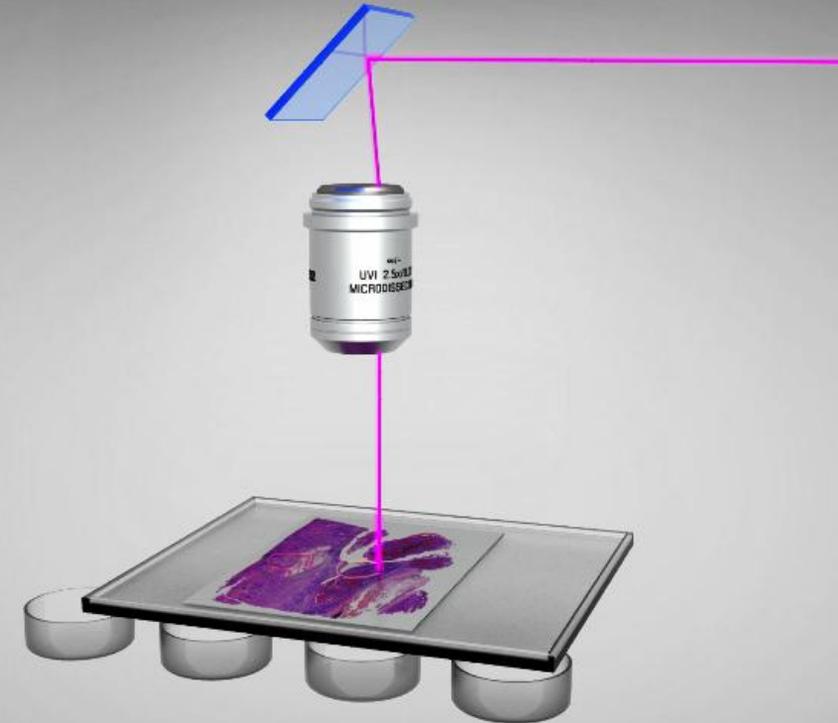


Laser Microdissection

University of North Carolina

Natasha Fernandez Diaz Granados
Spatial Biology Specialist
September 2025



From Eye to Insight

Leica

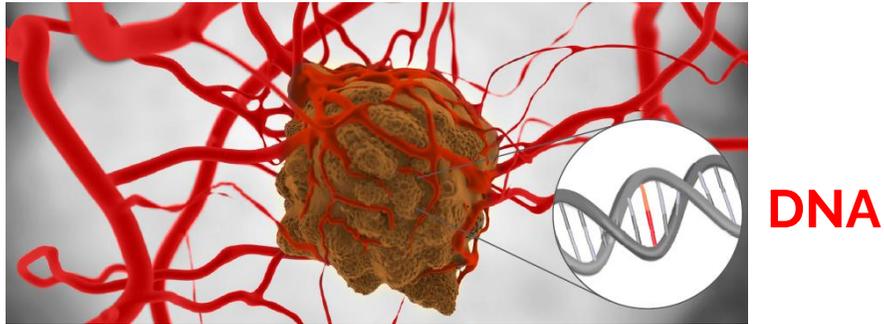
Leica Laser Microdissection

AGENDA:

- Why Laser Microdissection?
- Leica LMD Workflow and Sample Preparation
- Q & A

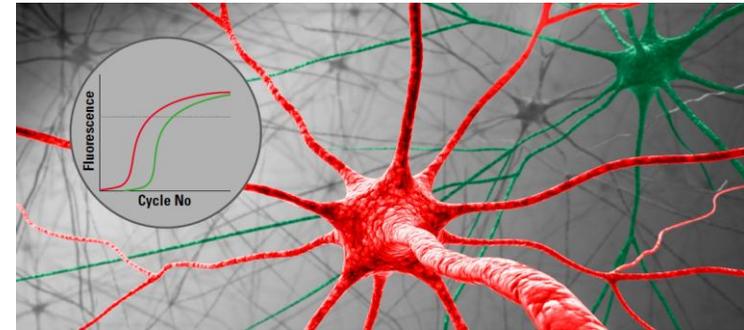


Cancer research: Tumors, Metastases
Which are the underlying mutations?



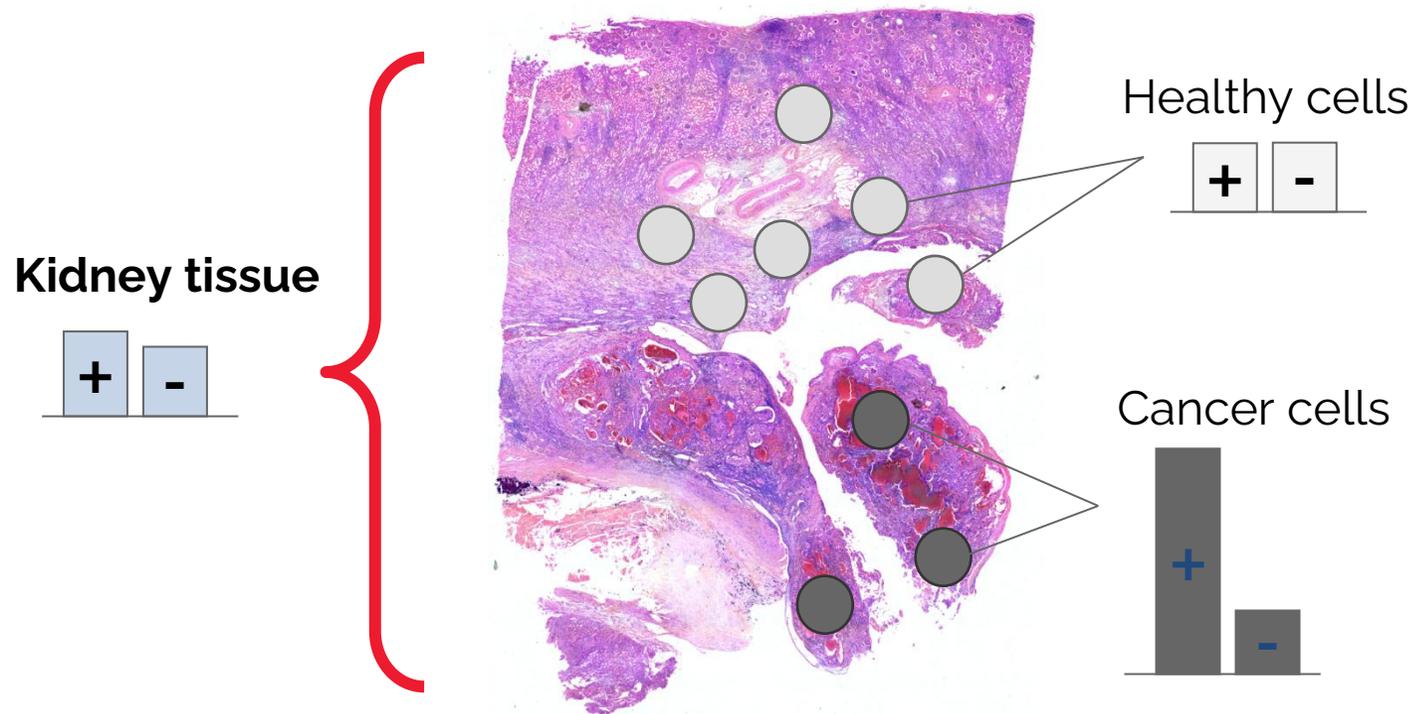
Parkinson's Disease: Dopaminergic Neurons
What is the difference in gene expression patterns?

RNA

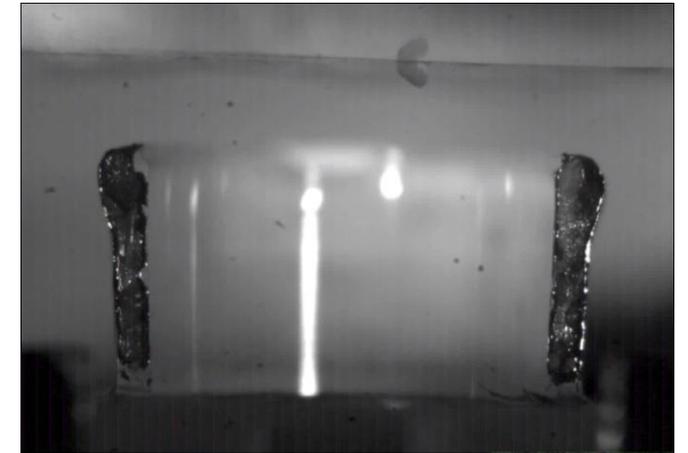


Alzheimer's Disease: Alzheimer's Plaques
What is the content of Alzheimers' Plaques?



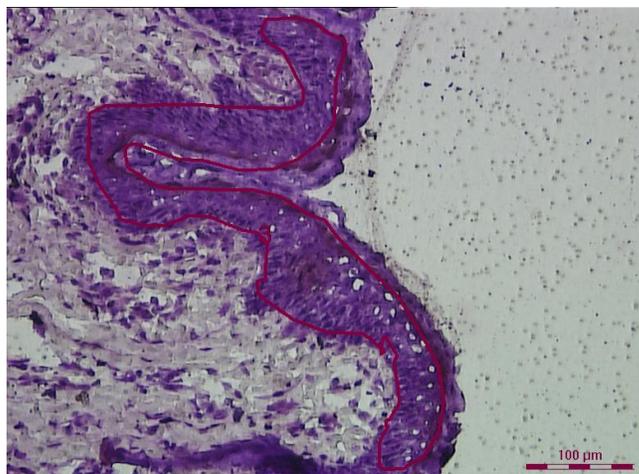


Purify a **homogeneous** population from **heterogeneous** tissues!



