Review Article

Laser capture microdissection in lung cancer: a narrative review

Davide Seminati[1], Gabriele Casati[1], Fabio Pagni[1], Filippo Fraggetta[2]

[1]Department of Pathology, University of Milano - Bicocca (UNIMIB), Monza, Italy; [2]Department of Pathology, Cannizzaro Hospital, Catania, Italy

Contributions: (I) Conception and design: D Seminati, F Pagni; (II) Administrative support: D Seminati, F Pagni; (III) Provision of study materials or patients: D Seminati, F Pagni; (IV) Collection and assembly of data: D Seminati, F Pagni; (V) Data analysis and interpretation: D Seminati, F Pagni; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Fabio Pagni, MD. Department of Pathology, University of Milano - Bicocca (UNIMIB), Monza, Italy.
Email: fabio.pagni@unimib.it.

Objective and Background: Lung cancer is still the most common cause of cancer mortality worldwide. Nowadays, precise identification of predictive biomarkers plays an unavoidable role in the treatment of non-small-cell lung cancer (NSCLC). In the interest of molecular and protein analysis efficiency, the proper isolation of the neoplastic cells from the surrounding stroma may be performed with laser capture microdissection (LCM), an accurate device based on laser cutting blended with a high quality resolution microscope. In the course of time, LCM has been progressively improved, leading to its full automatization and/or its pairing with more modern tools, such as mass spectrometry (MS) with matrix-assisted laser desorption/ionization (MALDI) technique.

Methods: We performed a literature search in PubMed (Medline) for studies written in the English language and published from January 1, 1995 to December 20, 2021 using a predefined search strategy combining the following search terms: "lung cancer" and "laser capture microdissection".

Key Content and Findings: This narrative review provides an overview of recent years LCM technological innovations regarding the attempt to make it more usable in the clinical practice daily-routine or either push its performances up to a single cell spatial resolution.

Conclusions: LCM is a reliable method for the investigation of specific areas of interest, especially crucial nowadays in the characterization of lung cancer molecular signatures for their associated customized treatments. In the future, its reliability and ease of use will make it an essential step in the application of every type of increasingly sophisticated downstream analysis that will be developed in this scientific field.

Keywords: Laser capture microdissection (LCM); lung cancer; molecular pathology

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Introduction

Lung cancer is the most common cause of cancer mortality worldwide and its treatment is particularly challenging since most patients are diagnosed in advanced tumor stages without surgical resection option, with either metastatic disease or unresectable tumor (1).

The advent of increasingly sophisticated molecular characterization techniques, aimed at identifying predictive and prognostic biomarkers, and the progressive development of new targeted drugs have now widely paved the way to the new precision treatment era of non-small-cell lung cancer (NSCLC) (2).

A correct mutational analysis is dependent on the quantity and quality of nucleic acids retrieved from the pathological samples. Frequently, the only material available for molecular testing is a cytological specimen (effusion fluids, liquid-based preparations, conventional fine needle aspirations or cell blocks). In cytopathological samples, which account for around 40% of NSCLC biopsied cases, tumor cells are scattered and mixed with normal elements

