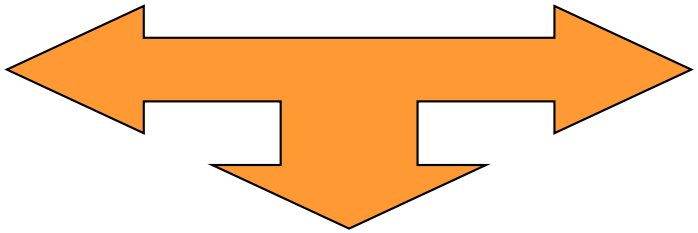
FIXED AND PARAFFIN EMBEDDED TISSUES



DNA

•Search for mutations and genetics tech

RNA

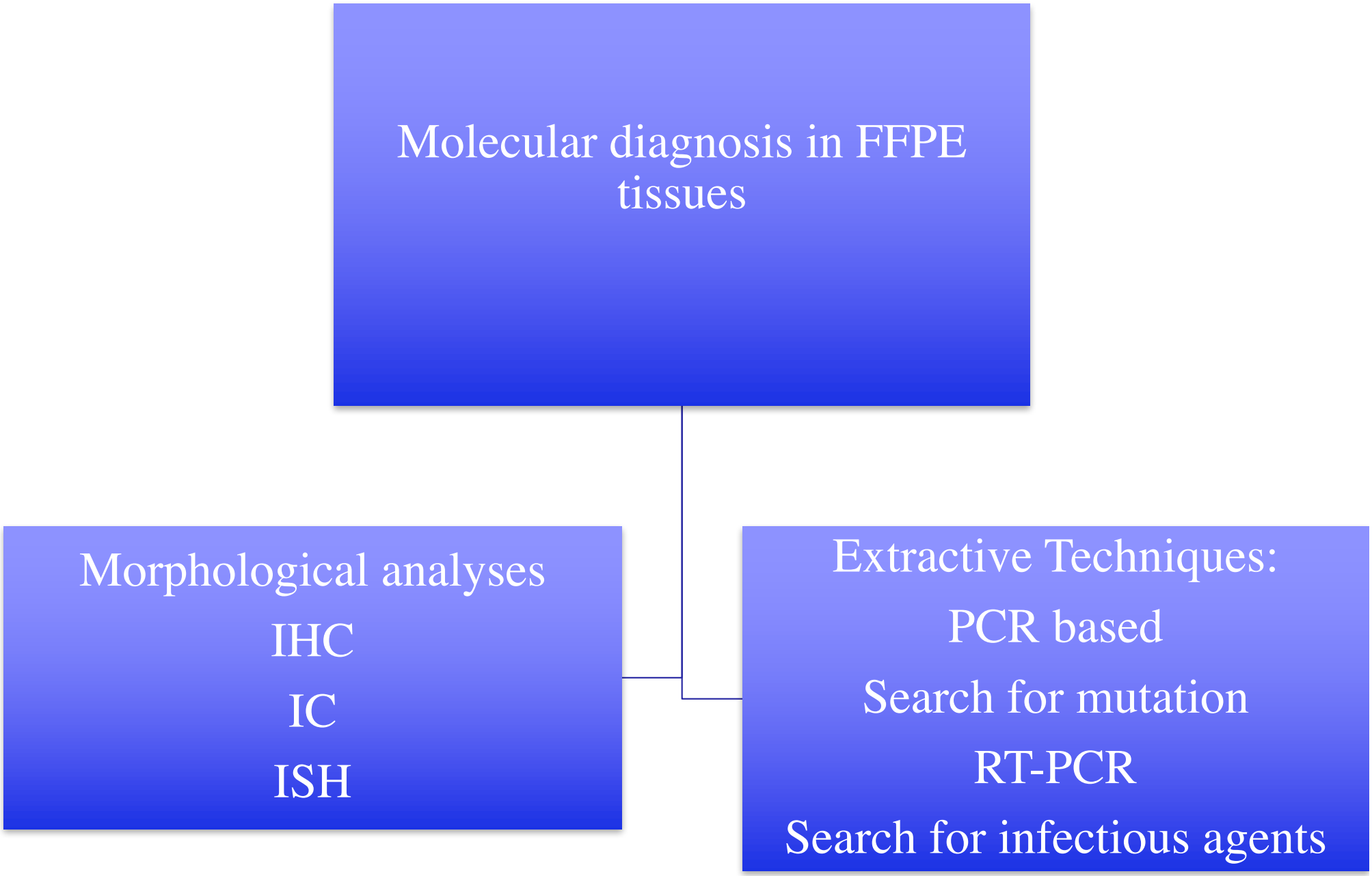
•Functional genomics
•methods

Proteins

•IHC
•WB
•Arrays

Nucleic acids isolation from FFPE, why?

