Autoimmune diagnostics

Indirect immunofluorescence technics

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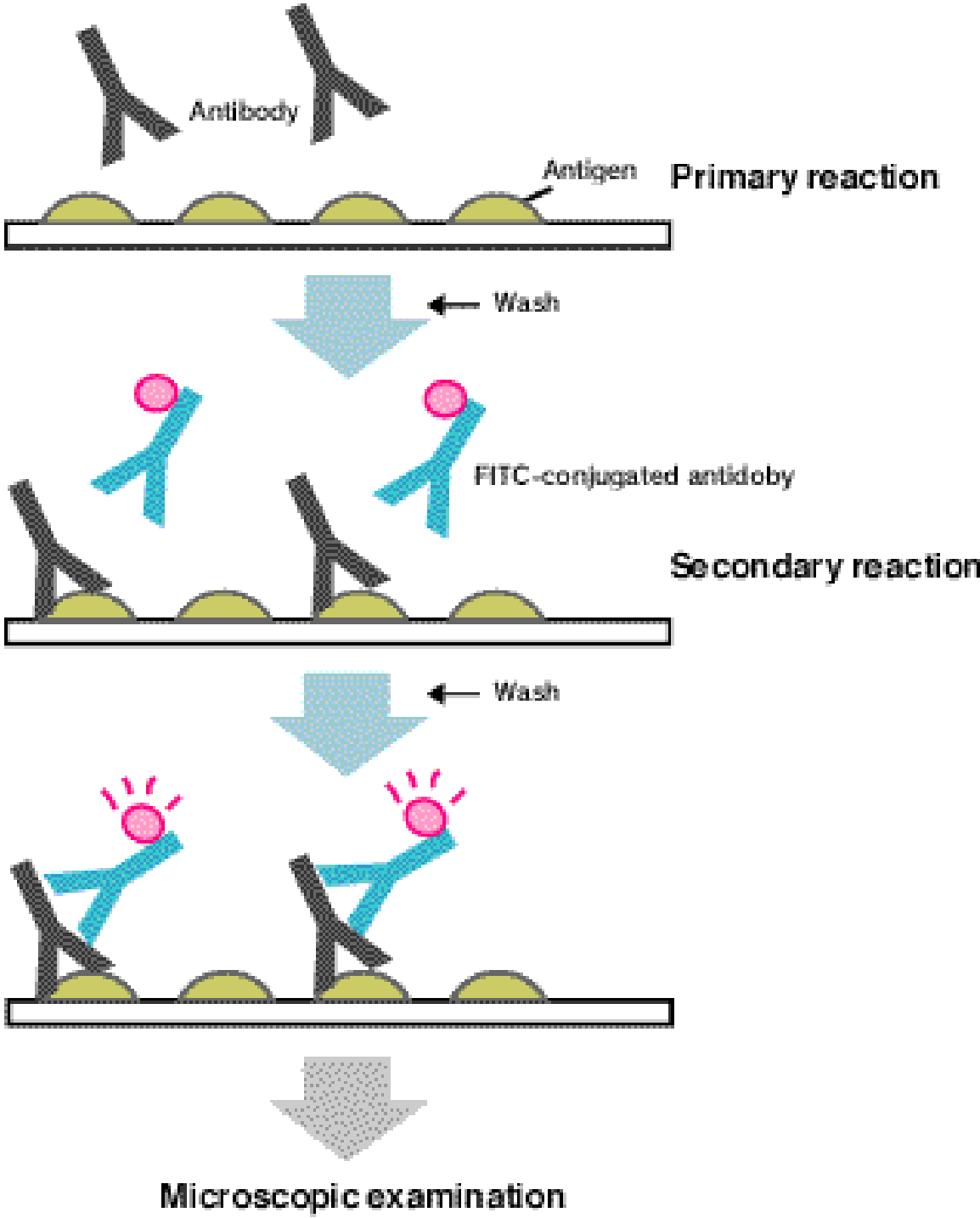
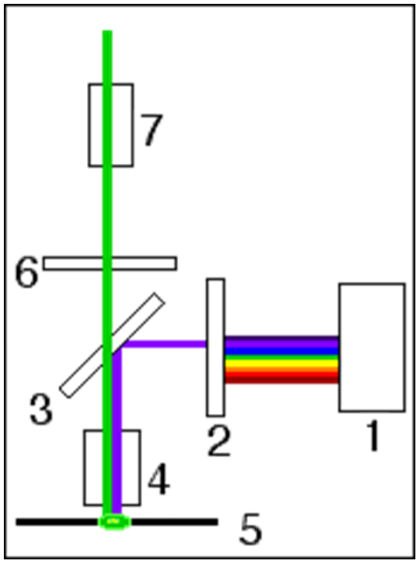
Institute of Laboratory Medicine

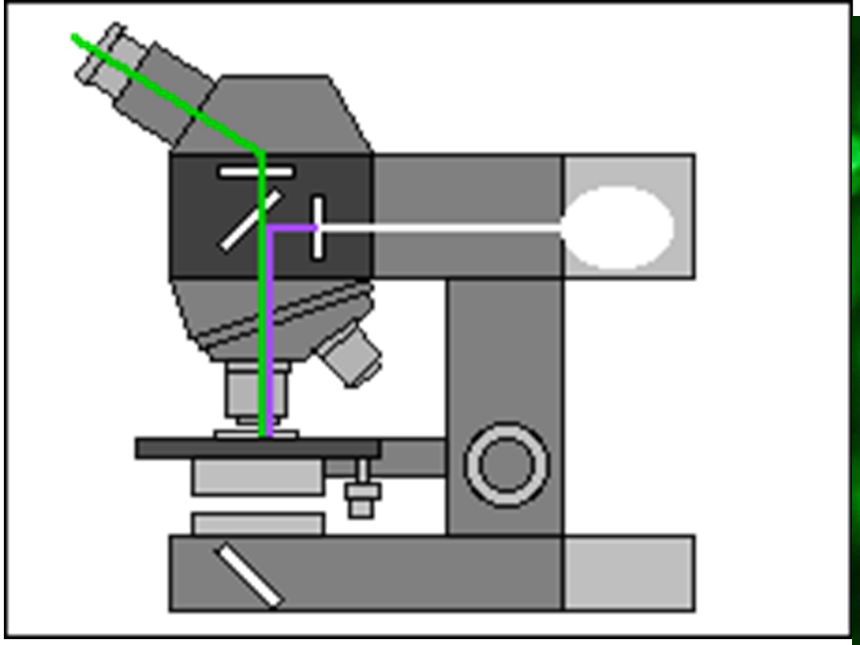
Indirect immuno-fluoreszence

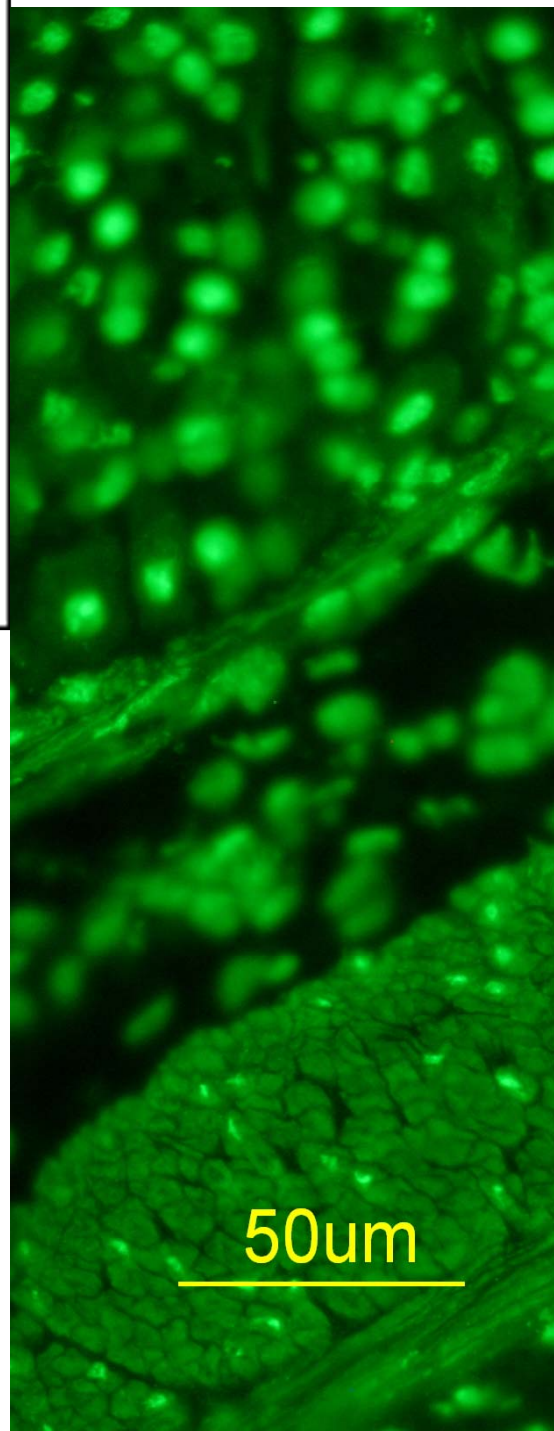
Substrate:

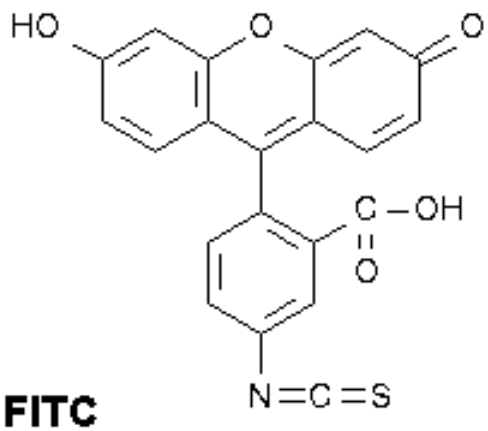
- Cells
- Tissue
(organ specific)

