# LECTURE

# MODERN METHODS USED IN PATHOLOGY

- **some of the following schemes, photographs and text were taken from www and Sandritters Color Atlas & Textbook of Histopathology.**

# ROUTINE STAININGS

- **HEMATOXYLIN-EOSIN**
- van GIESON – yellow = muscle
- - red = connective tissue
- ELASTICA – elastic fibers (black)
- FAT STAIN (SUDAN III) - red
- CONGO RED – amyloid - red
- PRUSSIAN BLUE – Calcium - blue

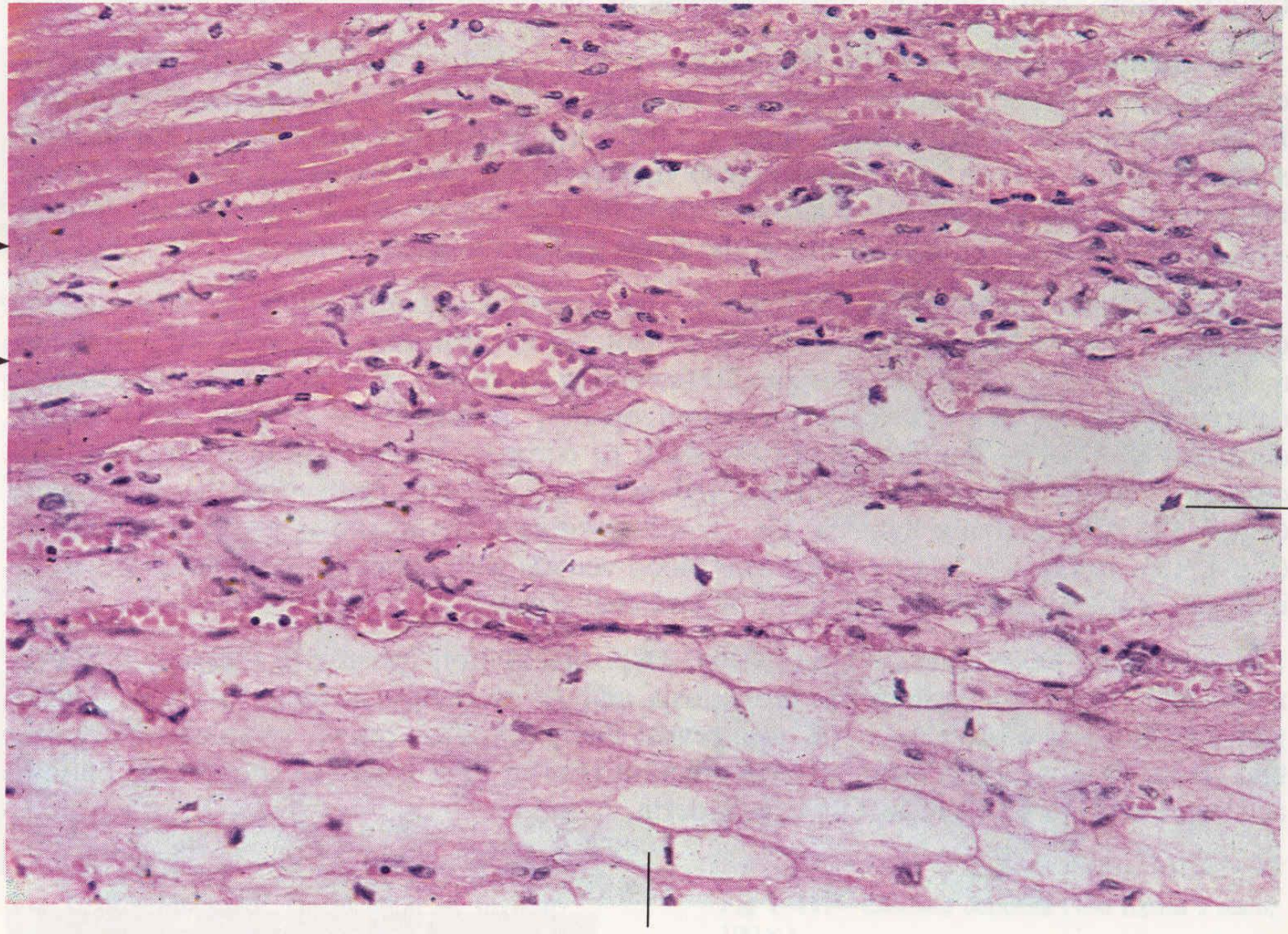



**Fig 1–15.**—Fresh necrosis of heart muscle showing sarcolysis (hematoxylin-eosin; 225×).

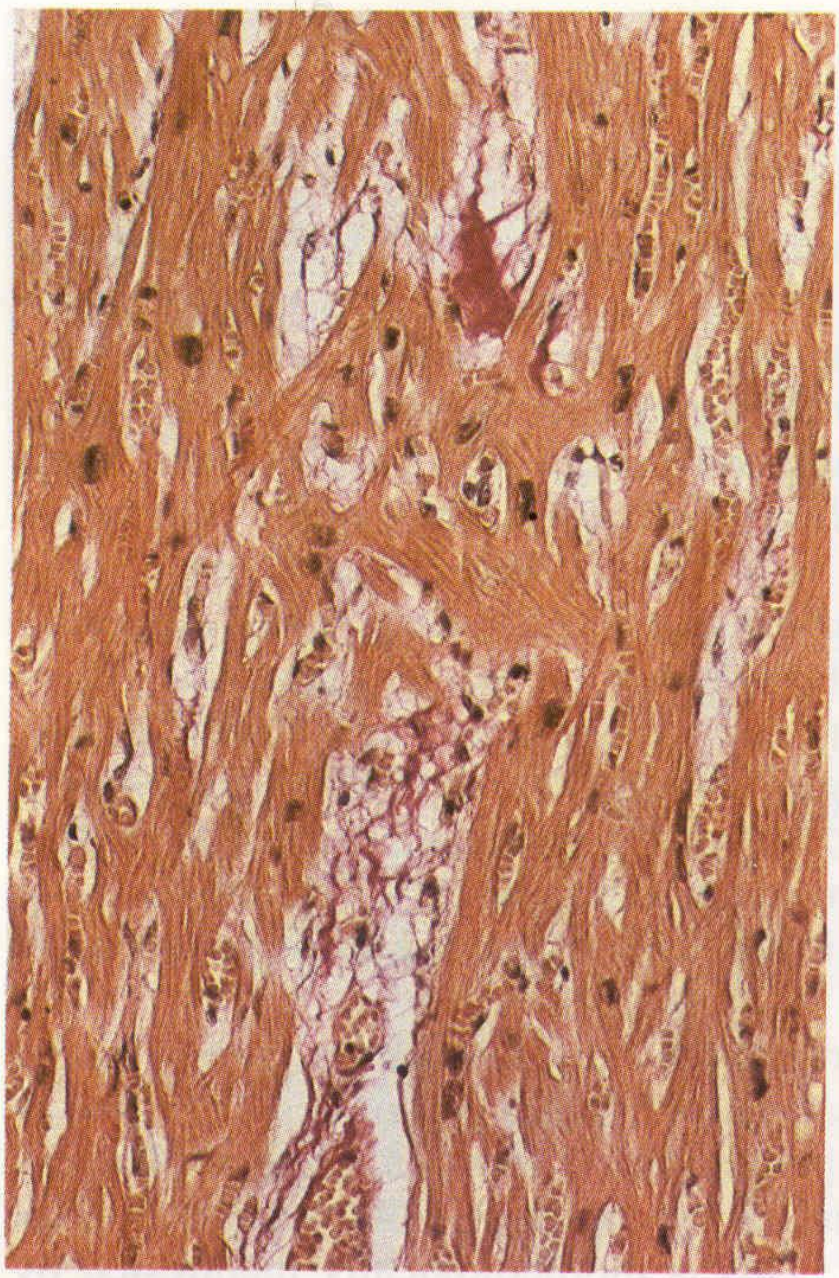


**Fig 1–6.**—Cardiomyopathy (van Gieson's stain). (Prof. Knierem)

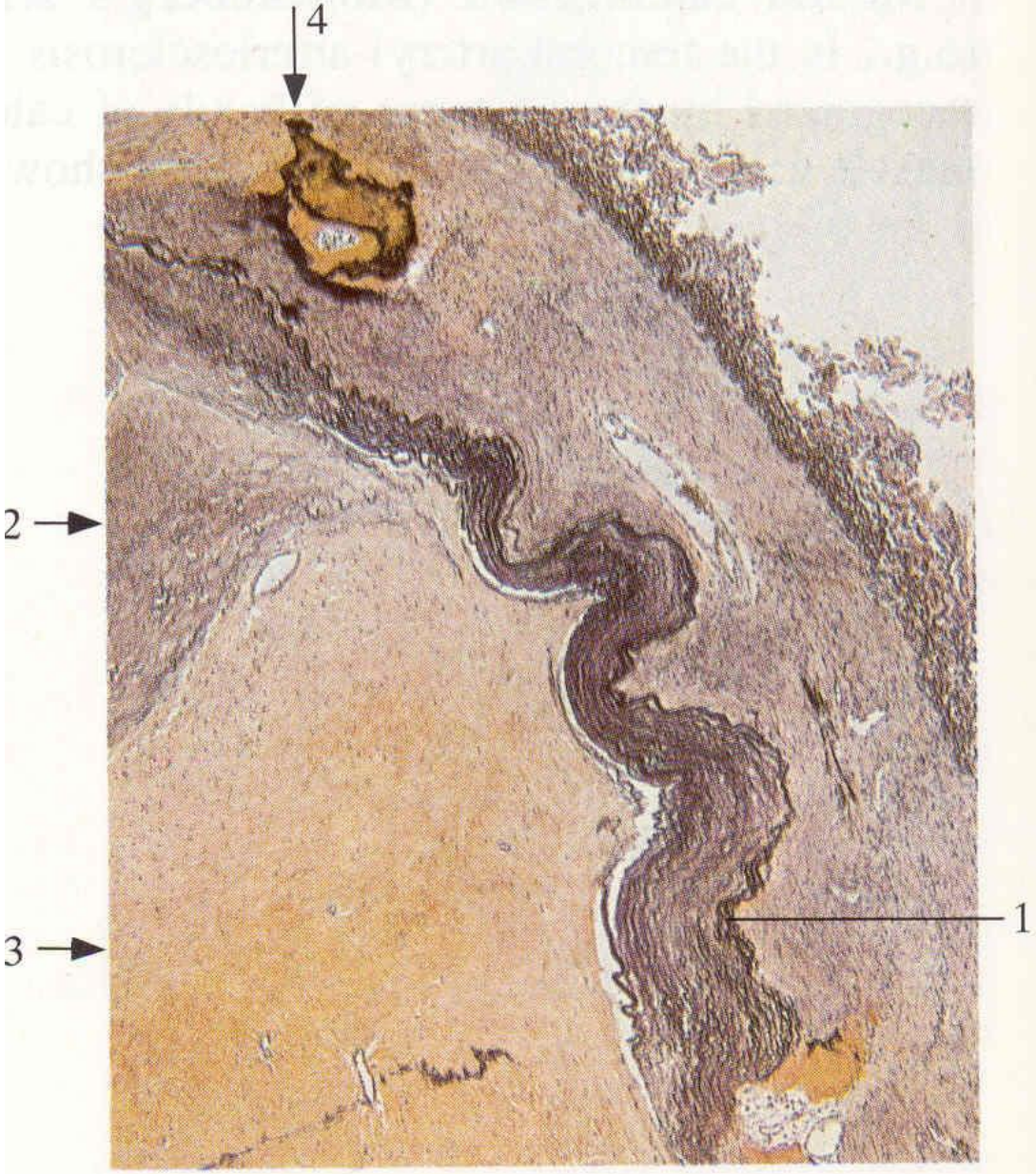



**Fig 2–7.**—Elastosis of coronary artery with old thrombus (elastica–van Gieson's stain).

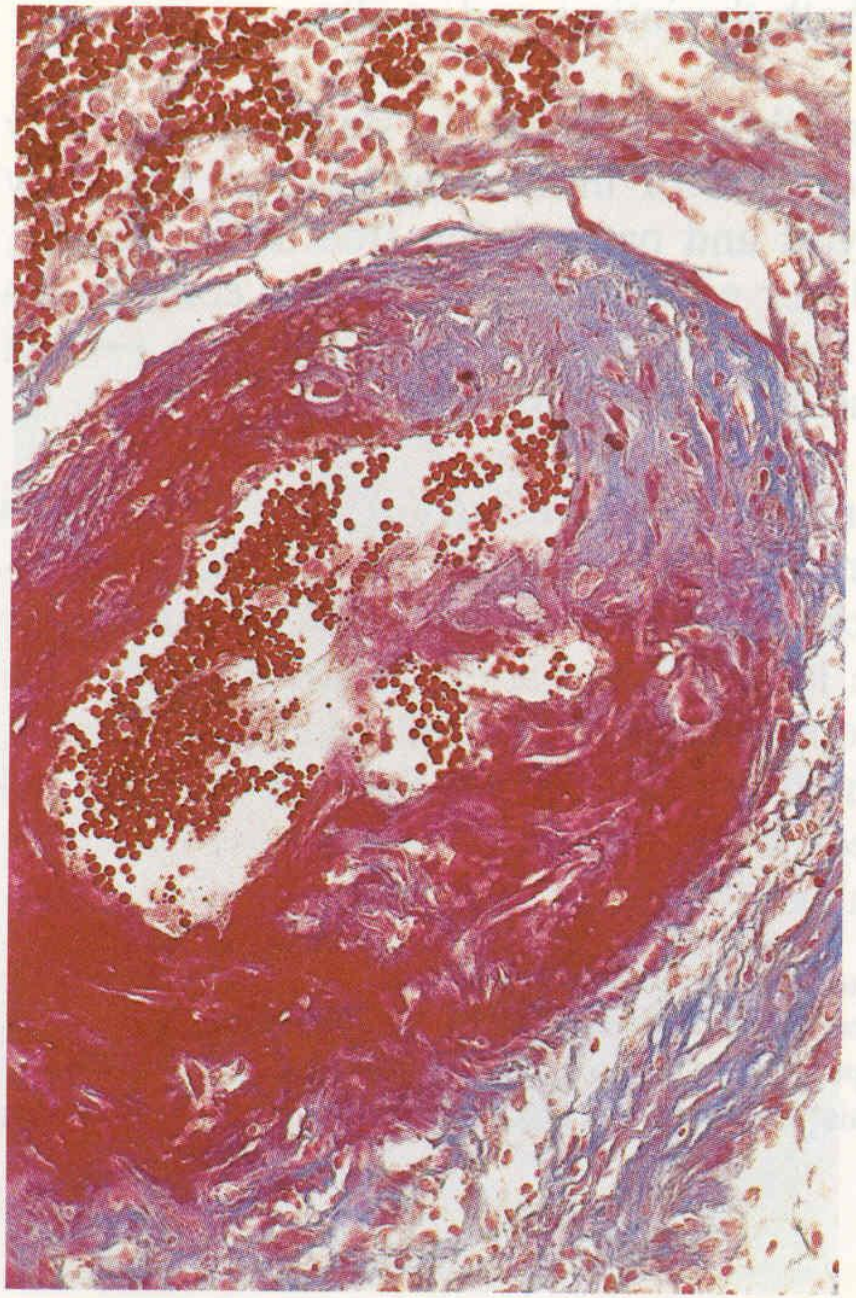


**Fig 2–10.**—Fresh fibrinoid necrosis (azan stain; 40×).

Fibrinoid necrosis – red;

