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VT 1200S Customer Training

Introduction & Sectioning Technique
2024/06/20
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Agenda

VT1200S Introduction

Hardware, operation & maintenance

Sectioning technique

Hands-on practice

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VT1200S Introduction

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What is Vibratome?

- A Vibratome is a microtome with a vibrating blade to section soft tissue under physiological conditions
- Special sectioning technique: the blade vibrates laterally and moves slowly forward through the specimen to reduction of cutting forces
- Limitation in section thickness compared to cryostats or rotary microtomes
  - Fixed tissue – down to 10µm
  - Unfixed
  - Fresh tissue mainly 100 – 300µm

Why using a Vibratome?

Advantage of sectioning under physiological conditions

- No thermal effect
- No chemical treatment necessary
- No embedding necessary
- Chilled physiological buffer surrounds the tissue while sectioning: Antigen protection / reduction of enzyme activity

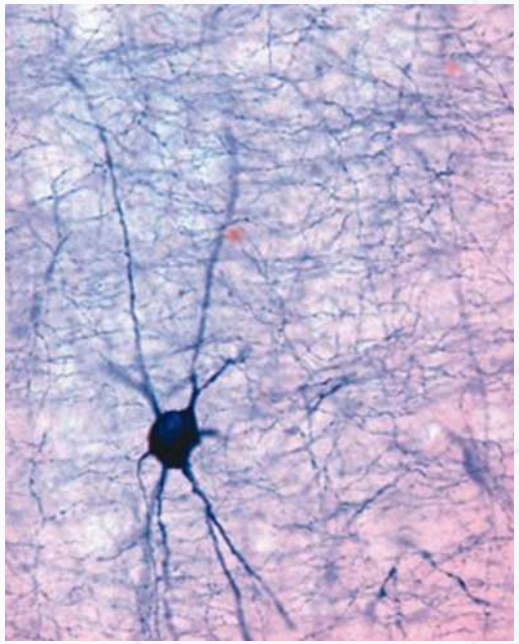
VT1200S – Intended use & Features

- RESEACH USE ONLY! Not for diagnosis!
- For SOFT fixed / unfixed tissue
- Knife holder technology: magnetic
- Adjustable amplitude up to 3mm
- Fixed frequency (85 Hz)
- Clearance angle adjustable: 15 ° , 18 ° and 21 °
- Sectioning Mode: semiautomated and automated (single and continuous)
- Vibrocheck technology

VT1200S – Application

Soft fixed / unfixed tissue in buffer

- Brain tissue
  - Fixed or unfixed
  - Electrophysiology / patch clamping
    - Rather thick (min. 30µm) sections needed
    - Vibrocheck technology ensures that the blades moves absolutely parallel through the tissue
    - Living cells, even on section surfaces
  - Fluorescence work
  - Immuno-staining
  - Cell cultures
- Other tissue
  - Mainly cell cultures
    - Liver, Heart, Kidney



VT1200S – Application

- Vibratomes are not designed to produce “thin” sections
  - The advantage of avoiding embedding / freezing however limits the “thinness” of the section – for living tissue to record cells – too thin sections would result in dead cells (cell size approx. 30µm)
  - If you are looking for thin sections below 10µm – we recommend:
    - rotary microtomes (embedded tissue)
    - cryostats (frozen tissue)
    - sliding microtomes with freezing attachment (fixed – frozen tissue)

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VT 1200S Hardware & operation

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VT1200S – Hardware overview

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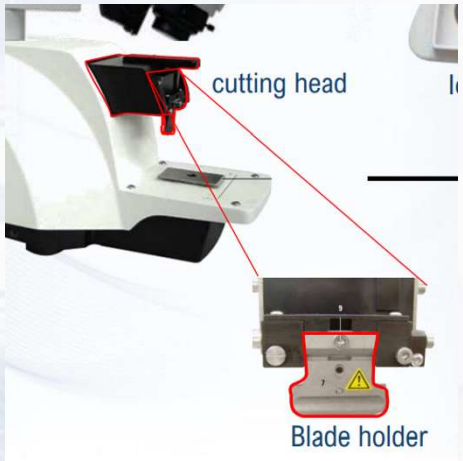
Socket (right hand side)