Purification under **visual control**

Leica Laser Microdissection



Define Region of Interest



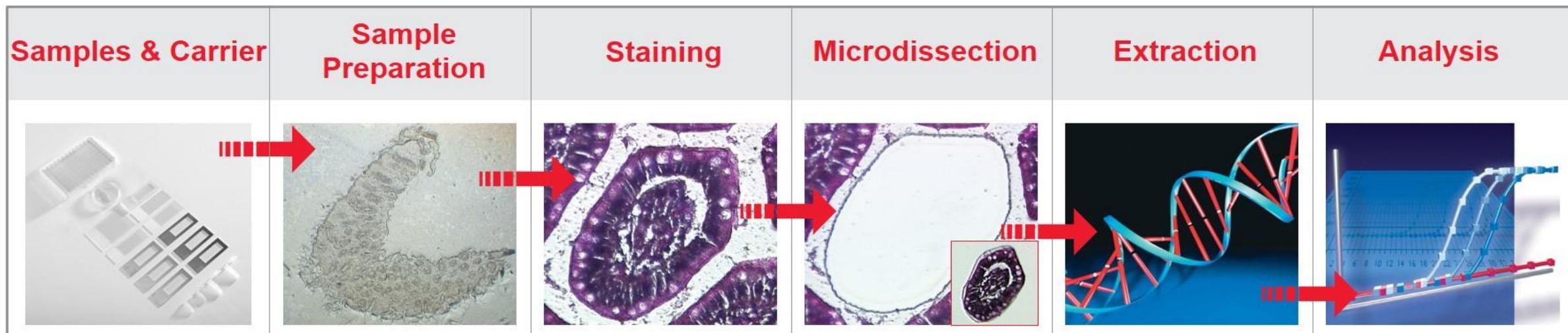
Sample after Cut

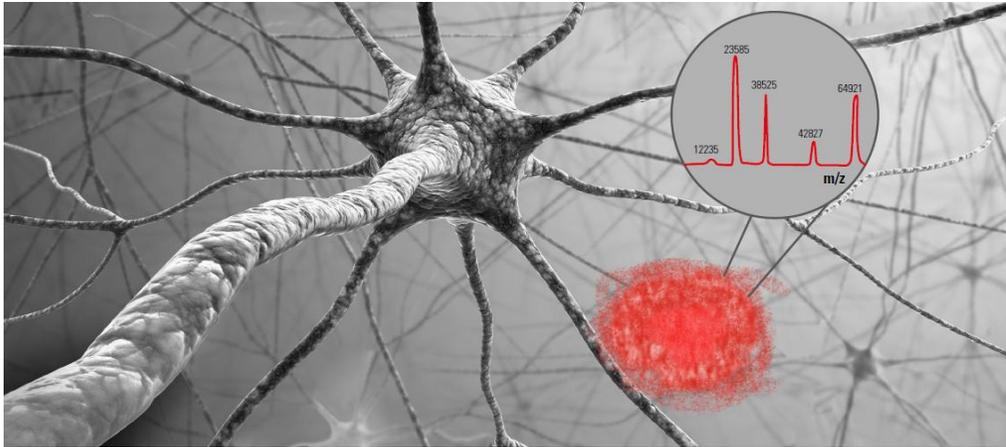


Specimen in collection vessel

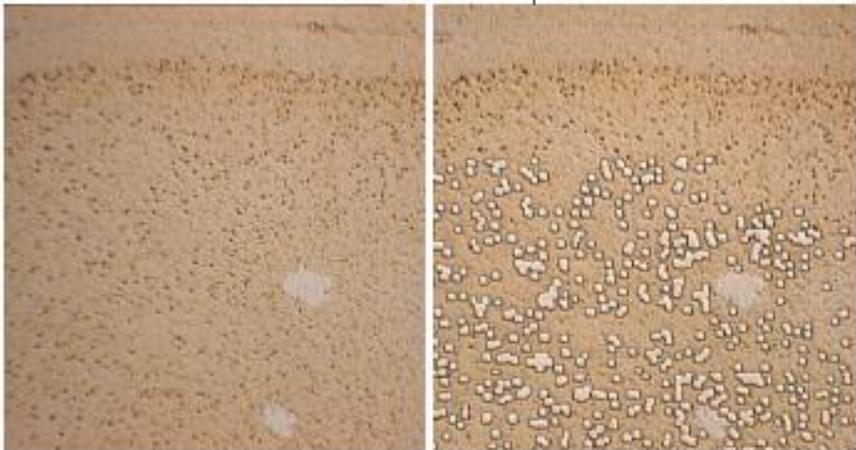
LMD Workflows in...

- Cancer Research
- Pathology/Disease Research
- Neuroscience
- Developmental Research
- Plant Research
- Living Cells
- Forensics
- Clinical Research
- Pharmaceutical Research
- Climate Research
- and many more





Alzheimer's Plaques



Before dissection

After dissection

Purification
under visual
control



Sample preparation

Cryo-sectioning with a cryostat and preparation of sections on special LMD slide.



Fixation and staining

Fixation and staining of tissue for microscopy and LMD application. Can be automated with a stainer.



Visualization and ROI definition

Automatic generation of sample overview. The regions of interest (ROI), can be identified and marked automatically or easily manual.



Laser microdissection

The ROIs will be automatically dissected and collected via gravity into standard, cost-effective consumables such as PCR tubes or 8-strip tubes (e.g. Leica LMD7).



Extraction of proteins

Preparation for downstream analysis can be performed with commercially available protein extraction kits (e.g. QIAGEN).

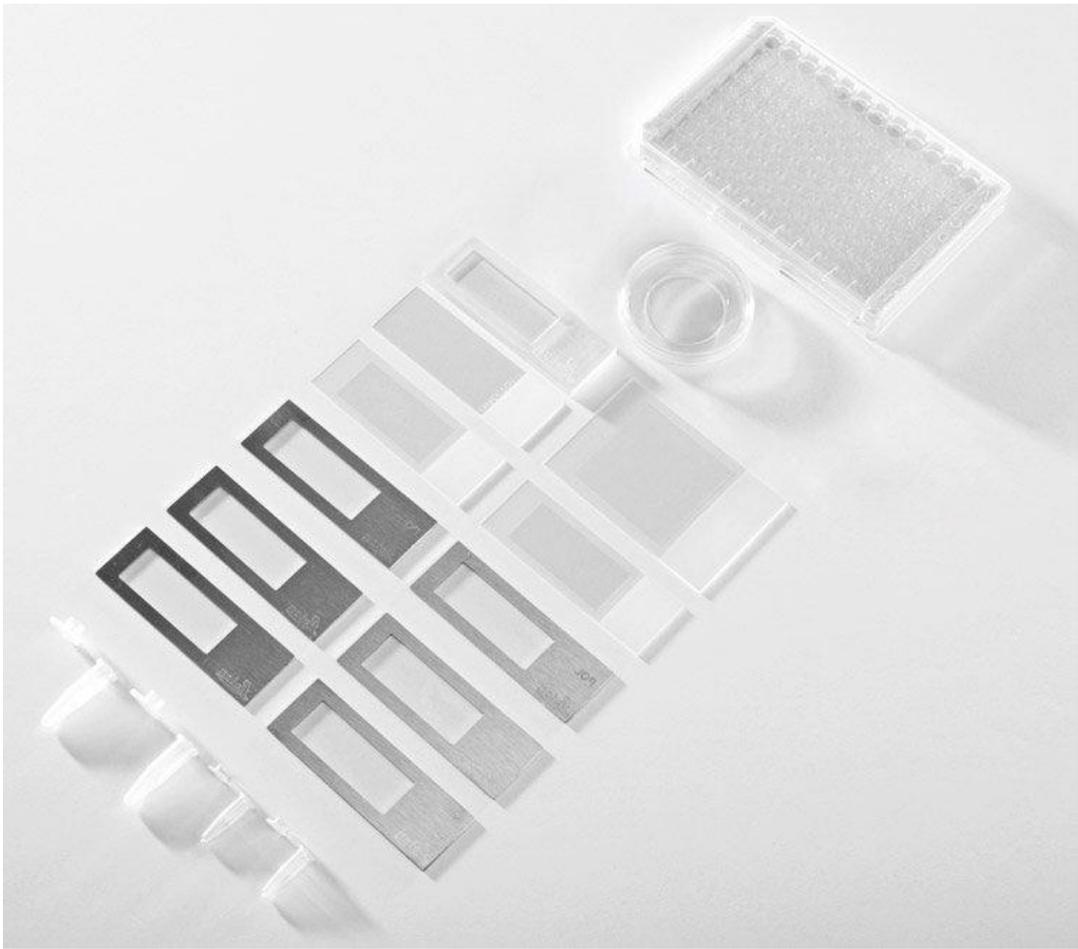
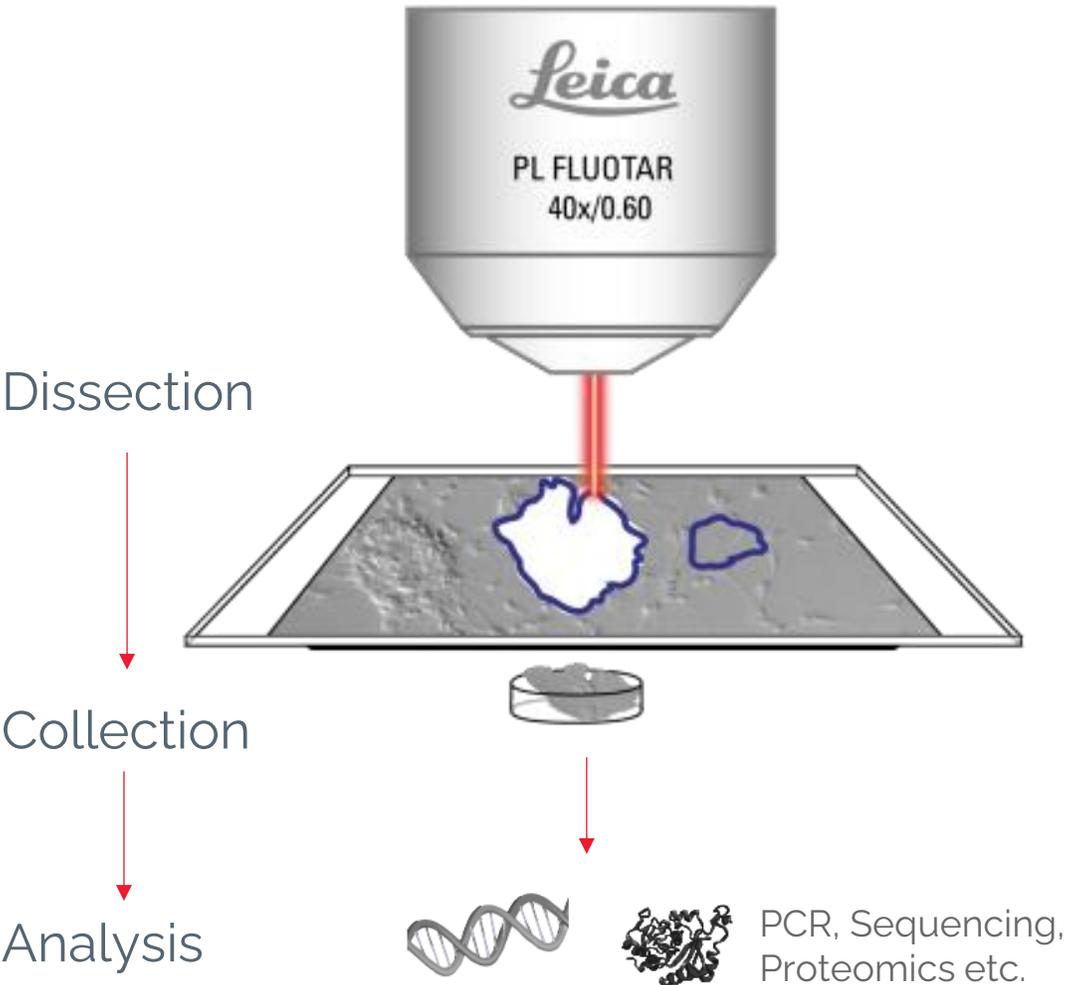


