Tissue dissection: the missing link in modern molecular pathology

by Dr Reinhold Wimberger-Friedl

Histopathology has benefitted hugely from the digitization of whole slide imaging, allowing remote discussions (telepathology), image archiving which facilitates storage and retrieval, comparison of areas on different slides, and direct slide annotation. However, until recently, the selection of tissue from the slide for further downstream molecular analysis has still been a time-consuming, manual process. This article discusses the advantages of integrating digitally guided automated tissue dissection into the pathology lab workflow.

By 2040, the number of new cancer cases are expected to reach 27.5 million worldwide [1]. Molecular diagnostics is heralding a way forward, offering the promise that clinicians will be able to target treatment to suit a patient's specific biological status. Unprecedented advances in PCR and next-generation sequencing technology have seen these innovations become a mainstay for guiding personalized cancer therapy. On the histopathology side, artificial intelligence (AI)-based algorithms are now becoming available to assess tissue morphologies, detect and classify cancer subtypes and segment tumour areas.

Representation of AI-based automatic transfer of ROI marking to dissection slides
(Source: Xyall BV)

Yet, there is an unrecognized barrier to the drive towards personalized medicine: the actual process for tumour tissue selection. The increase in targeted therapies is driving up demand for molecular diagnostic tests to inform treatment decisions for cancer patients. These tests demand a certain sample quality that can only be achieved by dissecting selected areas from tumour tissue. Selecting tumour tissue samples for molecular testing remains a labour-intensive manual process, based on subjective judgements, with limited precision, risk of contamination and error. This critical link in the chain of precision medicine needs an overhaul to catch up with the advances made in other aspects of this process. In this article, we explore automated solutions that have emerged to turn a highly manual craft into a more precise science.

Dangers of subjective sample assessment

Molecular pathologists themselves have been calling for novel tools to enable them to deliver greater precision and accuracy. Recent global research confirms that accuracy and cross-contamination were two of the biggest concerns of molecular diagnostic labs worldwide [2]. A report on the findings indicated that with workloads for handling breast, lung and colon cancers set to increase exponentially over the next three years, labs are calling out for new solutions – identifying lack of automation of tissue dissection as a glaring gap in the otherwise automated process.

Current practices of manual dissection of tissue were seen as a hindrance to efficiency and turnaround time, with around a third of respondents wanting this process to be automated. Finding qualified people is the next biggest challenge, as was quality control.

In the traditional way of working, histopathologists review the tumour tissue under a microscope to identify regions of interest (ROIs). Using their experience, they then make an educated guess on the tumour cell percentage, marking the outline of these likely ROIs on a hematoxylin-and-eosin stained slide with a pen.

The lab technician manually transfers the annotated area from the reference slide to a number of unstained dissection slides, matching these by visual judgement. The dissection operation itself is often done with a scalpel, with the lab technician attempting to follow the annotation as accurately as possible. This process determines the cellular composition of the sample and therefore the reliability of the test results, and it falls short for several reasons.

Because manual dissection is time and labour intensive, technicians must work very carefully and precisely to safeguard the quality of the test results. This can be tedious work. There is already a worldwide shortage of technicians, with labs increasingly finding it hard to attract and retain experienced technicians. New ways of added-value working are required.

With the development of minimally invasive biopsy procedures, tumour samples are becoming smaller. However, for some cancers, e.g. lung cancer, biopsies are still invasive and painful because specimens are difficult to obtain. The tumour areas are typically very small, and the smaller the sample, the more challenging it is to draw with a pen on a glass slide with sufficient precision, let alone scrape a tiny area of

tissue. Poor tissue selection increases the risk of a false-negative result, with the patient then not receiving timely treatment.

Genomic aberration-based tests need a minimum amount and percentage of tumour cells in the sample, but currently there is no standard means to measure that. The contribution of the immune infiltrate can easily be underestimated owing to the comparably small size of T-cells. With immunotherapy becoming the treatment of choice for many advanced cancers, diagnostic tests targeting the immune compartment are expected to emerge and will require different, more precise ROI segmentation. Tests based on gene expression panels require high precision, as these tests are more likely to be affected by the overall cellular composition compared to tests merely based on the presence of a genomic aberration.

Need for automated dissection solution

Following best practice is largely left to the individual technician. Without a traceability system in place to record the steps taken, quality control is poor and variability is expected. If a lab technician needs to check something with the pathologist, this still tends to be done in person as annotations on the steps taken are not being digitally stored. Although remote working may be possible in other areas, this is less easy when dealing with physical slides.

An automated dissection solution, making it possible to obtain a digital image of the tissue slide, would transform how clinical labs carry out this process, improving traceability, reliability and quality. This would speed up workflow, making more effective use of experienced technicians while freeing up histopathologists to examine outlier samples that may need additional investigation.

Convenient case loading into an instrument supports efficient lab workflow
(Source: Xyall BV)

Although automated tissue dissection solutions [such as laser capture micro-dissection (LCM)] provide high precision for research purposes, they are not suitable for routine diagnostic workflows, as they require highly trained staff, are time-consuming to run and cannot fully integrate into the lab's workflow.