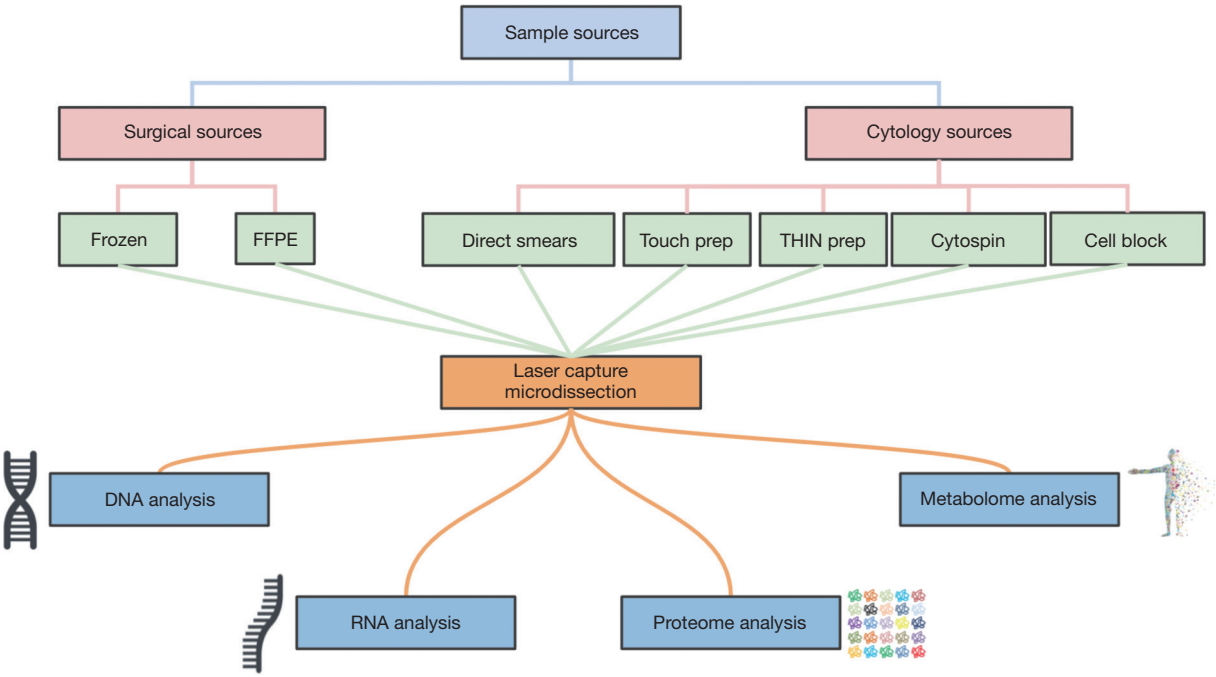

Figure 1 Sample sources and application fields of laser capture microdissection. FFPE, formalin-fixed paraffin-embedded.

Figure 2 Example of laser capture microdissection workstation (11).

making selecting for tumor enrichment difficult (3). On the other hand, small lung biopsies often contain only few available tumor cells, as they are usually consisted for the most part of non-neoplastic cells such as fibroblasts and endothelial cells of the tumor stroma, adjacent normal tissue, inflammatory infiltrate, histiocytes, mesothelium and other cells among the more than 42 identifiable lung cell types (4). In addition, nucleic acids and proteins extracted from formalin-fixed paraffin-embedded (FFPE) specimens are often highly fragmented and/or chemically modified (5).

Since the 90s, the role played by laser capture microdissection (LCM) is precisely isolating tumor cells from the surrounding elements in order to increase the genomic and proteomic diagnostic test performances, especially enhancing sensitivity, and reliably overcoming the cellular heterogeneity, starting either from smear cytology samples, cell block specimens and fresh, frozen or FFPE tissues (*Figure 1*) (5-9).

Furthermore, it was found that in non-FFPE samples it is still feasible to maintain a good nucleic acid integrity for LCM up to 3 days if the tissue is kept at –80 ℃ (10). The procedure involves a laser excision (a laser cuts around the boundary of a selected area and successively a laser pulse forces the cells into a collecting device) and a high-resolution microscope usually coupled with a video system (*Figures 2,3*).

Due to the increasing necessity for lung cancer (and not only) molecular characterization in routine practice, there is as well an urgent need for an efficient total automatization of this procedure, in fact currently LCM has a daily-routine little use owed mostly to high costs and long cells selection and collection times, with rather a more extensive employment in the multi-omics research fields (13-15).