- *The only tissues available for any patient are formalin fixed paraffin embedded*
- *These tissues are available at the clinical level for any molecular analysis*
- *Today prognostic and predictive biomarkers are assessed in these tissues and tomorrow heterogeneity will*
- *The FFPE tissues stored in hospitals are the widest collections of human tissues with any type of even rare lesions available for clinical research*


Molecular diagnosis in FFPE tissues
Morphological analyses
IHC
IC
ISH
Extractive Techniques:
PCR based
Search for mutation
RT-PCR
Search for infectious agents

Extractive Methods

- *Search for infectious agents*

  CMV, EBV, HPV, MT, Leishmania, Borrelia, HCV……

- *Mutation detection*

  K-Ras, EGFr, B-Raf

- *Analysis of microsatellite instability*

  MSI

- *Molecular profiling of cancers*

  OncoType DX

ADVANTAGES OF ARCHIVE TISSUES

- ✓ Analyses in morphologicall defined tissues
- ✓ Study of rare diseases
- ✓ Retrospective clinical studies
- ✓ Molecular epidemiology study
- ✓ Endogenous Rnases are inactive

Limits

- Degraded Nucleic acids
- Limited techniques for proteins
- Amplification of short fragments

The morphological classification represents the diagnostic basis for tumor diseases, however

Tumors of the same histo-morphological group can have different clinical behavior because the different lesions have different biological characteristics.

There is the possibility of evaluating the biochemical properties by means of different techniques in archive tissues in order to be able to answer to the major clinical requests:

? What is the prognosis of a patient, not of the pathological group

? What is the probability of recurrence and metastasis at the time of resection and diagnosis

? What is the response of each individual to anticancer treatments whether they are traditional or addressed to particular biological targets

There is the need of standardized analytical methods characterized by known values of specificity, sensitivity and predictivity

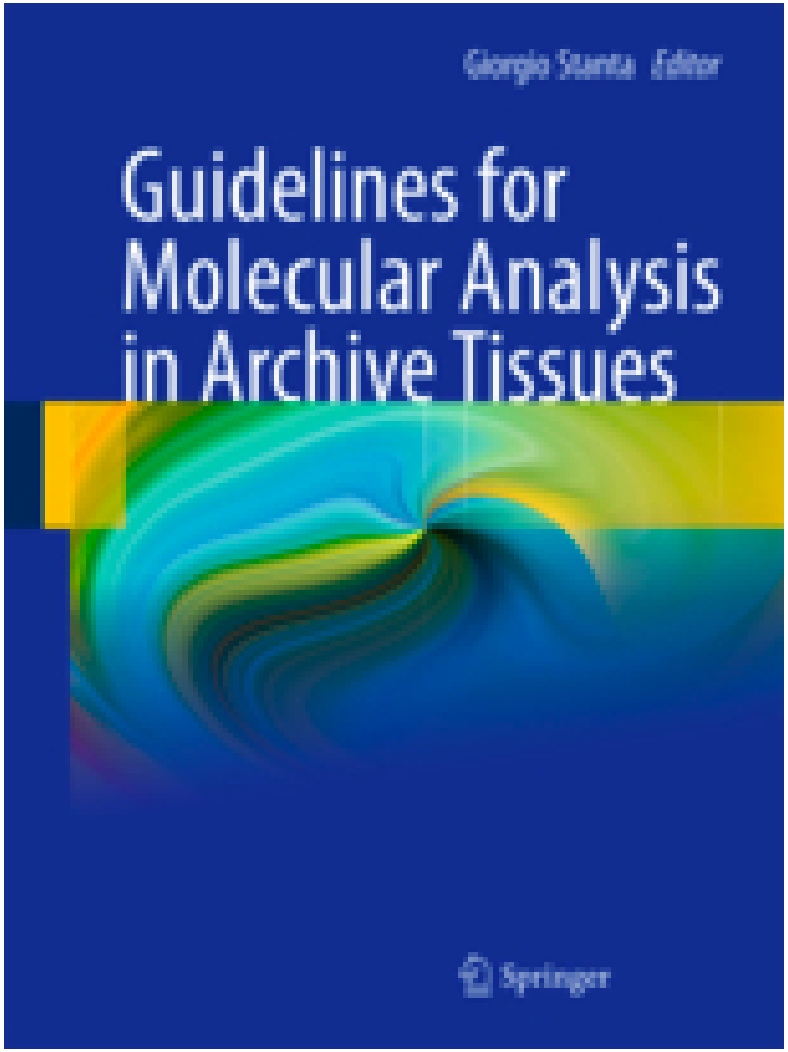
	MALATTIA		
TEST	PRESENTE	ASSENTE	
positive	a	b	a+b subjects with positive test
negative	c	d	c+d subjects with negative test
	a+c sick patients; b+d healthy		

