

Surgical Pathology Overview

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Objectives

- To understand the operation of diagnostic pathology services
- To understand the clinicopathologic dynamics of patient care
- To learn the importance of specimen handling and submission to pathology, and its relevance to diagnostic work-up

Objectives

- Stains and examination techniques
- What to expect from pathologic examination
- To understand the importance and uses of techniques and advances in diagnostic pathology

Pathology overview

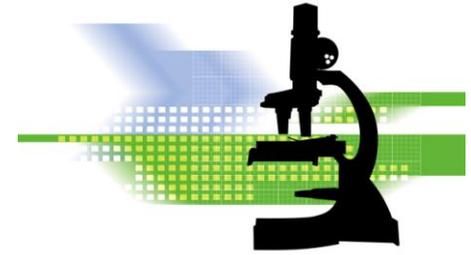


Pathology: Pathos+logos

- The study of suffering
- Bridges clinical practice and basic science
- Investigates underlying causes and mechanisms of diseases



Pathologists



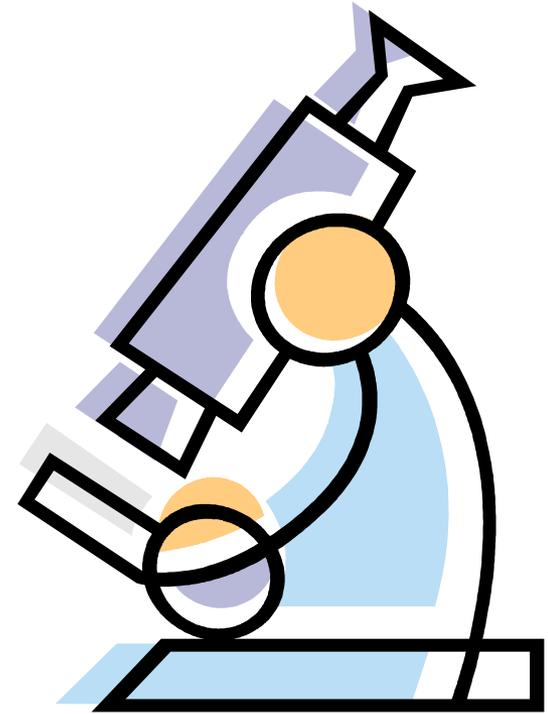
- Render a diagnosis and guide therapy in clinical setting
- Identify changes in the gross and microscopic appearance of cells and tissues
- Use contemporary molecular, microbiologic and immunologic techniques



Pathology overview

Discipline is divided into 2

- 1- General pathology
- 2- Systemic pathology



General pathology

SURGICAL PATHOLOGY

- Genitourinary pathology
- Gynecologic pathology
- Breast pathology
- Gastrointestinal pathology
- Bone and soft tissue pathology

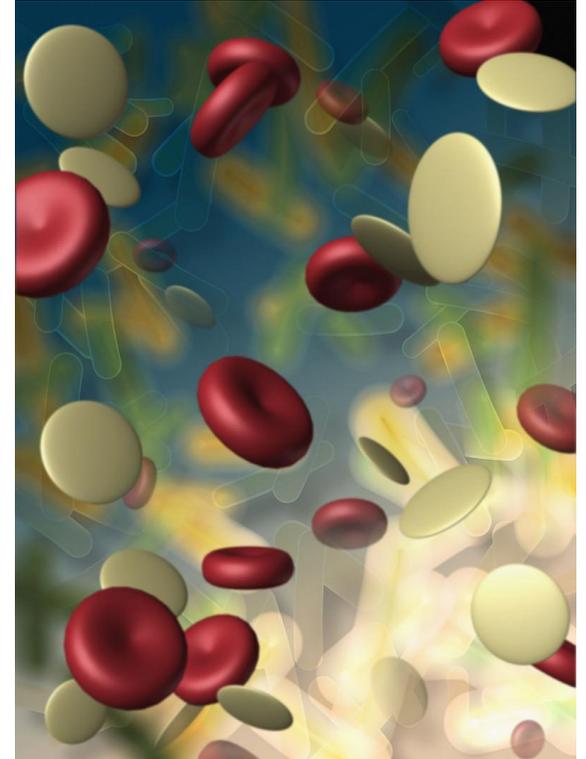
Systemic pathology-subspecialties

- Hematopathology
- Cytopathology
- Neuropathology
- Dermatopathology
- Renal pathology
- Molecular pathology

Hematopathology

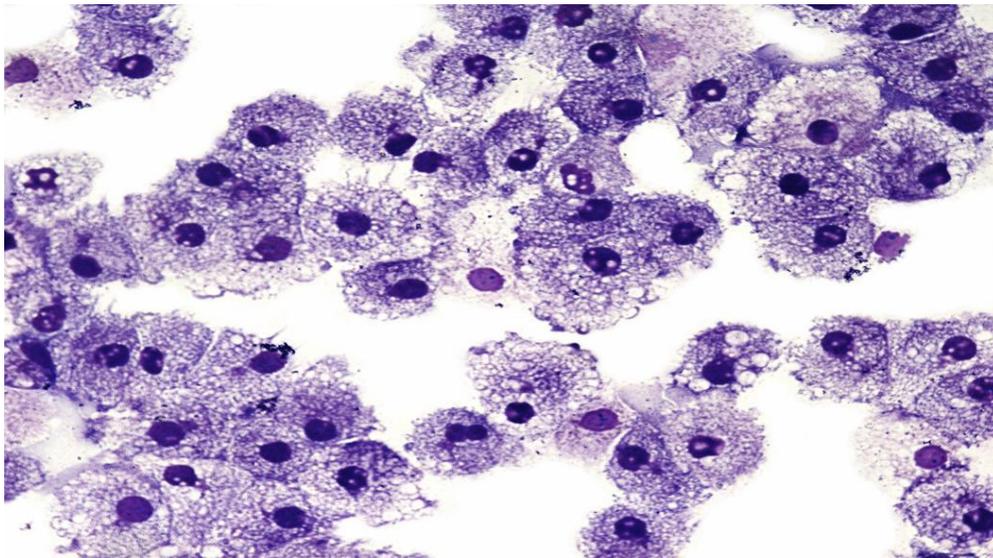
Examines;

- bone marrows
- lymph nodes
- spleen
- blood smear

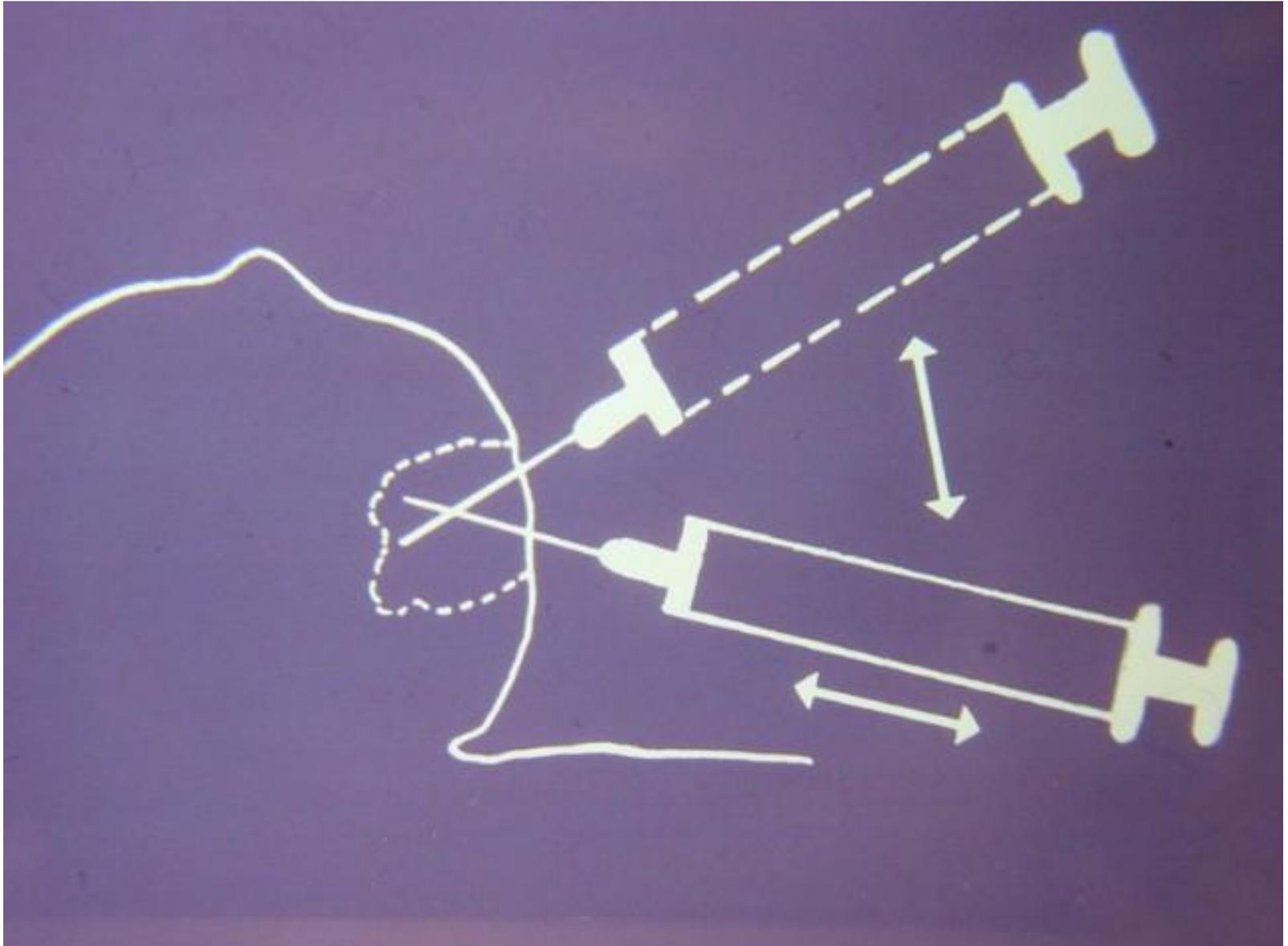


Cytopathology

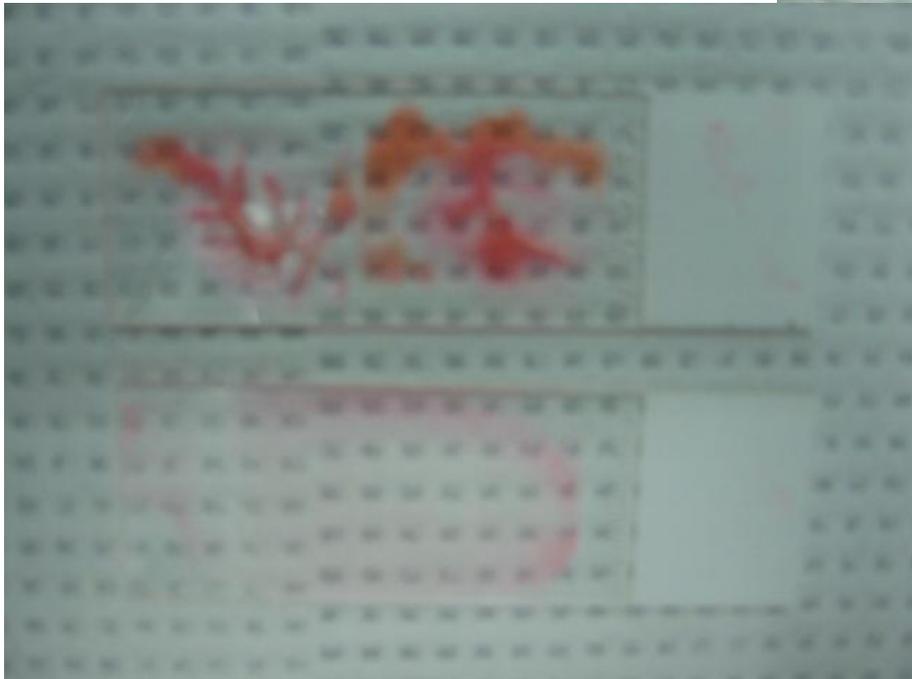
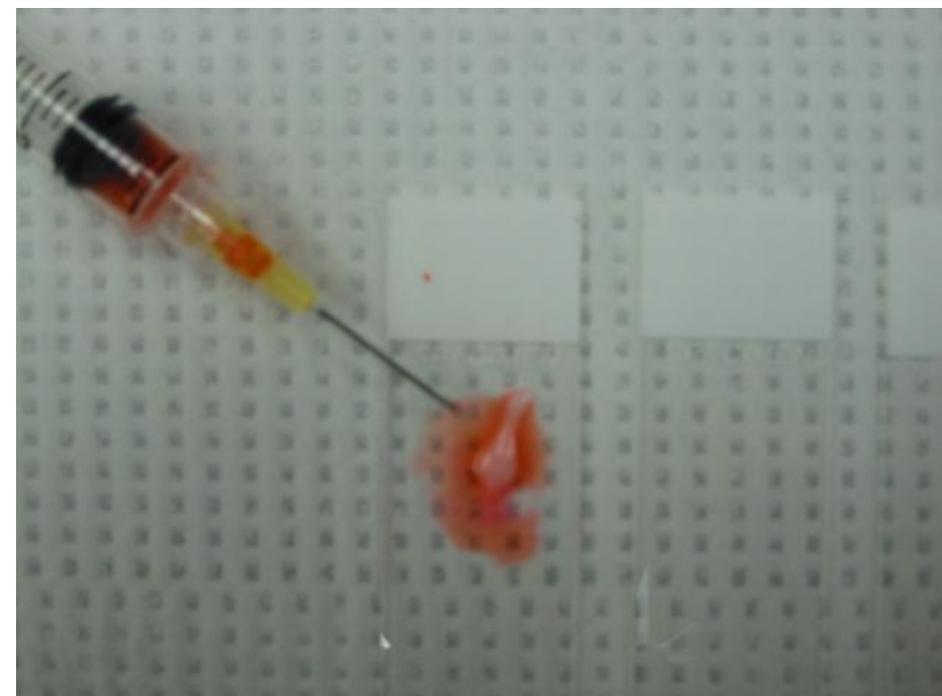
- PAP smears (cervix, vagina, vulva)
- Non-gynecological smears (body fluids, urine)
- Fine needle aspirations for any body site