Magnifier (add-on)

Microscopy (add-on)

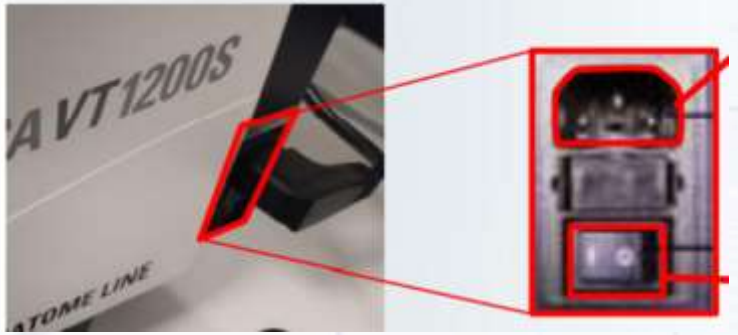
Adjustable 2-arm LED light (add-on)

Cutting head



Blade holder

Power socket & power switch

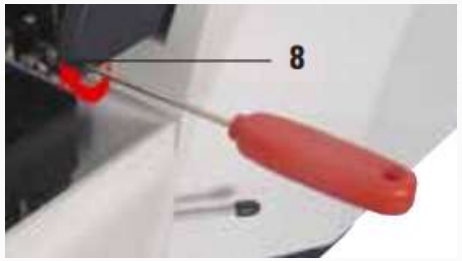


Control panel

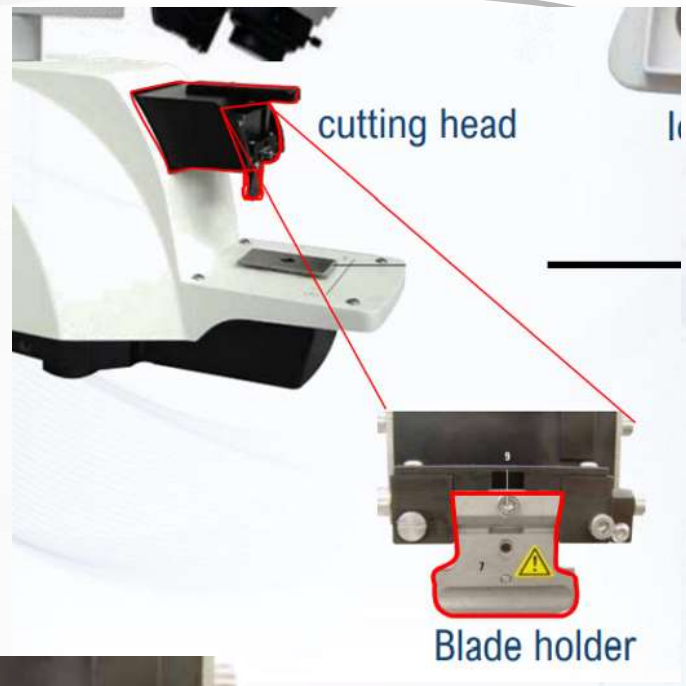
Buffer tray and ice bath

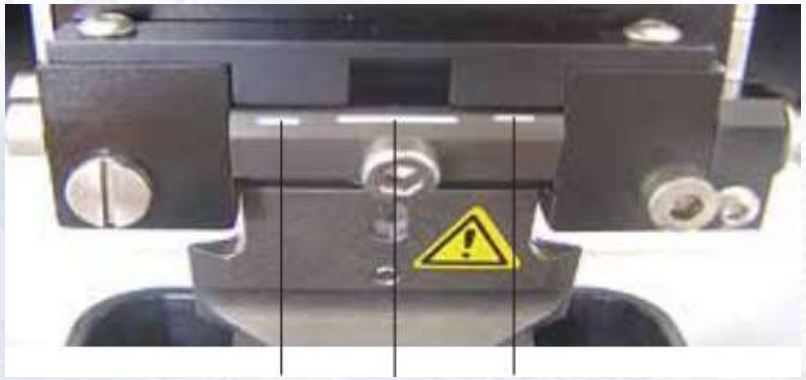
Hardware – Cutting head & blade holder

- Magnetic knife holder system
- Specimen retraction
- Usable for razor: injector /sapphire blades
- Clearance angle adjustable: 15 ° , 18 ° and 21 °
