Microdissection: Rationale and Applications

Yıldırım KARSLIOĞLU, M.D.
Gülhane Military Medical Academy
and
School of Medicine

Disclosure

- I DO NOT have any connection with the brand names, manufacturers and their representatives seen and/or mentioned in this presentation

Overview

- Definition
- Basic idea of microdissection
- History
- Types of microdissection
- Laser-assisted microdissection
- Available platforms
- Specimen preparation and expected yield of nucleic acids and proteins
- Selected applications in pathology and examples of downstream analyses
- Future perspectives

Definition

- Seperation of cells or groups of cells having **specific features** from the “background” population

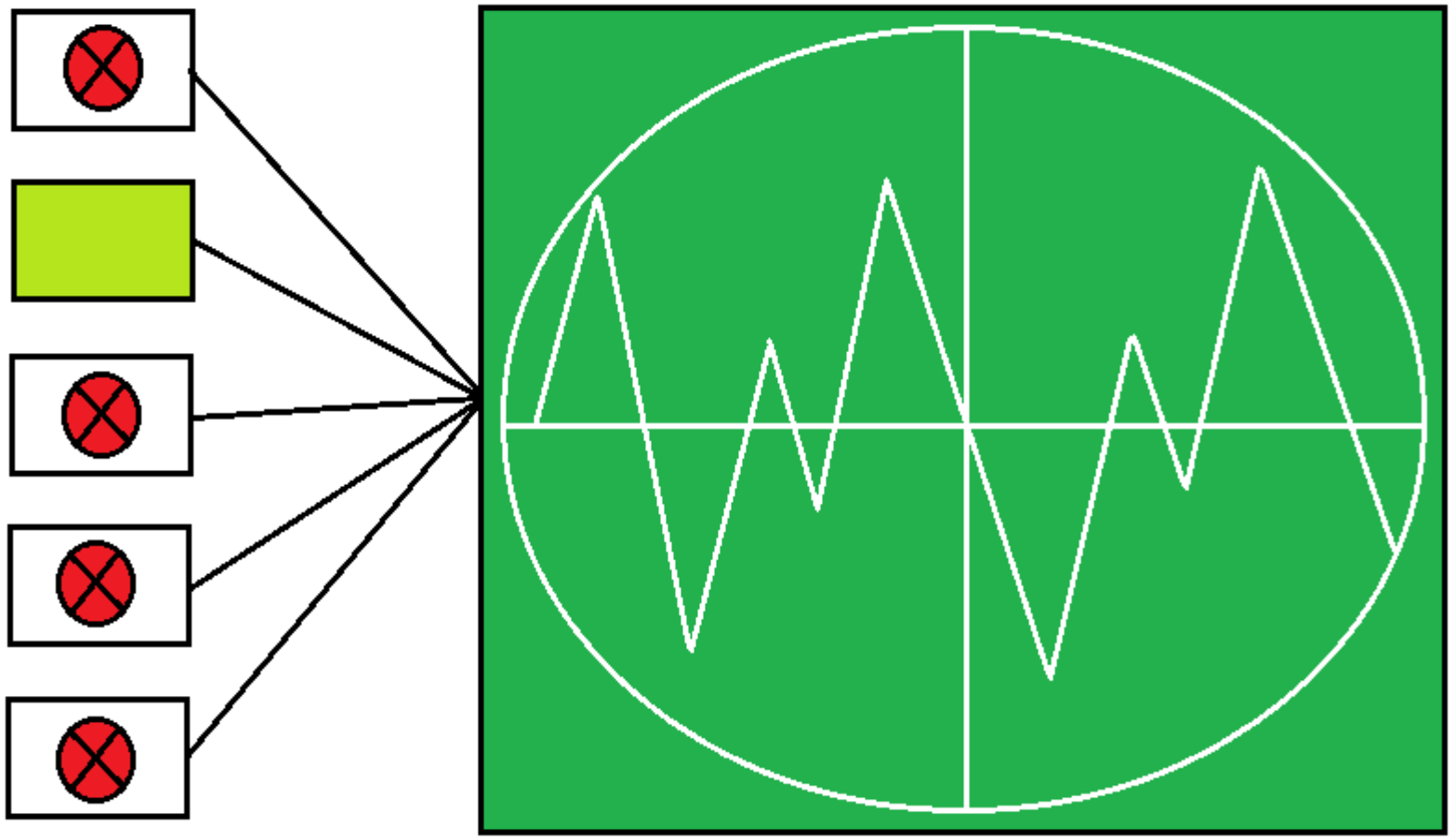
Definition

- These specific features can be:
  - Morphological properties only
  - Morphology + histochemistry / immunohistochemistry
  - Functional (biological behavior)

Definition

- Aim:
  - To obtain “pure” raw material for downstream analysis
  - To eliminate unwanted components from the section on the slide
  - To enrich selected types of cells for genomic or protemic studies

Basic Idea of Microdissection



History

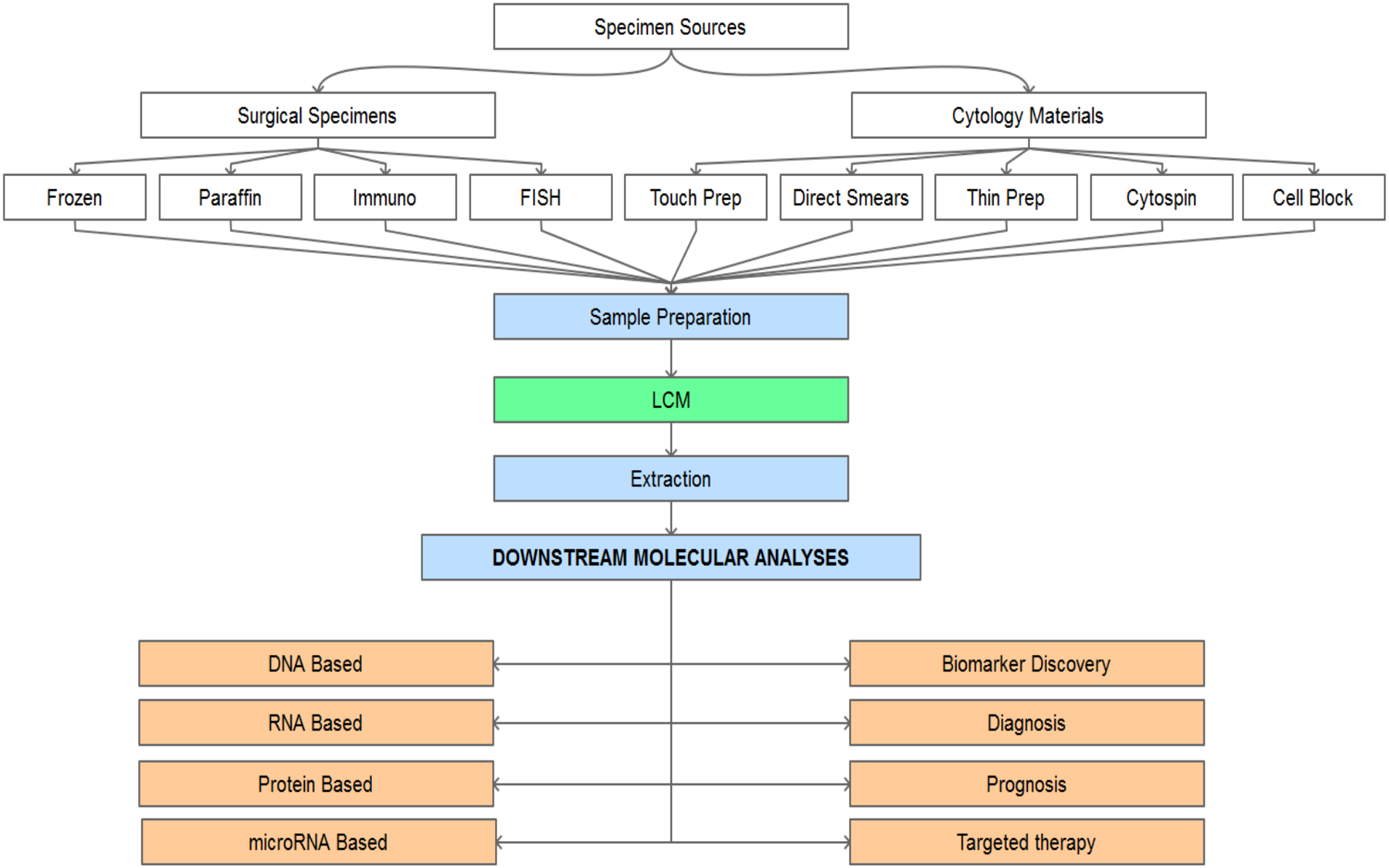
- The idea is not new
- First attemps were based on the usage of razor blades under a dissecting microscope
- Biochemical analysis on specific cell types from lyophilized tissues (Lowry and Passoneau, 1972)
- Manual extraction from formalin-fixed paraffin-embeded (FFPE) tissues by Goelz et al. (1985)

