

262 Zoo- Microtome knives and problems encountered with sectioning tissue blocks

Lecture 6

p 18-21

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Microtome knives

KNIFE DESIGN AND CUT TYPES

- There are many different types of microtome knives (e.g., stainless steel, carbide, diamond, glass, or disposable blades)
- Wedge-shaped stainless steel knives are used for most paraffinembedded specimens.
- kept clean and well-oiled or lubricated.

Knife Profiles



Planar Concave



Wedge



Chisel shaped

◉ Planar concave

- extremely sharp, but are also very delicate and are therefore only used with very soft samples.

◉ wedge profile knives

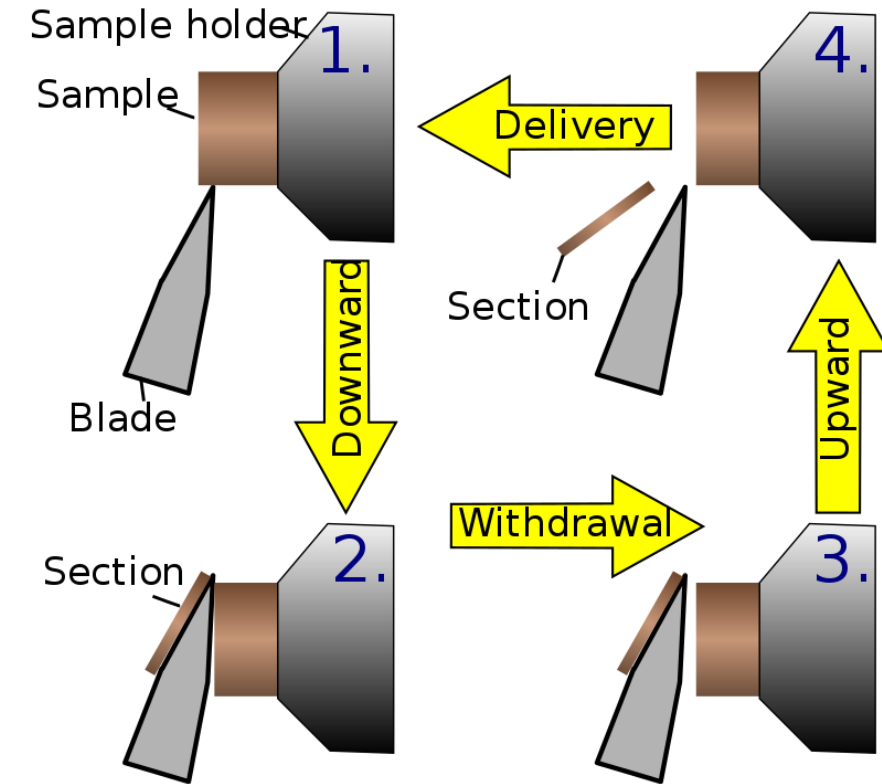
- more stable and find use in moderately hard materials, such as in epoxy or cryogenic sample cutting

◉ chisel profile

- with its blunt edge, raises the stability of the knife, whilst requiring significantly more force to achieve the cut.

