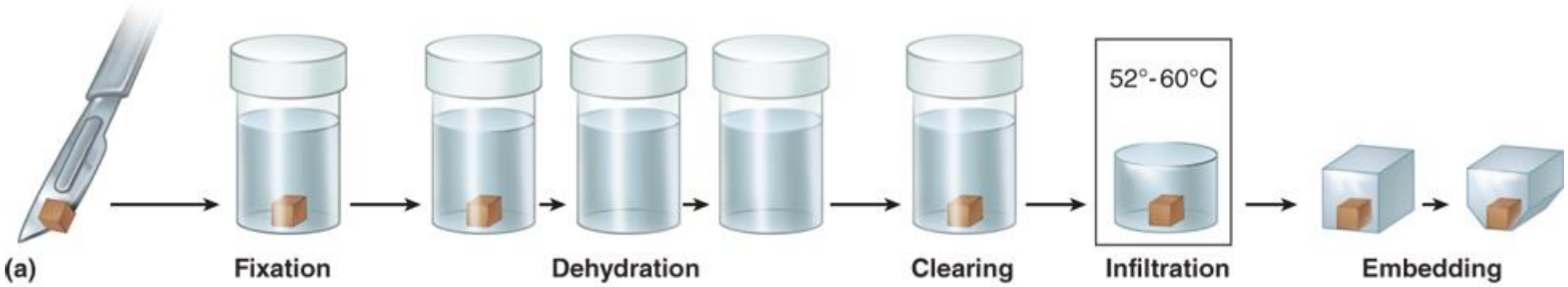
262 Zoo-
Embedding

Lecture 5

p 14-18

Dr. Nouf Alyami

Histology preparation steps



Embedding

- After the infiltration process has been completed, it is necessary to obtain a solid block containing the tissue.
- Coat a stainless steel histological base mold of suitable size to fit the tissue with glycerol or “mold release” to prevent adherence of the wax block.
- Pre-warming of the metal block is advised to prevent premature solidification of the wax block.
- Prior to beginning the infiltration process, an embedding cassette should be placed on top of the mold and labeled with the name of the tissue, fixative, and date.

histology cassette

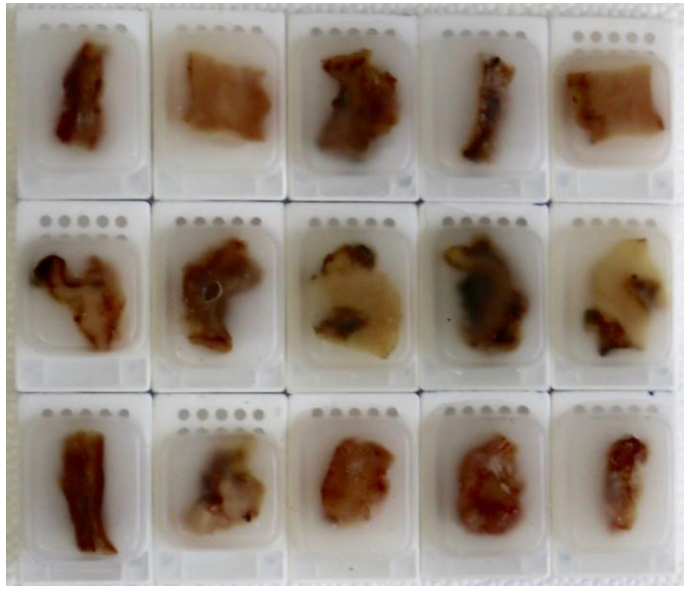
stainless steel histological base mold

Embedding

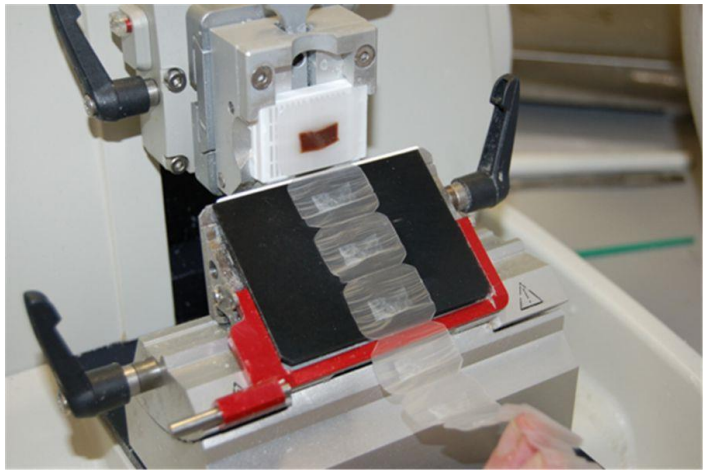
- After sufficient time, the cassette and mold should be separated and the paraffin block should be placed in the microtome in preparation for sectioning.

- If the tissue has been thoroughly fixed, dehydrated, cleared, and infiltrated, tissues embedded in paraffin wax provide good cutting qualities.

- On average, paraffin blocks remain durable and retain their good cutting qualities and staining characteristics indefinitely.