FNA Sampling



On-site Slide Preparation



Neuropathology

- Brain, spinal cord, peripheral nerve and muscle
- Dementia autopsies



Dermatopathology

- Skin



Specimen Types

- Biopsy (shave, needle core, incisional, excisional, stereotactic, endoscopic, punch, curetting, cone)
- Excision
- Resection
- Radical resection
- Amputation
- Cytologic specimen
- Autopsy

Submitting Specimens to Pathology

- By default in 10% phosphate buffered formalin (formalin-fixed, paraffin-embedded tissue)
- 2 % glutaraldehyde for EM
- In Michel's solution for fluorescent microscopy
- 100 % (absolute) ETOH for glycogen storage disease
- Fresh and immediately for intraoperative consultation
- Muscle biopsy for medical disease in damp gauze and immediately
- Lymph node fresh and immediately, may later be fixed in Bouin's solution

Fixation of specimens

- Fixation preserves the morphology and prevents decomposition, autolysis, microbial growth
 - Neutral buffered 10% formaldehyde-
 - Adequate fixation is important- specimen should be covered at least 10 times its volume of the fixative
 - Fixative penetrates 1 mm per hour

Journey of Specimens

Accessioning

- patient and specimen identifications
- give identification number
- entered in the computer system





- Large specimens



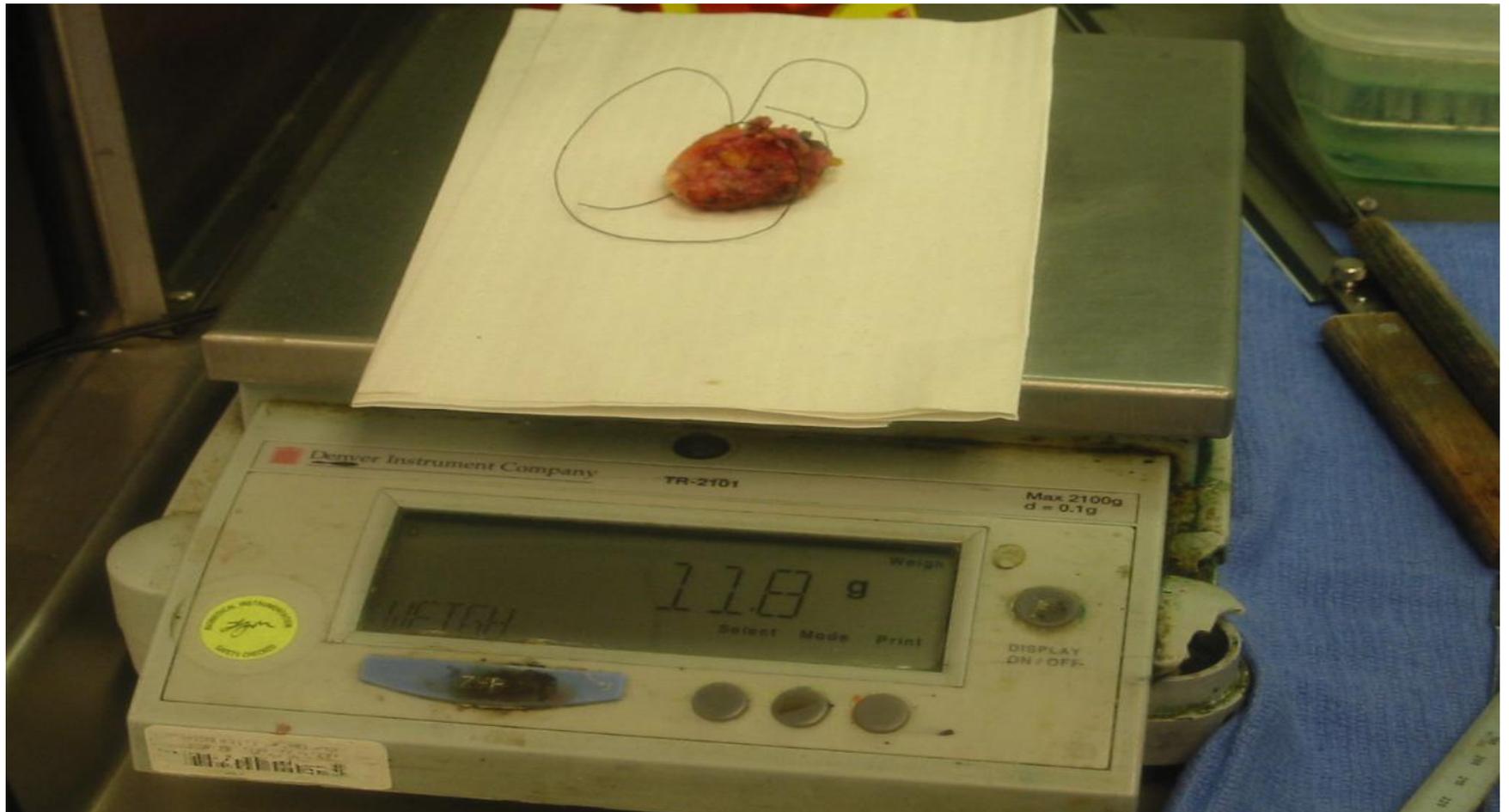
Work station



Gross examinations

- Specimens are described, measured and examined in gross room
- Pictures of specimens are taken
- Sections are taken from specimens and put into cassettes
- Intra-operative consultation- freezing method for quick result in 15 minutes while patient waiting in OR

Gross examinations



Gross examinations



HAZARD



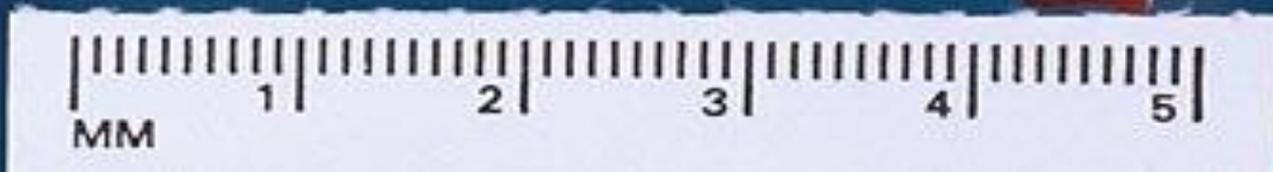
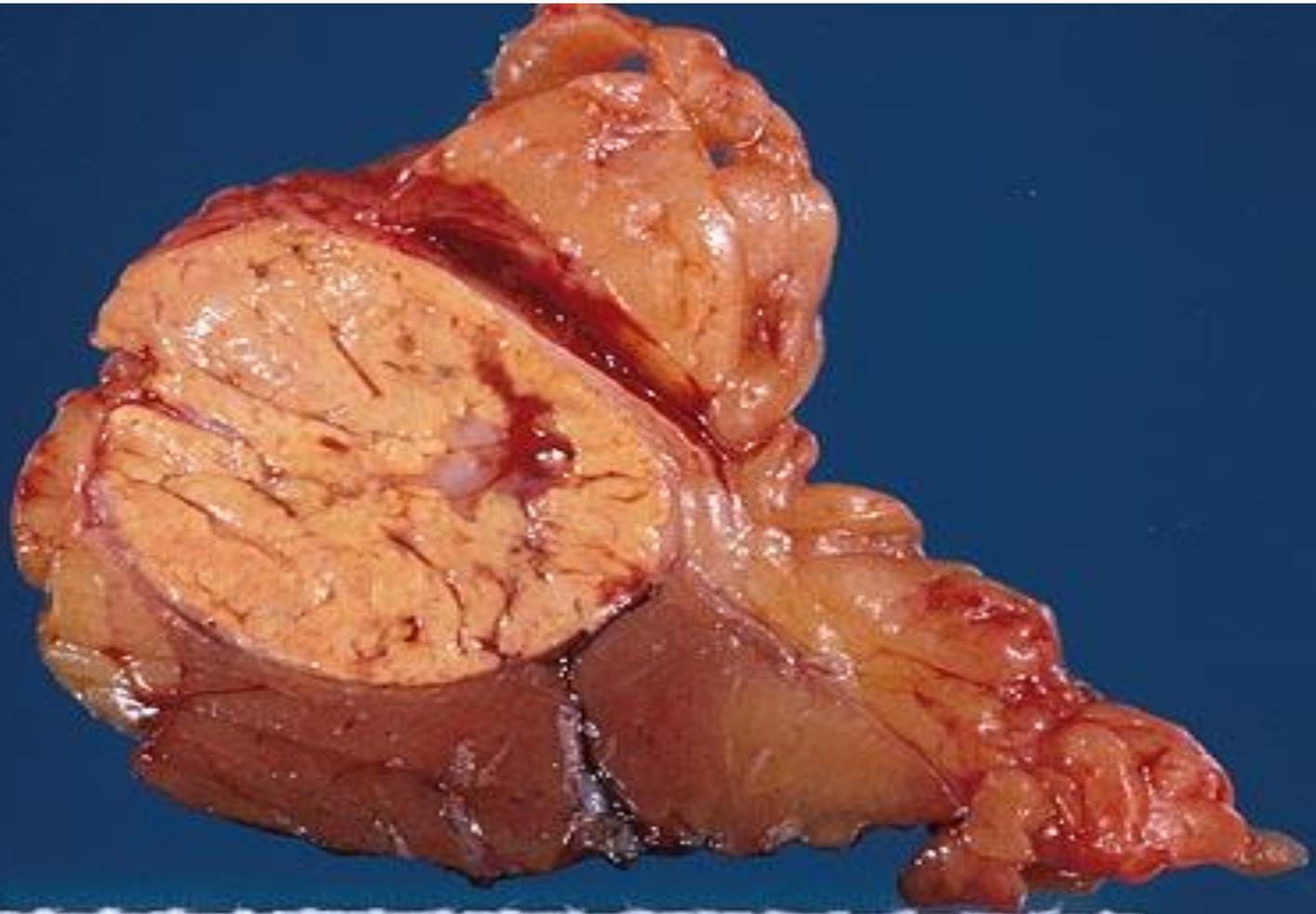
Gross examinations



