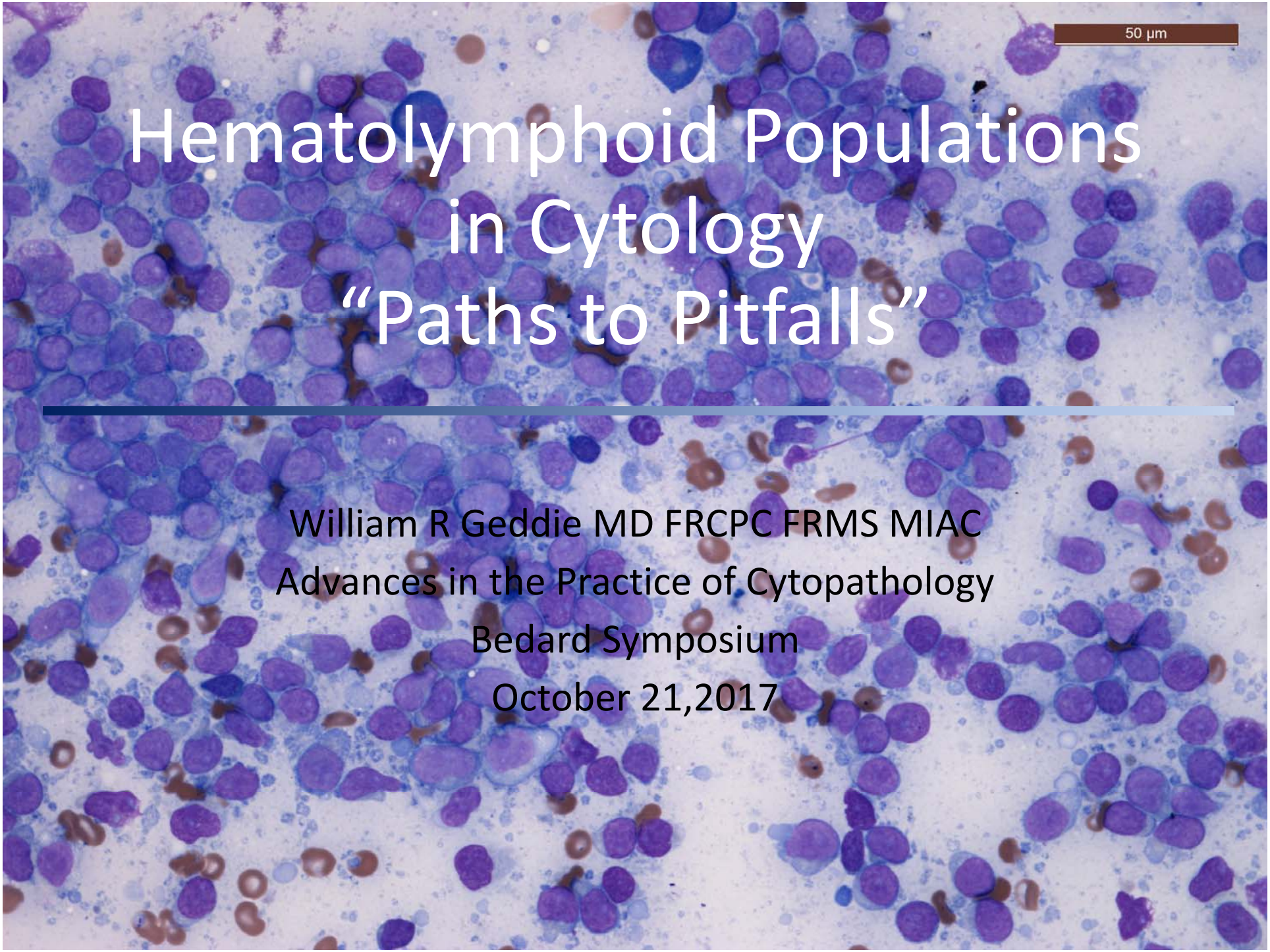


Hematolymphoid Populations in Cytology "Paths to Pitfalls"

William R Geddie MD FRCPC FRMS MIAC

Advances in the Practice of Cytopathology

Bedard Symposium

October 21,2017

Conflict of Interest

Nothing to Declare

Cytology of Lymphoid and Hematopoietic Tissues

- Immense topic
- “The immune and hematopoietic systems are complex and the reactive states and neoplasms of the both systems are correspondingly complex”
  - (Nancy Harris)
- Most diseases involving hematolymphoid populations have both a morphologic and immunophenotypic definition

Don't request a fine-needle aspirate (FNA) for the evaluation of suspected lymphoma.

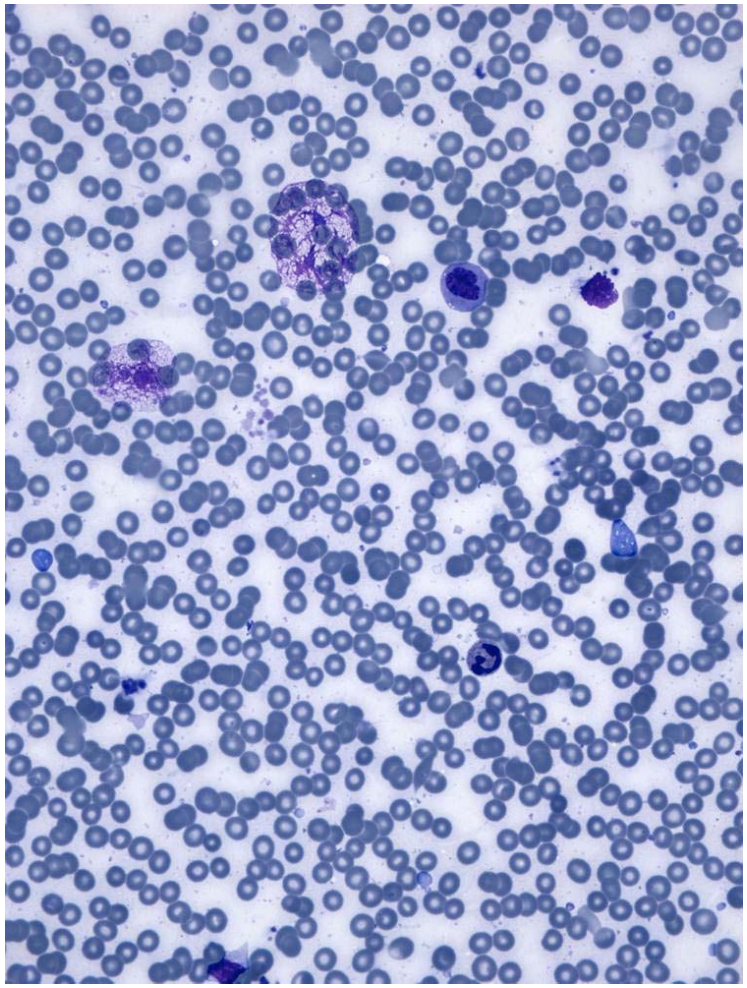
The diagnosis of lymphoma requires specimens with intact cellular architecture for accurate histopathologic and immunophenotypic classification. FNA is associated with a low sensitivity and potentially results in delays in lymphoma diagnosis. Although excisional biopsy is the gold standard for lymphoma diagnosis, depending on the lymph node location, excisional biopsy may be associated with complications and the need for general anesthesia. At a minimum, an imaging-guided core biopsy should be obtained to improve the accuracy and timeliness of lymphoma diagnosis.

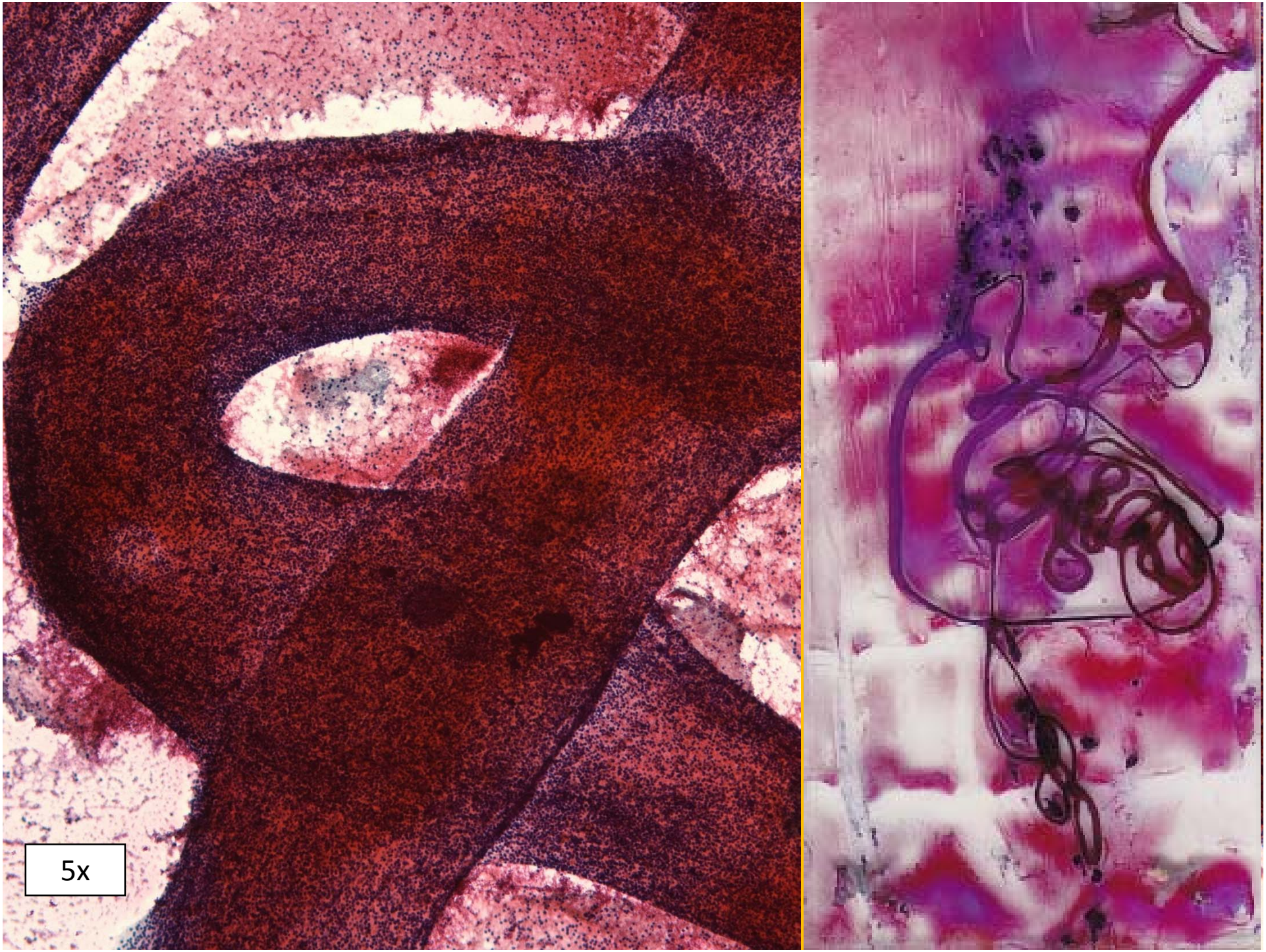
Objectives

- To review morphologic features of some benign lymph node cell types that can be mistaken for alien cells in cytology preparations
- To review features of some benign lymphoid proliferations that can be mistaken for lymphoma
- To review features of lymphomas that can be mistaken for epithelial neoplasms
- To suggest possible lab routines or additional procedures that can help avoid errors

Unsat and Sub-Optimal Samples

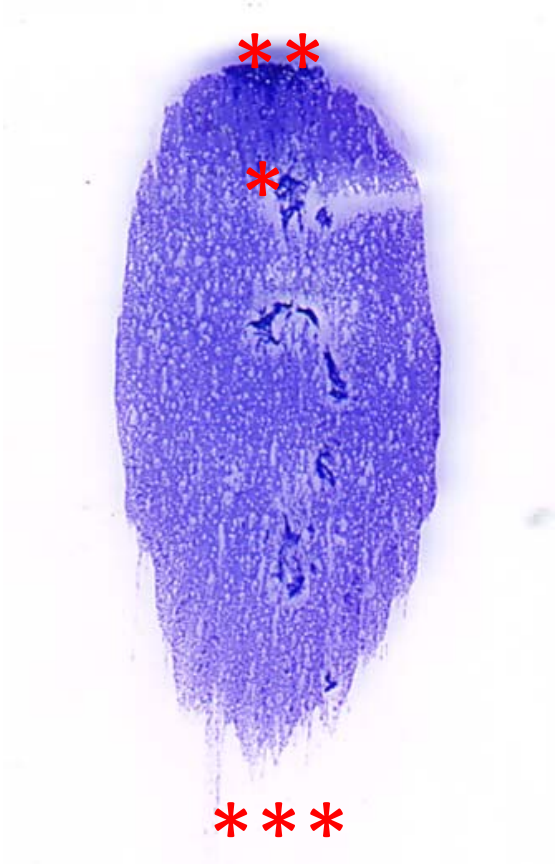
- Usually hemodiluted
- Determine what cells are there and look for abnormal morphology
- Correlate with findings in peripheral blood and immunophenotyping
- These samples are never satisfactory as a sole basis for treatment decisions.




5x

The Lymphoid Smear

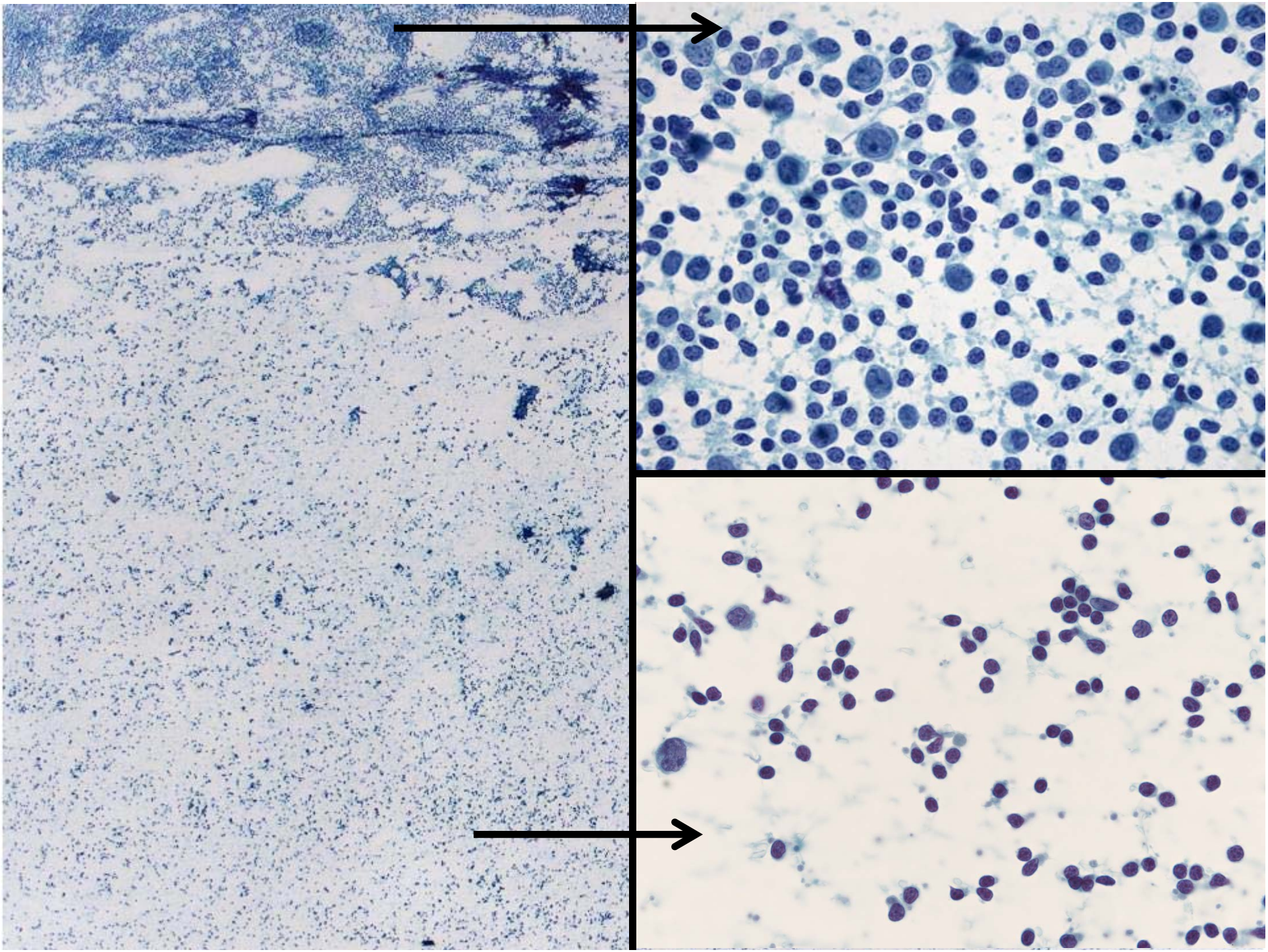
- Dispersed cells in a oval pattern with a feathered edge
- Often clumps due to lymphovascular tangles in the center of the smear



34 year old woman

FNB of 9 mm cervical lymph node to rule out metastatic papillary carcinoma

“Mobile” smaller elements such as RBC’s and small lymphocytes are pushed to the periphery and tissue fragments remain central.