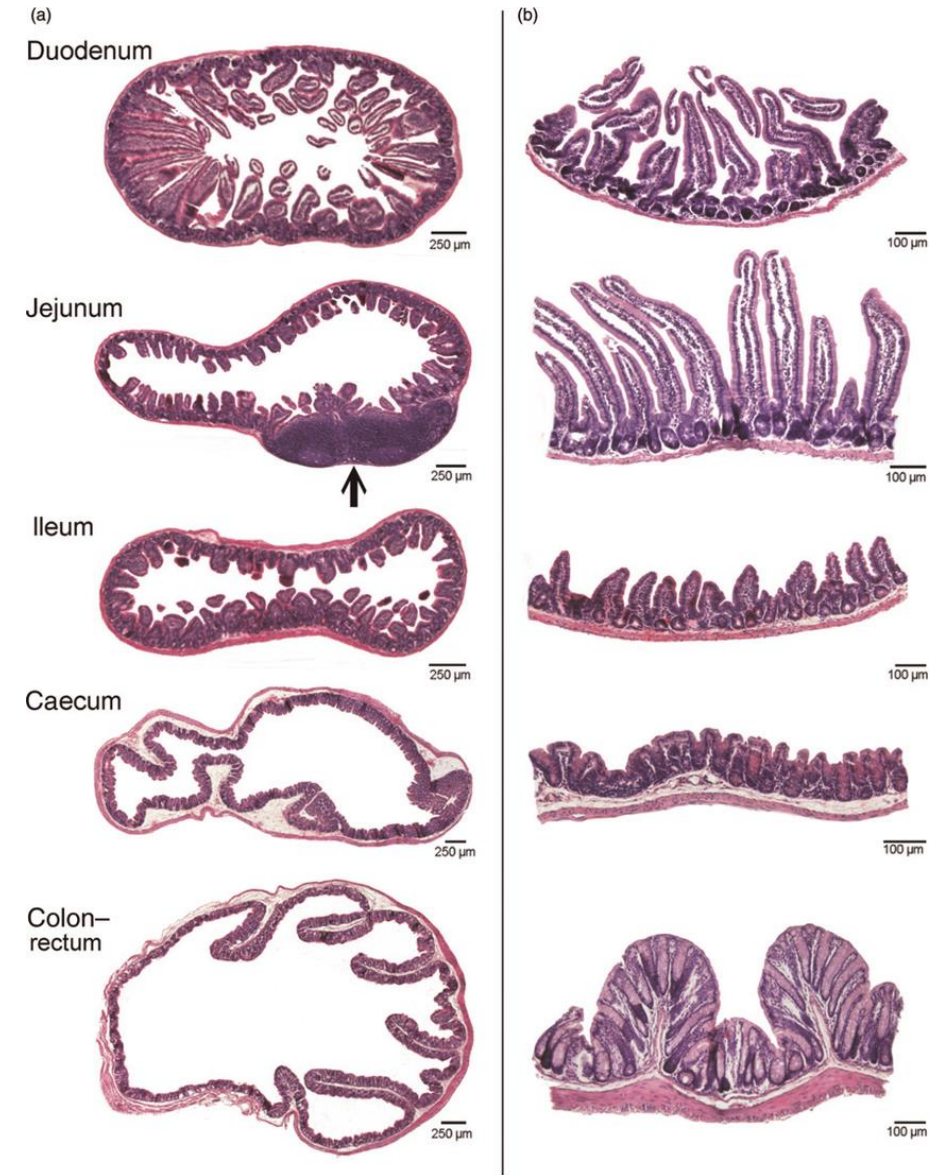
Microtome knives

- As an alternative to wedge-shaped stainless steel knives, disposable blades provide an excellent cutting edge for paraffin sectioning and are available in different sizes and thicknesses.
- Glass, sapphire, and diamond knives are used for specimens embedded in hard resin plastic (e.g., epoxy, glycolmethacrylate).



Section thickness and rough cutting

- A thickness of 6 μm is standard for histological tissue sections.
- For highly cellular tissues (e.g., lymph nodes), 4 μm is used most often. For thicker sections, 10 μm is used.
- For neurological tissues, 6–20 μm and myelinated nerves and 15–20 μm .



Section thickness and rough cutting

- Excess paraffin should be trimmed away from each side of the tissue block to create a trapezoid shape. The longer edge should be parallel with the knife edge.
- The tissue block should be roughly cut by advancing the block manually and sectioning until the entire surface of the tissue is exposed.