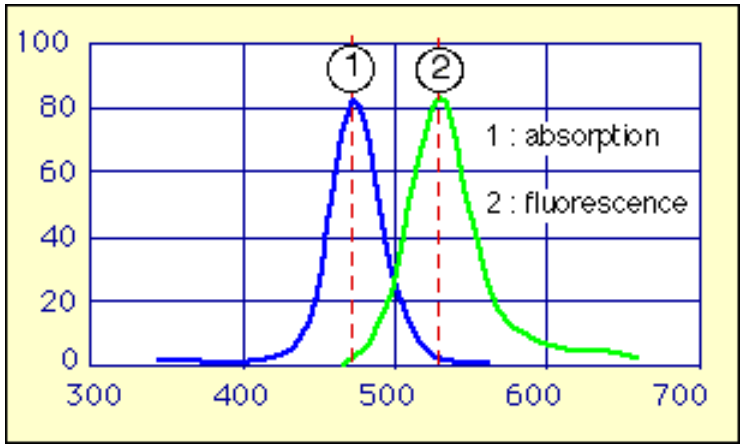



7
6
3
2
1
4
5




50um

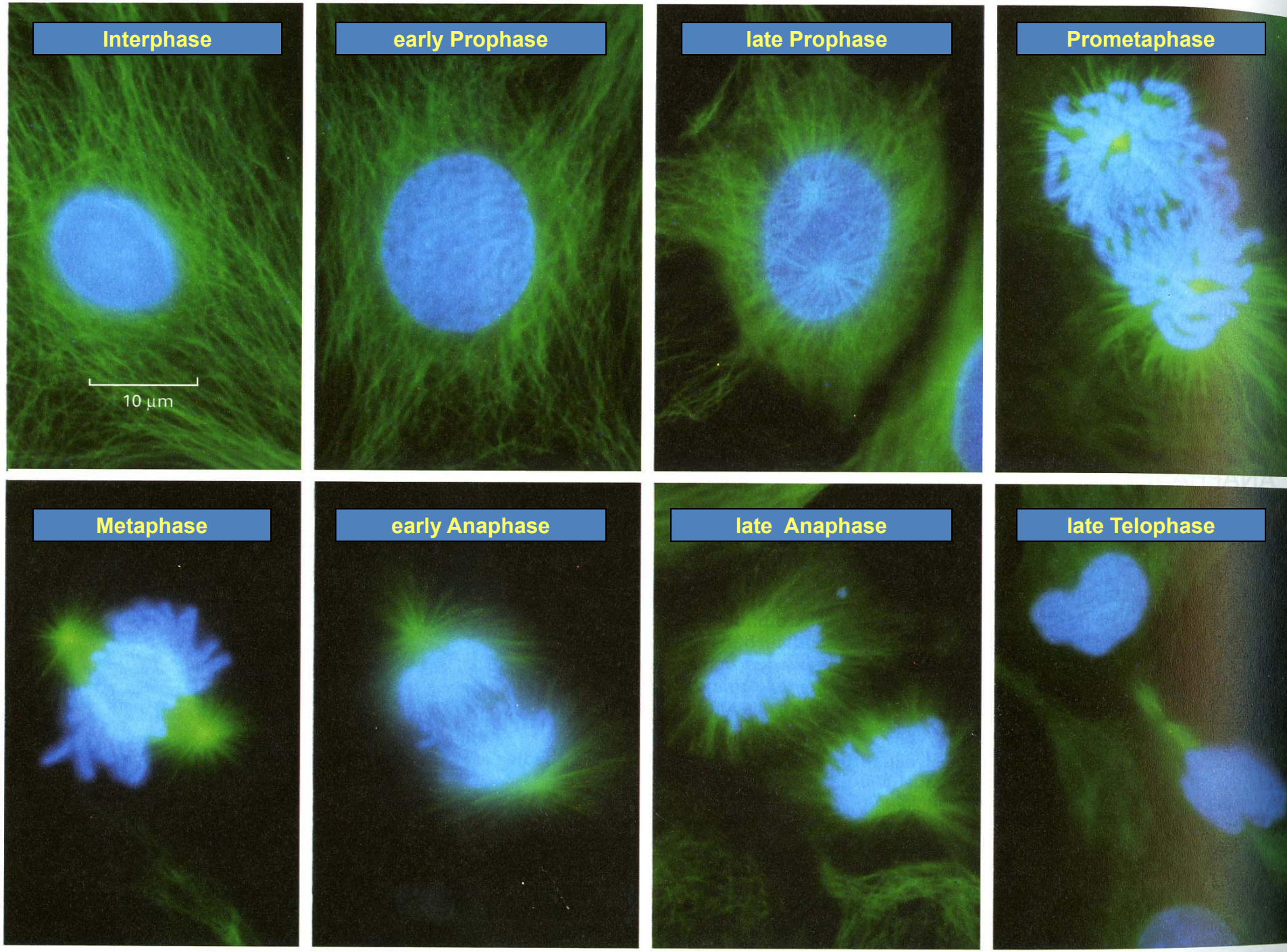

HO
O
O
C – OH
O
N=C=S
FITC

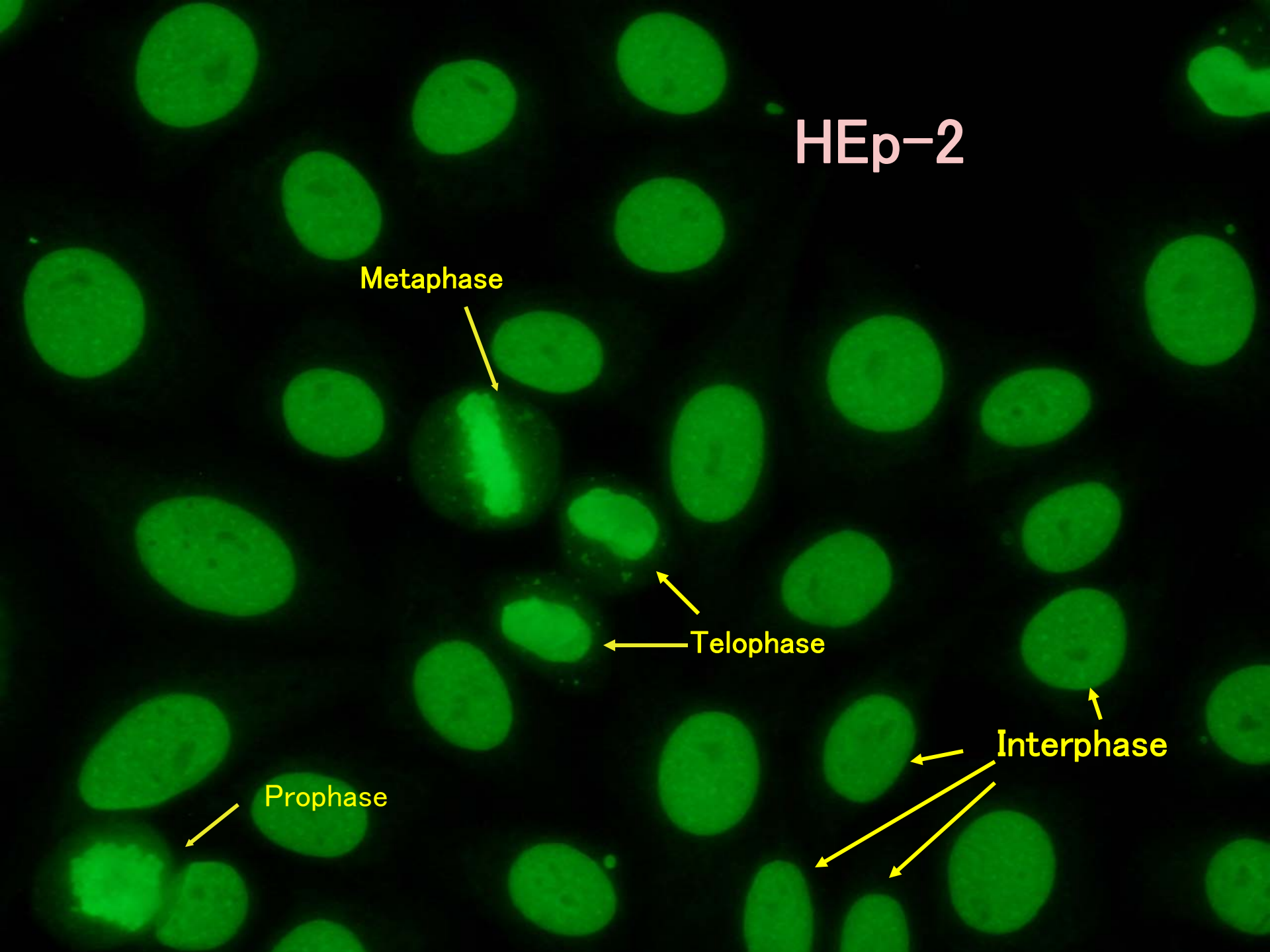

100
80
60
40
20
0
300
400
500
600
700
1
2
1 : absorption
2 : fluorescence

Antinuclear antigens ANA

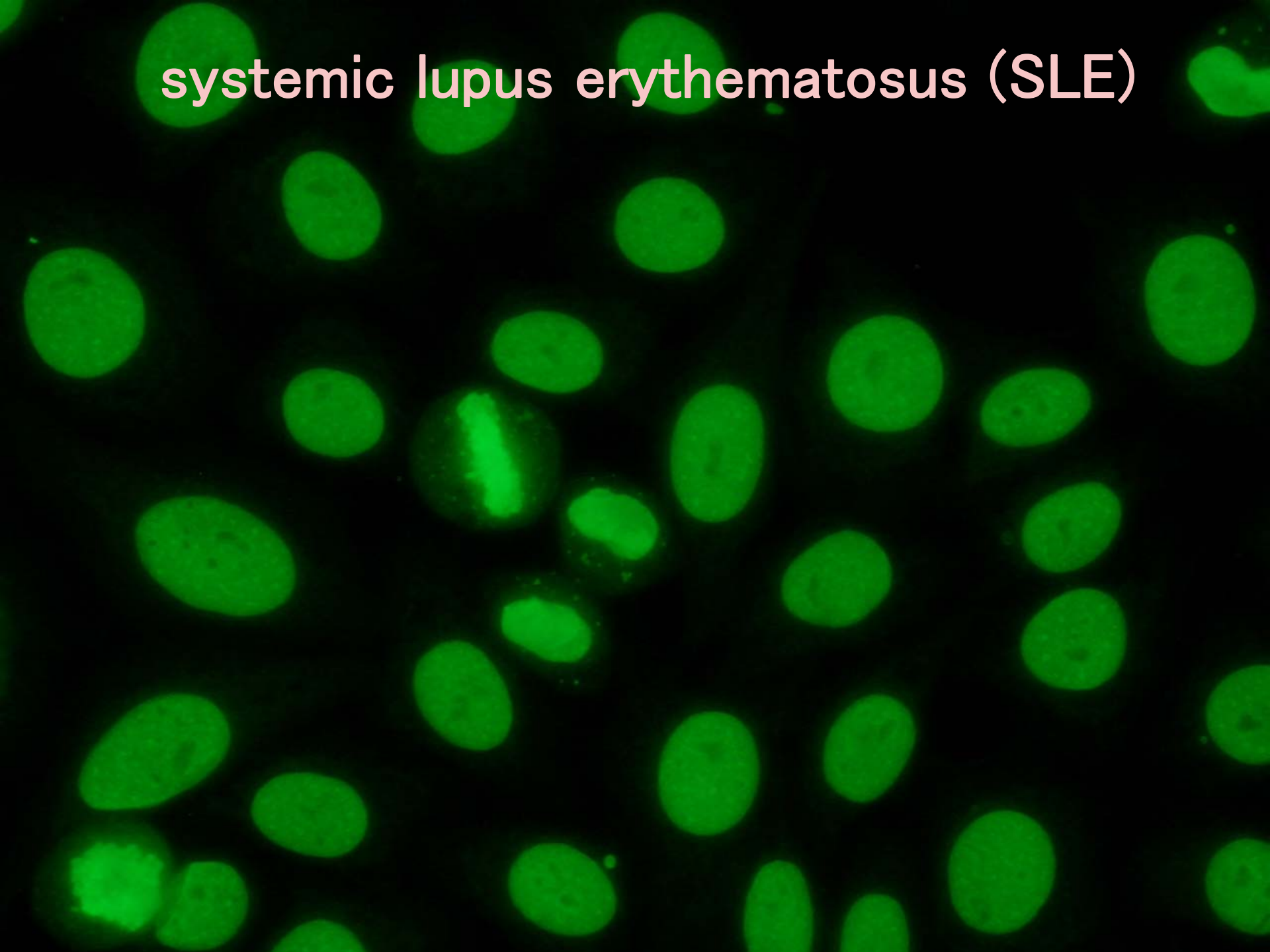
screening method

- pattern of interphase cells
- mitosis in metaphase
- *cytoplasmic staining (extra information)*

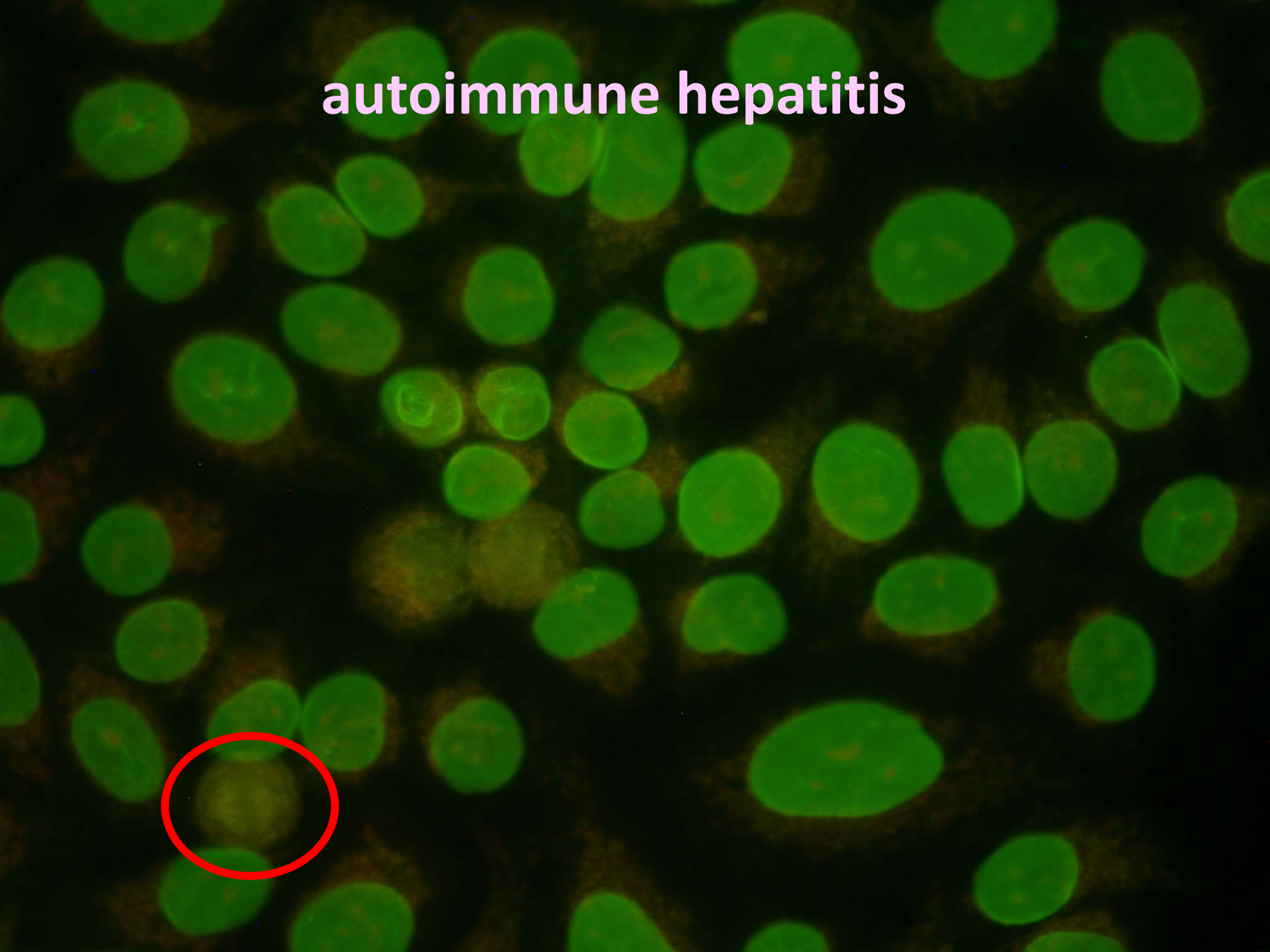

Interphase
10 µm
early Prophase
late Prophase
Prometaphase
Metaphase
early Anaphase
late Anaphase
late Telophase


HEp-2
Metaphase
Telophase
Interphase
Prophase

systemic lupus erythematosus (SLE)



autoimmune hepatitis



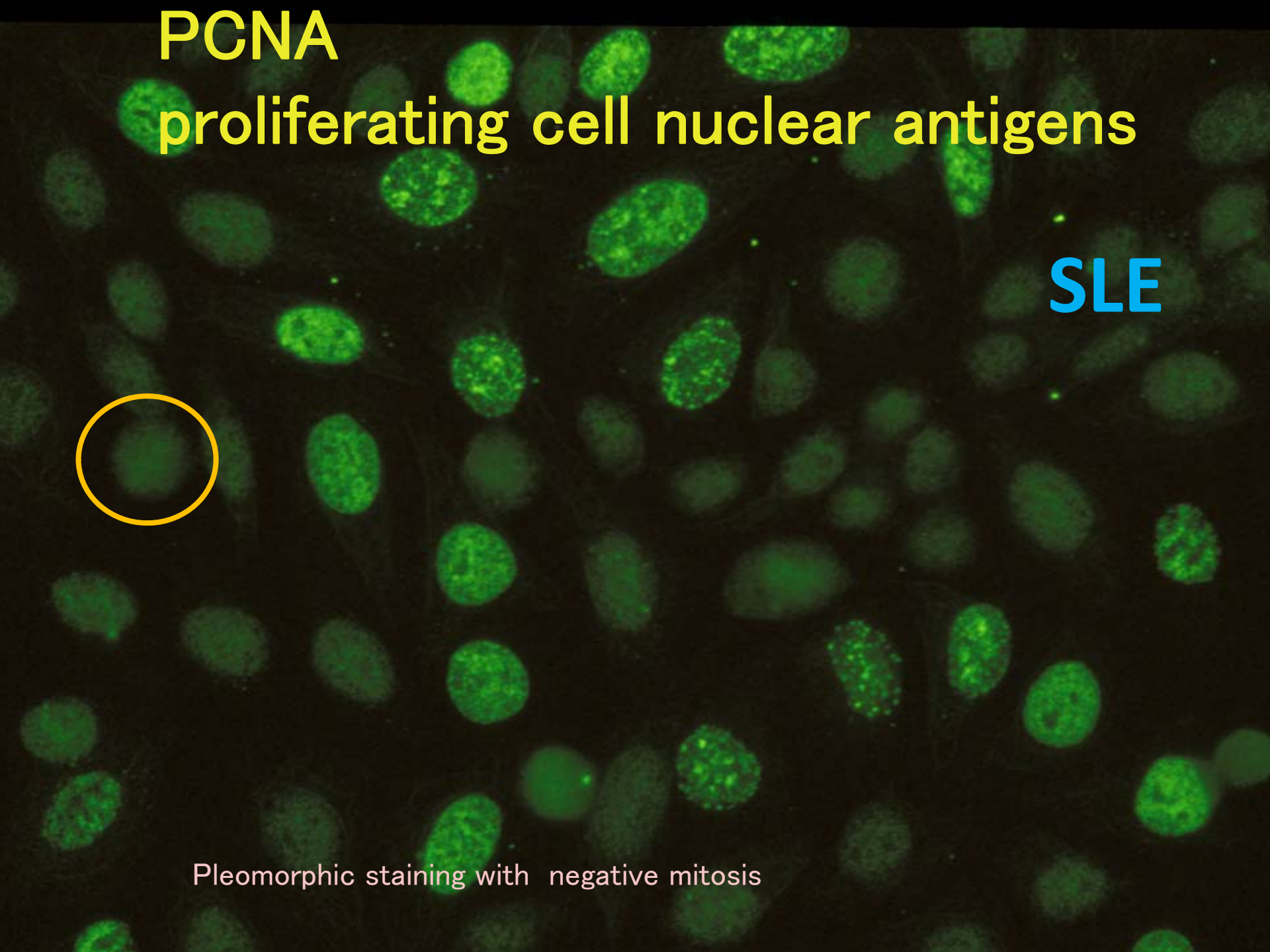
Why the mitotic cells are important?