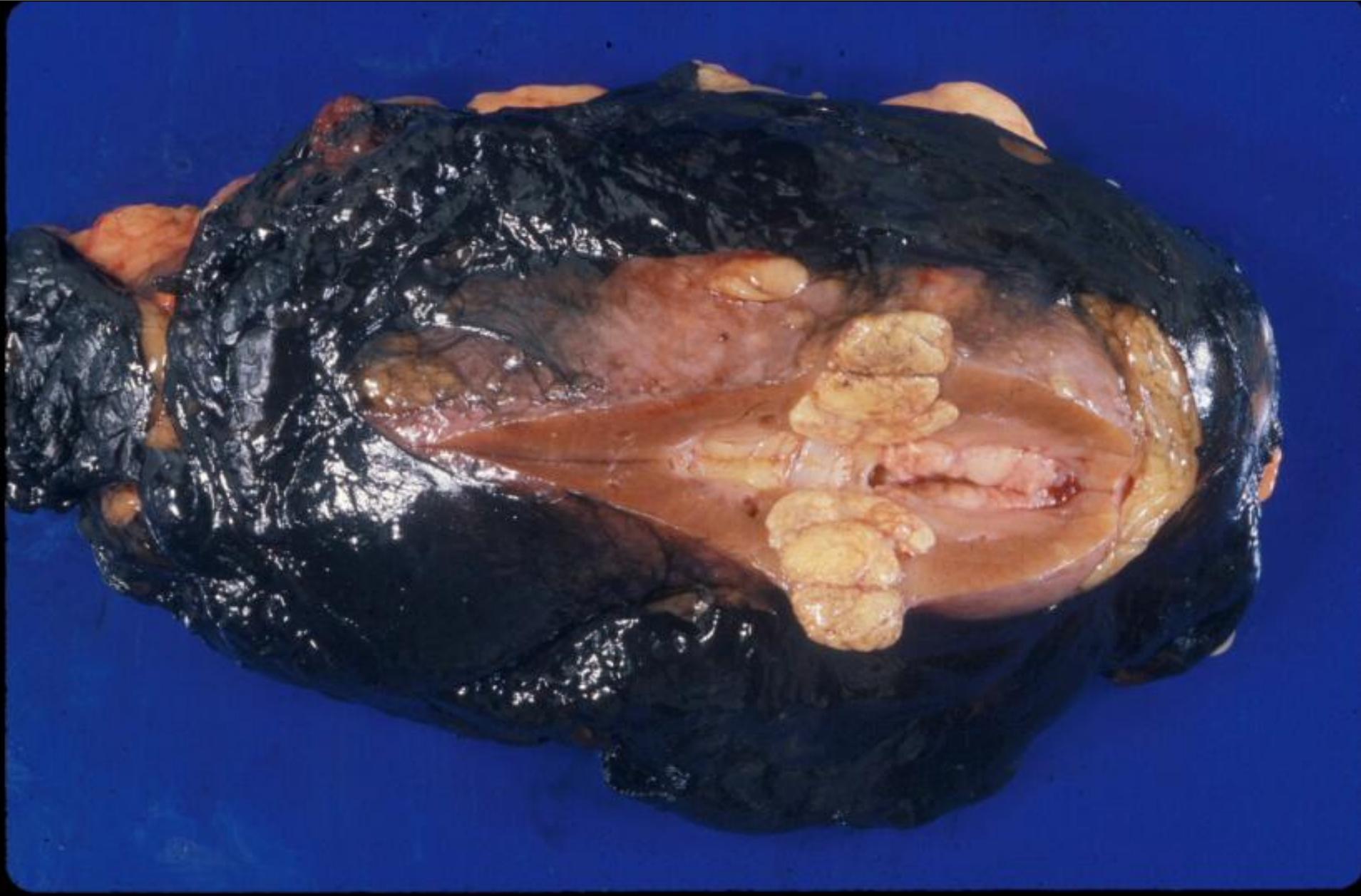
Gross examination



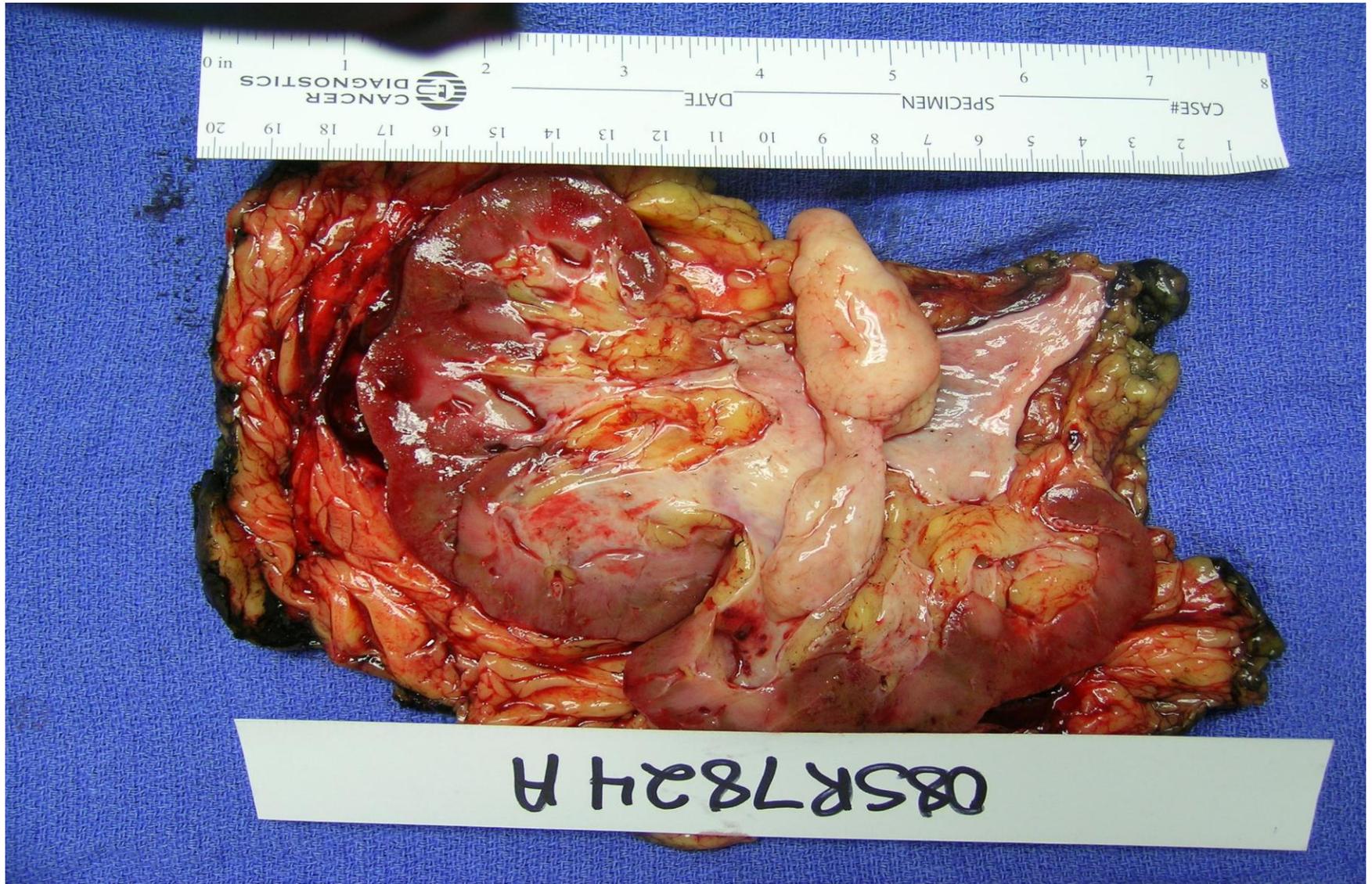
Partial nephrectomy



Radical nephrectomy



Radical nephrectomy



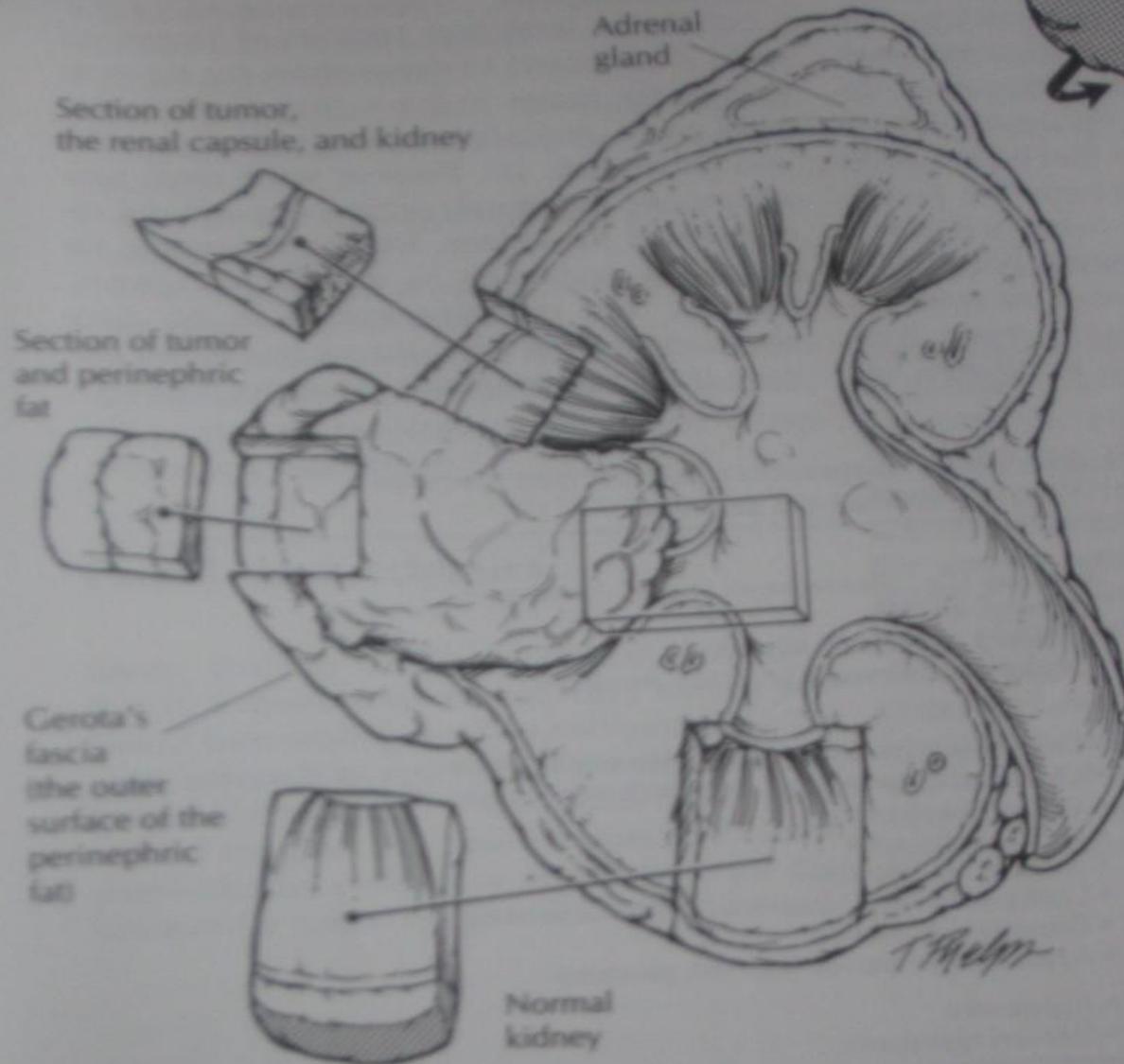


← Photography unit

Bone saw →



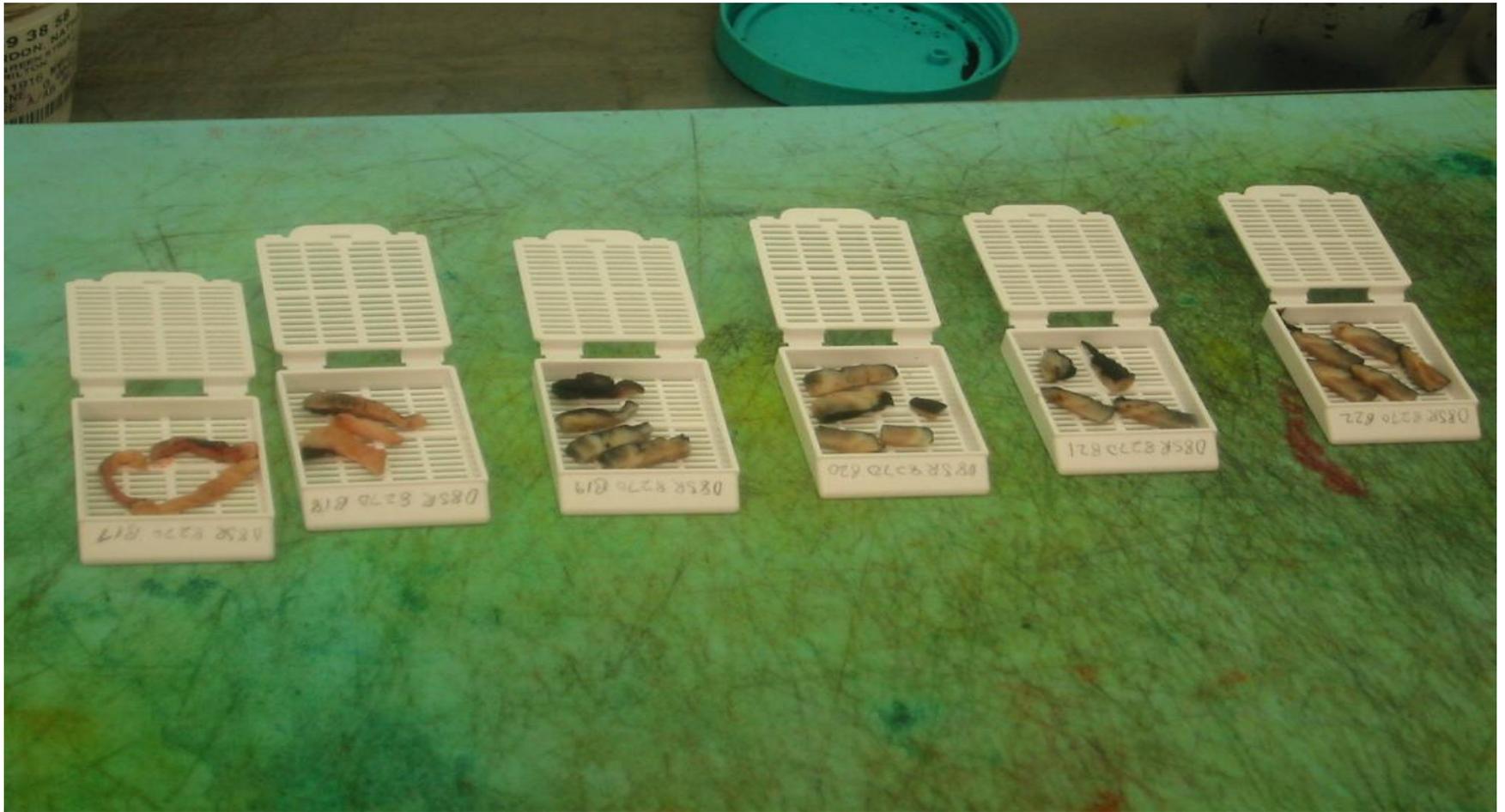
Kidney sections



Types of cassettes



Gross examinations



Tissue processor



Tissue processing

- Designed to remove all water from tissue replacing it with medium (paraffin) that provides sufficient rigidity to enable sectioning of the tissue without damage or distortion.

Stages of tissue processing

- **Dehydration**- removal of water and fixative from tissues
- **Clearing**- removal of dehydrating solutions making tissue components receptive to infiltrating medium
- **Infiltrating**- permeating the tissue with a support medium (paraffin)
- **Embedding**- orienting the tissue in paraffin and allowing it to solidify



