Microdissection—An Essential Prerequisite for Spatial Cancer Omics

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The problem with cancer tissue is that its intratumoral heterogeneity and its complexity is extremely high as cells possess, depending on their location and function, different mutations, different mRNA expression and the highest intricacy in the protein pattern. Prior to genomic and proteomic analyses, it is therefore indispensable to identify the exact part of the tissue or even the exact cell. Laser-based microdissection is a tried and tested technique able to produce pure and well-defined cell material for further analysis with proteomic and genomic techniques. It sheds light on the heterogeneity of cancer or other complex diseases and enables the identification of biomarkers. This review aims to raise awareness for the reconsideration of laser-based microdissection and seeks to present current state-of-the-art combinations with omic techniques.

1. Introduction

Laser-based microdissection was first described in its present form in 1996 as laser capture microdissection (LCM),[1] and as the noncontact Laser microdissection and pressure catapulting (LMPC) technology in 1997.[2] In spite of its promising qualities it has not become a widespread or common technique in the following decades. According to publications listed in pubmed, microdissection reached a plateau with 70–80 studies in 2007. In the same year we speculated in our review “Microdissecting the Proteome”[3] that microdissection was soon to become a standard technique that would be used in a self-evident routine. We hypothesized then and are still convinced scientists understand that a spatially resolved genomics or proteomics is indispensable to elucidate complex diseases,[4] as it is necessary to reduce the complexity and important to use defined cells for analysis. Looking back, we believe that the reasons for the lack of a widespread implementation of microdissection remain the same: i) The necessary histopathological competence is not always available for the evaluation of tissue, particularly with microdissection. ii) Laser-based microdissection is time consuming. iii) Laser-based microdissection devices are expensive, although lasers are now available at lower prices. iv) Sensitivity of most downstream applications could be increased, terms like nano- or micro-proteomics were coined.[5] Nevertheless, in spite of increased sensitivity small tissue areas or even single cells remain problematic regarding sensitivity.

In this review, we will cite and discuss the appropriateness of starting material for microdissection, the microdissection process itself as well as compatible omic techniques with regard to sensitivity and their benefit. We will focus mainly on cancer as a paradigm for a complex heterogeneous disease.

2. Technical Approaches for Laser Based Microdissection

All microdissection approaches have a microscope coupled laser able to cut defined areas of a tissue section (Figure 1) in common. The transfer of the cut-out areas is solved in different ways. Figure 2 illustrates the results of downstream omic experiments with and without microdissection in a simplified sketch. It is obvious that the result without microdissection is at least misleading.

In the following we will describe the four different technical approaches.

2.1. Laser Capture Microdissection

The laser capture microdissection (LCM) was developed at the National Cancer Institute of the National Institutes of Health, Bethesda.[1,6] It allows the isolation of tissue areas or single cells from sections mounted on a glass slide. The main difference to other systems lies in the transfer of cut cells for further processing. In the actual version of the system special caps with a thermoplastic film are used. Stand-off rails keep the film separated from the tissue. An infrared laser extends the film to the cells. The adhesion force of the tissue to the activated membrane exceeds that off the glass slide and hence allows the selective removal of the targeted tissue fragment. Although the laser raises the temperature of the transfer membrane to 90 °C DNA or proteins...
in cells should not be affected. The minimum size theoretically limits the dissection precision, especially if single, irregularly shaped cells have to be removed without running the risk of contamination. However, many studies based on single cells obtained by LCM seem to be without contamination. The LCM system was used until now for several, mostly genomic, studies. In a more recent study it was used to analyze microbiota in crypts of colon cancer patients. The primary Arcturus LCM system is now distributed by ThermoFisher (thermo Fisher.com). A derived and enhanced system was launched by Fluidigm as AccuLift LCM in 2020 (www.fluidigm.com).