  - Recommended setting: 18 °
  - For 15°, the effective clearance angle is "0"

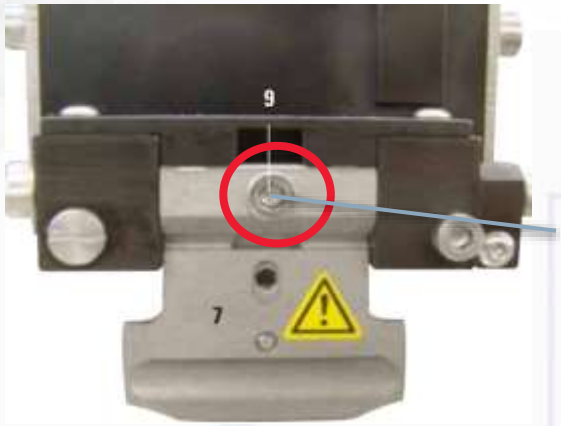


Hole for tile up blade holder





15 ° 18 ° 21 °

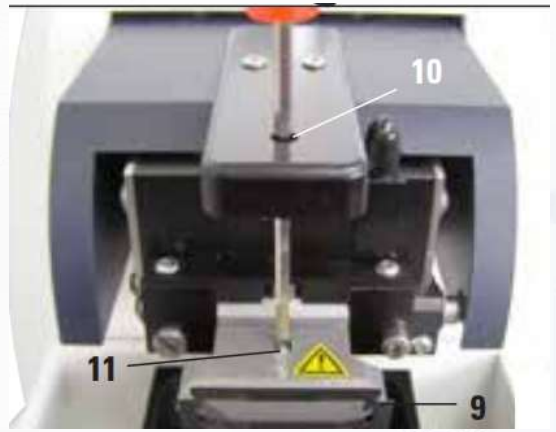


Hole for remove the blade holder

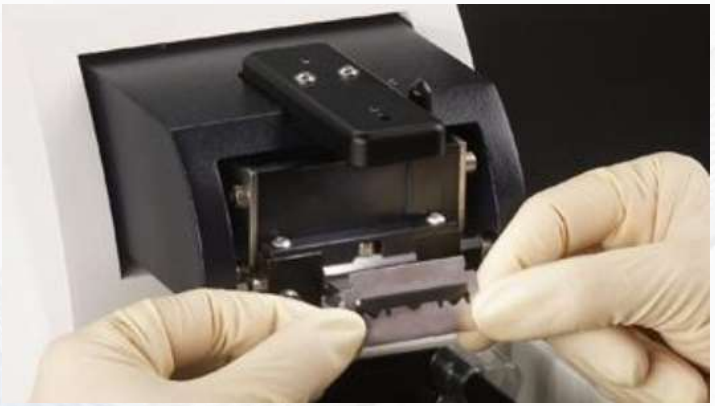
Hardware – Insert a blade

1. rotate it 90° clockwise (tile up) with allen key

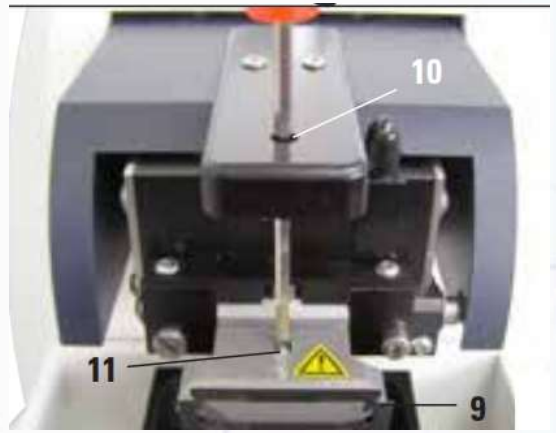
2. insert allen key from the top through the opening (10) into the blade holder (11) and open the blade holder