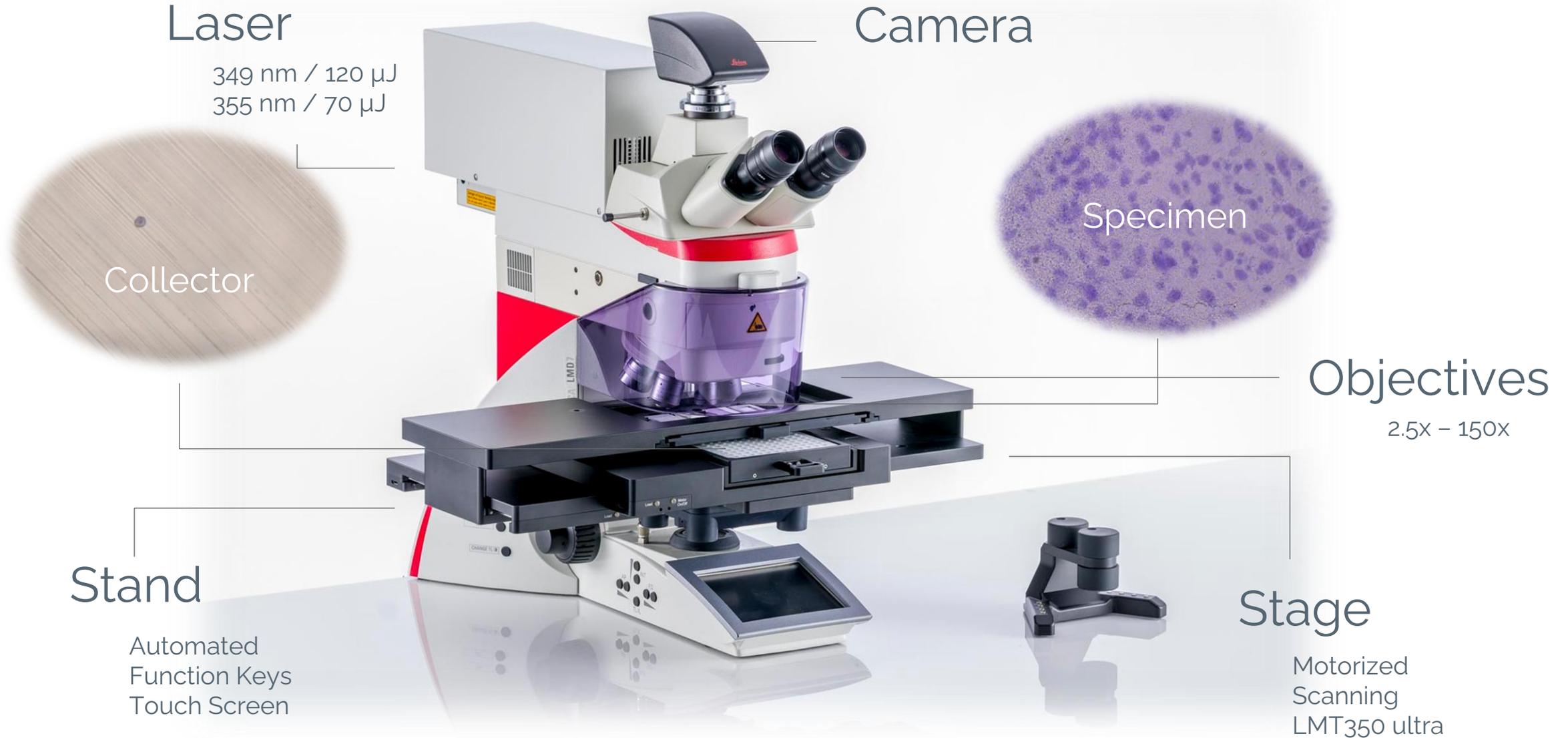
Protein expression analysis

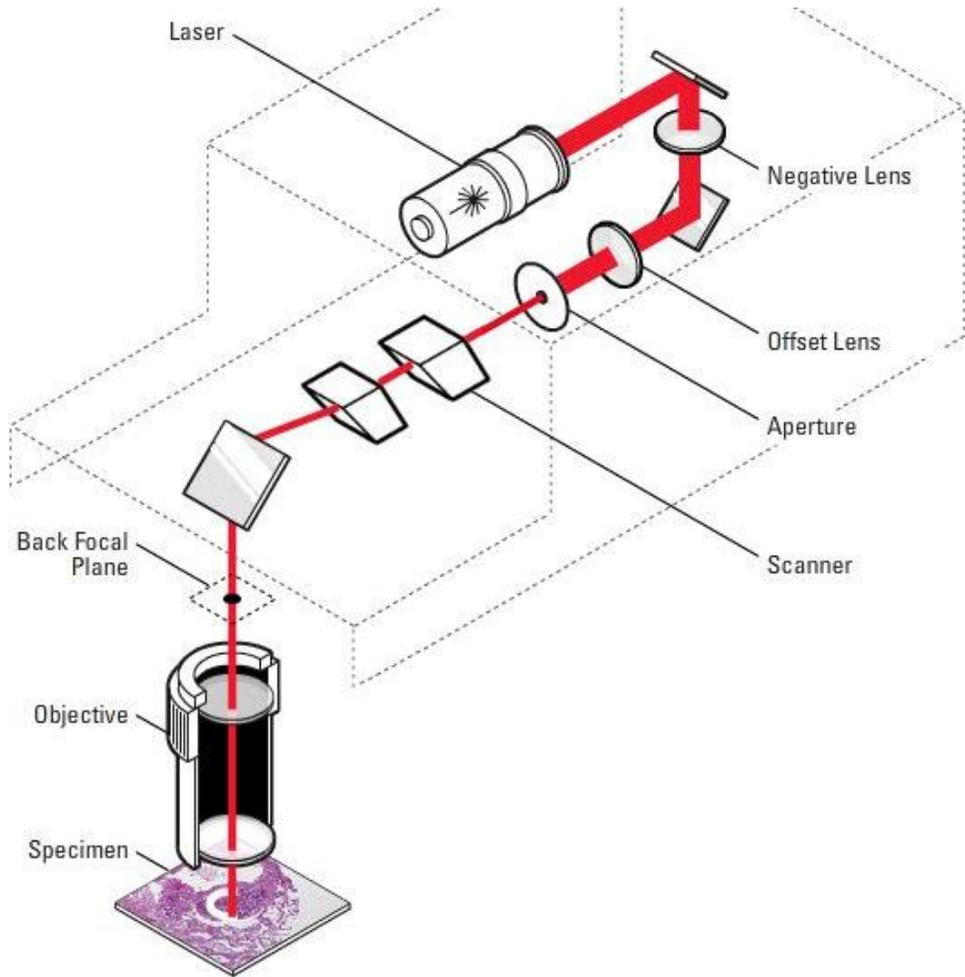
Obtain reproducible and specific results of protein expression using a LC-MS/MS system (e.g. Sciex).



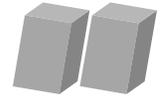
Laser Microdissection – The Principle







Laser beam movement **via optics**

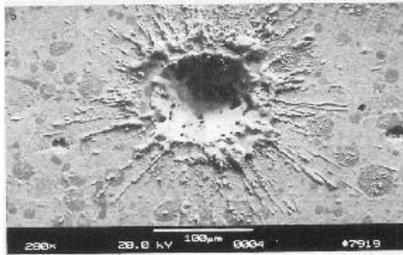


- High precision (objective depending)
- Real-time cutting (Wacom Pen)

Leica Laser Microdissection

- Vaporization of material, ***cold ablation***
 - Surrounding tissue is not affected or heated

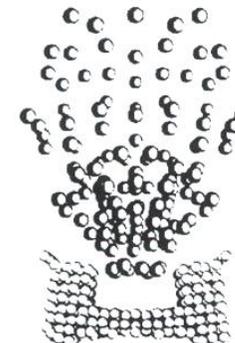
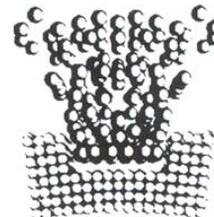
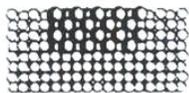
Vaporization