2.2. Laser Microdissection and Pressure Catapulting

With the laser microdissection (LMD) and pressure catapulting (LMPC; Zeiss PALM MicroBeam), technically developed by Schütze et al., the non-contact capture of specimen for functional DNA, RNA, and protein analysis became possible with the option to trace back results to the morphology of the investigated cell samples. This technology is now based on a diode laser with 355 nm and an optional optical tweezer that is coupled into a motorized inverse microscope. The laser first cuts the desired area. This area is then ejected from the object plane with a single laser pulse—generating a microplasma—against gravity into a microfuge cap. Depending on the desired cutting size and used objectives laser, spot sizes down to 0.5 µm in diameter are possible. This offers the possibility to select and analyze individual cells as well as entire tissue areas for different omic applications. Fluorescence and sophisticated software allow image analysis, navigation, cell recognition, and automatic sampling. Various targets, from parts of chromosomes to invadosomes and entire living organisms can be isolated. A unique feature of the LMPC technology of particular interest for proteomic research is the possibility to collect samples directly from routine glass-mounted tissue sections without the need of special preparation techniques. This is of high importance especially for formalin fixed and paraffin embedded (FFPE) tissue archived on glass slides for histopathological routine diagnostic. Nevertheless, in contrast to normal glass-mounted tissue, membrane-mounted sections allow the preservation of the specimen’s morphology after catapulting. In more recent studies LMPC was also combined with MALDI MS imaging (see Section 4.2.3).

2.3. Laser Microdissection

The LMD system of Leica instruments is technically similar to the Zeiss PALM system. Both systems use a UV diode laser, in case of LMD coupled in an upright microscope. Here the cut probe is not catapulted into the lid of a tube, but instead, overcoming adhesive forces, falling into a vessel underneath the slide. This process is also contamination free. The transfer process from membrane coated slides is non-destructive and cell morphology is retained together with the record of the cell’s original location. In the “Draw and Scann”-mode defined regions or single cells can also be transferred from normal glass slides. Until now several studies have been published using this system with a laser wave length of 355 or 349, respectively. The Leica LMD6/7 uses high-precision optics to steer the laser beam along the desired cut line on the tissue with prisms. In contrast to Zeiss PALM system, the sample table is not moved during the cutting processes. It is also possible to cut directly in real-time with the so called “Move and Cut”-mode. The LMD was used until now in numerous studies including forensic, plant research and different omic applications.

2.4. Laser Microbeam Microdissection

The CellCut laser based instruments from MMI AG (MMI—Molecular Machines and Industries, Eching, Germany) allow high-precision microdissection of samples applying the following process: First, the tissue is mounted on a glass slide and covered by a membrane. Next, the UV laser cuts areas of interest by “cold ablation”. An adhesive lid is then placed on the selected area and the dissected structures are mechanically lifted with the lid.

Further, this technology is especially useful for live cell manipulations. For this purpose, cells grown in a cell chamber
Figure 1. Principle of laser-based microdissection shown for an inverse microscope. A) The laser is coupled in the objective and separates the desired area from the surrounding tissue. B) The sample capture is initiated using a single laser pulse or by just falling down using an upright microscope. C) The sample is transferred to the desired capture device (modified from zeiss.com).

Figure 2. If a tissue sample is analyzed without microdissection the resulting proteomic profile is an intricate conglomerate of at least two different functional tissues. After dissection of different tissue areas an assignment of a specific peak (e.g., X and Y) can be made for tissue component A (normal tissue) and/or component B (tumor tissue).

are placed in a Petri dish coated with a special adhesive material to seal the dish. It is then put on the microdissection system and a laser is used as an optical tweezer to trap, move and position single cells. This laser technology has also been used to create DNA double-strand breaks in defined sub-nuclear volumes allowing the observation of DNA repair dynamics in live cells in different cancer studies.[23] Most recent studies deal with FFPE tissue,[24] DNA methylation,[25] and miRNA analysis Wilczynski.[26]

3. Tissue Samples

In general, tissue biopsies have to be handled with specific precautions to maintain the quality and to fix the current state of life. After surgery of human or animal tissue it has to be fixed by cold, ethanol, or formalin or a combination of either them and further processed by, for example, paraffin embedding. Each of these fixation methods is fit for different approaches conditioned only by the intended down-stream analysis. In the following we will describe the two most commonly used fixation techniques: FFPE and cryo fixation.
3.1. Formalin Fixed Paraffin Embedded Tissue

Formalin fixation with subsequent paraffin embedding is a universal method of tissue stabilization and preservation especially for light microscopy. It has emerged to the gold standard as it enables storage for decades under ambient conditions preserving both the histological and morphological architecture of tissue. Proteomic analysis of FFPE tissue therefore enables retrospective biomarker investigations of worldwide existing archives replete with pathologically characterized clinical samples. Nevertheless, at least for proteomic assays formalin fixation is problematic. It is a known fact that formalin mediates covalent cross-linking. However FFPE tissue can be deconvoluted by tryptic digestion and antigen retrieval as it is used in immunohistochemistry. Generally, the chemistry of formalin fixation is still not completely understood.