Embedding tissues in paraffin blocks



One of the blocks fixed on the microtome and sectioning

Other Embedding media

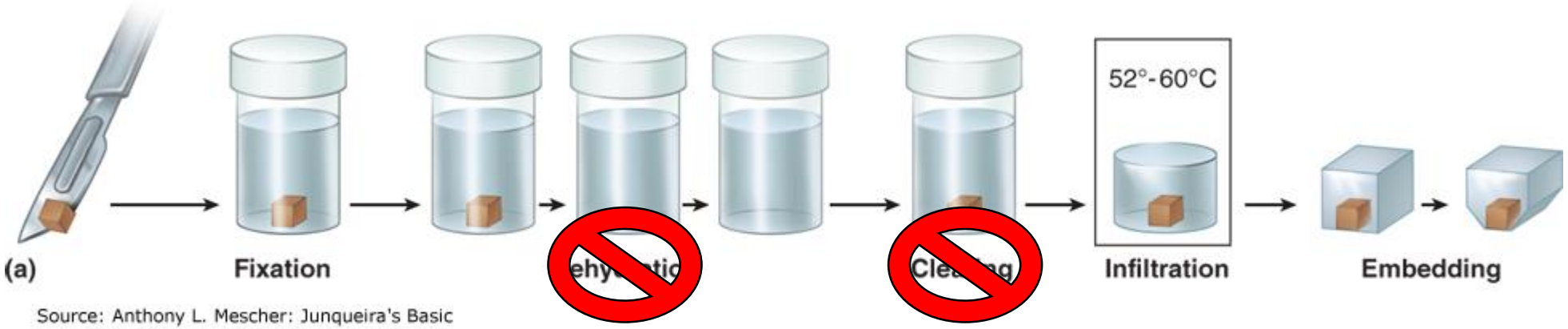
- The most common infiltrating agent and embedding medium is paraffin wax .

- Ester wax offers a lower melting point than paraffin wax and tends to be harder when solid, allowing this medium to be suitable for cutting thinner (i.e., 2–3 µm) sections with minimal tissue shrinkage.

Other Embedding media

- When water-soluble waxes (i.e., polyethylene glycol waxes) are used, tissues are transferred directly from aqueous fixatives to wax for infiltration without dehydration or clearing.

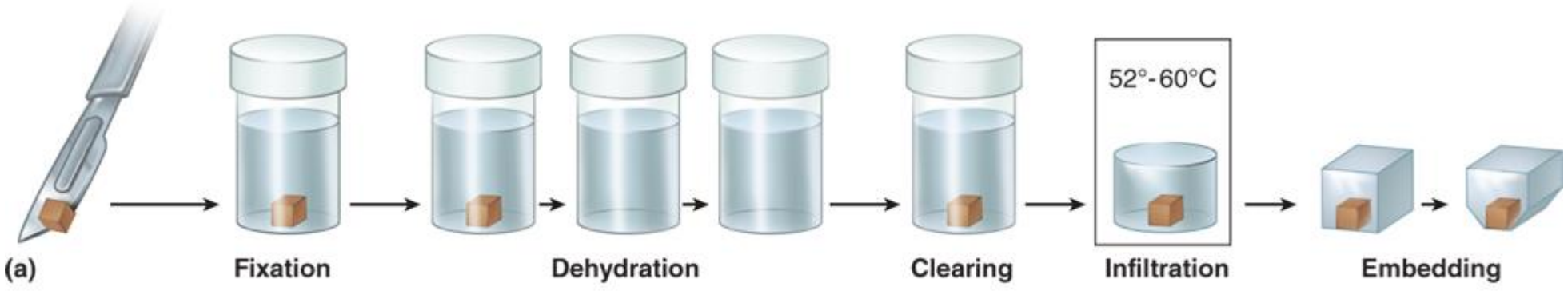
- But sectioning is more difficult than with paraffin wax.



Other Embedding media

- If cellulose nitrate (i.e., celloidin/low-viscosity nitrocellulose) as an embedding medium, tissues must be dehydrated and embedded with solutions of cellulose nitrate dissolved in an alcohol/ether mixture.

- This medium is used typically for large pieces of, for example, bone and brain tissues.



Microtomy

- Microtomes are used to cut the tissue into thin sections for microscopic viewing.
- The type of specimen will determine the type of microtome to be used.

- Microtomes have a feed mechanism to advance the specimen (or knife) to a predetermined thickness for sectioning (i.e., typically 5–10 µm) and can produce serial sections.

https://www.youtube.com/watch?v=KnMdSgd5mts

Cryostat

- A cryostat or freezing microtome is used for obtaining thin sections of unfixed tissues.
- It can be used, additionally, for observing fatty tissues.
- The microtome is maintained at −15 to −20°C in a refrigerated chamber.

- The tissue block can be mounted in a high-viscosity water-soluble gel, such as 1% glucose, gelatin, or cellulose on the platform and must be frozen immediately.
- Sections are fixed in 5% acetic acid in absolute alcohol and then subsequently stained (e.g., with hematoxylin and eosin). Frozen sectioning is typically used for rapid preparation and diagnosis by a pathologist.

https://www.youtube.com/watch?v=d43LFVV3h6w