History

- The concept of using laser beam goes back 1969 (Berns-Rounds)
- First UV-laser equipped microscope in 1976 (Isenberg, Meier-Ruge)
- First prototypes of laser-based systems were massively space-occupying ☺
- First advanced laser-microdissection instrument in 1996 (Emmert-Buck)

History

- This prototype was commercialized by Arcturus Engineering
- Then, several competitors were appeared
- Most widely used systems are Laser Capture Microdissection instruments
- Several modifications and improvements became available on the growing market


Specimen Sources
Surgical Specimens
Cytology Materials
Frozen
Paraffin
Immuno
FISH
Touch Prep
Direct Smears
Thin Prep
Cytospin
Cell Block
Sample Preparation
LCM
Extraction
DOWNSTREAM MOLECULAR ANALYSES
DNA Based
RNA Based
Protein Based
microRNA Based
Biomarker Discovery
Diagnosis
Prognosis
Targeted therapy

Tissue Conditions

Healthy ↔ Diseased

Treated ↔ Untreated

Microdissection

Sample Isolation

DNA

RNA

Protein

Need > 500 / 2000 cells

Analyte Amplification

++

+

-

Analysis

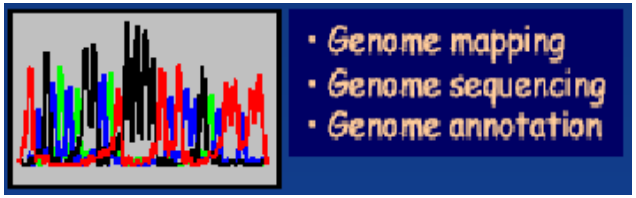
Structural Genomics

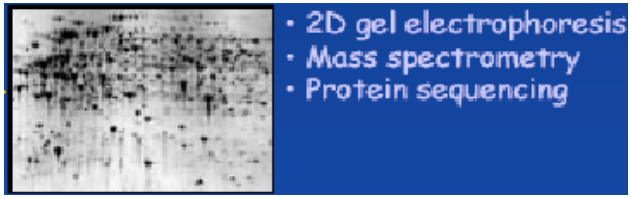
Functional Genomics (Biochips)