To deconvolute the FFPE material tissue sections were deparaffinized and trypsin digested leaving proteins available for downstream MS analysis. Interestingly Hood et al. could show that digested proteins contain only low amounts of formylated lysine residue. The oxidized methionine residues or peptides containing missed tryptic cleavages are not significantly different from those observed in proteomic studies of fresh cells.

Becker et al. used the commercially available Qproteome FFPE Tissue Kit from Qiagen to test FFPE for western blot and for protein microarray analysis. They succeeded in isolating non-degraded, soluble, and immunoreactive proteins and were able to detect membrane, cytoplasmic, and nuclear proteins at their expected molecular weight. Also, the protein yield and abundances seem to be equal in fresh frozen and formalin fixed tissues. It might therefore be ideal for microdissected tissue in conjunction with sensitive proteomic techniques. Nevertheless, results from cryo or formalin fixed tissue are not fully comparable. An attempt has been made to use ethanol instead of formalin (PAXgene tissue, Qiagen). It seems to be easier in handling, is not toxic and, most importantly, preventing covalent cross linking. During sample preparation lipids and small metabolites are washed out like in the case of the FFPE procedure and cannot be analyzed in downstream experiments. In case of genomic analysis formalin fixation is also not without problems as DNA quality and quantity decreases depending on the formalin concentration and incubation time. The need for improvement and standardization of using FFPE tissue for genomic and proteomic analysis therefore remains.

In conclusion FFPE material is a stable source for genomic and proteomic analyses. In contrast to frozen tissue it is extraordinarily good starting material for microdissection due to its superior fixed morphology. Furthermore, FFPE samples are the ideal source for retrospective studies with a large sample cohort.

3.2. Cryo Tissue

The best material for proteomic and genomic analysis up to now is freshly snap-frozen, unfixed and unstained tissue. The distribution, quantities, and structure of molecules of the actual in-vivo conditions are expected to be best represented by cryo sections. Cryo samples require less sample processing for genomic and proteomic analysis in comparison to FFPE tissue. The widespread use of cryofixed tissue is due to the fact that samples of frozen tissue can be easily pulverized and lysed in a mortar cooled with liquid nitrogen. Disadvantageous for subsequent microdissection is the poorer rigidity of morphology compared to FFPE tissue. It has to be kept in mind that disintegration of tissue starts at once when it is resected from animals or humans. Therefore a prompt fixation in liquid nitrogen—best with isopentan to better maintain the morphology—has to be carried out. Additional pre- and post-resection variables such as the time under ischaemic conditions during surgery is critical for molecular degradation. It was shown that 18S and 28S rRNA species were completely degraded after 4 h under ischaemic conditions at 37 °C. Protein expression changes can already be observed after 5–8 min after resection. Such parameters cannot be controlled during surgery but should be precisely recorded. Cryo samples need to be stored at −80 °C and can be utilized for several month. Thawing and freezing cycles are, of course, to be avoided. Until now most of the proteomic studies have been performed with cryofixed tissue or cryofixed cells. For reviews see.

4. Omic Techniques Compatible with Microdissection

Although it seems obvious that microdissection of tissue is a prerequisite for proper results, only a few studies perform a direct comparison between whole and microdissected tissue in proteomic profiling studies. De Marchi et al. compared high resolution MS data from a cohort of 38 whole and microdissected breast cancer tissues. They found that the microdissected tissue better reflects the biology of breast tumor epithelial cells due to lower interference from surrounding tissues and highly abundant proteins. Surprisingly, even a higher amount of proteins could be detected in the microdissected fraction than in the whole tissue.