Melting

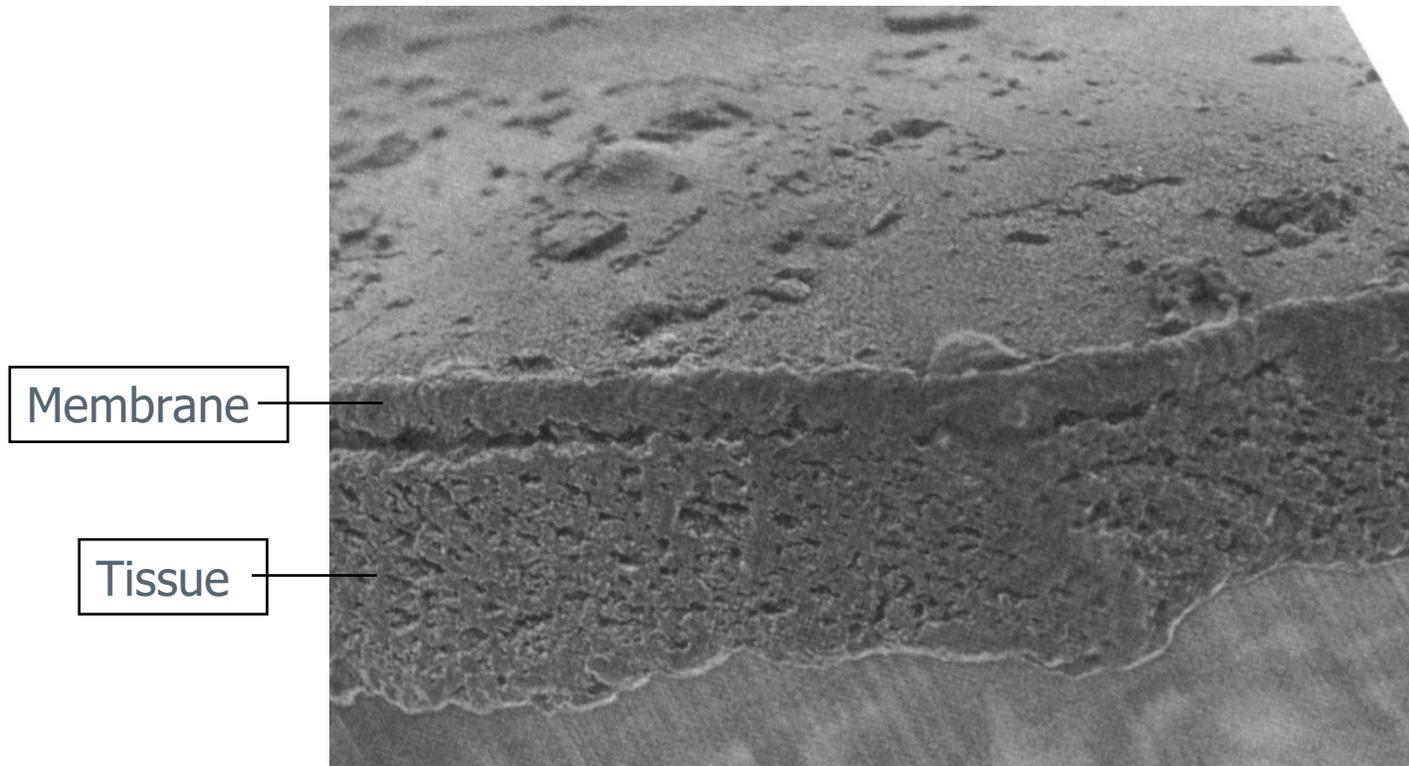


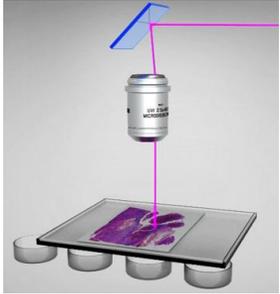
- Scheme, molecular level



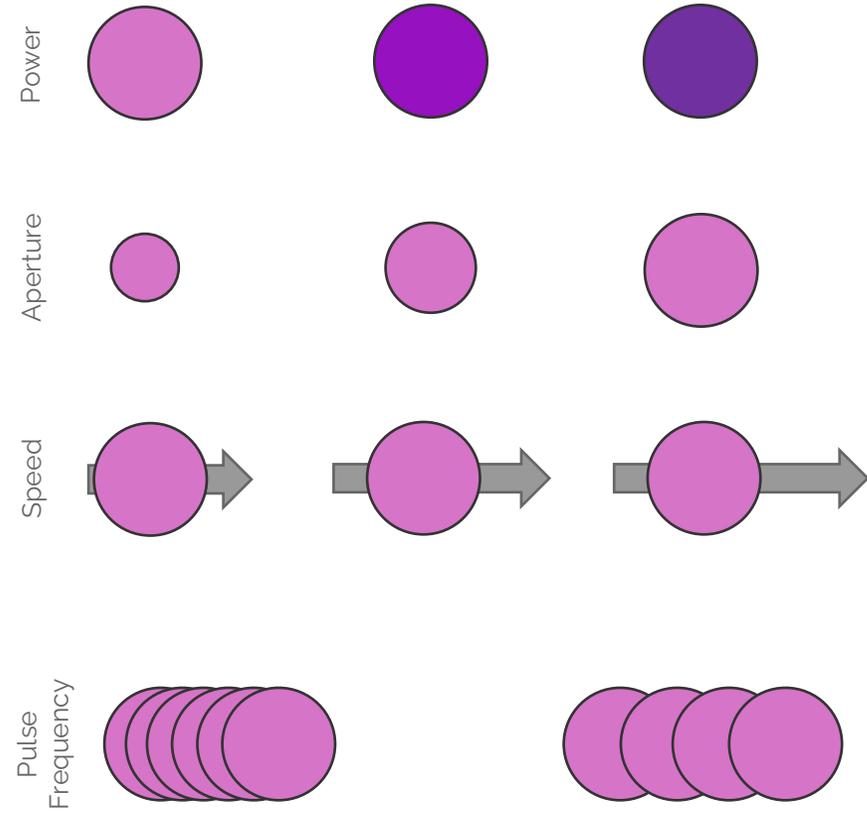
Leica Laser Microdissection

- Image of laser cut line
 - Membrane and tissue are evenly cut
 - Smooth and fine cut line



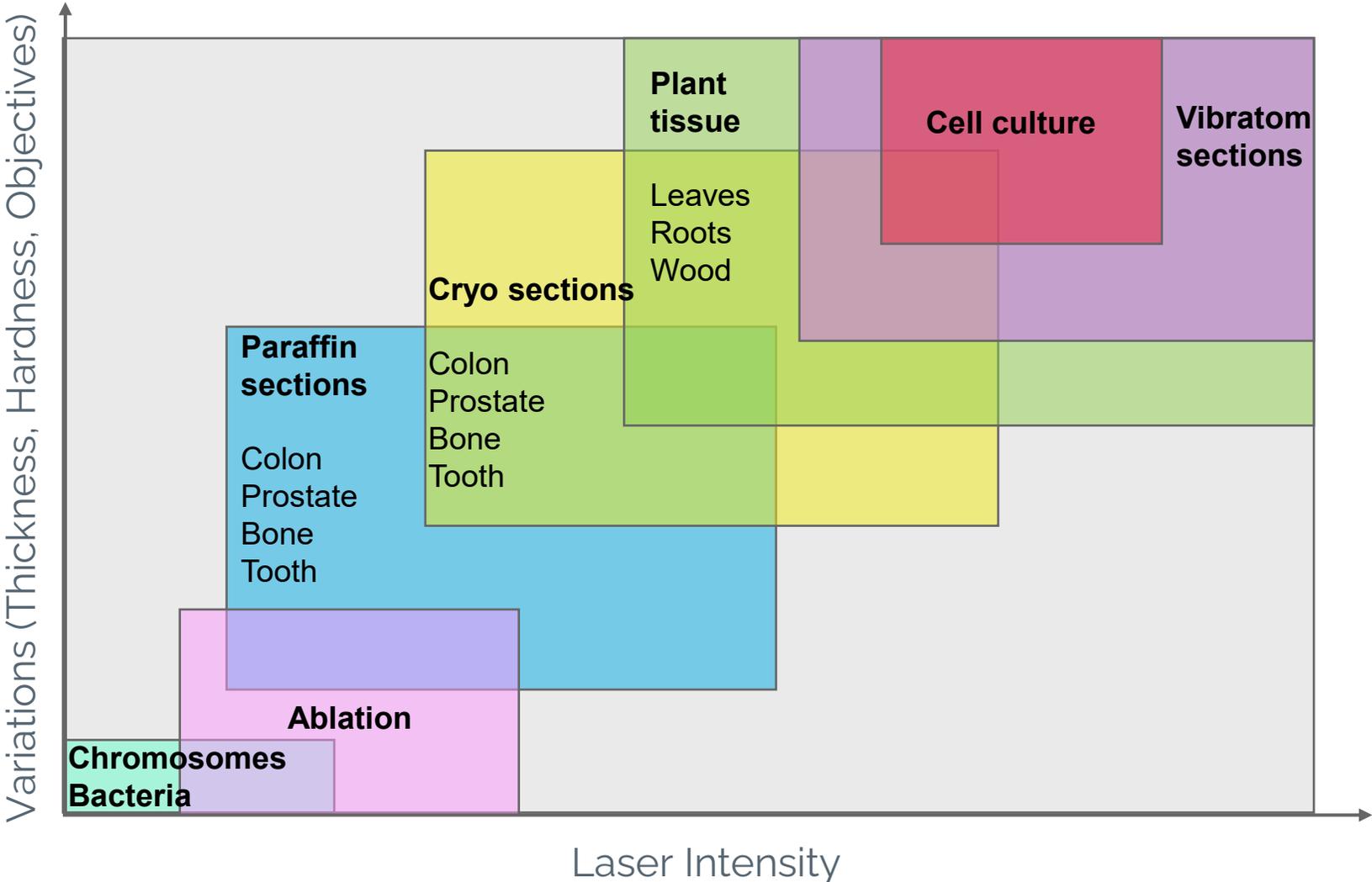


**Diode pumped
solid state laser**



	LMD6	LMD7
Wavelength	355 nm	349 nm
Pulse frequency	80 Hz	10-5000 Hz
Average pulse energy	70 μJ	120 μJ

Adjust the laser to your sample, not the other way round!



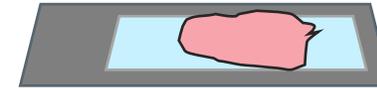
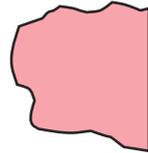
Applications – Sample Preparation

Specimen Carrier



UV absorbing membrane

Sections (Paraffin, Cryo)



