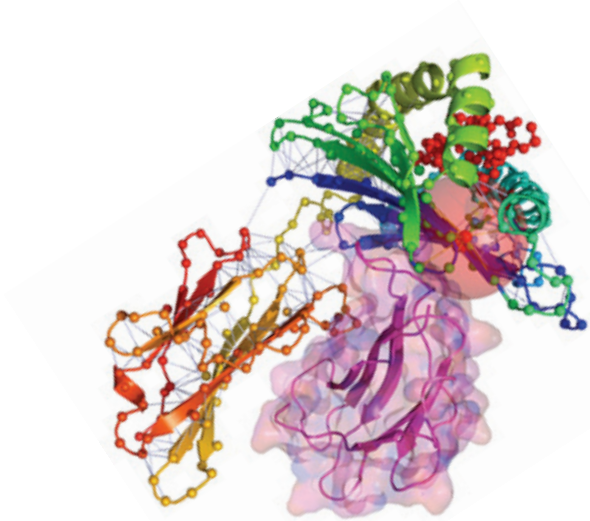
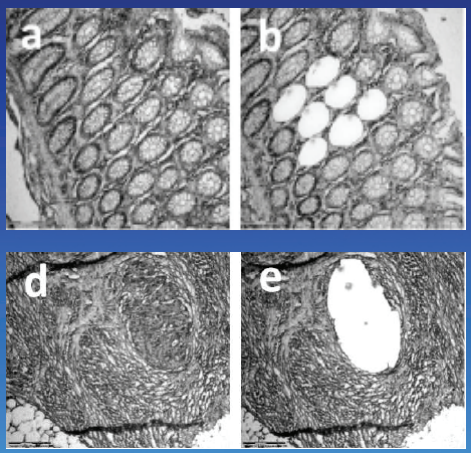
Laser Microdissection: A Key Technology for Microproteomics

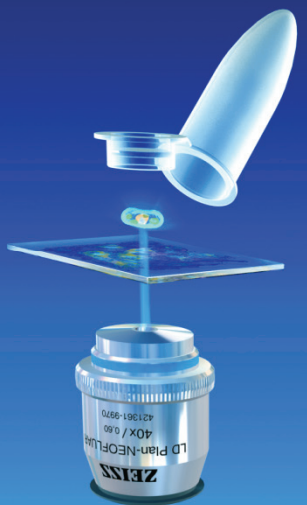


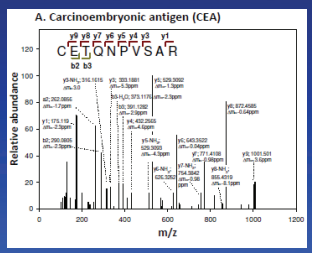
Most proteomic applications face two major challenges. One is the requirement of substantial amounts of tissue for a comprehensive characterization. The second is to guarantee tissue specificity in order to obtain significant results.

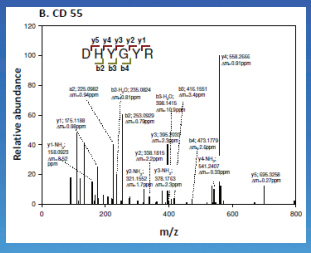
Recent developments are focusing on the improvements of sensitivity and selectivity of conventional sampling techniques. One enabling key technology is Laser Capture Microdissection (LCM). Already proven in genomics as a precise tissue separating and collecting technology, Carl Zeiss now offers a fast collection method for large amounts of material. In combination with new improvements e.g. in mass spectrometric resolution and sensitivity, LCM clears the way for definite "microproteomics".

Here you will find different examples using LCM as major enabling technology pointing out the benefits of tissue specific proteomic characterization.









Wisniewski JR et al., J Proteome Res, 2011

Isolate a single cell population

Isolate 30.000 cells in one step within minutes

Seamless integrate LCM into your workflow

ZEISS

We make it visible.

EXAMPLE

Quantitative proteomics in formalin-fixed paraffin-embedded tissue using LCM combined with high-resolution mass spectrometry

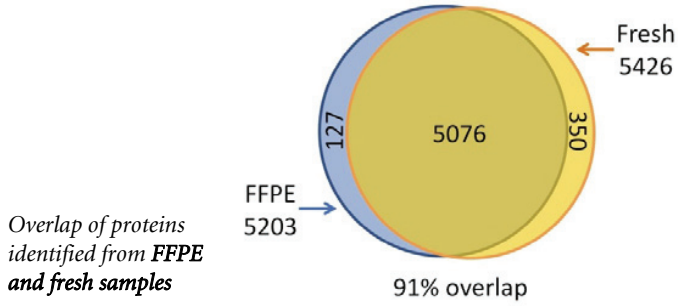
Tissue samples in biobanks are typically formalin-fixed and paraffin-embedded (FFPE) and routinely prepared onto glass slides. It has only recently been shown that proteins in FFPE tissues can be identified by LCM combined with mass spectrometry-based proteomics.