3. Hold the entire razor blade (not separated) on the left and right with both hands and insert it into the blade holder. Hook in the blade over bottom pressure plate



4. insert allen key from the top through the opening (10) into the blade holder (11) and tighten the blade holder

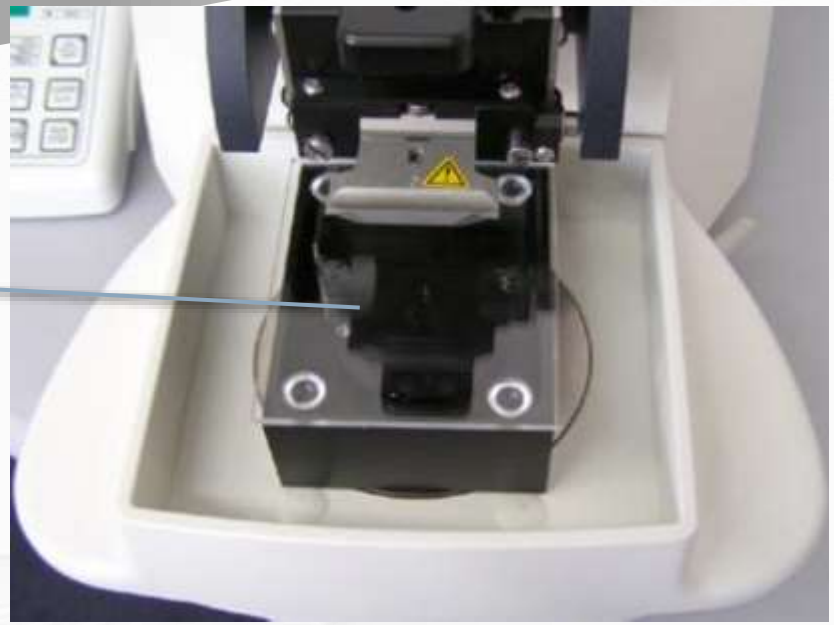


Hardware – Specimen plate, Buffer tray & Ice tray

Metal buffer tray (cannot be autoclaved)

Plastic buffer tray (can be autoclaved)

Lid



Ice tray

Manipulator and specimen plate

Hardware – Install ice tray & buffer tray

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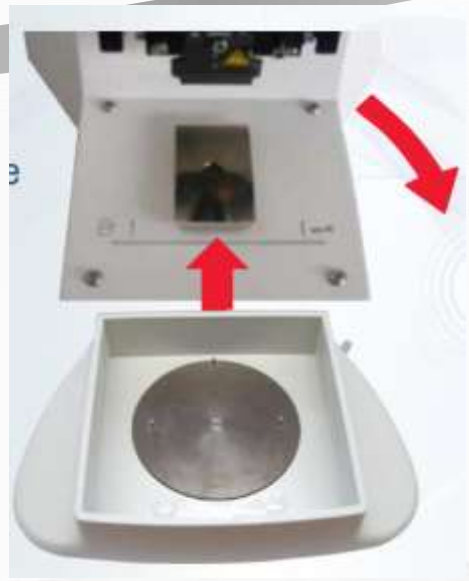

1. Insert buffer tray onto ice tray
2. Cover the buffer tray with the acrylic lid
3. fill the ice tray with crushed ice
4. Uncover the buffer tray and fill it with cooled buffer solution
5. Pulled the ice tray forward for clamping on device
6. Clamp it down by pushing the lever towards the rear