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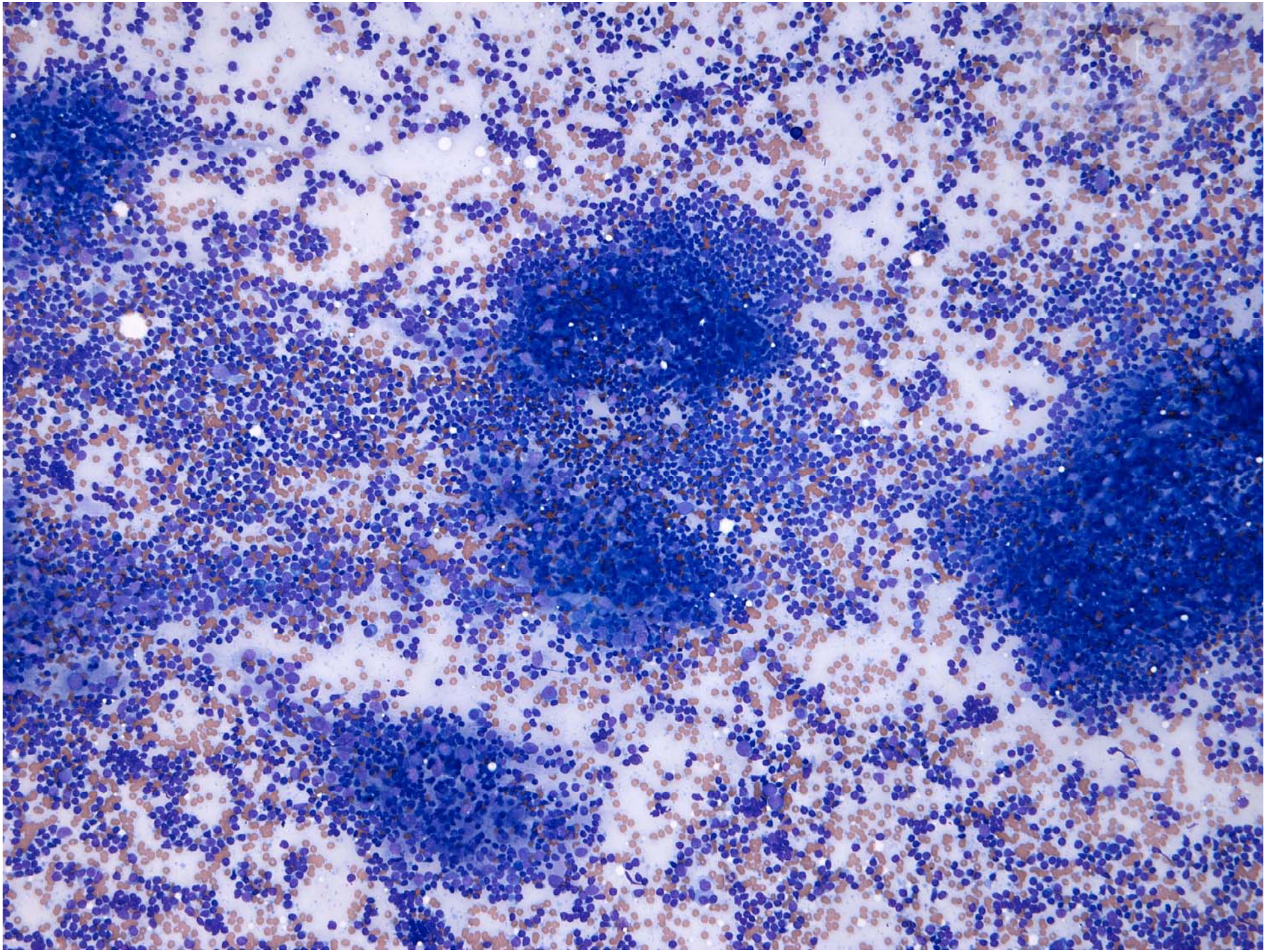
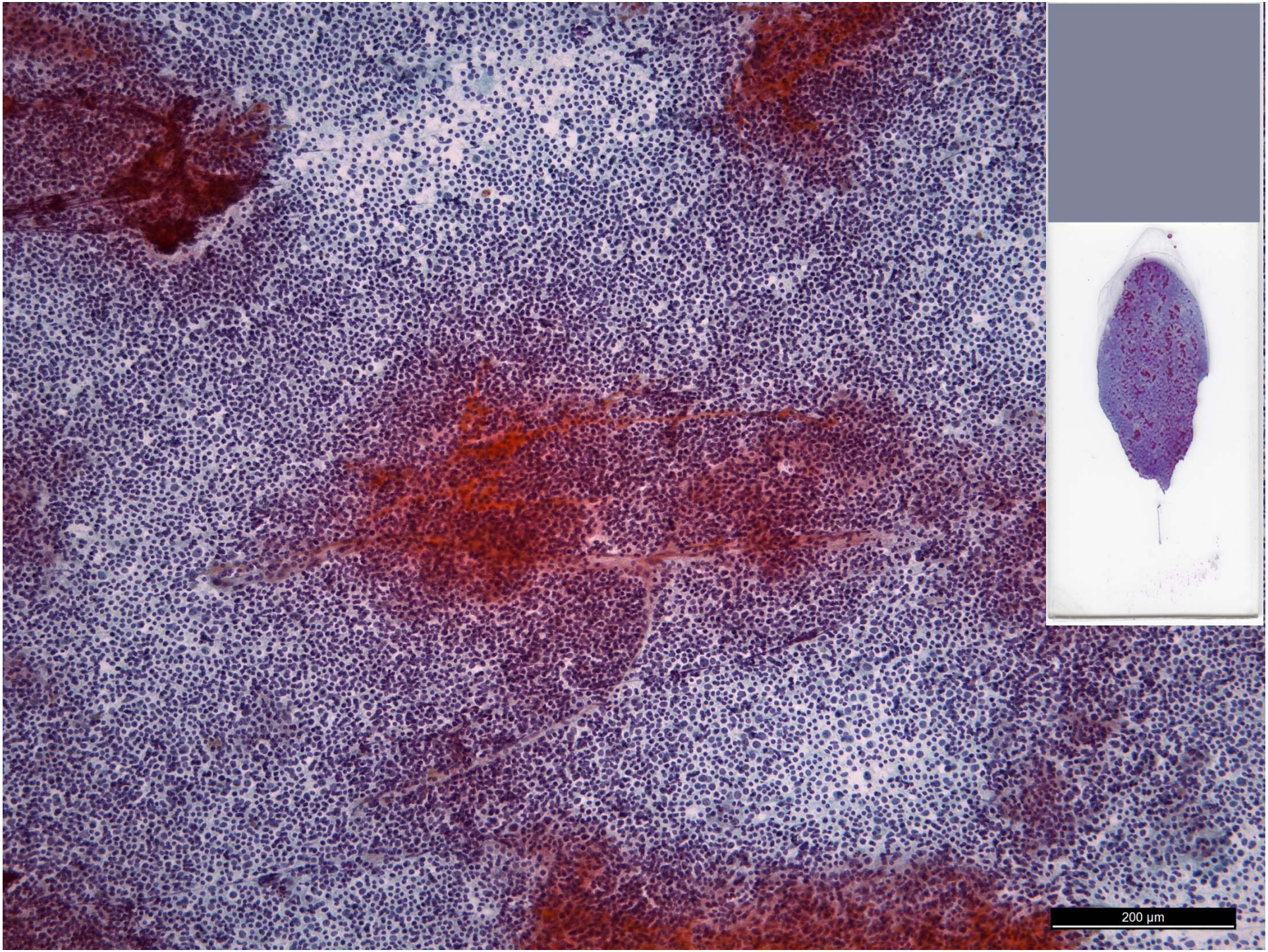
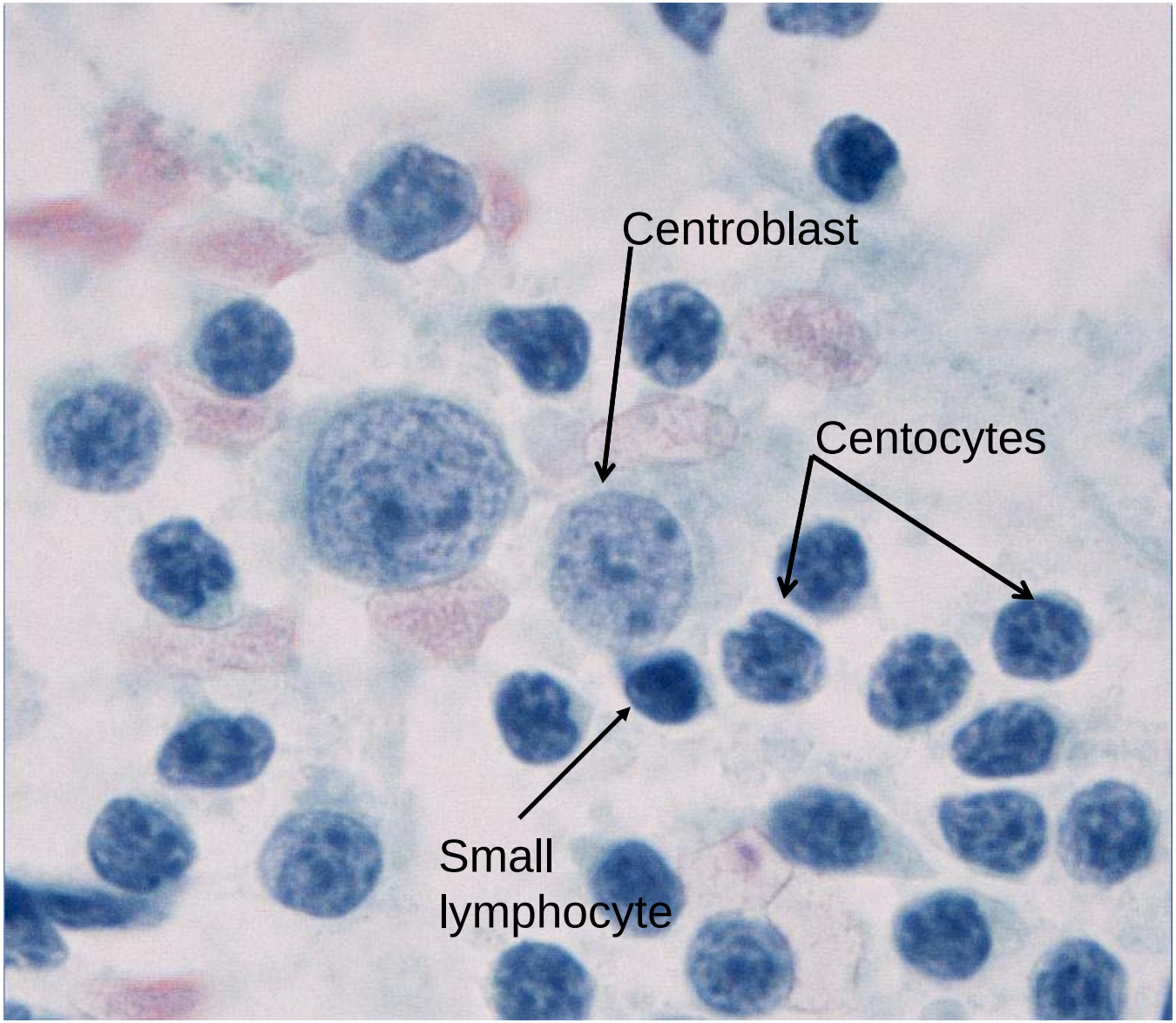


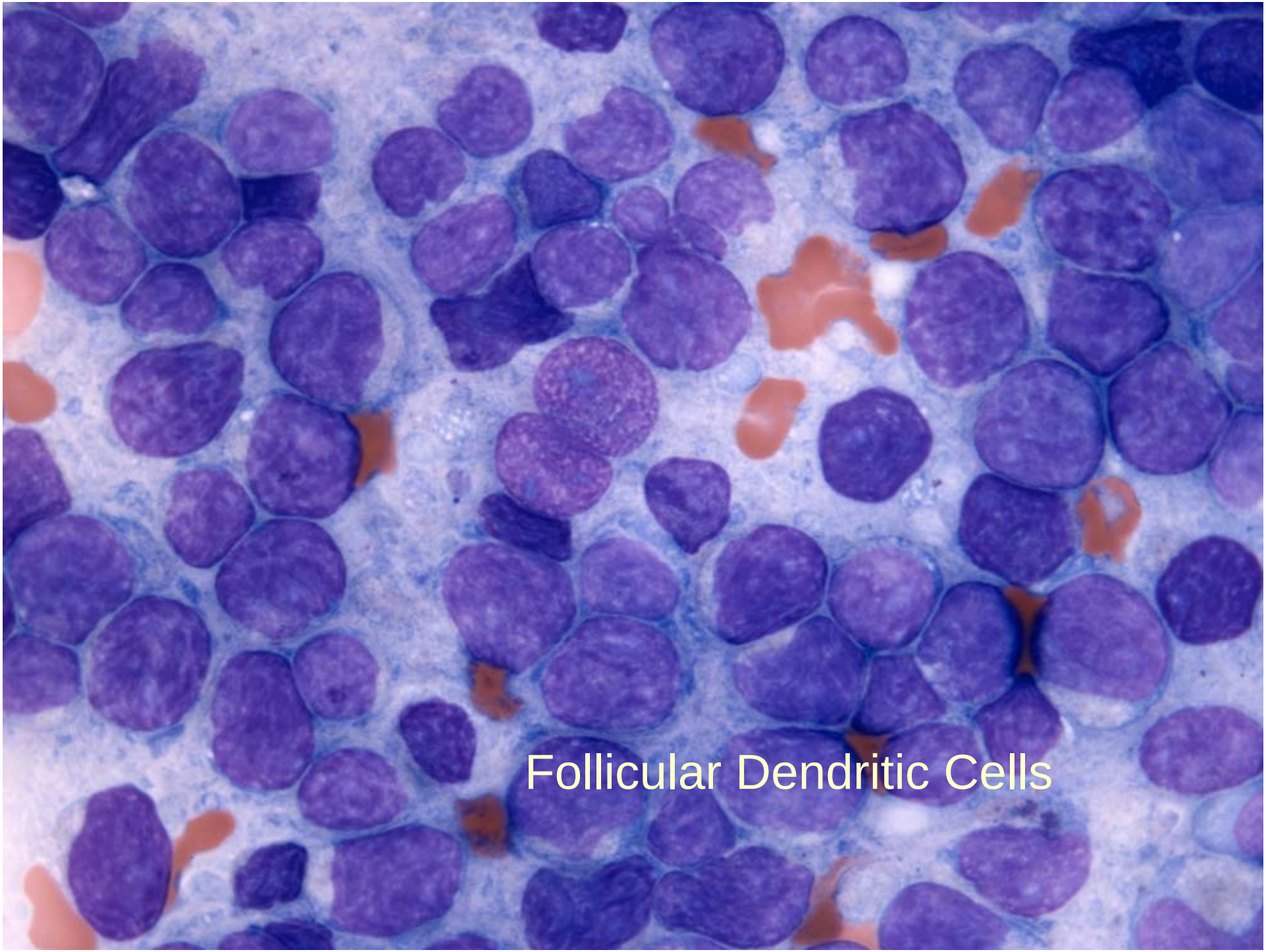

200 µm

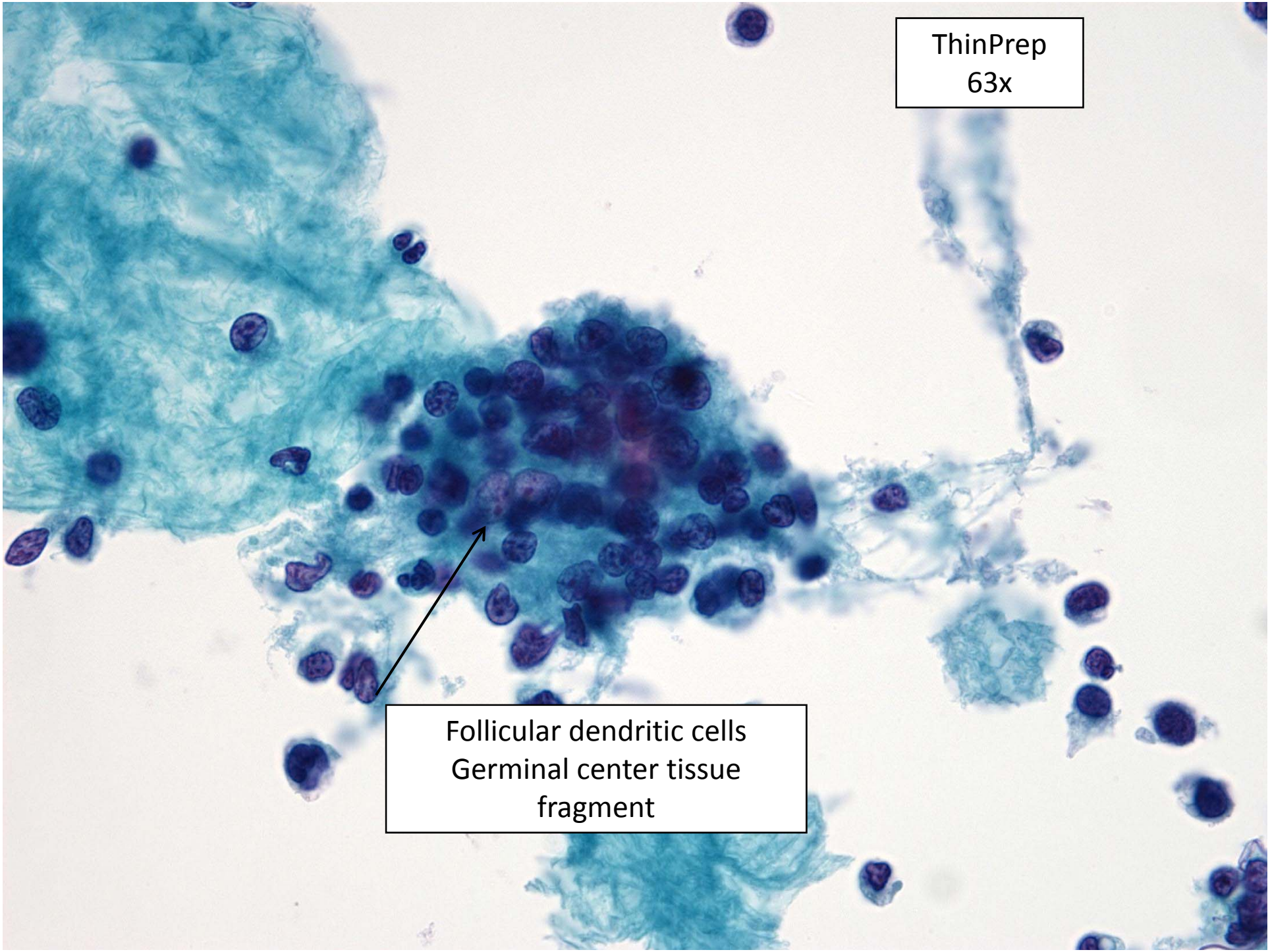


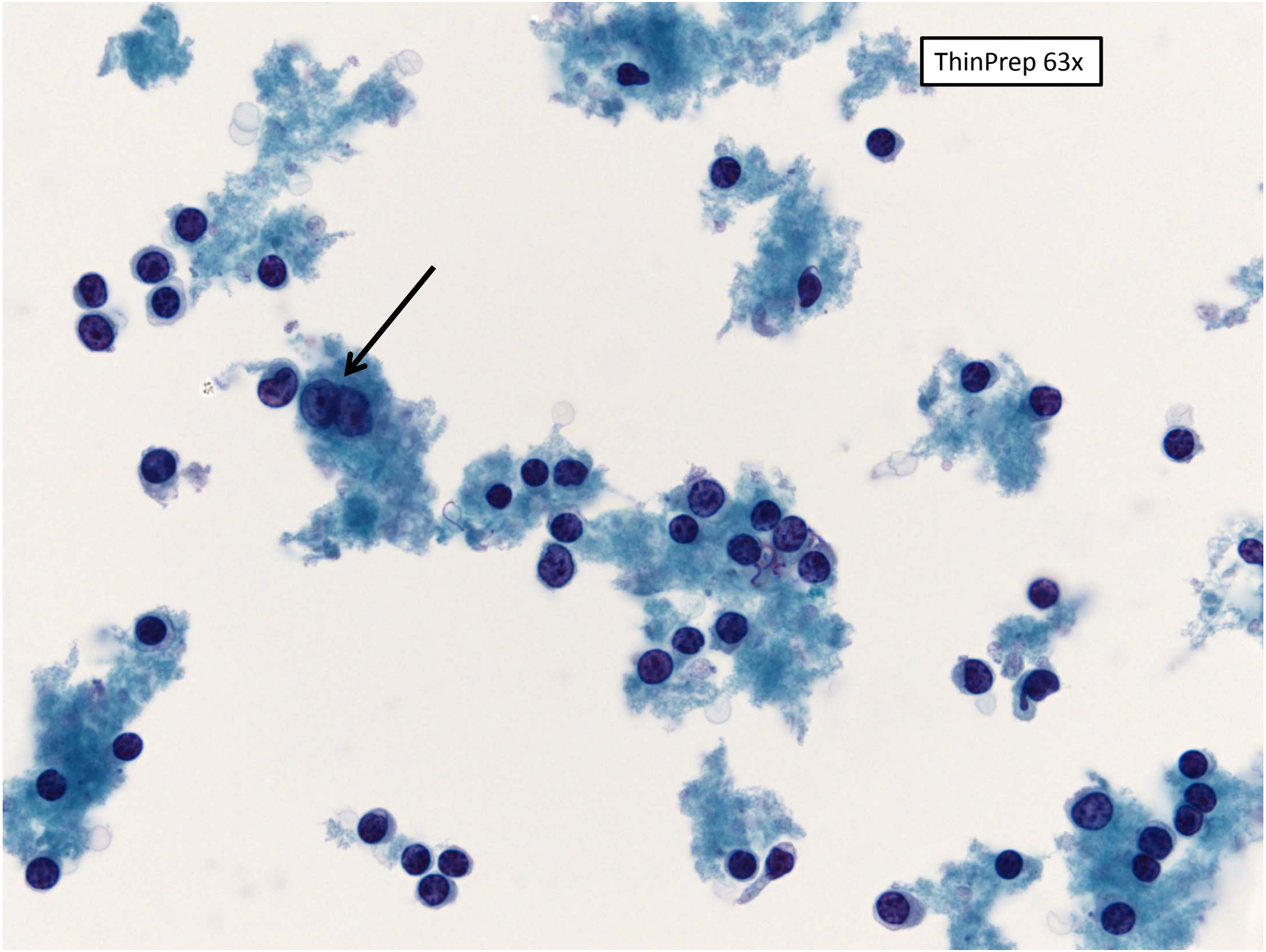
Centrocytes
-Clumped chromatin
-May or may not show an indentation
-Do not have conspicuous nucleoli