In this narrative review, we will provide a brief report about the feasible various applications of LCM in routine clinical practice lung cancer scenarios and we will take

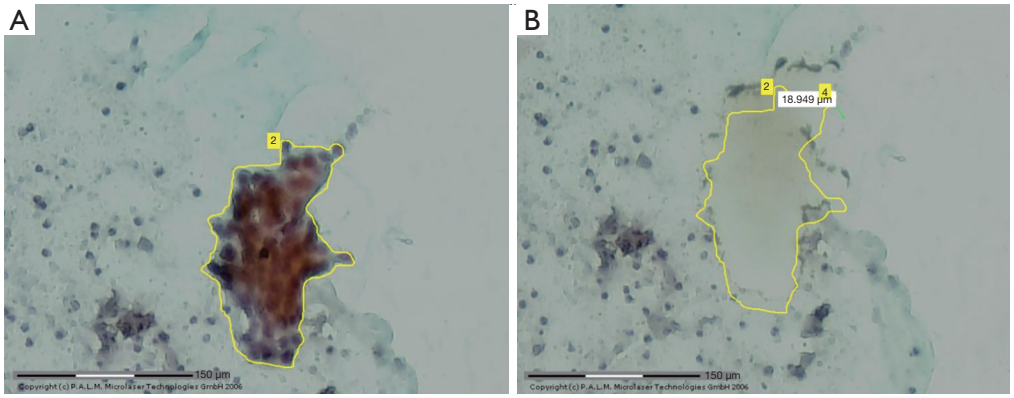

Figure 3 Laser capture microdissection performed on a NSCLC Papanicolaou-stained ThinPrep slide (PAP, ×40, yellow numbers stand for the count of manual dashes employed for the ROI selection, figure courtesy of the authors). (A) Manual ROI selection, the yellow line delineates a tumour cells aggregate. (B) Corresponding area after dissection (12). NSCLC, non-small-cell lung cancer; ROI, regions of interest.

stock of the situation about the attempts to combine it with some newer diagnostic techniques. Finally, we summarize in Table S1 the main recent LCM progress in lung setting.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://jxym.amegroups.com/article/view/10.21037/jxym-21-55/rc).

Methods

We performed a literature search (date of search: December 21, 2021) in PubMed (Medline) for studies published from January 1, 1995 to December 20, 2021 using a predefined search strategy combining the following search terms: "lung cancer" and "laser capture microdissection" requiring the term "laser" to appear in either the title or the abstract of the papers.

Articles satisfying the following inclusion criteria were included in our review (regardless of the study design): (I) study was written in English language; (II) the full article could be obtained.

Articles satisfying the following exclusion criteria were excluded in our review: (I) study was written in non-English language; (II) the full article was not available; (III) study was not related to lung cancer; (IV) study was not published in a peer-reviewed journal.

The literature review and the data extraction were conducted independently by two reviewers (D.S. and F.P.). A secondary search of the literature was manually conducted from the references of our primary search included papers by the application of the same inclusion and exclusion criteria. Doubts or disagreements regarding the inclusion or exclusion of manuscripts were resolved through a discussion between the reviewers until a consensus was reached (search strategy summary at *Table 1* and detailed Medline search strategy at Table S2).

Discussion

Undissected samples with traditional tissue-block homogenization contain a tangled mixture of tumor and non-neoplastic cells. This heterogeneity and the usual low tumor content are the two major problems in the investigation of lung cancer molecular signatures in cell blocks because they determine the inability to perform an efficient neoplastic cells selection for molecular characterization, unlike what occurs with macrodissection carried out on histological sections from surgical resections. Hence cell blocks are typically used in their entirety by whole slide scrapes for DNA/RNA extraction, thus strongly diluting the tumor DNA/RNA content, obscuring signals from the malignant compartment and decreasing the sensitivity of the molecular assays by raising the limits of detection for genomic variants (16-18). To remedy this issue, it is possible to use a LCM system to precisely dissect the morphologically malignant cells and so enhance the desired cell population before subsequent nucleic acid or protein isolation. Manual microdissection (microscope plus sterile scalpel) is feasible with lower costs and greater temporal efficiency and throughput for tissue separation, although precision may not be as good as for LCM (19). Furthermore, both manual and laser