Hardware – Control panel

In semiautomatic mode, the desired section thickness is selected with Knob2. Values appears in µm. After each sectioning process has been completed, the µm display is set to "0"

SPEED:
blade feed rate
0.01 to 1.5 mm/s

AMPLITUDE:
0 to 3 mm

***AUTO FEED:**
max. 1000 µm

LIGHT ON/OFF

ROTARY KNOB 1:
For speed, amplitude, auto feed

ROTARY KNOB 2:
For moving specimen receptacle vertically

CLEAR:
Display for the current specimen holder position / reset the display to 0 at any point

STEP SIZE (use with knob 2)
Choose 1, 10 or 100 µm

MENU:
User profile

Hardware – Control panel

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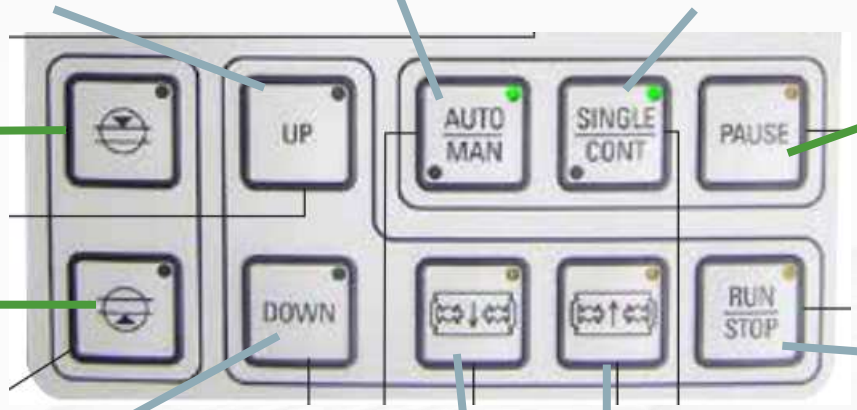
UP:
Press and hold: specimen receptacle moves upward
Release: stop moving
lights up when pos. is the highest

AUTO / MAN:
Switch between auto and manual (semiauto) sectioning

SINGLE / CONT:
Switch between single and continuous sectioning

PAUSE:
Stop immediately
Press again to restart the current process
Press RUN / STOP to discontinues the sectioning process

***CUTTING WINDOW:**
Set the cutting path edge
Holding the key down for 3s to activate / deactivated
LED lights up when edge is set



DOWN:
Press x1: specimen receptacle auto moves to lowest pos.
lights up when pos. is the lowest
Press again: stop moving

BLADE FORWARD / BACK:
Press and hold: blade moves forward / back
LED lights up when reach the end point

RUN / STOP:
Press to start
In CONT mode, blade will stop after the current sectioning is done

Operation – Retraction between auto & semiauto sectioning

What is retraction?

- specimen is lowered to the desired retraction value after each cut to prevent the specimen surface and the blade from coming into contact while the blade is being retracted.
- **Recommended for section soft tissue**
- **In semiautomatic sectioning mode**, a manual feed to the desired section thickness must be carried out before each cut. There is no automatic specimen retraction; however, retraction can be performed manually.
- **In automatic mode**, an automatic feed (AUTO FEED) to the selected section thickness is carried out before each cut, and the specimen is lowered to the desired retraction value after each cut.

