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OVERVIEW OF LASER CAPTURE MICRODISSECTION (LCM)

- LCM is a technique developed by the National Institute of Health employing a laser to dissect individual cells or small clusters of cells selected by concurrent light microscopy
- Objective: to obtain a pure sample comprised only of the specific cells of diagnostic, prognostic, or research interest

Principles and Procedural Overview

- The cells of interest are identified using light microscopy (Figure 1)
- A specialized centrifuge cap with an attached thin thermoplastic transfer film is placed over the area of interest with the film coming in direct contact with the tissue on an uncoverslipped, uncharged glass slide
- A near infrared laser with a thin beam is passed through the transfer film and the underlying cell(s) of interest
- The transfer film rapidly heats and focally melts
- The melted transfer film permeates the empty spaces in the tissue immediately under the laser beam
- The transfer film cools to form a new polymer with the selected cells
  - The heating and cooling process completes in milliseconds
  - The new bond between the selected cell(s) and the transfer film is stronger than the bond between the glass slide and the cell
- The cell-film polymer is retracted from the surrounding tissue by lifting the cap
- The transfer film can be moved to multiple additional areas to harvest many areas of interest on a single film
- When all desired harvesting is complete, the cap and attached transfer film are removed from the setup and placed on a standard 0.5-mL microcentrifuge tube containing a lysis buffer or a digestion buffer
- The lysis or digestion buffer digests the cells in the film on the underside of the cap and thereby releases a pure sample suspension with macromolecules (DNA, RNA, and proteins) suitable for molecular analysis

Conceptual Importance of Procuring Pure Cell Populations

- It was originally asserted by Virchow (1821–1902) that the cell, rather than the tissue, represents the most basic unit of disease
- A single cell cannot be adequately analyzed without a means of reliably isolating it from adjacent cells
- Accurate and sensitive detection of molecular changes in malignant or pre-malignant cells usually requires that only cells of interest are examined
  - Genetic material from contaminating cells can mask a finding with conflicting data
    - Even rare unintended cells’ genetic material can become amplified during polymerase chain reaction techniques
    - Using non-microdissected material often underestimates the actual incidence of genetic alterations
  - Human tumors are heterogeneous with admixed cell populations
    - Normal parenchyma that the tumor is invading or in which the tumor developed
    - Blood vessels supplying the tumor
    - Inflammatory cell infiltrate that often accompanies malignancy
    - Stromal cells and/or desmoplastic connective tissue in response to an invasive tumor
- LCM is capable of extremely selective sampling (Figure 2)

LASER CAPTURE MICRODISSECTION (LCM) SYSTEMS

Specimen Considerations and Handling

Types of Specimens (Figure 3)

- Paraffin-embedded specimens
  - Formalin-fixed
    - Most common archival tissue in surgical pathology
    - Neutral buffered formalin fixation is acceptable; however, it causes extensive cross-linking of nucleic acids and protein, which makes polymerase chain reaction (PCR) amplification more difficult
    - Typical DNA fragments range from 100 to 1500 bp
    - RNA may be too poor a quality to be useful, though recent advances have overcome some technical difficulties
    - Over-fixation worsens macromolecule quality
      - Biopsies should fix <12 hours
      - Large specimens should fix <48 hours
    - Metal salt-based fixatives should be avoided
    - Bouin’s and B5 fixatives are even more damaging than formalin and should be avoided whenever possible
    - Alcohol fixation is the most desirable method for paraffin-embedded specimens