Table 1 Search strategy summary

Items	Specifications
Date of search	21-Dec-2021
Databases and other sources searched	PubMed (Medline)
Search terms used	Search terms: "lung cancer" and "laser capture microdissection"
Timeframe	From January 1, 1995 to December 20, 2021
Inclusion and exclusion criteria	Inclusion criteria:
	(I) Study was written in English language;
	(II) The full article could be obtained
	Exclusion criteria:
	(I) Study was written in non-English language;
	(II) The full article was not available;
	(III) Study was not related to lung cancer;
	(IV) Study was not published in a peer-reviewed journal
Selection process	The literature review and the data extraction were conducted independently by two reviewers (D.S. and F.P.)
	A secondary search of the literature was manually conducted from the references of our primary search included papers by the application of the same inclusion and exclusion criteria
	Doubts or disagreements regarding the inclusion or exclusion of manuscripts were resolved through a discussion between the reviewers until a consensus was reached

techniques are subjected to a time-consuming and tedious user-dependent cell-by-cell selection of regions of interest (ROI) under direct microscopic visualization accomplished by a pathologist or cytotechnologist (20). In addition, laser-associated heat as well as the presence of nucleases or proteases tissue-specific (e.g., lung, pancreatic, spleen) may accelerate DNA, RNA and protein degradation processes, thereby a safety margin laser application and different protocols depending on the tissue type are employed (10,21).

A rapid and eventually user-independent ROI selection is achievable with the immunoguided LCM, based on cancer specific immunostaining (e.g., anti-cytokeratin-7 primary antibody for lung adenocarcinoma), even by the use of handheld and computer-aided laser devices (21-24).

Immunoguided LCM, compatible with both immuno-cyto/histochemistry or immunofluorescence targeting approaches, may be either human operator-based, computer-assisted via stain recognition algorithms or expression-based (16). In particular, this last user-independent method relies on a semiautomated identification and dissecting software in need of minimal supervision due to its ability to properly judge antibody staining. In immunoguided LCM the stained slices may also be previously covered with an ethylene-vinyl-acetate (EVA) membrane and then a laser irradiation can be performed on the whole slide: the heat derived from the localized energy absorption by the dark DAB (diaminobenzidine) stained tumor cells leads to the corresponding melting of the EVA membrane at the sites of most intensive staining. Subsequently, when the complete EVA membrane is removed, the attached tumor cells are isolated from the non-neoplastic elements, with an efficiency strongly related to immunostaining intensity and laser energy (19,23). An attempt to further optimize this process is the use of Vektor Black as chromogen, which provides to positive cells a dark black staining able of increasing the absorbed energy of the infrared laser irradiation better than slides stained with DAB (19). The immunoguided LCM has been even combined with a digital whole-slide scanning and image analysis performed before and after microdissection as a quality control protocol (19). However, it should be considered that the immunoguided LMC brings with it time, cost and technical issues related to the immunostaining steps, as well as their potential deleterious effects on the nucleic acid quality (25).