Connective tissue - blue

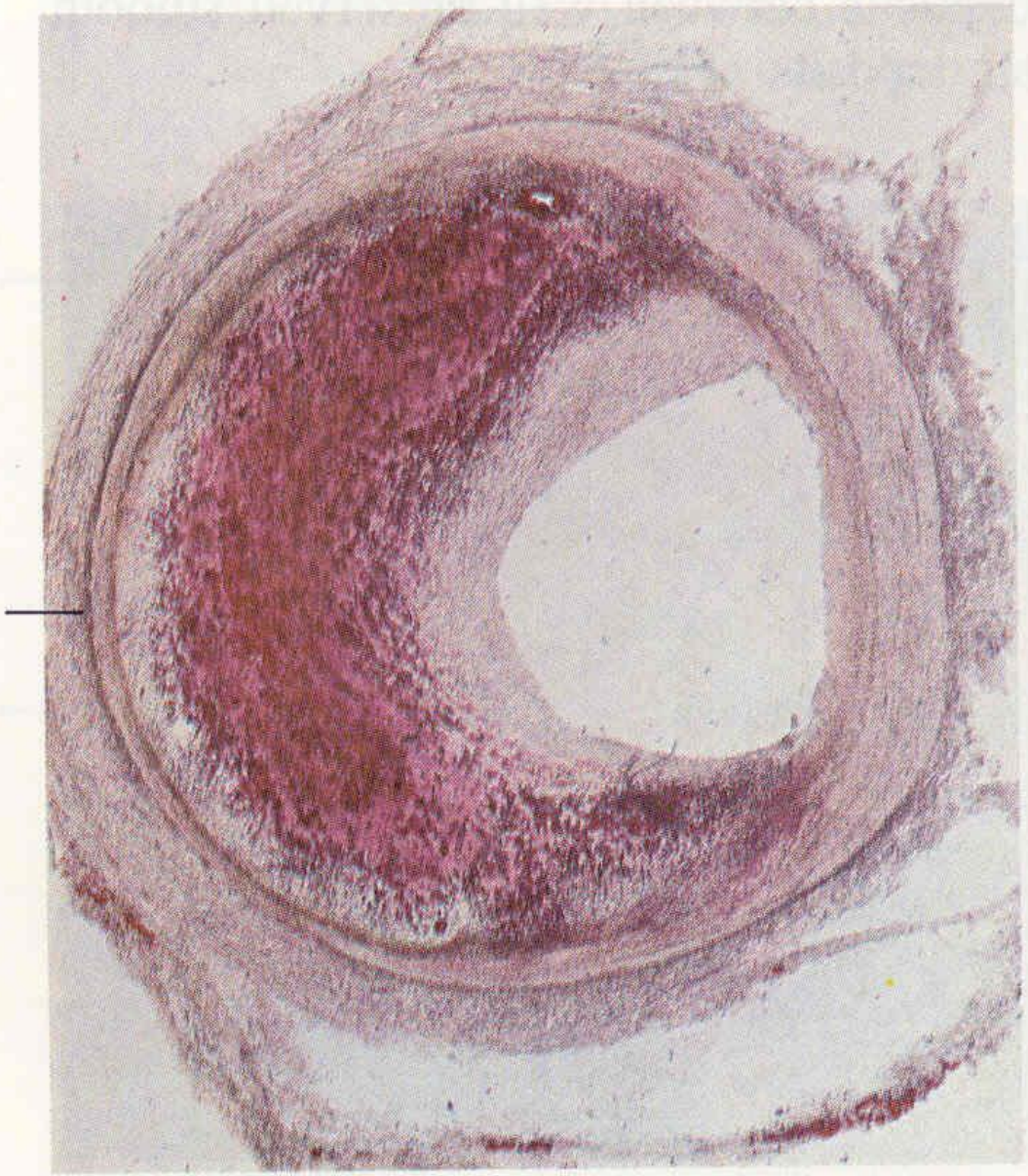


**Fig 2–6.**—Coronary arteriosclerosis with atheroma (Sudan-hematoxylin; 14×).

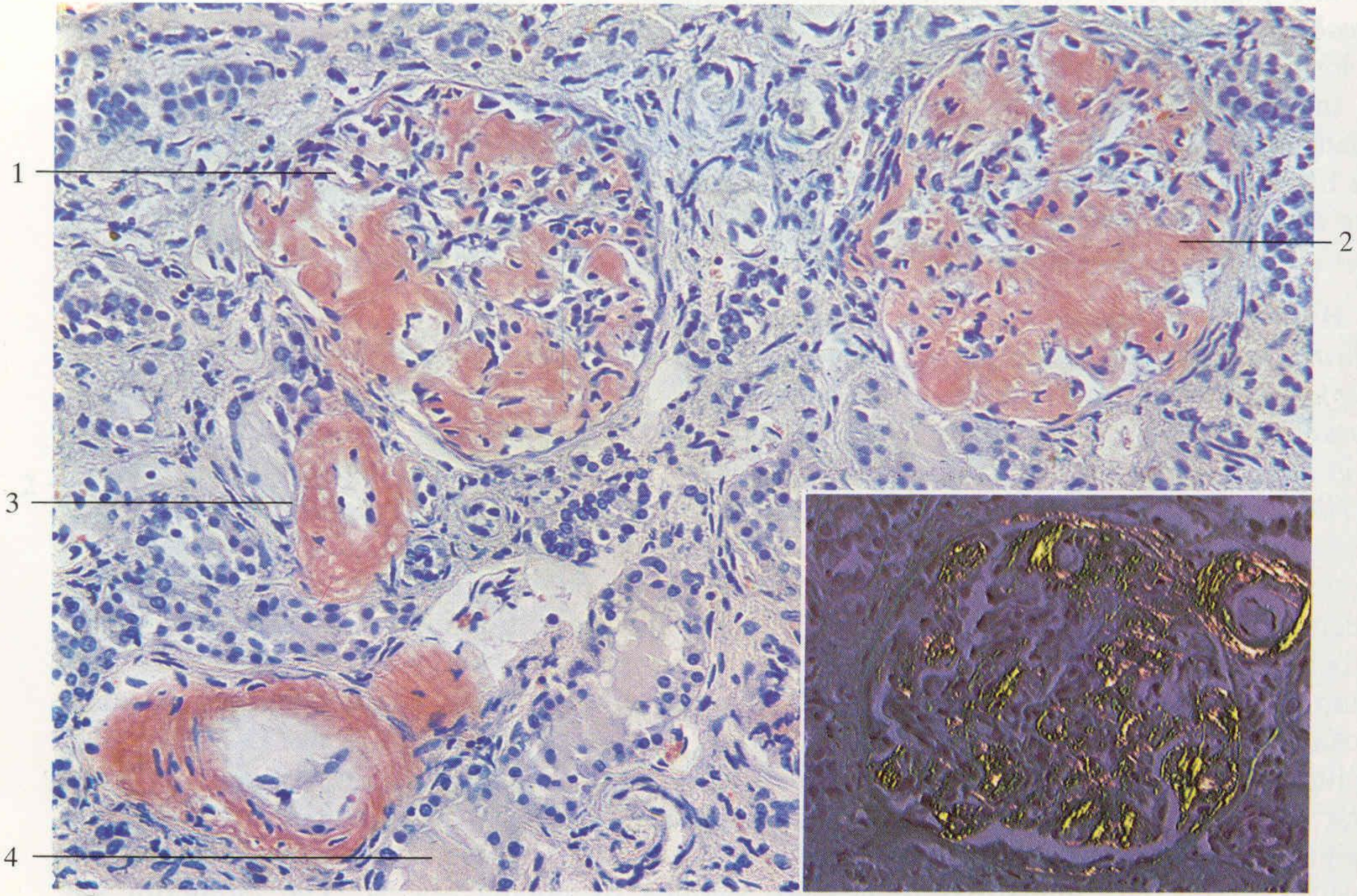



**Fig 6–7.**—Amyloid nephrosis (Congo red–hematoxylin; 200×). *Inset:* Amyloid under polarized light.

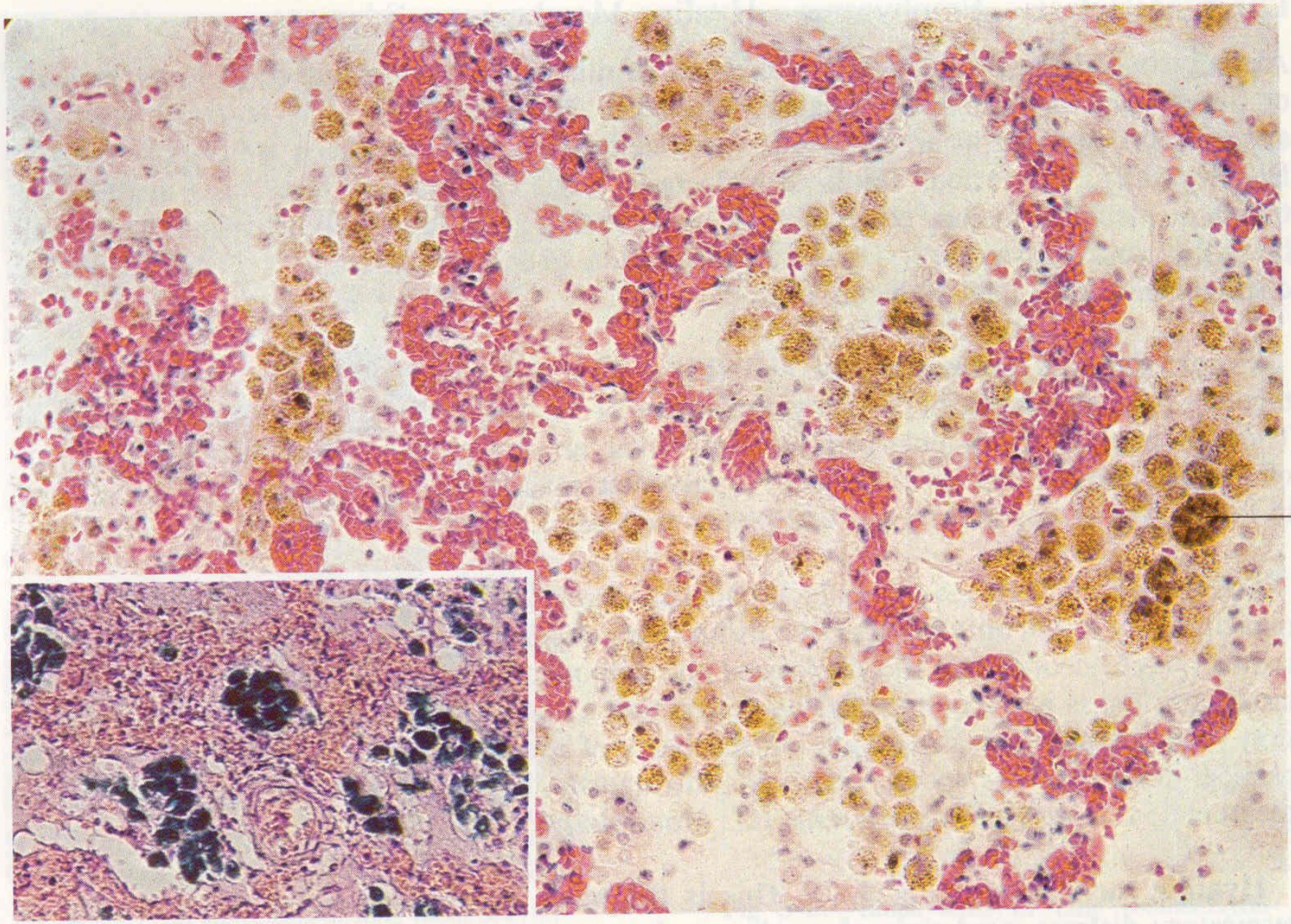


**Fig 3–9.**—Congestion of the lung (passive hyperemia or stasis) (hematoxylin-eosin; 240×). *Inset,* Prussian blue reaction; 132×).

