Peer Review File

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Comments 1 and 2
The review is too concise and do not discuss the pro/cons of this expensive and time-consuming method.

Basically, no pathologist adopts LCM in routine practice to determine predictive biomarkers. This fact should be emphasized. LCM may have a role in research setting, if any.

Reply 1 and 2
Thank you for your comments.
According to your suggestions, we added in Discussion a recent new insight on LCM and PDL-1 evaluation.

Interestingly, LCM may also have a role in lung adenocarcinoma PDL-1 (programmed death ligand-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].

Among the Discussion we had assessed the pros/cons of laser capture microdissection in a discursive manner, as follow:

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

tissue type are employed [10,21]. … 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].

In order to properly point out these aspects, we added in Discussion a new clarifying table (Table 2) that summarizes 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 cytotechnologist expertise (especially if manual LCM)
	Nucleases and proteases tissue-specific presence
	Immunostaining issues (if immunoguided LCM)

Table 2: Main advantages and disadvantages of laser capture microdissection.

Then, in Introduction we stated the current scarce use of laser capture microdissection in routine clinical practice.

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].

Furthermore, we concluded the Discussion pointing out the actual limited use of laser capture microdissection in clinical practice because of its well-known limitations, but also its potential utility if properly automated or if wisely coupled with high resolution techniques.

In conclusion, the laser capture microdissection 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].

Comment 3
Illustrative images are limited to 1 figure not very explicative of LCM. Have the authors the possibility to catch some other images before and after LCM?

Reply 3
Thank you for pointing this out.
We added in Introduction three explicative figures about laser capture microdissection usage scenarios (Figure 1), workstation (Figure 2) and microdissection process (Figure 3A-B).

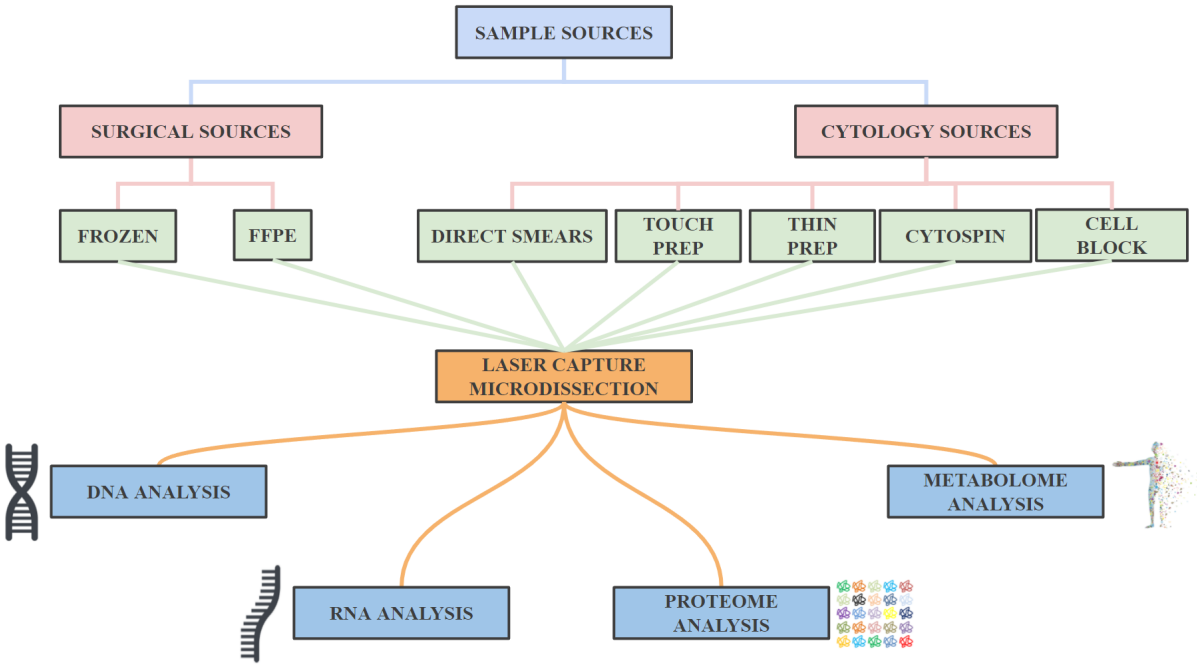

Figure 1: Sample sources and application fields of laser capture microdissection.

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

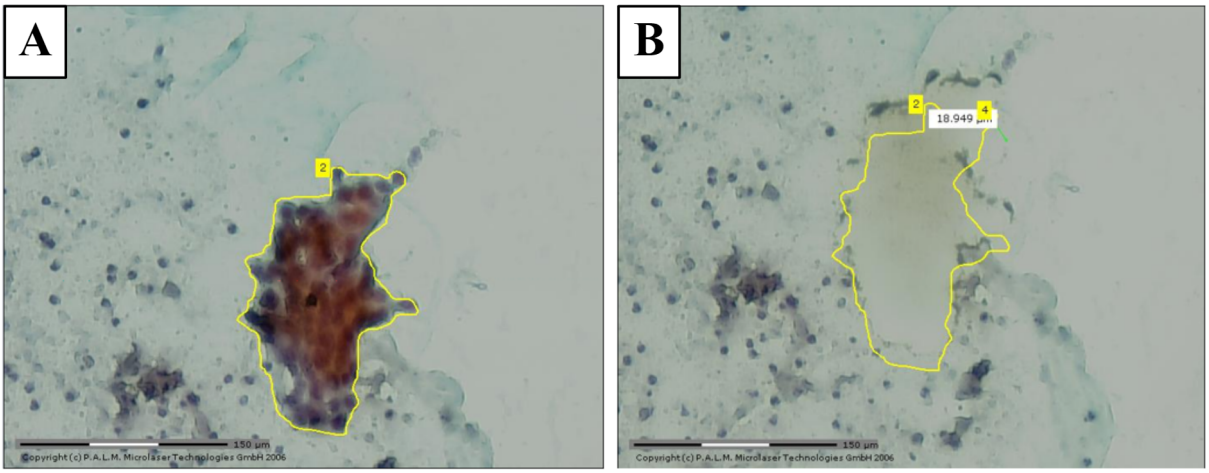

Figure 3: laser capture microdissection performed on a NSCLC Papanicolaou-stained ThinPrep slide (PAP, x40). (A) Manual ROI selection. (B) Corresponding area after dissection (12).

COMMENT 4
The authors could provide a Table summarizing the most important papers using LCM in molecular characterization of lung cancer.

REPLAY 4
Thank you for the comment.
We added in Introduction a Supplementary Table 1 (SEE SUPPLEMENTARY TABLE 1 FILE) with a collection of papers concerning the main laser capture microdissection progress in lung setting.

In Introduction we stated:

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 stock of the situation about the attempts to combine it with some newer diagnostic techniques. Finally, we summarize in Supplementary Table 1 the main recent LCM progress in lung setting (Supplementary Table 1).