Genome

Transcriptome

Proteome





Markets

Molecular Pathology

Oncology

Neuroscience

Toxicogenomics

Pharmacogenomics

Neuroscience

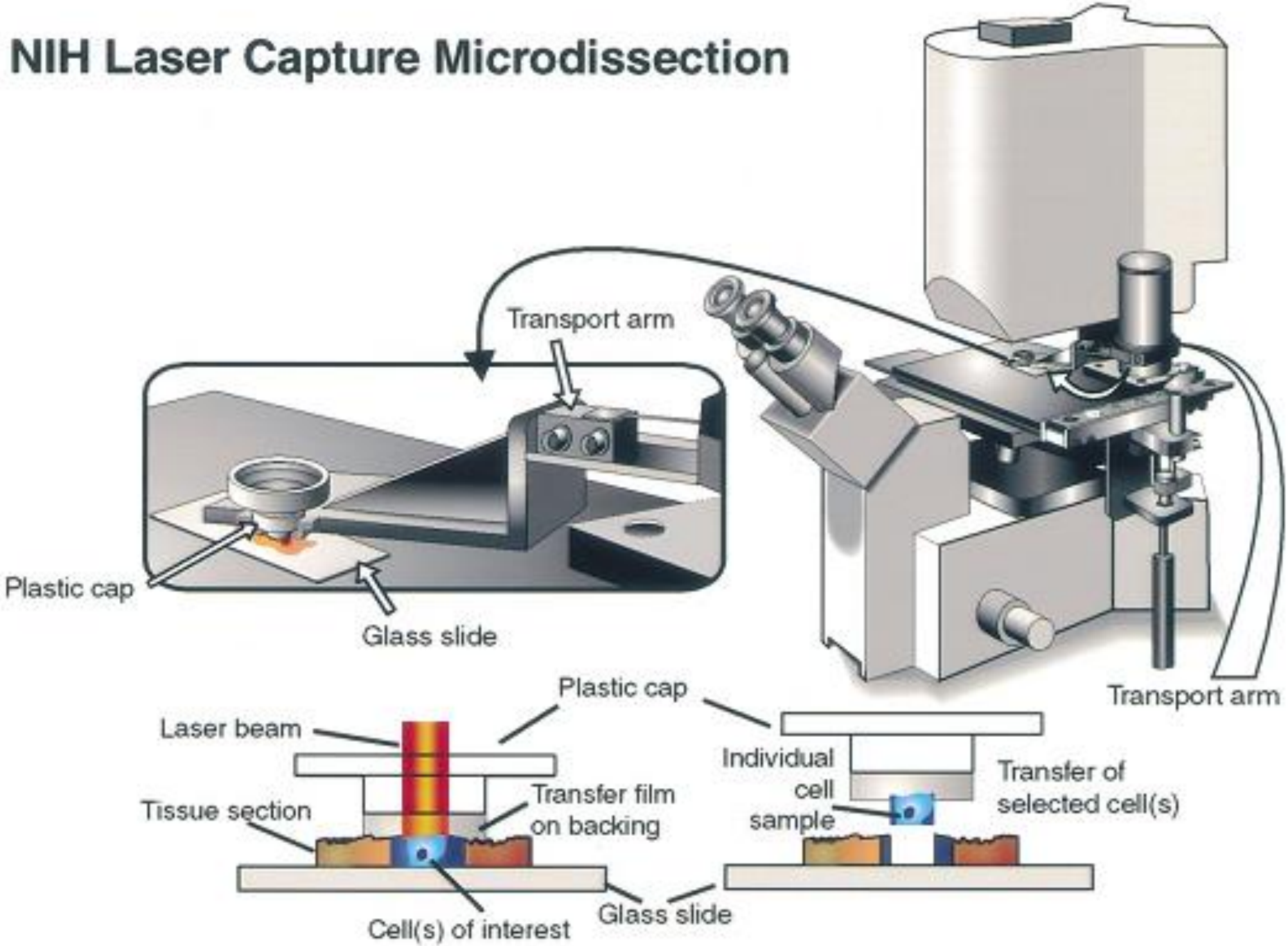
Types of Microdissection

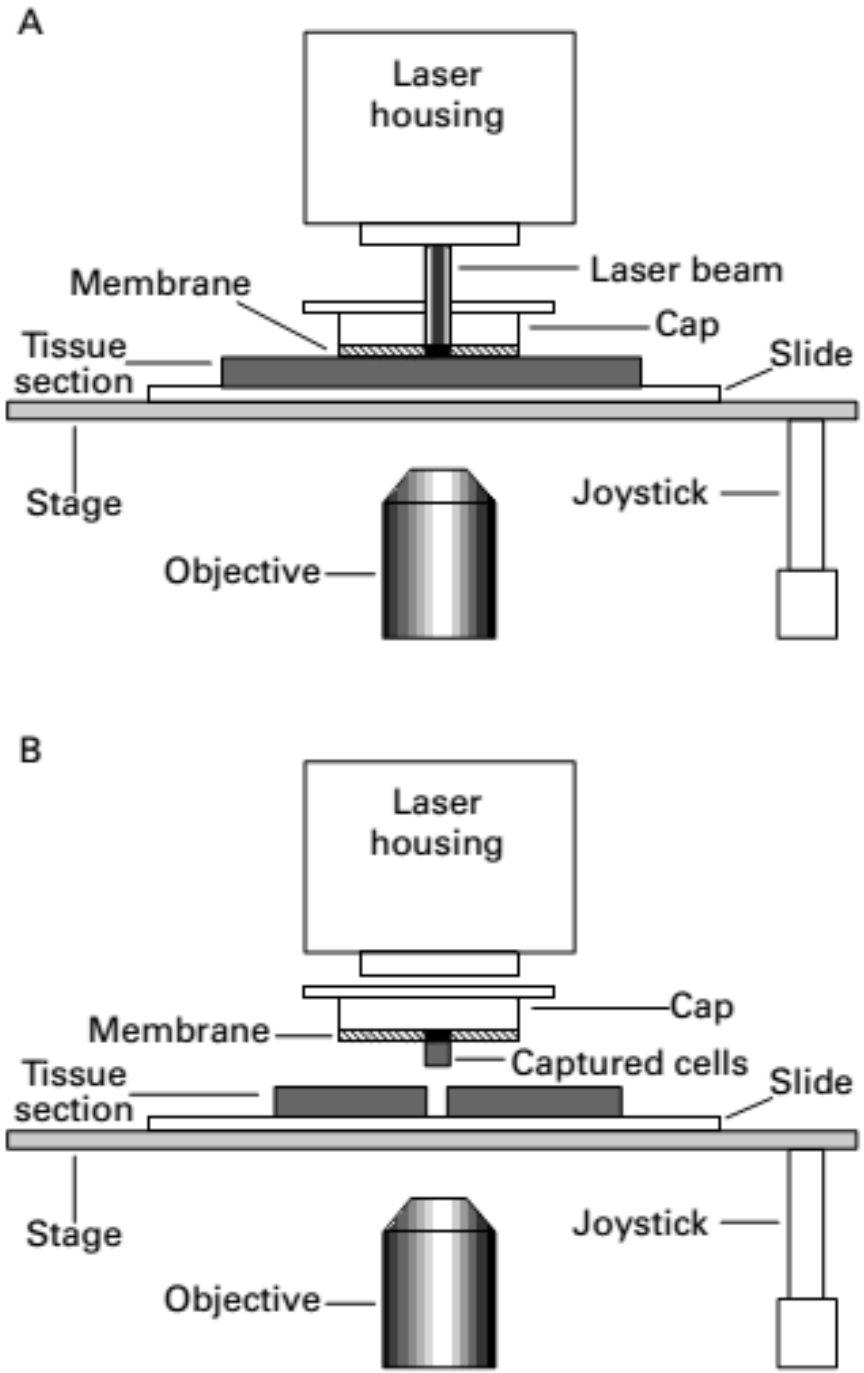
- Manual microdissection
  - Scalpels, razor blades, needle-tips
  - Dissection microscope + micromanipulator
- Instrument-assisted microdissection
  - Devices using needles with ultrasonic vibrations
  - **Laser capture microdissection**
  - Laser cutting microdissection

Laser-capture microdissection (LCM)

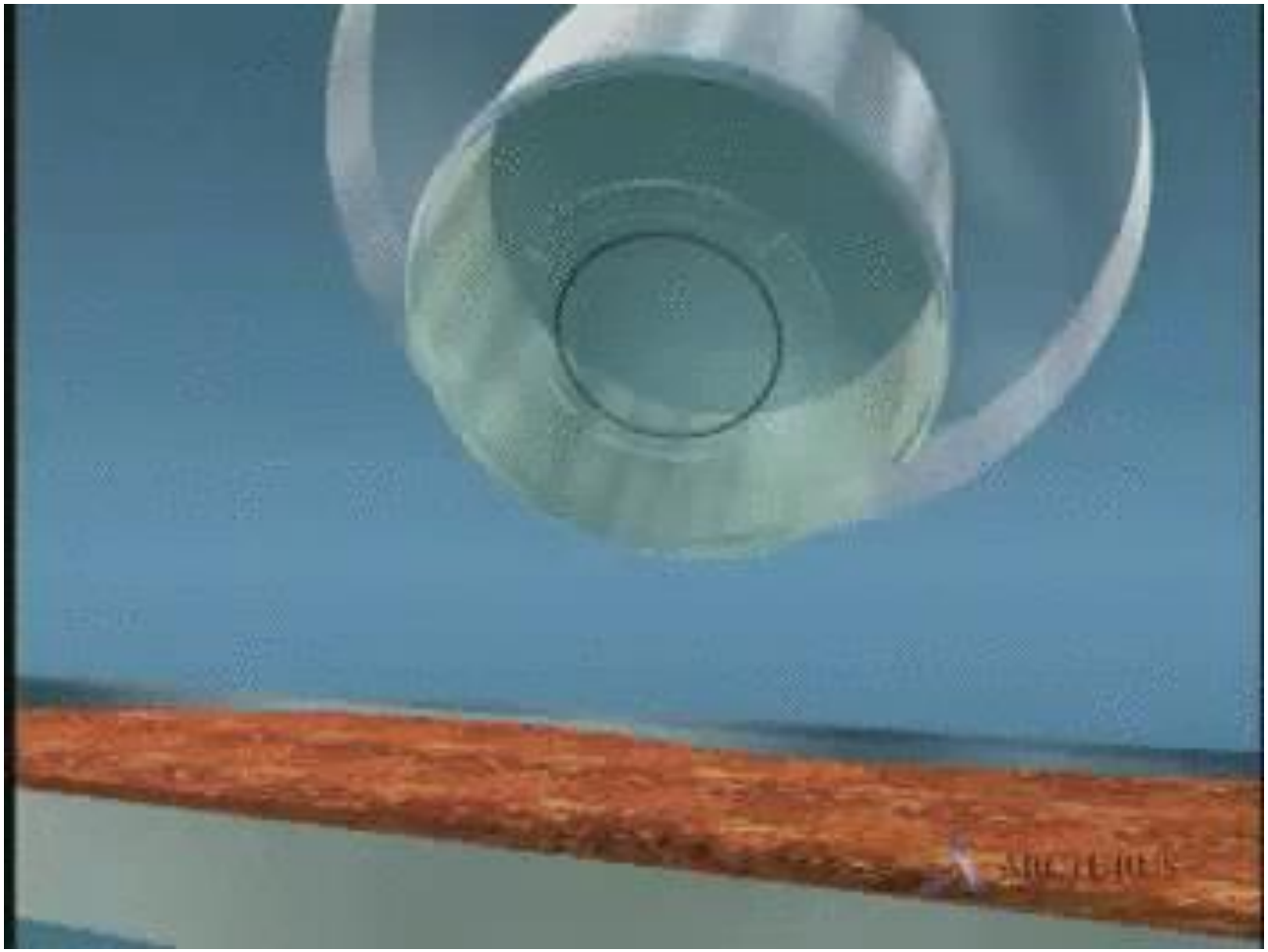
- Inverted bright-field light microscope
- Thermoplastic membrane-mounted cap
- Cap overlies the cells to be microdissected
- Low-power **infrared** (IR) laser beam
- Laser melts a spot of membrane on cells
- Melted membrane attached the cells
- As the plastic cools, the cells are lifted off