The effectiveness of digitally guided tissue dissection compared with the traditional manual tissue dissection method has already been highlighted by a study from the USA involving pancreatic adenocarcinoma specimens [3]. It found that the KRAS mutant allele fraction and the estimated neoplastic cell fraction were significantly higher in samples obtained by digitally guided tissue dissection. In almost a quarter of the samples, a detectable mutation could only be found by the digital method. This indicated the diagnostic potential for digitally guided tissue dissection, and its ability to improve testing capabilities and diagnostic accuracy.

However, for this technology to be adopted into routine clinical testing it must also operate efficiently into the lab's workflow and conform to cost requirements for molecular pathology labs.

Requirements for automated tissue dissection platforms
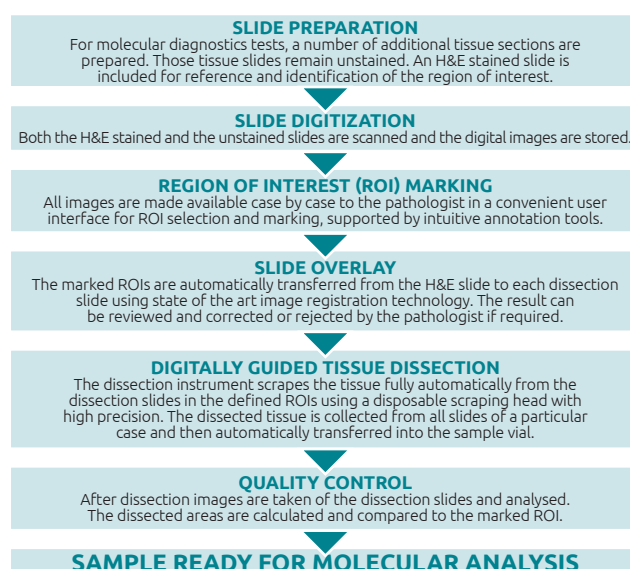
Workflow
Ideally, such a platform should allow the pathologist to annotate on a screen at any desired resolution. The marked ROIs would then be automatically transferred to the dissection slides and the selected tissue scraped into the sample tube by a robot with a disposable knife.

Additionally, such a device should also exploit the recent availability of image registration algorithms. These would be used to transfer annotations with a far greater accuracy than a technician carrying this out manually. The result could always be verified and adjusted by a pathologist where a second opinion is required.

Having registered digital images of the ROI allows the use of AI algorithms to calculate both the number and purity of tumour cells of the sample going into molecular testing. Such algorithms are available for research use already and should be integrated in the workflow. Having access to information on cellular composition is not only useful for verifying sample quality but provides the bioinformatician with vital data for analysing sequencing results.

Speed and precision
A platform designed for routine clinical practice would not need the high spatial resolution of LCM (<1μ), but an accuracy of <0.1mm would be appropriate for the application, and is more reproducible than manual dissection, particularly with very small specimens and samples with very small populations of tumour cells. Additionally, an automated device can run continuously, creating higher throughput than manual tissue dissection. Automatic disposal of the scraping head would minimise the risk of cross-contamination and the process should avoid the use of liquids to make it fully compatible with all molecular sample prep protocols. Figure 1 shows a schematic for an automated workflow.

SLIDE PREPARATION
For molecular diagnostics tests, a number of additional tissue sections are prepared. Those tissue slides remain unstained. An H&E stained slide is included for reference and identification of the region of interest.

SLIDE DIGITIZATION
Both the H&E stained and the unstained slides are scanned and the digital images are stored.

REGION OF INTEREST (ROI) MARKING
All images are made available case by case to the pathologist in a convenient user interface for ROI selection and marking, supported by intuitive annotation tools.

SLIDE OVERLAY
The marked ROIs are automatically transferred from the H&E slide to each dissection slide using state of the art image registration technology. The result can be reviewed and corrected or rejected by the pathologist if required.

DIGITALLY GUIDED TISSUE DISSECTION
The dissection instrument scrapes the tissue fully automatically from the dissection slides in the defined ROIs using a disposable scraping head with high precision. The dissected tissue is collected from all slides of a particular case and then automatically transferred into the sample vial.

QUALITY CONTROL
After dissection images are taken of the dissection slides and analysed. The dissected areas are calculated and compared to the marked ROI.

SAMPLE READY FOR MOLECULAR ANALYSIS

Figure 1. Workflow for automated histopathology
H&E, hematoxylin-and-eosin; ROI, region of interest.
(Source: Amended from Xyall BV)

Final comments
In other areas of the clinical lab, automated workflows have become the norm – replacing time-consuming, subjective and manual processes. Further, they make the best use of scarce and valuable staff resources. The overall result is increased efficiency, faster turnaround time, high-throughput sample processing, and increased reproducibility. If we are to get smarter about beating cancer, we need to change the way we handle test samples. Automated tissue dissection offers a compelling way forward.

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