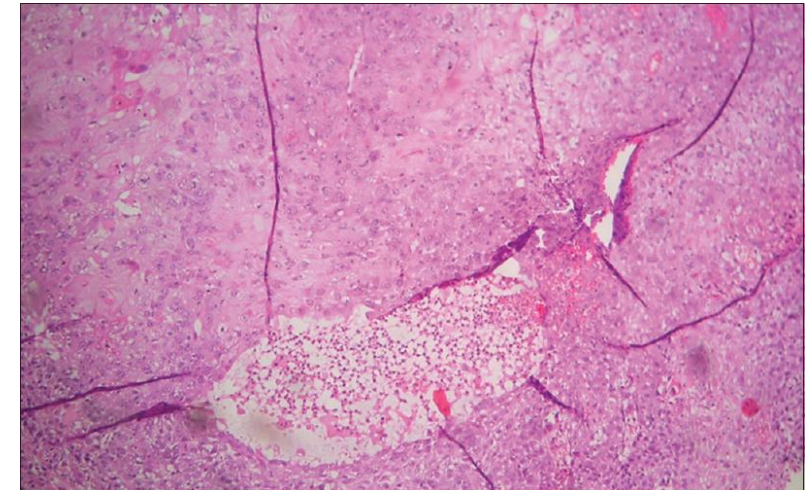
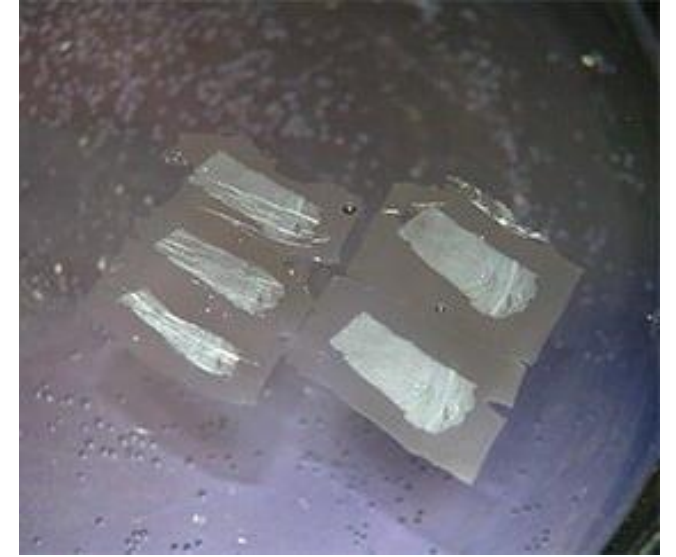
Section adhesives, sectioning tissues, and sealing of blocks

- Section adhesives, such as gelatin, casein glue, starch, and albumin.
- Gelatin can be added to the water bath.
- Daily cleaning of the water bath with sodium hypochlorite solution (e.g., Clorox soap) is necessary to prevent contamination.
- Alternatively, a thin coat of albumin or treatment of the slide with a reactive silicon or polylysine compound chemically changes the glass.



Section adhesives, sectioning tissues, and sealing of blocks

- If the sections are wrinkling, a 70% alcohol solution can be added to the water bath prior to section collection.
- Once the desired sections have been cut, the block can be removed from the block holder and sealed with molten paraffin wax to ensure that the tissue will not dry out and become brittle (blocks can last for weeks, months, or years).