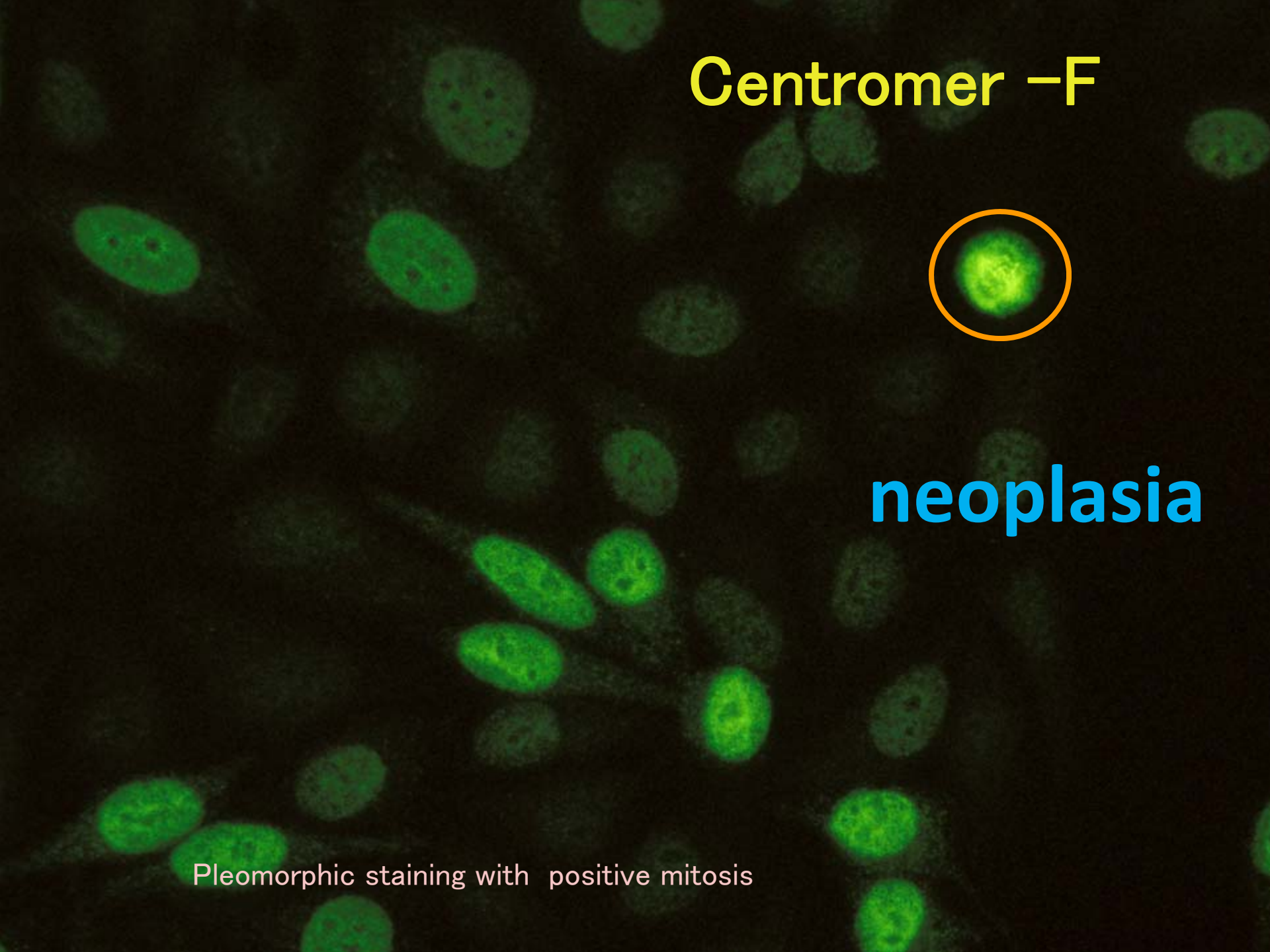
Tumor cells have high proliferation rate.

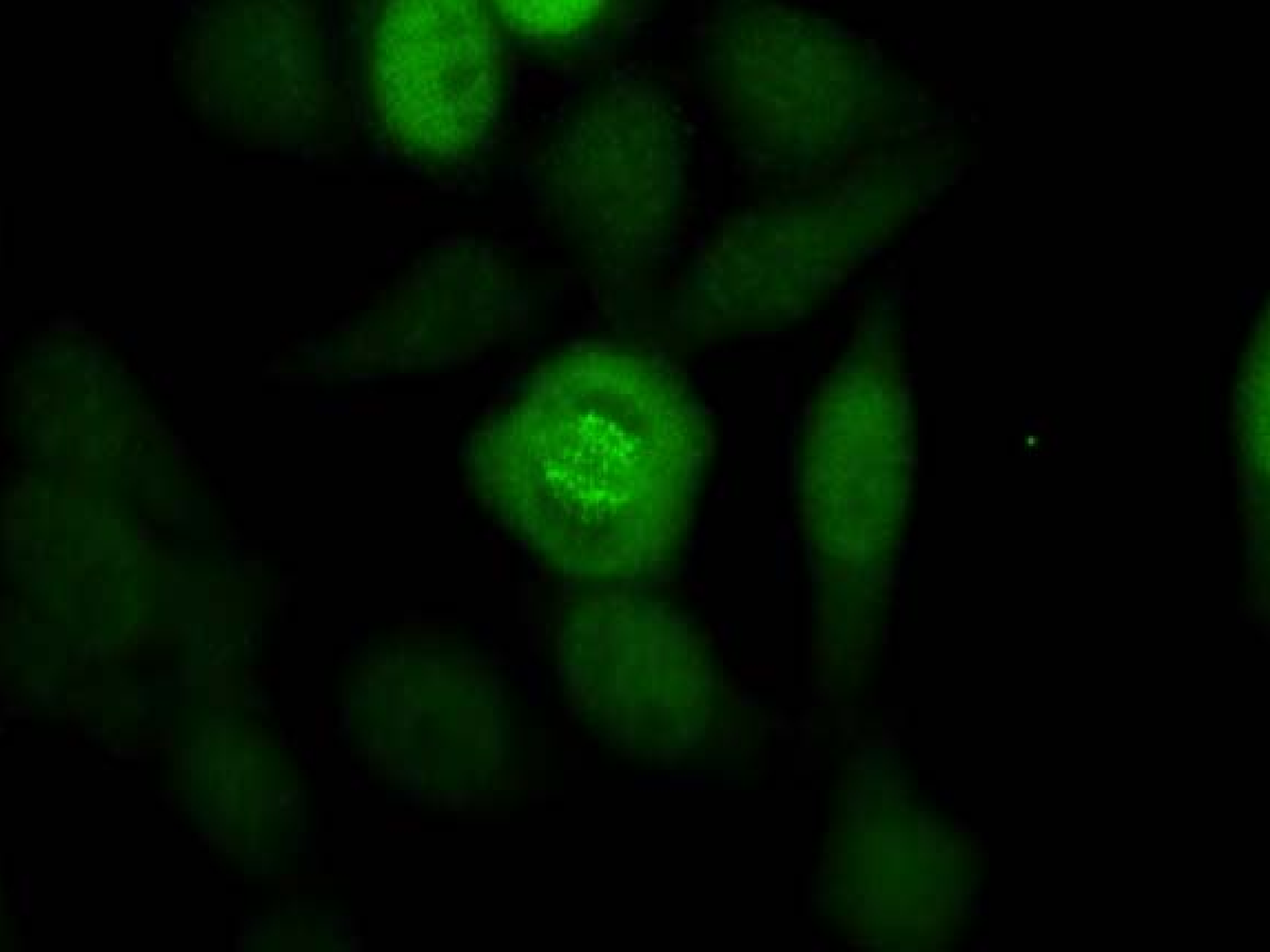
More proliferation associated protein can be expressed.

More or other potential autoantigens can be presented in ***mitotic cells in patients with cancer***.

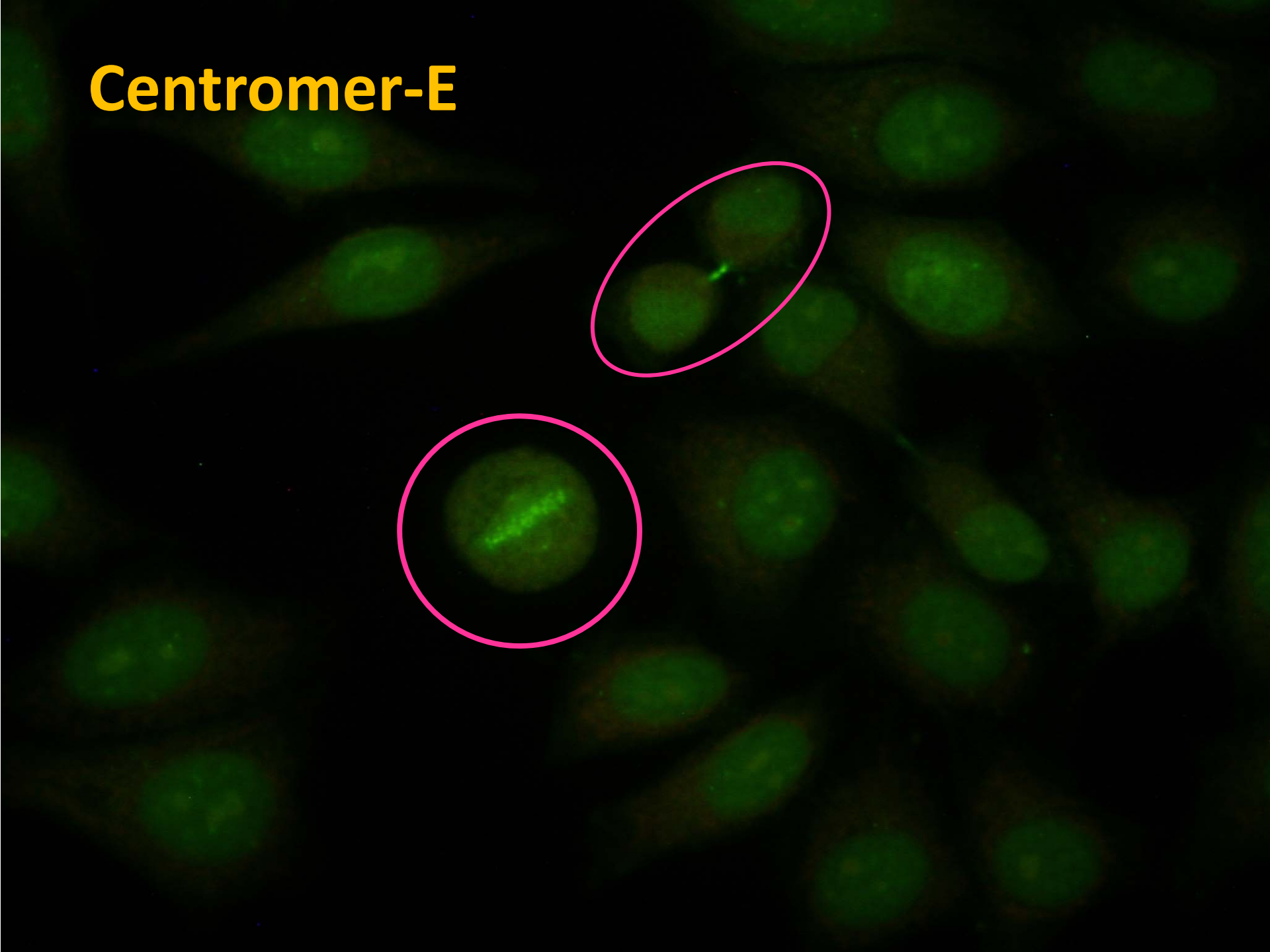
Mitosis associated autoantibodies might be a diagnostic value in neoplasia!

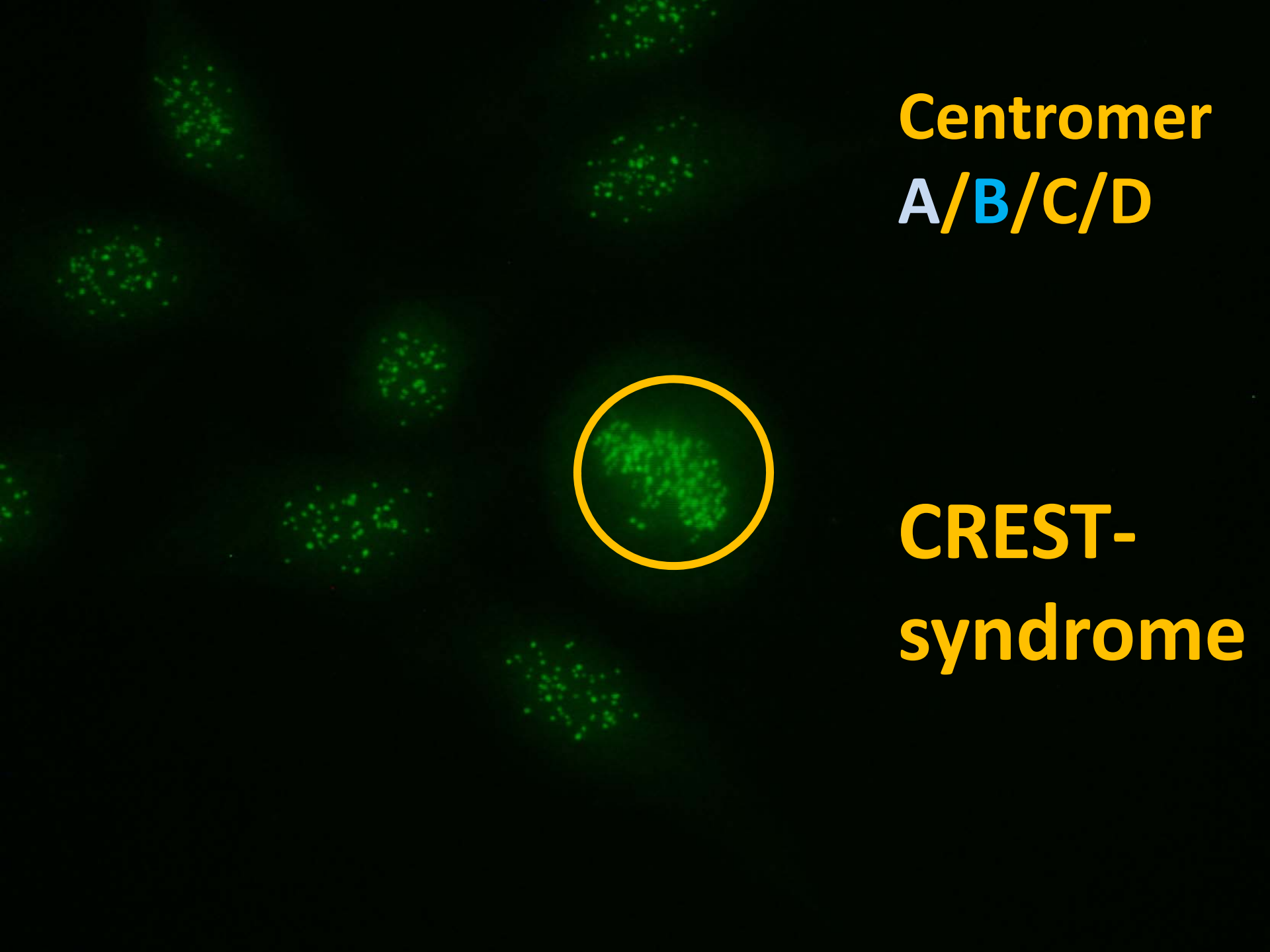

PCNA
proliferating cell nuclear antigens
SLE
Pleomorphic staining with negative mitosis