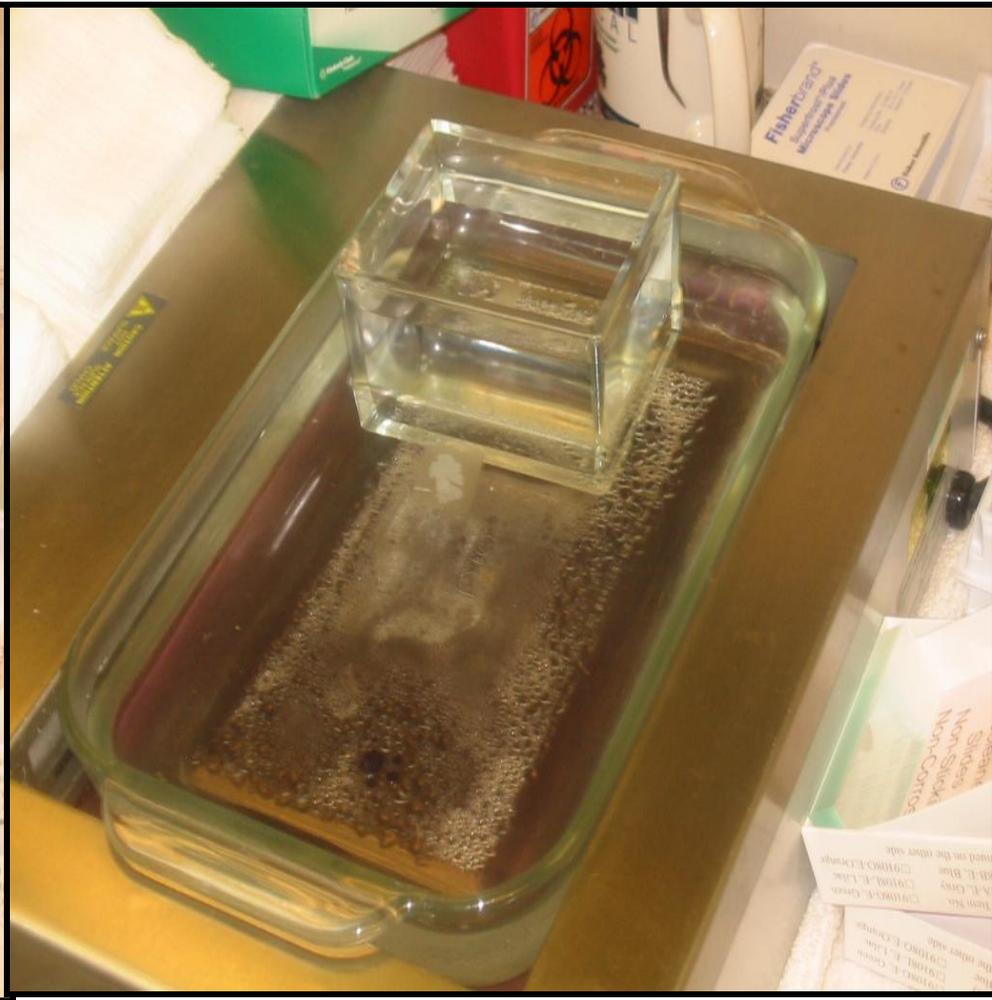
Paraffin blocks



Cutting of blocks-4 micron sections



Sections in water bath



Taking section onto glass slide



Autostainer

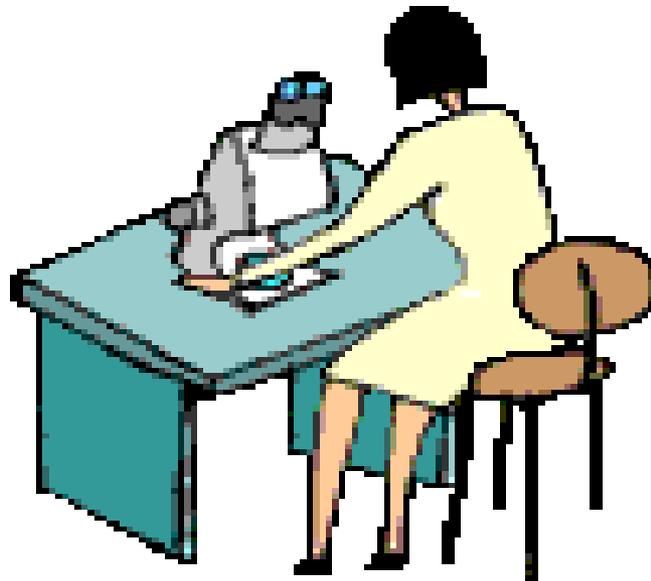


Slides ready for review in trays





Microscopic Evaluation







Storage for specimens

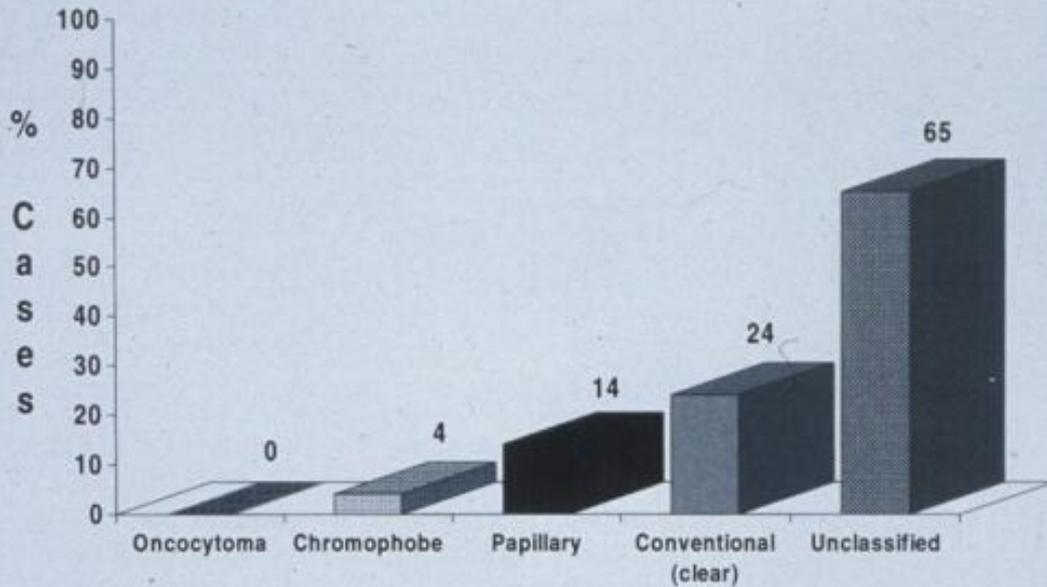


Reporting of tumors

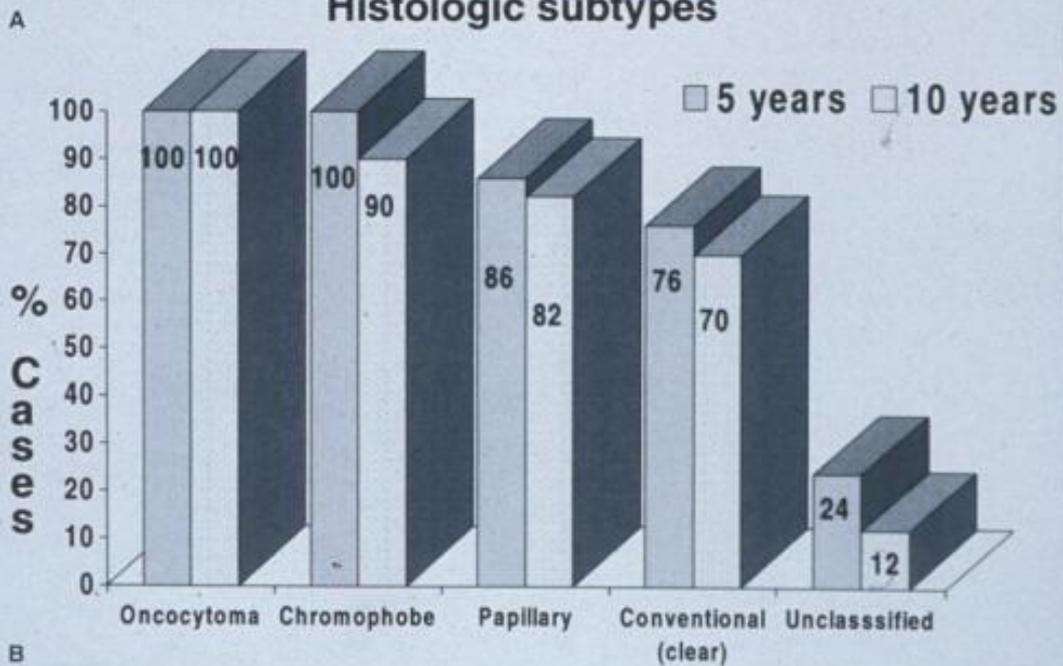
- **Nomenclature**- type and name of tumor
- **Grading** of tumor cells
- **Staging** of tumor

Renal cell tumors-WHO,2004

- Clear cell renal cell carcinoma
- Multilocular renal cell carcinoma
- Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma
- Collecting duct carcinoma
- Medullary carcinoma
- Unclassified
- *Papillary adenoma; metanephric adenoma*
- *Oncocytoma*



Histologic subtypes



Grading and staging of tumors

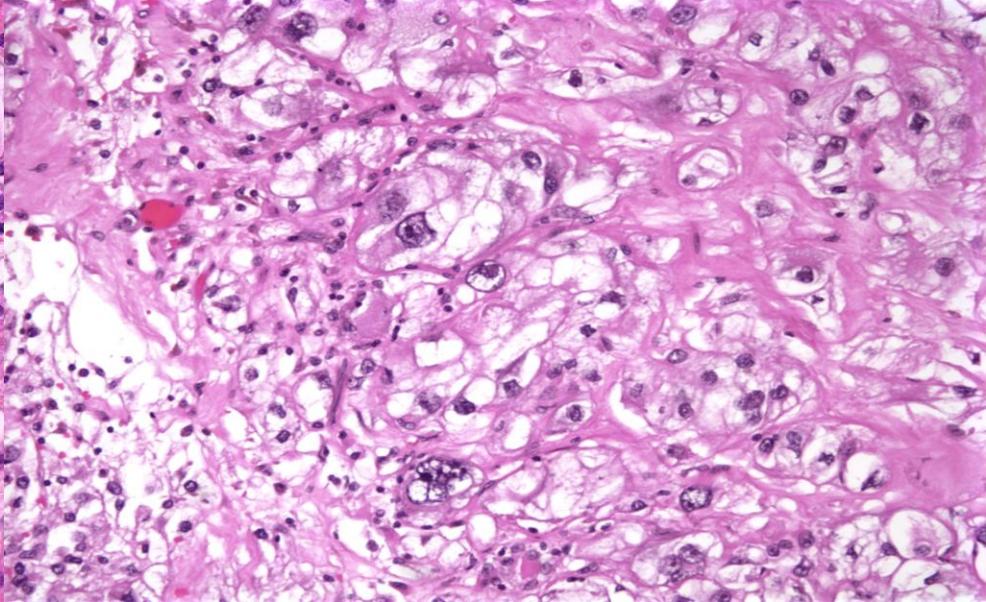
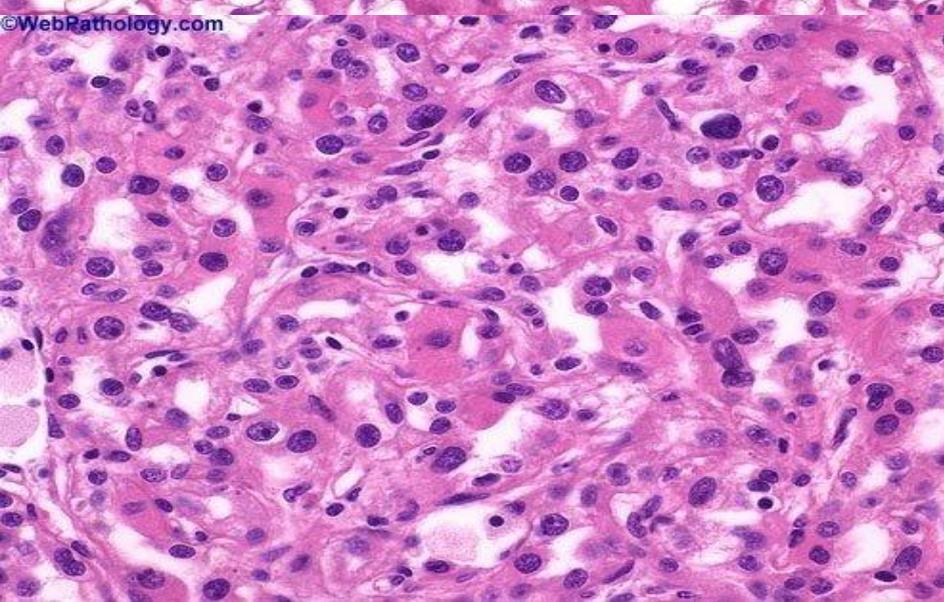
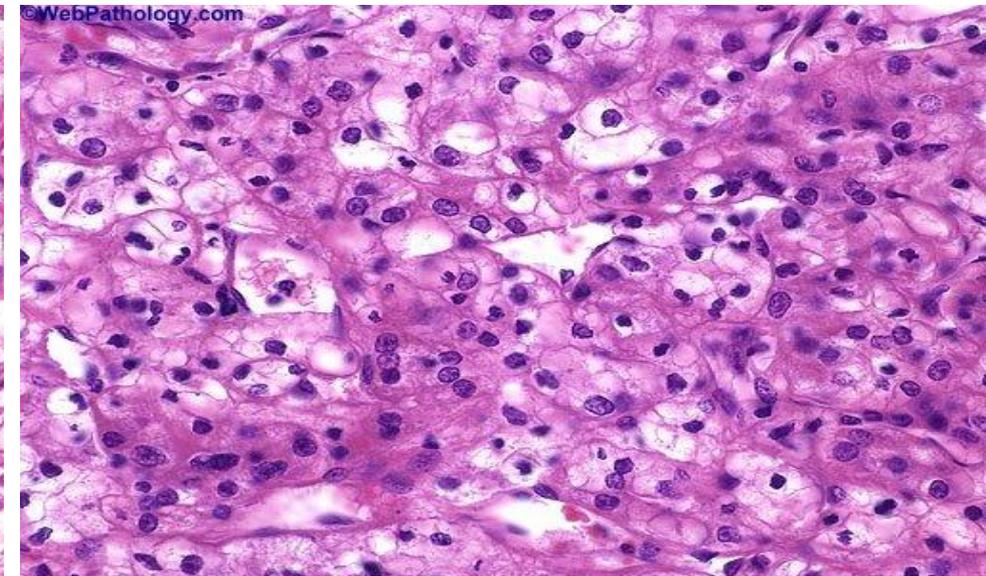
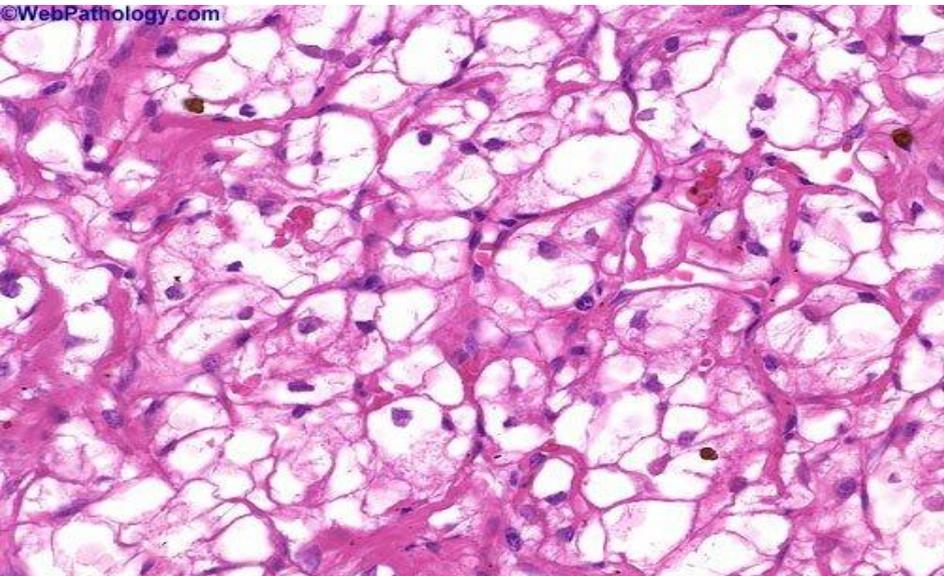
- **Grade**: level of differentiation in tumor cells
- **Stage**: extent of spread of cancer-
2 systems; 1- UICC 2- American Joint
Committee
- **UICC**: TNM system-different for each
cancer

