

**Troubleshooting:** Common issues encountered during the workflow of tissue embedding, sectioning, microdissection, and DNA extraction, with likely causes, corrective actions, and preventive measures.

| Step / Problem  | Likely cause(s)  | Corrective action  | Preventive measure   |
|---|--|--|--|
| <b>OCT embedding – bubbles or tissue floating</b>     | OCT poured too fast; residual RNAlater; air trapped beneath tissue | Gently reposition tissue; tap mold; repour OCT if large bubbles persist          | Blot RNAlater tissues thoroughly; pour OCT slowly along mold edge        |
| <b>OCT block cracks during storage</b>                | Direct exposure to freezer air; rapid temperature change           | Re-embed if possible; discard cracked regions                                    | Wrap in aluminium foil; maintain cold chain during transfer              |
| <b>Cryostat sections curl / wrinkle</b>               | Incorrect temperature; dull blade; anti-roll plate misaligned      | Adjust cryostat temp (–20 to –25 °C); replace blade; recalibrate anti-roll plate | Use new blade per specimen; equilibrate block before cutting             |
| <b>Sections detach during staining</b>                | Non-adhesive slides; incomplete drying                             | Use Superfrost™ Plus slides; ensure ≥10 min air-dry before fixation              | Pre-label and pre-cool slides; standardize drying time                   |
| <b>Uneven staining or washed-out eosin</b>            | Over-rinsing; aged reagents  | Reduce rinses; use fresh solutions   | Avoid rinsing after eosin; replace stains monthly                        |
| <b>Poor cryostat adhesion between block and chuck</b> | Chuck too cold   | Warm chuck to RT before adding OCT drop  | Always use room-temp chuck to ensure bonding                             |
| <b>Macrodissection tissue mis-oriented</b>            | Inaccurate alignment to pathology slide                            | Compare to WSI; mark orientation before cutting                                  | Use visible alignment mark on block and print WSI with orientation arrow |
| <b>LCM tissue burning or poor catapulting</b>         | Laser overpowered; thick section (>15 µm); poor focus              | Reduce laser energy; verify focus; adjust slide position                         | Maintain section thickness ≤15 µm; recalibrate laser daily               |
| <b>Cross-contamination between samples</b>            | Blade reuse; cryostat not decontaminated; mislabeled slides        | Replace blade; wipe surfaces with DNA-OFF; verify IDs before each run            | One blade/sample; record decontamination log                             |