Centromer -F
neoplasia
Pleomorphic staining with positive mitosis



Centromer-E



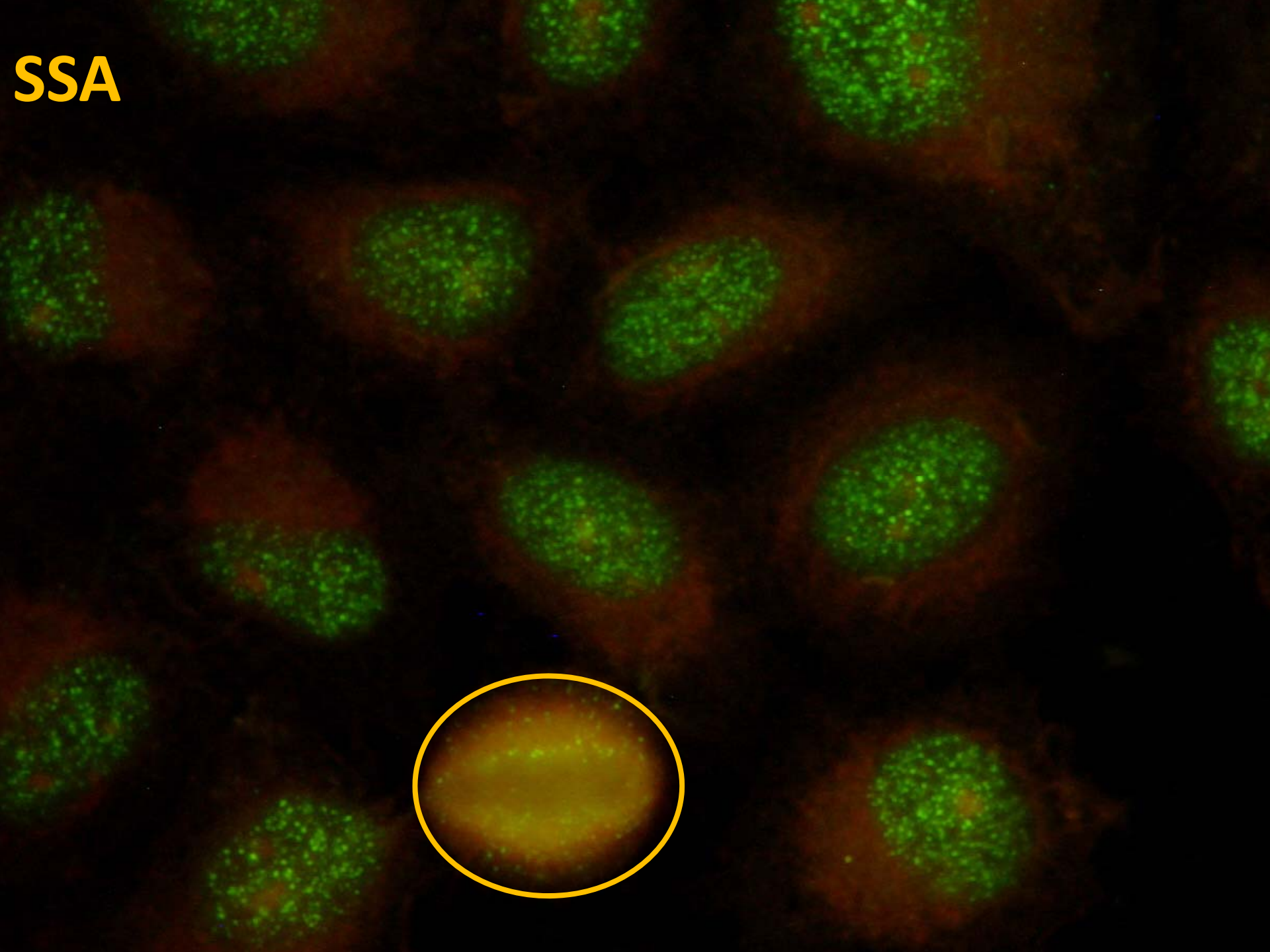

Centromer
A/B/C/D
CREST-
syndrome

ENA is a subgroup of ANA

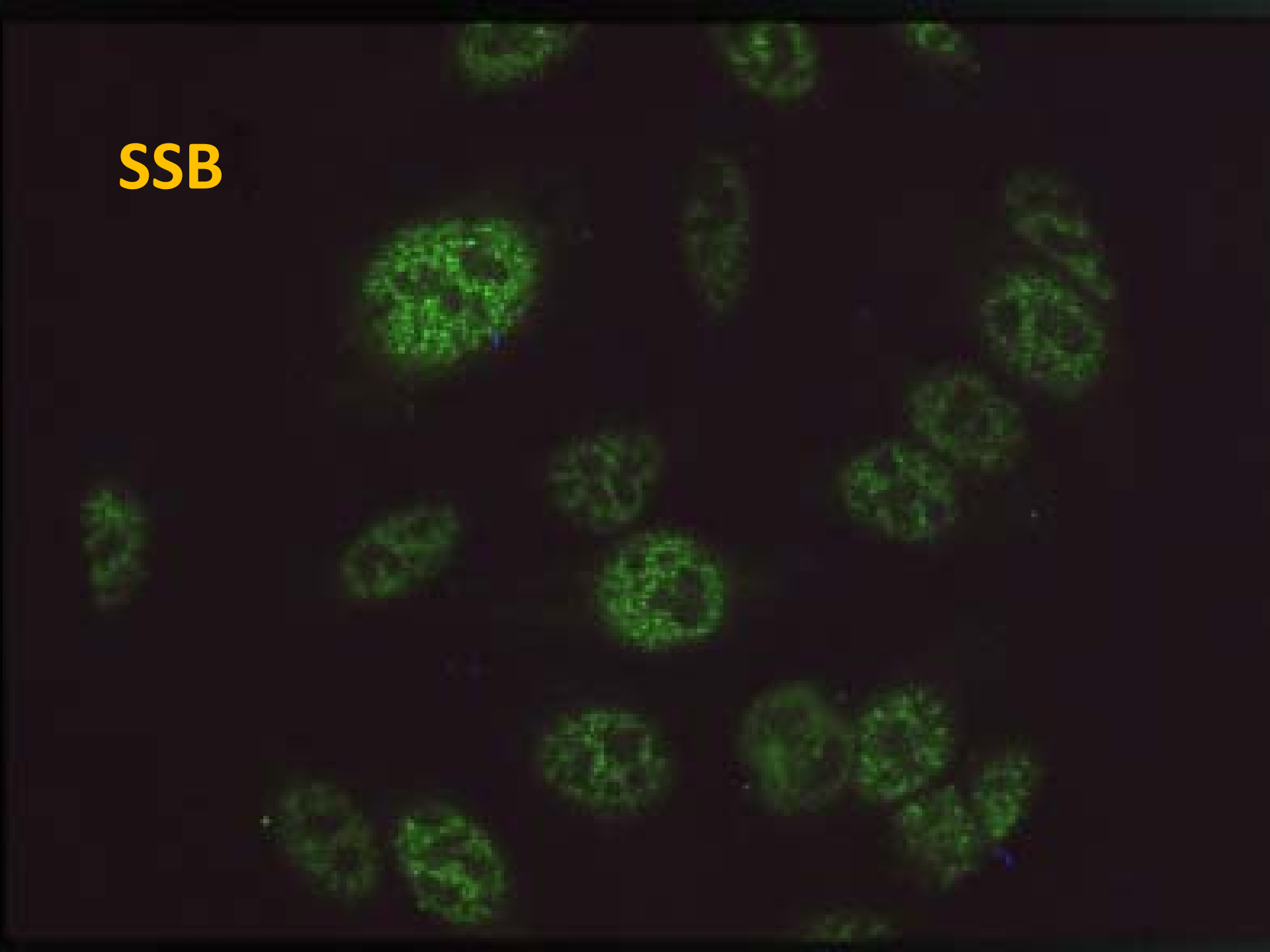
extractable **n**uclear **a**ntigen
from the nucleus by salt solution

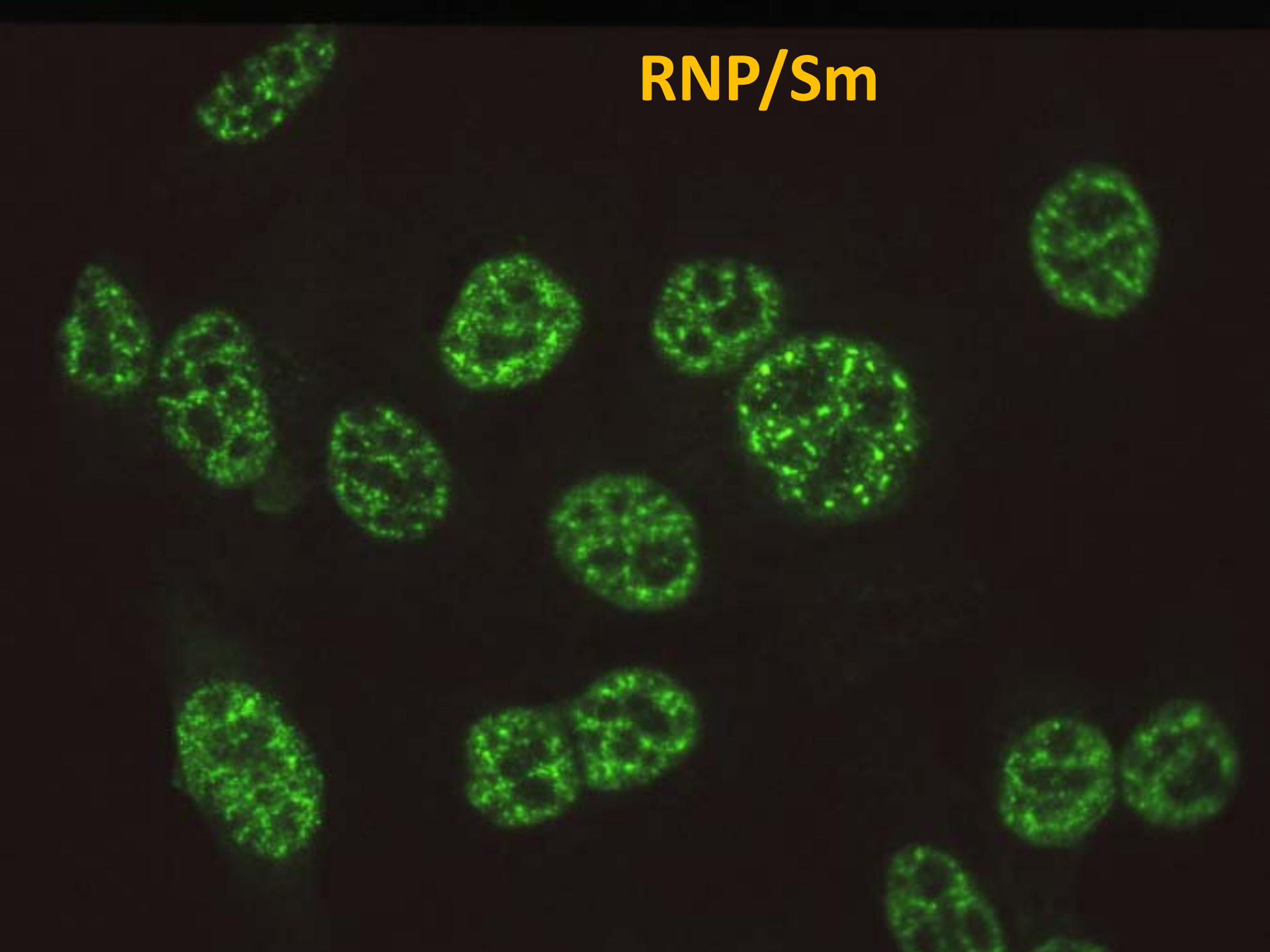
- 1961 **SSA/Ro** (60 kDa) - *SLE, Sjögren syndrome*
- 1961 **SSB/La** – *lupus by new born, Sjögren syndrome*
- 1966 **Sm**(ith Stephanie) - *SLE*
- 1970's **RNP** (ribonucleoprotein) – *MCTD*
- 1979 **Scl-70** (topoisomerase I) – *scleroderma*
- 1980 **Jo**(e)**-1** - *polimyositis*

not a homogenous disease group!