T: primary tumor size

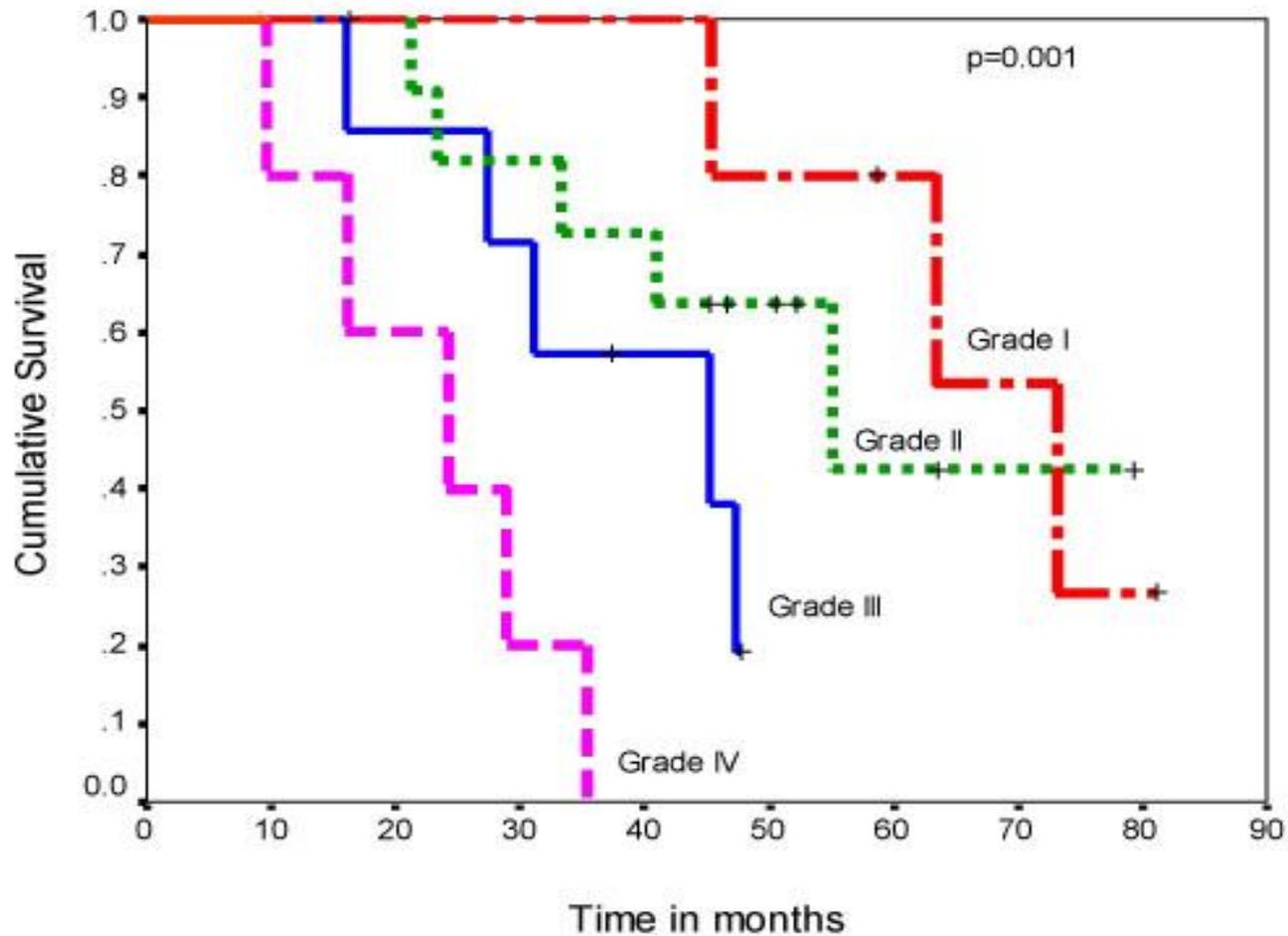
N: regional lymph node involvement

M: metastasis

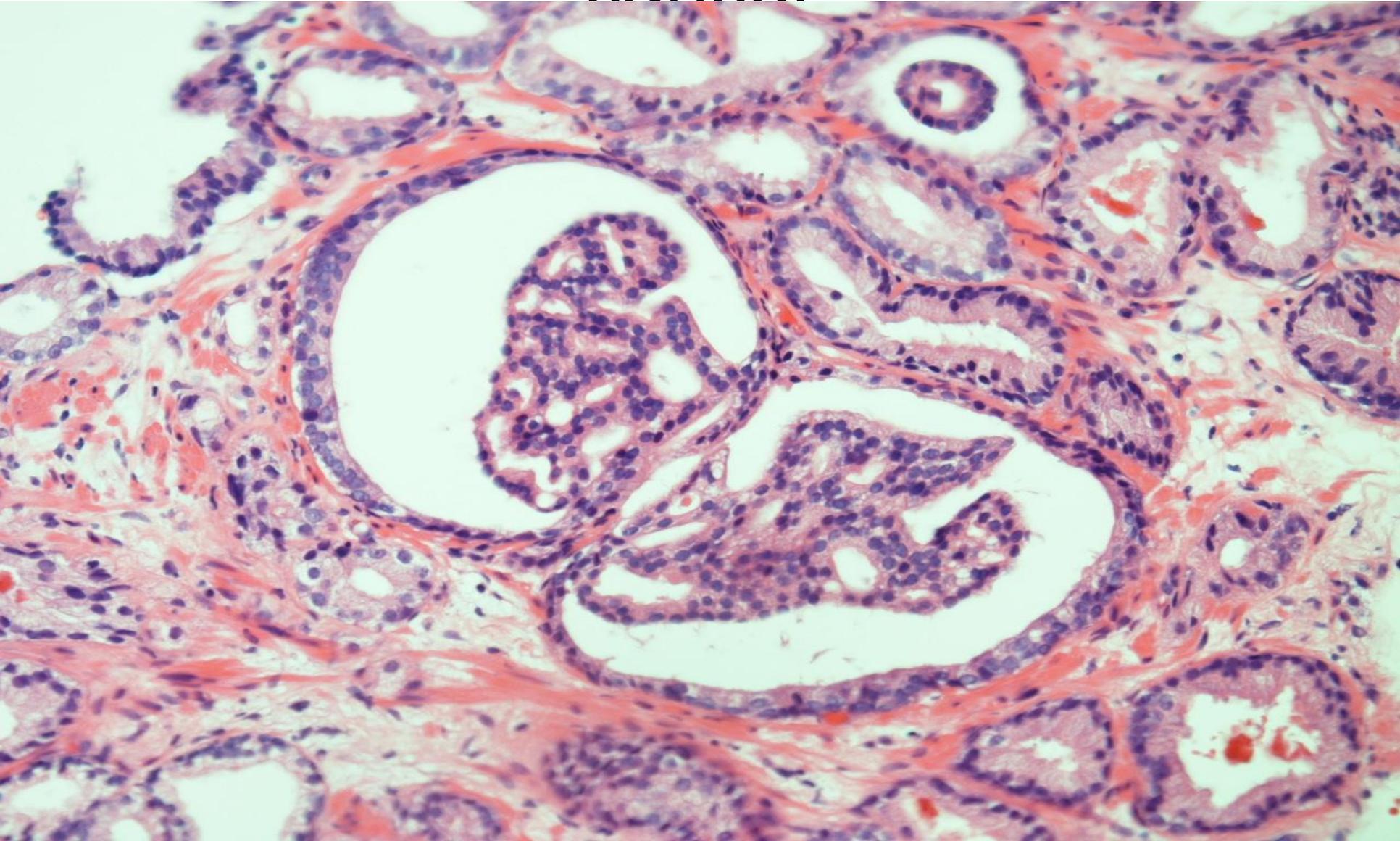
Fuhrman grading for kidney cancer



Fuhrman grading



Gleason grading for prostate cancer



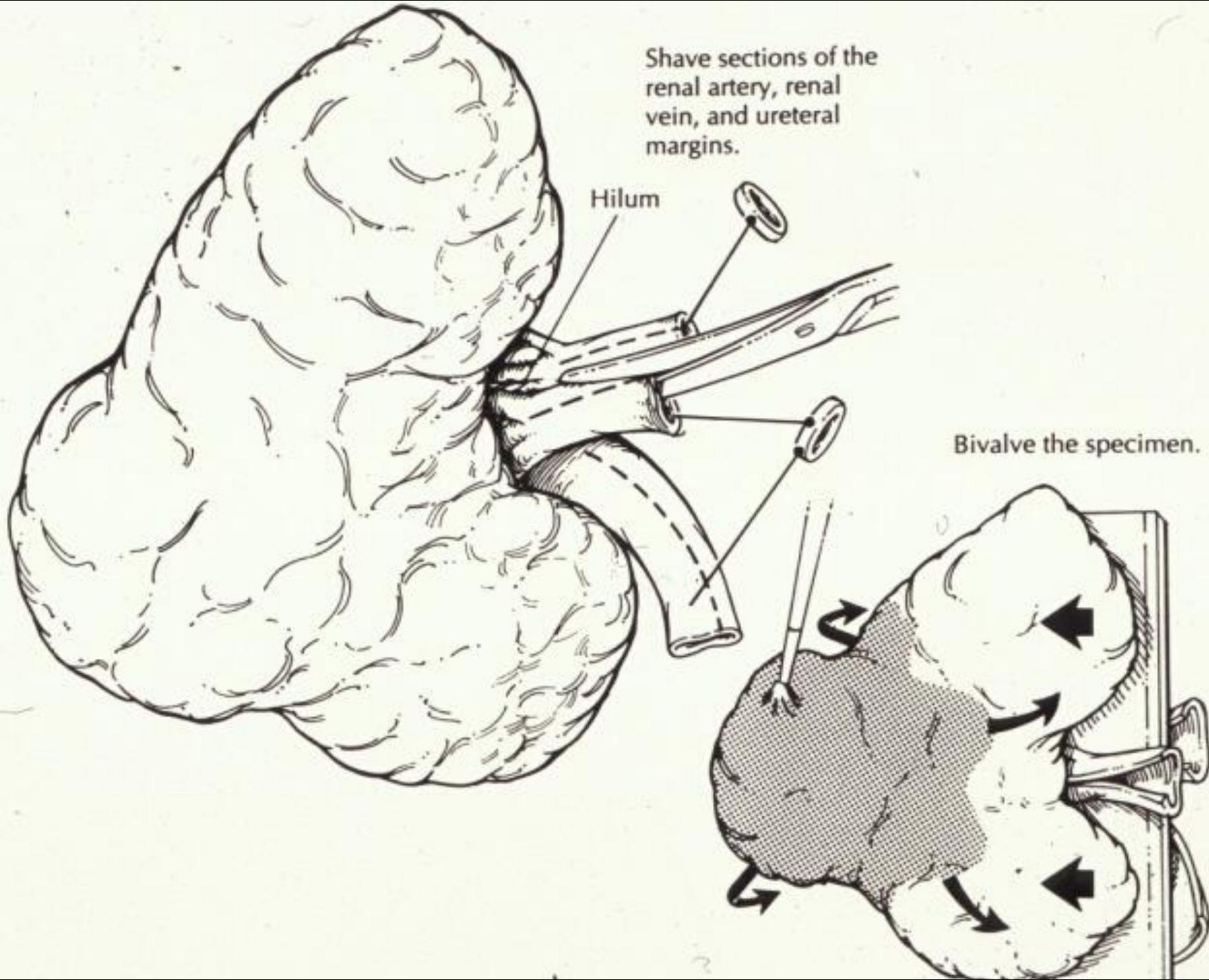
TNM staging of Kidney tumors

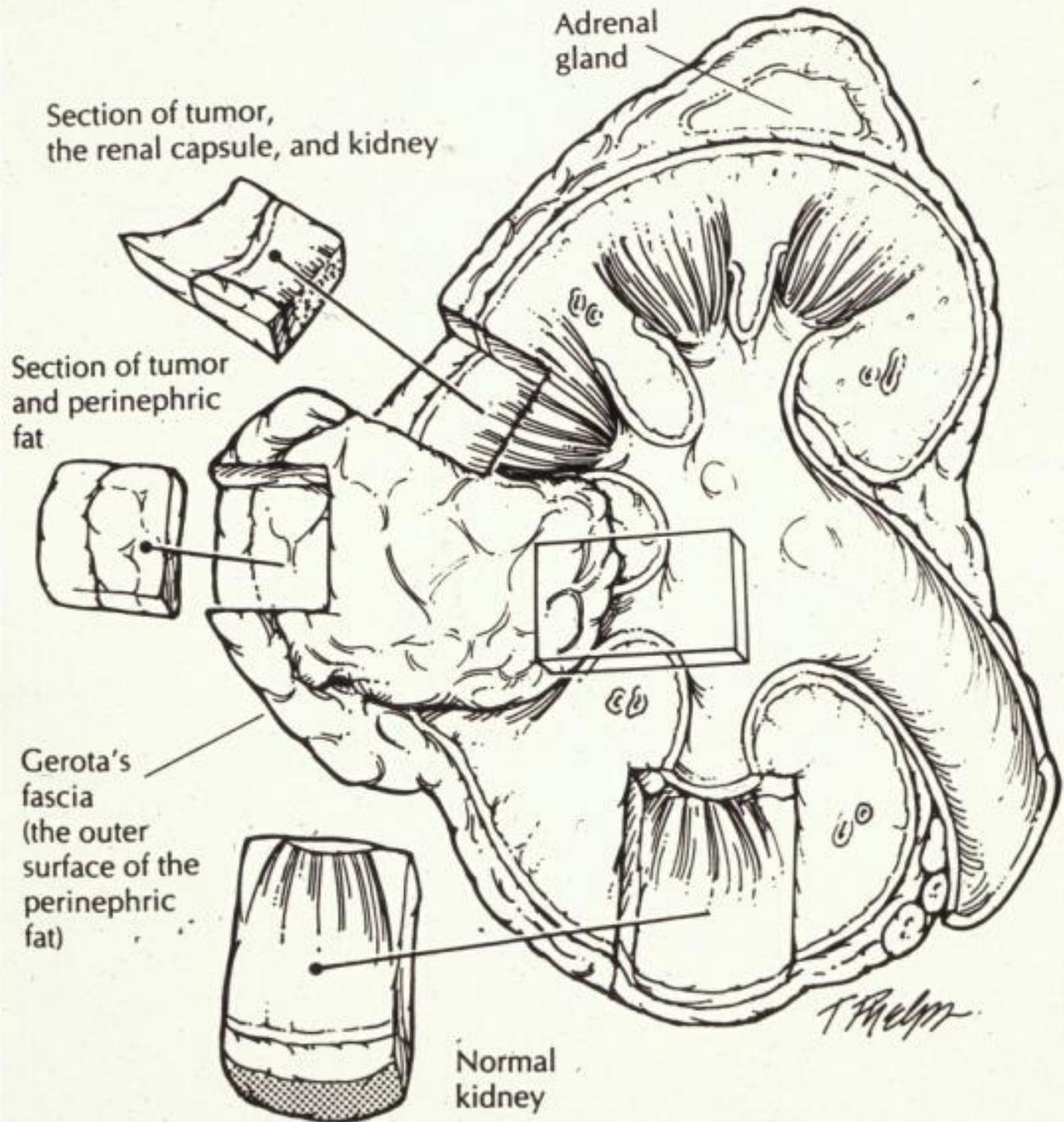
- **T1**- 7 cm. or less, confined to kidney
- **T2**- >7cm, confined to kidney
- **T3**- a-invades into adrenal , perirenal and/or sinus fat, but not beyond Gerota's fascia
 - b-grossly extends into renal vein, or v. cava below diaphragm
 - c-extends into v. cava above diaphragm or invades the wall of v. cava
- **T4**- invades Gerota's fascia
- **N**- N0, N1-single regional LN, N2->1 regional LN

Shave sections of the renal artery, renal vein, and ureteral margins.