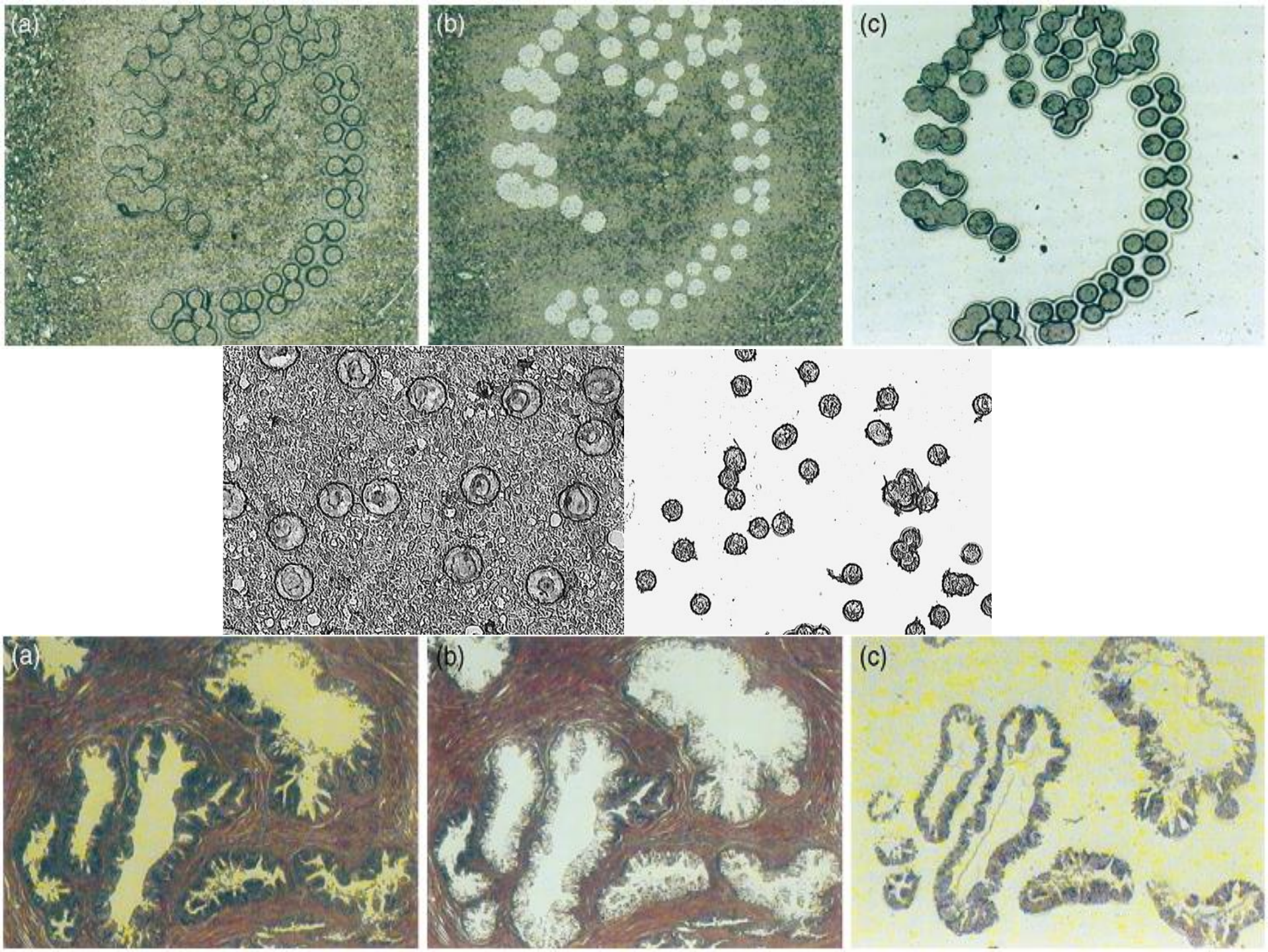
NIH Laser Capture Microdissection

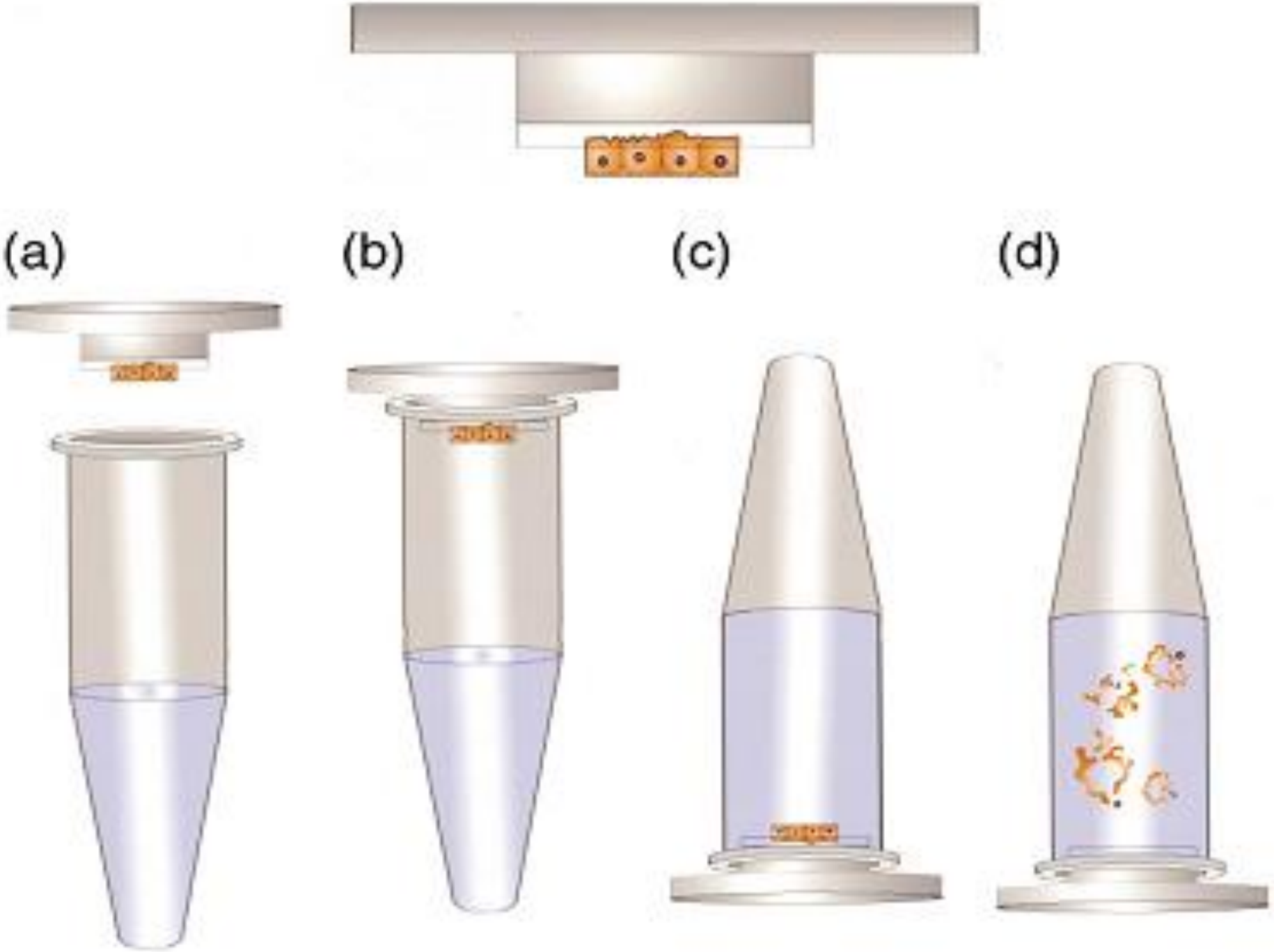



A
Laser housing
Laser beam
Membrane
Cap
Tissue section
Slide
Stage
Joystick
Objective
B
Laser housing
Cap
Membrane
Captured cells
Tissue section
Slide
Stage
Joystick
Objective

