

Observations and Procedures on Vibratome Sectioning

4% Paraformaldehyde: Cuts best using low speed (1) and low amplitude (1) settings. Short time fixations seem difficult to cut, 6 hrs. appears to be the minimum fixation time for brainstem to get 30 μm sections. If having problems at 30 μm , shifting to 40 μm or 50 μm may help. Softer tissue usually needs higher amplitude with slow speed.

Bouins/Zamboni's: Cuts well at speed/amplitude settings between 1 and 5 (try 3 to start). Overnight fixation is often necessary for immersion fixed tissues. 30 μm sections are easily obtained.

Methacarn: Higher cutting speed/amplitude of 3 to 5 necessary. Does not cut well at 30 μm . Tissue seems to bend and resist more than other fixatives (even after 24 hour fixation). Spinal cord does not cut at all and there is irregular cutting in brainstem. This fixative does not harden tissue the way that aldehyde-based fixatives do.

Be sure to rehydrate brains before sectioning by removing the chloroform with 2 changes of absolute alcohol, then taking the tissue through 95% and 70% ethanols, then into buffer. The waste Methacarn fixative, containing chloroform, with the absolute ethanol washes, must be discarded in the Waste Chloroform jug.

Cutting angle:

There are general angles that usually work. The cutting angle usually only needs to be varied a couple of degrees in very small increments. Sections that alternate between skipped sections and overly thick sections may indicate low knife angle. A high angle is usually tearing and digging into the tissue. However, loose knives, clamps, tissue support blocks, poorly attached tissue and poor fixation will also lead to alternating skipped sections. Some combinations of tissue and fixation require different angles or settings for amplitude and speed (rate of blade advance).

Vibratome:

Knife angle - An angle in the range of 15 to 20° generally works best. Use the angle plate to determine the angle when you adjust. There is a small metal pointer on the blade holder that points to the angle.

Leica VT-1000:

Knife angle – The angle indicator is a small metal bar that extends upwards at the right hand side of the blade clamp. There are 3 small holes in the bar. The leading edge of the knife holder frame should bisect the middle hole. Small variations in angle by aligning to each edge of the middle hole are usually sufficient to ensure consistent cutting.