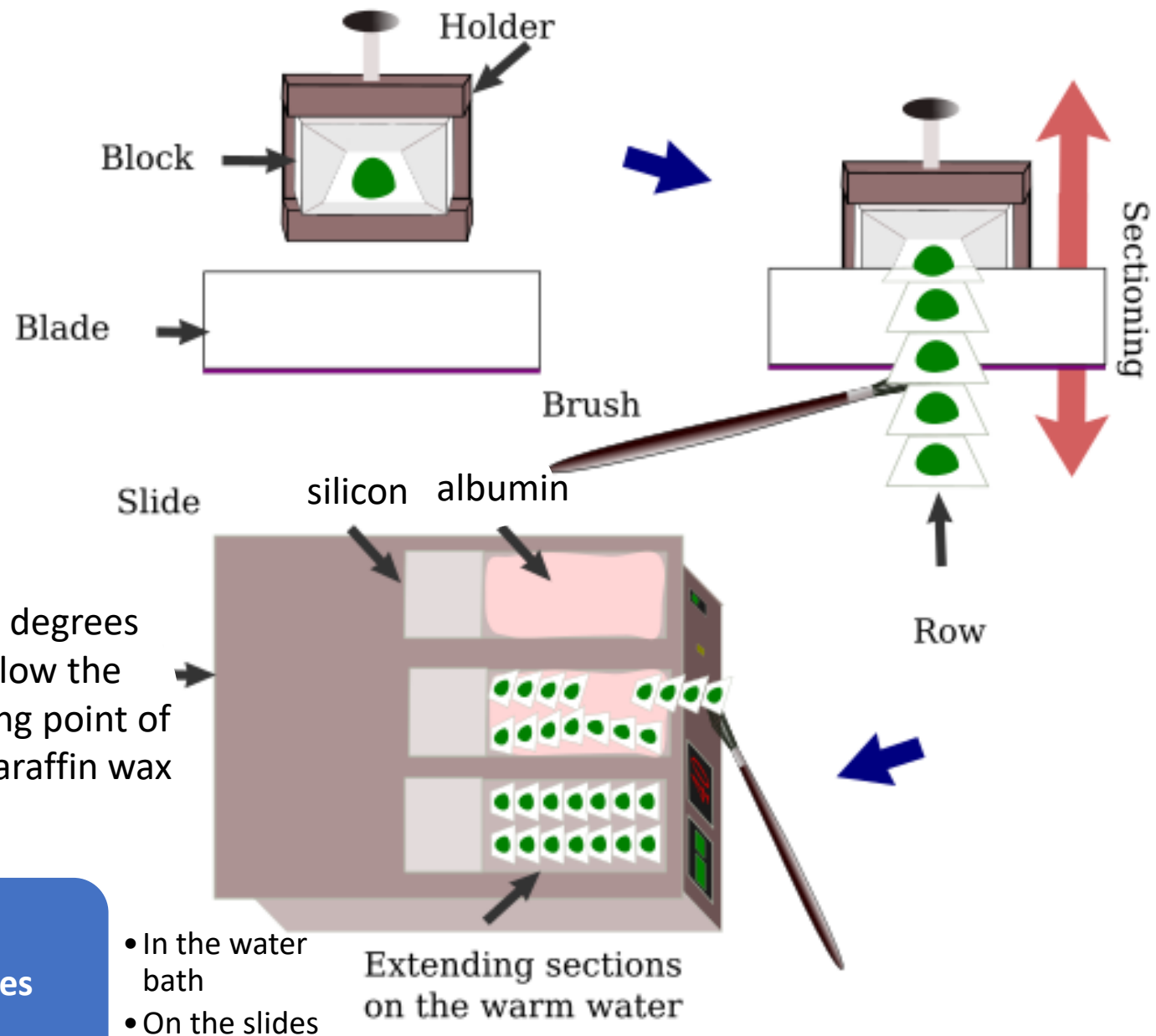


Embedded block

Trimming block
>> how it is
oriented and
shape?

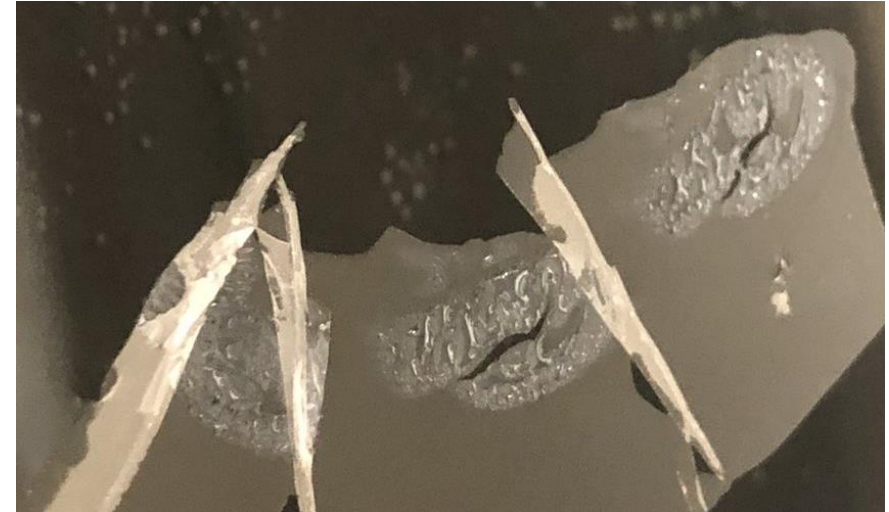
Cutting the
block>>type
knife and
thickness

adhesives



Problems encountered with sectioning tissue blocks

- what will make the sectioning not easy?
- for example, brittle or shrunken tissue, improperly infiltrated tissues, or sections with, for example, **holes or scratches in them**>>>>> **fix it by adding 10% diluted ammonium hydroxide solution (soaking).**



Problems encountered with sectioning tissue blocks

- If sections have holes in them, this can be indicative of incompletely infiltrated tissue >>> fixed by placing the tissue block back in the heated wax bath to melt it and then proceed to re-embed the block.
- If artifactual scratches or tears occur across the tissue sections, caused by indicative of flaws or dirt on the cutting edge of the knife >>> fixed by repositioning or replacing the blade.