Ostasiewicz et al. used the filter aided sample preparation (FASP) method to analyze microdissected samples solubilized in high concentrations of SDS and extended this feature to develop a simple protocol for FFPE analysis.

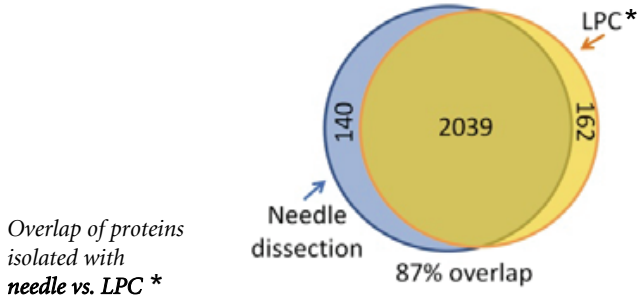
Results from the FFPE-FASP procedure do not indicate any discernable changes due to storage time, hematoxylin staining or LCM. Comparison of fresh against FFPE tissue presented no significant qualitative or quantitative differences between these samples neither at the protein nor at the peptide level.

Application of the FFPE-FASP protocol to phosphorylation and N-glycosylation pinpointed nearly 5,000 phosphosites and 1,500 N-glycosylation sites. Analysis of FFPE tissue revealed that these posttranslational modifications were quantitatively preserved.

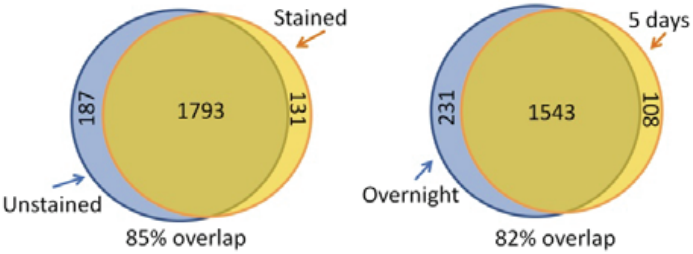
Thus FFPE biobank material can be analyzed by combination of LCM and microproteomics.



*Overlap of proteins identified from **FFPE and fresh samples***



*Overlap of proteins isolated with **needle vs. LPC ***



Effect of key processing steps in FFPE analysis
- hematoxylin staining
- fixation time

***** *LPC = Laser Pressure Catapulting*
- after Laser microdissection the segregated samples are lifted into a collection device by a single laser pulse

Ostasiewicz P et al., *J Proteome Res,* 2010, 9,7: 3688-3700

EXAMPLE

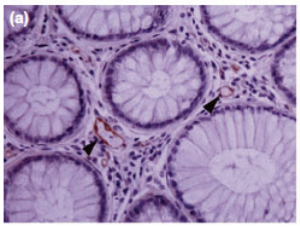
Identification of biomarkers of colorectal cancer by combination of LCM and quantitative proteome analysis

Tumor tissues obtained from surgery represent ideal material for biomarker searching by proteomic analysis. However, tissue heterogeneity complicates the identification of tumor markers and the results obtained through proteomic analysis of whole tissue may be considered controversial.

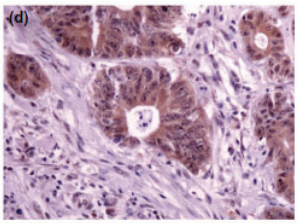
Laser Capture Microdissection (LCM) can overcome the heterogeneity problem by isolating individual tumor cells as shown by Zhang et al.

They analyzed LCM purified cells from CRC tissue and adjacent normal mucosa using D0 / D3 acetylation labeling combined with high-performance LTQ-FT MS.