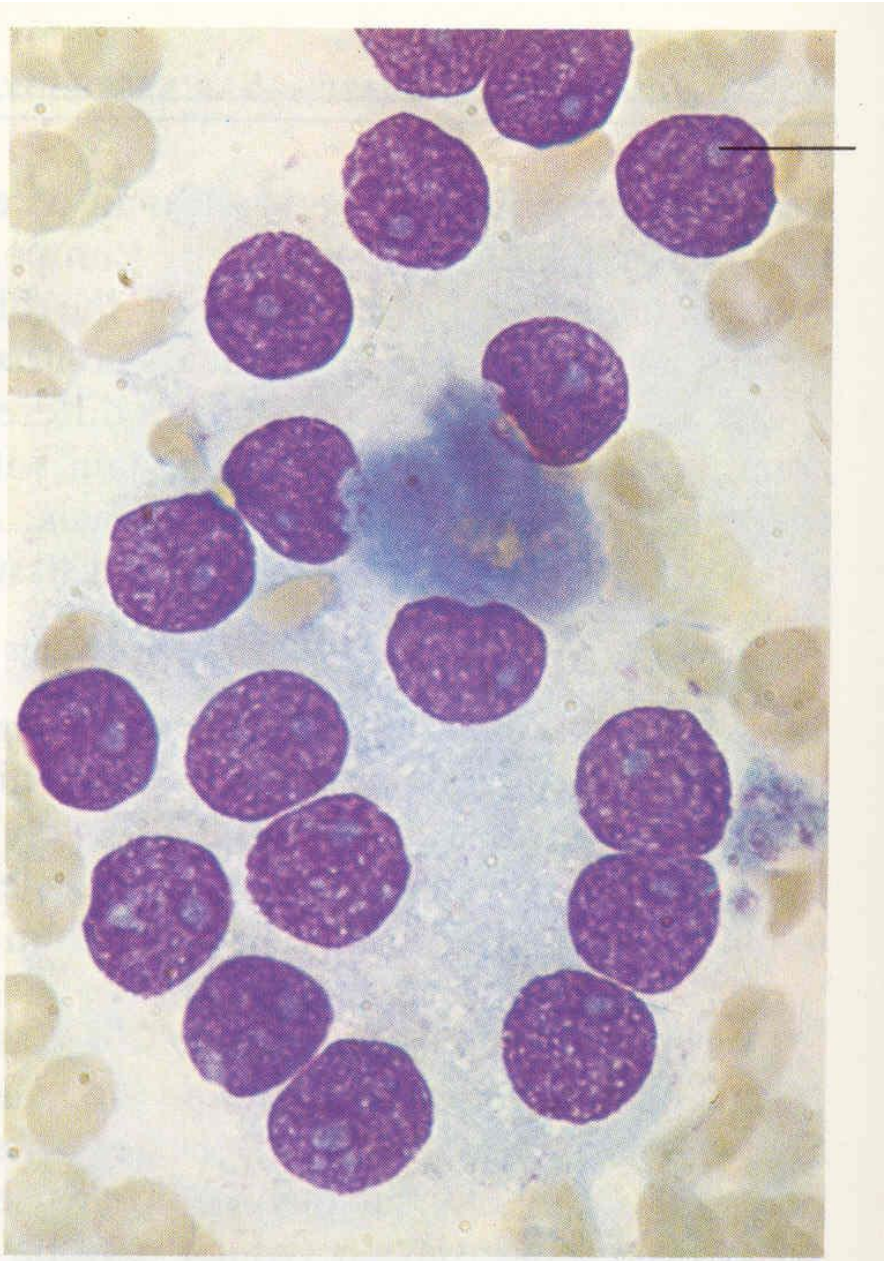


**Fig 16–2.**—May-Grünwald-Giemsa stain; normal thyroid epithelium (800×).

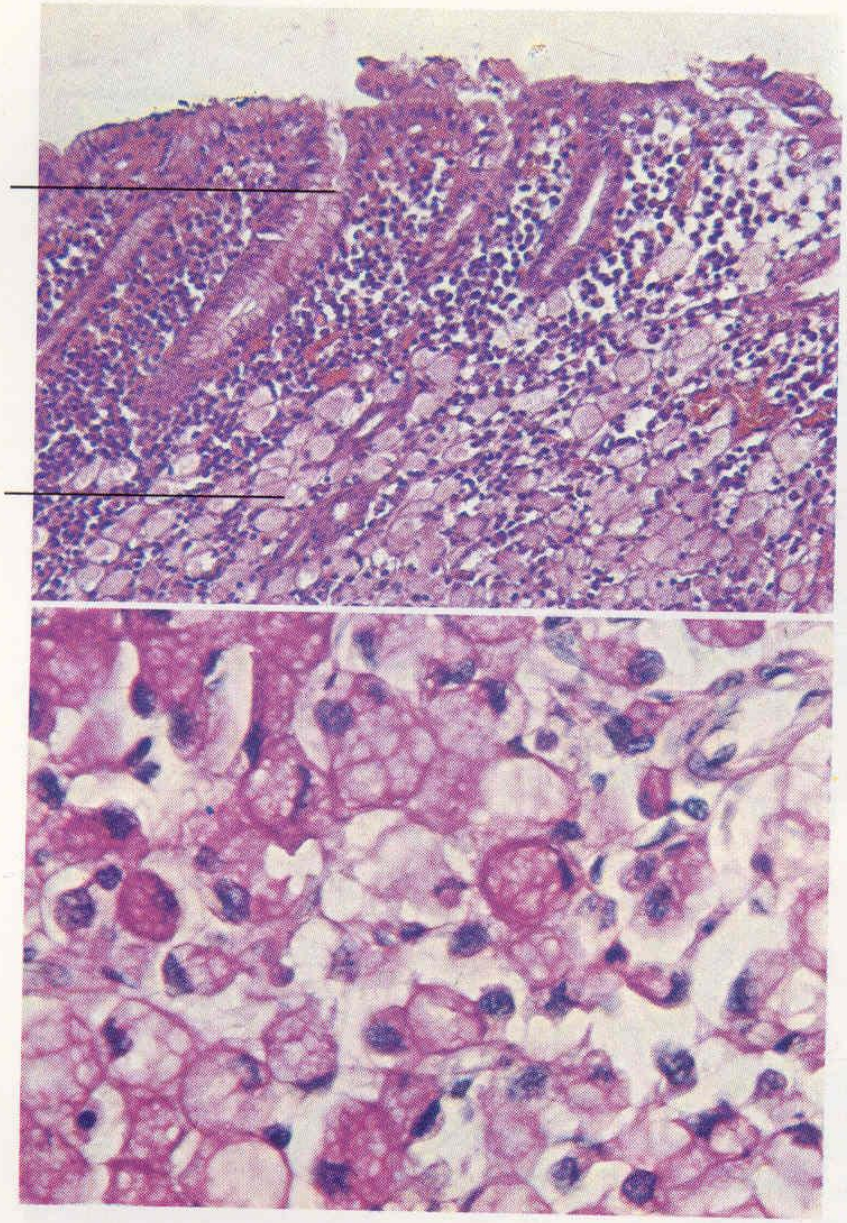


**Fig 4–28.—Top,** early infiltrating carcinoma of stomach (hematoxylin-eosin). **Bottom,** mucinous carcinoma of stomach with signet ring cells (PAS; 320×).

# IMMUNOENZYMATIC REACTIONS

**DIRECT REACTION (ONE ANTIBODY)**

**INDIRECT REACTION (TWO ANTIBODIES)** – MORE EFFICIENT, MORE TIME CONSUMING, SEVERAL NON-SPECIFIC REACTIONS POSSIBLE → DIRECT/INDIRECT IMMUNOFLUORESCENCE

**ENZYME-ANTIENZYME COMPLEX REACTION (3 ANTIBODIES**: SPECIFIC, ANTIGLOBULIN AND DETECTION COMPLEX ENZYME-ANTIENZYME)

# ENZYMATIC MARKERS OF ANTIBODIES

1. PEROXIDASE IN A COMPLEX WITH ANTIPEROXIDASE (PAP)
2. ALKALINE PHOSPHATASE (AP)
3. b-D-GALACTOSIDASE
4. GLUCOSE OXIDASE

- **IMMUNOFLUORESCENCE IS LABELING OF ANTIBODIES OR ANTIGENS WITH FLUORESCENT DYES.**
- THIS TECHNIQUE IS OFTEN USED TO VISUALIZE SUBCELLULAR DISTRIBUTION OF BIOMOLECULES OF INTEREST.
- Immunofluorescent-labeled tissue sections or cultures are studied using **FLUORESCENCE MICROSCOPE OR BY CONFOCAL MICROSCOPY**

# IMMUNOFLUORESCENCE (IMF)

- **QUALITATIVE METHOD** (IE. ANTIGEN + ANTIBODY IN SITU) IN TARGETED OBJECTS
- **IMF** CAN BE DONE IN VIVO, ON **UNFIXED MATERIAL**

## • MAJOR APPLICATIONS:

- RECOGNITION OF ANTIGEN AND RECEPTOR EXPRESSION IN DIFFERENT STAGES OF DEVELOPMENT EG. **IN VIRUSES**
- EXPLANATION OF PATHOGENESIS OF NUMEROUS DISEASES, INCLUDING THEIR DIAGNOSTICS
- IN CELL BIOLOGY TO IDENTIFY AND TOPOGRAPHY OF CELLULAR ORGANELLES, RECEPTORS, LIGANDS, SITES OF HORMONE SYNTHESIS, GROWTH FACTORS ETC.

# IN SITU HYBRIDIZATION, ISH (or FLUORESCENCE ISH, FISH)

- **CYTOCHEMICAL TECHNIQUE**
- ALLOWS DETERMINATION OF SINGLE GENES OR ITS FRAGMENT IN CHROMATIN/CHROMOSOME AND ITS SPECIFIC EXPRESSION IN CELL/TISSUE
- BASED ON RELATIVELY STABLE HYDROGEN-BINDING BETWEEN SPECIFIC PARTS OF **DNA OR RNA** CALLED **MOLECULAR PROBES OR PROBES**

## TYPES OF PROBES:

- DOUBLE STRANDED OR MONO STRANDED DNA (cDNA OR ssDNA)
- MONO STRANDED OLIGONUCLEOTIDE (DNA)
- MONO STRANDED RNA PROBES RNA (cRNA)

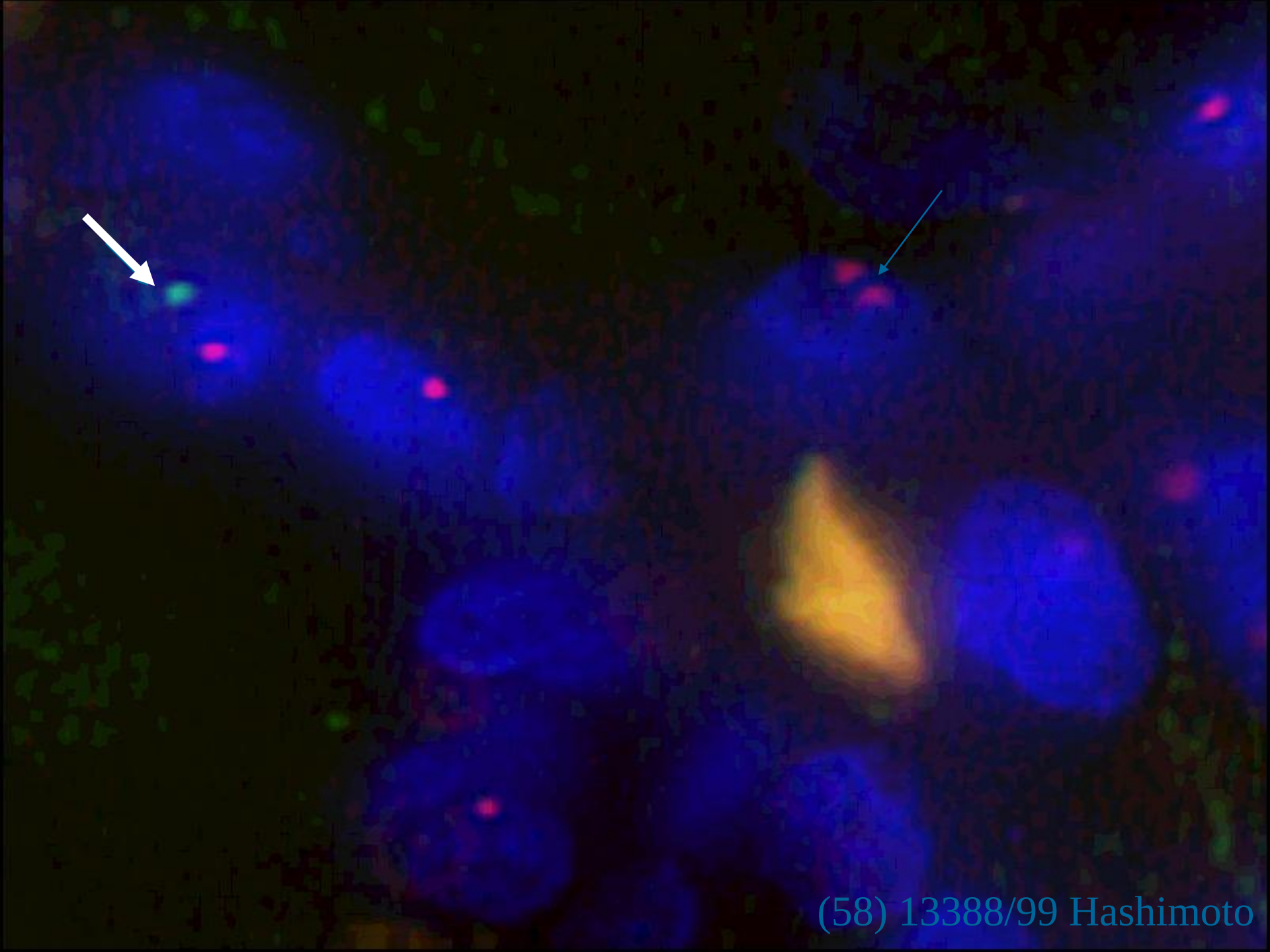
## MARKERS TO THE PROBES:

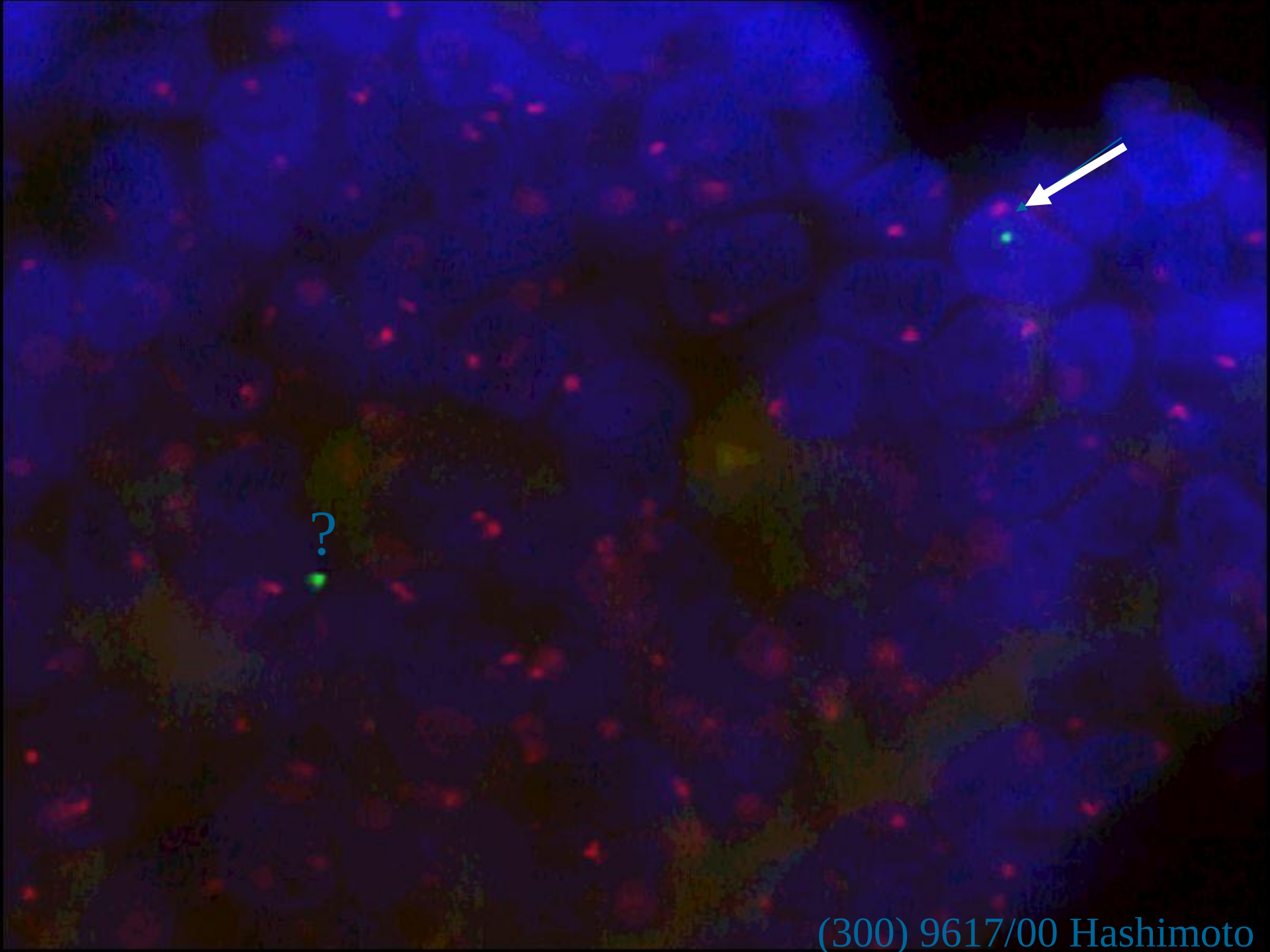
- RADIOACTIVE, EG. **$^{3}H$, $^{32}P$, $^{33}P$ i $^{35}S**
- NON-RADIOACTIVE, EG. **BIOTIN**
- BIOTINYLATED PROBES – **OFTEN USED**