LCM in Action



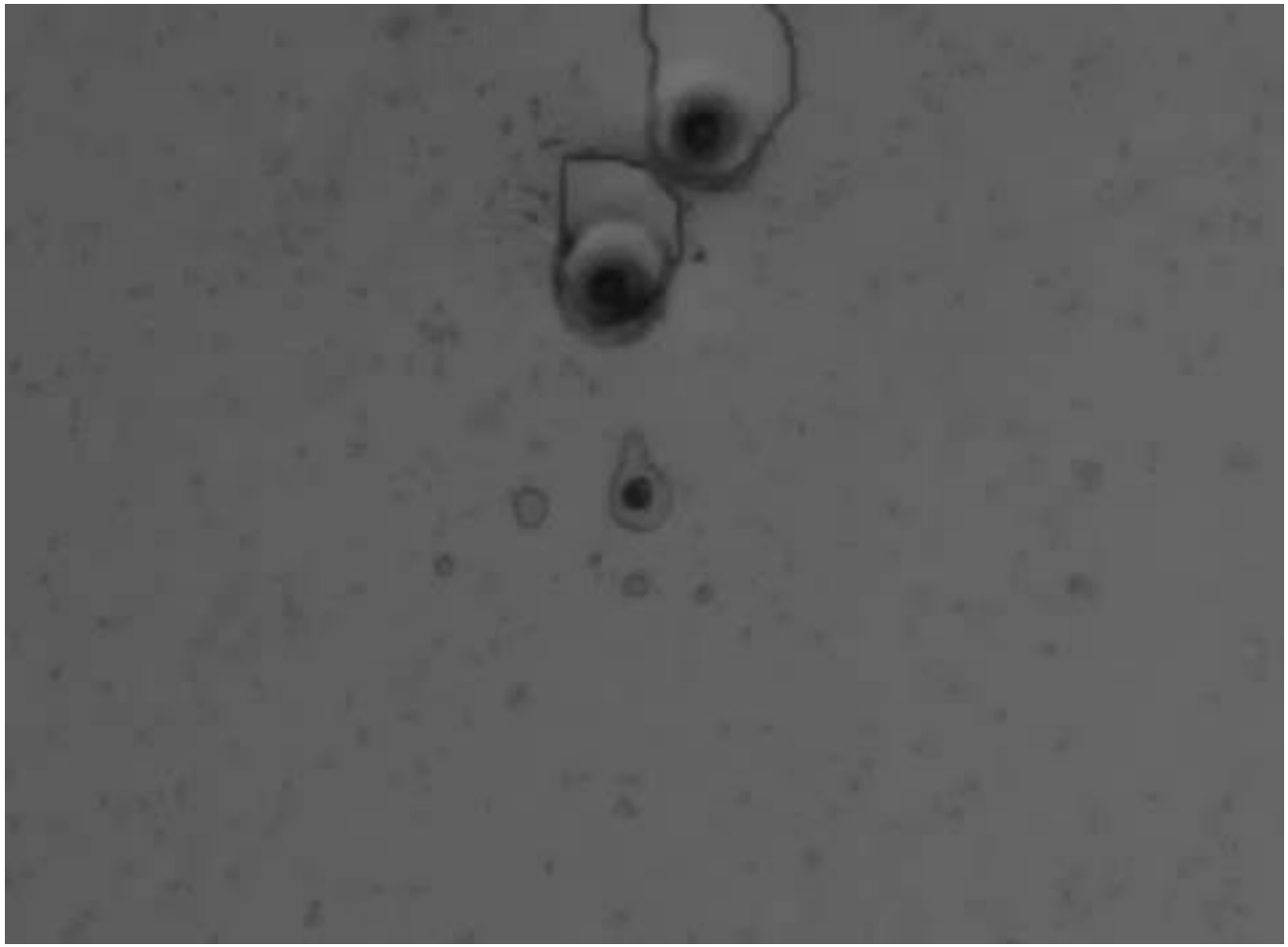

(a)
(b)
(c)
(a)
(b)
(c)

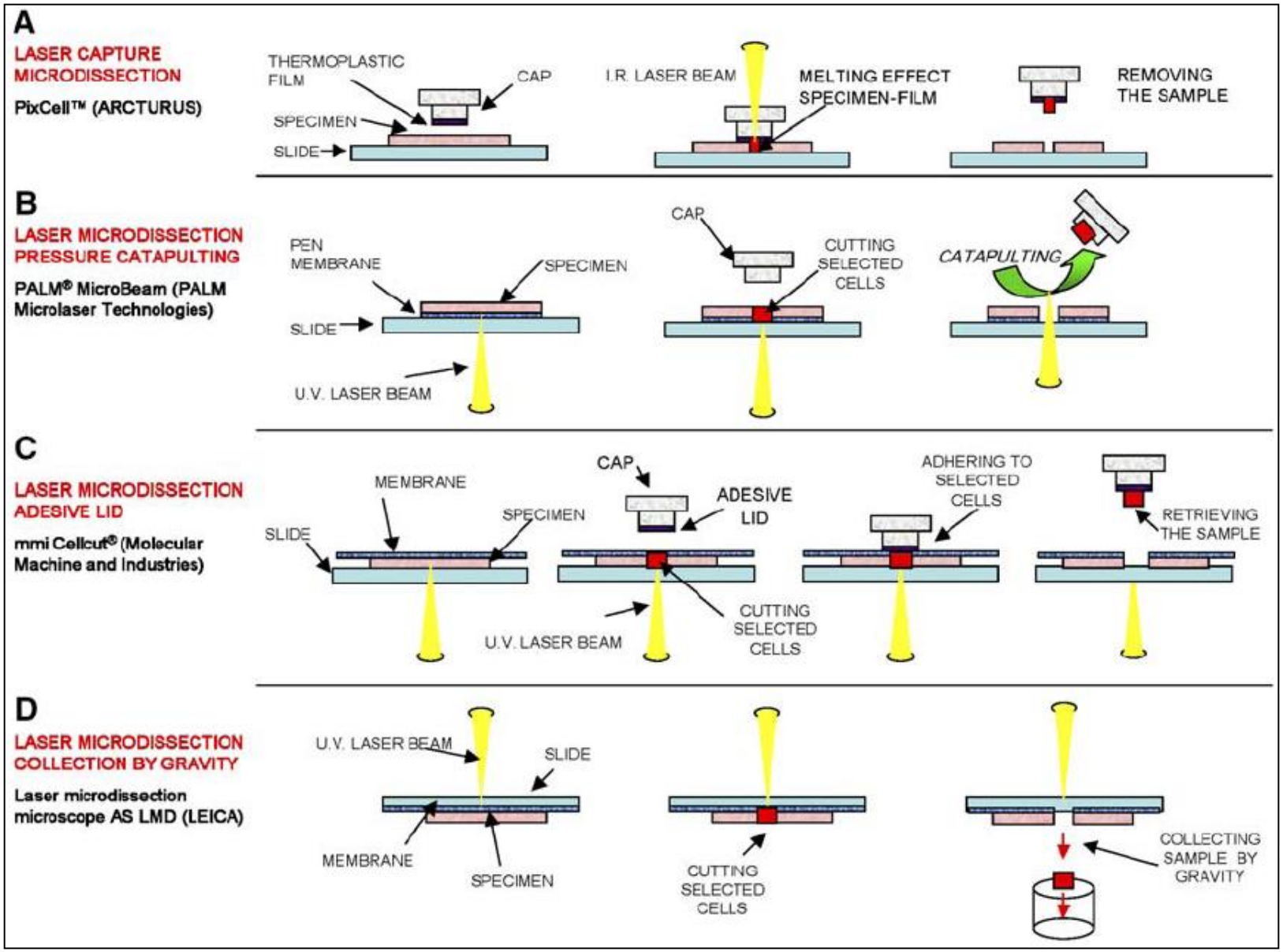

(a)
(b)
(c)
(d)

