and Fixatives (2) – Factors influencing chemical fixation, formaldehyde and glutaraldehyde, Leica Biosystems