

# Detection of low copy number changes and deletions in lung adenocarcinoma is enhanced by laser capture microdissection.

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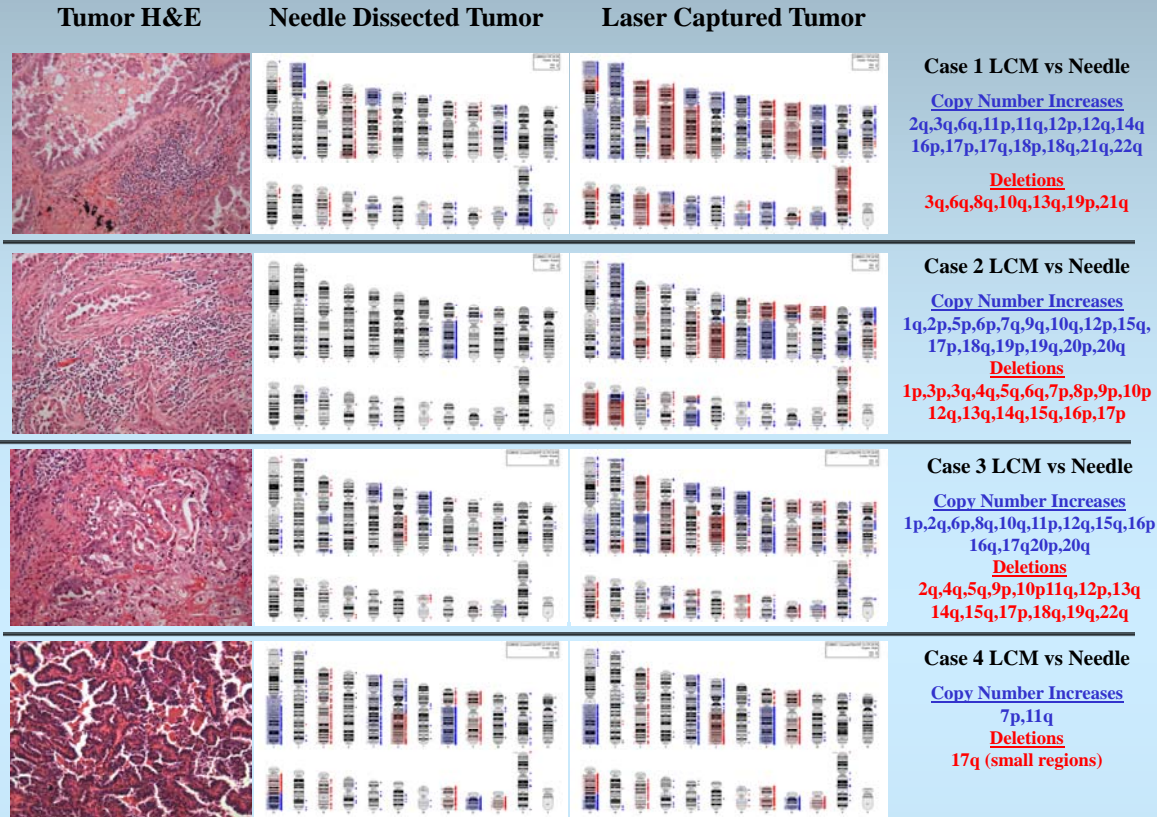
## Abstract

Mixed subtype pulmonary adenocarcinomas frequently have an admixture of tumor cells, desmoplastic stroma and inflammation, as well as areas of residual alveolar walls in areas of lepidic growth. Sections that contain over 70% tumor are preferentially used for studies of DNA copy number imbalances. However, many tumor sections have far less representation of tumor cells (<< 70%) or they contain significant numbers of benign inflammatory cells. The net effect is a reduction in the sensitivity of methodologies for detection of copy number gains and losses in the tumor specimen.

Frozen sections of mixed subtype adenocarcinoma were either manually needle dissected or laser capture microdissected using a PALM Zeiss Microbeam System to obtain a minimum of 500 ng of genomic DNA for study on Affymetric 6.0 SNP microarrays. Laser capture microdissected material identified all alterations that were detected by needle microdissection, confirming that laser capture did not result in poor DNA quality. Laser capture microdissected tumor allowed for detection of increases and decreases in copy number in multiple chromosomal arms; overall, alterations in 19 chromosomal arms in one pair of samples and 11 chromosomal arms in the other pair were detected in the LCM samples only. The majority of these alterations comprised small deletions that would not be expected to be detected in a specimen of heterogenous cell populations.

## Materials and Methods

Frozen tissue was acquired from 4 adenocarcinomas of lung and frozen sections were cut at 6 microns thickness. Manual needle dissection of tumor rich areas was performed with an 18 gauge needle on an Olympus microscope using at 40X magnification and collected by vacuum suction into a pipette tip. Laser capture was performed on serial frozen sections using a Palm Zeiss Laser microscope. A total of 500 ng of DNA was collected by both methods and subsequently prepared for hybridization to Affymetric 6.0 SNP arrays.



## Results

Tumor sections were over 70% tumor; however the tumors themselves contained variable amounts of non-neoplastic cells such as fibroblasts and inflammatory cells. While needle dissection enriched the sample for tumor, lymphoid aggregates and desmoplasia could not be removed by that method. LCM captured tumor cells with less stromal cell contamination.

The SNP karyograms demonstrate copy number gains and losses detected by both methods, indicating successful tumor sampling by both needle biopsy and LCM. The majority of changes seen in needle dissected samples were seen in the LCM derived samples.

In 3 of 4 samples, numerous regions of copy number gains and losses were detected by the LCM samples only, and in many cases, regions detected by needle dissection became better defined. In one sample the effect of laser capture dissection was less evident; this was likely due to the higher relative percentage of tumor cells in that mass, especially relative to inflammation.

## Conclusions

- Laser capture microdissection of tumors improves detection of low copy number increases and deletions using Affymetrix SNP arrays.
- Laser capture does not damage the DNA sample.
- Overall cellularity of the tumor associated stroma variably affects the sensitivity of copy number analyses in lung tumor samples.