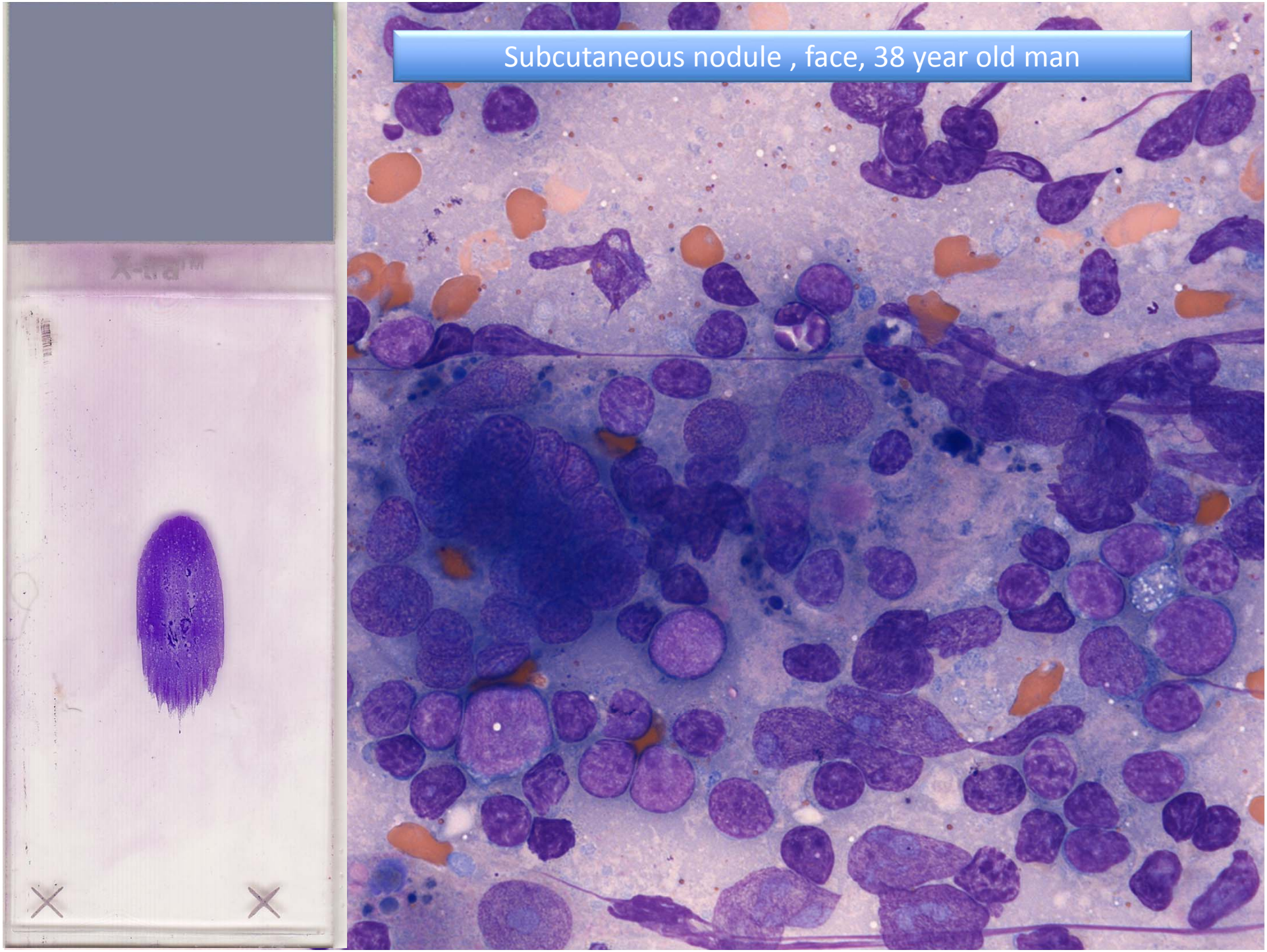
Centroblasts
-Finely dispersed chromatin
-May show a cleft or indentation
-2-4 peripheral nucleoli

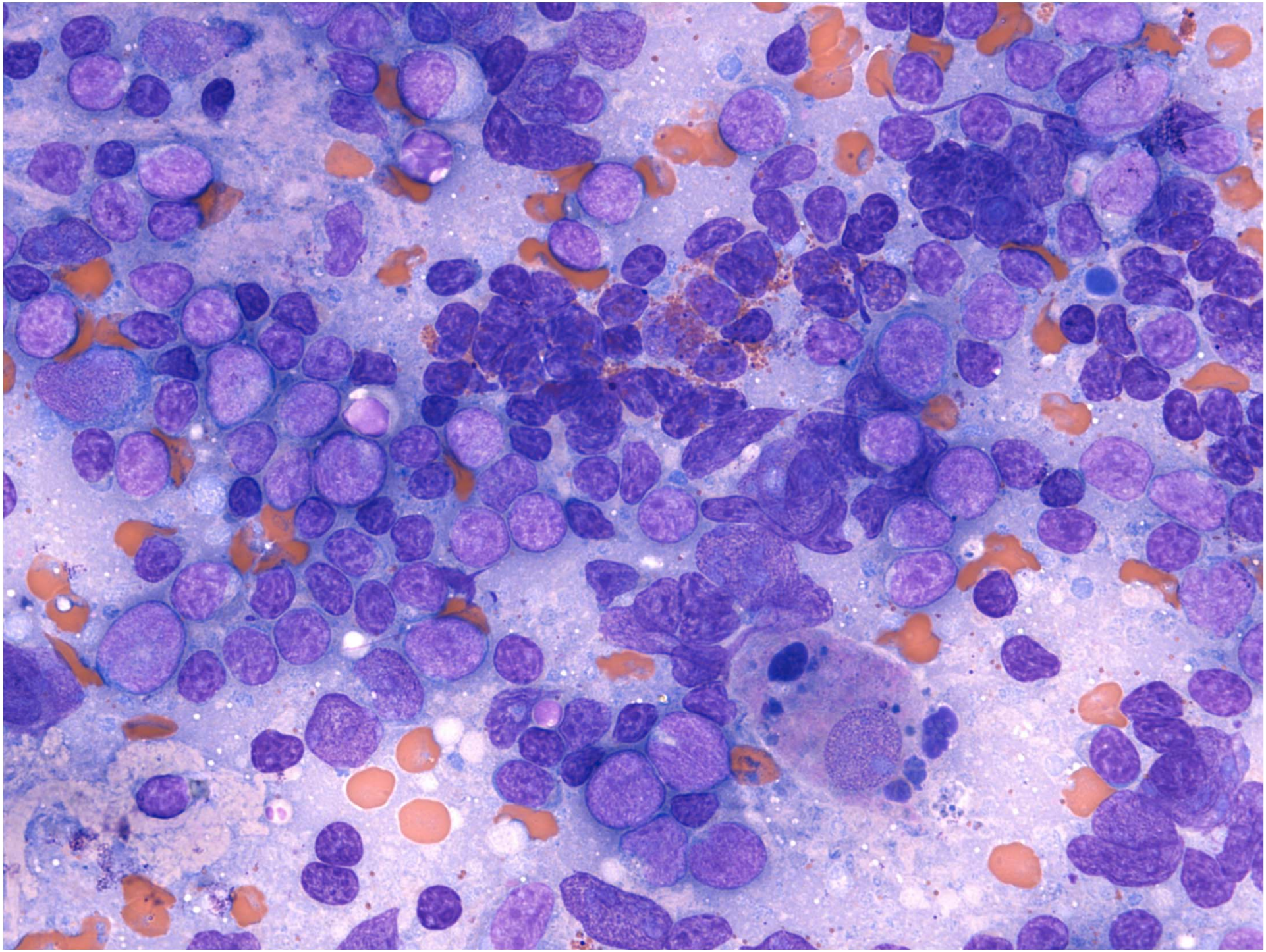
Centrocytes and Centroblasts - “large centrocytes” may overlap in size with “small centroblasts”.


Follicular Dendritic Cells


ThinPrep
63x
Follicular dendritic cells
Germinal center tissue
fragment


ThinPrep 63x


Subcutaneous nodule , face, 38 year old man



"Warthin-Finkeldey" type giant cells

- Actually first described by Italian pathologist Carmelo Ciaccio and ENT surgeon Gaspare Alagna as giant cells in the lungs and tonsils of children who died in a measles epidemic in Palermo in 1908

Ciaccio C. *Beitrag zur pathologischen Anatomie und zur Mikrobiologie der Masern*. Virchows Arch 1910; 199: 378-400

Alagna G. *Histopathologische Veränderungen der Tonsille und der Schleimhaut der ersten Luftwege bei Masern*. Arch Laryng Rhin 1911;25:527-530

WFL Giant Cells

- Reactive lymphoid conditions
  - Reactive lymphoid hyperplasia, lymphoid tissue around metastatic deposits
- Autoimmune conditions
  - Hashimoto's, SLE
- HIV related lymphadenopathy
- Kimura disease
- Neoplastic conditions
  - Follicular lymphoma, NLPHL, HL, follicular dendritic cell sarcoma

WFL Giant Cells

- By immunophenotyping orign thought to be T-cell, B-cell and follicular dendritic cells
- Perhaps different in measles and other conditions
- By morphology polykaryosomes appear to be of follicular dendritic cell origin.
- Not to be mistaken for Langhans, Touton, foreign body, osteoclastic GC's or megakaryocytes, or multinucleated malignant cells of any kind

Diagnostic Cytopathology

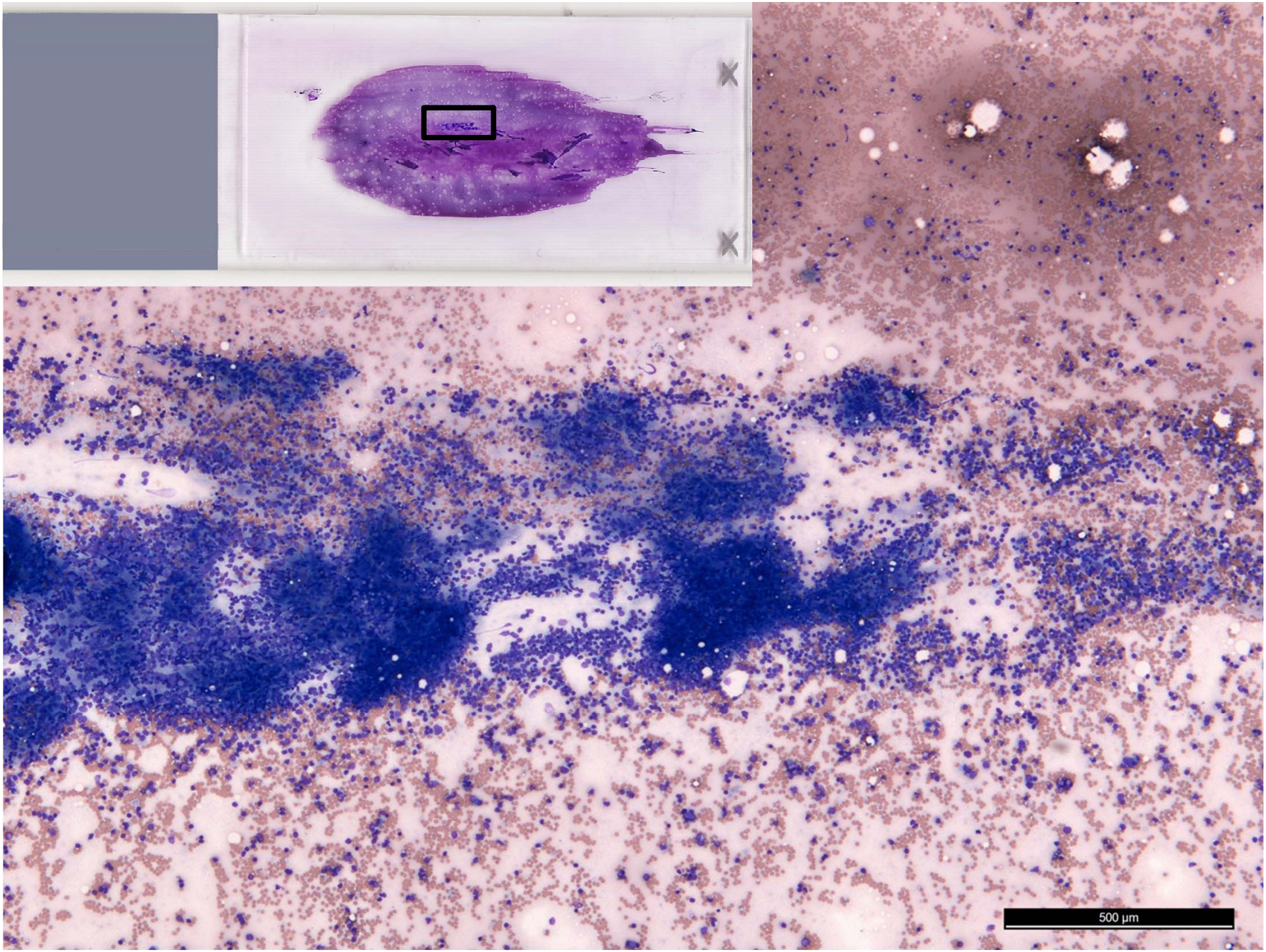
Explore this journal >

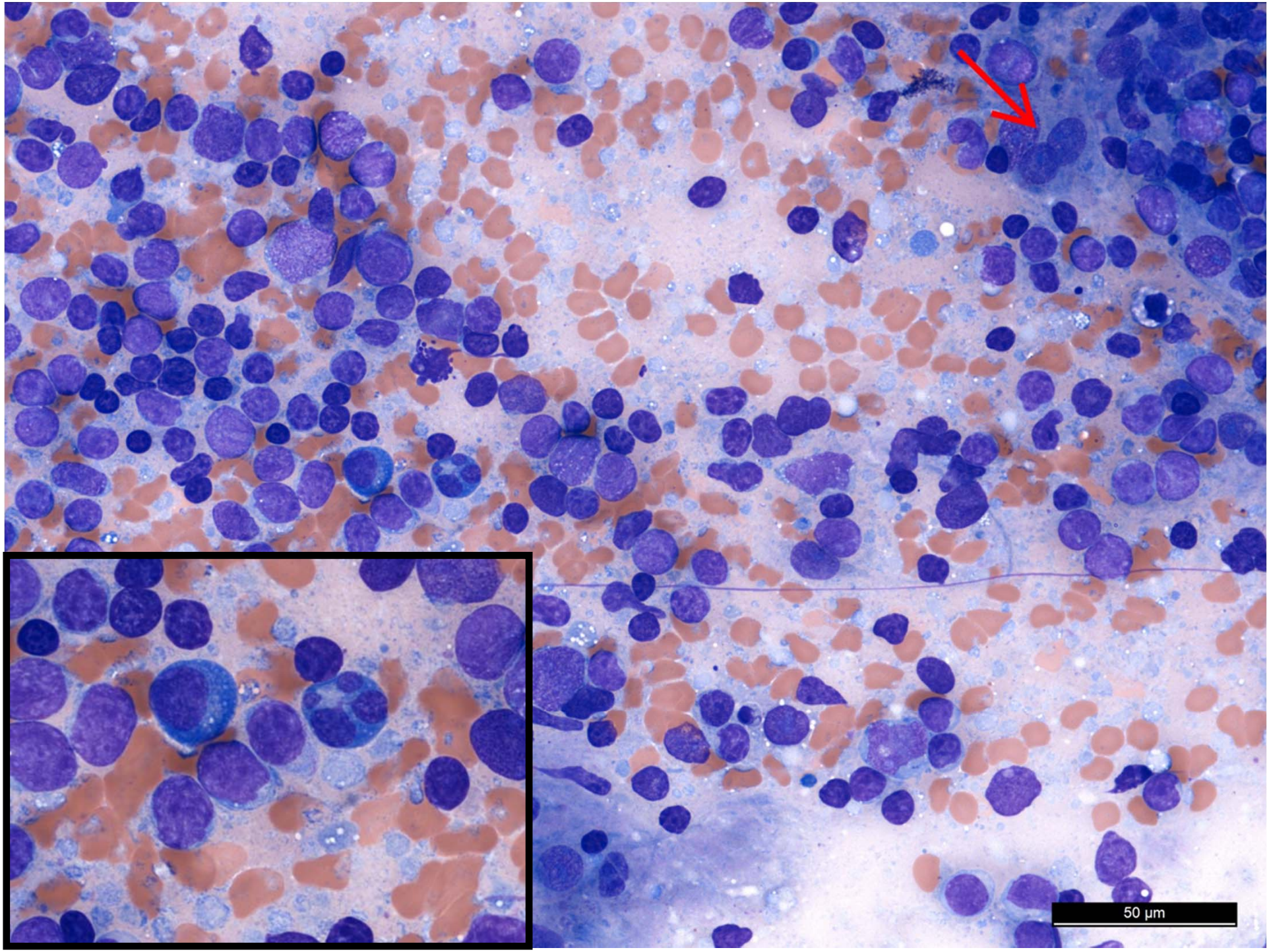
Images in Cytology

Mulberry cells in the thyroid: warthin–finkeldey-like cells in hashimoto thyroiditis-associated lymphoma