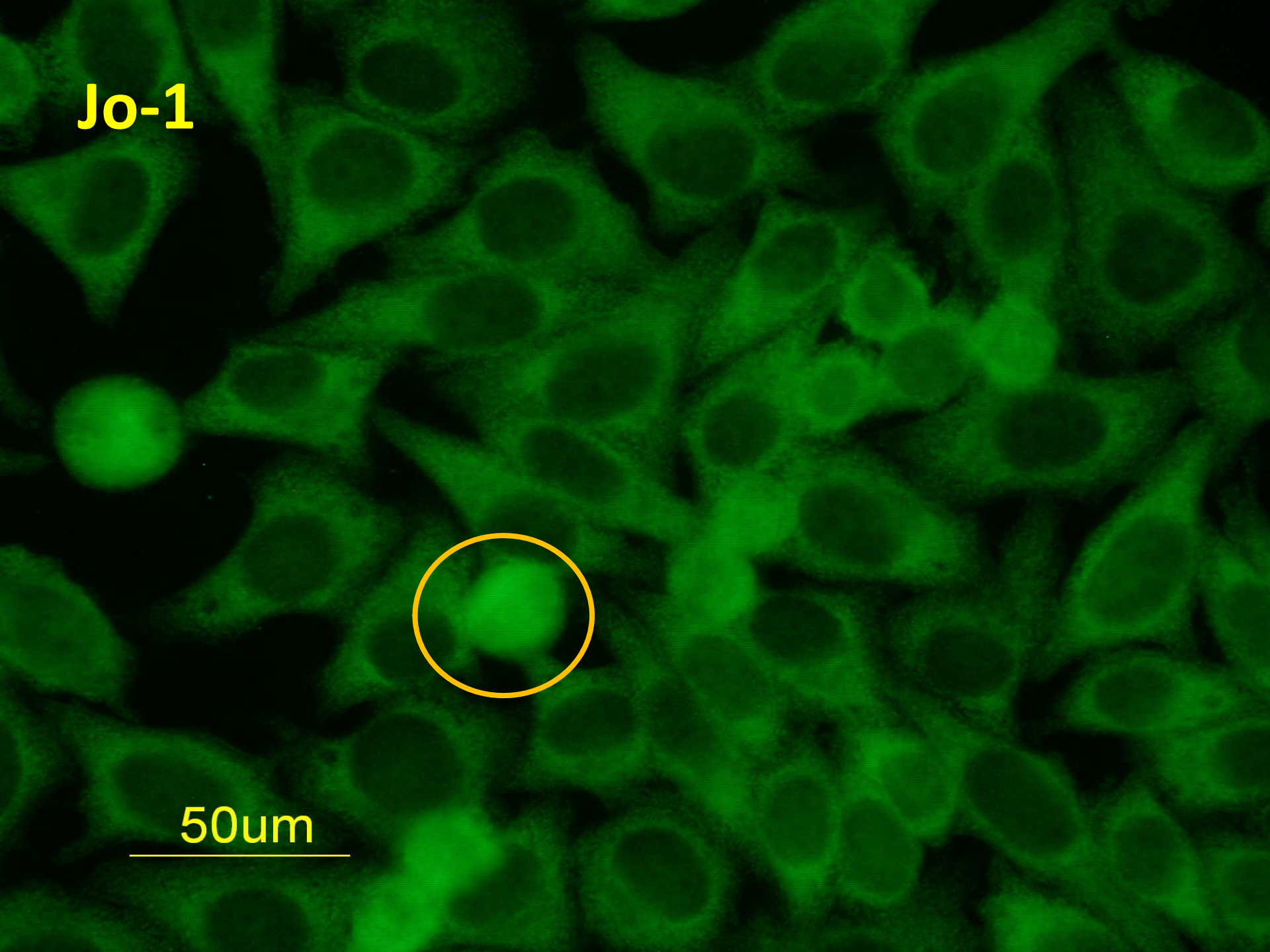

SSA

SSB



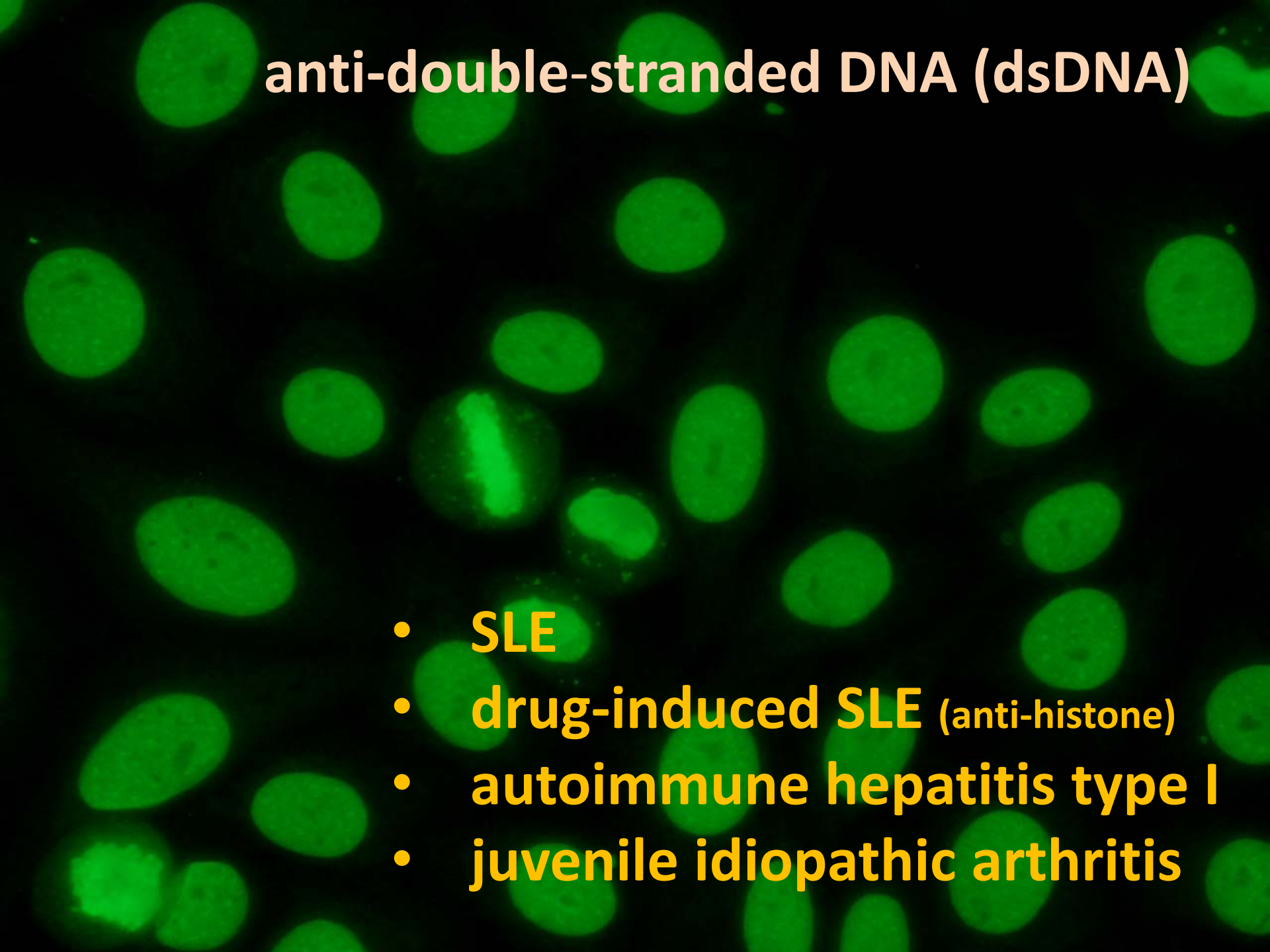

RNP/Sm

ScL-70


Jo-1
50um

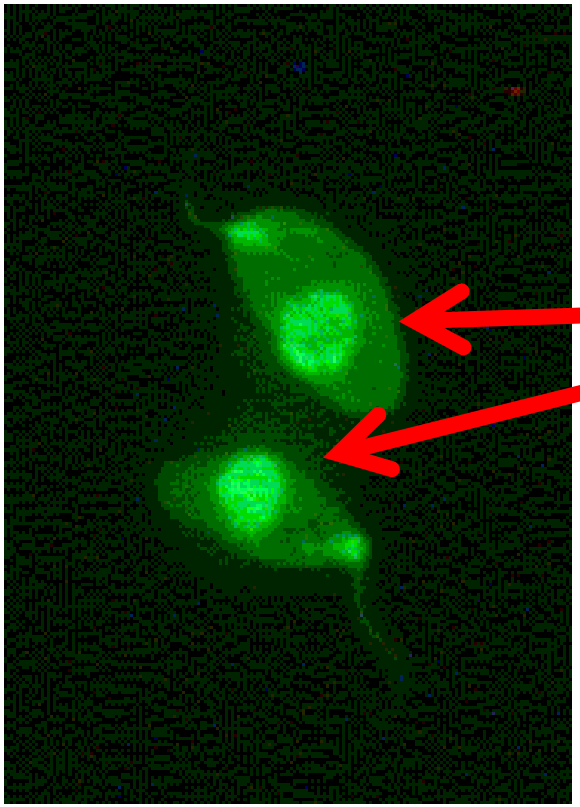
Anti-dsDNA - does not belong to ENA’s

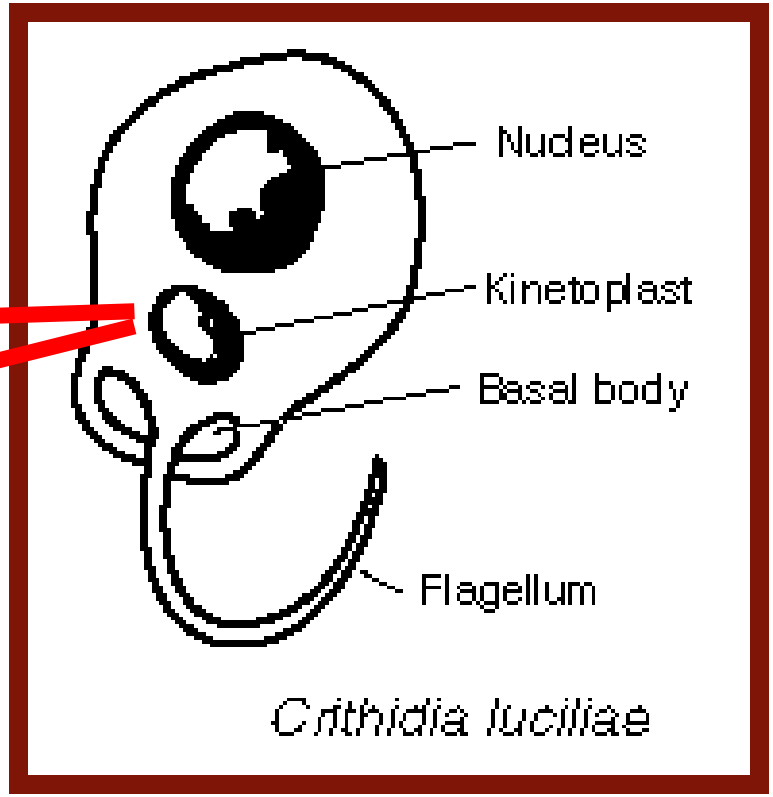
anti-double-stranded DNA (dsDNA)



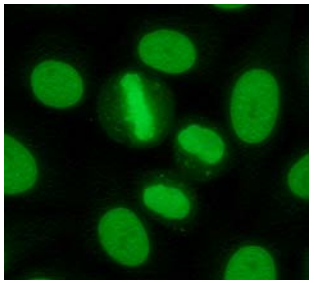
- SLE
- drug-induced SLE (anti-histone)
- autoimmune hepatitis type I
- juvenile idiopathic arthritis

Crithidia luciliae

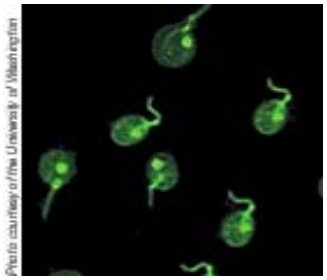




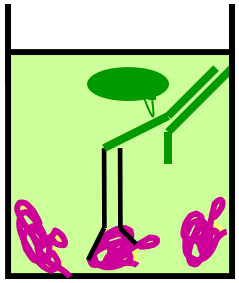
Different detection methods of anti-dsDNA in SLE patients



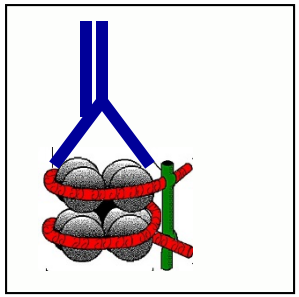
HEp-2
IIF



Crithidia IIF



ELISA
dsDNS
(Nukleosome)



FARR
Assay

Sensitivity	Specifity	Cut-off
76	45	1:320
13	99	1:10
29 (85)	96	200 U/ml
32	96	-

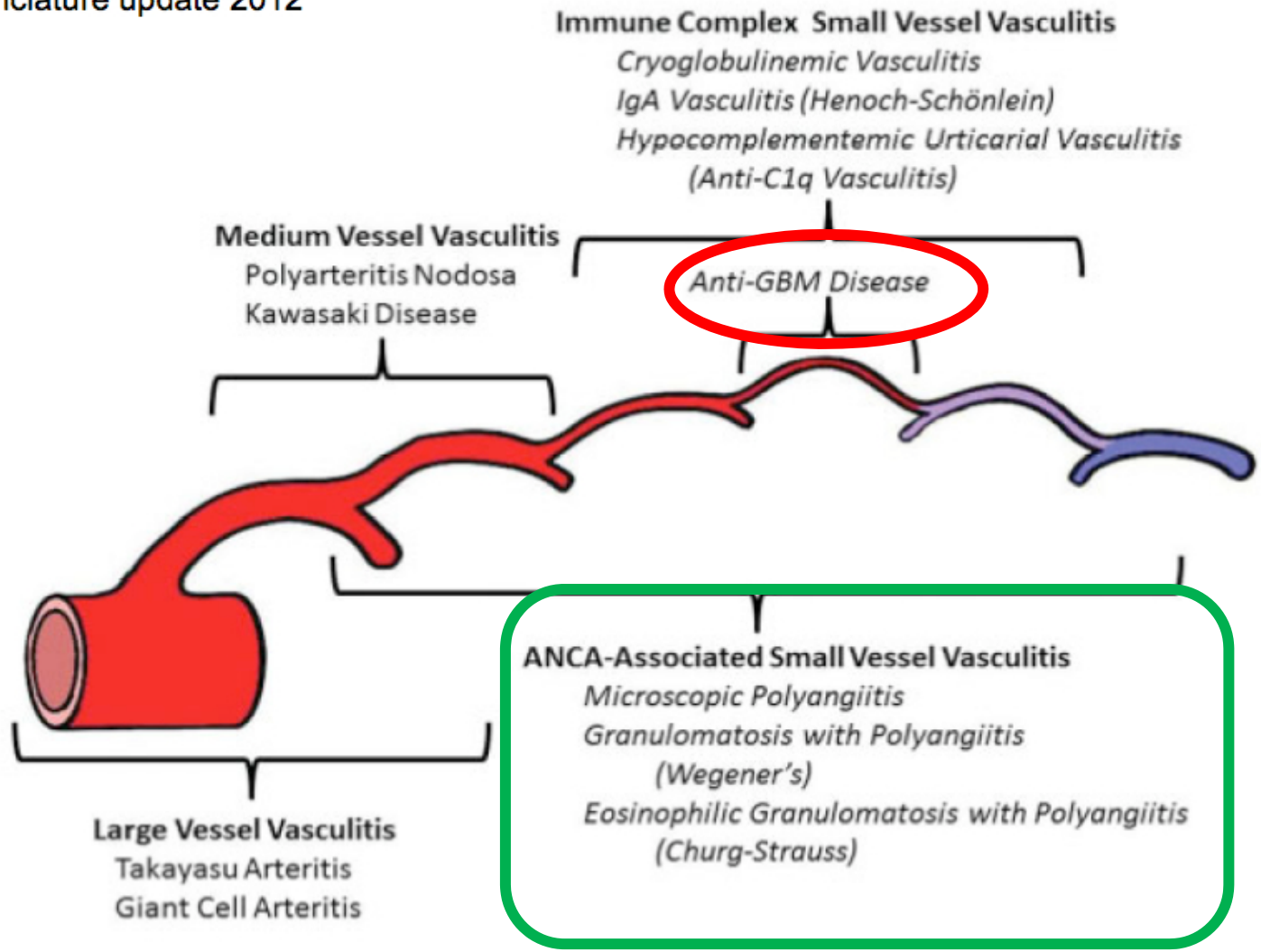
Autoimmune vasculitis

ANCA-associated vasculitis

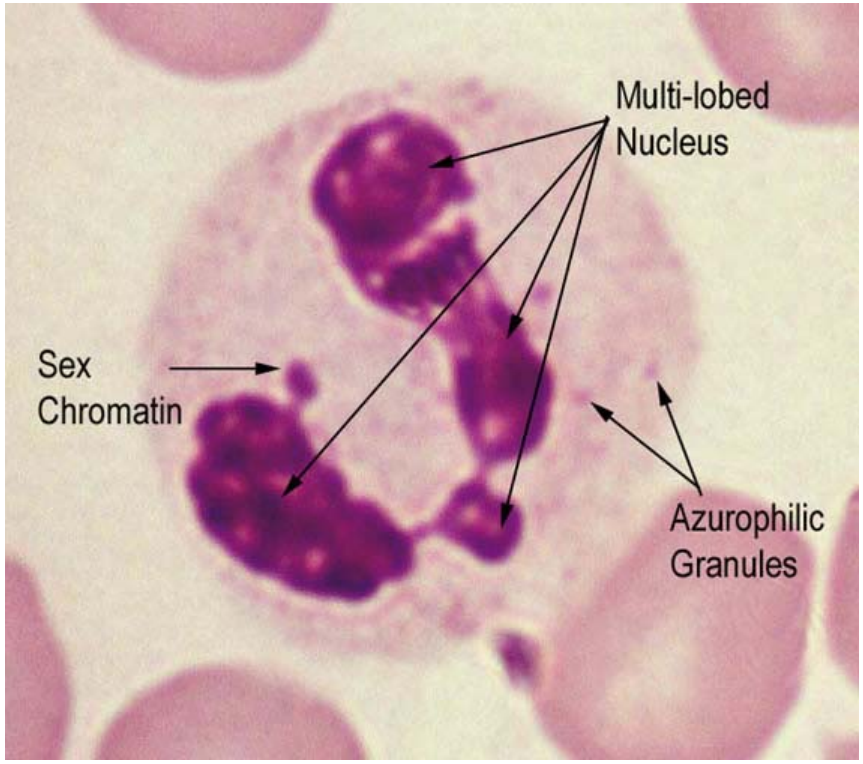
Anti-Neutrophil Cytoplasmic Autoantibody

Classification of Vasculitis

Chapel Hill Consensus Criteria
Nomenclature update 2012



Azurophilic granules in neutrophyl granulocytes



Myeloperoxidase (cationic)

Serine protease e.g.: **Proteinase-3**

BPI (bactericidal permeability increasing protein)

Elastase

Cathepsin G

other granule: Lactoferrin

c-ANCA - p-ANCA

indirect immunofluorescence:
cytoplasmic reaction

Immunoassay
antigen specificity:

proteinase 3
(PR3-ANCA)

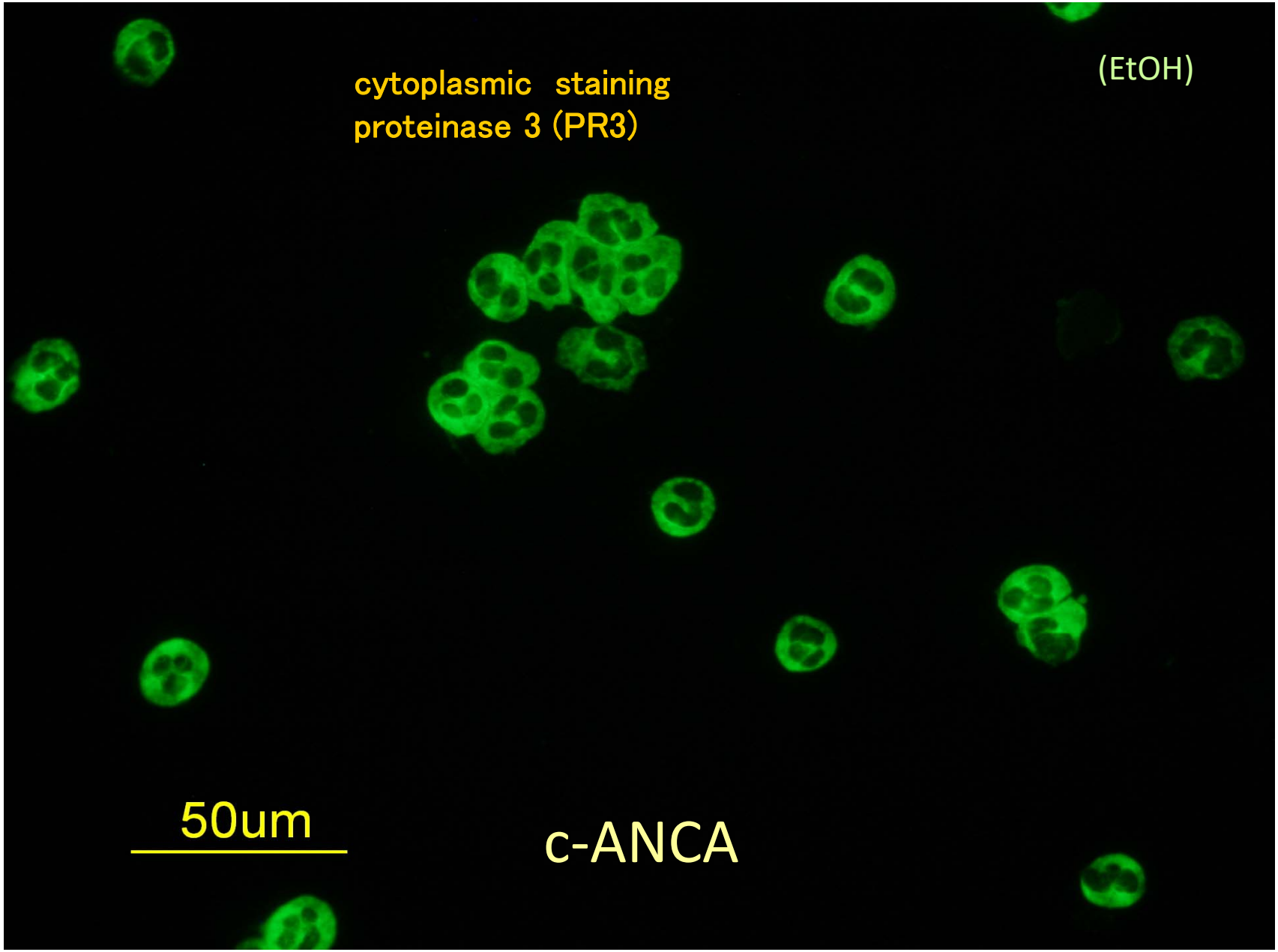
Wegener`s granulomatosis
(50 –95 %)