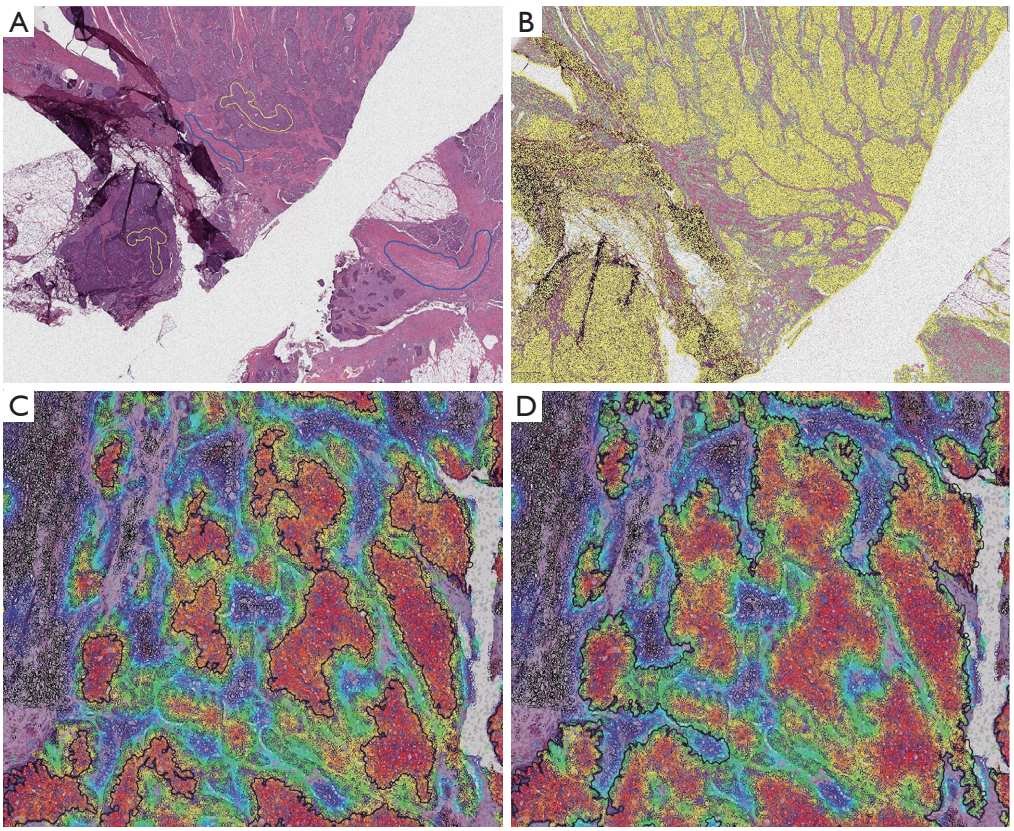

Figure 4 Example of semi-automated LCM procedure (H&E, ×5). (A) ROI are manually selected. Here, yellow lines delineate tumour areas, the blue ones normal stroma. (B) The algorithm then segments the whole-section to identify cell boundaries and automatically classify them based on the previous ROI detection. (C,D) This generates a heat map of the tumour probability score assigned by the classifier to each cell. The user can choose different confidence contours with constant tumour probability for the subsequent dissection (26). LCM, laser capture microdissection; ROI, regions of interest.

Conversely, a method that does not necessarily require immunostaining is the spatially invariant vector quantization, a pattern-matching algorithm for identification of specific cell types based on an iterative testing and real-time evaluation of match quality (16,24). With this kind of platform, the pathologist just has to identify the cell type or the morphologic pattern of interest and then the machine learning algorithm performs a whole-slide research to find similar features, including cell size, shape, nucleus and nucleolus (*Figure 4*).

Selbach *et al.* in 2021 conceived a hyperspectral infrared microscopy LCM procedure, feasible on label-free or even on hematoxylin and eosin (H&E) stained tissue sections (13). This method is based on the Fourier transform infrared (FTIR) imaging technique, which assigns a vibrational spectrum to each tissue component at high spatial resolution (27). This fully automated approach relies on a trained random forest classifier able to correctly recognize each infrared pixel spectrum, distinguishing different types of tissue (e.g., normal, tumoral, inflamed) as well as various subtypes of thoracic tumors, and thus proceed to a 95% success rate ROI dissection (28).

A combined protocol of LCM and mass spectrometry (LCM-MS) on FFPE specimens of lung tissue, applicable even on the matrix-assisted laser desorption/ionization (MALDI) technique, has been recently proposed (29). The LCM-MS has the advantage of investigating the content of cells within their morphological context, even at single cell resolution (30). Nevertheless, it must be stated that larger sampling is needed in case of LCM for protein identification, since their analysis usually needs a larger amount of template compared to the few nanograms required by either PCR assays and next generation sequencing (NGS) technology, with which a

Table 2 Main advantages and disadvantages of laser capture microdissection

Pros	Cons
Single cell precision	Expensive
Combination with single cell resolution techniques (e.g., MALDI)	Time-consuming
Semi/fully automated ROI selection (if computer-assisted LCM)	Laser-associated heat degradation
Single fluent diagnostic and molecular digitized workflow	Tedious user-dependent selection (especially if manual LCM)
Compliance with the traceability criteria (synoptic report)	Requires a pathologist or a cytotechnologist expertise (especially if manual LCM)
	Nucleases and proteases tissue-specific presence
	Immunostaining issues (if immunoguided LCM)

MALDI, matrix-assisted laser desorption/ionization; ROI, regions of interest; LCM, laser capture microdissection.

wide range of molecular analysis (genomic, epigenomic and transcriptomic) can be performed with low quality/quantity material (31,32). In order to reduce sample loss and therefore improve sensitivity of LCM-based proteomics, different preparation protocols have been developed in the years (21,33-36).