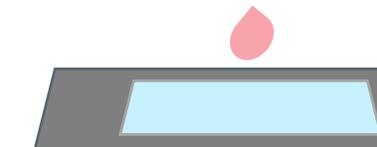
Histology
Pathology

Smear Preparation



Forensics

Live Cells



Cell Biology

Plant Tissue

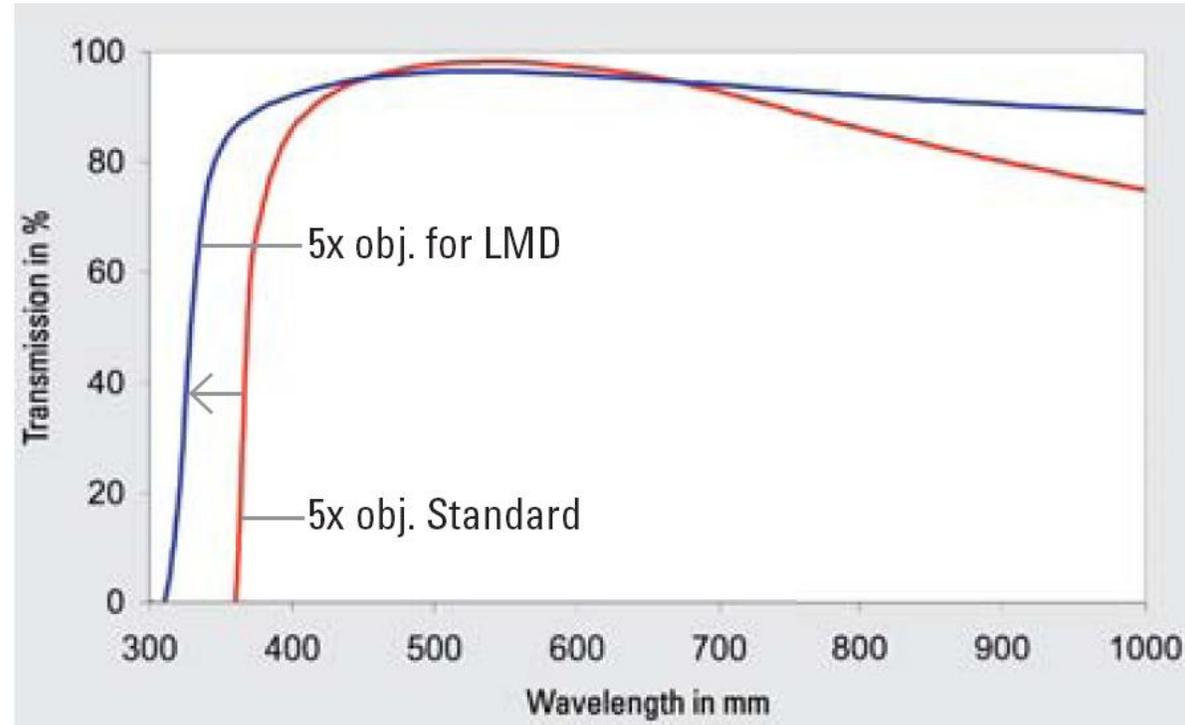


Plant Biology

2.5X – 150X

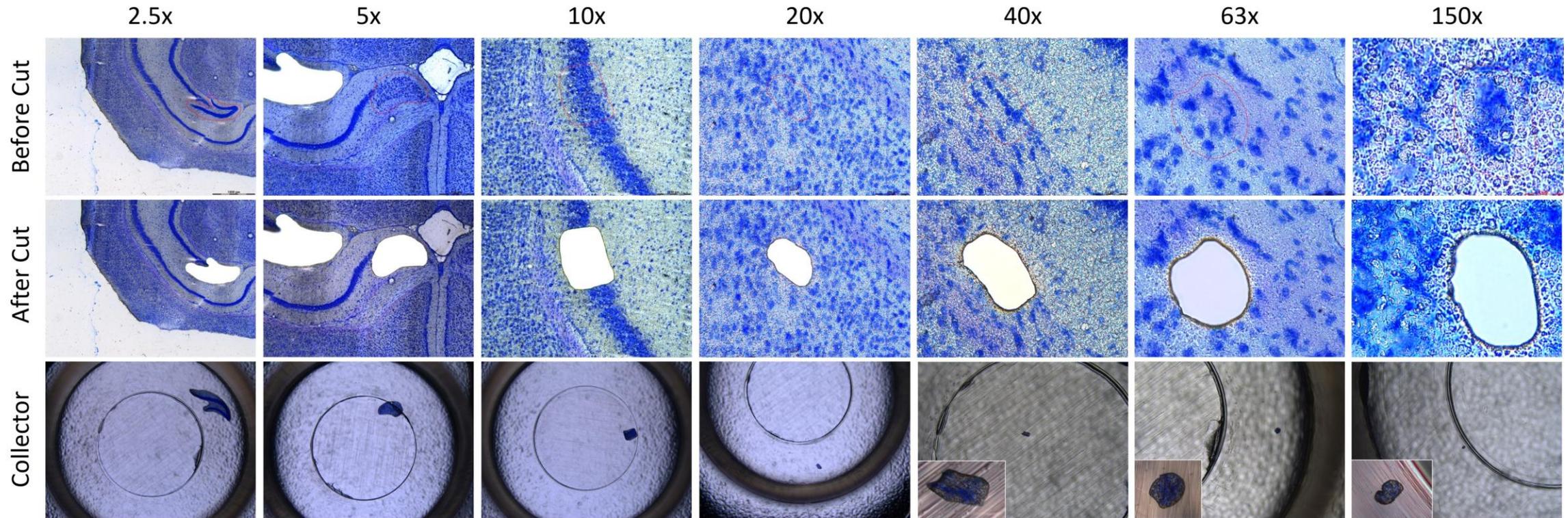


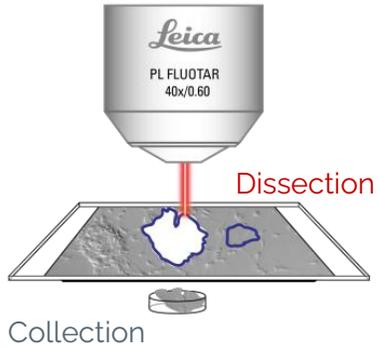
Up to 83% transmission at 350 nm



- Specially designed **DRY** for laser microdissection
- Much **higher UV transmission** than standard objectives
 - More laser power on the tissue

Laser Microdissection – Magnifications





Ibidi® Slides

Petri Dishes

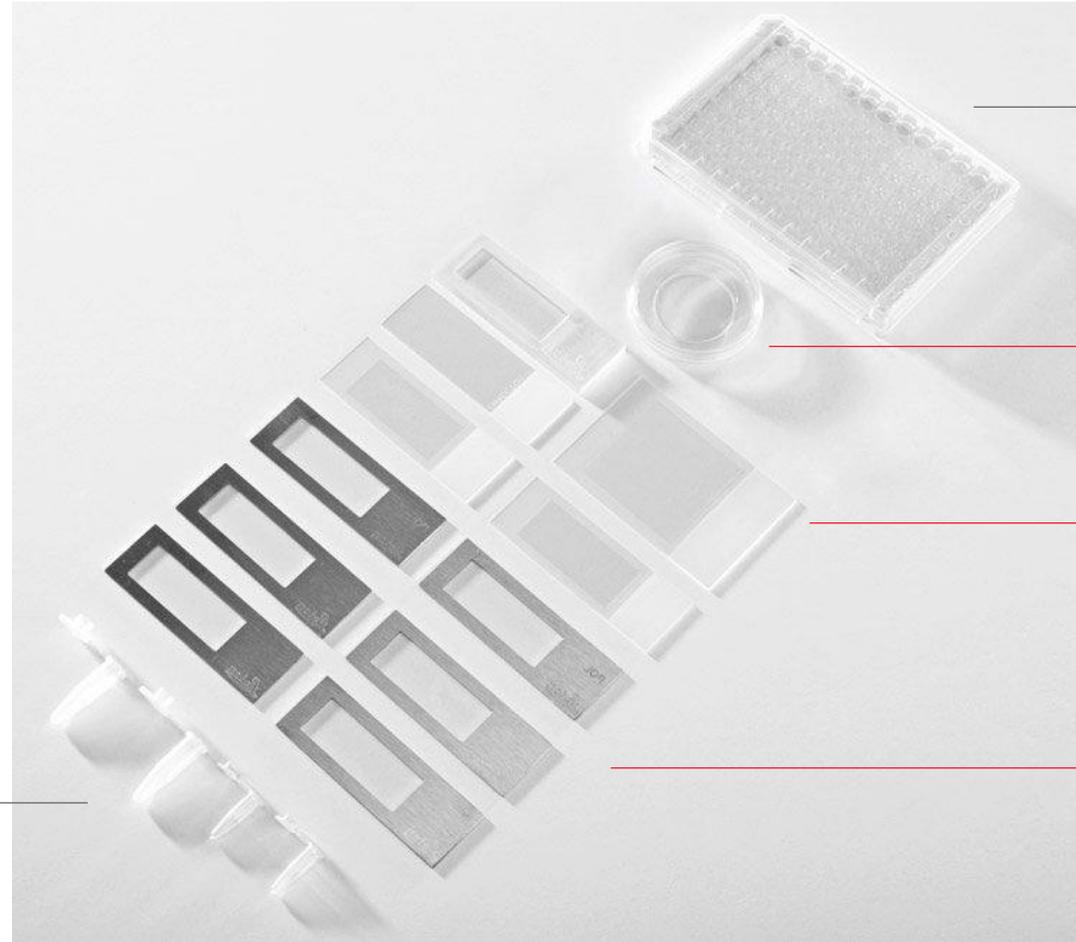
8 Well Stripes

12 Well Stripes

0.2 mL PCR tube caps

0.5 mL PCR tube caps

1.5 mL PCR tube caps



96 Well Plates

Petri Dishes

Glass Slides

Metal Frame Slides

		Motor Stage	Scanning Stage	LMT350 ultra
Holder	Classic Slides (25 mm x 76 mm) 	2 ^a	3	4
	Large Slide (50 mm x 76 mm) 	1	1	1
	Petri Dish 	1	1	2
	18 Well ibidi Slide 	1 ^b	1	1
	18 Well ibidi Slide Stack 2x 	1 ^b	1	1
Collector	0.2 mL PCR Tube 	4	4	4
	0.5 mL PCR Tube 	4	4	4
	1.5 + 2.0 mL PCR Tube 	-	-	1
	1.5 mL filled cap tube 	-	-	1
	8-Well Strip Caps 	-	2	1-2
	12-Well Strip Caps 	-	-	1
	48-Well Collector (6x8-Well Strip Caps) 	-	1	-
	96 / 384 -Well Plate (PCR, LCC) 	-	-	1
	Chamber Slide 	-	1	-
	Petri Dish 	-	1	2
	8-Strip LCC 	1	2	12 ^c
	18 Well ibidi Slide 	-	1	-
	14 x 24 mm PCT μTubes (Barocycler) 	-	-	1

^a You can place 2 classic slides in the holder for large slides
^b no dedicated holder for this set up, fits into standard slide holder
^c up to 12 strips can be mounted in a 96 well frame

Order No. (*RNase-free Slides)	Product Description/ Quantity	Usable LMD area (approx.)	Membrane Thickness	Objectives ^a Recommended Objective CORR Settings for high-quality Images								Fluorescence Applicability	Remarks
				5x	6.3x ^a	10x ^a	L20x CORR ^a	L40x CORR ^a	L63x CORR ^a	150x			
11600316 50 slides/box	Glass, PPS-membrane (25 x 76 mm)	19 mm x 41 mm	4 µm	++	++	+++	+++ D = 1.0	+++ D = 1.0	+++ D = 1.0	-	++	Suitable for almost all applications (except DIC); PPS is semi-permeable for water, do not dip into water/water-containing solution	
11600294 50 slides/box	Steel frames, PPS-membrane (25 x 76 mm)	16 mm x 45 mm	4 µm	++	++	+++	+++ D = 0	+++ D = 0	+++ D = 0	+	++	Suitable for almost all applications (except DIC)	
11505158 *11505189 50 slides/box	Glass, PEN-membrane (25 x 76 mm)	19 mm x 41 mm	2 µm	++	++	+++	+++ D = 1.0	+++ D = 1.0	+++ D = 1.0	-	*	The most popular slides; standardly used in 95% of all applications	
11600288 50 slides/box	Glass, PEN-membrane (25 x 76 mm)	19 mm x 41 mm	4 µm	++	++	+++	+++ D = 1.0	+++ D = 1.0	+++ D = 1.0	-	+	The most popular slides improved for Leica LMD applications	
11600289 50 slides/box 11600288	Steel frames, PEN-membrane (25 x 76 mm), Glass	16 mm x 45 mm	4 µm	++	++	+++	+++ D = 0	+++ D = 0	+++ D = 0	+	*		
11505151 *11505190 50 slides/box	Steel frames, PET-membrane (25 x 76 mm)	16 mm x 45 mm	1.4 µm	++	++	+++	+++ D = 0	+++ D = 0	+++ D = 0	+	+	Recommended for MALDI-TOF	
11505188 *11505191 50 slides/box	Steel frames, POL-membrane (25 x 76 mm)	16 mm x 45 mm	0.9 µm	++	++	+++	+++ D = 0	+++ D = 0	+++ D = 0	+++	++	Recommended for chromosome spreads	
11505215 (sterile) 100 slides/box	LARGE Glass PEN-membrane (50 x 76 mm)	39 mm x 45 mm	2 µm	++	++	+++	+++ D = 1.0	+++ D = 1.0	+++ D = 1.0	-	*	For large tissue sections (e.g. in neurobiology for brain slides), requires optional slide holder	
11532860 (sterile) 100 slides/box	LARGE Glass PPS-membrane (50 x 76 mm)	39 mm x 45 mm	1.2 µm	++	++	+++	+++ D = 1.0	+++ D = 1.0	+++ D = 1.0	-	++		
11600250 5 slides/box	Steel frames, FLUO-Membrane (25 mm x 76mm)	16 mm x 45 mm		-	-	+	+ D = 0	+ D = 0	+ D = 0	+	+++	For Fluorescence and DIC-contrast	
11505172 20 dishes/ box	50 mm Petri dish with PEN-membrane	30 mm diameter	2 µm	+	+	+++	+++ D = 0	+++ D = 0	- D = 0	-	+		
11505240 (sterile) 15 slides/box	µ-Slide, 18-well with PEN-membrane	30 µl vol. per well	2 µm	+	+	+++	+++ D = 0	+++ D = 0	- D = 0	-	+	For live cell cutting; direct LMD of adherent cells for recultivation or analysis	
11600249 (10 slides/box)	EP-DIRECTOR [®] slides	50 mm x 20 mm	membrane free	++	++	+++	+++ D = 1.0	+++ D = 1.0	+++ D = 1.0	-	+++	Recommended for Proteomics Optional for true fluorescence and DIC	