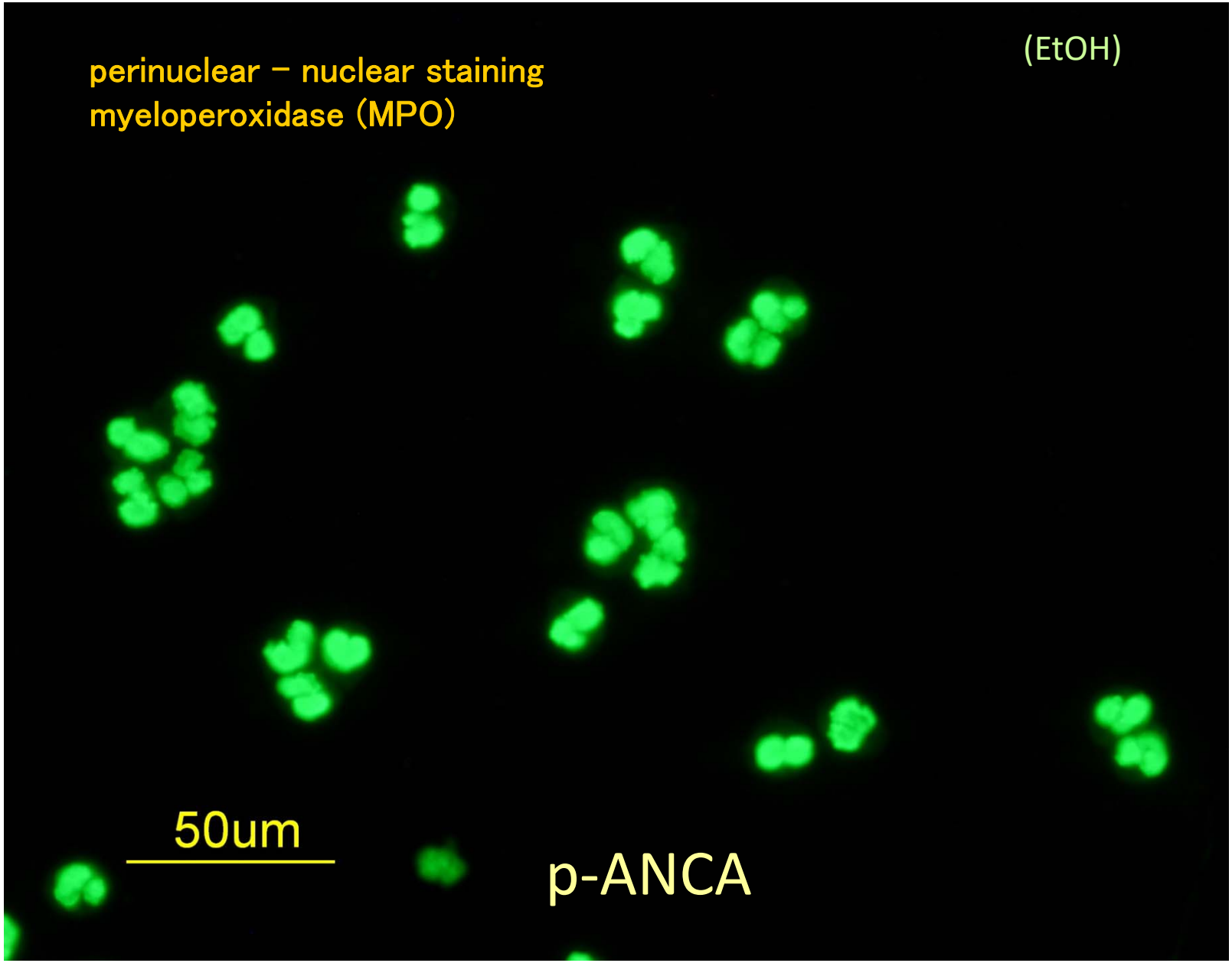
indirect immunofluorescence
perinuclear reaction

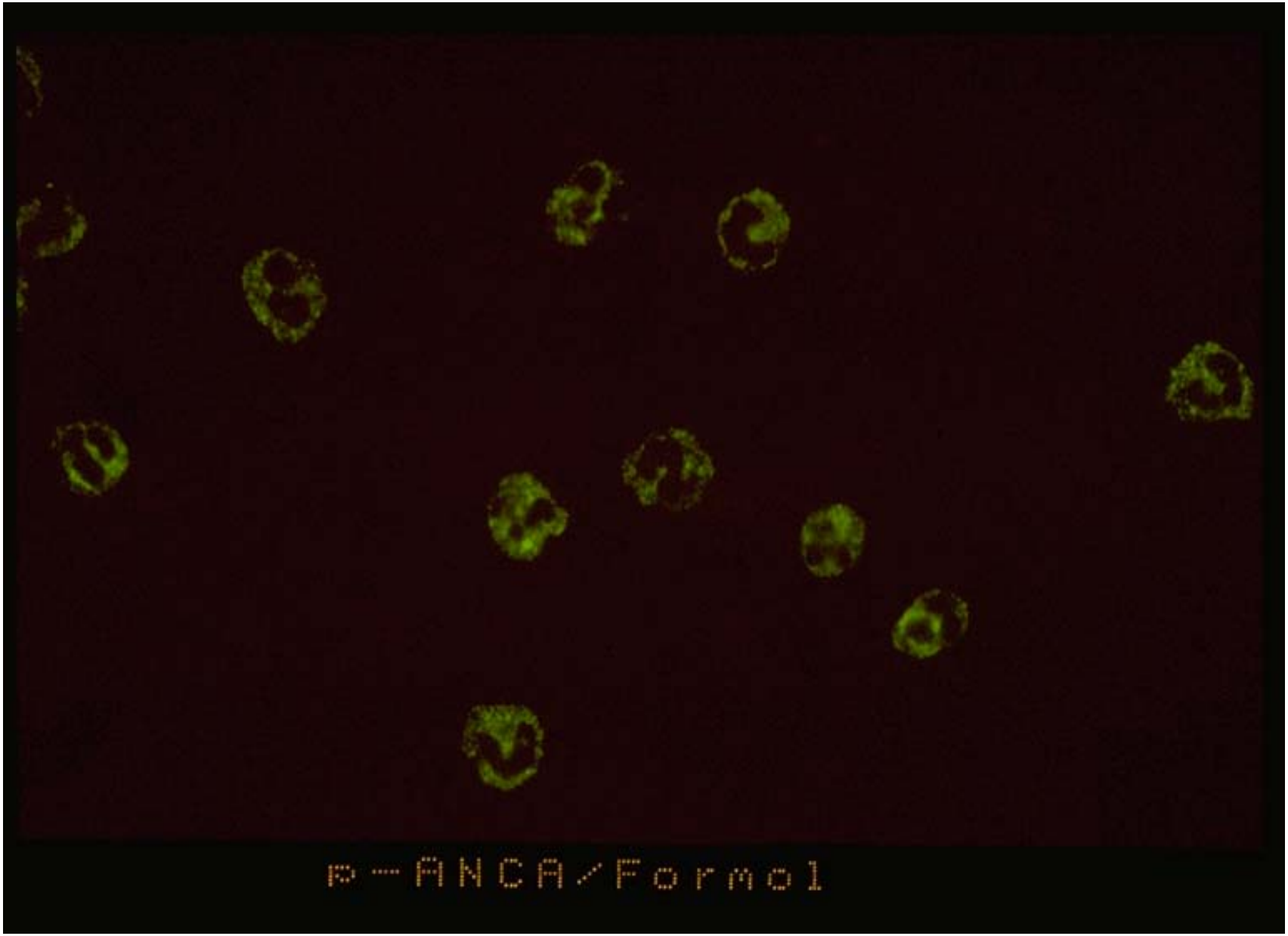
Immunoassay
antigen specificity:

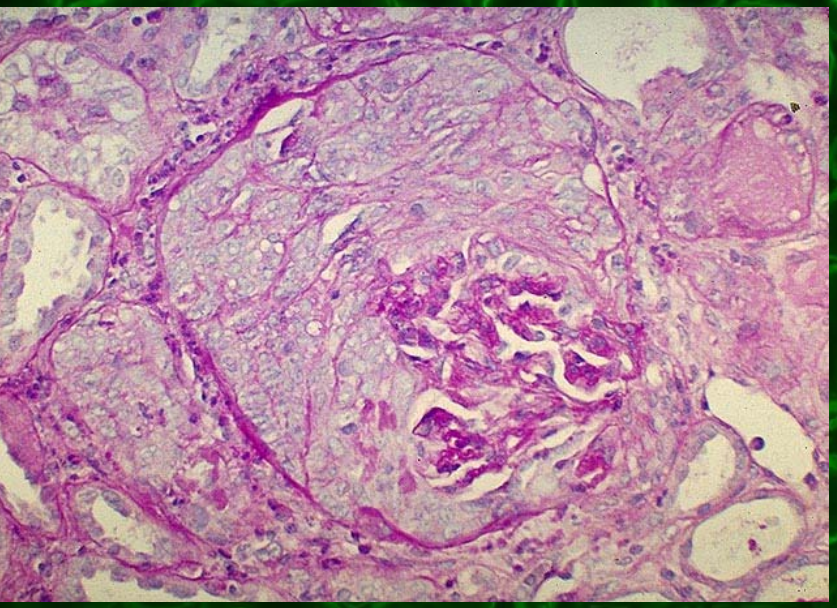
myeloperoxidase
(MPO-ANCA)

Churg–Strauss syndrome
(ca. 60%)
microscopic polyangiitis
(ca. 70%)

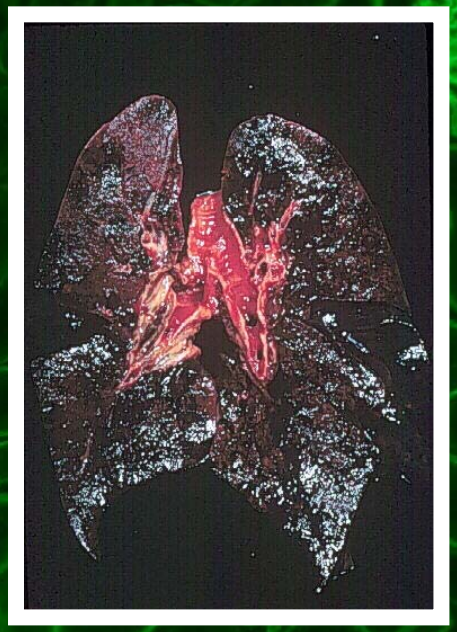

cytoplasmic staining
proteinase 3 (PR3)
(EtOH)
50um
c-ANCA


perinuclear – nuclear staining
myeloperoxidase (MPO)
(EtOH)
50um
p-ANCA


P — ANCA/Formol



Goodpasture syndrome
anti-GBM syndrome



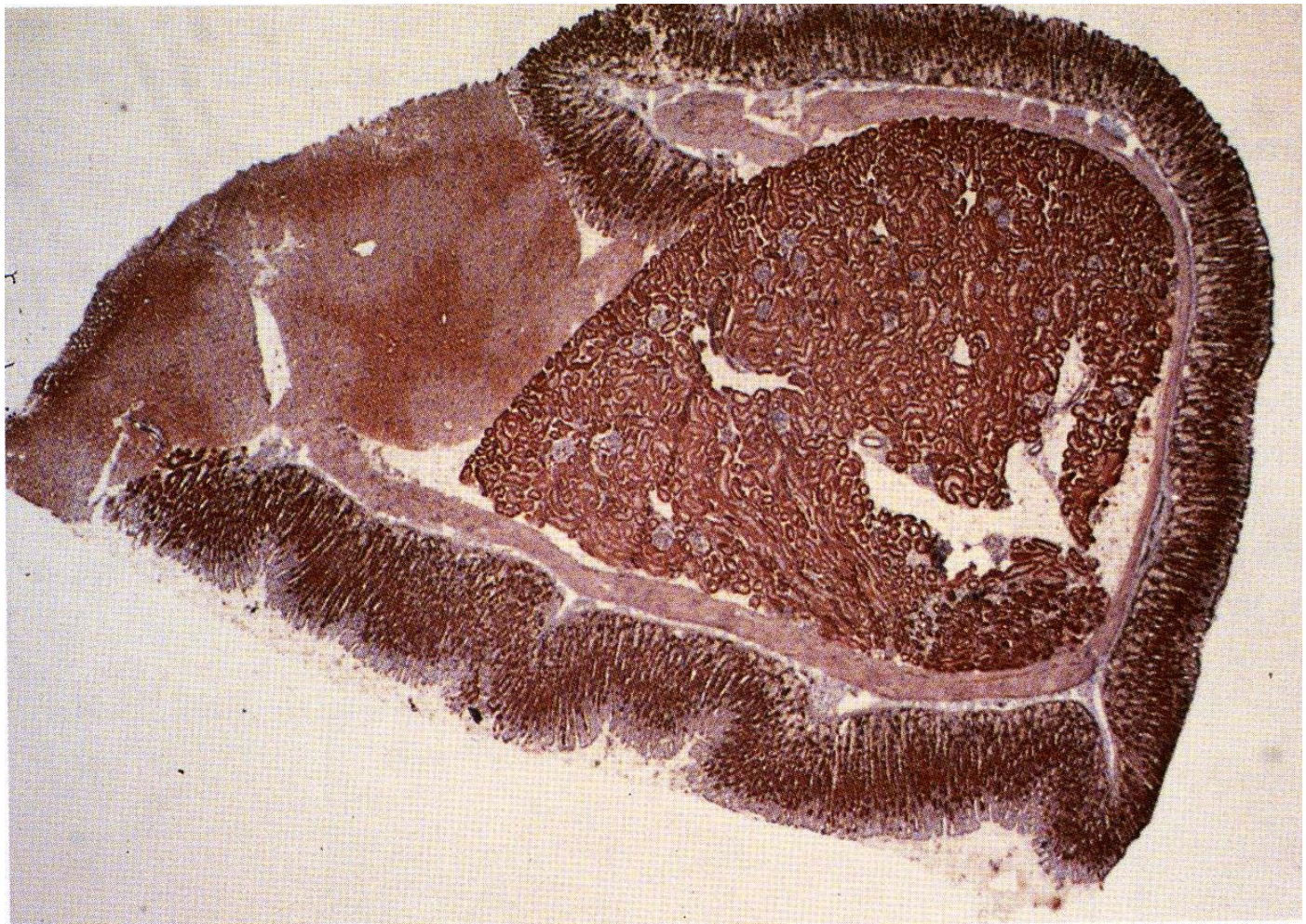
glomerular basment membrane
α3 chain of the Type IV collagen