Figure 3 depicts the pivotal point of microdissection. Microdissection is the link between the visualization of molecules within their spatial context and theirs identification. Tissue with histopathological stains, tissue analyzed by spectroscopic and/or spectrometric techniques can be used as starting material for microdissection. Microdissection in general reduces the complexity enabling an enhanced specificity of the downstream analyses. The downstream analyses are genomic and proteomic techniques sensitive enough for minute amounts of DNA, RNA, and proteins. Microdissection per se does not influence the mass spectrometry based identification of proteins. Even post-translational modifications on proteins such as glycosylation were not affected. But in case of selection of cells performed on stained sections minimal effects could be observed during subsequent immunostaining. The same applies for DNA and RNA analysis. H&E and Papanicolaou (Pap) staining result in low DNA recovery and some degree of DNA fragmentation. To overcome these limitations the usage of consecutive stained and unstained slides or the adaption of the staining protocols by reducing incubation times and solvent concentration are appropriate. Another option for performing cell
Figure 3. From spatial visualization to molecular identification. Microdissection is central for tissue-based analyses. The tissue section can be analyzed by H&E staining, Immunohistochemistry, spectroscopic analysis like Raman or Mass spectrometric (MS) Imaging. Microdissected regions can be transferred to down-stream techniques like LC-MS/MS for identification of proteins or genomic techniques such as next generation sequencing (NGS). Imaging data can be analyzed directly or in combination with downstream techniques for deep learning algorithms.

4.1. Microdissection and Genomics

The fact that RNA and DNA can be amplified by PCR based techniques from a very low amount like the content of one cell gives genomics a major advantage over proteomics. Therefore, the combination of microdissection with genomics should be easier to achieve than its combination with proteomics as problems with sensitivity are avoided. Nevertheless, it is not being used as a routine technique. This would, however, be of utmost importance as an exponential amplification leads to minor impurities with a major impact in the genomic results. Bevilacqua and Ducos summarized in a review the need as well as the genomic studies using microdissection. [43] The so called microgenomic techniques, working with low amounts of RNA or DNA, are next generation sequencing (NGS) of DNA or RNA sequencing (RNAseq), real-time quantitative PCR (qPCR) and microarrays:

NGS allows analyzing the whole DNA of a tissue area or a single cell. [44] “Targeted” NGS is often applied either due to the low amount of DNA from microdissected material or because it is easier to interpret. For instance only ten to hundreds of cancer related genes are sequenced. [45] In contrast to “targeted NGS” the sequence of the whole genome or at least all exons is analyzed in whole-genome sequencing (WGS). [46]

The real-time quantitative PCR (qPCR) is an ideal and affordable high-throughput approach in case only few genes (e. g. to monitor infections) have to be analyzed. [47] If the expression of a
higher number of genes or even the whole transcriptome has to be analyzed microarrays are used.

Due to the rapid development in next generation sequencing and today’s low cost RNAseq is the widely used approach. It allows the sequencing of all RNA present in a single cell or tissue region. Therefore, RNAseq can discover and analyze new transcripts (also miRNA) and is not limited to a fixed set of RNAs as with microarrays. The small amounts of RNA from microdissected tissue however need a pre-amplification before analysis. This can lead to a bias of quantification of transcripts.

On the DNA level epigenetic changes play an important role in cancer. As in the case the expression of RNA epigenetic changes like methylation or histone acetylation are highly dependent on their location. Therefore, different studies have combined it with microdissection to get a precise image of the spatial distribution in tissue.

4.2. Microdissection and Proteomics

It is a well-known fact that there is no linear or fixed relationship between gene expression at the mRNA and protein level. The existence of a DNA or even mRNA sequence does not guarantee the synthesis of a corresponding protein, nor is it sufficient to describe its function and cellular location. Moreover, proteins with their post-translational modifications and isoforms are the real actors in the cells. This of course implies that translation from DNA to protein is highly depended on the function of the cell and for this reason space dependent. In this chapter we present the most prominent formerly (4.2.1 + 4.2.2) and currently (4.2.3 + 4.2.4) used proteomic techniques.

4.2.1. 2D Protein Gel Electrophoresis

Before the term “Proteomics” was defined the 2D protein gel electrophoresis (2-DE) had been developed in the 70’s by Klose. Even with sensitive silver or fluorescent stains big amounts of protein were needed. But there were several attempts to raise sensitivity as well by modified stainings as in subsequent analyses by mass spectrometry (MS). In two tumor based studies up to 70 000 cells containing about 50 µg protein were used. Microdissection took up to 14 h. Kondo et al. used sensitive fluorescent dyes for 2-DE. They were able to run a 2D gel with 1500 cells containing <6.6 µg.

Differential 2-D gel electrophoresis, also known as DIGE, either with Cy-dyes or adapted to the ampholyte technique with Alexa-dyes allows the run of two different stained samples in one gel avoiding problems with matching protein spots from different runs. It seems to have advantages in visualizing the proteins. Sítke et al. performed a study to identify new molecular markers for the precursor lesions of pancreatic ductal adenocarcinoma. They developed a procedure with fluorescence dye saturation labeling. In this approach proteins extracted from 1000 microdissected cells resulted in a high-resolution gel of up to 2500 protein spots or with ten glomeruli from kidney. In the latter case the protein amount was sufficient to visualize 900 spots.