PEN
(Polyethylene naphthalate)
Most applications

PPS
(Polyphenylene sulfide)
Less autofluorescence

PET
(Polyethylene terephthalate)
Mass spec applications

POL
(Polyester)
Chromosome applications

FLUO
(Fluorocarbon)
Fluorescence, PH and DIC



Dissection



Collection



NEW: 384-well-plate
1536-well-plate





The screenshot displays the Leica Laser Microdissection (LMD) software interface. The main window shows a live image of a specimen with a 1000 µm scale bar. The interface includes a menu bar (File, View, Camera, Image, Laser, Auto Detection, Options, Help) and a toolbar with icons for Save, Live, Freeze, MicCtrl, Specimen, Collector, Focus, Unload, Laser, Edit, and New. On the left, there is a vertical toolbar with icons for Select, Draw, Ellipse, Rect, PtoP, Reuse, Text, Cut Txt, and Detect. The central image is surrounded by several control panels:

- Laser Control:** Includes sliders for Revolver Position (1), Magnification (2.5x), Power, Aperture (60), Speed (19), Specimen Balance (5), Line Spacing for Draw + Scan (34), Head Current (4), and Pulse Frequency (100%). It also has an Offset slider (12) and buttons for Calibrate, Auto Calibrate, and Factory Settings.
- Microscope Control:** Features Contrast Methods (TL-BF, FLUO, TL-POL), Magnification (2.5x, 5x, 10x, 20x, 40x, 63x, 150x), Focus (7803 µm), Light Intensity (255), and Stage Control (directional arrows and 100% zoom).
- Right Panel:** Contains Zoom Factor (1.0), Draw Shape(s) (Close Line(s), Single Shape, Multiple Shapes), Cut Shape(s) (Shadow Cuts, Final Pulse, Draw + Cut, Draw + Scan, Move + Cut, Laser Screen), X-Y-Z Precision (Fine, Coarse), Database (Connect to database), Specimen ID (jutta), and a Shape List table.

At the bottom, there is a Collection Device grid (HydraFourSlides) with columns 1-12 and rows A-H, a Slide Holder, and Stage Position Memory (2). The status bar shows 'Ready', 'HydraFourSlides', 'x-y-live: 831 / 175 x-y-stage: 43441µm / 52237µm', and 'Magnification: 2.5x'. The Leica LMD7 logo is visible in the bottom right of the interface.





Multifruit mixed smoothie



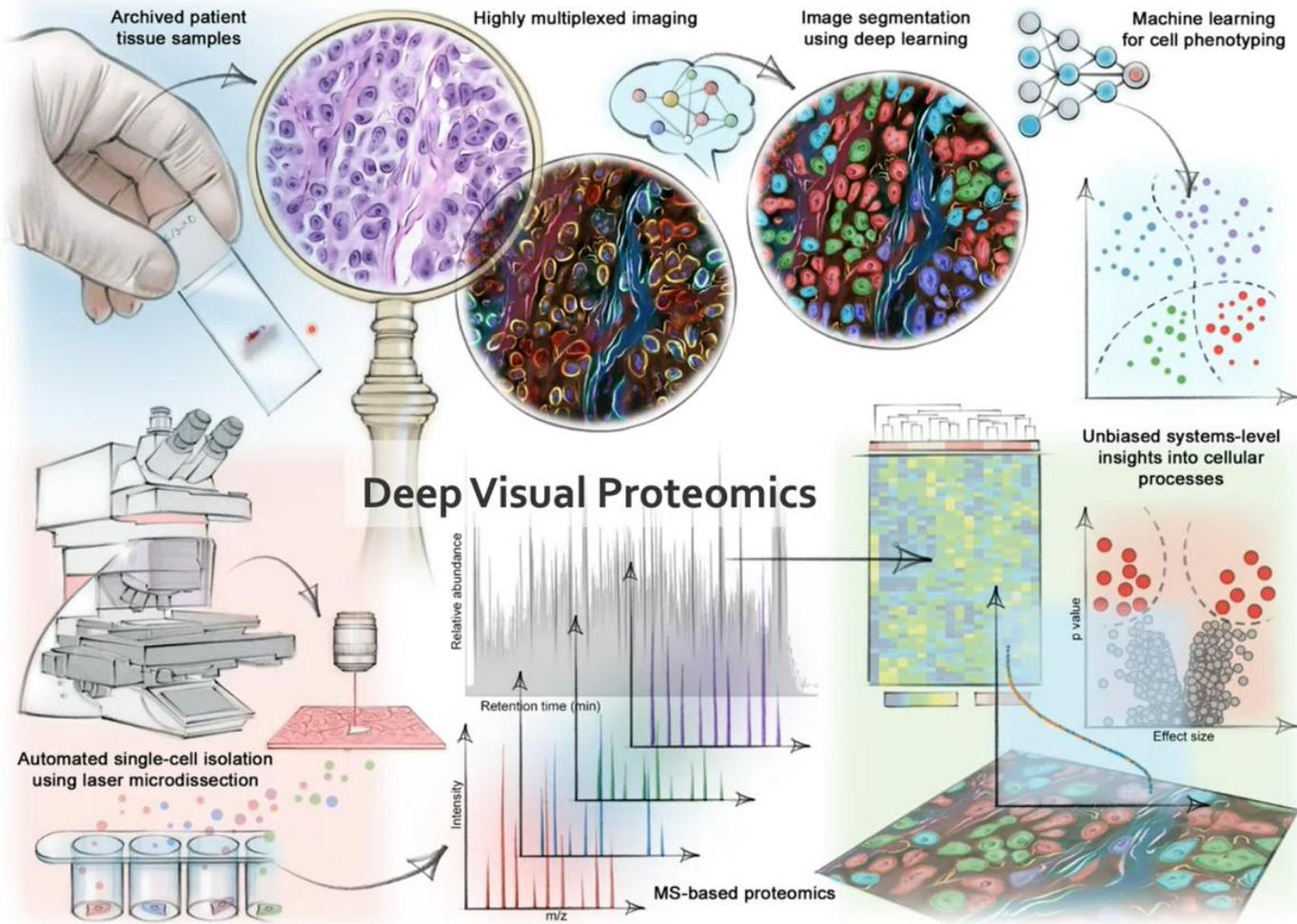
[nature](#) > [nature methods](#) > focus

Focus | 10 December 2024

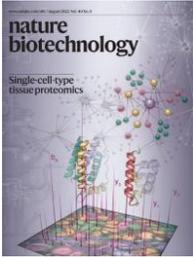
Method of the Year 2024: spatial proteomics

Spatial proteomics is our pick for Method of the Year 2024, for the impact that these technologies have had on the understanding of the organization, structure and function of complex tissues, including in global tissue atlas projects.





Mund, A., Coscia, F., Kriston, A., ..., Matthias Mann
Deep Visual Proteomics defines single-cell identity and heterogeneity.
Nat Biotechnol **40**, 1231–1240 (2022).
<https://doi.org/10.1038/s41587-022-01302-5>