Laser cutting microdissection

- Conceptually different than LCM
- Mostly "**non-contact**"
- Narrow beam of **ultraviolet** (UV) laser
- Laser draw around and cut out the cells
- Dissected cells are collected either by:
  - pressure "catapulting", OR
  - gravity, OR
  - mechanically acquired by a needle

Pressure “catapulting”




A
LASER CAPTURE MICRODISSECTION
PixCell™ (ARCTURUS)
THERMOPLASTIC FILM
CAP
SPECIMEN
SLIDE
I.R. LASER BEAM
MELTING EFFECT SPECIMEN-FILM
REMOVING THE SAMPLE
B
LASER MICRODISSECTION PRESSURE CATAPULTING
PALM® MicroBeam (PALM Microlaser Technologies)
PEN MEMBRANE
SPECIMEN
SLIDE
U.V. LASER BEAM
CAP
CUTTING SELECTED CELLS
CATAPULTING
C
LASER MICRODISSECTION ADESIVE LID
mmi Cellcut® (Molecular Machine and Industries)
MEMBRANE
SPECIMEN
SLIDE
CAP
ADESIVE LID
U.V. LASER BEAM
CUTTING SELECTED CELLS
ADHERING TO SELECTED CELLS
RETRIEVING THE SAMPLE
D
LASER MICRODISSECTION COLLECTION BY GRAVITY
Laser microdissection microscope AS LMD (LEICA)
U.V. LASER BEAM
SLIDE
MEMBRANE
SPECIMEN
CUTTING SELECTED CELLS
COLLECTING SAMPLE BY GRAVITY