Razvan Lapadat ✉, Moon Woo Nam, Swati Mehrotra, Milind Velankar, Stefan E. Pambuccian

Diagn. Cytopathol., 45: 212–216. doi:10.1002/dc.23652


500 µm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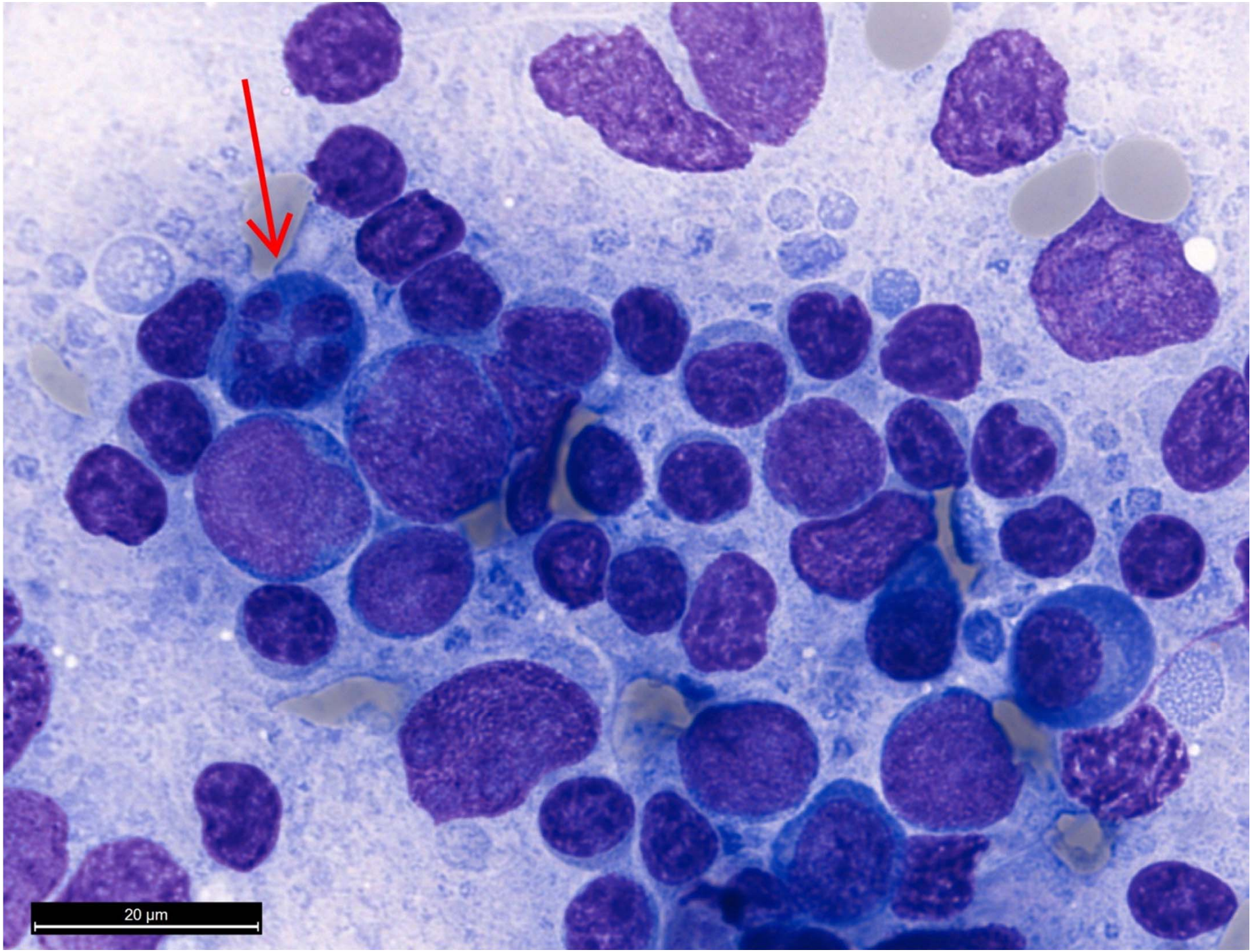
20 µm

IMAGES IN CYTOLOGY

Section Editor: Stephan Pambuccian, M.D.

Plasma Cells With Segmented Nuclei in Benign Lymph Node Follicular Hyperplasia

Philippe Benjamin Stephenson, M.D.,[1]* and
William Robert Geddie, M.D., F.R.C.P.C.,[2]

Diagn Cytopathol. 2016 Apr 1. doi: 10.1002/dc.23478. [Epub ahead of print]

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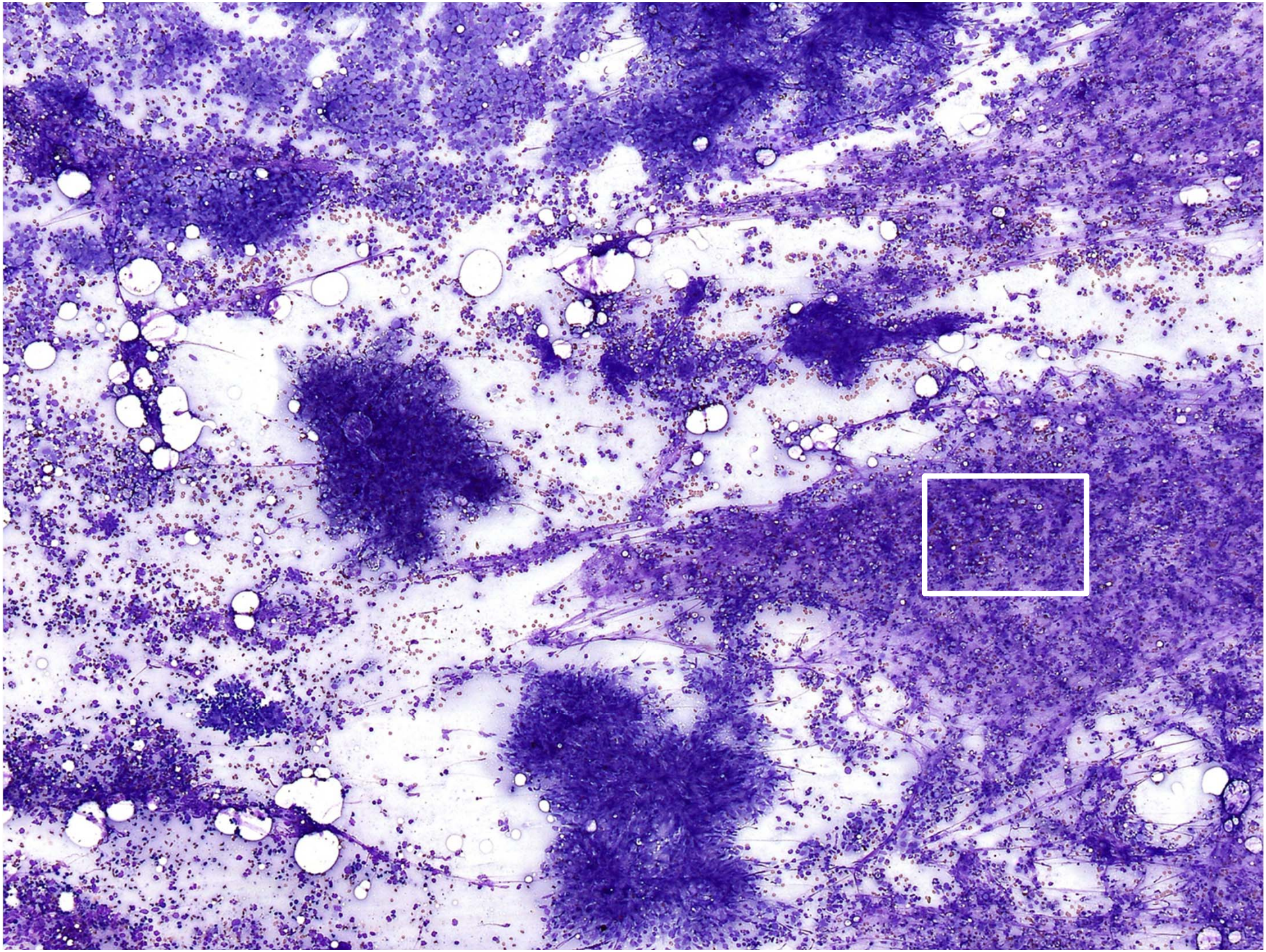
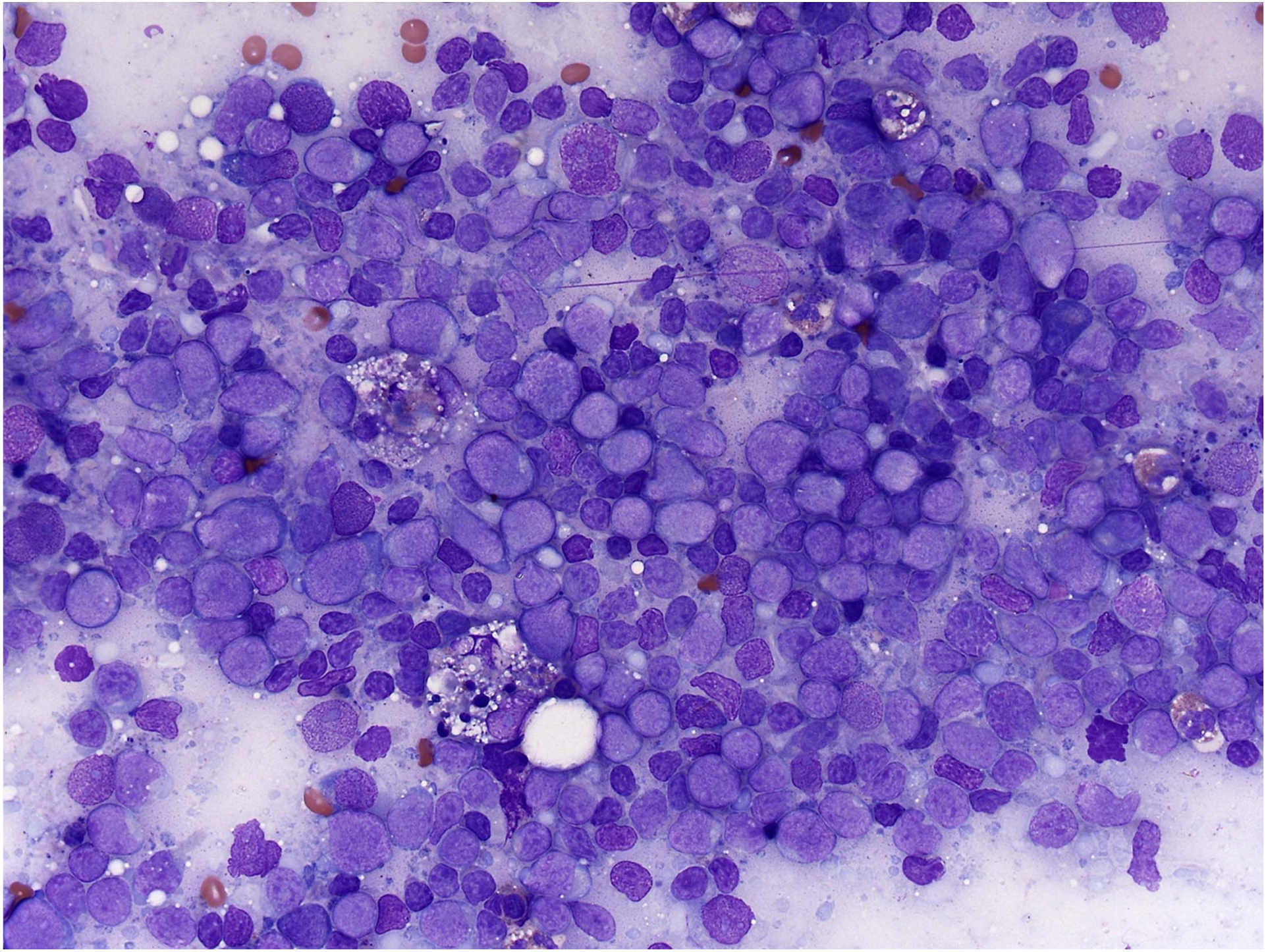
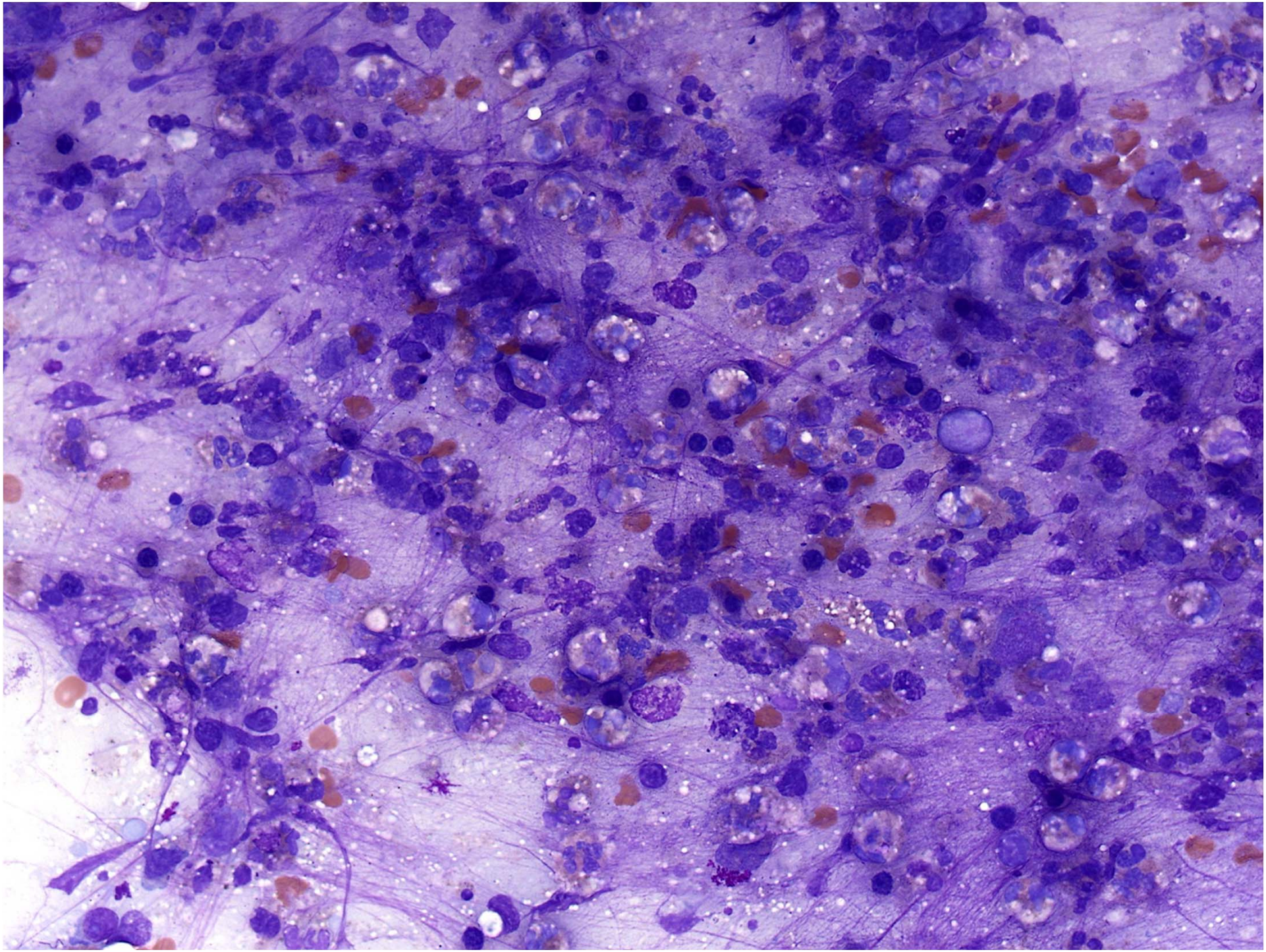
45 yo male,HIV+, CD4/CD8 normal – tender inguinal lymph node

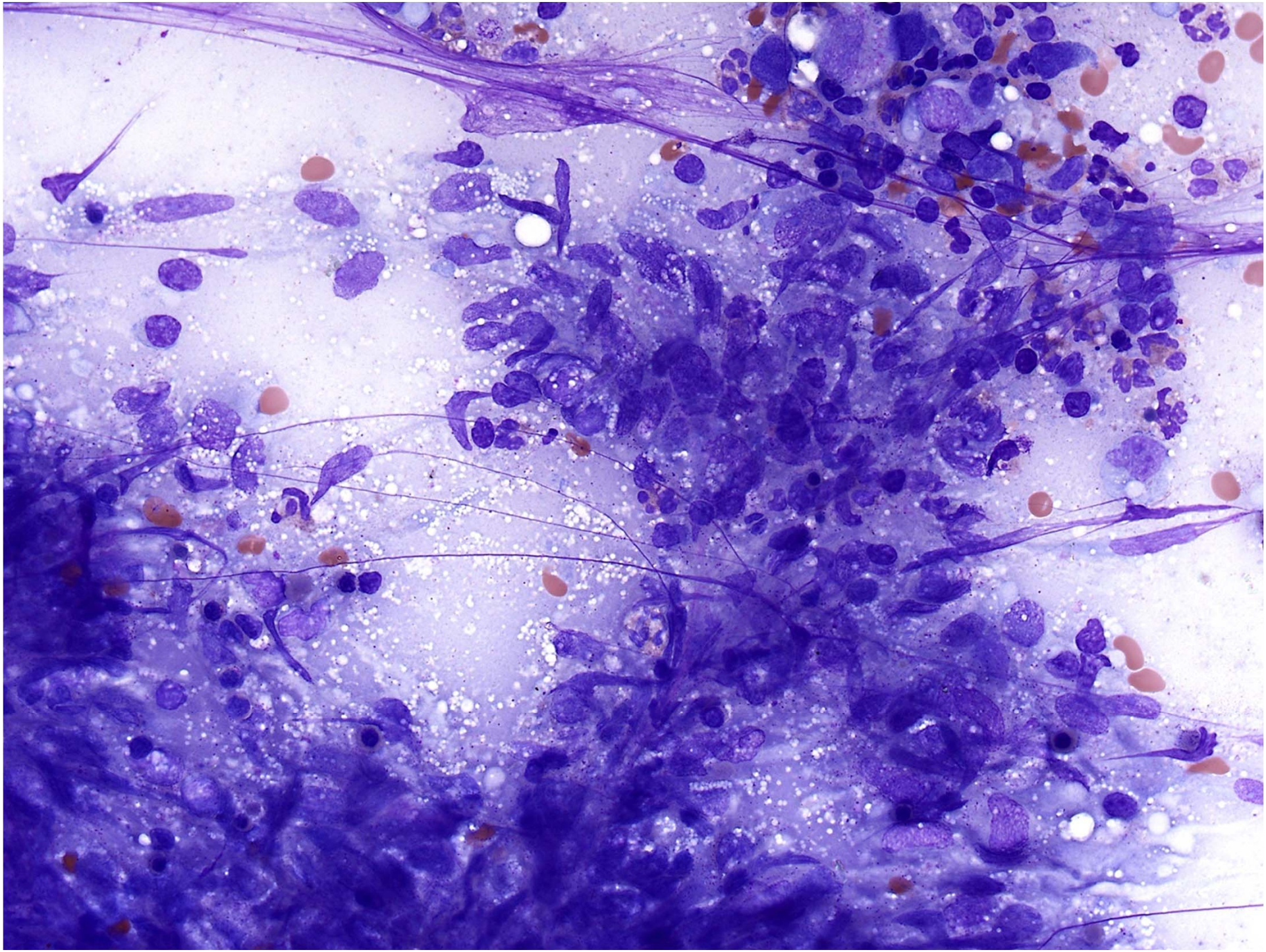
ROSE requested to ”collect sample for flow”