anti-GBM autoantibody -
only urgency analysis

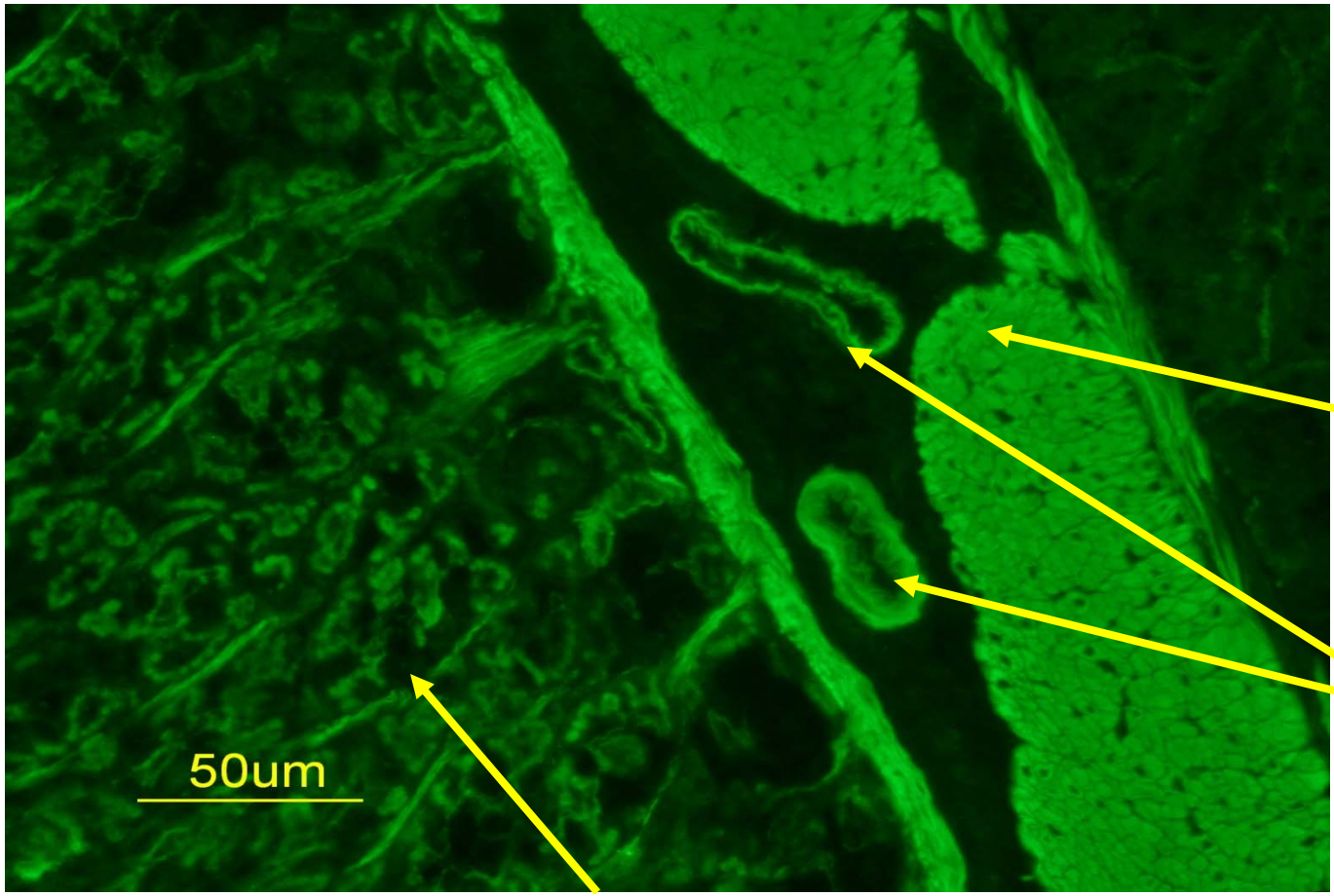
Organ-specific autoantibodies

Liver/Kidney/Stomach (LKS) Substrat

Autoimmune hepatobiliary disease



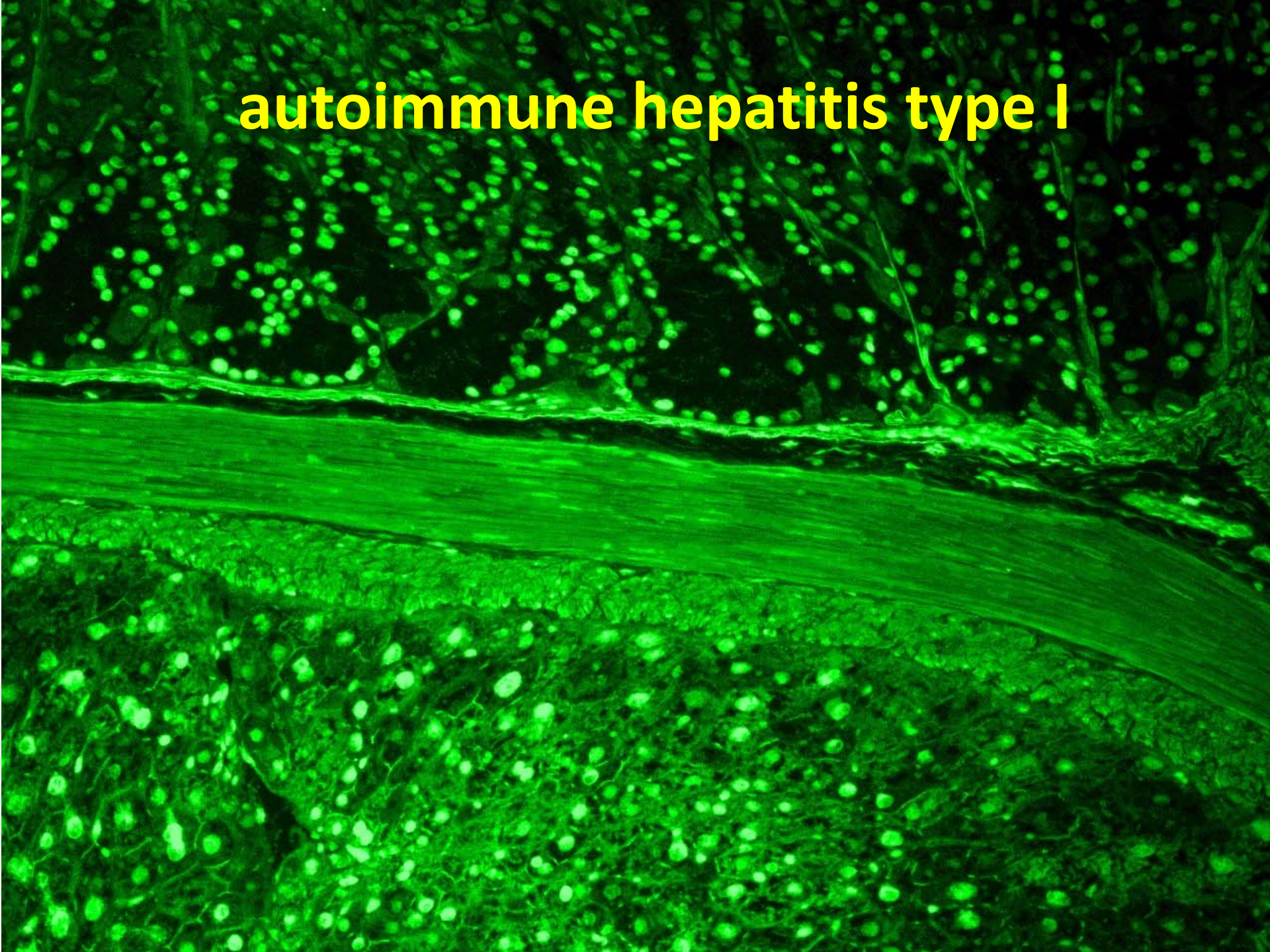
Anti-smooth muscle antibody (ASMA) - *autoimmune hepatitis type I*



smooth muscle in stomach

blood vessels wall

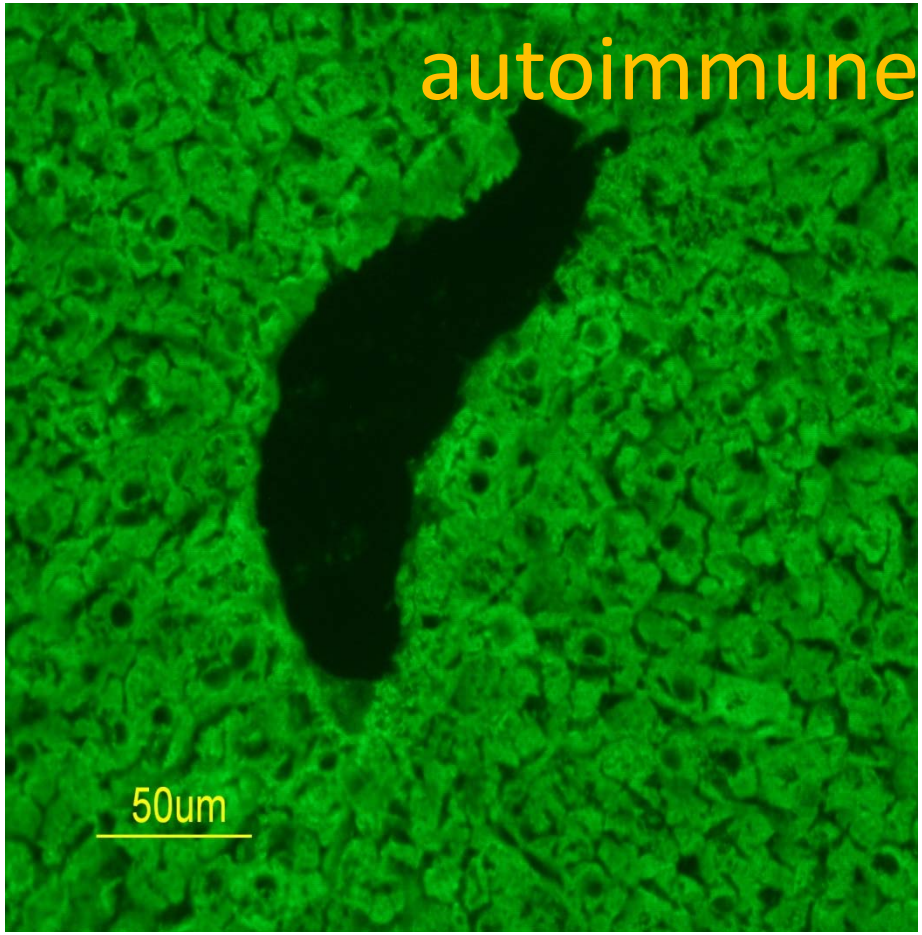
intergrandular fibers in Lamina propia of gastric mucosa

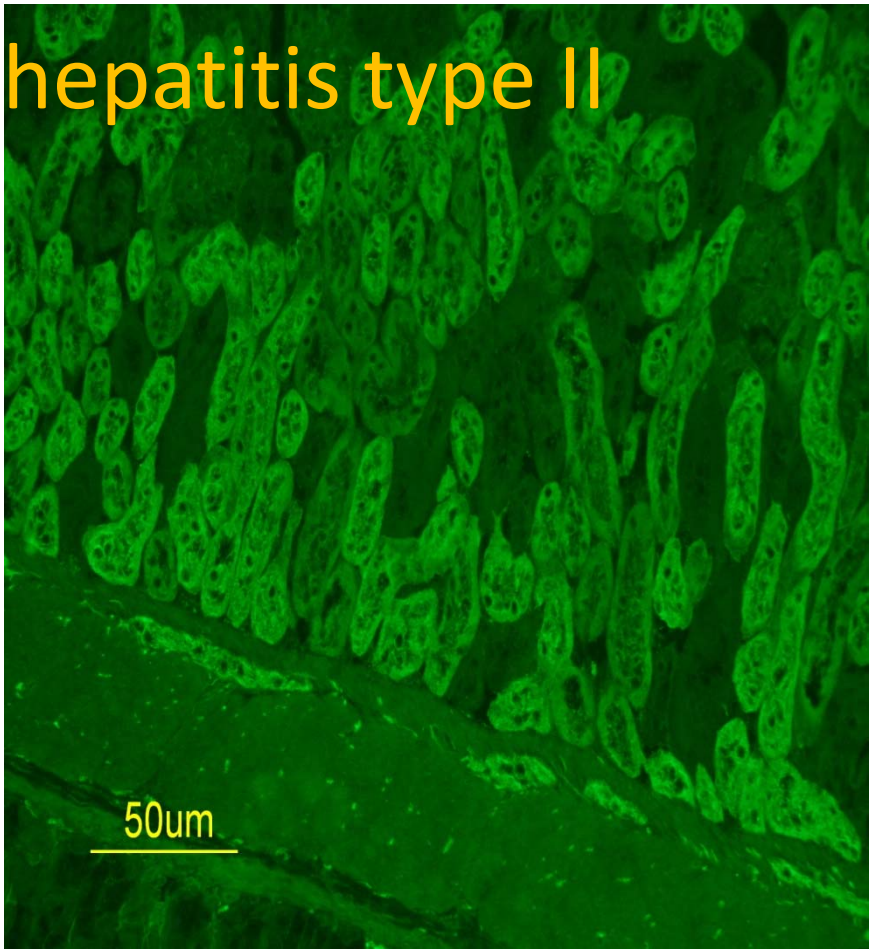

autoimmune hepatitis type I

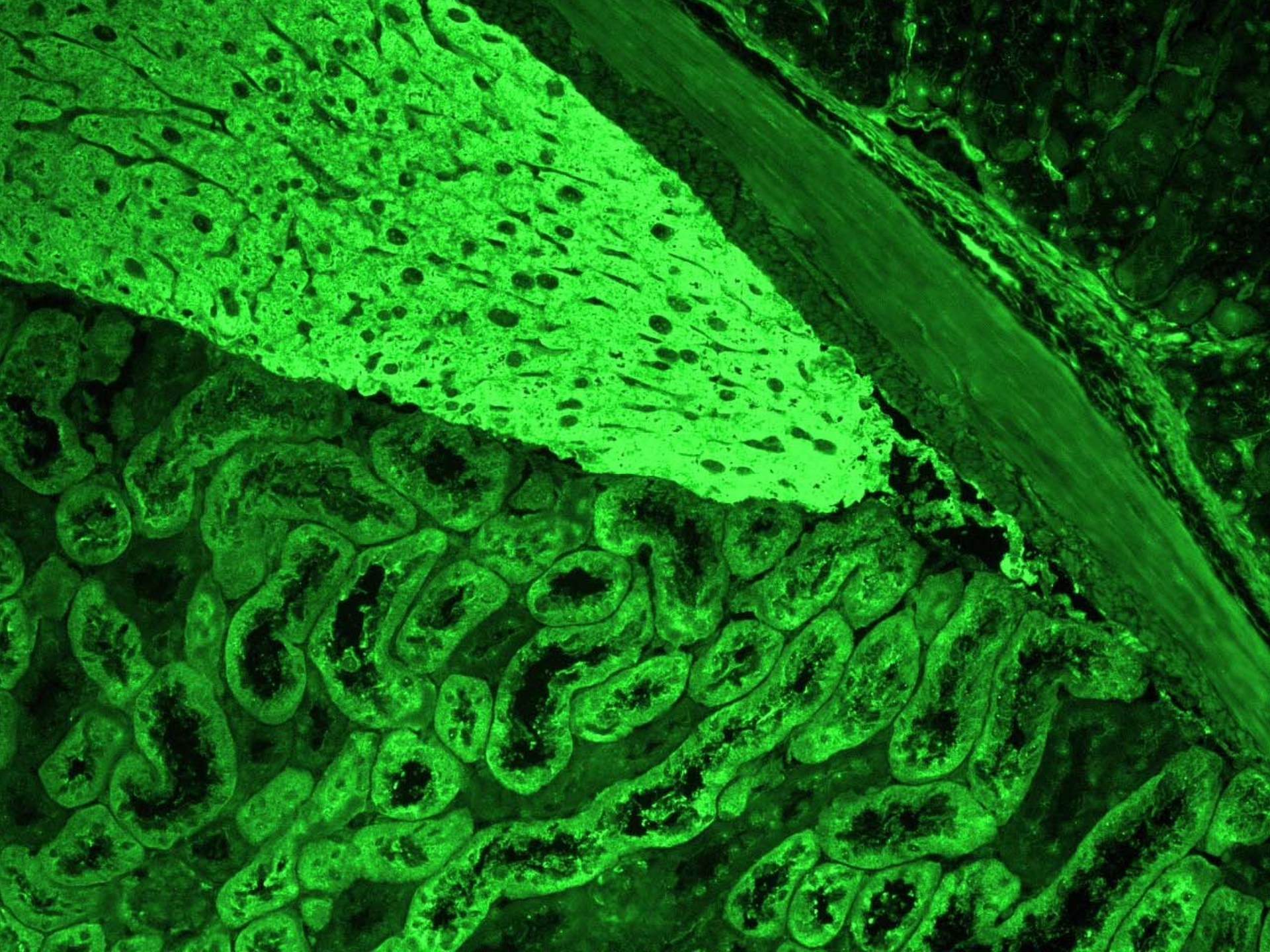
ASMA verification on intestinal epithel cells