Interestingly, LCM may also have a role in lung adenocarcinoma programmed death ligand-1 (PDL-1) expression assessment through Reverse Phase Protein Microarrays (RPPA), in fact this combination allows a continuous quantitative scaled detection with performances comparable to the immunohistochemistry (IHC), but potentially less dependent upon subjective operator evaluations or IHC clones employed, with an improved insight on immune cells classes and their spatial relationship with the tumour cells (37).

Finally, the opportunity to have LCM tools under the direct Pathology Department Laboratory Information System (LIS) control may allow the pathologist to speed up the procedure by immediately selecting the ROI, in the strength of his expertise and from what he has already investigated in the course of the diagnostic phase, thus being able to promptly provide a correct estimate of either the quantitative and qualitative adequacy of the specimen sent to the downstream molecular analyzes. Additionally, the progressive digitalisation of all the pathologist's activities will lead to the development of a single fluent comprehensive diagnostic and molecular workflow, while the insertion of a proper LCM section in the pathology report would allow the full respect of the traceability criteria (synoptic report).

In conclusion, the LCM daily-routine application in clinical practice, after an initial great enthusiasm, is currently heavily constrained by numerous and well-known limitations (*Table 2*), nevertheless the development of tailor-made digital pathology tools and machine learning algorithms may lead to an efficacy and reliable, as well as rapid and sensitive, automatization of the LCM workflow and therefore result in a potential large-scale use, while the LCM combination with advanced high resolution techniques (e.g., MALDI) may open up new scenarios in the research setting (38-40).

Conclusions

LCM is a reliable procedure for the investigation of protein and gene expression in specific areas of interest, especially crucial nowadays in the characterization of lung cancer molecular signatures. In the future, its reliability and ease of use will make LCM an essential step in the application of the numerous available downstream analyses.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jxym.amegroups.com/article/view/10.21037/jxym-21-55/coif). The series "Predictive Molecular Pathology in Lung Cancer" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 Main laser capture microdissection progress in lung setting