- MANY USES OF **IMF** WERE OUTMODED BY THE DEVELOPMENT OF RECOMBINANT PROTEINS CONTAINING FLUORESCENT PROTEIN DOMAINS, E.G. **GREEN FLUORESCENT PROTEIN (GFP).**
- USE OF SUCH „TAGGED" PROTEINS ALLOWS MUCH BETTER LOCALIZATION AND LESS DISRUPTION OF PROTEIN FUNCTION

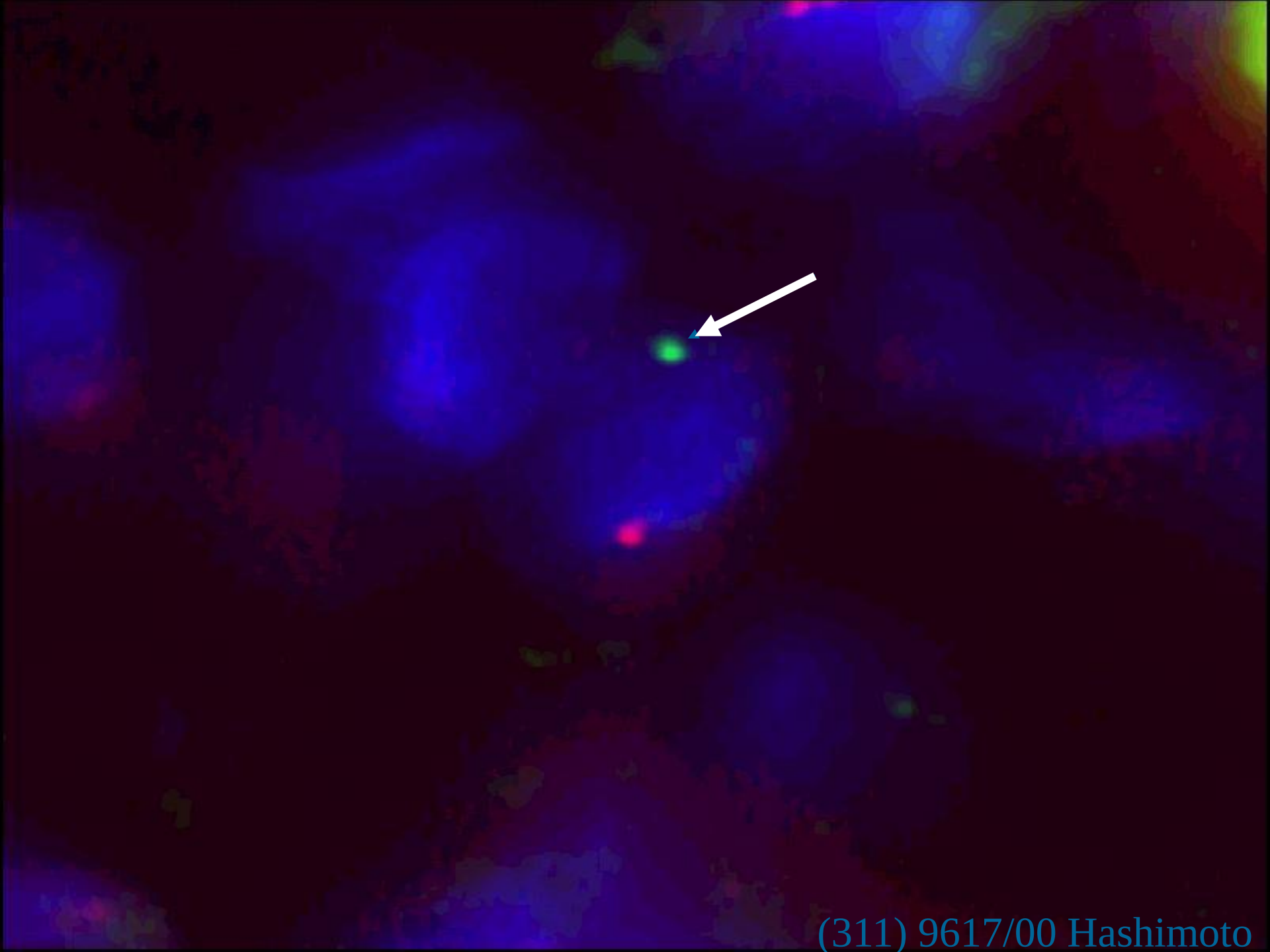
# EXAMPLES OF IMF

- In thyroid gland disorders
- May help in diagnosis of autoimmune diseases


(58) 13388/99 Hashimoto




(300) 9617/00 Hashimoto



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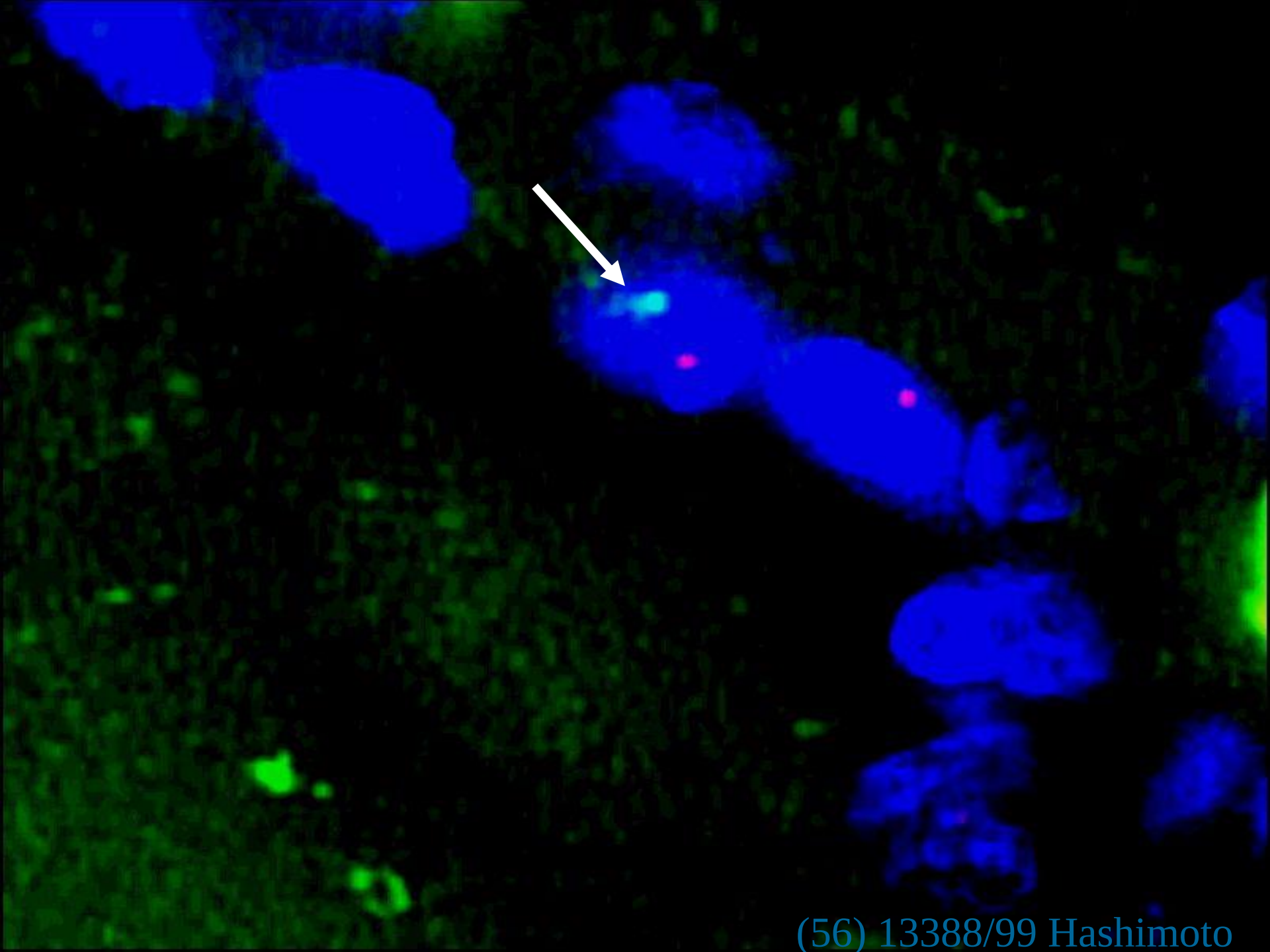

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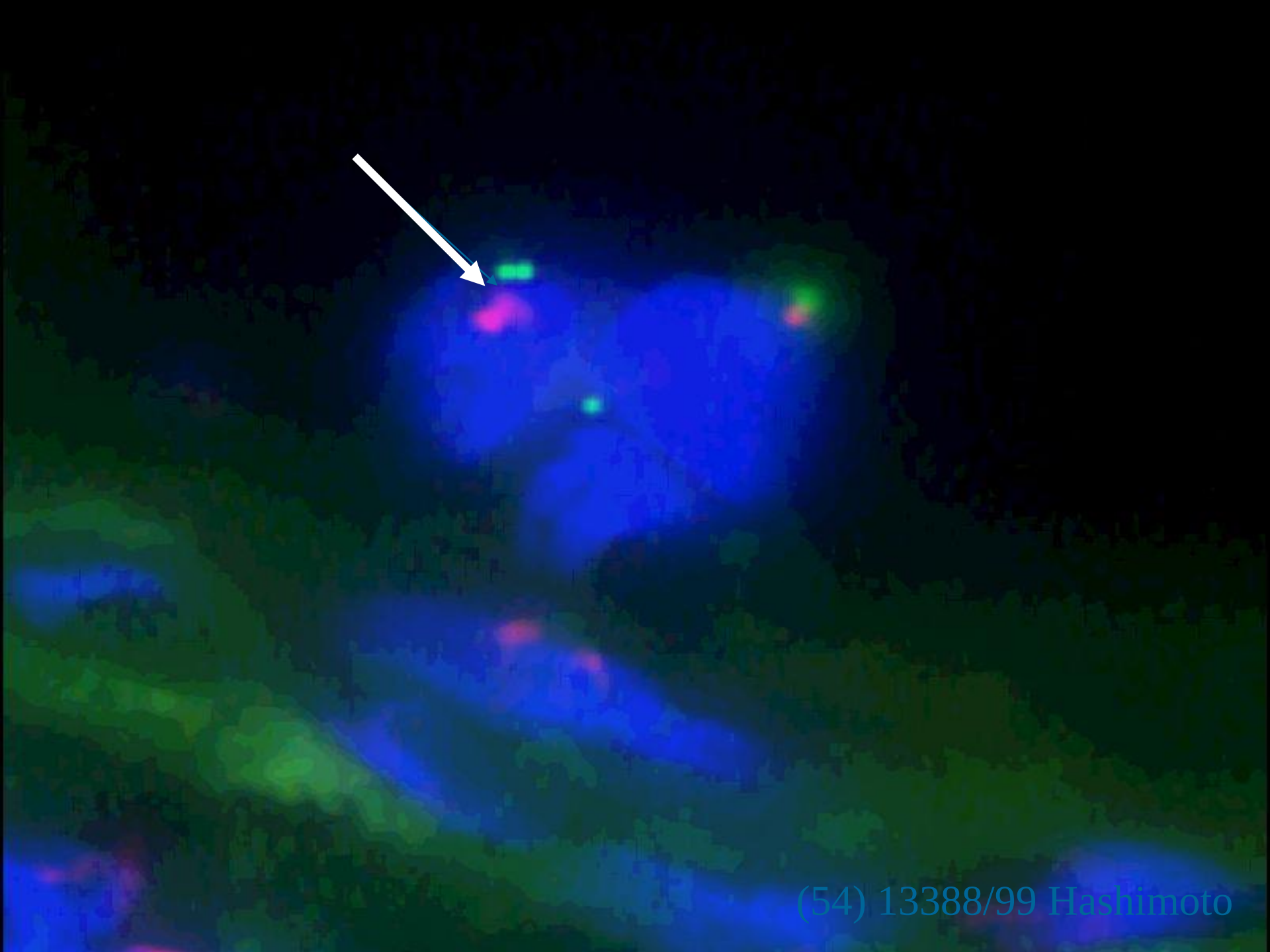
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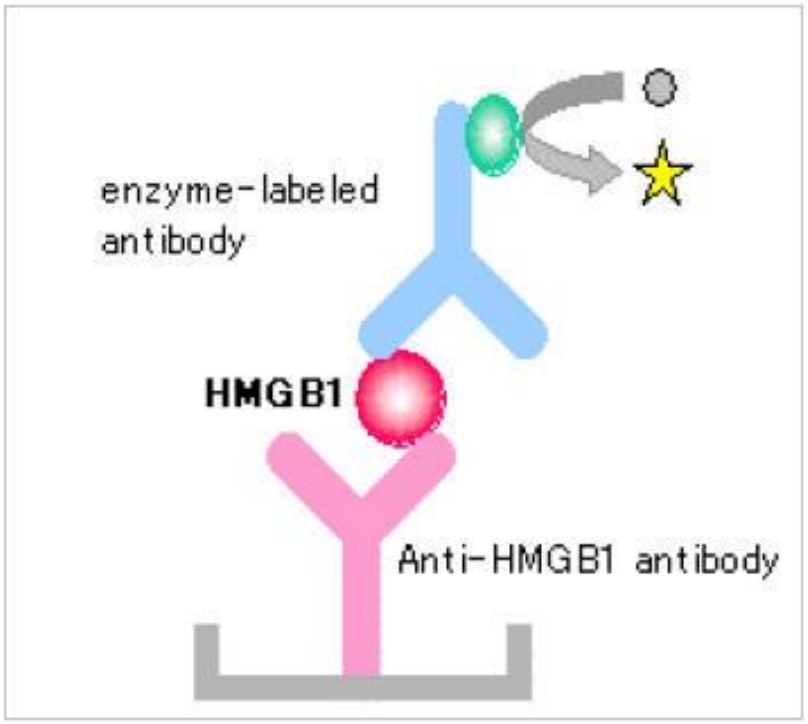
# METHOD OF IMMUNOENZYMATIC TEST (ELISA)