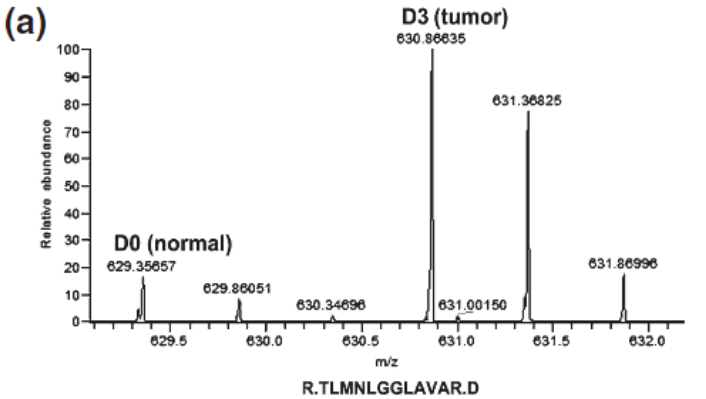
LTQ-FT MS possesses the obvious advantages of both allowing small amounts of samples to be analyzed and providing high proteome coverage for complex biological samples.
Using a pooling strategy, they found a total of 137 differentially expressed proteins between CRC and normal mucosa.



Normal mucosal epithelia



hepatic metastasis of colo-rectal cancer (CRC)

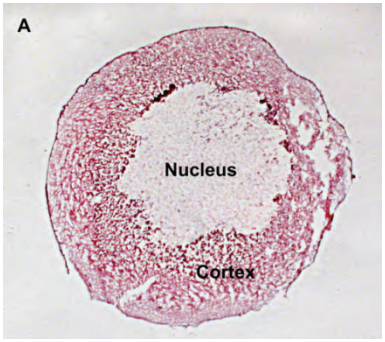


Quantification and validation of protein overexpression in pooled colorectal cancer (CRC) samples. (a) The expression level of transgelin-2 (TAGLN2) was quantified to be four-fold higher by calculating the areas under the mono-isotopic peaks of the heavy isotopic (D3) versus light isotopic (D0) peptides.

Zhang Y et al., *Cancer Sci,* 2010, 101,2: 523-529

EXAMPLE

Identification of protein profiles in the human lens cortex using LCM combined with 2D-DIGE, MALDI-TOF and ESI-QTRAP LC-MS/MS

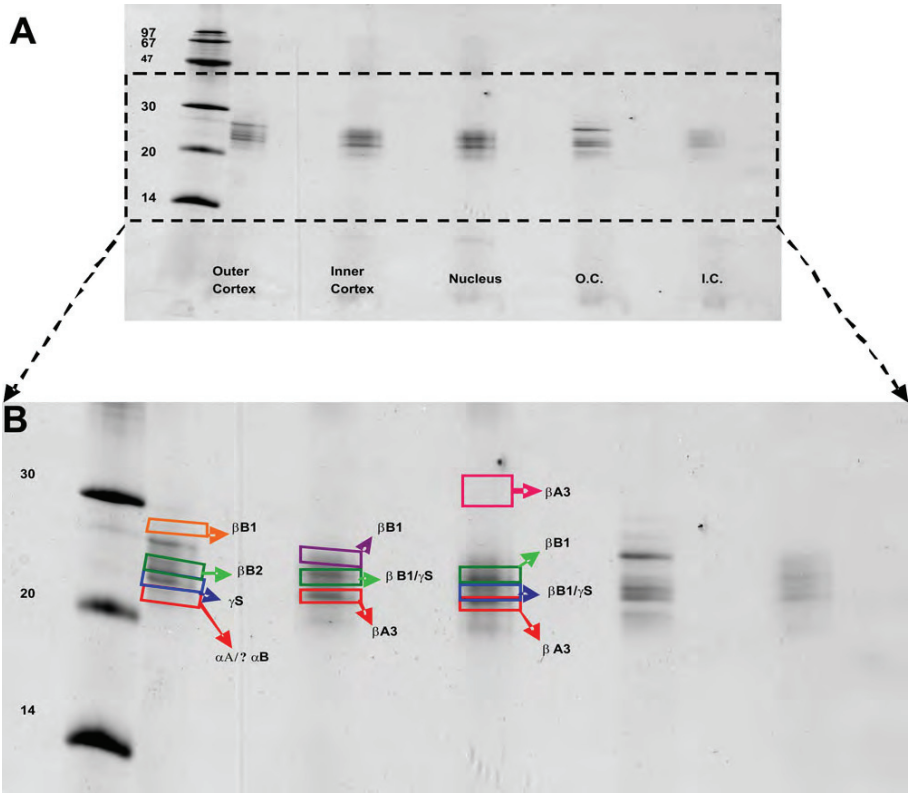


Tissue section of a 69-year-old human lens
LCM can easily isolate the irregular shaped cortical and nuclear regions for detailed proteomic analysis

With aging, human lens crystallines undergo post-translational modifications (PTMs) and these modifications are believed to play a major role in age-related cataract development.