Operation – Automatic sectioning

1. Specimen preparation (Put specimen in buffer tray in ice tray)
2. Switch on VT1200S
3. Tile up blade holder to insert the blade,
4. Load the ice tray (with specimen inside) to VT
5. Tile down blade holder and to the desired clearance angle
6. Choose auto mode & single / cont, set speed, amplitude, auto feed thickness
7. According to the specimen position, set the cutting window and specimen receptacle position
8. Start the run
9. When finish sectioning, lower the specimen receptacle, replace with another specimen plate with the manipulator or remove the whole ice tray
10. Remove the blade

Operation – Semiautomatic sectioning

1. Specimen preparation (Put specimen in buffer tray in ice tray)
2. Switch on VT1200S
3. Tile up blade holder to insert the blade,
4. Load the ice tray (with specimen inside) to VT
5. Tile down blade holder and to the desired clearance angle
6. Choose semiauto mode, set speed, amplitude
7. According to the specimen position, set specimen receptacle position (=thickness) and blade position
8. Start the run
9. Lower a bit the specimen receptacle with knob 2, move the blade back, move up the specimen receptacle (µm display is set to "0“) (manual retraction)
10. Repeat the step 7 to 10
11. When finish sectioning, lower the specimen receptacle, replace with another specimen plate with the manipulator or remove the whole ice tray
12. Remove the blade

Maintenance

Before each cleaning, carry out the following preparatory steps:

1. Switch off the main switch on the side of the instrument.
2. Place the magnifier cover on the magnifier.
3. Remove the blade from the blade holder and dispose of it safely.
4. Pull the ice tray and buffer tray off of the dovetail guide and place them on the stage.
5. Remove and empty out the buffer tray. Dispose of the contents of the buffer tray properly.
6. Remove the specimen plate and lay it flat on the stage.
7. Remove the specimen using a single-sided blade and remove any cyanoacrylate adhesive residue from the specimen plate.

Maintenance

- Instrument & outside surfaces
  - Cleaned with a mild commercial household cleaner or soap water (DO NOT USE SOLVENT CONTAINING XYLENE / ACETONE) and then be wiped with a cloth.
  - completely dry before use
- Specimen plate / buffer tray / ice tray / Allen key / manipulator
  - Wash thoroughly with de-ionized water and finally with 100% Ethanol
  - Dry the parts on a tissue paper before re-installing them
- Cleaning the blade holder
  - Remove and spray with alcohol. Wiped down with a piece of cellulose and placed on a cellulose towel to dry completely
- Cleaning the sapphire blades
  - using an alcohol-based solution or acetone

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Sectioning technique

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Sectioning technique

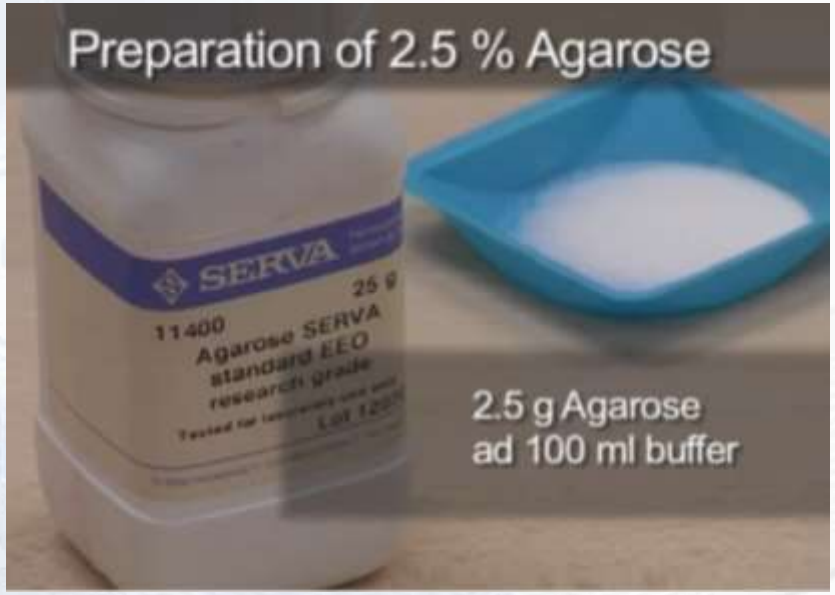
- Sectioning speed
  - select a moderate knife travel forward speed to avoid that the specimen is pushed while sectioning
  - The “Rule of thumb” is that softer tissues require slower forward speed, higher amplitude and frequency, steeper blade angle and cold buffer
  - Fresh brain tissue: amplitude 1.5 - 1.8 mm / speed: very slow
  - Fixed brain tissue: amplitude 0.8 –1.0 mm / speed: slow
  - Liver: amplitude 3mm / speed: 10µm/s –the slowest speed needs to be used
- Sectioning under physiological conditions
  - work with cold buffer
    - Firm specimen is easier to be cut
    - Crushed ice can be used to keep the buffer cooled or more elegantly, a double walled buffer with a circulation Julabo Chiller device
- When sectioning fresh tissue,
  - oxygenate the tissue while sectioning to ensure viability of the cells