INVOLVES ENZYMES TO DETECT AND TO QUANTITATIVE DETERMINATION OF ANTIGENS AND ANTIBODIES

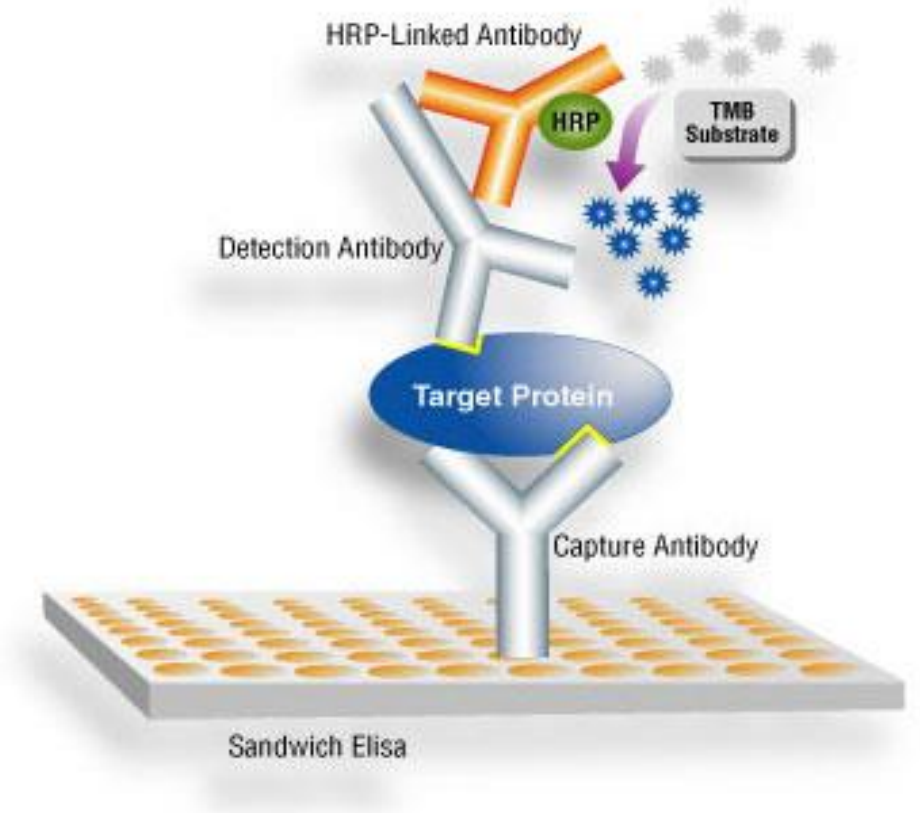
**SINCE 1966** – WIDELY USED

- **PERFORMING ELISA INVOLVES AT LEAST:**
- ONE ANTIBODY WITH SPECIFICITY FOR A PARTICULAR ANTIGEN.
- THE SAMPLE WITH UNKNOWN AMOUNT OF ANTIGEN IS IMMOBILIZED ON SOLID SUPPORT (POLYSTYRENE MICROTITER PLATE) EITHER NON-SPECIFICALLY (VIA ADSORPTION TO THE SURFACE) OR SPECIFICALLY (VIA CAPTURE BY ANOTHER ANTIBODY SPECIFIC TO THE SAME ANTIGEN, IN A „SANDWICH” ELISA).
- AFTER THE FINAL WASH THE PLATE IS DEVELOPED BY ADDING ENZYMATIC SUBSTRATE TO PRODUCE VISIBLE SIGNAL WHICH INDICATES THE QUANTITY OF ANTIGEN IN THE SAMPLE.

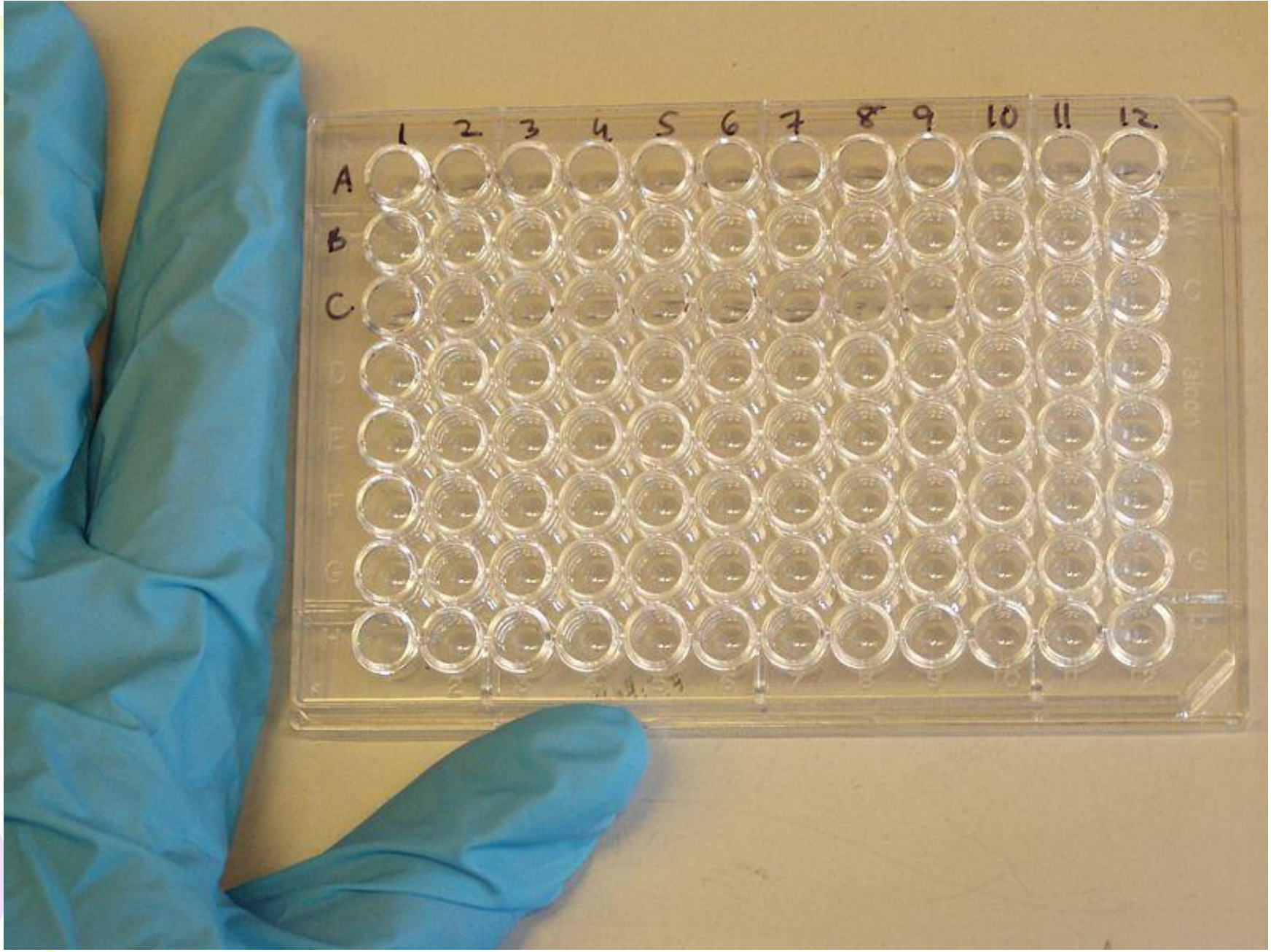
# 2 ANTIBODIES vs 3 ANTIBODIES sandwich




*The HMGB1 ELISA Kit is a 2-step sandwich ELISA.*




# SCHEMES OF ELISA



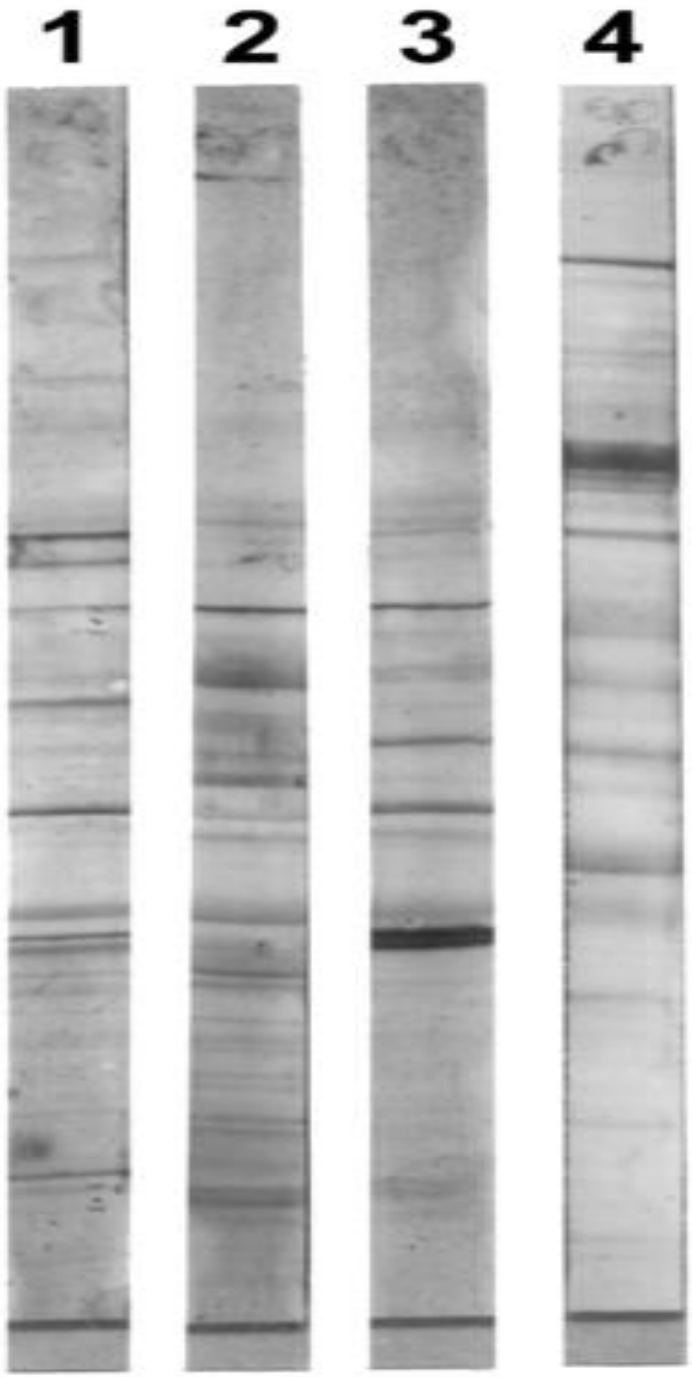

# ELISA PLATE

- **WESTERN BLOT** (ALTERNATIVELY, PROTEIN IMMUNOBLOT)

- ANALYTICAL TECHNIQUE USED **TO DETECT SPECIFIC PROTEINS IN A GIVEN SAMPLE** OF TISSUE HOMOGENATE OR EXTRACT. IT USES GEL ELECTROPHORESIS TO SEPARATE NATIVE OR DENATURED PROTEINS BY THE LENGTH OF POLYPEPTIDE (DENATURING CONDITIONS) OR BY THE 3-D STRUCTURE OF THE PROTEIN (NATIVE/NON-DENATURING CONDITIONS).

- PROTEINS ARE THEN TRANSFERRED TO A MEMBRANE (TYPICALLY NITROCELLULOSE OR PVDF, POLY(VINYLIDENE FLUORIDE), WHERE THEY ARE DETECTED (PROBED) USING ANTIBODIES SPECIFIC TO THE TARGET PROTEIN

- THE METHOD ORIGINATED FROM THE LABORATORY OF **GEORGE STARK** AT STANFORD. THE NAME **WESTERN BLOT** WAS GIVEN TO TECHNIQUE BY W.NEAL BURNETTE AND IS A PLAY ON THE NAME **SOUTHERN BLOT**, A TECHNIQUE FOR DNA DETECTION DEVELOPED EARLIER BY EDWIN SOUTHERN. DETECTION OF RNA IS TERMED **NORTHERN BLOTTING** AND DETECTION OF POST-TRANSLATIONAL MODIFICATION OF PROTEIN IS TERMED **EASTERN BLOTTING**