Hilum

Bivalve the specimen.





Adrenal gland

Section of tumor, the renal capsule, and kidney

Section of tumor and perinephric fat

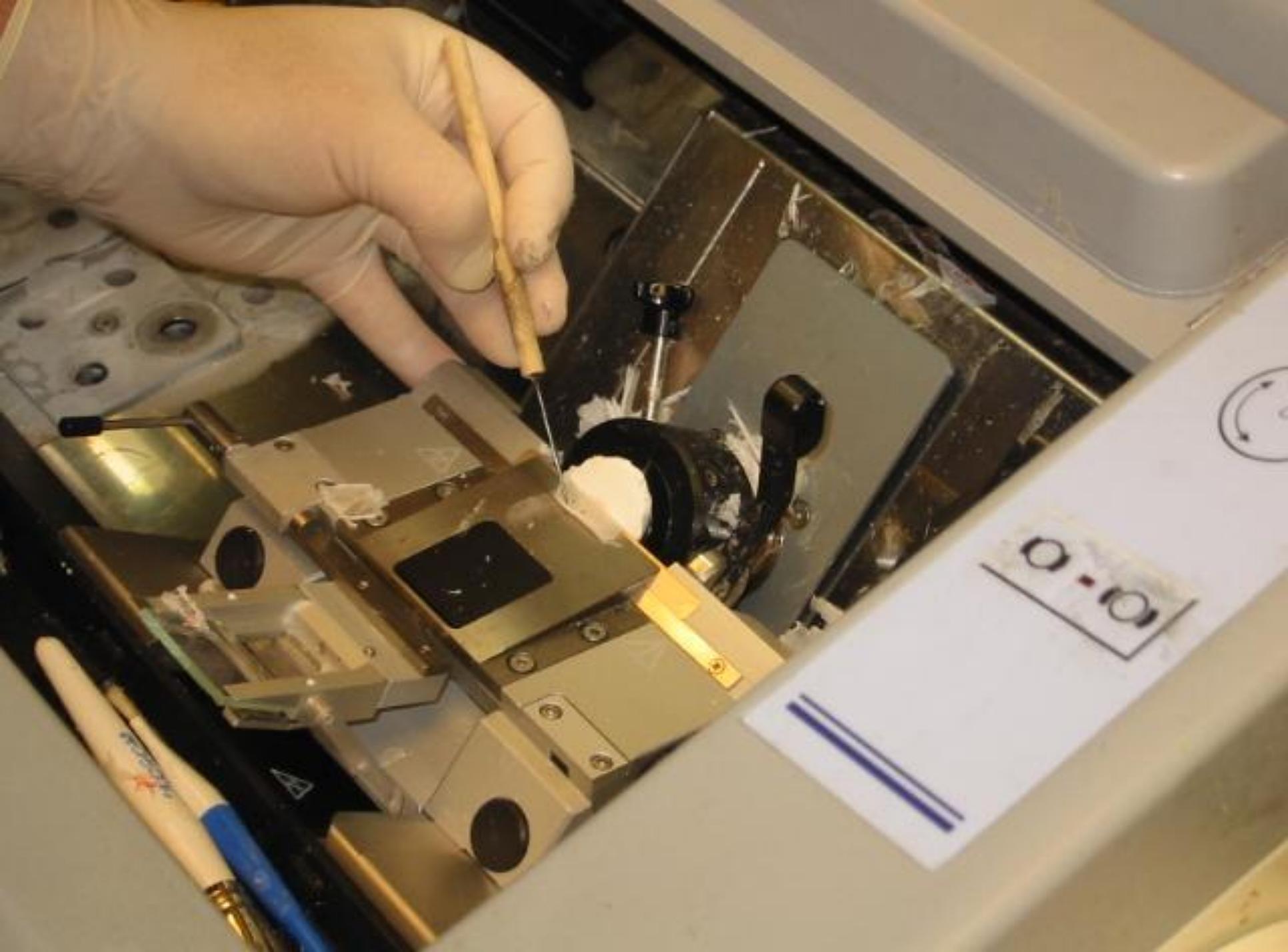
Gerota's fascia (the outer surface of the perinephric fat)

Normal kidney

T. Phelps

Intra-operative consultations-frozen sections

- Rapid diagnosis to guide immediate surgical decisions, such as evaluating surgical margins of resection for tumor
- Gross and/or microscopic and/or cytologic (intraoperative smears/imprints)
- Staging of cancers



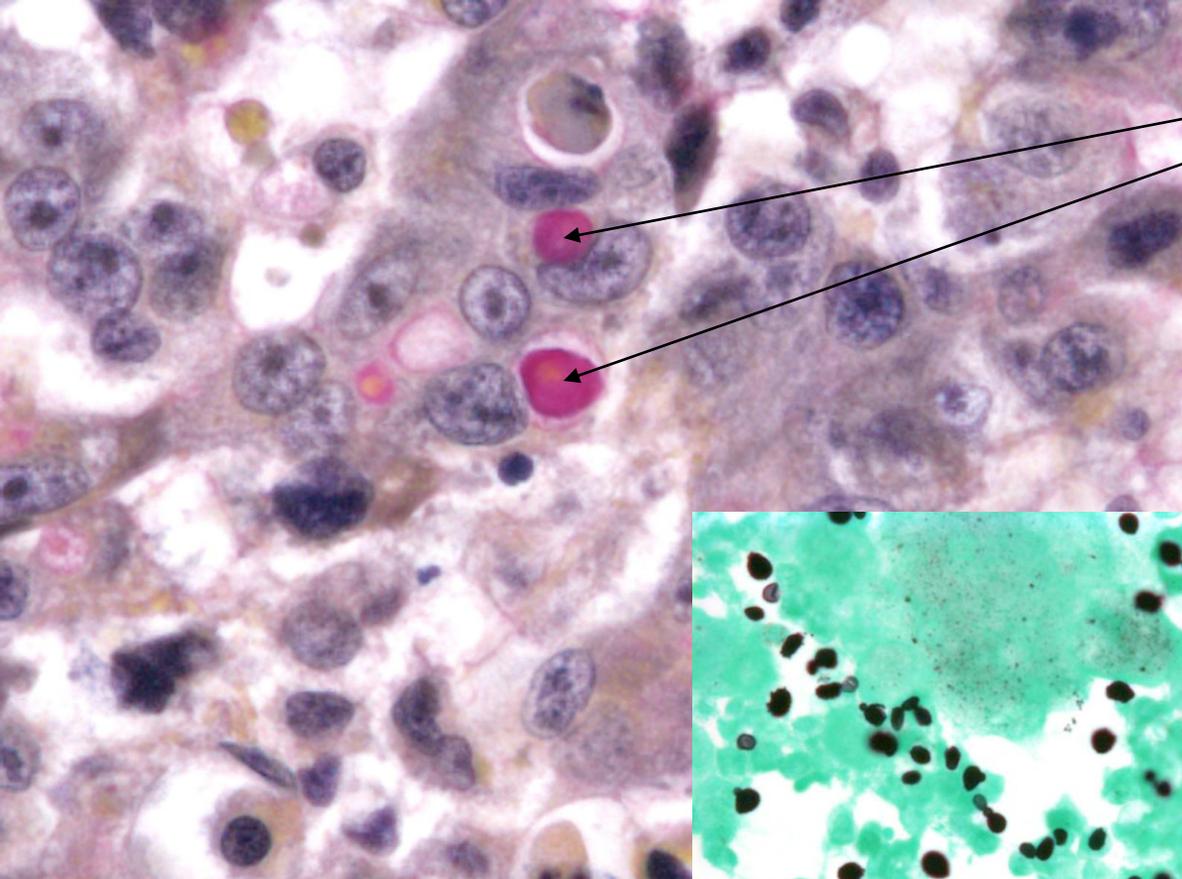
Special Stains

Histochemical Stains

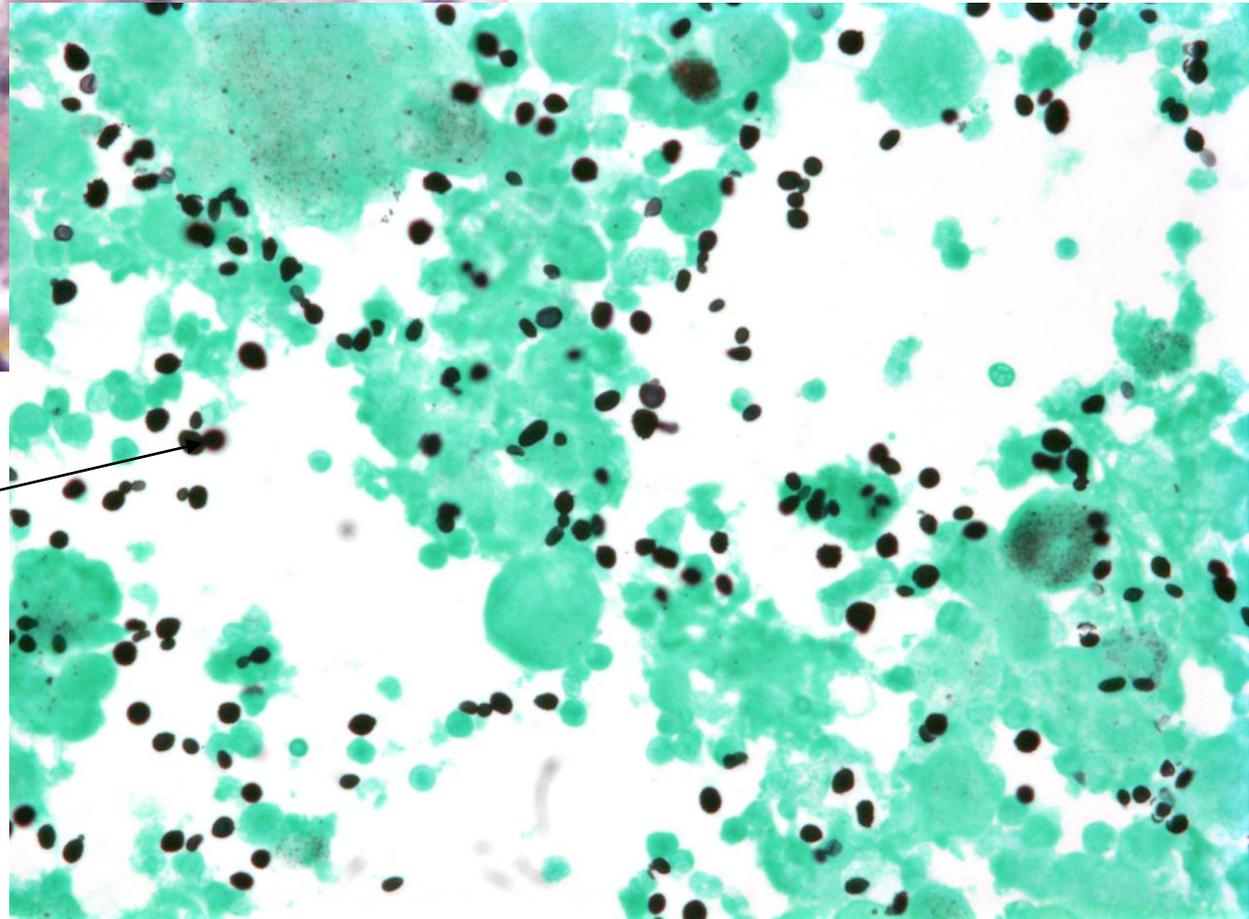
- Reticulin – Reticulin fibers, as in liver
- Mucicarmine – Epithelial mucin, as in adenocarcinoma
- PAS – Glycogen, as in glycogen storage disease; glycoproteins, as in basement membranes, fungus cell walls
- Masson's trichrome – Collagen, as in fibrosis

Histochemical Stains, *cont'd*

- GMS (Grocott's/Gomori's methenamine silver) – Fungi
- Verhoeff- van Gieson – Elastic tissue, as in vessel wall
- Fontana-Masson – Melanin
- Congo red - Amyloid



Mucin in adenocarcinoma cell
Mucicarmen stain



GMS stain for
fungus

Histochemistry

Immunohistochemistry-brown stain

- Categorization of undifferentiated malignant tumors
- Categorization of leukemias/lymphomas
- Determination of site of origin of metastatic tumors.e.g. PSA, thyroglobulin.
- Detection of molecules that have prognostic and therapeutic significance. e.g. ER, PR receptors and her2-neu in breast cancer