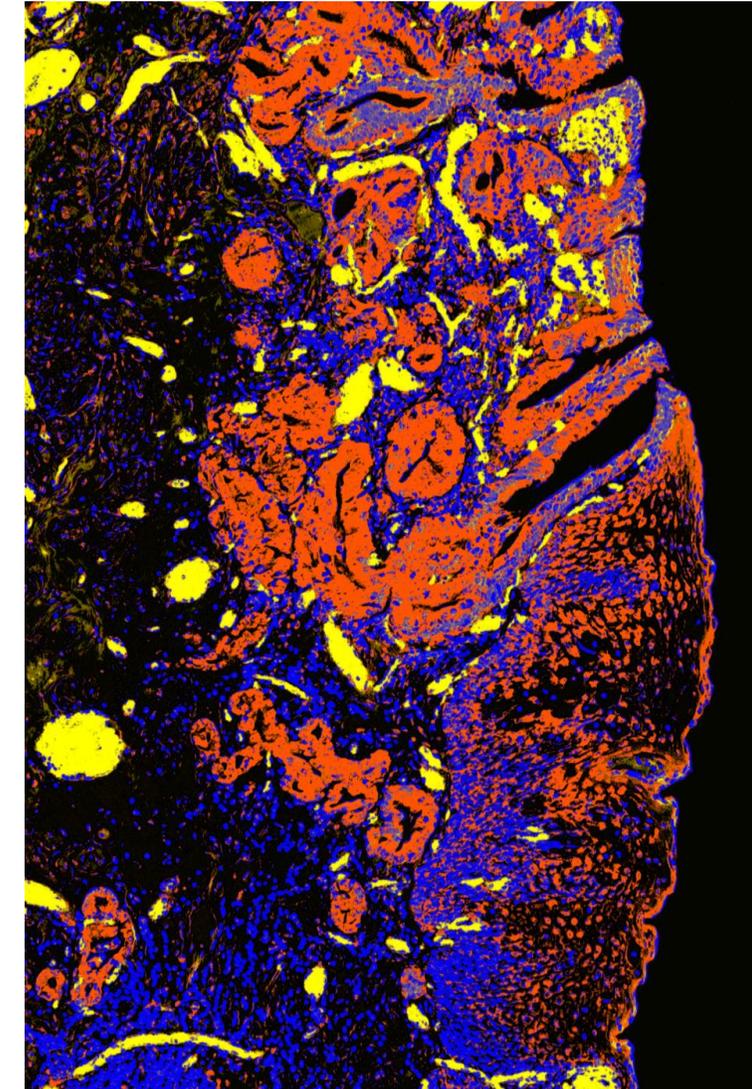
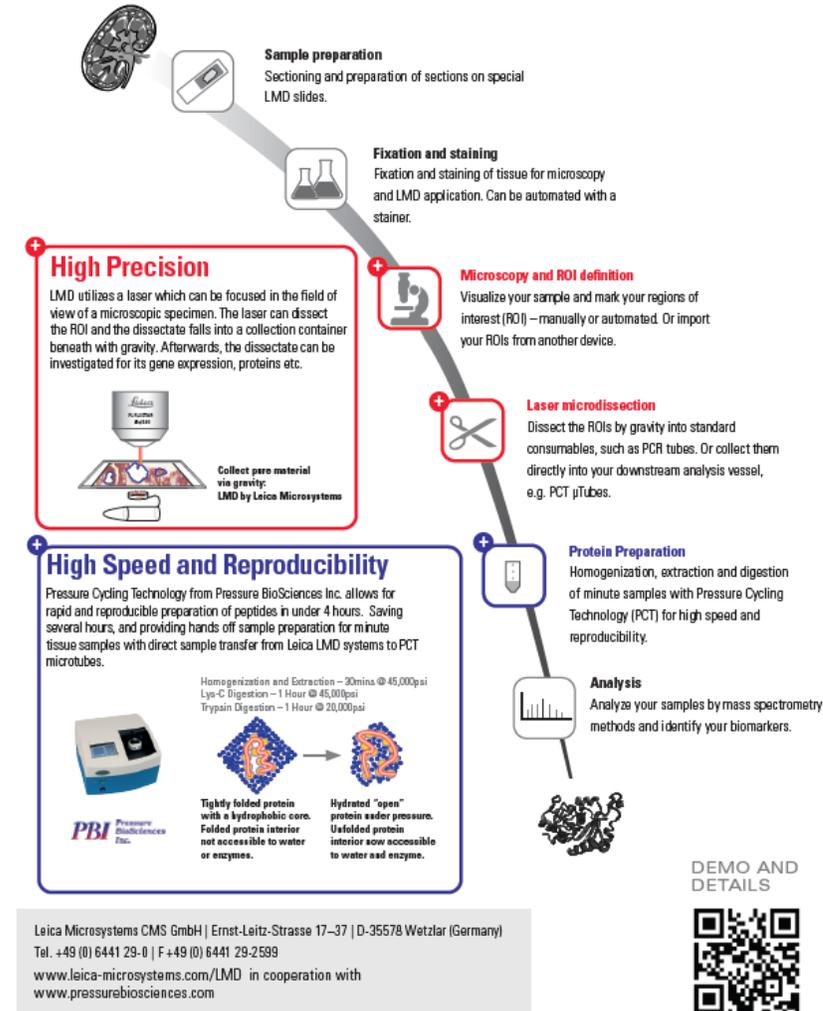


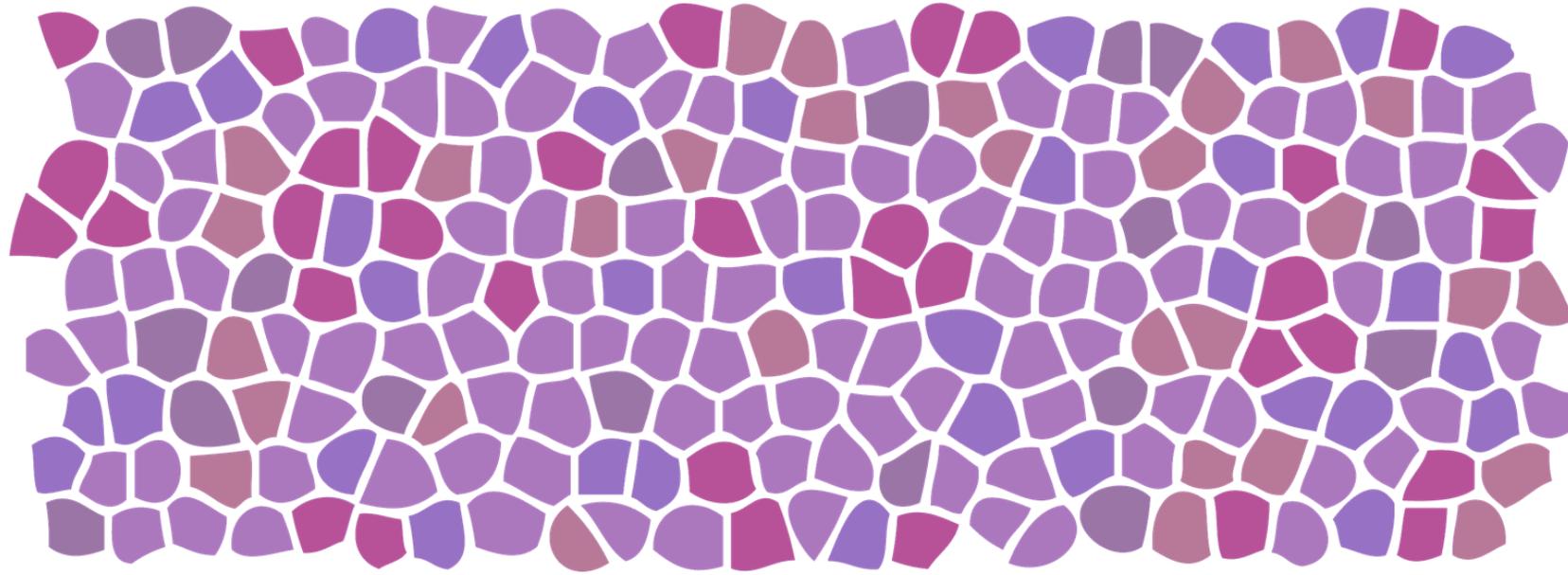
-
- 
Matthias Mann - Germany
 - 
Andreas Mund - Denmark
 - 
Fabian Coscia - Germany
 - 
Peter Horvath - Hungary

LMD

- Areas of interest identified by AIVIA
- AIVIA maps ported to LMD for dissection and tissue collection.

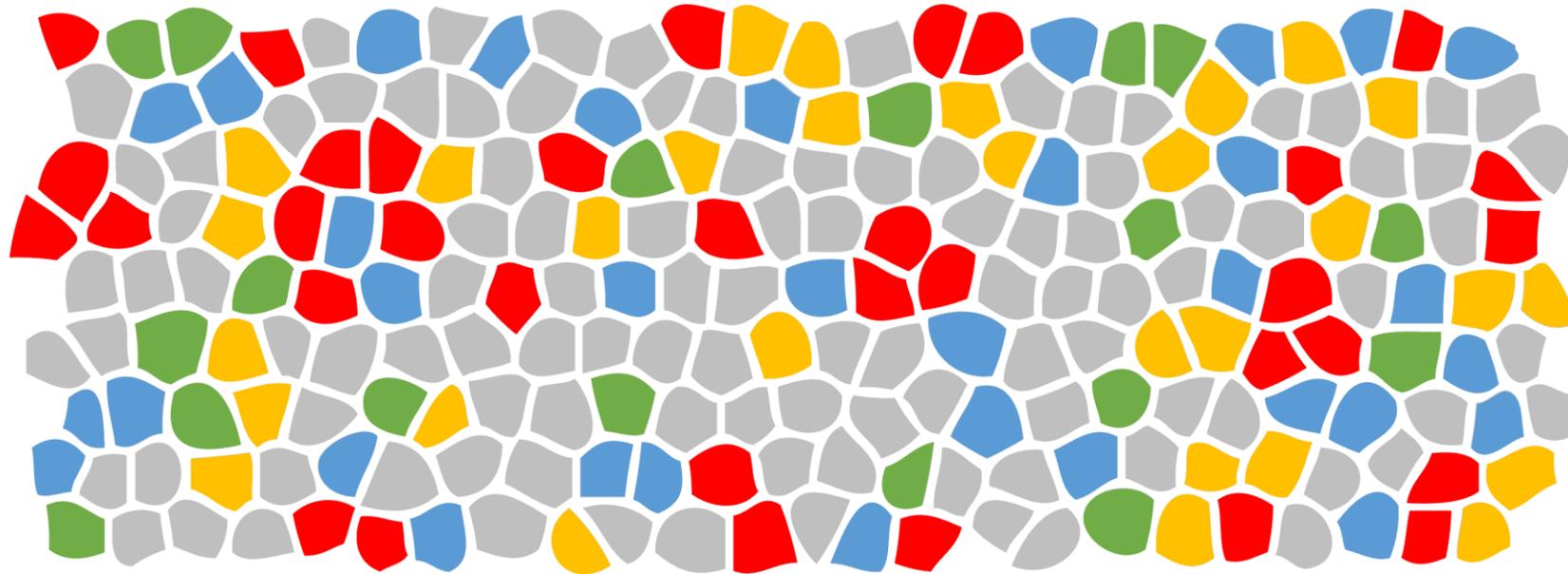
Fast and pure Sample Preparation for Biomarker Discovery





Microscope





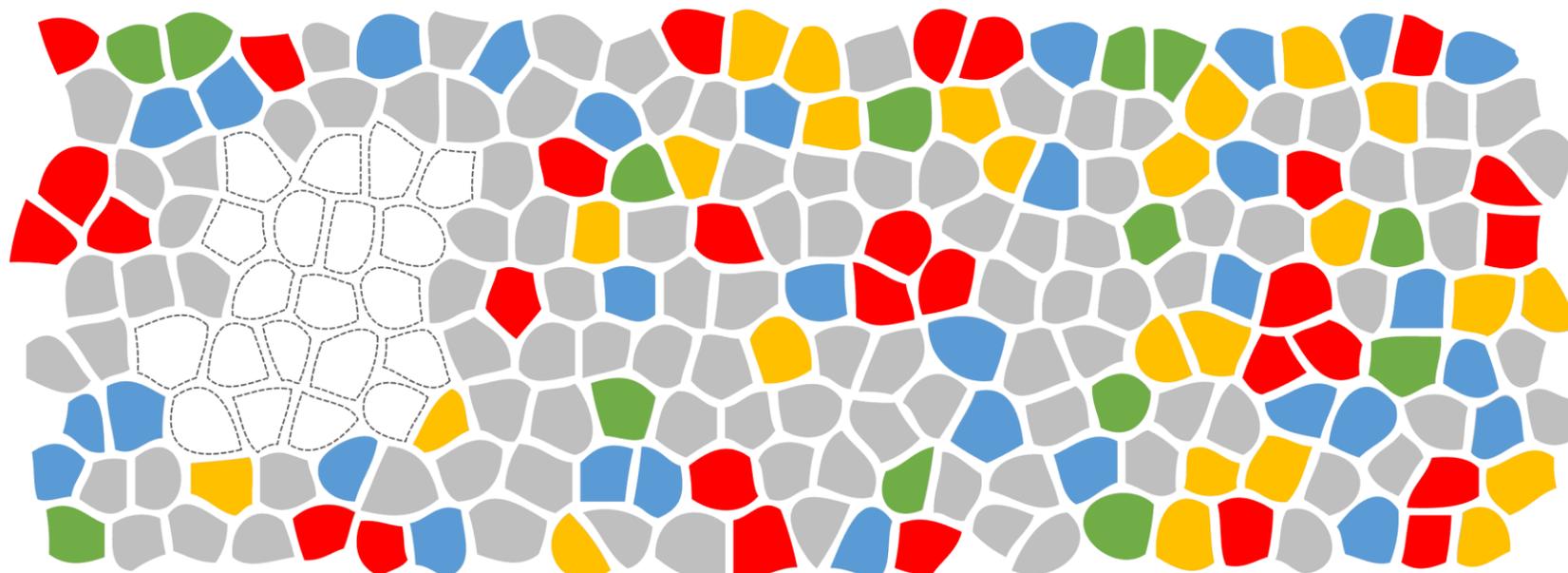
Microscope



AI



Imaging → Segmentation → Laser Microdissection



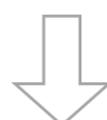
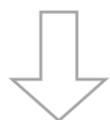
Microscope