# DETECTION OF PROTEINS BY WESTERN BLOT

## ELECTRON MICROSCOPY

- KNOWN FROM 1931 !!!
- MAJOR REMARK:
- USELESS IN ROUTINE DIAGNOSTICS; MORE USEFUL IN RESEARCH

## ADVANTAGES:

- BETTER OPTICAL PARTITION

## DRAWBACKS:

- TIME CONSUMING
- HIGHLY EXPENSIVE
- LOW APPLICATION IN ROUTINE DIAGNOSTICS
- BRIEFLY: IT IS NOT ECONOMIC

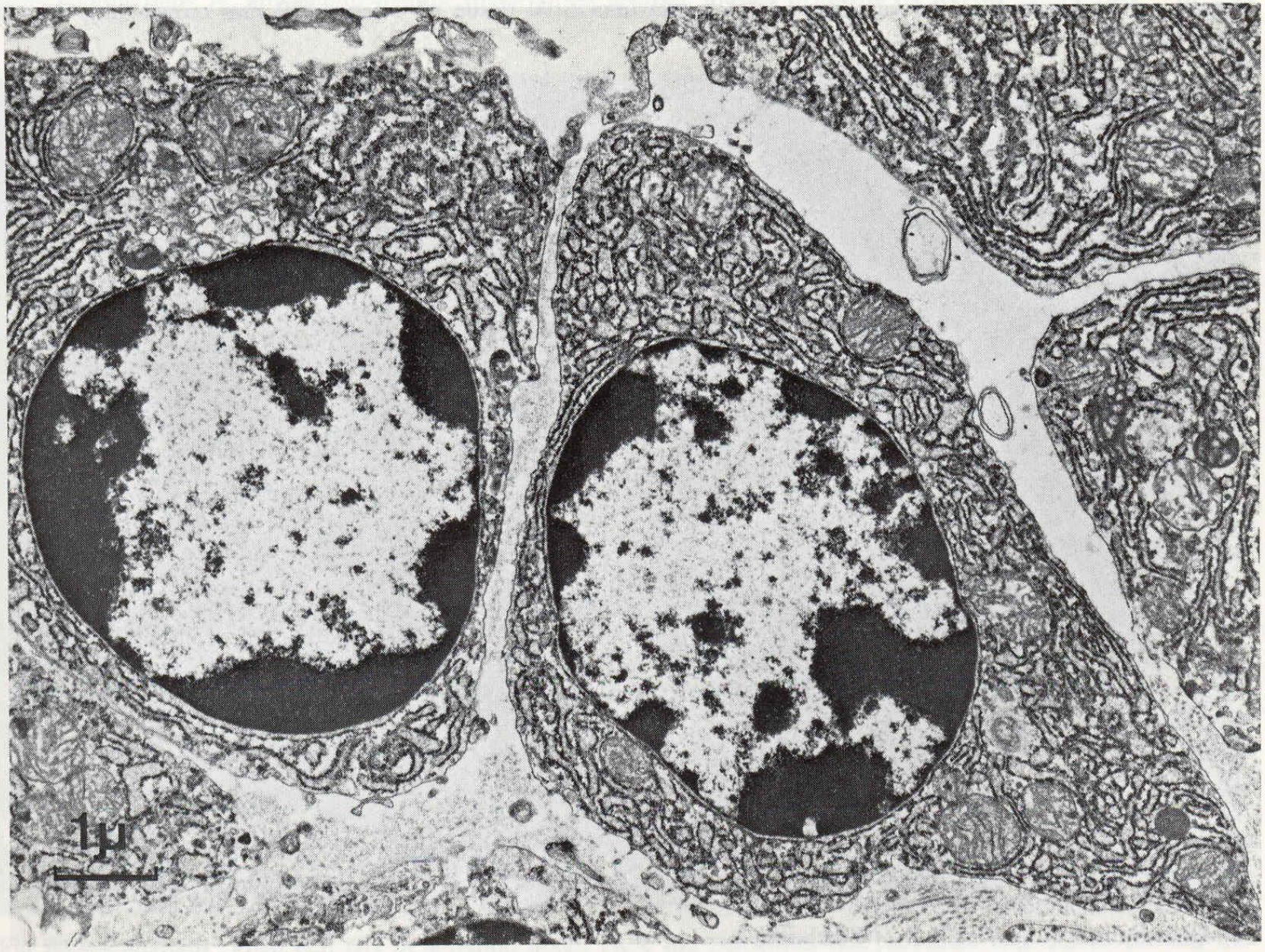


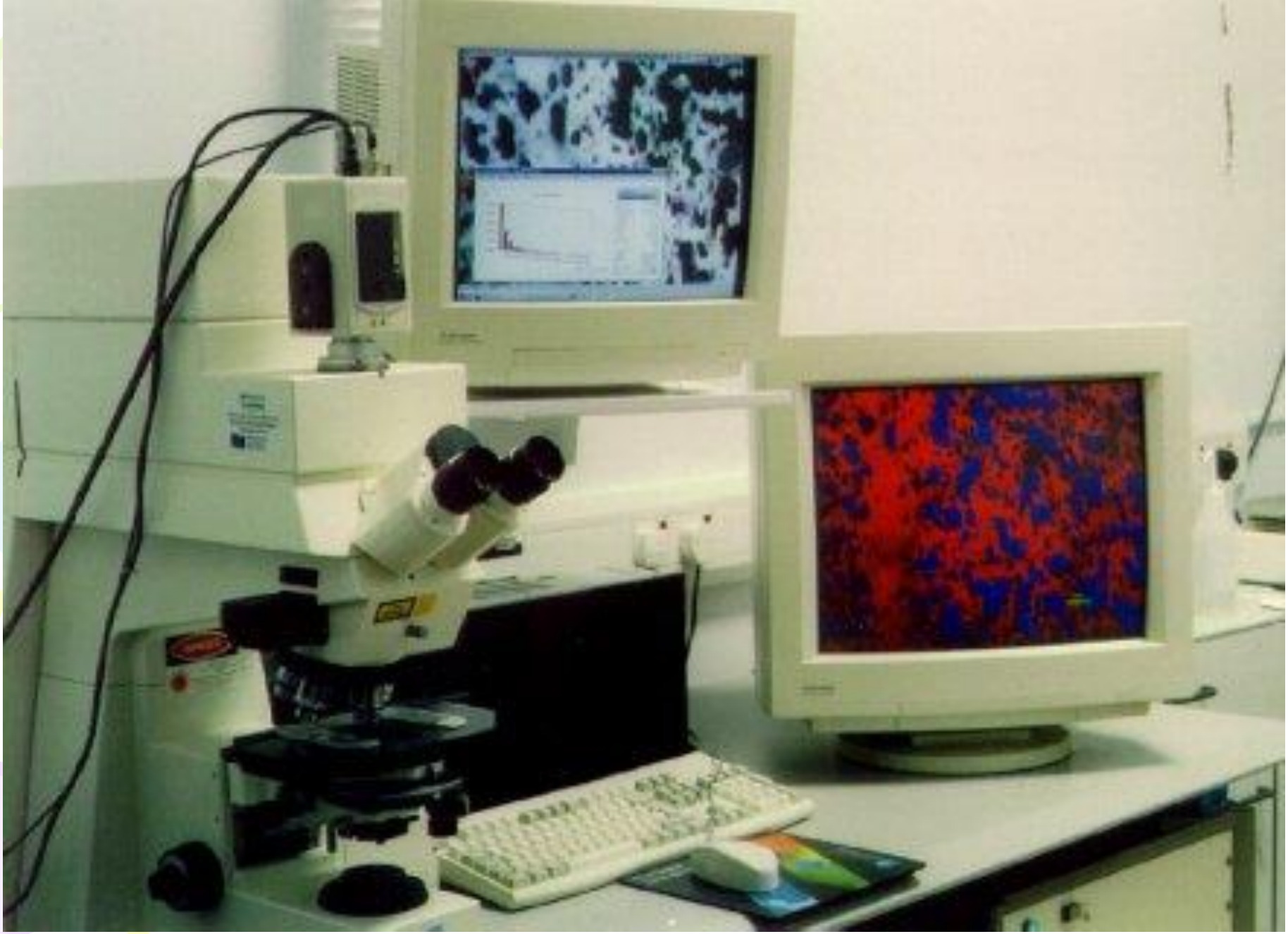

**Fig 29.**—Plasma cells from a bronchogenic carcinoma. Note structure of the nucleus as well as the distended rough endoplasmic reticulum. (Magnification about 10,500×.) (Kistler)

- **CONFOCAL MICROSCOPY IS AN OPTICAL IMAGING TECHNIQUE USED TO INCREASE OPTICAL RESOLUTION AND CONTRAST OF A MICROGRAPH BY USING A SPATIAL PINHOLE TO ELIMINATE OUT-OF-FOCUS LIGHT IN SPECIMENS THAT ARE THICKER THAN THE FOCAL PLANE.**
- **IT ENABLES THE RECONSTRUCTION OF THREE-DIMENSIONAL STRUCTURES FROM THE OBTAINED IMAGES. THIS TECHNIQUE GAINED POPULARITY IN LIFE SCIENCES (AND MEDICINE), SEMICONDUCTOR INSPECTION AND MATERIAL SCIENCE.**

## CONFOCAL MICROSCOPY

- **AIM:** IMPROVEMENT IN THE QUALITY OF IMAGING
- **BASIC DEVICE:** CONFOCAL SHUTTER MADE OF MATERIAL THAT DOES NOT ALLOW LIGHT TO PASS THROUGH, SUPPLIED WITH A SMALL PINHOLE IN THE CENTRE

- **LIGHT SOURCE IS A LASER** (THAT IS WHY WE CALL THIS - Laser Scanning Microscope – **LSM or Confocal LSM, CLSM)**



# CONFOCAL LASER SCANNING MICROSCOPE

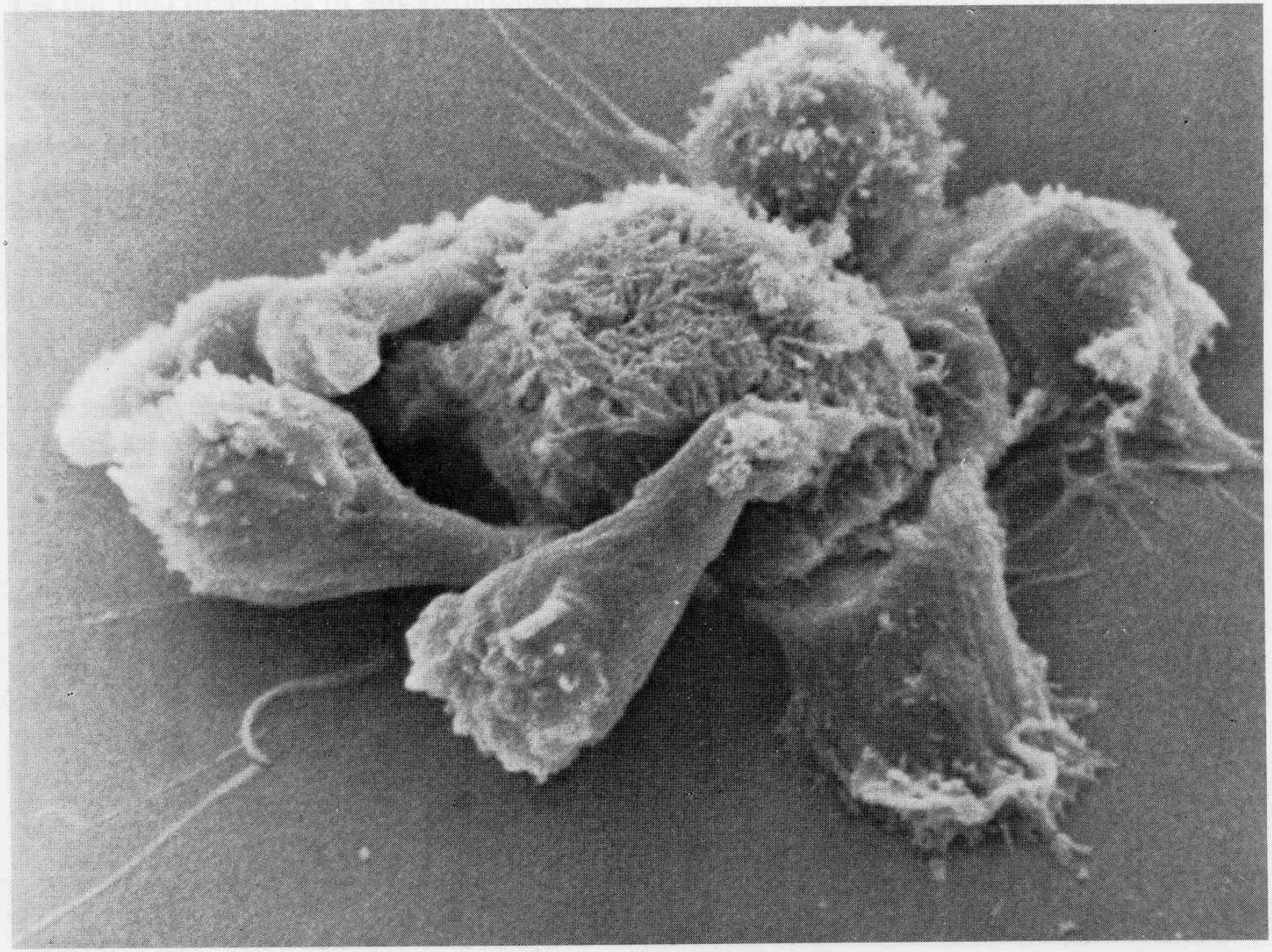
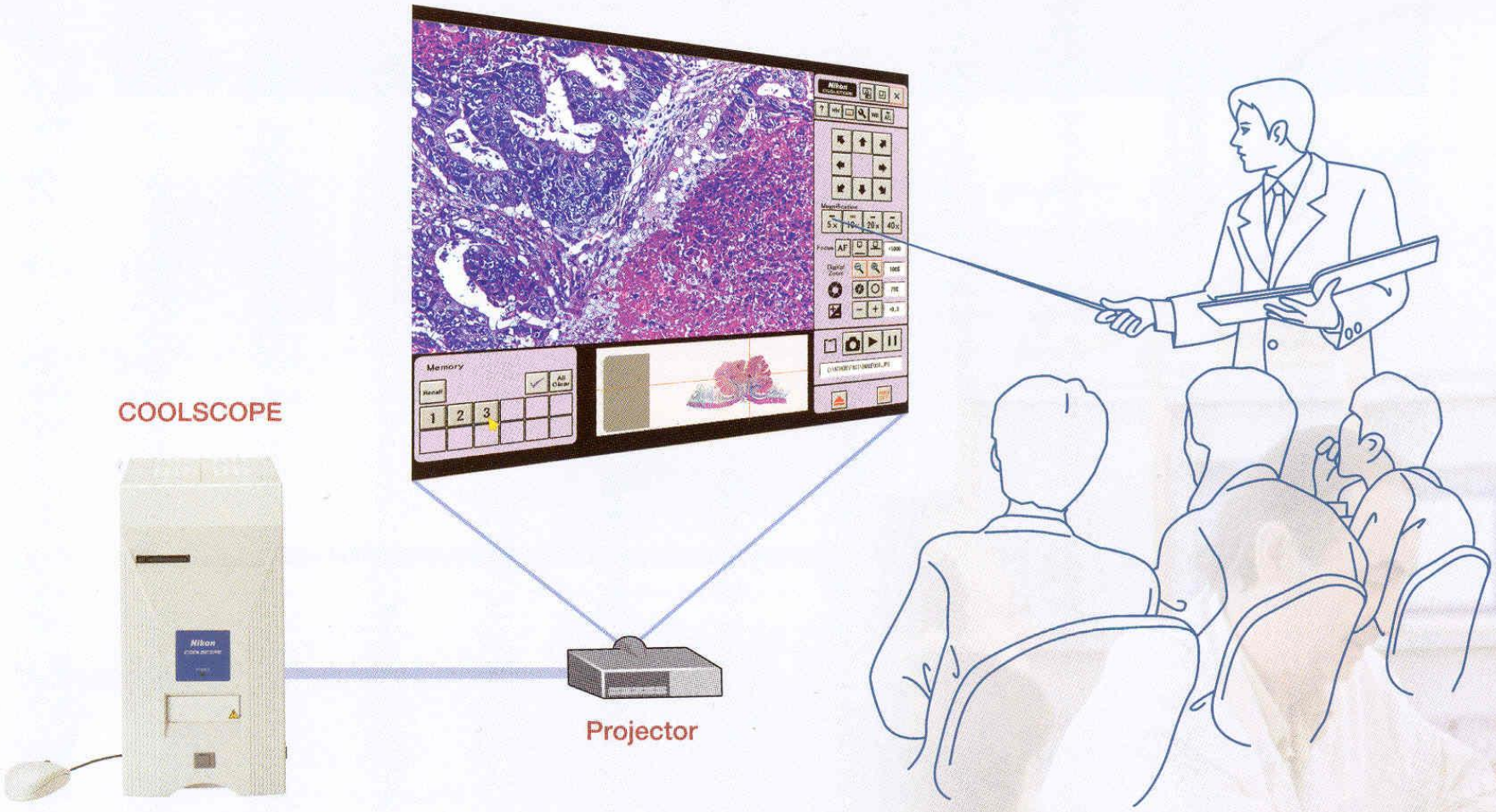