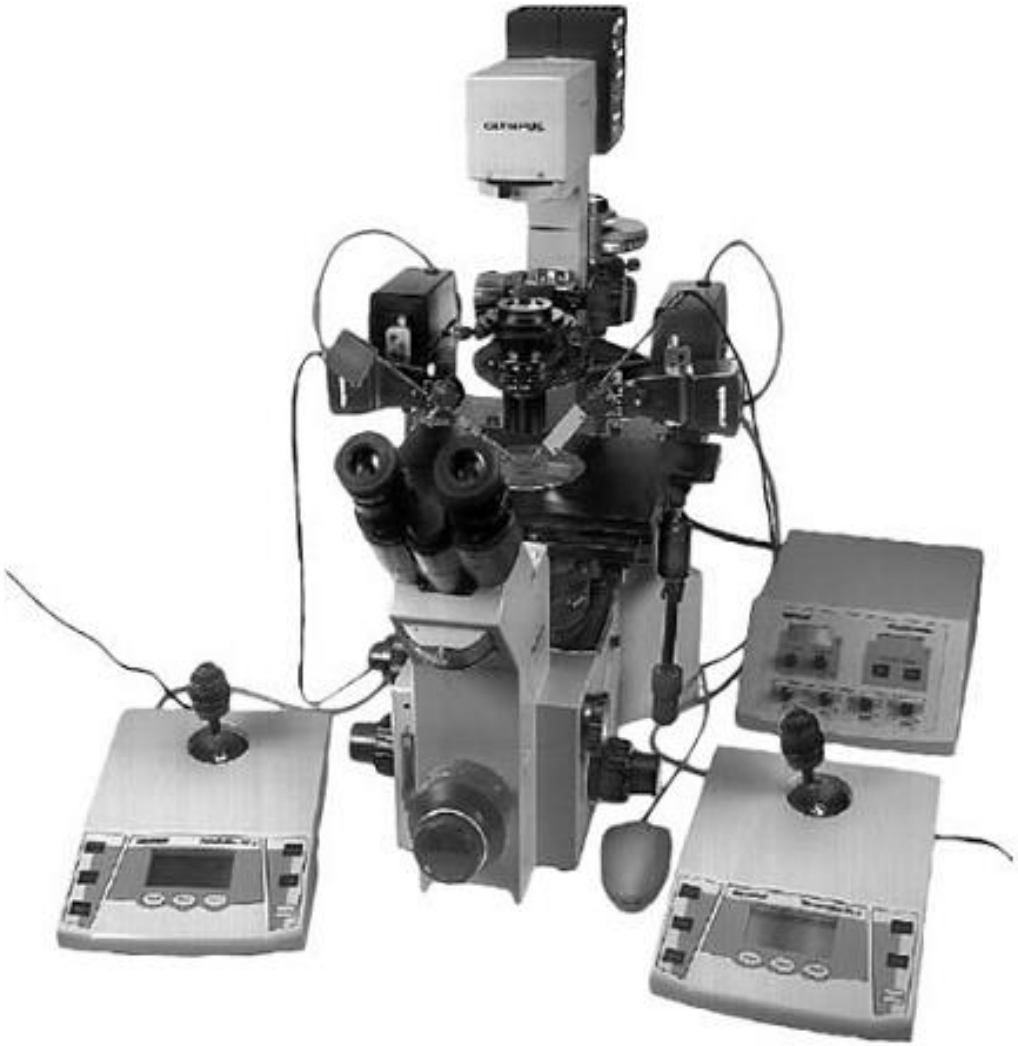
Arcturus PixCell IIe

A

B

A

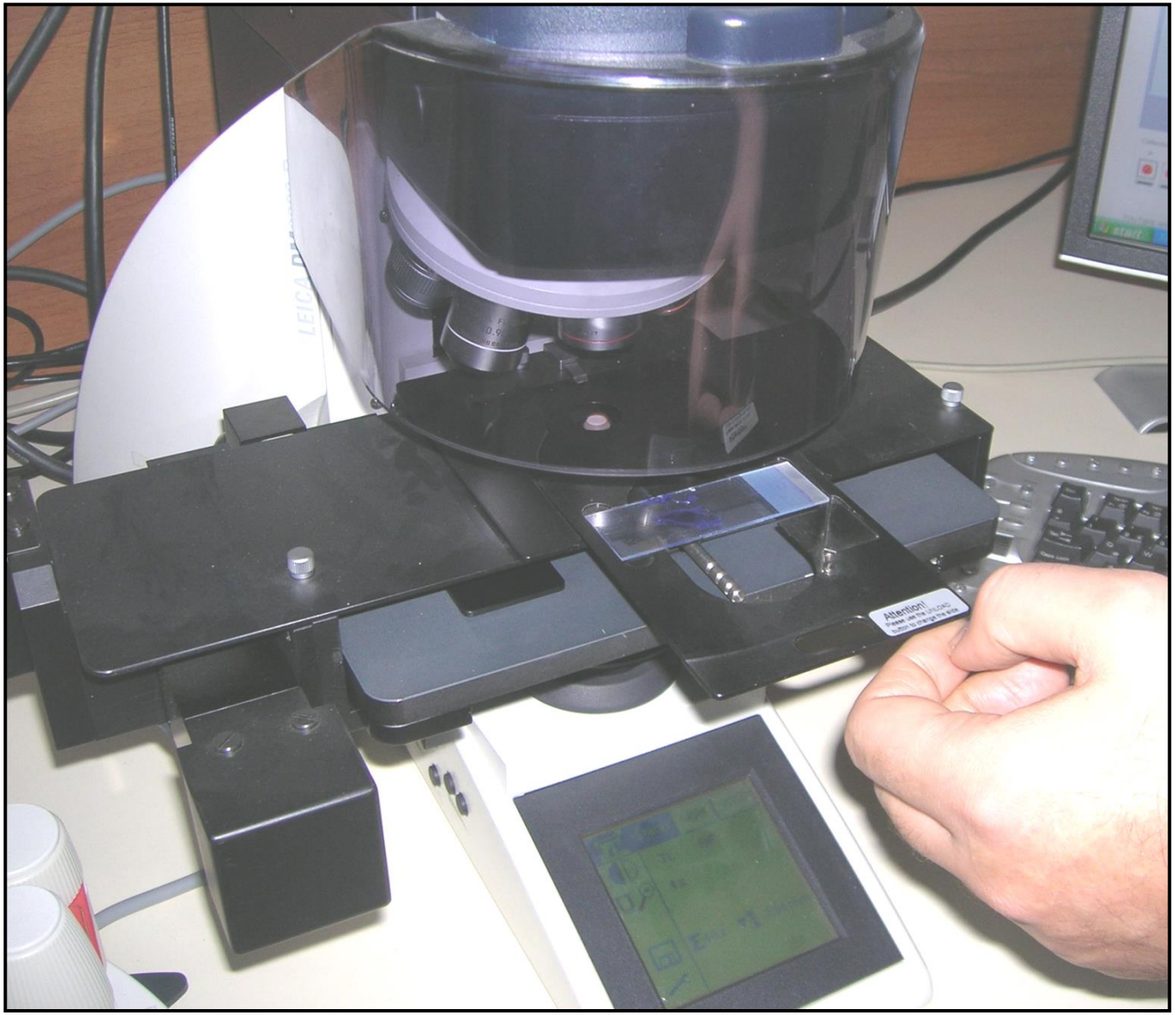
B

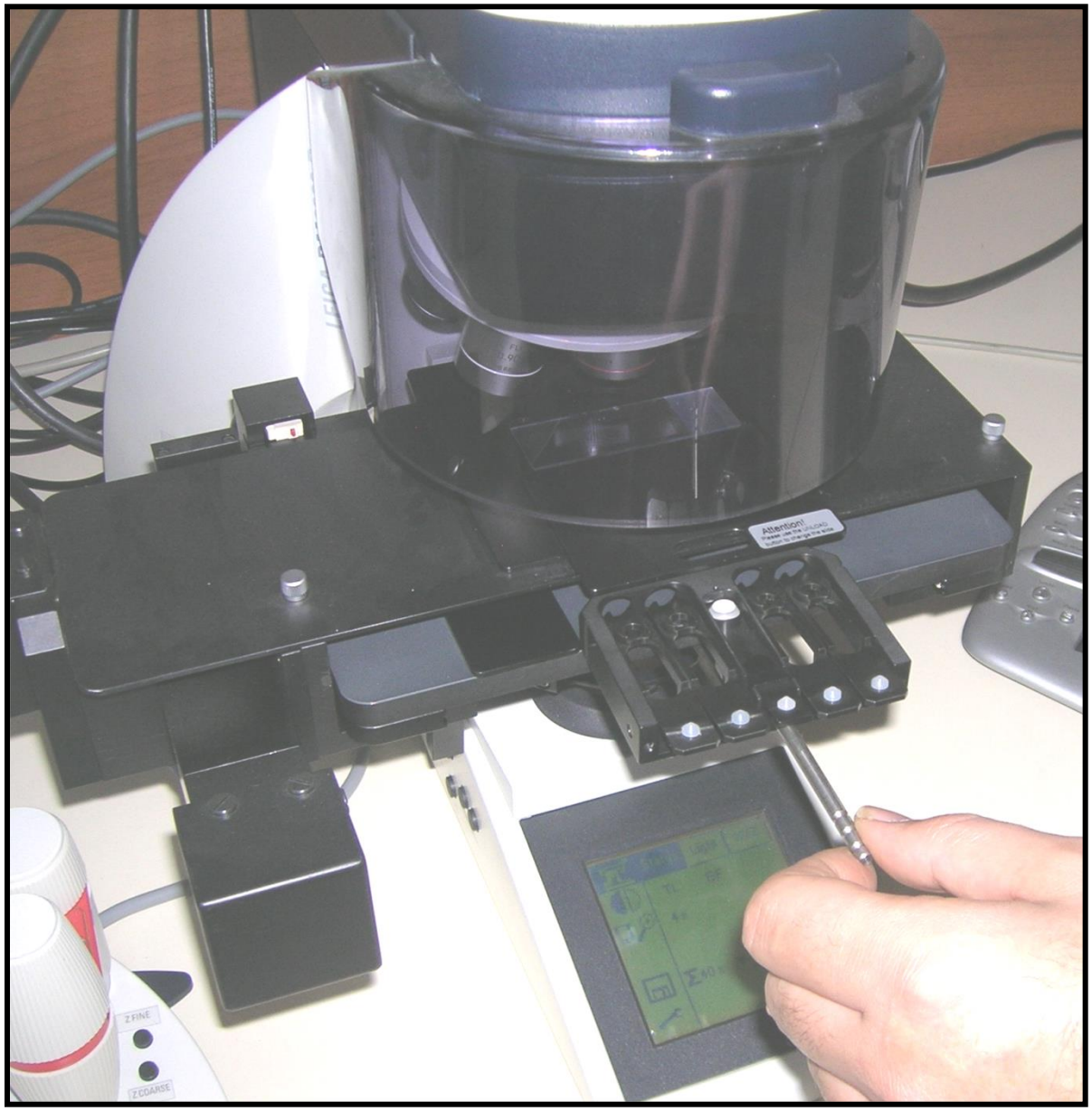


Specimen Preparation

- Any kind of material can be used
- Fresh-frozen tissues or using non-cross linking fixatives are advisable
- Thickness of the section matters (thicker sections require more laser energy)
- Quick staining methods should be used for reducing the possible artifacts
- Adhesive slides may interfere the specimen collection greatly