Multiple cores for morphology









Lymphogranuloma Venereum

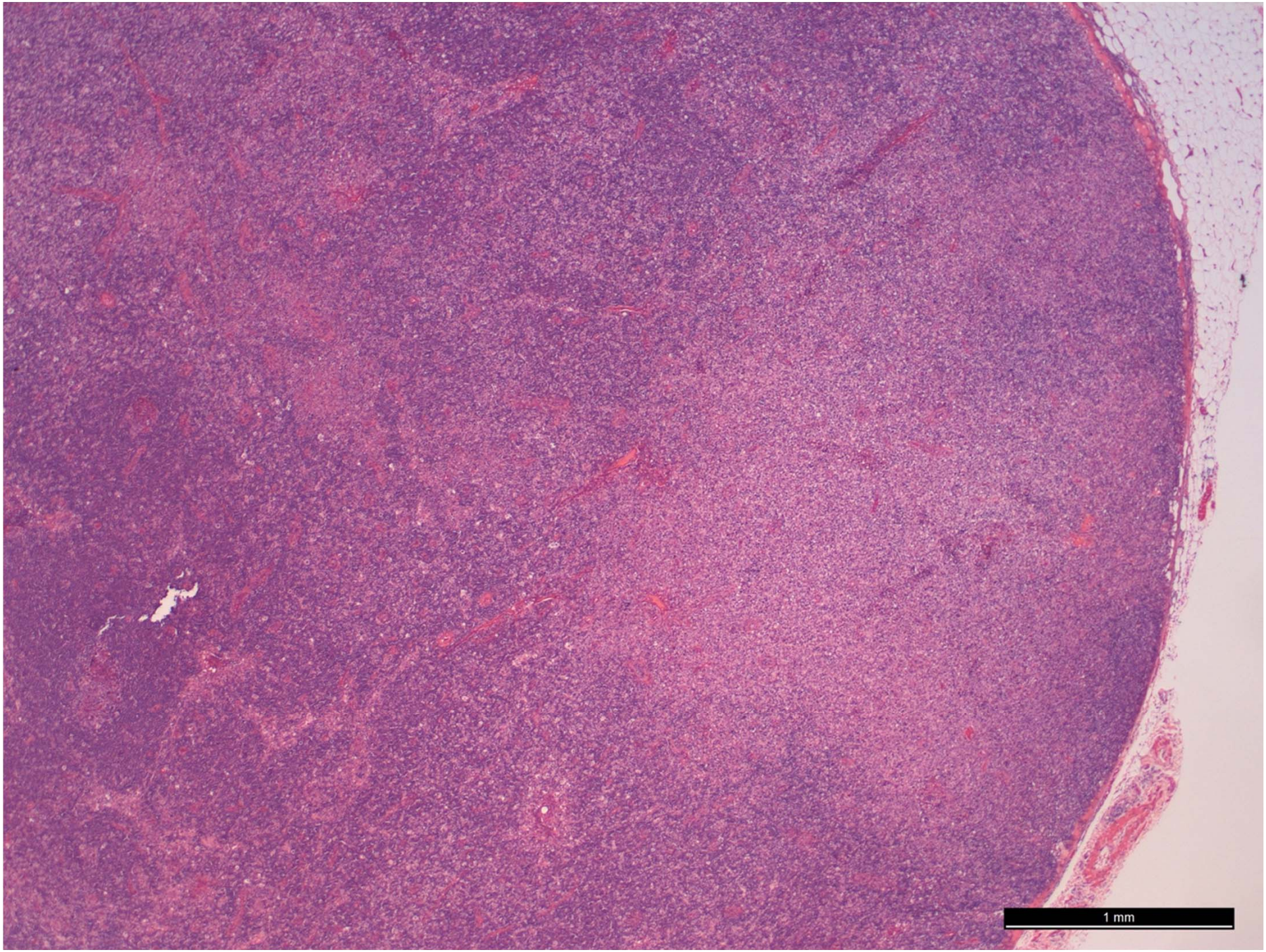
- PCR positive for C. trachomatis (same day)
- The core? - Skeletal muscle only
- Flow not done
  - If it turns out to be necessary later on, FNB can be repeated

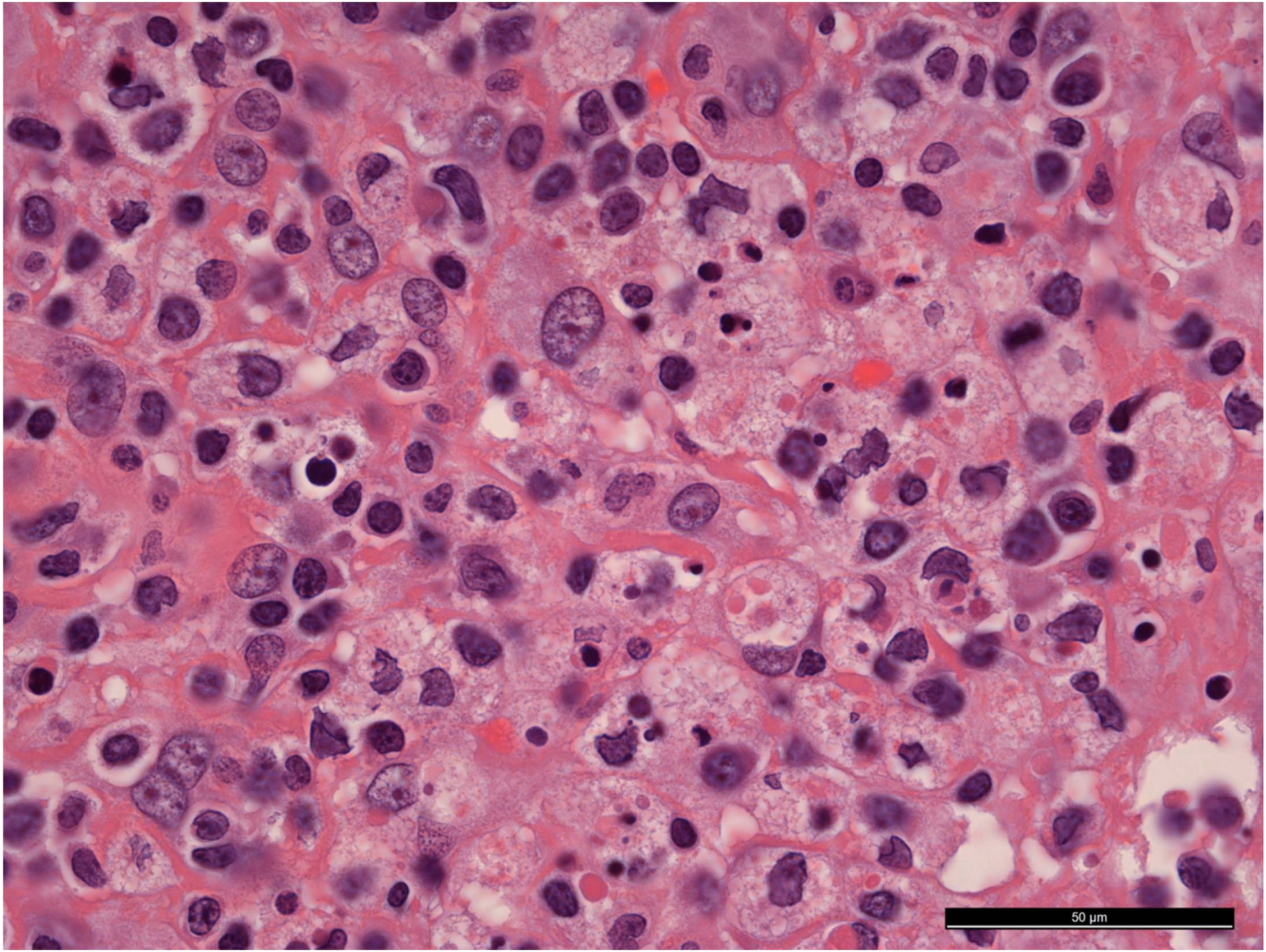
It can be a mistake to send small amounts of sample in too many directions.

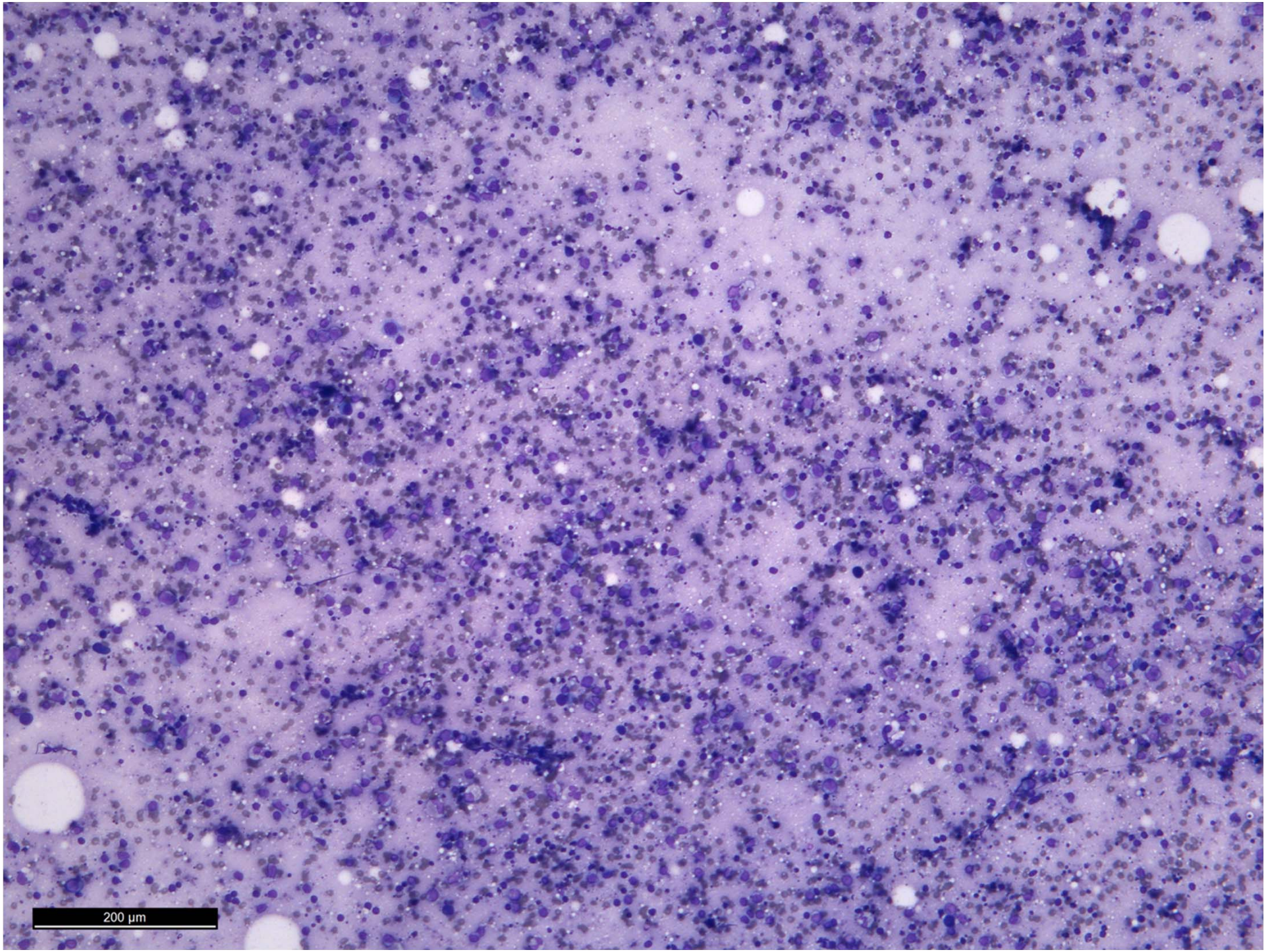
Best to send the bulk of the sample for the test with the greatest chance of being diagnostic.

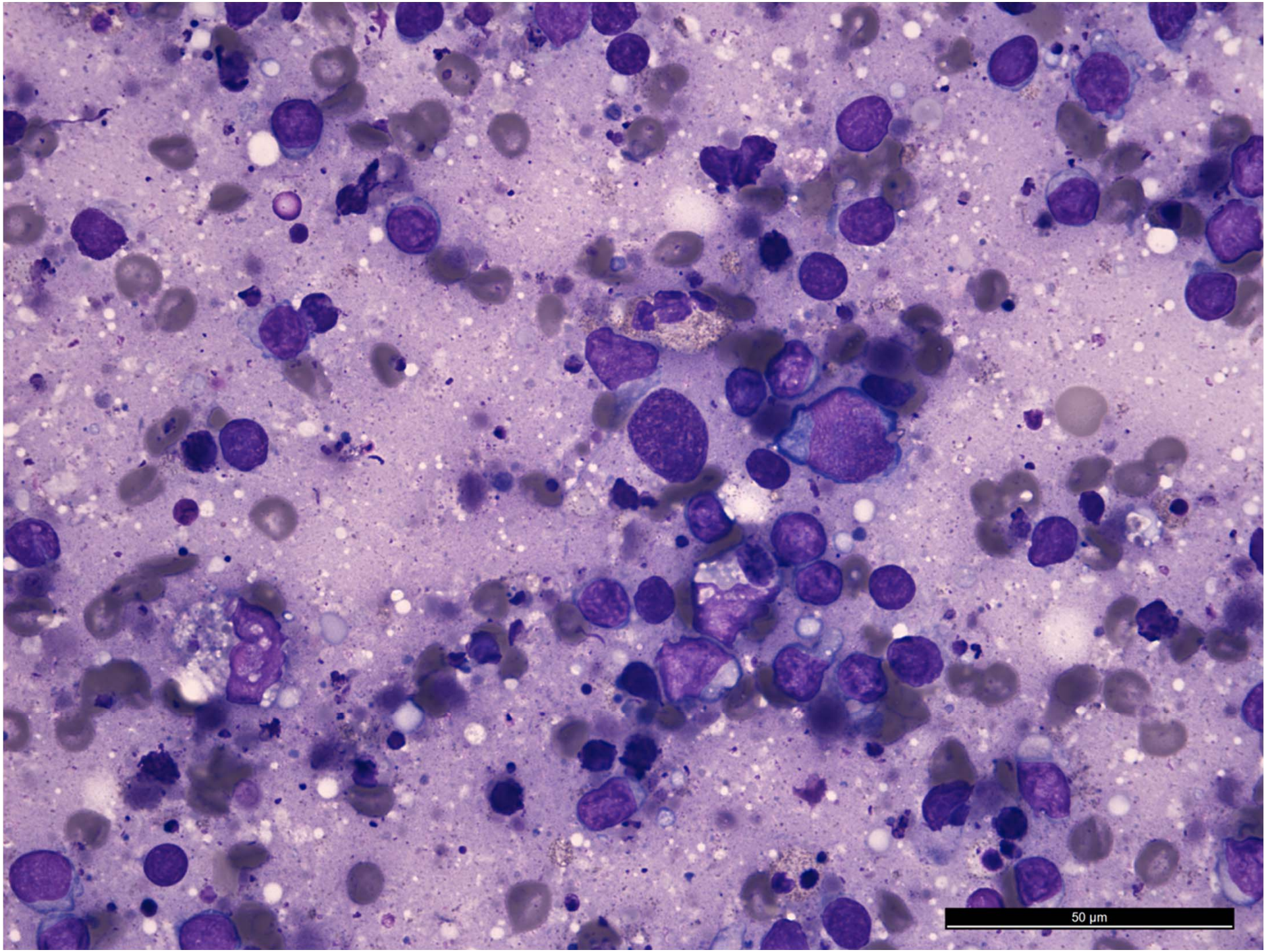
Kikuchi's - Histologic Features

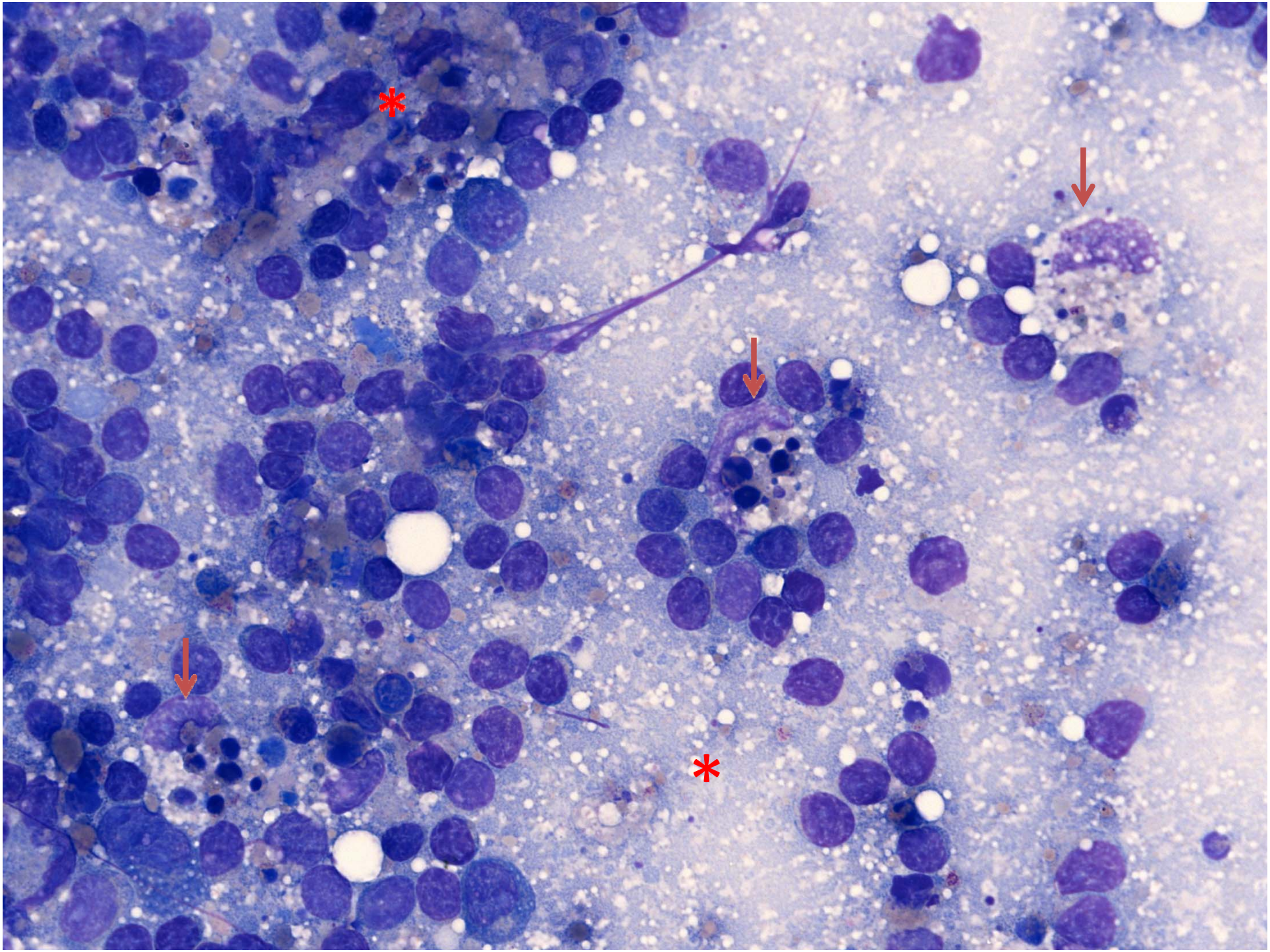
- Paracortical, well demarcated "wedges" of necrosis - starry sky appearance
- Karyorrhexis, fibrin
- "plasmacytoid" monocytes (?CD4+) that may resemble lymphoma
- Histiocytes
- No (or rare) plasma cells and neutrophils
- Absence of follicular hyperplasia
- Occasionally no overt necrosis