Asomugha et al. combined the accurate techniques, LCM and 2D-DIGE, with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) and Electrospray Ionization Quadripole Linear Ion-Trap Liquid Chromatography (ESI-QTRAP LC-MS/MS) mass spectrometry to determine the protein profiles in cortex and nucleus of a single lens.

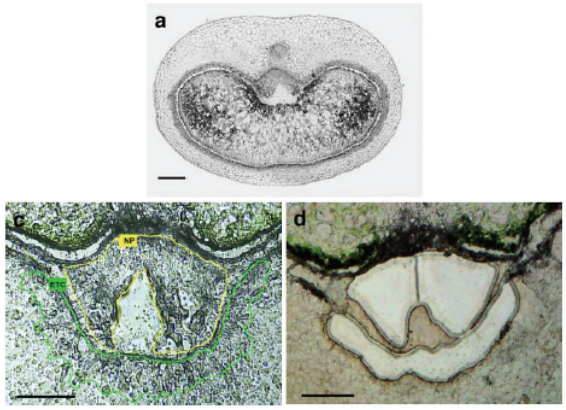
Laser microdissection was shown to be a advantageous technology to differentiate the in situ regional distribution of crystallines and intermediate filament proteins in cortical and nuclear regions of the same lens. It was superior to a similar isolation procedure using sequential solubilization of different regions of a human lens.



LCM as enabeling technology finally leads into more specific results
Separation of 69-year-old human lens proteins using 15% polyacrylamide gel by SDS–PAGE analysis (A) and identification of excised bands by MALDI-TOF mass spectrometry (B)

EXAMPLE

LCM-based micromethod for the analysis of amino acid concentrations in plant tissues



The transcriptome and metabolite analysis from micro-amounts of plant tissues demands the extension of the capabilities to extremely small, physiologically distinct cell types.
The nucellar projection (NP)/endosperm transfer cell (ETC) complex represents the link between maternal and filial seed tissues in barley. Cells of NP function as metabolic interface to precondition amino acid supply of the endosperm.

To understand metabolism, interconversion and transfer of amino acids at the maternal–filial boundary, Thiel et al. applied a combined transcriptome and metabolite approach based on LCM and ultra performance liquid chromatography (UPLC).

This approach finally enabled Thiel et al. to suggest that amino acid degradation observed in NP largely occurs within mitochondria, consistent with their role in controlling amino acid homeostasis and metabolism.

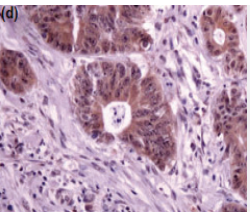
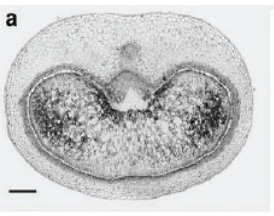
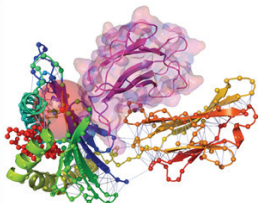
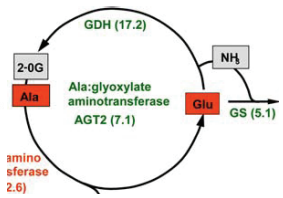
	Amino acid content (amol µm^{-3})	
Amino acid	ETC	NP
Histidine	0.08 ± 0.06	0.35 ± 0.11**
Asparagine	13.05 ± 1.33	11.96 ± 1.41
Serine	13.76 ± 3.85	12.43 ± 7.14
Glutamine	7.29 ± 1.20	14.75 ± 4.30***
Arginine	0.76 ± 0.23	3.21 ± 0.73***
Glycine	3.46 ± 0.79***	1.92 ± 0.47
Aspartate	4.54 ± 0.68	4.67 ± 1.38
Glutamate	13.46 ± 1.42***	8.93 ± 1.76
Threonine	3.01 ± 0.75	3.60 ± 1.3
Alanine	40.24 ± 5.26**	28.62 ± 8.13
GABA	0.76 ± 0.23	1.22 ± 0.21**
Proline	8.66 ± 2.61***	2.44 ± 0.23
Lysine	0.00 ± 0.00	0.81 ± 0.08*
Methionine	0.87 ± 0.04	1.23 ± 0.27*
Valine	1.40 ± 0.51	1.48 ± 0.55
Isoleucine	0.37 ± 0.13	0.50 ± 0.12
Leucine	0.25 ± 0.1	0.39 ± 0.11
Phenylalanine	0.15 ± 0.02	0.22 ± 0.06