**Fig 38.**—Cellular aggression against a rounded-off cancer cell (HeLa cell) by cativated killer lymphocytes (scanning electron micrograph; 5,500×). (Paweletz, Cancer Center, Heidelberg)

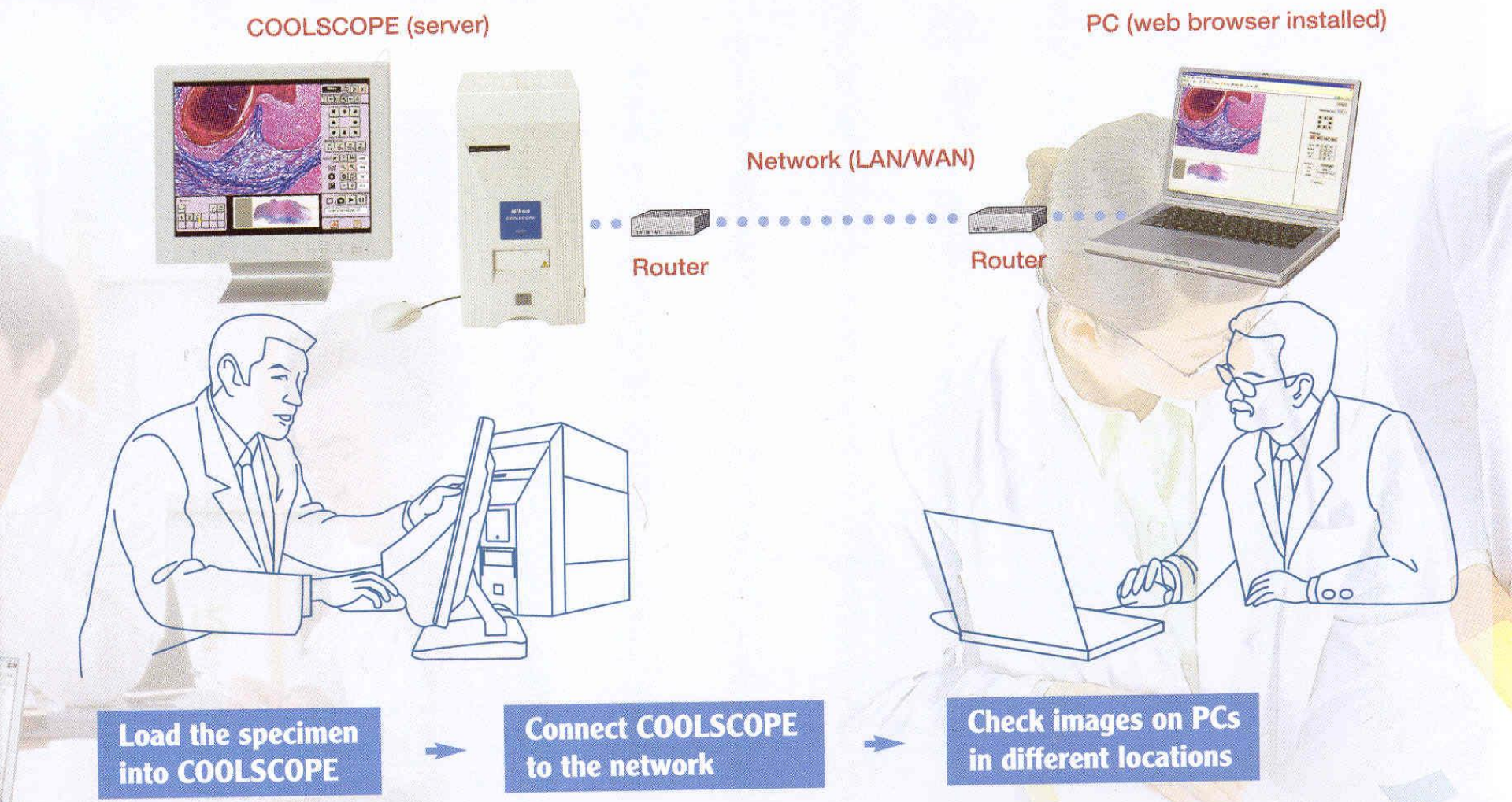
# Projection and conferencing at scientific congresses and study groups




**Connect the projector** → **Load the specimen into COOLSCOPE** → **Project the images for all to see**

The time and trouble of adjusting the microscope and manipulating various settings are dramatically eliminated. Changing the specimen is also fast and easy.

# Consultation via the network



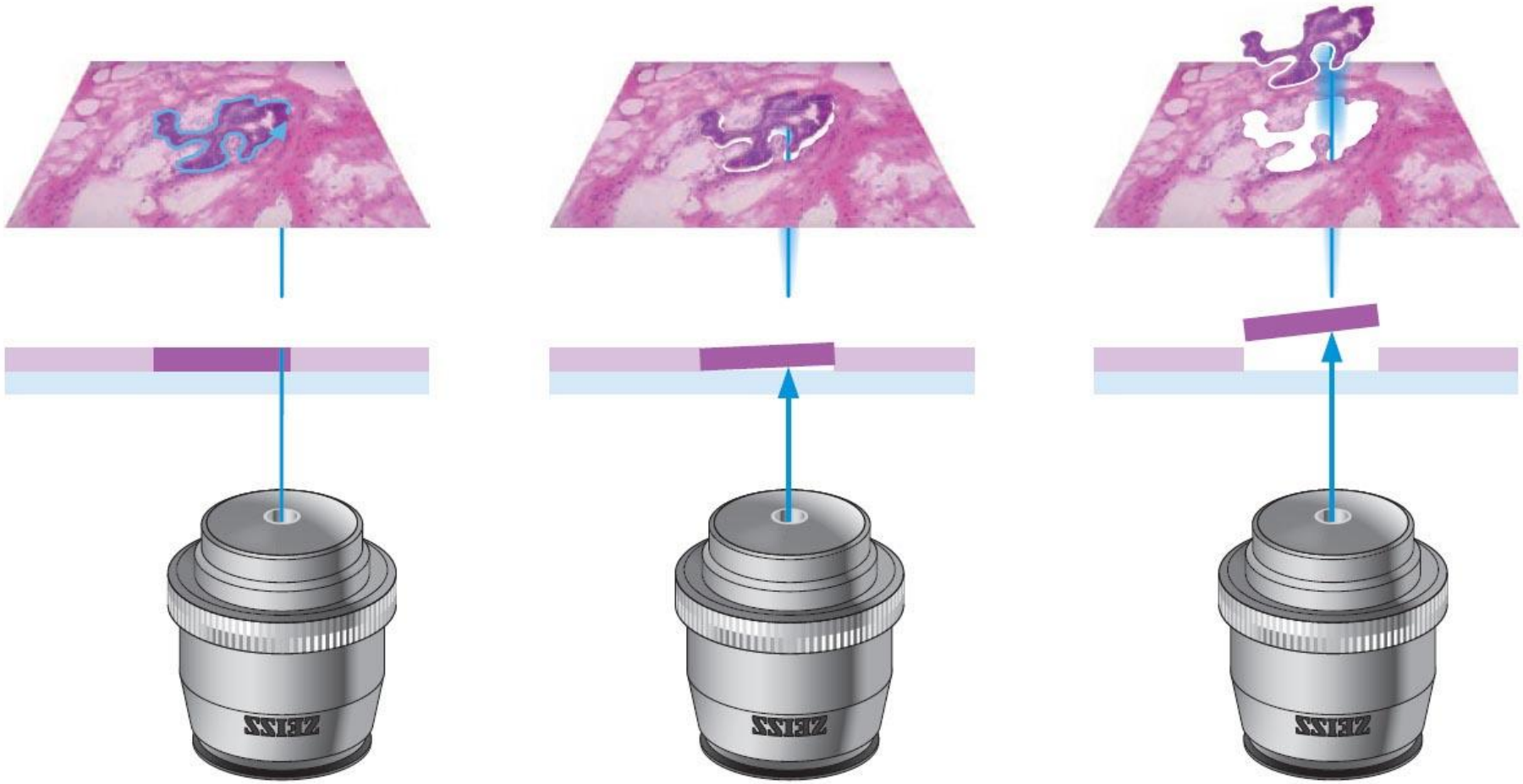

**Load the specimen into COOLSCOPE** → **Connect COOLSCOPE to the network** → **Check images on PCs in different locations**

The same pathology specimen can be viewed on networked PCs in different locations, enabling consultation within a hospital or beyond it. Control of the image, e.g. changing of the magnification, is possible from remotely networked PCs.

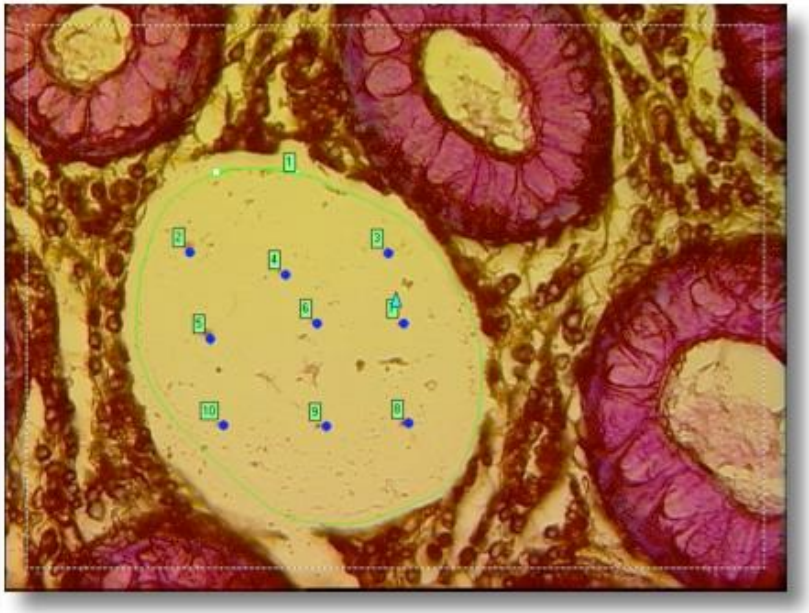
# LASER MICRODISSECTION (LM)

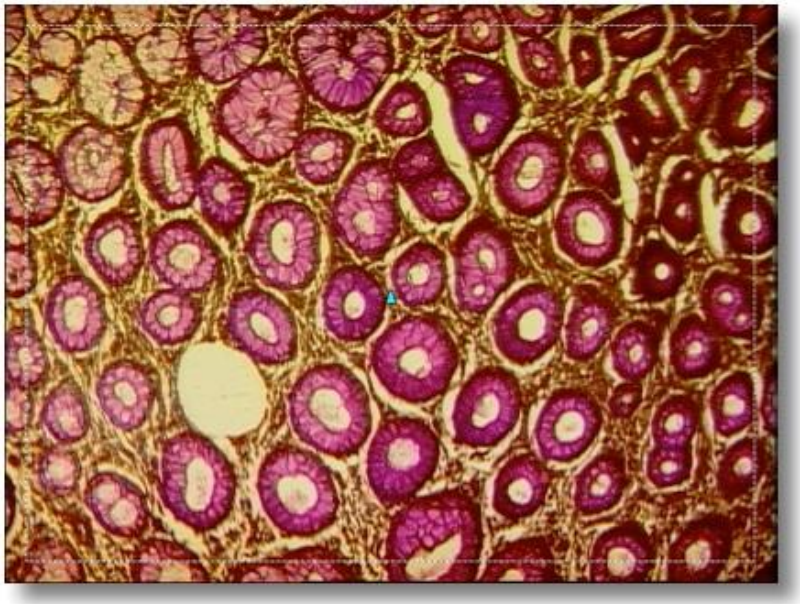
DEFINITION: ISOLATION OF CHOSEN FRAGMENTS OF TISSUES OR CELLS OR ORGANELLES. ALLOWS TO TARGET OBJECTS 1 MICROMETER IN SIZE WITHOUT A RISK OF CONTAMINATION

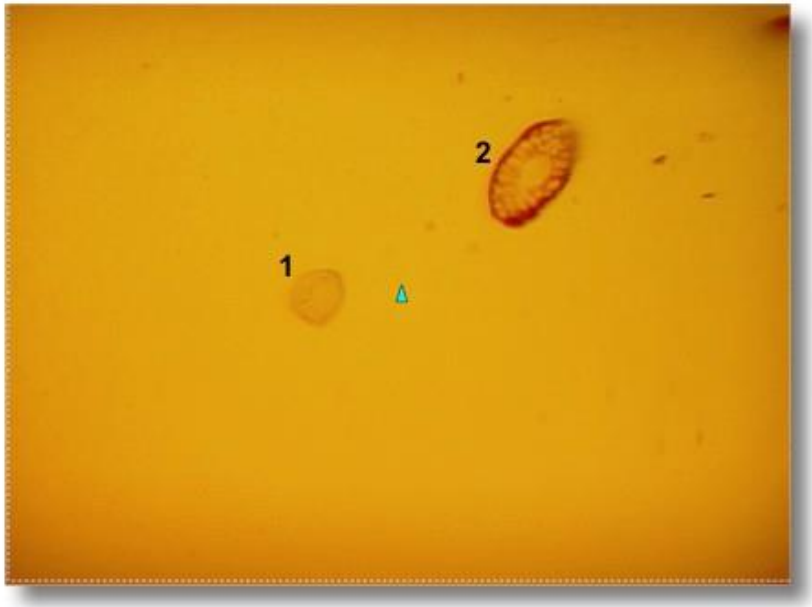
- **LM – „COOL EVAPORATION" THAT PROTECTS CELLS FROM DAMAGE (NO DAMAGE OF DNA/RNA/PROTEIN)**