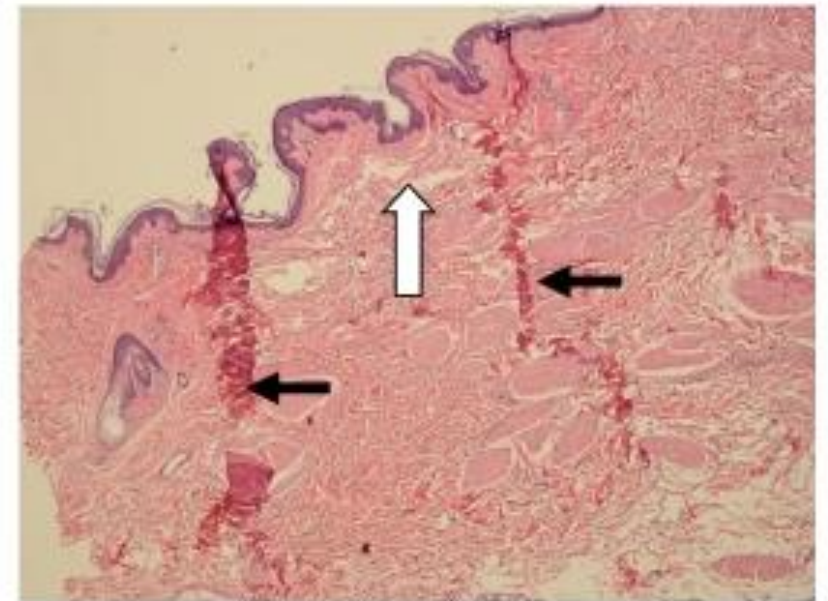
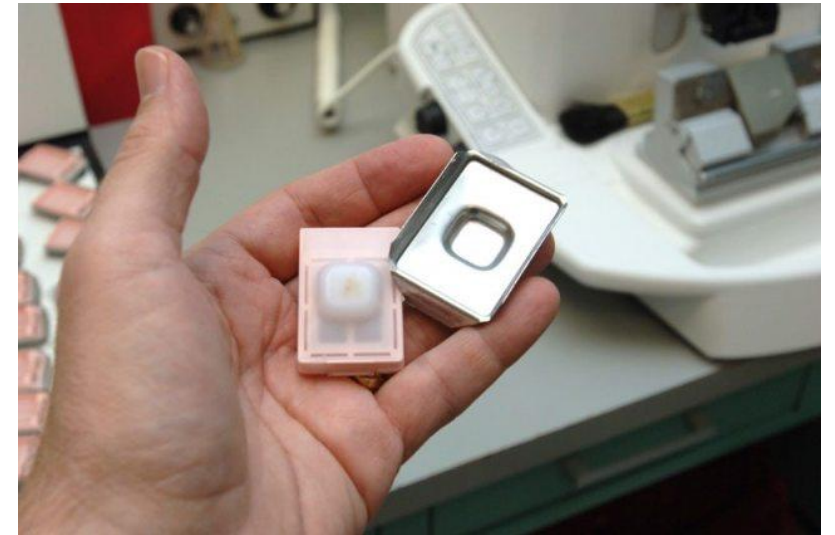


Figure 1. Skin, H&E stain
Original magnification x100
Solid arrow – wrinkles
Open arrow - tear

Problems encountered with sectioning tissue blocks

- The tissue block appears to be **too soft** or **too hard**.
- If **too soft**, a remedy may be to place the block tissue side down on several sheets of **Kimwipes or paper towel in the freezer (-15°C) or a refrigerator ($0-4^{\circ}\text{C}$) (chilling times may vary), prior to sectioning.**
- If **too hard**, a piece of wet cotton/Kimwipe may be placed in **lukewarm water and then placed over the surface of the block (times may vary)**.



These solutions are temporary and may allow only a few successful sections to be cut.