1 mm


50 µm

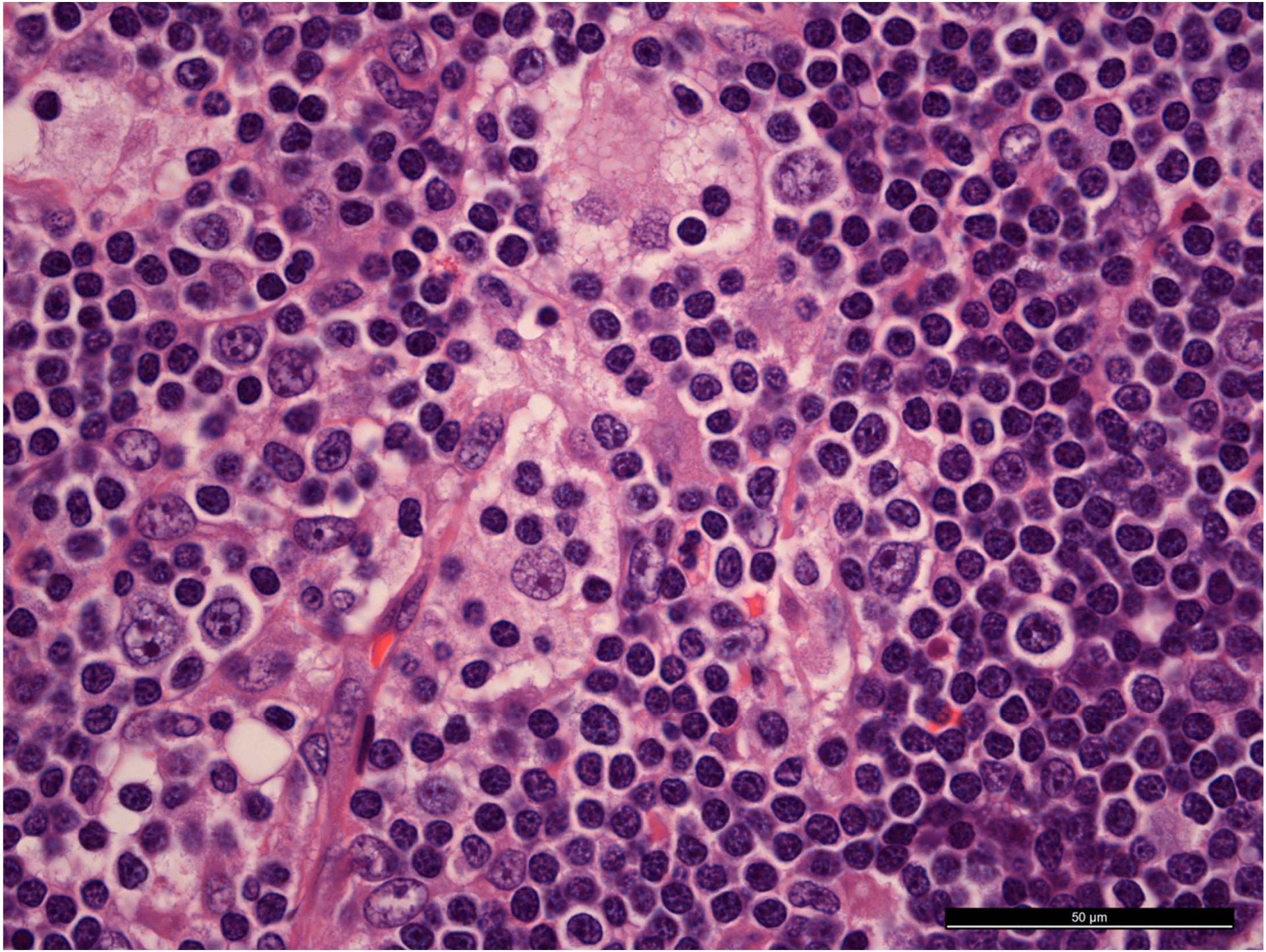

200 µm


50 µm



Rosai-Dorfman Disease

- Capsular inflammation and fibrosis
- Dilated sinuses with large histiocytes showing emperipolesis
- Histiocytes show large round vesicular nuclei with prominent nucleoli - can be multinucleation or "atypia"
- Background of polyclonal plasma cells and lymphocytes - PCs may surround high endothelial venules
- Russell bodies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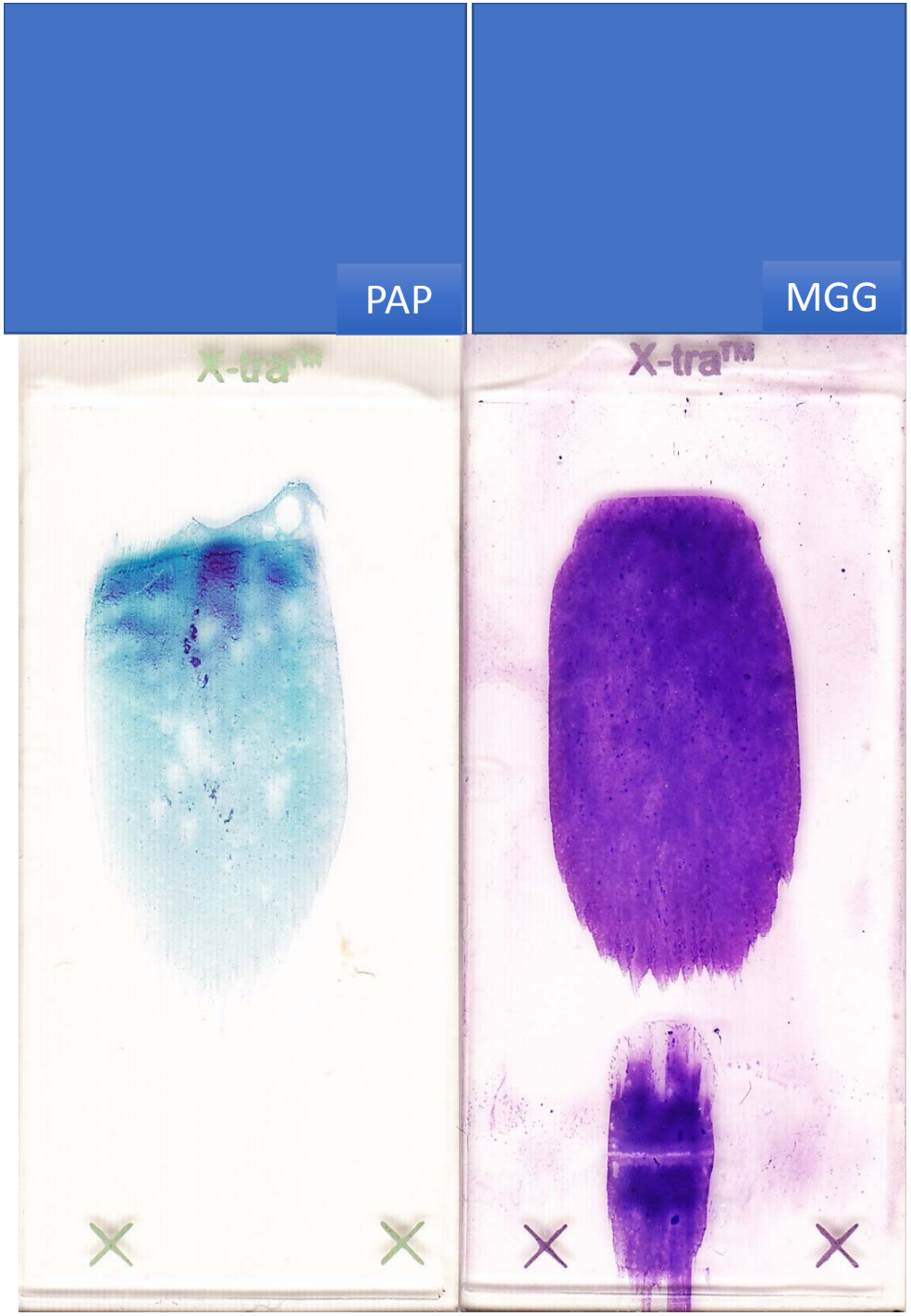
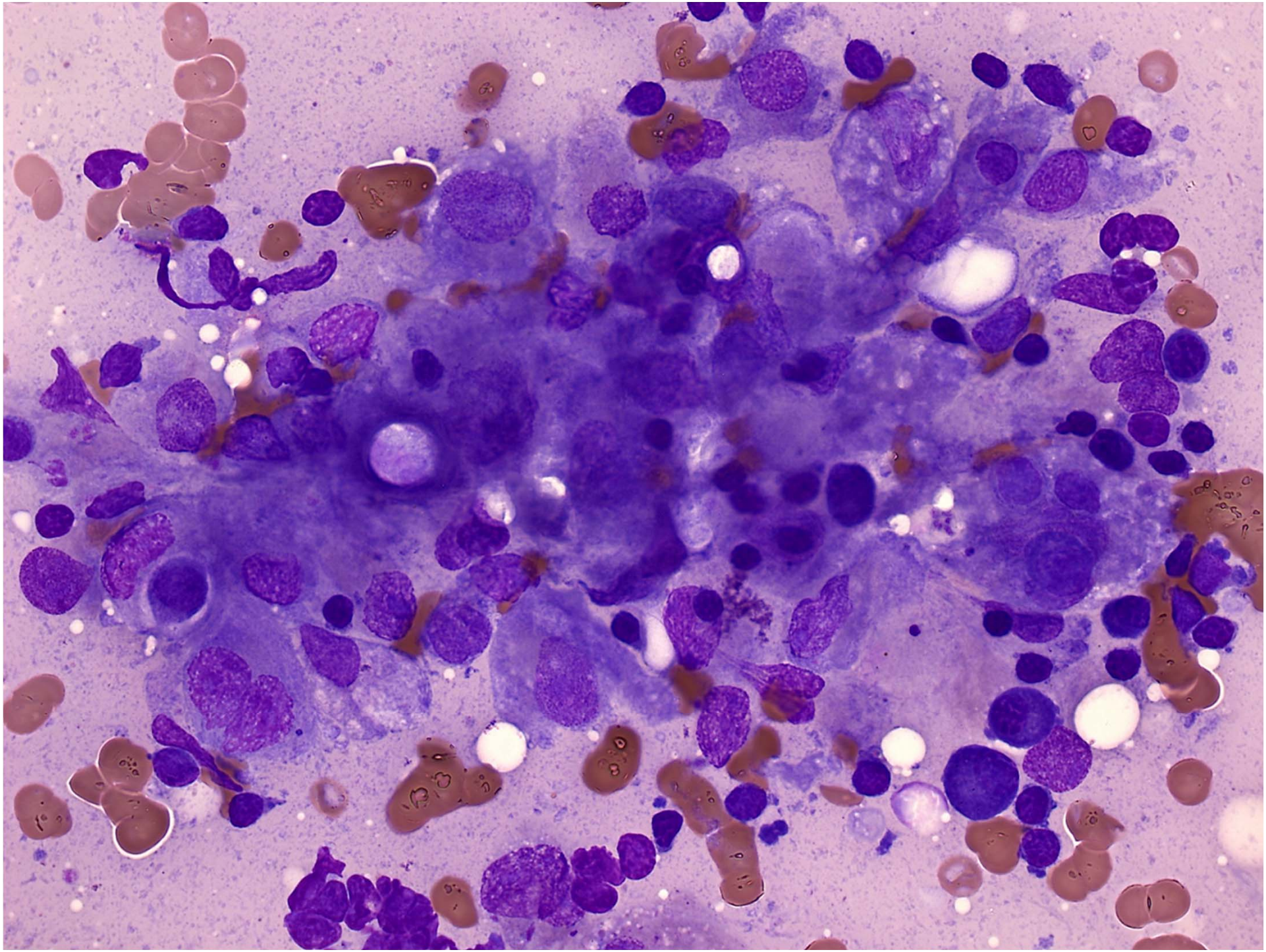
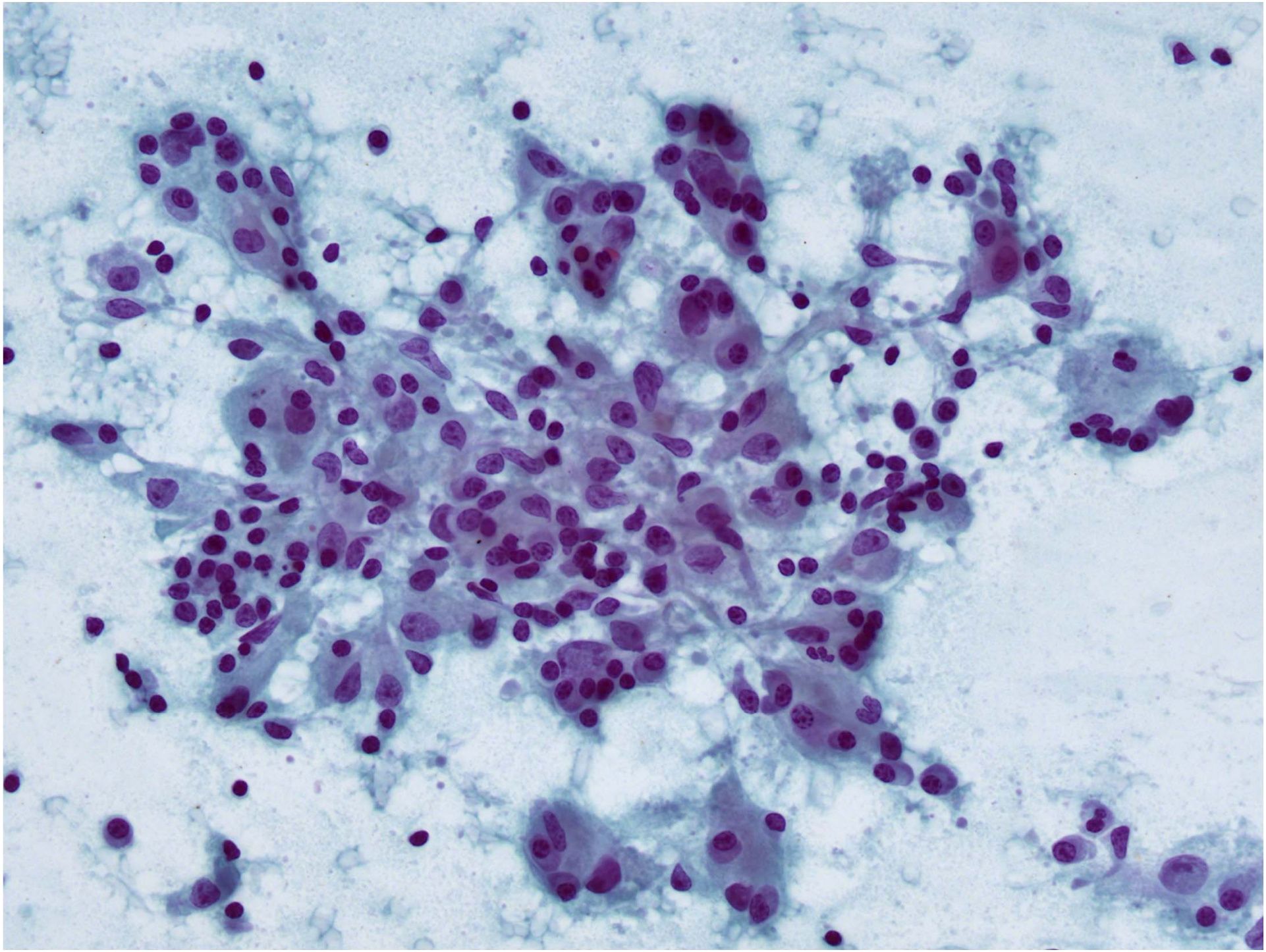
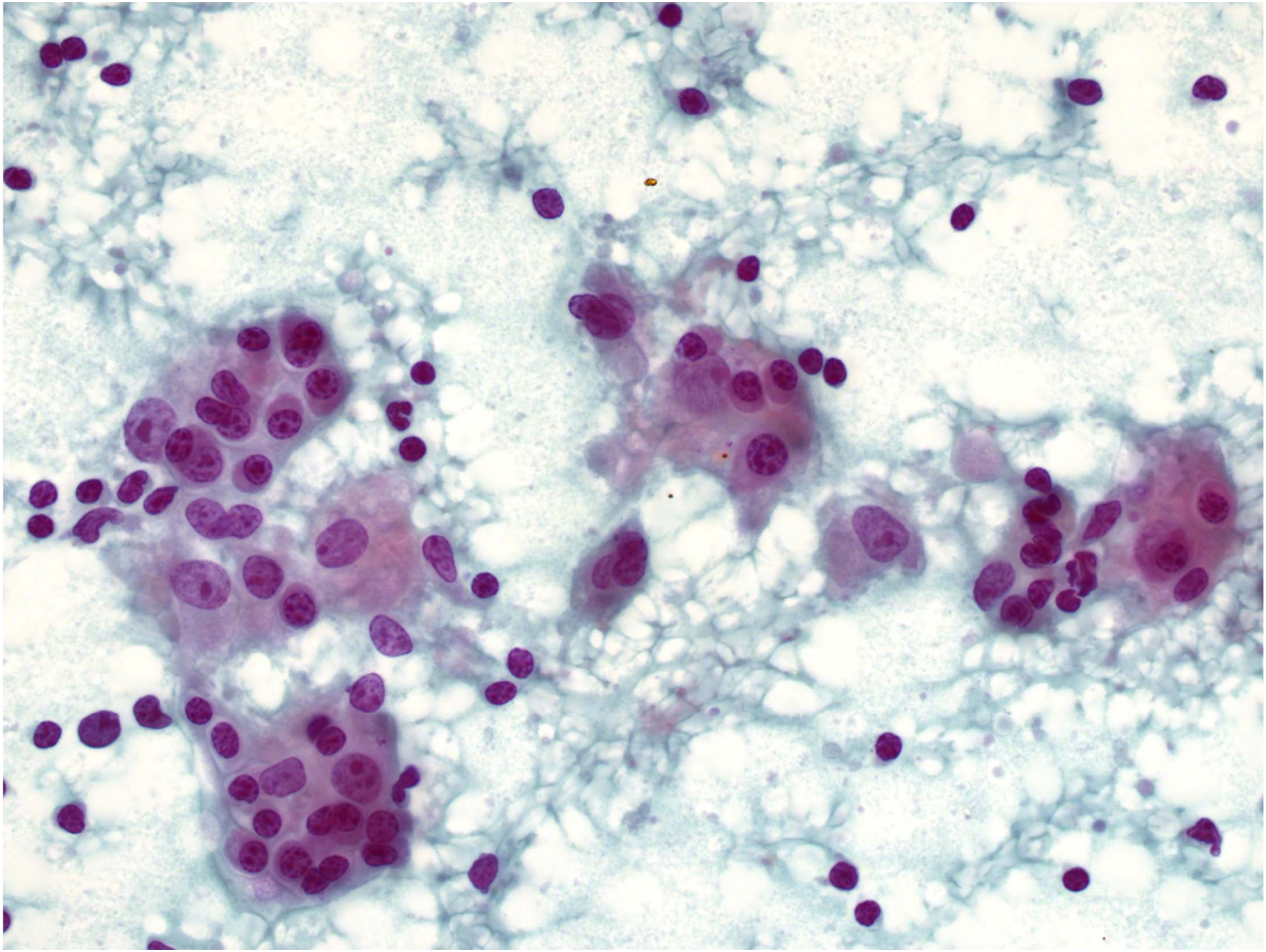
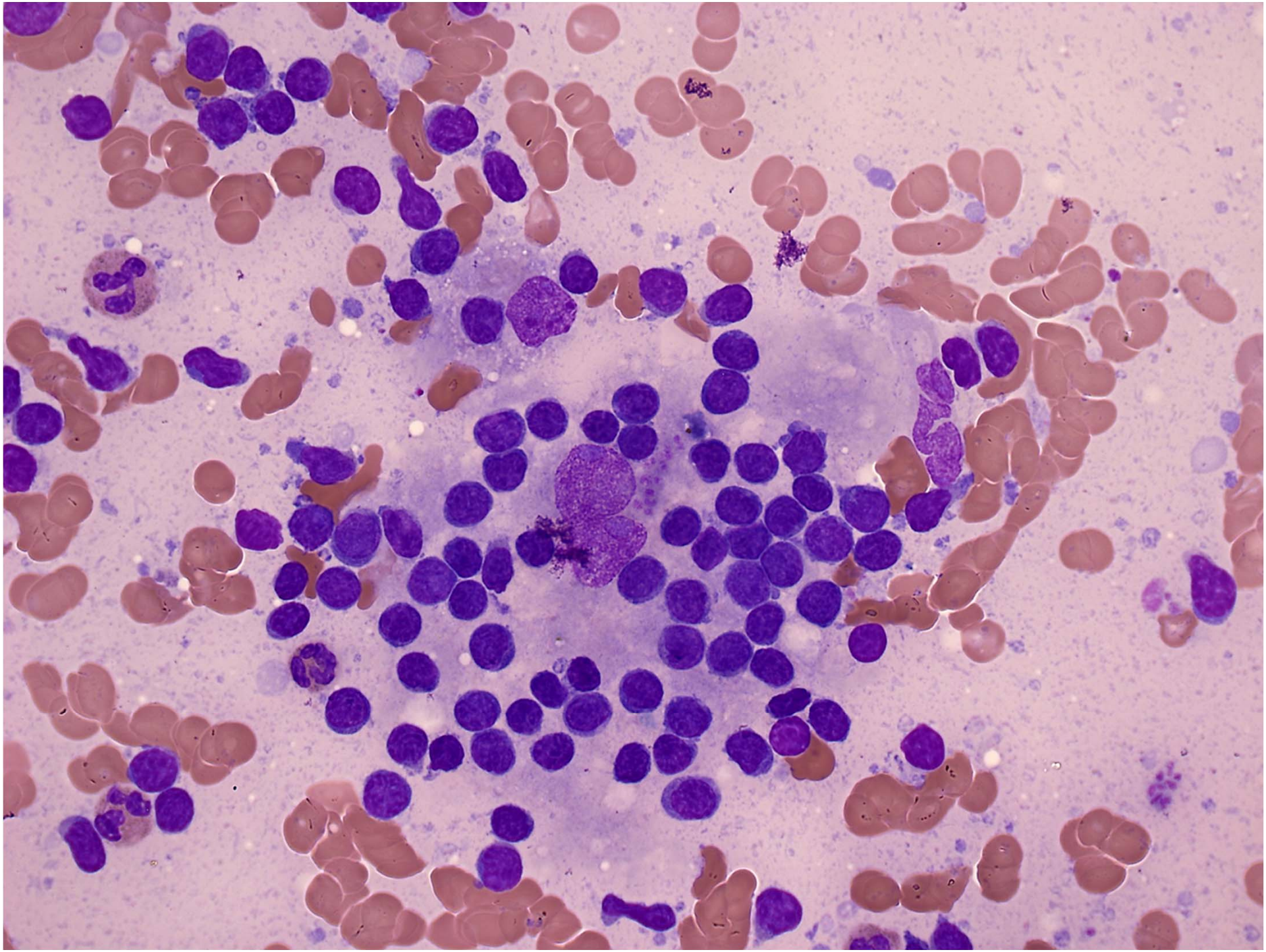