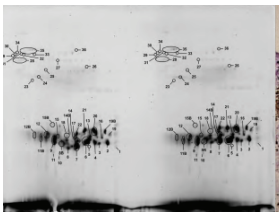
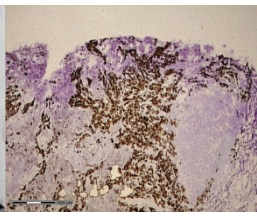
Values are the mean of 4–5 biological replicates obtained from 10 to 20 tissue sections

Increased at the significance level * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$

Amino Acid concentration *in nucellar projection (NP) and endosperm transfer cells (ETC)*

Thiel J et al., *Planta*, 2009, 278,6: 633-638

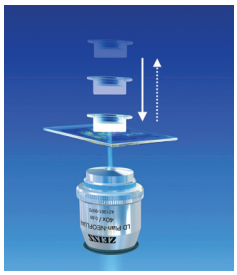



It's your choice - How LCM works

• Collect ultrapure, selected material from heterogeneous regions or from e.g. single cells or live cells with the well established precise and unique non-contact Laser Capture Microdissection (LCM). A defined laser pulse transports the selected specimen out of the object plane into a collection device – against gravity.

• Collect large samples from homogeneous regions captured in one piece by pick-up LCM without dividing up and start directly, e.g. proteomic or metabolic profiling.

Non-contact LCM

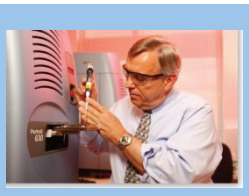


Pick-up LCM

For more details please visit:
http://www.zeiss.de/microdissection

Prof. Jacek Wisniewski
Max-Planck Institute for Biochemistry
Martinsried, Germany

"Our lab uses PALM MicroBeam in combination with a streamlined filter aided sample preparation (FASP) workflow that allows efficient analysis of lysates from low numbers of cells. We were able to analyze formalin fixed and paraffin embedded human tissues prepared by LCM to a depth of 5000-6000 proteins per sample.
This setup has the potential for exploration of clinical samples for biomarker and drug target discovery."

