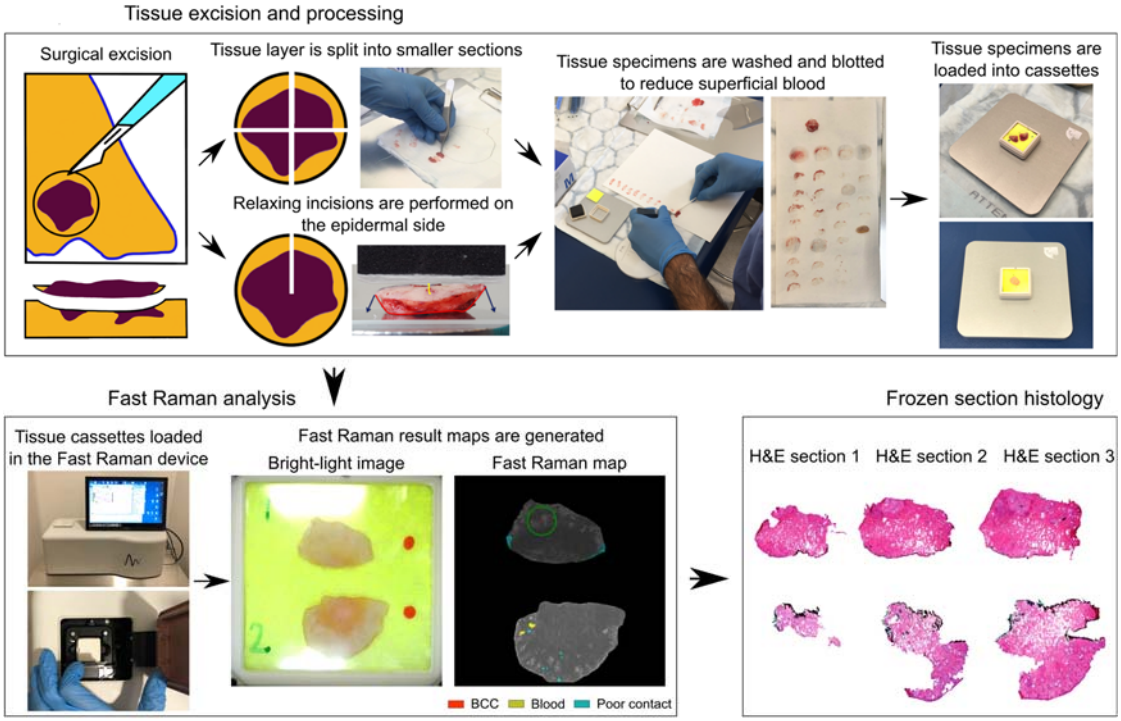
Fast Raman instrument operation and sample preparation protocol

Total Fast Raman measurement time: 30 minutes.
Total specimen pre-processing time (steps 3-9): < 5 minutes.

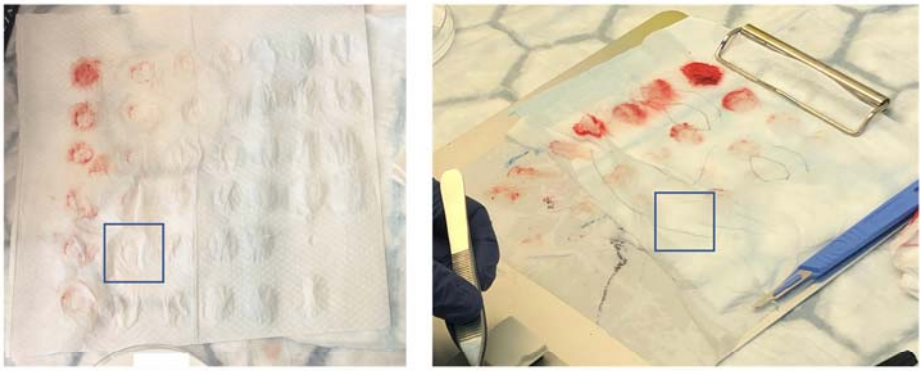
1. Resect tissue specimen from patient as per standard Mohs procedure.
2. Perform 2-3 mm deep knicks on the edges of the tissue specimen (2-4 knicks depending on the size and structural integrity of the specimen).
3. Immerse specimen in 1X RBC lysis buffer for 10 seconds.
4. Blot specimens with tissue paper in order to remove the majority of the superficial blood. Blotting consists of applying downwards pressure with a finger on the epidermal side of the sample, with the sample being placed between two layers of tissue paper.
5. Alternate between immersion in RBC lysis buffer and blotting for a minimum of 10 times and a maximum of 25 times. After a minimum of 10 blots, blotting may stop if no further blood imprints can be observed on the tissue paper or if there is risk of damaging the tissue.
6. Immerse specimen in saline for 10 seconds to remove remaining traces of the RBC lysis buffer and debris.
7. Blot specimen one more time to remove excess saline.
8. Place specimens in the cassette with the epidermal side facing upward, away from the coverslip and the resection surface towards the coverslip, ensuring whey are within the working area indicated by the cassette loading tool. Ensure that the epidermis is in contact with the coverslip for all tissue samples.
9. Mark the cassette coverslip with coloured marker pens to indicate the sample ID and the edges that need to be stained for each of the specimens within the cassette.
10. Place the cotton substrate with the yellow, fluorescent side facing downwards on top of the tissue samples and close the cassette lid.
11. Tighten the cassette lid screws to increase the tissue surface area in contact with the cassette coverslip.
12. Load the cassette into the Fast Raman instrument with the coverslip facing downward, input the patent ID and specimen ID in the required fields and start a measurement.
13. After the measurement has completed, remove each specimen and mark it with positioning ink according to the markings on the cassette coverslip.
14. Send the samples to the histopathology lab to be processed for frozen section H&E.

Fast Raman measurement workflow



Fast Raman – Pre-processing examples

Examples of acceptable imprints to stop blotting process



Examples of clean specimens loaded in cassette

Tissue processing quick-checklist

1. Perform 2-4 ~2mm deep knicks for orientation.
2. Wipe off excess blood with tissue paper.
3. Immerse specimen in RBC lysis solution for ~10 seconds.
4. Blot specimen using tissue paper: 10-25 blots (alternate between blotting and immersion of specimen in RBC lysis solution).
5. Immerse specimen in saline solution for ~10 seconds and blot it one more time.
6. Place specimen in Fast Raman cassette.
7. Mark cassette with coloured marker pens, add fluorescent substrate and close cassette lid.