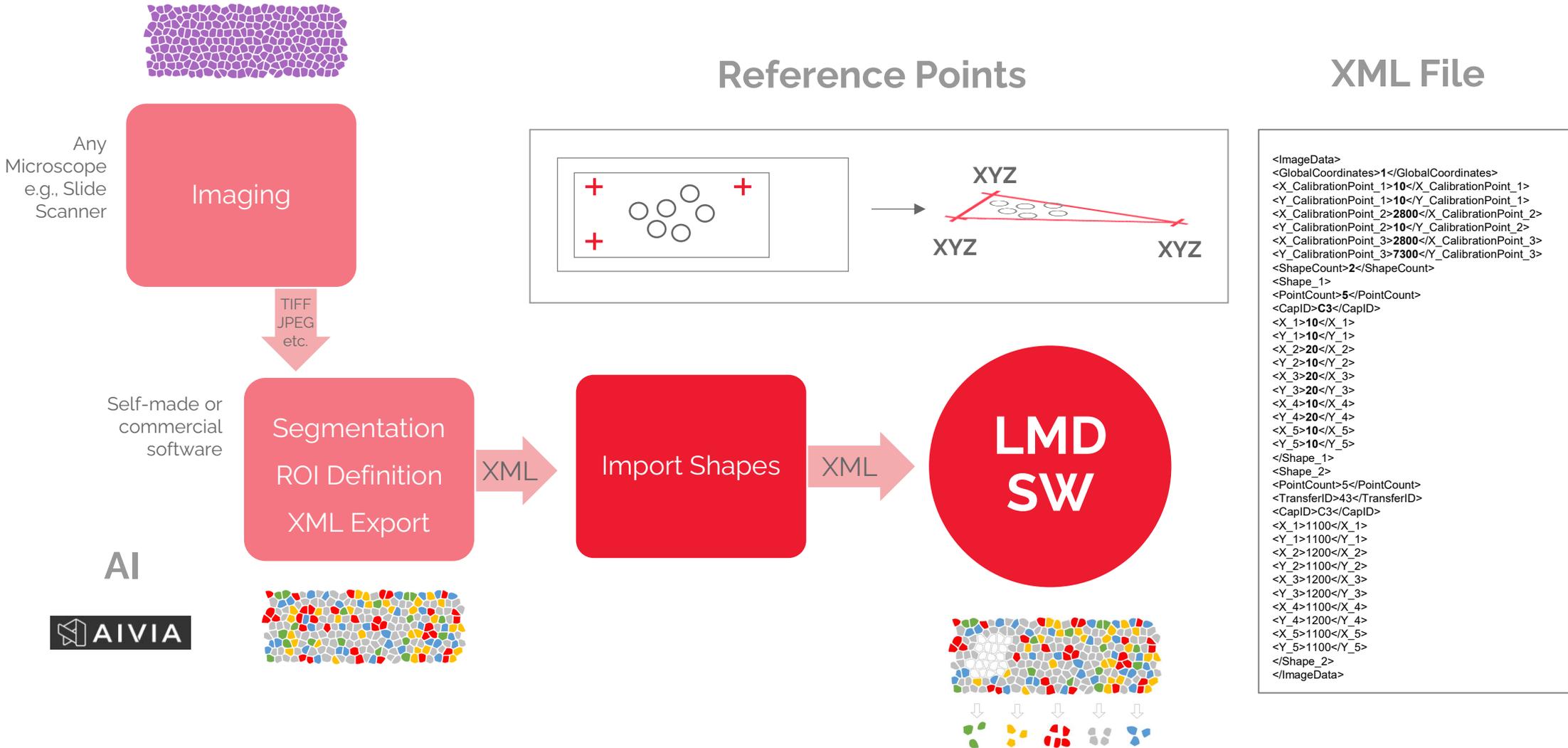


AI



Laser Microdissection

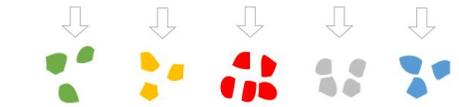
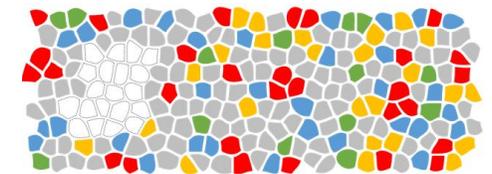
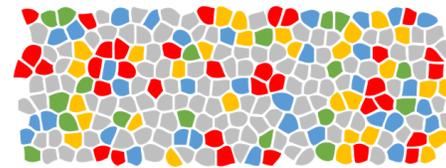
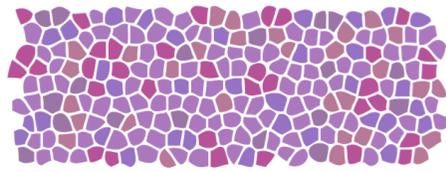




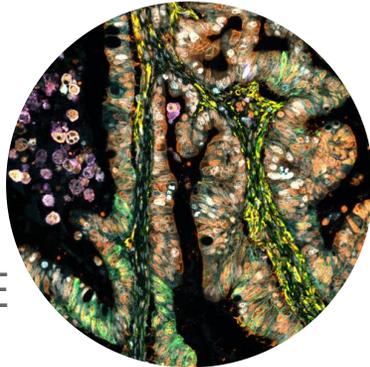
Multiplexing

Automatic ROI detection

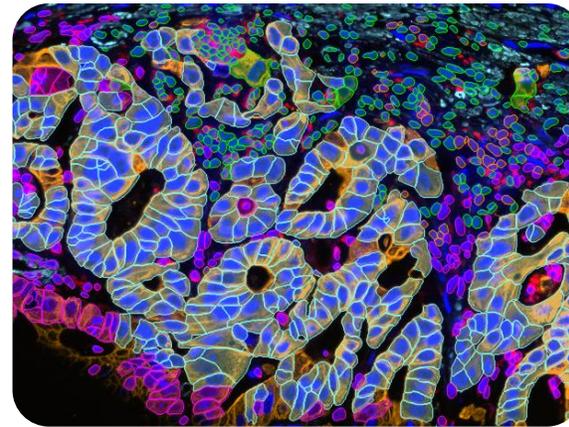
Single cell isolation



Cell DIVE



SpectraPlex



<https://www.leica-microsystems.com/science-lab/life-science/consumables-for-laser-microdissection/>

Order No. (Please-free Slides)	Product Description/ Quantity	Usable LMD area (approx.)	Membrane Thickness	Objectives ¹ Se S Star 100x	Recommended Objective CORRS Settings for high-quality images			Fluorescence Applicability	Remarks
					100x CORRS	100x CORRS	100x CORRS		
11600316 50 slides/box	Glass, PPS- membrane (25 x 76 mm)	19 mm x 41 mm	4 µm	**	***	***	***	***	Suitable for almost all applications (except DIC). PPS is semi-permeable for water, do not dip into water/water-containing solution
11600294 50 slides/box	Steel frames, PPS- membrane (25 x 76 mm)	16 mm x 45 mm	4 µm	**	***	***	***	***	Suitable for almost all applications (except DIC)
11505138 *11505189 50 slides/box	Glass, PEN- membrane (25 x 76 mm)	19 mm x 41 mm	2 µm	**	***	***	***	***	The most popular slides, standardly used in 90% of all applications
11600288 50 slides/box	Glass, PEN- membrane (25 x 76 mm)	19 mm x 41 mm	4 µm	**	***	***	***	***	The most popular slides improved for Leica LMD applications
11600289 50 slides/box	Steel frames, PEN- membrane (25 x 76 mm)	16 mm x 45 mm	4 µm	**	***	***	***	***	
11600288	Glass	16 mm x 45 mm	1.4 µm	**	***	***	***	***	Recommended for MALDI-TOF
11505193 *11505190 50 slides/box	Steel frames, PEN- membrane (25 x 76 mm)	16 mm x 45 mm	0.9 µm	**	***	***	***	***	Recommended for chromosome spreads
11505188 *11505191 50 slides/box	Steel frames, POL- membrane (25 x 76 mm)	16 mm x 45 mm	0.9 µm	**	***	***	***	***	
11505215 100 slides/box	LARGE Glass PEN- membrane (50 x 76 mm)	39 mm x 45 mm	2 µm	**	***	***	***	***	For large tissue sections (e.g. in neurobiology for brain slides), requires optional slide holder
11532860 100 slides/box	LARGE Glass PPS- membrane (50 x 76 mm)	39 mm x 45 mm	1.2 µm	**	***	***	***	***	
11600290 50 slides/box	Steel frames, FLUO- Membrane (25 mm x 76mm)	16 mm x 45 mm	-	-	-	D=0	D=0	D=0	For Fluorescence and DIC-contrast
11505172 20 slides/box	50 mm Petri dish with PEN- membrane	30 mm diameter	2 µm	+	***	***	***	***	
11505340 15 slides/box (sterile)	µ-Slide, 18 well with PEN-membrane	30 µl vol. per well	2 µm	+	***	***	***	***	For live cell cutting: direct LMD of adherent cells for recultivation or analysis
11600249 100 slides/box	EP-DIRECTOR® slides	50 mm x 20 mm	membrane free	**	***	***	***	***	Recommended for Proteomics Optional for true fluorescence and DIC

From Eye to Insight



SAMPLE PREPARATION FOR LEICA LASER MICRODISSECTION

Protocol Guide for Leica Microsystems Laser Microdissection Systems

Any Questions?

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Thank You!



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