Immunohistochemistry

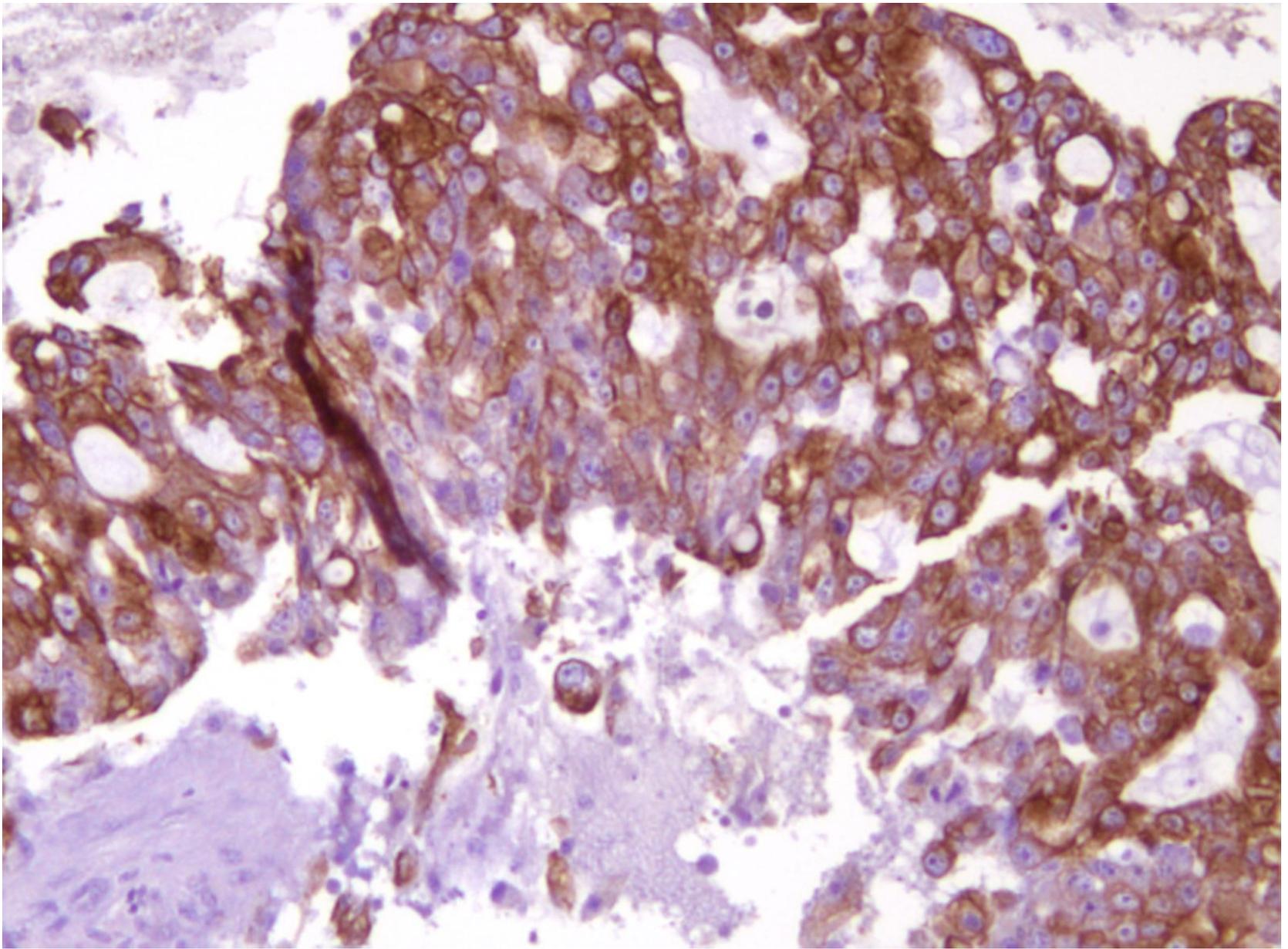
- Cytokeratin: Epithelial cells
- CK7/CK20, CK5/6, etc.
- Thyroid transcription factor-1 (TTF-1):
Thyroid, lung
- Hep-Par-1: Hepatocytes
- Thyroglobulin: Thyroid follicle cells
- Chromogranin, synaptophysin:
Neuroendocrine/neural differentiation

Immunohistochemistry, *cont'd*

- Estrogen receptor, progesterone receptor
- HER2/Neu
- Ki-67 (Mib-1)
- p53

Automated immunostainers



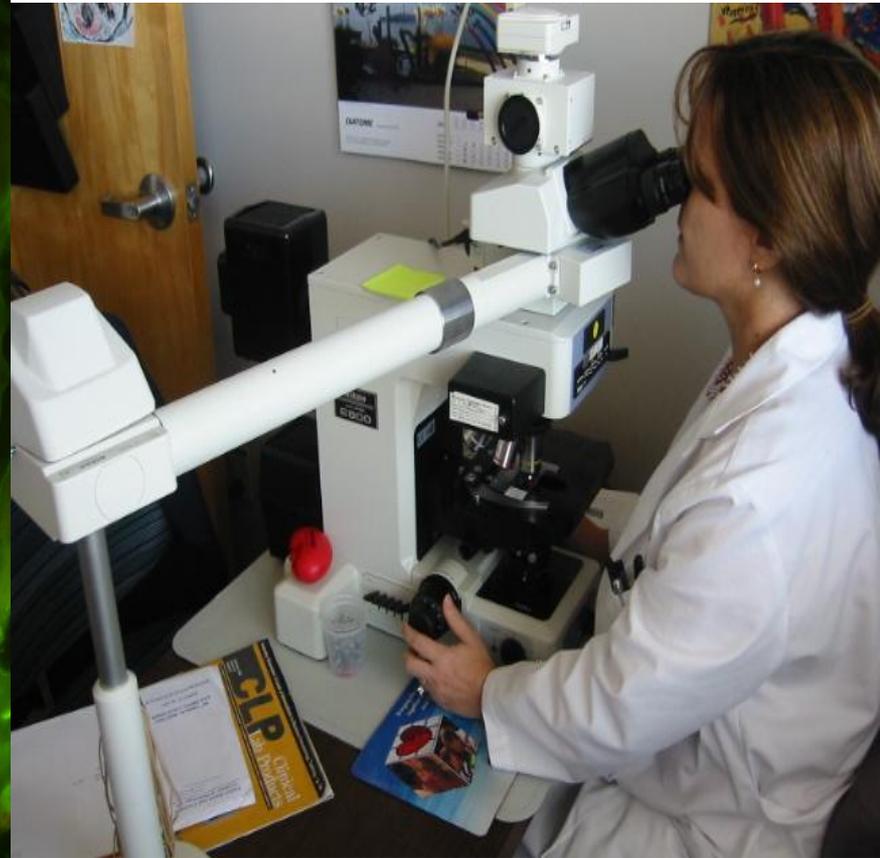
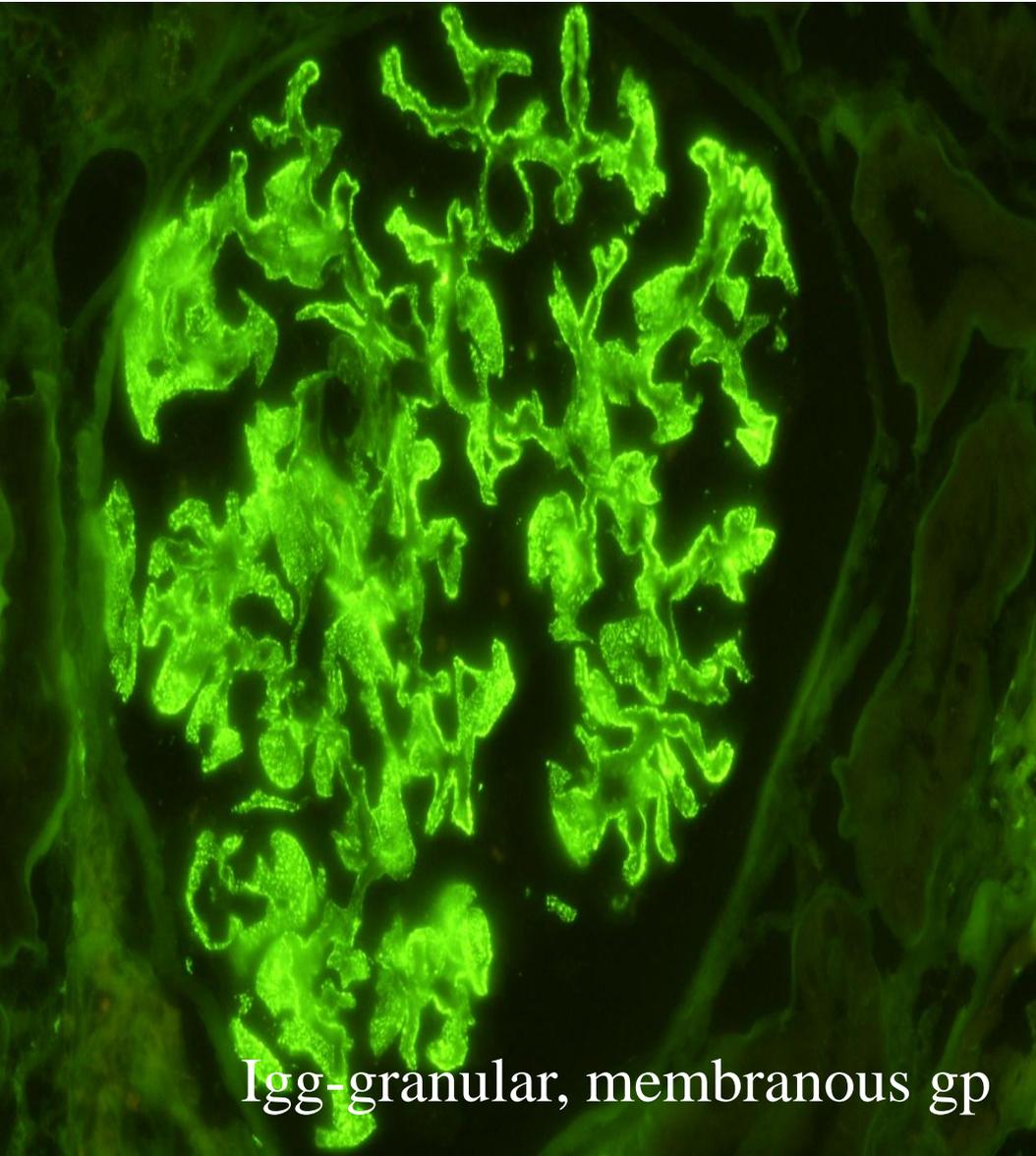


Immunohistochemistry-keratin stain for carcinoma

Special Techniques

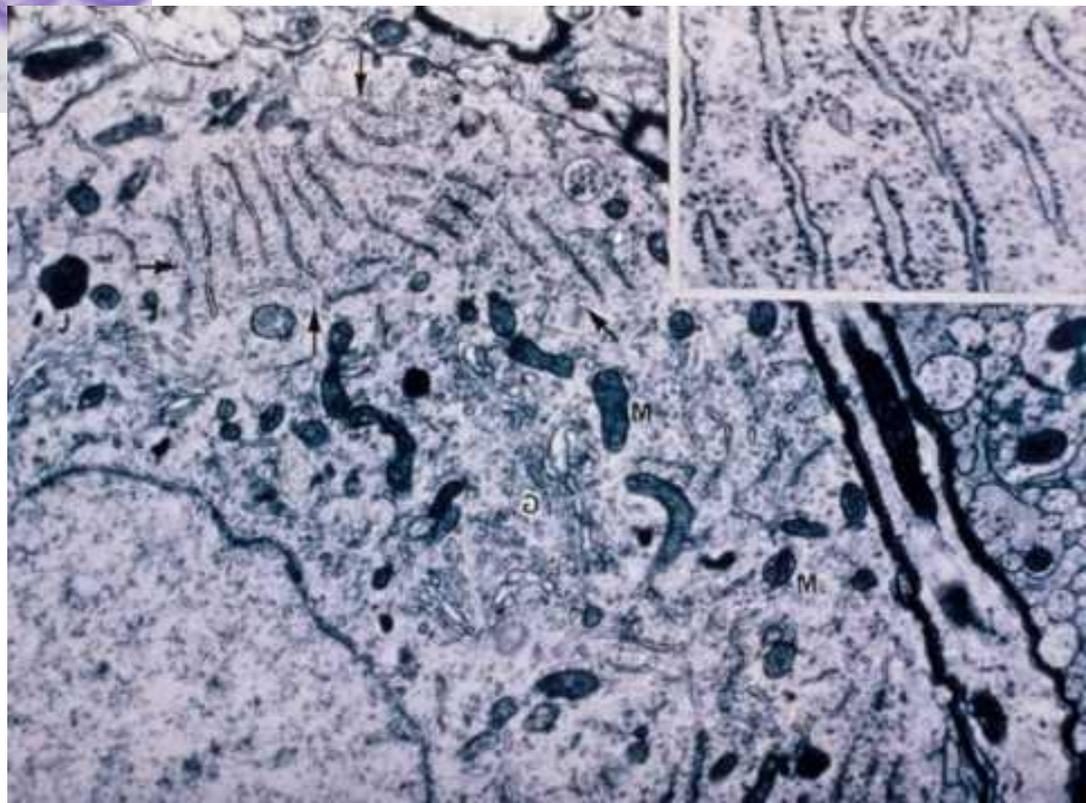
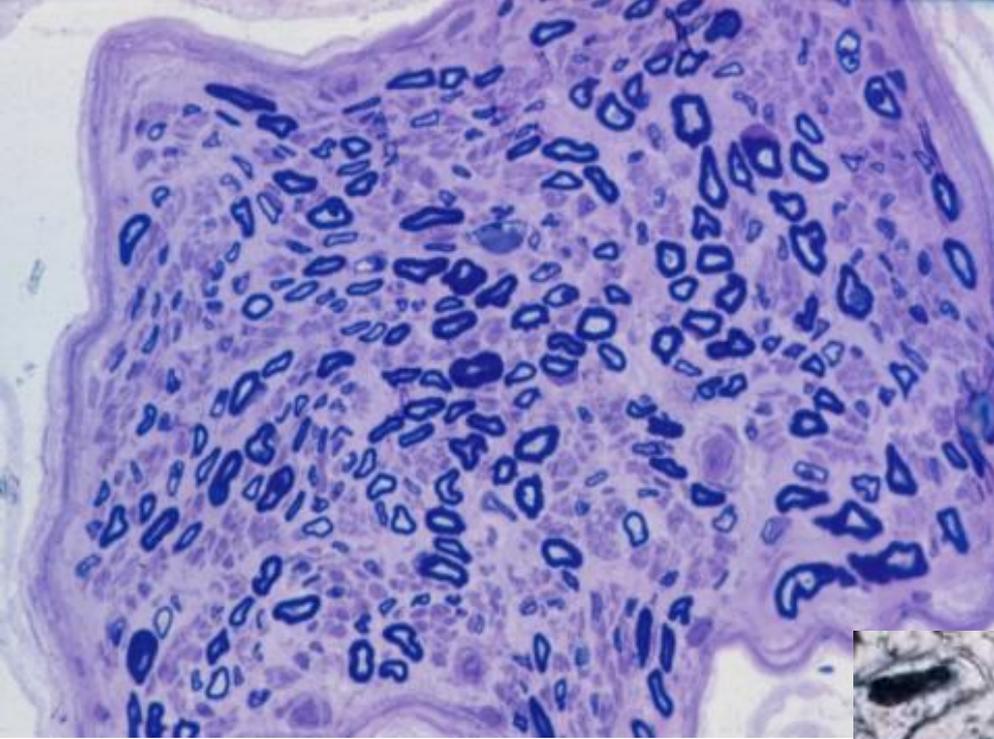
- Fluorescent microscopy
- Polarization microscopy
- Electron microscopy (EM)
- Flow cytometry (FCM)
- In-situ hybridization (FISH, CISH)
- Polymerase chain reaction (PCR)
- Cytogenetics