24 year old man, Caucasian, with a subpectoral mass.



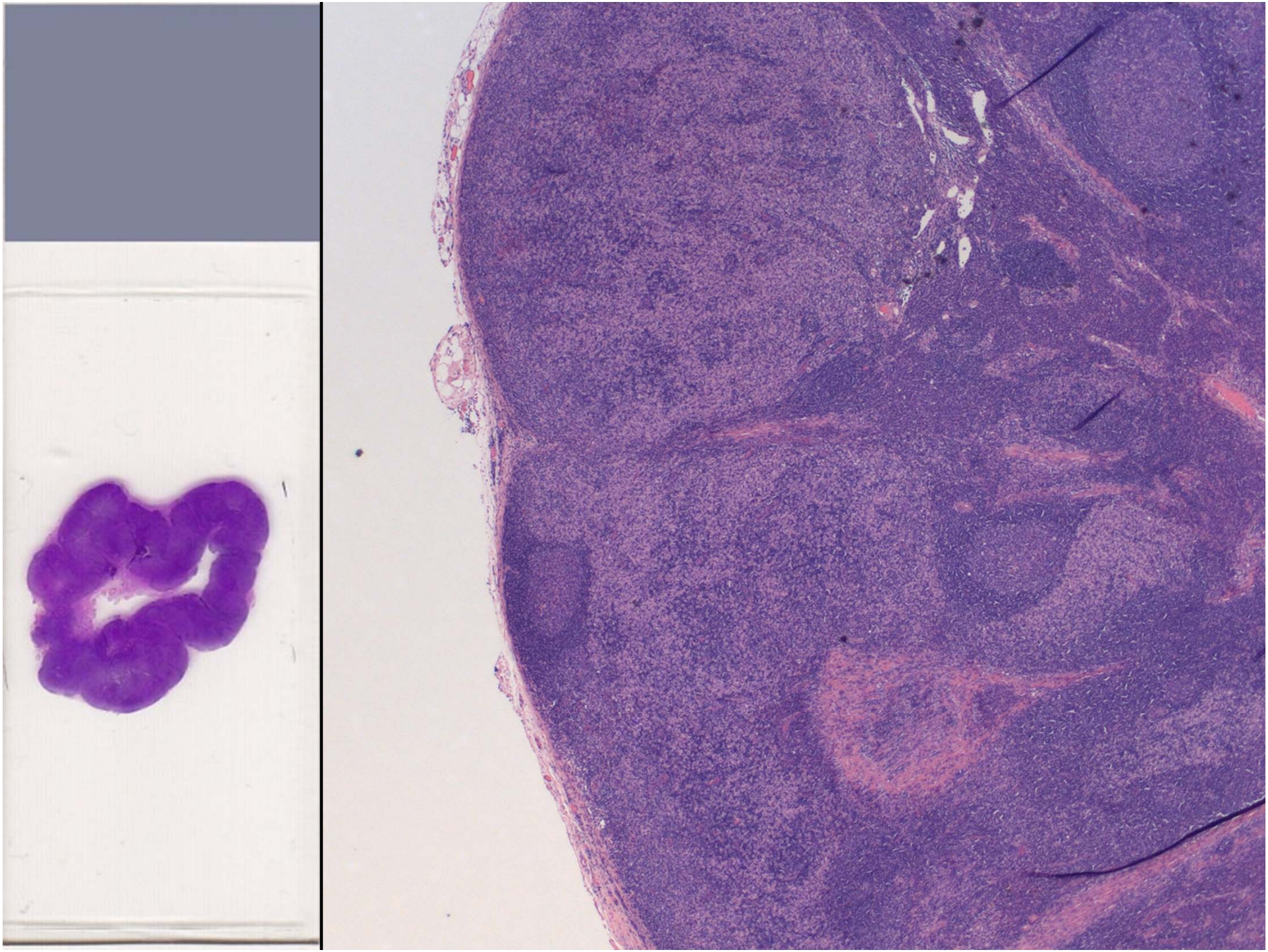


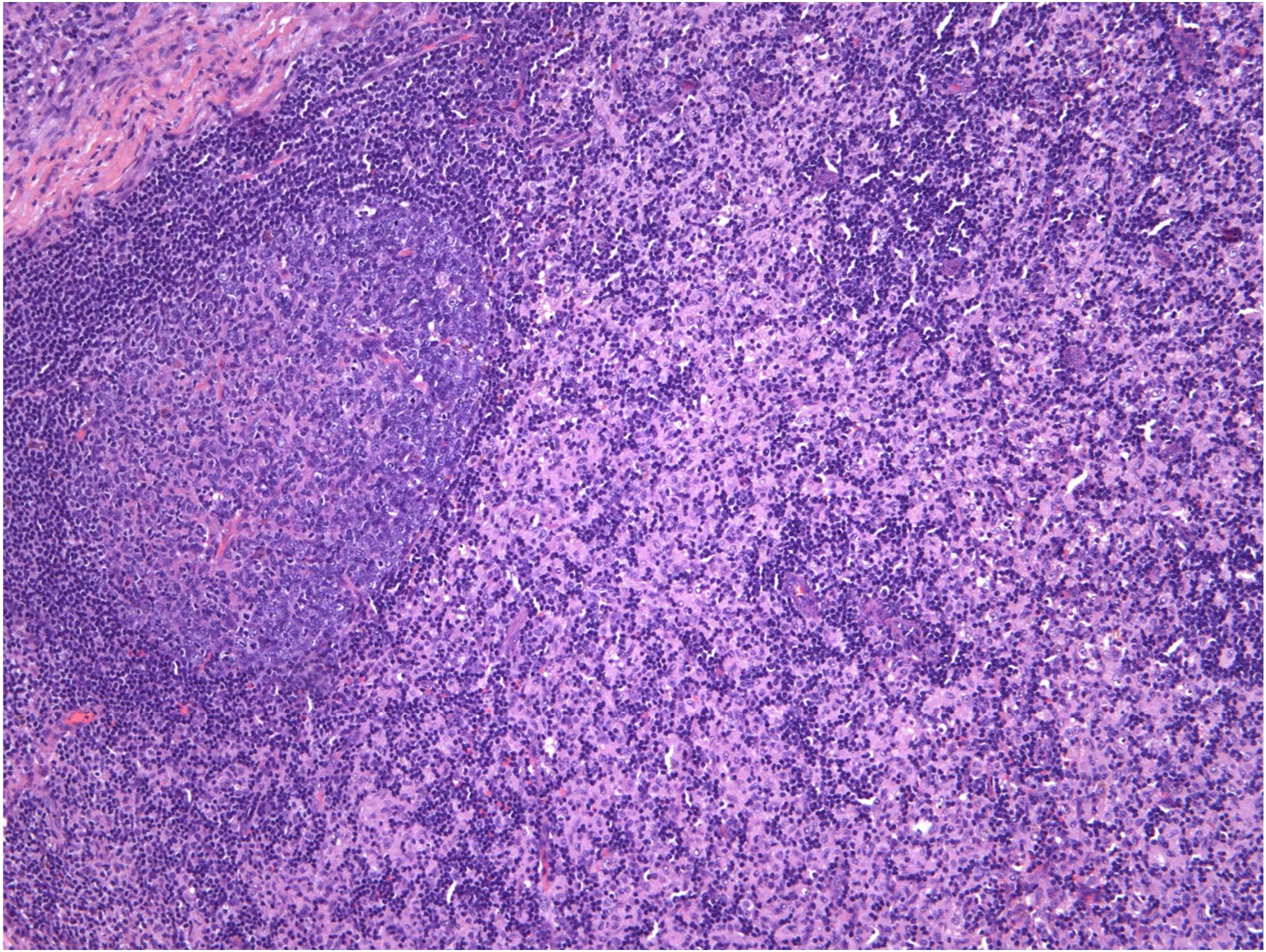


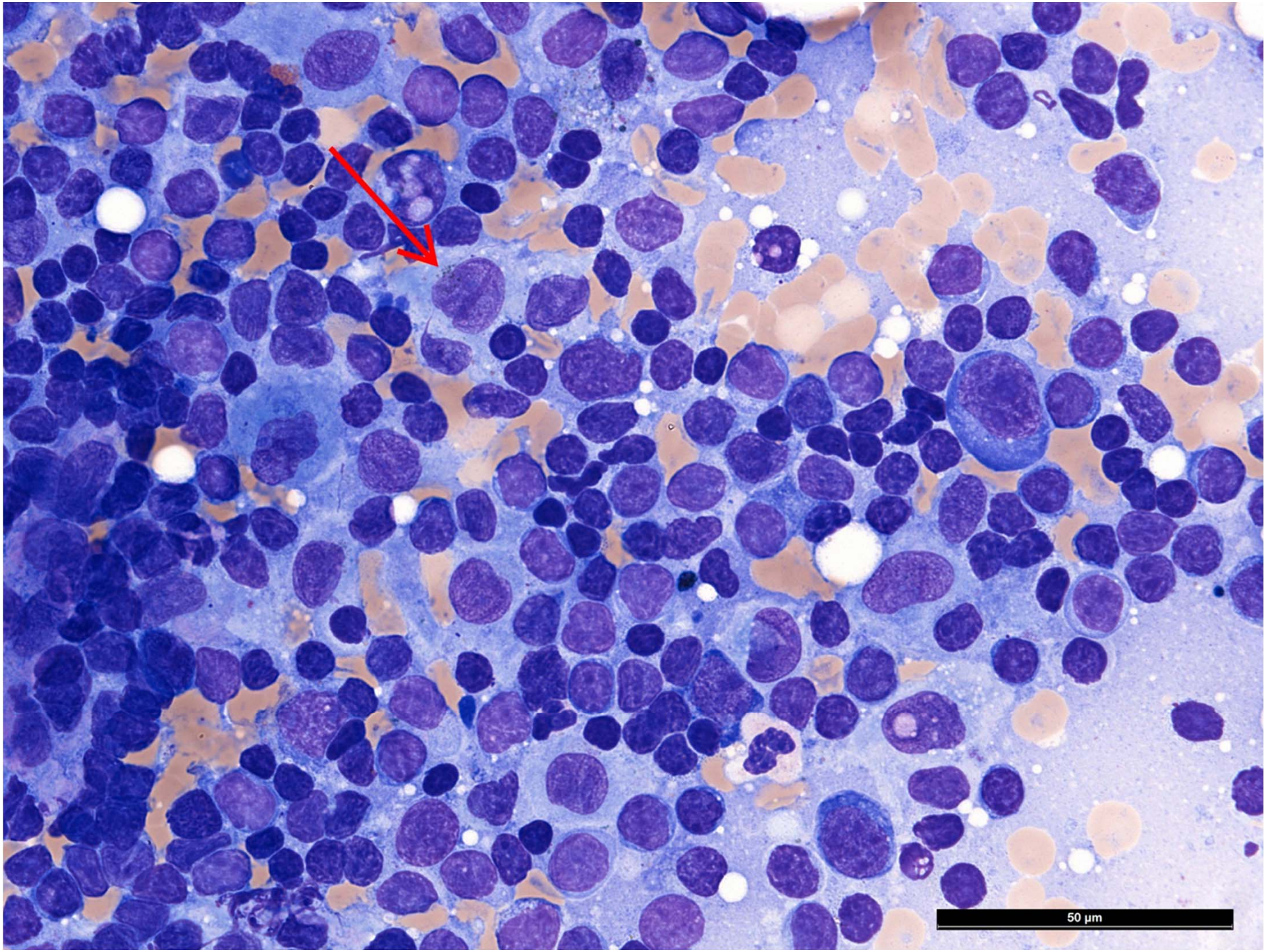


Dermatopathic Lymphadenopathy Histology

- "Lipomelanosis reticularis of Pautrier"
- Follicular hyperplasia
- Nodular expansion of interfollicular areas
  - Histiocytes containing fat and melanin pigment
  - Langerhan's cells
  - Interdigitating reticulum cells
  - Plasma cells and eosinophils
- Prominent post-capillary venules