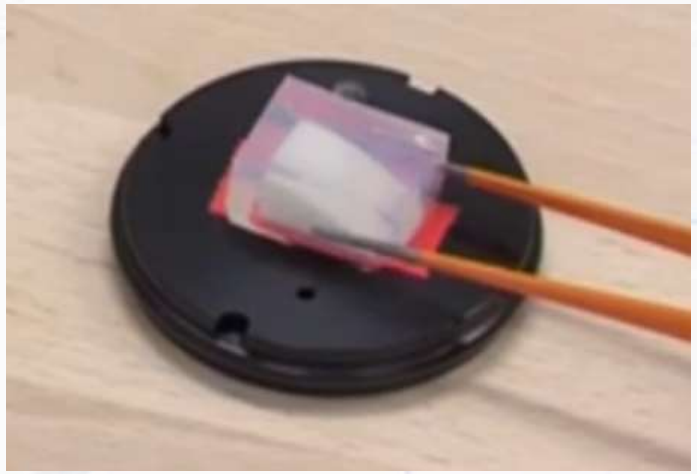
Sectioning technique

- Specimen preparation: Tape-mounted sectioning

Sectioning technique

- Specimen preparation: Agarose embedded block
  - For very small and soft tissue

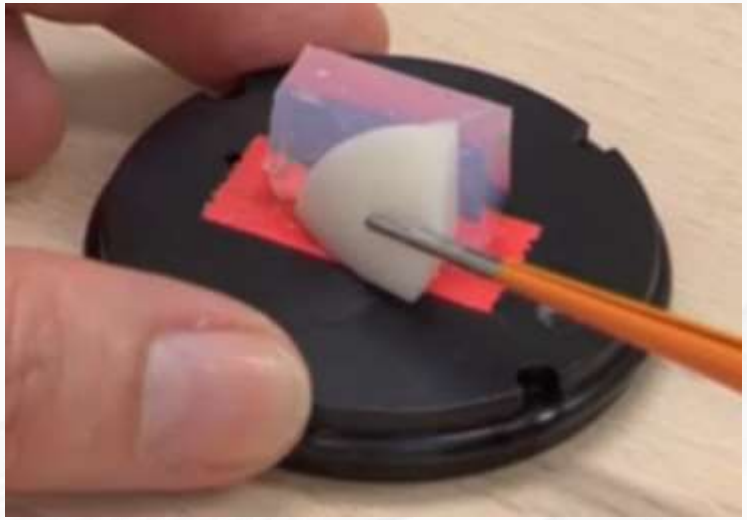