Immunofluorescence-kidney



Electron microscope





Electron Microscopy

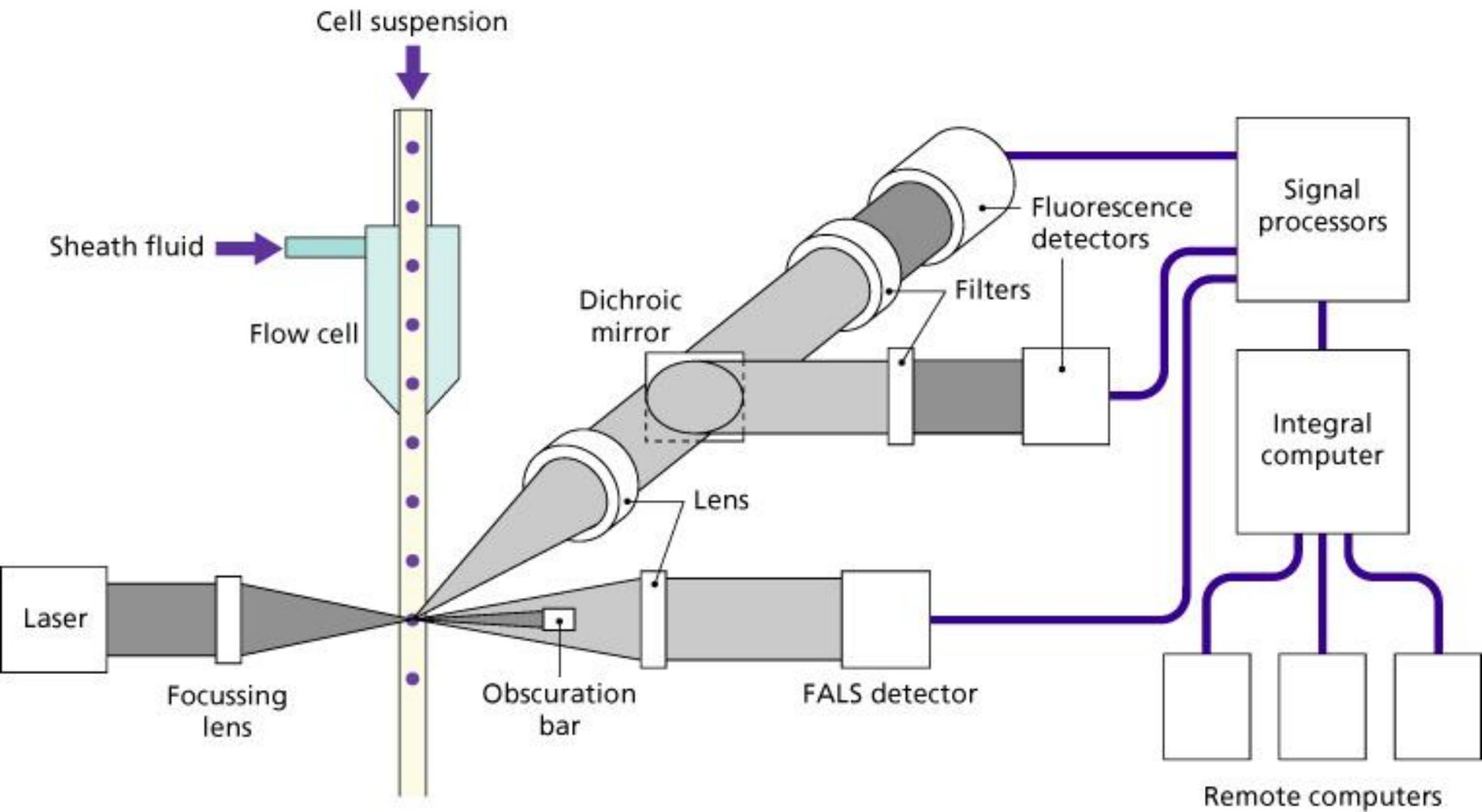
Flow cytometry

- Rapidly and quantitatively **measure cell membrane antigens**

Widely used in leukemias and lymphomas

- **DNA content of tumor cells-ploidy**

Breast ca, urinary bladder, lung and colorectal and prostate cancer.



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Flow Cytometry

Molecular diagnosis

- Detection of translocations in leukemias/lymphomas, sarcomas by
 - Routine **cytogenetic** analysis
 - FISH**-Fluorescence in situ hybridization
 - PCR**-Polymerase chain reaction-the most sensitive method
 - Spectral karyotyping**- all chromosomes are examined
 - Comparative genomic hybridization**- genome amplification and chromosomal gains and losses in tumor cells

Molecular testing for prognosis

- Detecting changes associated with poor prognosis such as:

N-MYC gene amplification in neuroblastoma; poor prognosis

Loss of chromosome **1p19q** in oligodendroglioma-respond well to therapy

Molecular detection of residual disease

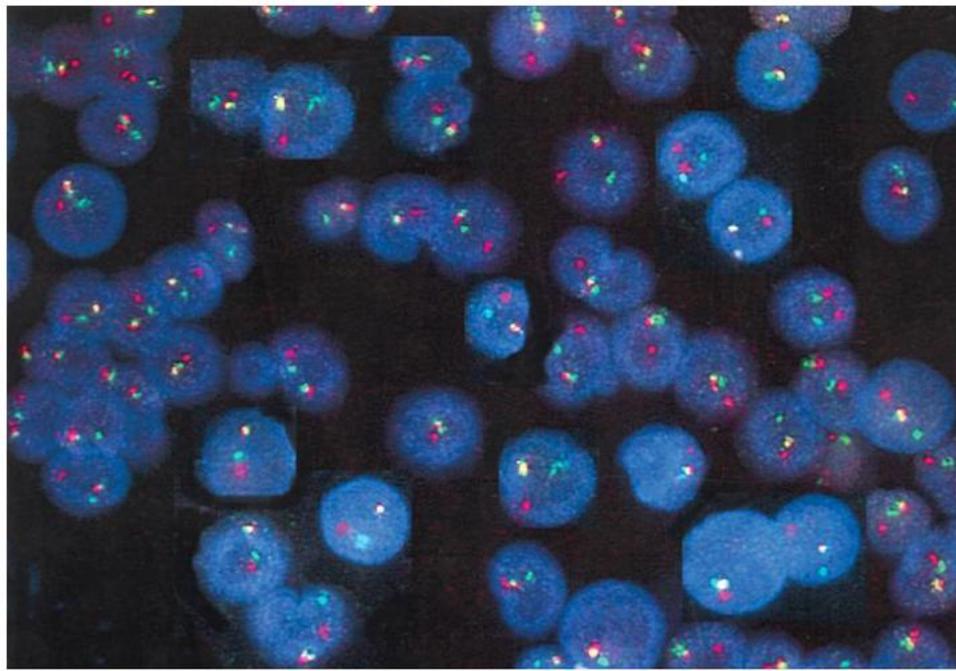
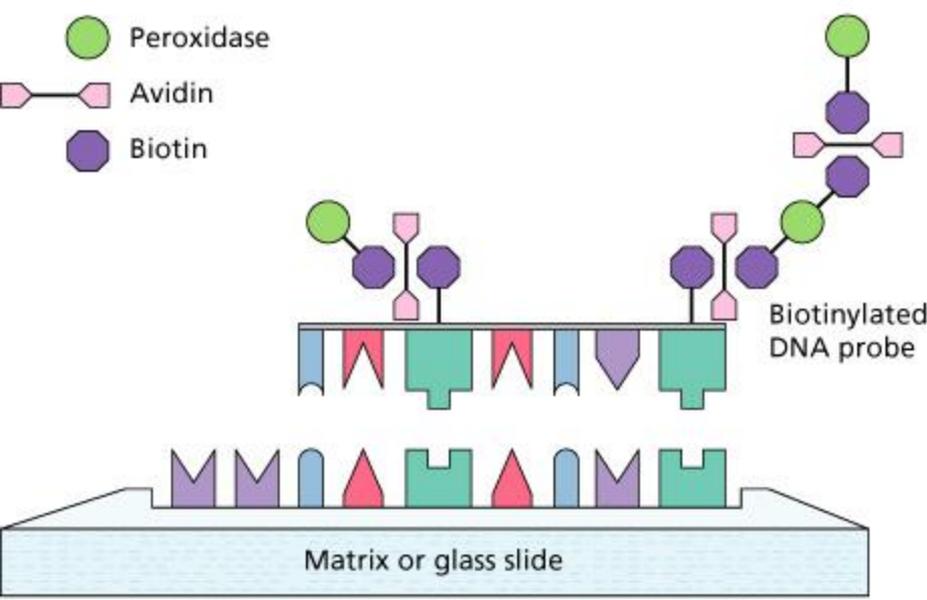
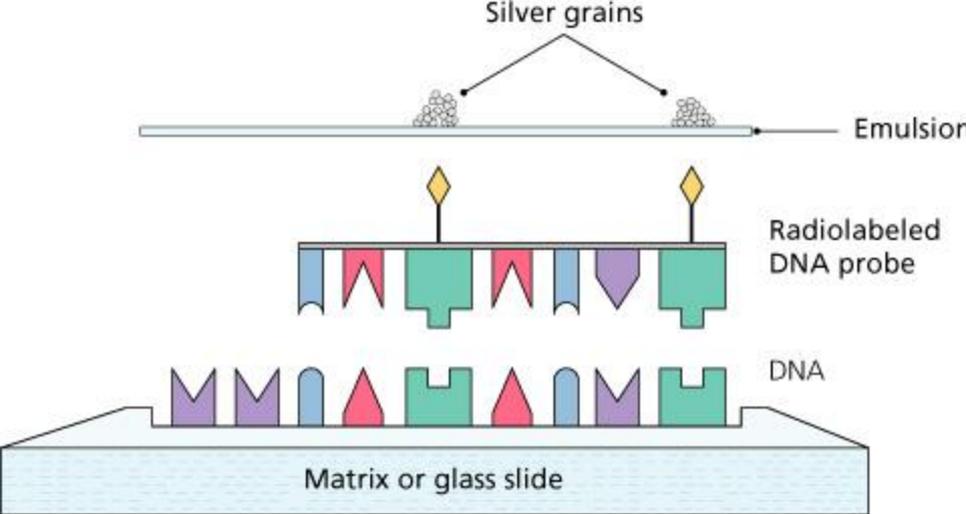
- Leukemia/lymphoma-relapse can be monitored by PCR-based amplification of unique nucleic acid sequences.
- **K-RAS** mutations in colon cancer

Diagnosis of hereditary predisposition to cancer

- Germ line mutations in tumor suppressor genes: BRCA1, BRCA2, RET

DNA microarray and proteomics

- Using gene chip technology in **DNA microarray**- can reveal different RNA expressions from 30000 different genes- identified new subtypes of cancers- leukemia, breast, and gliomas
- **Proteomics**- determines the protein profiles from 3000 genes

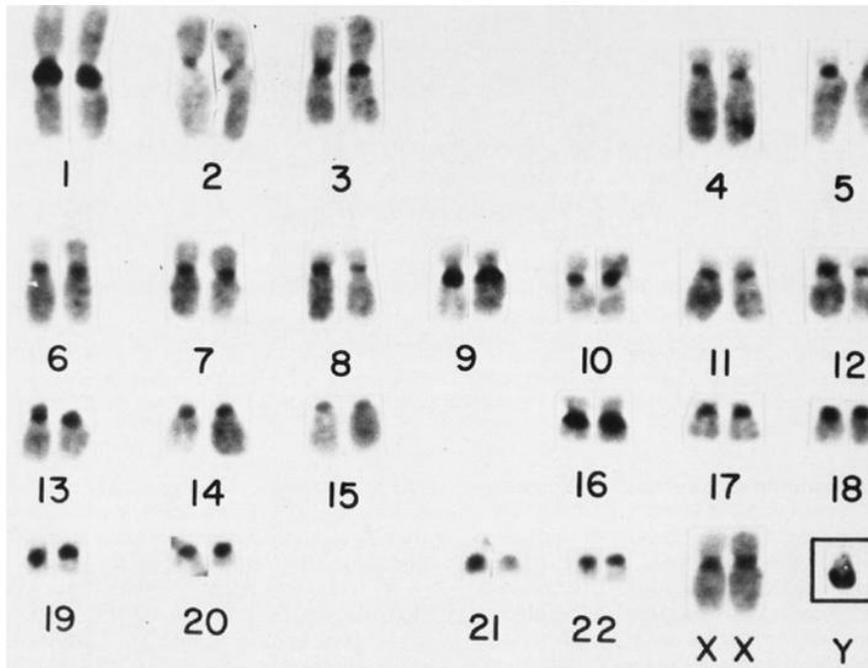


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FISH

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In-situ hybridization



Cytogenetics

Figure 4-18 C-banded karyotype of a normal female cell, with constitutive heterochromatin of the various chromosomes staining dark. Note in particular the relatively large C-bands in chromosomes 1, 9, and 16, with each showing polymorphism of these bands. The *inset* shows a Y chromosome from a male cell demonstrating the dark staining of its long arms with this procedure. (Courtesy of Dr. Avery Sandberg.)

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Figure 4-15A G-banded metaphase spread of a normal male cell.

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CAP INSPECTION

CAP formed in 1947. Since then, it has emerged as the leading voice for pathologists.

- **Laboratory inspection using the checklist every other year**
- **CAP Mission Statement**

The College of American Pathologists, the leading organization of board-certified pathologists, serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine.

Summary

- Specimen type, how to submit
- Stains and examination techniques
- What to expect from pathologic examination
- Subspecialties, advance techniques