✓Sensitivity: proportion of sick subjects with positive test : a/a+c

✓Specificity: proportion of healthy subjects with negative test : d/b+d

✓Predictive value of a positive test : proportion of sick subjects among those who have a positive test : a/a+b

✓Predictive value of a negative test: proportion of normal subjects among those who have a negative test : d/c+d


Giorgio Stanta Editor
Guidelines for Molecular Analysis in Archive Tissues
Springer

Microdissezione

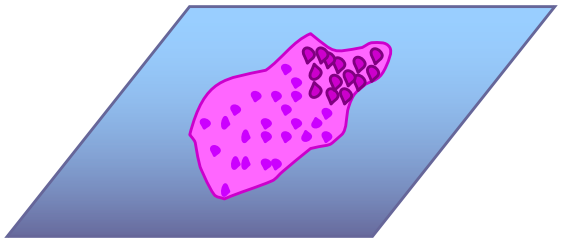
It is a method to obtain pure cells from a specific microscopic region of tissue section. Under the microscope, the tissues appear as heterogeneous structures with hundreds of cell types organised in morphological units. The cells of analytical interest (eg precancerous, neoplastic, metastatic) are flanked by heterogeneous tissue elements such as stroma, blood vessels, glandular and muscular components, fat cells or inflammatory cells. Microdissection can be indispensable when molecular biology techniques are applied to tissues.