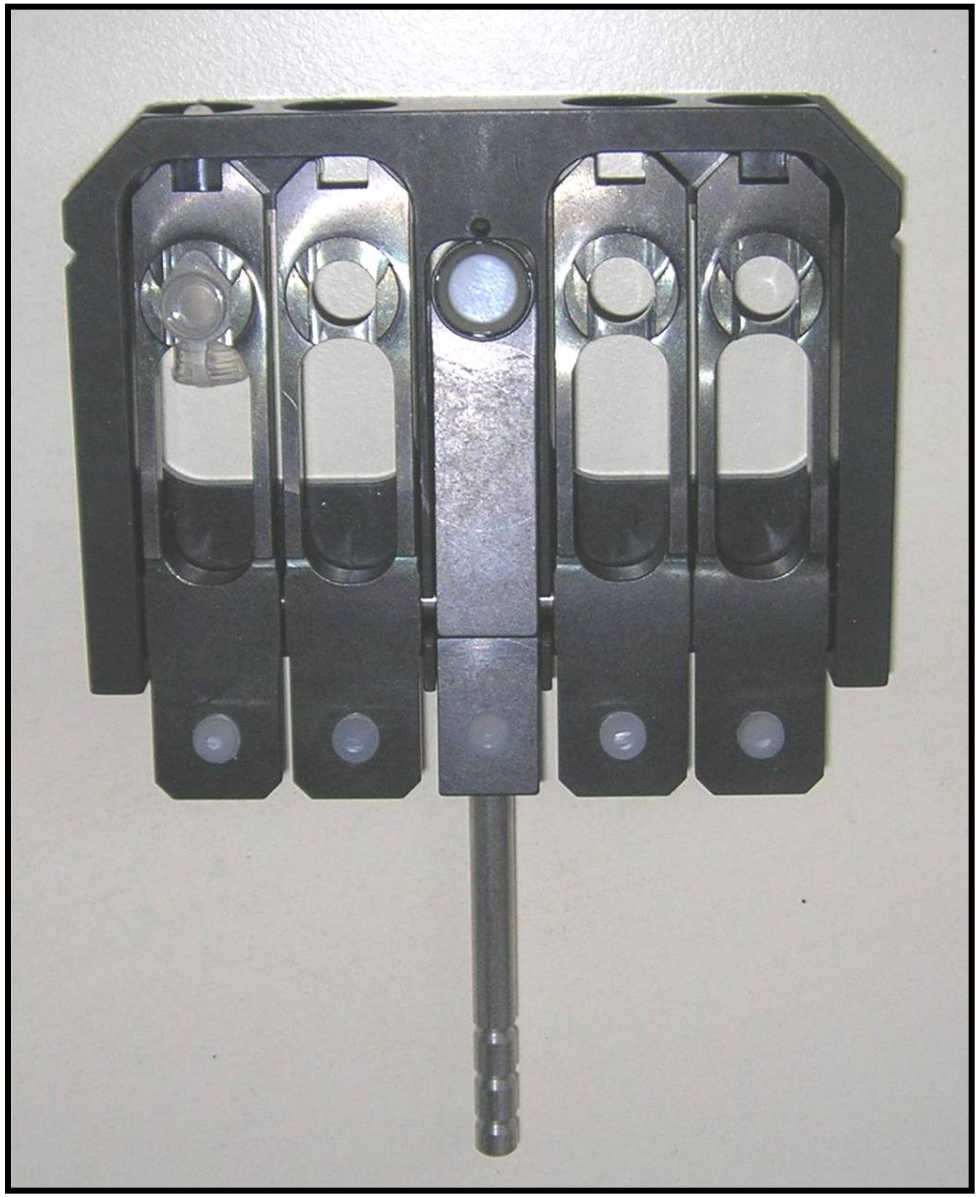

CryLaS
FTSS 355-50
DELL

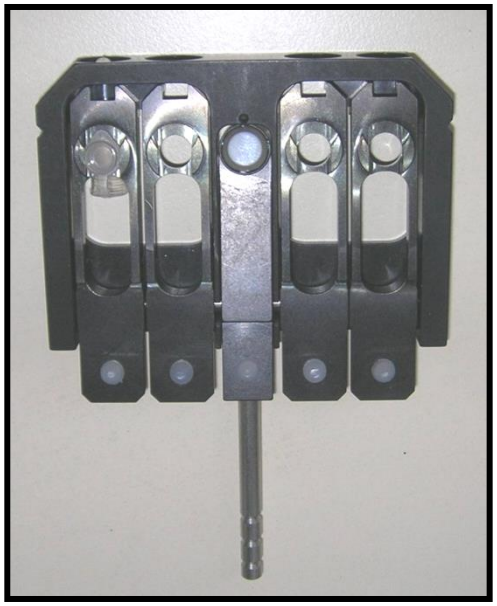

Attention!

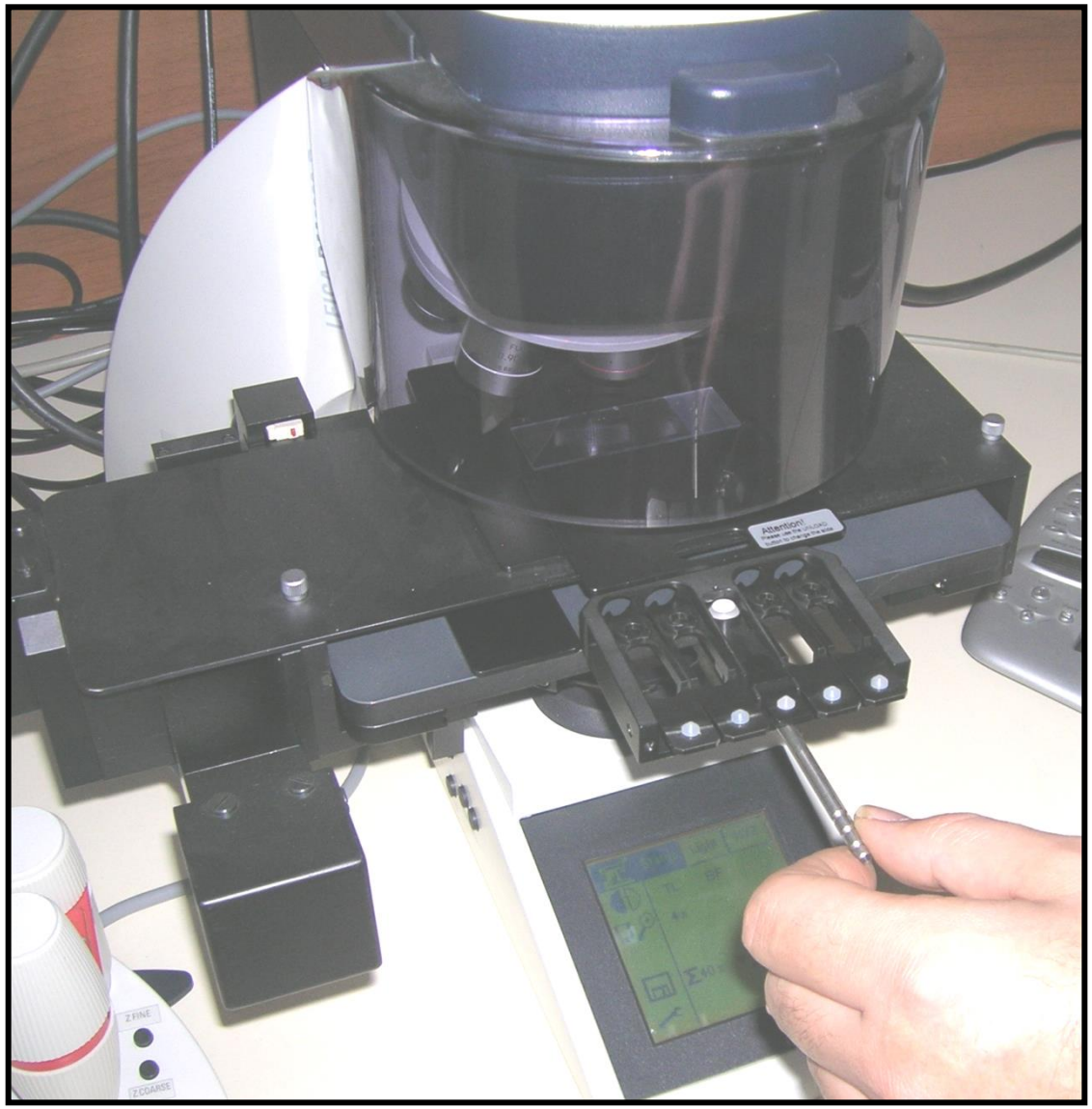

Attention!
Z FINE
Z COARSE


DELL


DELL






Attention!
Z FINE
Z COARSE

Expected Yields of Nucleic Acids and Proteins

- Each somatic cell contains 7 pg DNA
- 50-100 pg of DNA is enough for PCR
- Total RNA from a cell varies 10-30 pg
- Only 5% of it is mRNA
- Quality of RNA depends on the preservation
- Protein content is tissues specific
- 10-100 times more cells are needed for proteomics

Selected application in Pathology

- Breast cancer
- Colorectal cancer
- Prostate cancer
- Lung cancer

Selected application in Pathology

- Hepatocellular carcinoma
- Neuroblastoma
- Soft-tissue sarcomas
- Lymphoma
- Tuberous sclerosis

DNA-based analyses

- Mutation detection
- Epigenetic alterations
- Loss of Heterozygosity (LOH)
- RFLP
- Comparative genomic hybridization
- Microarrays
- ...

RNA-based analyses

- Gene expression profiles
- Representation difference analysis
- Expressed sequence tag
- Serial analysis of gene expression
- Gene chips
- ...

Protein-based analyses

- Western blotting
- Mass spectrometry
- Peptide sequencing
- Two-dimensional electrophoresis
- Protein microarrays
- ...

microRNA-based analyses

- Posttranscription regulation of genes
- Tissue specific markers
- Developmental lineage signature
- Chemosensitivity and response to treatment
- Diagnostic and prognostic markers
- ...

Future Perspectives

- Developing better tissue preservation methods
- Improved methods on the old FFPE tissues
- Nanomicrodissection (organelles, chromosomes, secretory granules)
- Accelerating various “omics”
- Supporting the individualized patient care

Thank you...