# LM








LM

- **LASER MICRODISSECTION**

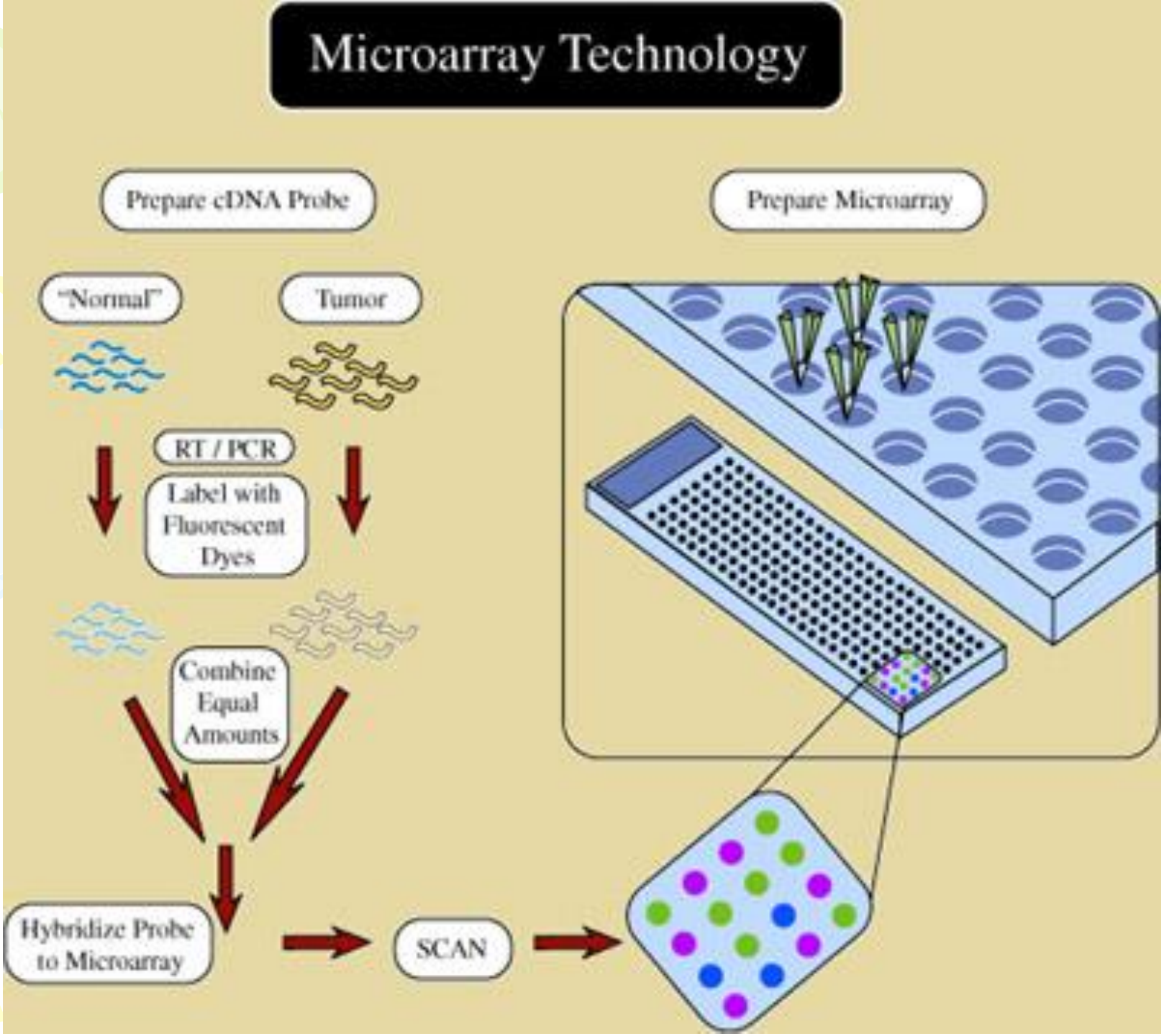
PROCESS DOES NOT ALTER OR DAMAGE THE MORPHOLOGY AND CHEMISTRY OF THE SAMPLE COLLECTED NOR SURROUNDING CELLS.

- LM IS A USEFUL METHOD OF COLLECTING SELECTED CELLS FOR DNA, RNA AND PROTEIN ANALYSIS.
- LM CAN BE PERFORMED ON A VARIETY OF TISSUE SAMPLES INCLUDING BLOOD SMEARS, CYTOLOGIC PREPARATIONS, CELL CULTURES AND ALIQUOTS OF SOLID TISSUE.
- FROZEN AND PARAFFIN EMBEDDED ARCHIVAL TISSUE MAY ALSO BE USED.

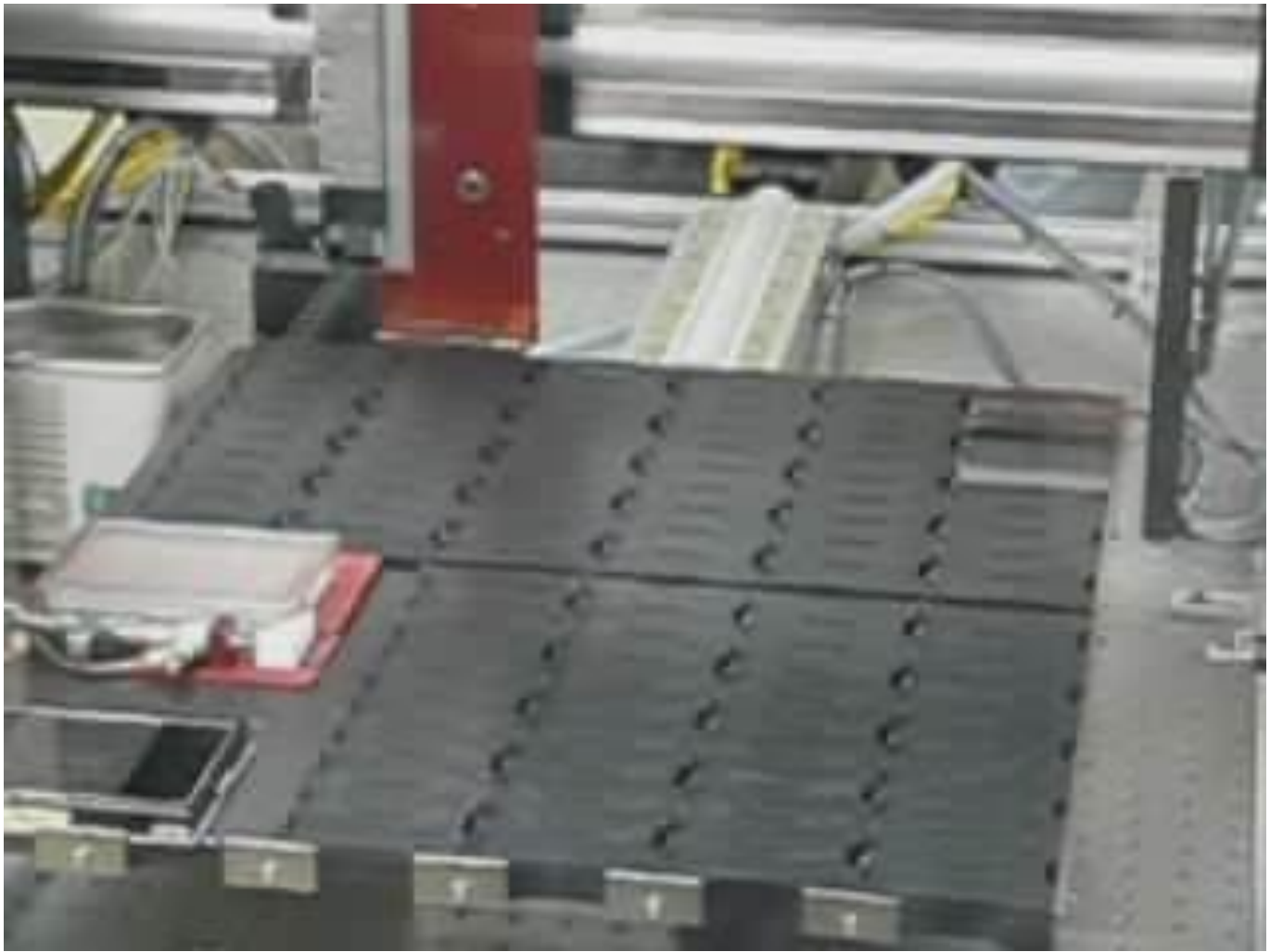
- **A METHOD CALLED**
- **DNA MICROARRAYS CAN BE USED TO**
- MEASURE CHANGES IN GENE EXPRESSION LEVELS,
- DETECT SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS),
- GENOTYPE OR RESEQUENCE MUTANT GENOMES.

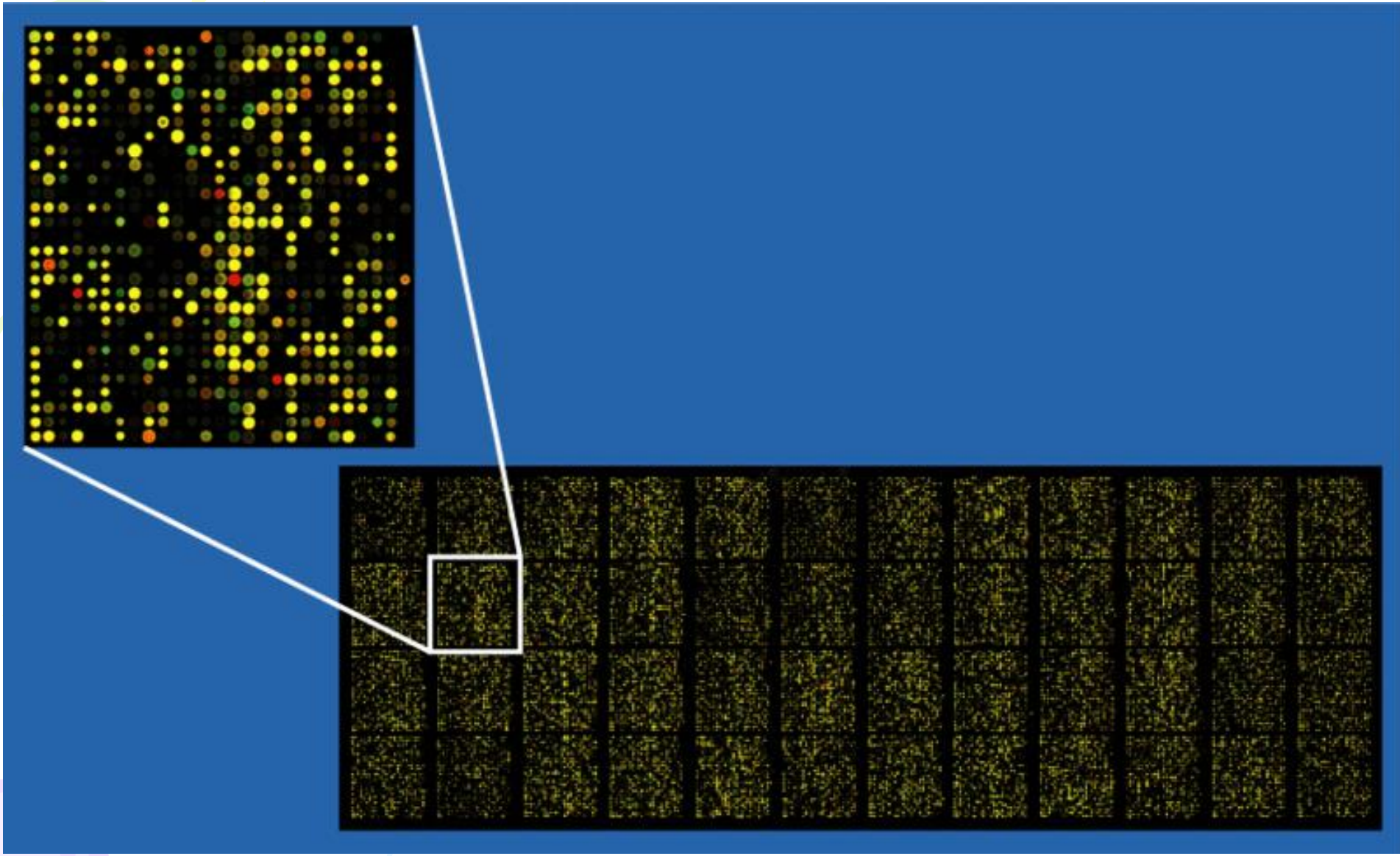
- IT CONSISTS OF ARRAYED SERIES OF THOUSANDS OF MICROSCOPIC SPOTS OF DNA OLIGONUCLEOTIDES, CALLED FEATURES, EACH CONTAINING PICOMOLES OF A SPECIFIC DNA SEQUENCE KNOWN AS PROBES (OR REPORTERS).

- SINCE AN ARRAY CAN CONTAIN TENS OF THOUSANDS OF PROBES, A MICROARRAY EXPERIMENT CAN ACCOMPLISH MANY GENETIC TESTS IN PARALLEL. THEREFORE ARRAYS HAVE DRAMATICALLY ACCELERATED MANY TYPES OF INVESTIGATION.


Microarray Technology
Prepare cDNA Probe
Prepare Microarray
"Normal"
Tumor
RT / PCR
Label with Fluorescent Dyes
Combine Equal Amounts
Hybridize Probe to Microarray
SCAN

- A DNA MICROARRAY BEING PRINTED BY A ROBOT AT THE UNIVERSITY OF DELAWARE





# DNA MICROARRAY

- **ANTIGENS AND MARKERS USEFUL IN DIAGNOSIS OF EPITHELIAL TUMORS**
- **(EXAMPLES)**
- **CYTOKERATINS – EPITHELIAL TUMORS (adenocarcinoma, urothelial carcinoma, SCC etc.)**
- **EMA (epithelial membrane antigen)** – MEMBRANE ANTIGEN, PRESENT IN EPITHELIAL TUMORS AND IN PHEOCHROMOCYTOMA, SYNOVIAL SARCOMA, CHORDOMA
- **CEA – (carcino-embryonic antigen)** MARKER OF COLON, LIVER, LUNG, BREAST AND PANCREATIC CARCINOMA
- **a-fetoprotein** – MARKER OF LIVER CIRRHOSIS, HEPATITIS, AND LIVER CANCER
- **CA 15-3** – MONITORING OF BREAST CANCER AND ITS METASTASES
- **CA 125** - MONITORING OF OVARIAN CANCER
- **PSA** – PROGNOSIS AND DETECTION OF PROSTATIC CANCER
- **ACID PHOSPHATASE – AS ABOVE MENTIONED**
- OTHERS: **CD (**cluster of differentiation) IN MALIGNANT LYMPHOMA


THANK
YOU