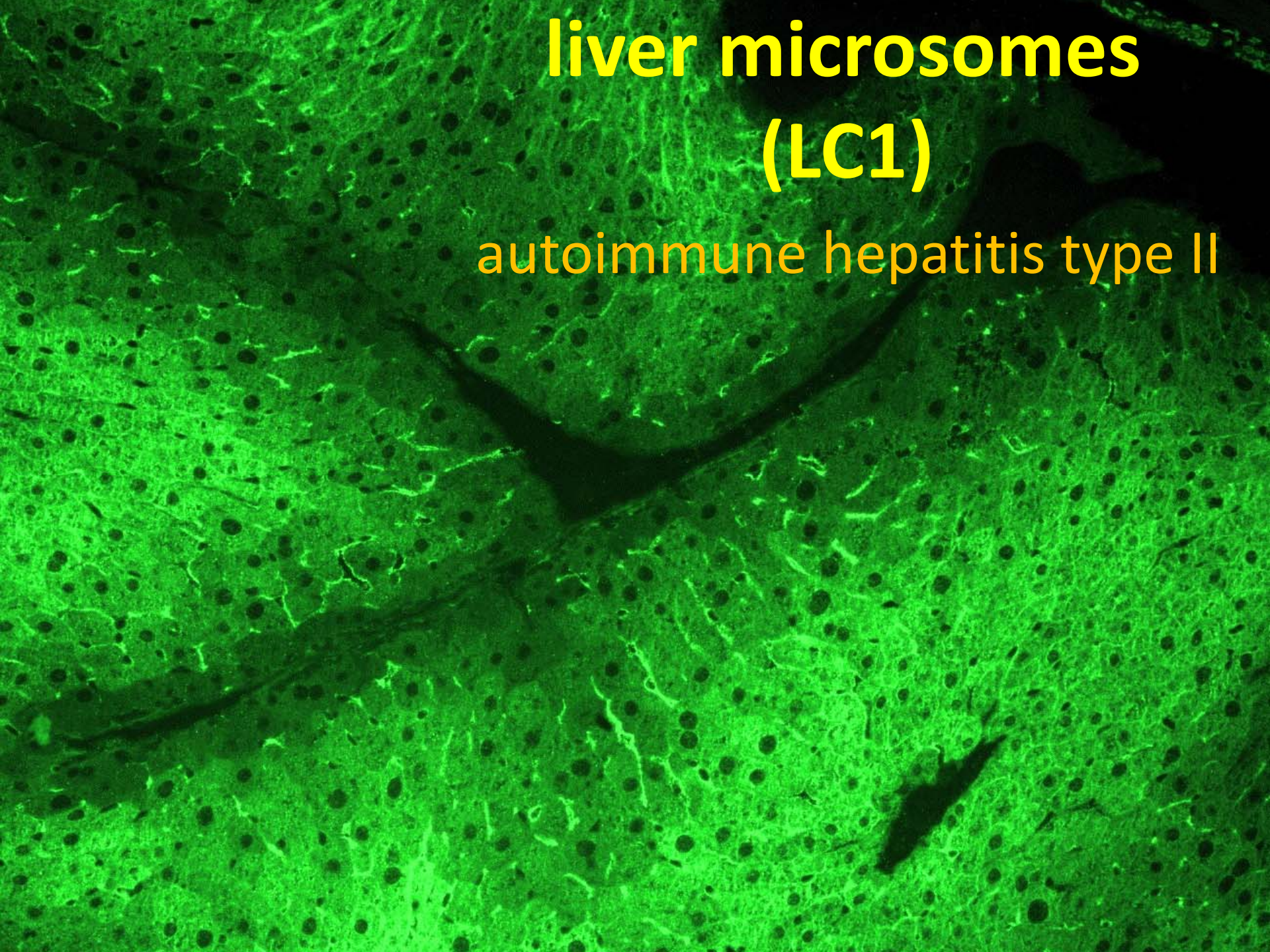
only autoantibodies against F-actin is specific for autoimmune hepatitis type I

Liver-kidney microsomes
LKM-1

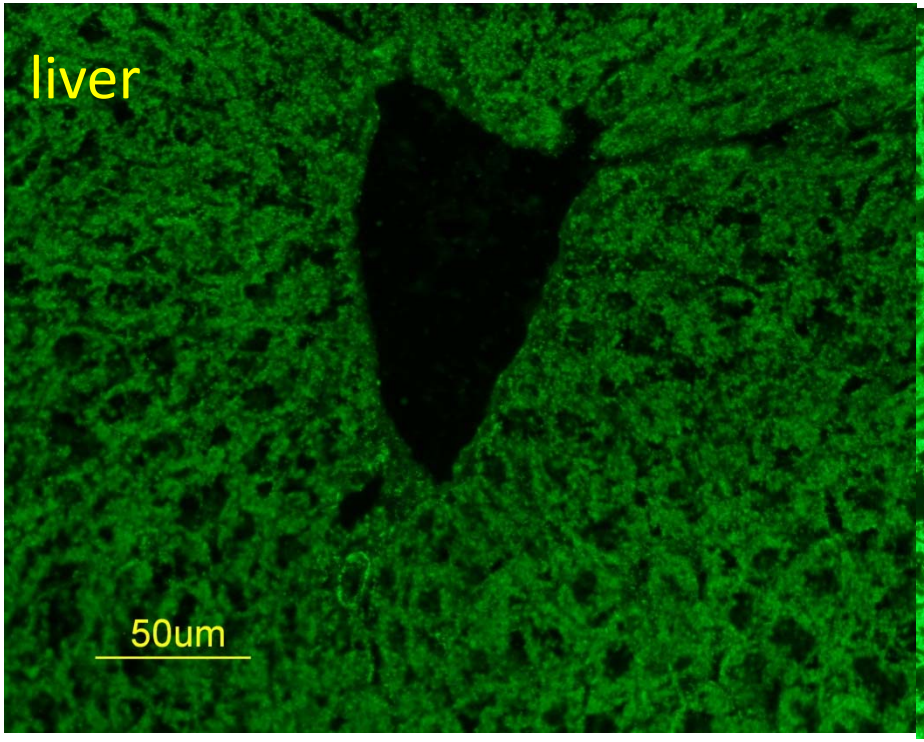


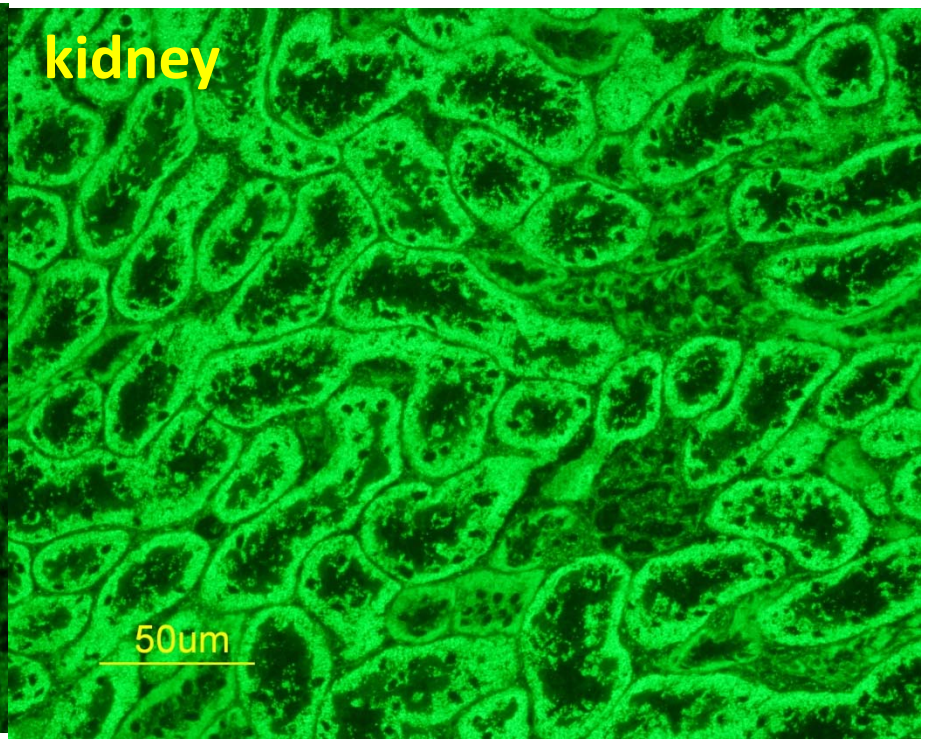


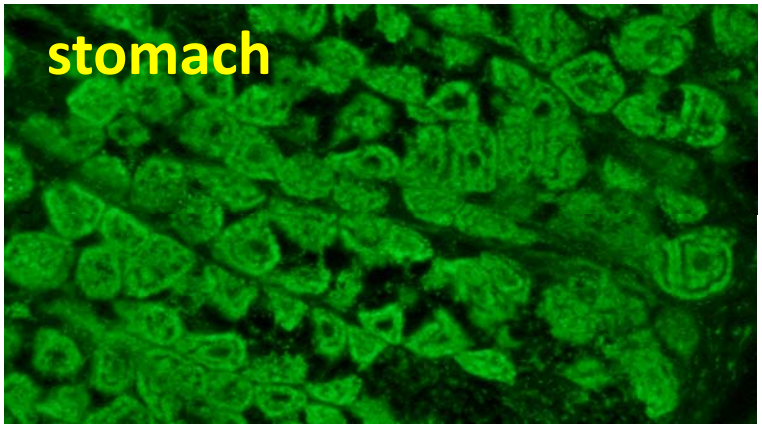


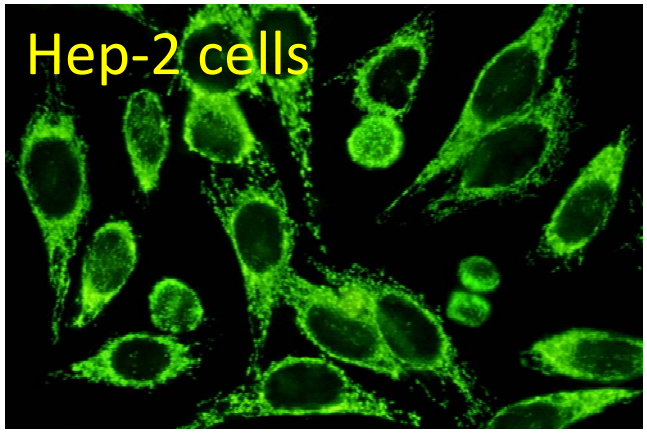

liver microsomes
(LC1)
autoimmune hepatitis type II

antimitochondrial antibody - *primary biliary cirrhosis*



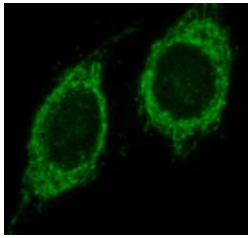






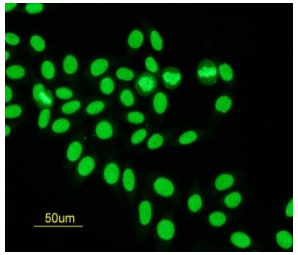
The role of autoantibodies

- predictive



antimitochondrial antibodies in PBS

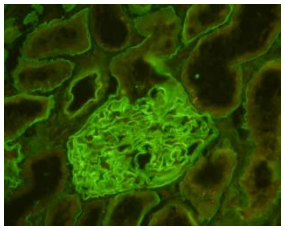
- preventive



natural autoantibodies

IgM anti-dsDNA in SLE

- pathogenic

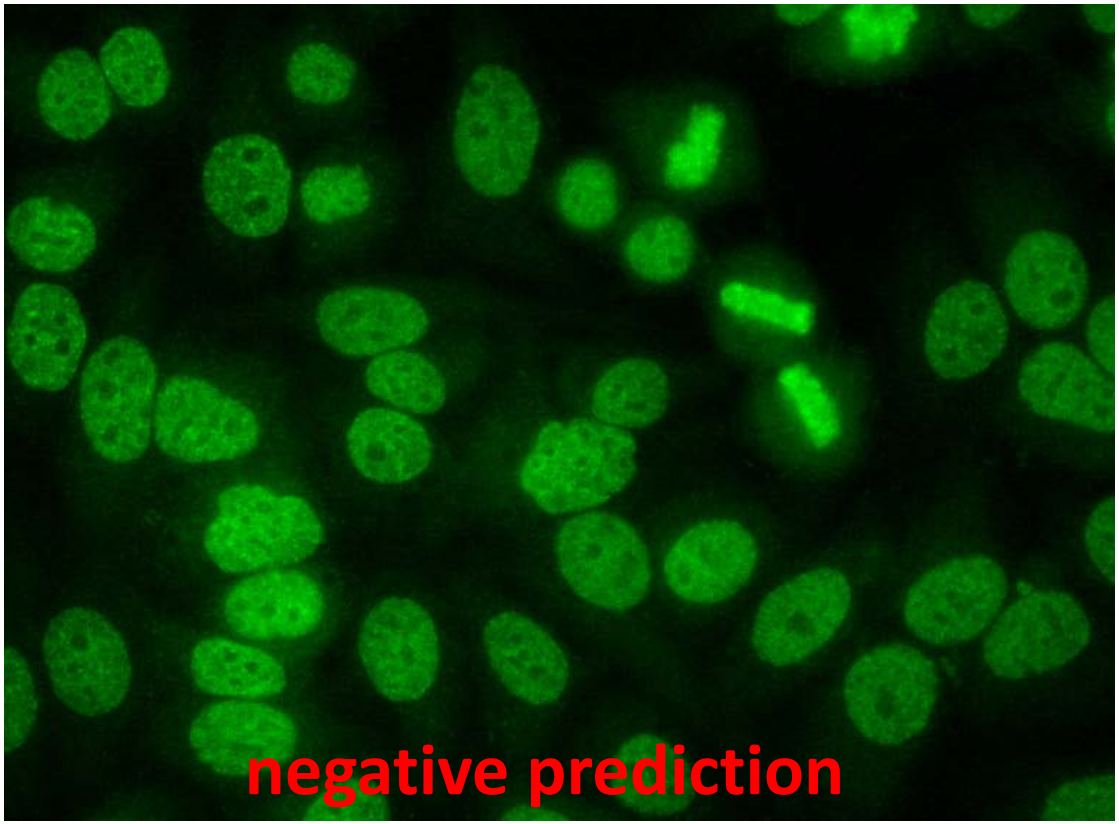


anti-GBM antibodies in Goodpasture syndrome

systemic autoimmune rheumatic diseases

- ANA positivity is a hallmark of SARD

but dense fine speckled (DFS-70) pattern:



IIF in autoimmune serology

- a good screening method
- high variety of antigens are present
- the antigens are close to native form
- rare antibodies do not have a verification methods
- automatization is in progress