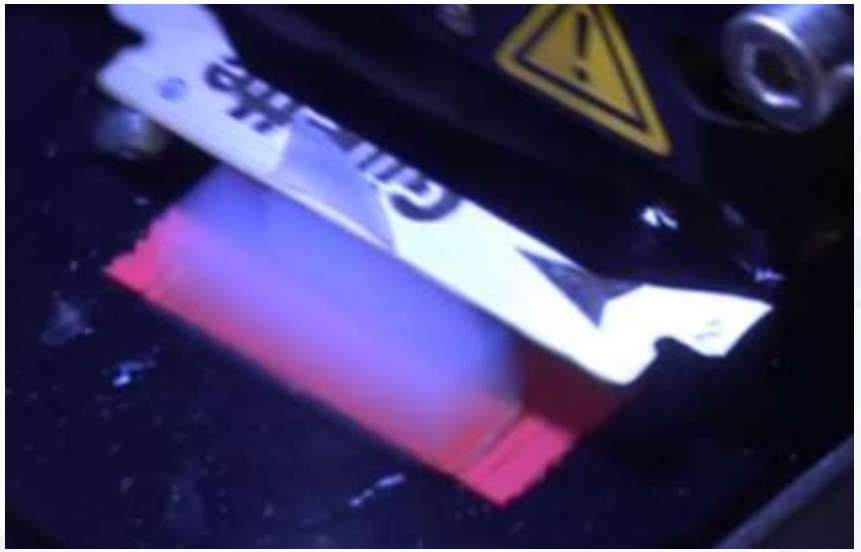


Recommended agarose with low melting point of 37 to avoid heating artifact

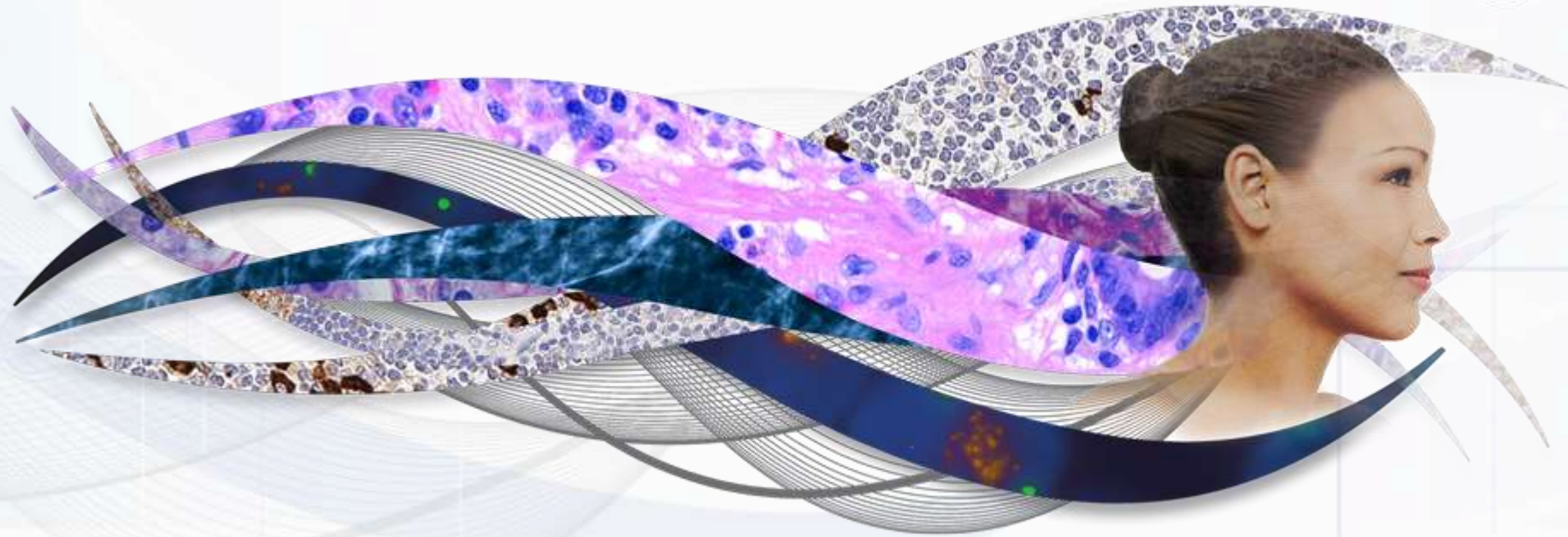
Sectioning technique

- Specimen preparation: Agarose barrier





Thank you



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