Year of publication	Authors	Title of papers	Summary
2004	Tangrea MA, Chuaqui RF, Gillespie JW, *et al.* (23)	Expression microdissection: operator-independent retrieval of cells for molecular profiling	Lung cancer immunoguided LCM with EVA membrane performed on FFPE cytomegalovirus (CMV) IHC positive cells from human lung tissue
2012	Chowdhuri SR, Xi L, Pham TH, *et al.* (8)	EGFR and KRAS mutation analysis in cytologic samples of lung adenocarcinoma enabled by laser capture microdissection	LCM enhanced sensitivity in EGFR and KRAS molecular evaluation in 19 cell block lung adenocarcinoma specimens
	Selamat SA, Chung BS, Girard L, *et al.* (20)	Genome-scale analysis of DNA methylation in lung adenocarcinoma and integration with mRNA expression	Genome-scale DNA methylation profiling on 59 matched lung adenocarcinoma/non-tumor lung pairs
	Roy Chowdhuri S, Hanson J, Cheng J, *et al.* (16)	Semiautomated laser capture microdissection of lung adenocarcinoma cytology samples	Semiautomated computer-guided LCM of a pleural effusion cell block specimen using spatially invariant vector quantization (SIVQ) and AutoScan
	Malapelle U, de Rosa N, Rocco D, *et al.* (12)	EGFR and KRAS mutations detection on lung cancer liquid-based cytology: a pilot study	NGS testing of EGFR and KRAS after LCM of 41 liquid-based cytology specimens
2013	Didelot A, Kotsopoulos SK, Lupo A, *et al.* (5)	Multiplex picoliter-droplet digital PCR for quantitative assessment of DNA integrity in clinical samples	Digital PCR for detection of DNA integrity and quantity from 12 FFPE laser dissected lung adenocarcinoma tissues
2015	Großerueschkamp F, Kallenbach-Thieltges A, Behrens T, *et al.* (28)	Marker-free automated histopathological annotation of lung tumour subtypes by FTIR imaging	Fully automated LCM coupled with label-free Fourier transform infrared (FTIR) imaging technique to distinguish lung tissue types and tumour subtypes through proteome analysis
2017	Grafen M, Hofmann TR, Scheel AH, *et al.* (19)	Optimized expression-based microdissection of formalin-fixed lung cancer tissue	Lung cancer immunoguided LCM with EVA membrane and Vektor Black as chromogen
2018	Pierobon M, Baldelli E, Hodge KA, *et al.* (37)	Development of a quantitative PD-L1 assay using laser capture microdissection (LCM)-based reverse phase protein microarray (RPPA) workflow: Implications for precision medicine	Combined LCM and Reverse Phase Protein Microarrays (RPPA) for an IHC-independent PDL-1 assessment
	Dong X, Shi M, Lee M, *et al.* (15)	Global, integrated analysis of methylomes and transcriptomes from laser capture microdissected bronchial and alveolar cells in human lung	Genome-wide bisulfite sequencing and RNA-seq of bronchial and alveolar cells isolated by LCM from 12 flash-frozen lung tissue samples
2019	Vu QD, Graham S, Kurc T, *et al.* (38)	Methods for Segmentation and Classification of Digital Microscopy Tissue Images.	Application of two computer algorithms for segmentation of nuclei and classification of whole slide NSCLC tissue images
	Mueller C, Davis JB, Liotta LA (40)	Combining the “Sibling Technologies” of Laser Capture Microdissection and Reverse Phase Protein Microarrays	Potential and pitfalls of LCM and Reverse phase protein microarrays (RPPA) combination
2020	Herrera JA, Mallikarjun V, Rosini S, *et al.* (29)	Laser capture microdissection coupled mass spectrometry (LCM-MS) for spatially resolved analysis of formalin-fixed and stained human lung tissues	LCM coupled to mass spectrometry (LCM-MS) to assess 1252 uniquely expressed proteins in three Idiopathic Pulmonary Fibrosis (IPF) specimens
	Wang S, Rong R, Yang DM, *et al.* [39]	Computational Staining of Pathology Images to Study the Tumor Microenvironment in Lung Cancer	Deep learning-based computation model to assess spatial organization of tumour microenvironment on H&E-stained tissue images in lung adenocarcinoma

Table S1 (*continued*)

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Table S1 (*continued*)

Year of publication	Authors	Title of papers	Summary
2021	Selbach L, Kowalski T, Gerwert K, *et al.* (13)	Shape decomposition algorithms for laser capture microdissection	Skeleton-based decomposition method for simple polygons as a novel approach to decompose disease-specific regions in NSCLC samples to optimize the amount of tissue obtained by LCM
	Mickler EA, Zhou H, Phang TL, *et al.* (32)	Low-Coverage Whole Genome Sequencing Using Laser Capture Microscopy with Combined Digital Droplet PCR: An Effective Tool to Study Copy Number and Kras Mutations in Early Lung Adenocarcinoma	Combination of LCM with digital droplet PCR (ddPCR) and low-coverage whole genome DNA sequencing (LC-WGS)

LCM: laser capture microdissection; EVA: ethylene-vinyl-acetate; FFPE: formalin fixed paraffin embedded; IHC: immunohistochemistry; EGFR: epidermal growth factor receptor; KRAS: Kirsten rat sarcoma viral oncogene homolog; NGS: next generation sequencing; NSCLC: non-small-cell lung cancer; H&E: hematoxylin and eosin.

Table S2 The detailed Medline search strategy

Step	Medline search strategy
#1	"lung cancer" AND "laser" [Title/Abstract]
#2	"laser capture microdissection" [Title/Abstract]
#3	"lung" AND "laser capture microdissection" [Title/Abstract]
#4	Inclusion criteria application (see Methods and *Table 1*)
#5	Exclusion criteria application (see Methods and *Table 1*)
#6	Secondary manual search from references of the primary search included papers by the application of the same #4 and #5 criteria
#7	Reviewers D.S. and F.P. consensus in case of doubts or disagreements

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