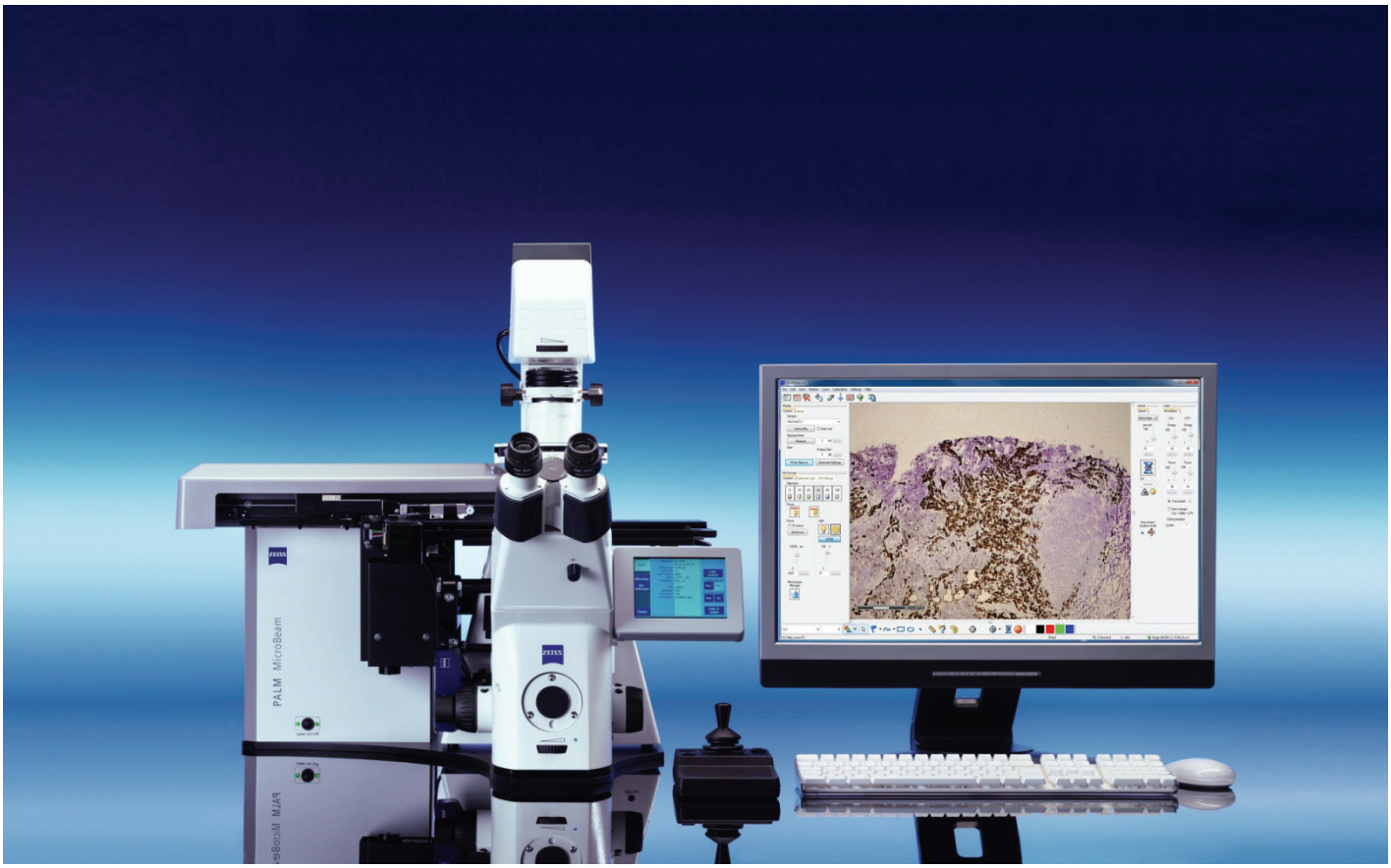


Richard Caprioli, Ph. D. [1]
Stanley Cohen Professor of Biochemistry
Director, Mass Spectrometry Research Center
Vanderbilt University School of Medicine

"Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) can profile proteins at high sensitivity up to 50 kDa in tissues. This technology can directly measure many peptides and proteins in tumor tissue sections and can also be used for high resolution imaging of individual biomolecules present in tissue sections.Coupled with laser capture microdissection (LCM), MALDI MS is an ideal approach for generation of separate protein profiles of the invasive tumor and normal epithelial components of breast tumors and tissues" [2]

[1] *Information taken from http://www.vanderbilt.edu/chemistry/faculty/caprioli.php*

[2] *Sanders ME, Dias EC, Xu BJ, Mobley JA, Billheimer D, Roder H, Grigorieva J, Dowsett M, Arteaga CL, Caprioli RM. Differentiating Proteomic Biomarkers in Breast Cancer by Laser Capture Microdissection and MALDI MS. J Proteome Res. 7(4):1500-1507, 2008*



PALM MicroBeam - A Unique Solution from Carl Zeiss

The System at a Glance

- Fully automated, high precision one-step LCM
- Perform LCM even from standard glass slides (archived material) or use standard reaction tubes
- Structured documentation with a reasonable data management (InformationCenter)
- Flexible collection devices - Catapult on customized holders, e.g. MALDI Slides

Combined LCM and High-End Imaging Technology

- Use Multichannel Fluorescence (MCF) to display different color channels individually or merged in one fluorescence image
- Combine microdissection with confocal imaging technologies, like Spinning Disk
- Extend LCM with optical tweezers, our contact free manipulation tool

Benefits for Proteomic Application

- Extracted sample size up to several mm^2
- Pooling of dissectates possible
- Image analysis for fast and fully automated sample recognition

Scientific Support

- Dedicated protocols for Proteomics available
- Ask for fast and competent support
  - an experienced group of dedicated scientists from the ApplicationLab (labs@zeiss.de) will accelerate your research

PALM MicroBeam is not intended for diagnostic use

Carl Zeiss MicroImaging GmbH
07740 Jena, Germany

BioSciences I Location Munich
Phone : + 49 89 90 9000 800
Telefax: + 49 89 90 9000 820
E-Mail : palm-info@zeiss.de
www.zeiss.de/microdissection

For questions please contact
E-Mail : labs@zeiss.de
Hotline: +49 89 90 9000 900

Subject to change.
60-3-0010 – issued 06.11