4.2.2. Surface Enhanced Laser Desorption and Ionization Mass Spectrometry

Surface Enhanced Laser Desorption and Ionization Mass Spectrometry (SELDI MS) is a proteomic technique using chromatographic surfaces to retain proteins depending on their physicochemical properties, followed by direct analysis via TOF MS. Several studies applying this technology have been carried out to analyze the protein profiles of biological fluids, especially serum samples in order to find biomarkers. Because this technique demands no more than a minute sample amount it is fit for the analysis of microdissected tissues. Next to the combination with microdissection SELDI MS has also been combined with 2-DE and immunohistochemistry (technical triad) to find markers in different cancer entities. Furthermore it has been used to analyze serum to define easily accessible biomarkers stemming from microdissected tissue analyses. The combination of microdissection, 2-DE, SELDI, and IHC successfully revealed several biomarkers.

4.2.3. Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging

In the last decades, matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) has developed to an imaging technique with the potential to characterize complex tumor tissue. The simultaneous, label-free detection of numerous biomolecules in intact tissue sections is an important advancement over current histopathological techniques. The combination with omic techniques was reviewed by Crecelius et al.

Since MALDI MSI is still a novel technique besides our own no studies have been published with regard to the analysis of FFPE tissues of head and neck squamous cell carcinoma (HNSCC). FFPE tissues are readily available in large quantities from pathology archives. Their storage is both easy and cost effective, even for long periods of time. Therefore, FFPE tumor samples are an ideal resource for MALDI MSI. Hoffmann et al. used MALDI MSI to investigate tumor tissue heterogeneity. The focus of their investigations was on biomarkers useful for detecting malignant cells in squamous cell carcinoma. By using FFPE and fresh frozen tissue samples they were able to find eight characteristic m/z values that let them distinguish between the tumor region and non-tumor regions. Several sections were used to identify the peptides with LC-MS/MS after testing. Finally IHC was carried out to verify the results on tissue sections. Bottek et al. used MALDI MSI in a spatial proteomic attempt to decipher the crosstalk between the infected urothelium and macrophages.

The number of studies in cancer may lead to the assumption that MALDI MSI can replace microdissection. It allows measuring a complete mass spectrum over every pixel of a tissue. Similar to microdissection in combination with LC-MS/MS it allows...
a spatially resolved proteomics. The main advantage of the latter combination lies in the possibility to identify masses and to assign them to a specific peptide by MS/MS or other fragmentation tools. In principle MALDI MSI also allows the identification by MS/MS. Nevertheless, the mass resolution is not sufficient for a direct identification of peptides or proteins from tissue sections. To our knowledge nothing has so far been published on the identification of peptides or proteins with MALDI imaging as the only approach applied. In the latest study of MALDI MSI microdissection was introduced again to build the link between imaging and identification.[17] After MALDI MSI analysis the result can be directly projected on the corresponding H&E stained section. This can be done by displaying the distribution of one single mass or by clustering regions of similar spectra.[77] Regions of high concentrations for a specific mass or clustered region (segments) can be used as guidance for the microdissection.

4.2.4. Liquid Chromatography–Mass Spectrometry

Tissue images generated with the aid of histological, spectroscopic and, in particular, spectroscopic techniques do not yet enable the direct identification of biomolecular markers according to their spatial distribution, because only stains defining histopathological features, mass signatures or vibrational modes of molecules can be displayed.

All three modalities need tools for the exact spatially resolved identification. With microdissection regions defined by these modalities can be cut, collected and transferred to liquid chromatography–mass spectrometry (LC-MS/MS) or other high resolution systems.

Most common are studies that use FFPE or frozen tissue sections with a normal histopathological stain as starting material for microdissection.[78] The identification is usually done on quadrupole Orbitrap mass spectrometer. The new parallel accumulation-serial fragmentation (PASEF) combined with the trapped ion mobility spectrometry (TIMS) technology has also the potential to identify masses from a few cells due to its high sensitivity. The results from MALDI MSI can be directly projected on the corresponding H&E stained section. This can be done by displaying the distribution of one single mass or of similar spectra.[77] Regions of high concentrations of a specific mass or clustered region (segments) can be used as guidance for the microdissection. Cut regions can then be identified by different LC-MS/MS systems.