Microdissezione in tessuti d'archivio

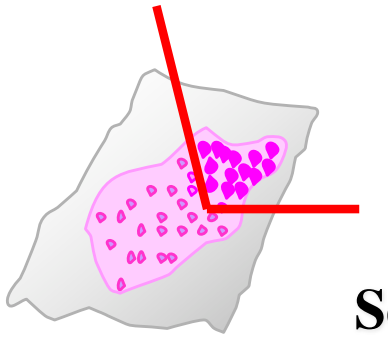
MECCANICA

Section for morphological analysis

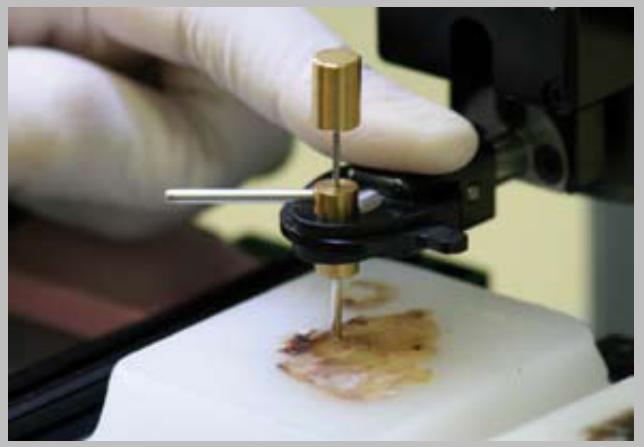
H&E

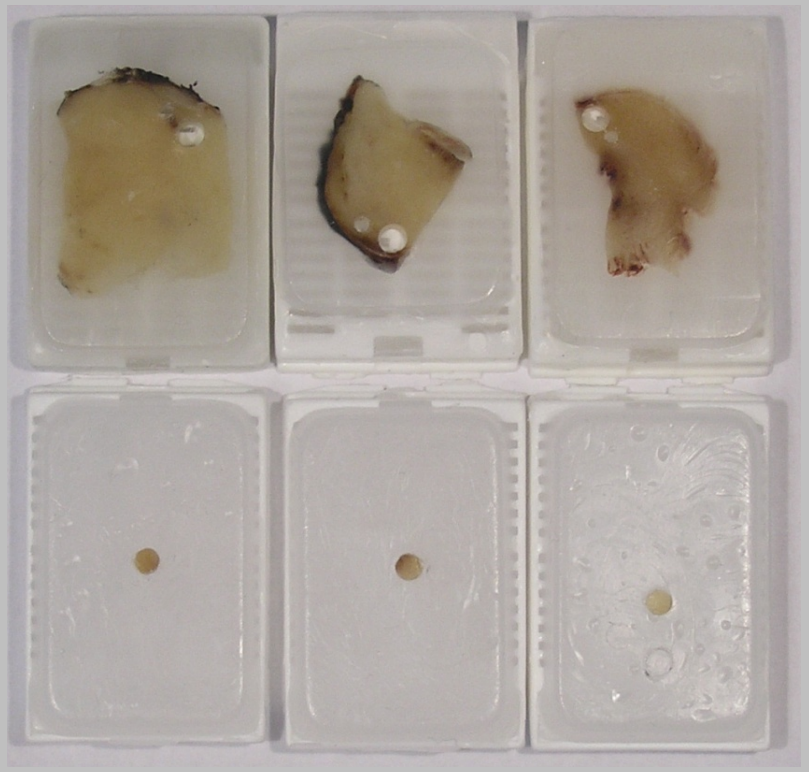


Following blank section



Selected area for microdissection

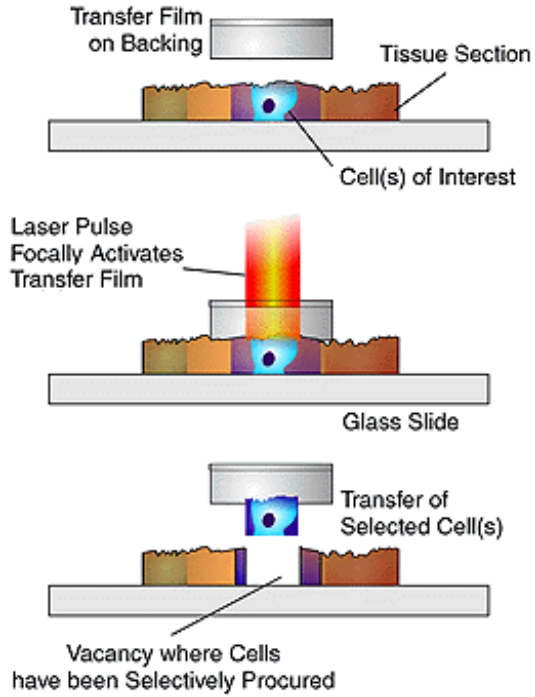




Laser Capture Microdissection

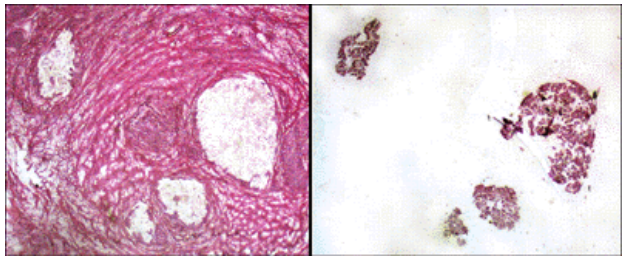
It is an automated technique useful for standardizing microdissection. A laser beam is used, a special film to transfer the cells of interest. The transfer film is applied to the surface of the tissue section.

The tissue covered by the transparent film is identified through the microscope and the cluster of cells to be isolated is selected



→Cells of interest in the center of the visual field

→The laser is swithed on by a button. At that point (laser beam) the film melts with the selected underlying cells. When the film is removed the chosen cells remain adhered to its surface, while the rest of the tissue is left on the slide.



Laser Capture Microdissection

Use: to process DNA, RNA or proteins from the selected area. The technique does not damage these macromolecules because the laser energy is absorbed by the film. The starting material can be fixed (in formalin or ethanol), included in paraffin or frozen. The sections can be colored with standard methods to enhance the cellular population of interest.

IT IS POSSIBLE TO ISOLATE NUCLEIC ACIDS FROM ARCHIVE TISSUES, BUT

Each sample has different levels of nucleic acid degradation

even if processed in the same laboratory

- ✓ **Non standardized procedures**
- ✓ **variable pre-fixation time**
- ✓ **use of different fixatives:**

  non buffered formalin (formic acid)

  Bouin's liquid (15 units saturated aqueous solution of picric acid, 5 units formalin 40% and 1 unit glacial acetic acid)
- ✓ **Temperature of paraffin embedding**