Spectroscopic techniques like Raman or Fourier Transform Infrared Imaging (FTIR) depict vibrational spectra from tissue sections. These are per se not specific but define a group of molecules like lipids. To our knowledge FTIR is the only spectroscopic technique that was combined with microdissection and subsequent identification by MS/MS.[17] For all identification done by MS/MS the results should be confirmed by immunohistochemically staining with an appropriate antibody.[81]

4.3. From Single-Omic to Multi-Omic Approaches

The need for tissue microdissection was stated for the single omic (4.1 + 4.2) techniques. This particularly applies to multi-omic approaches where at least two omic technologies are combined. Without microdissection conflicting results render this complex, time consuming and expensive analysis useless as it can only deliver a rather fuzzy image. Till now only a few studies have applied microdissection before multi-omics.[82]

Computational methods are imperative for the analysis of the resulting large biomedical omics datasets. The needed multi-view clustering necessary for multi-omics approaches is part of machine learning attempts.[83] In using multiple omic technology it is mandatory to analyze data with machine learning algorithms as described in the following paragraph.

5. Big Data, Image Analysis, and Machine Learning

Microdissection in combination with the presented omic methods yields complex and large data sets, way beyond the size of data generated in traditional processing applications. This makes it a typical Big Data use case. The main objectives in the evolution of microdissection are a better preselection of the ROI before microdissection as well as the reconstruction of the tissue and the identification of molecules after microdissection. In order to exploit the full potential of microdissection-combined omics innovative strategies especially for data and imaging analysis as well as for visualization are needed to maximize the potential of microdissection in tissue analysis. If imaging methods like MSI or spectroscopic imaging are used before microdissection an automated preselection based on the spectral information of the ROI is possible. These complex data sets require artificial intelligence.[84] Classical H&E-staining for histopathological annotations to define the area for microdissection is time consuming and needs high pathological knowledge. As a major advantage Machine Learning can automate this process. It has the ability to build predictive models based on known features from supervised methods or unknown features from unsupervised methods. Classification of cells or tissue areas and the choice for ROIs will improve by an increasing interpretability.[85]

In a study mimicking the routine pathology workflow it could be shown that deep learning algorithms achieved better performance in detecting metastases in H&E stained sections than a panel of pathologists.[86] In conclusion, a coevolution of microdissection, imaging, and AI-approaches is necessary. This will allow a better exploitation of the large and high-dimensional data sets. Regarding the field of digital pathology the combination of microdissection, with highly sensitive bottom-up analyzes and AI approaches will have a high impact. It can deliver additional molecular information and detect unknown morphological features supporting a precise and personalized medicine by improving diagnosis, prognosis, and prediction of therapy response.

6. Outlook

Routinely used microdissection techniques have to be robust. They have to enable the automated transfer of spatial information within a complete workflow. In case of laser-based microdissection this means that imaging data form histopathological stainings, spectroscopic and spectrometric imaging data (e.g., coordinates of ROI) have to be transferred to microdissection systems.
to cut out defined regions after co-registration with high precision.[87] The annotated spatial and functional regions can be transferred to genomic or proteomic downstream analyses. Finally, a multidimensional data set is produced in this process. This data set can be further analyzed by machine learning algorithms. Actual studies show the high potential of using AI approaches for tissue analysis. In future, these methods will support tissue analysis in an extended way. Innovative strategies like virtual microdissection[88] or virtual pseudo H&E-staining[89] will further support laser-based microdissection.

7. Conclusion

Microdissecting tissue to gain defined material for genomic and proteomic downstream analysis is indispensable now and in future. Non-microdissected tissue is a mixture of several transcriptomes and proteomes. It is therefore not useful to resolve specific interactions and the physiology of a functional tissue. In this review, we have presented different techniques for laser-based microdissection. We have also described established as well as new proteomic downstream techniques sensitive enough to analyze minute amounts of proteins. In the past years it seemed that new spectroscopic and mass spectrometric imaging techniques make microdissection redundant. We suggest, however, that microdissection is a necessary complementary technique to these new imaging techniques. Especially dealing with complex diseases like cancer there is no plausible excuse to perform tissue-based genomic and proteomic studies without microdissection. Not now and not in future.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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