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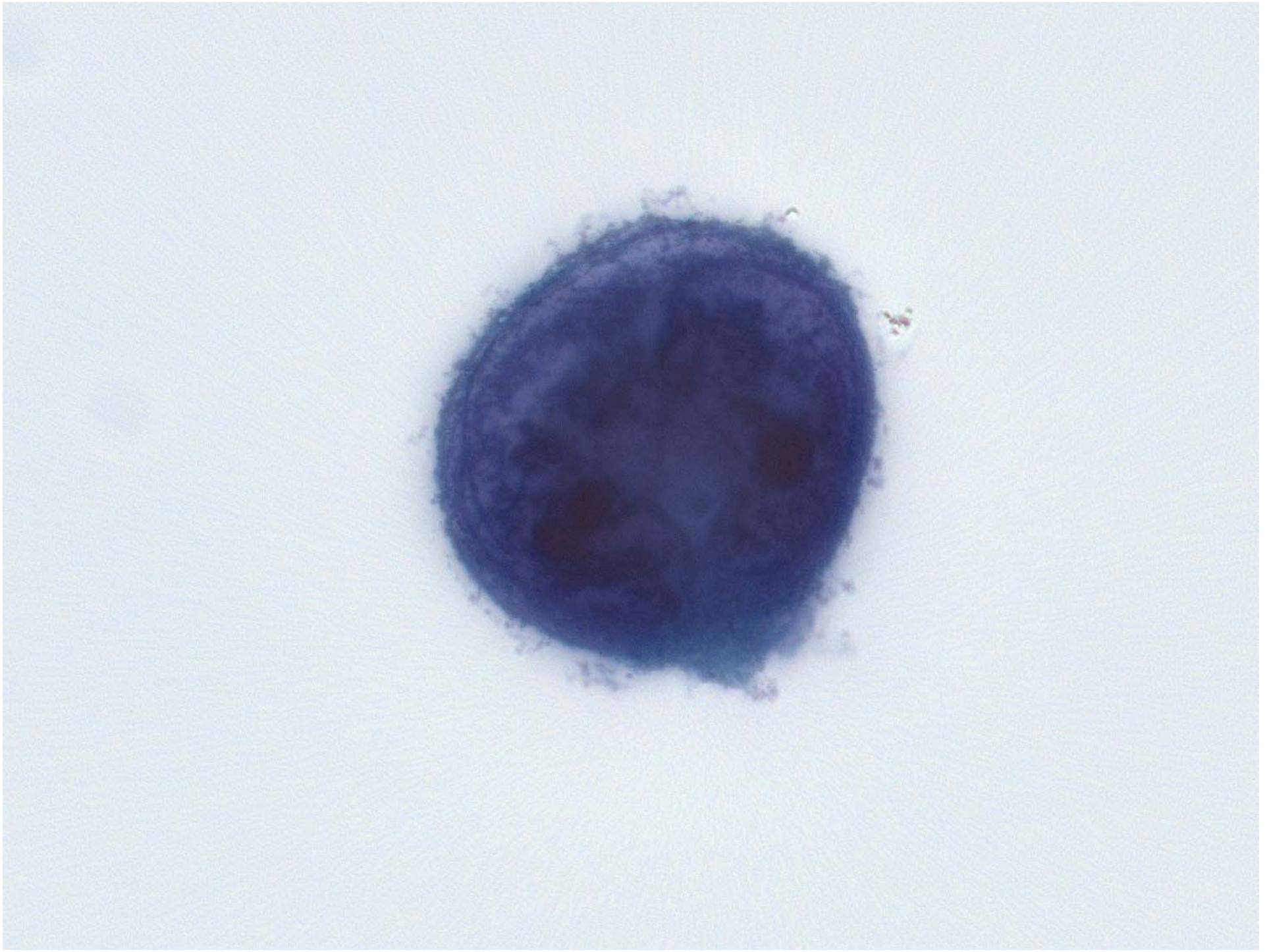
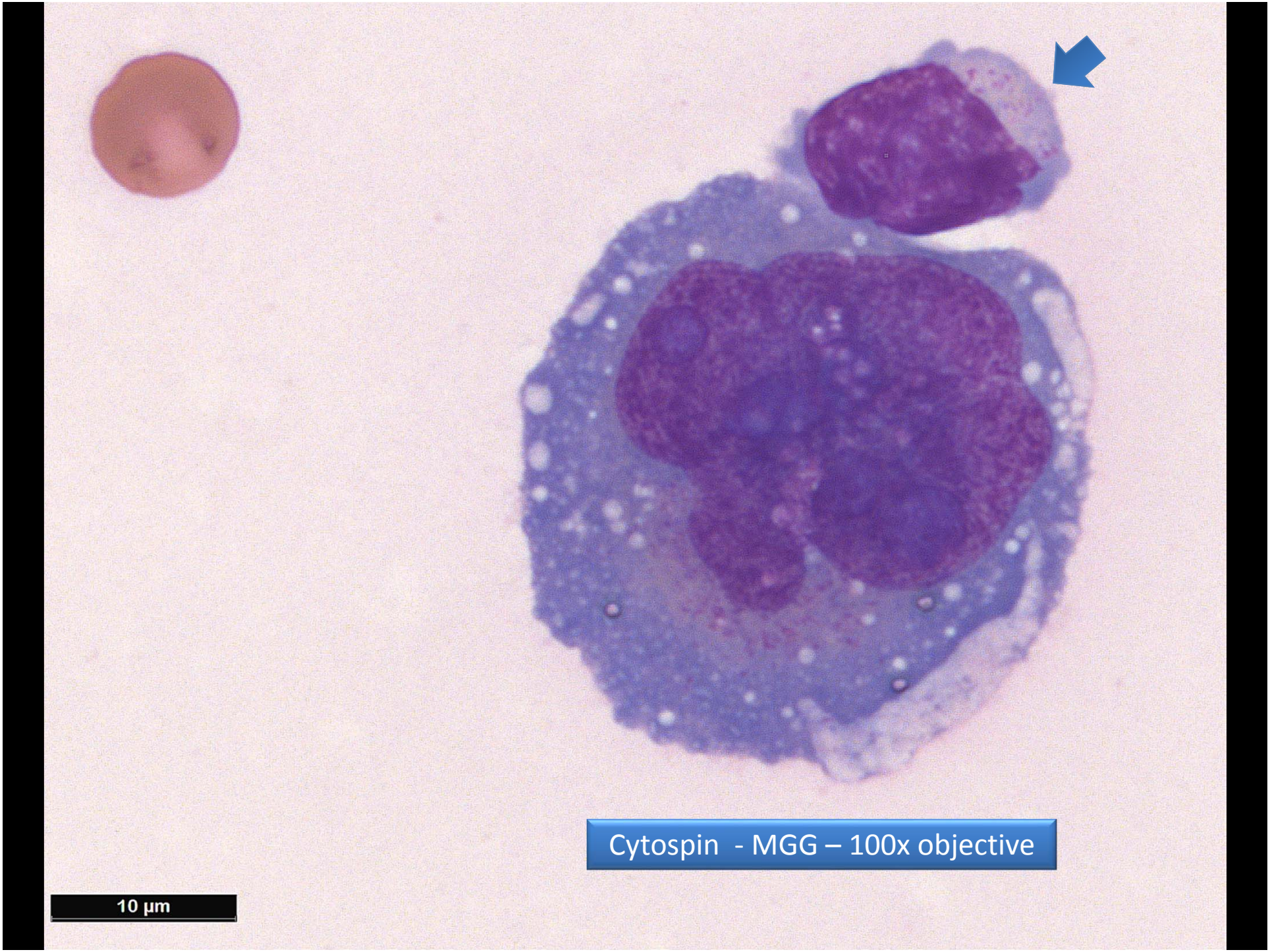
Sample submitted as “Breast Cyst Fluid” 45 yo woman

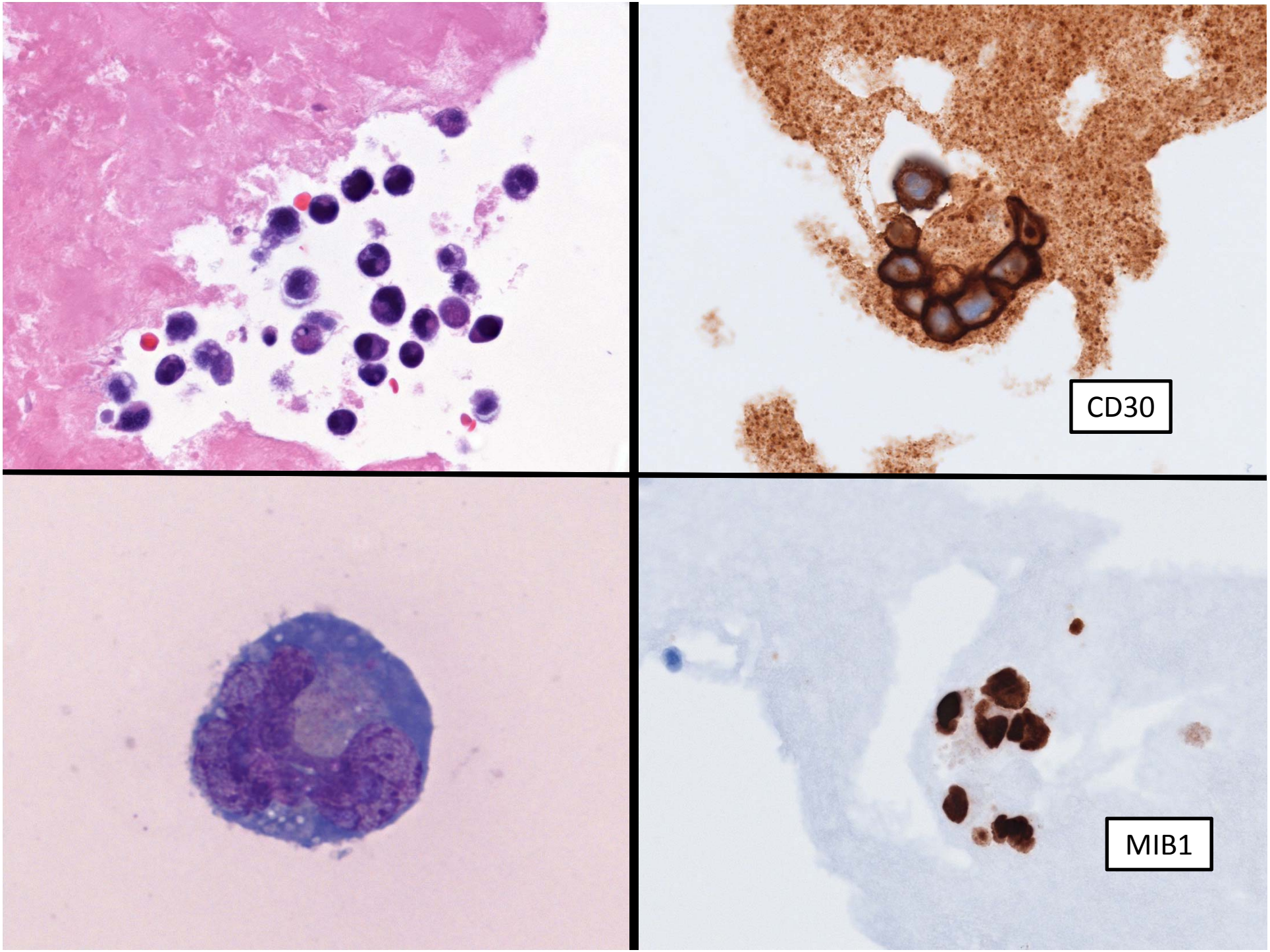
One Thin Prep made

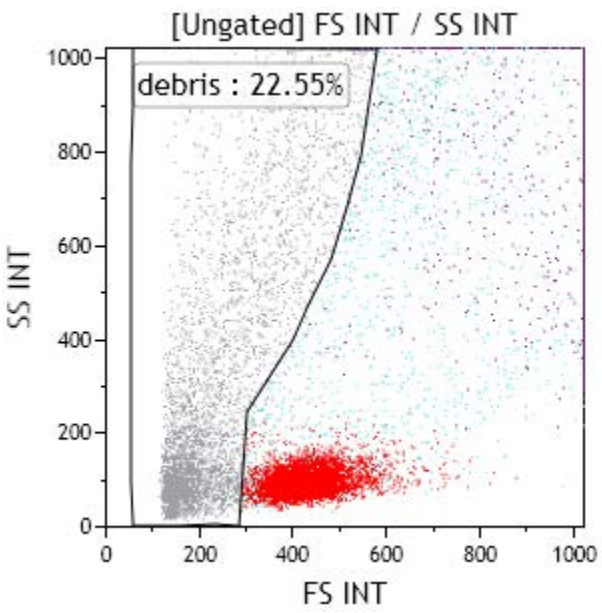
Residual 200 ml fluid in fridge

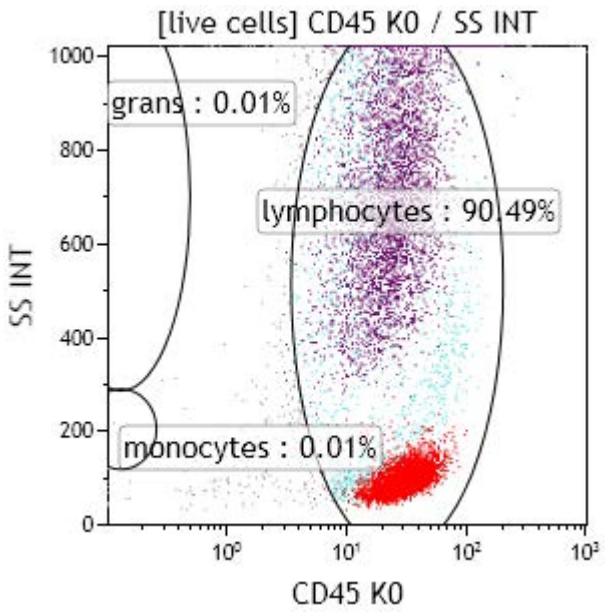

Thin Prep slide10x

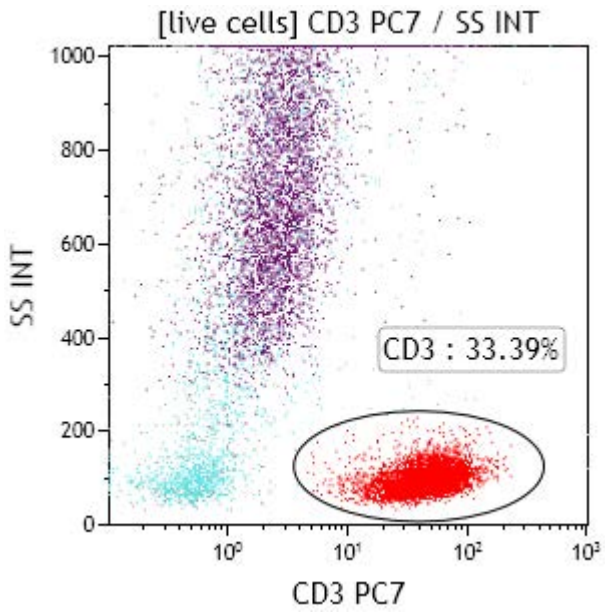


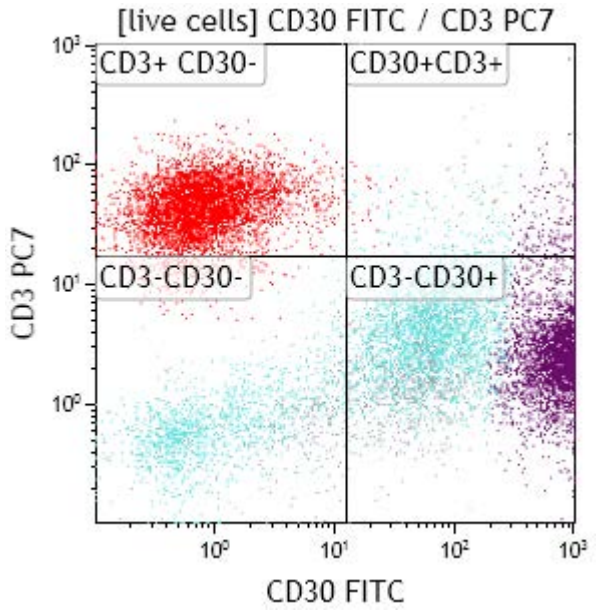

Cytospin - MGG – 100x objective
10 µm

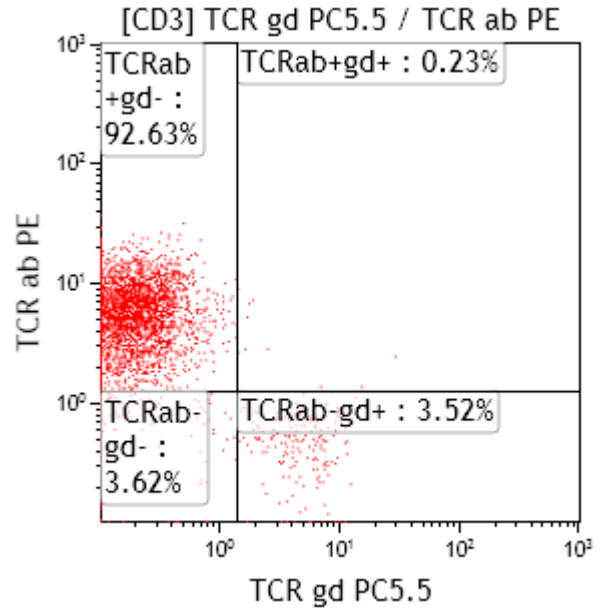

CD30
MIB1

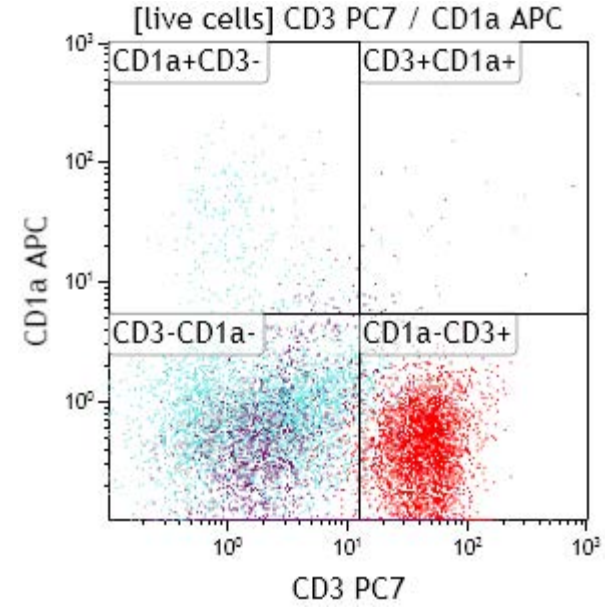

[Ungated] FS INT / SS INT
debris : 22.55%
SS INT
FS INT

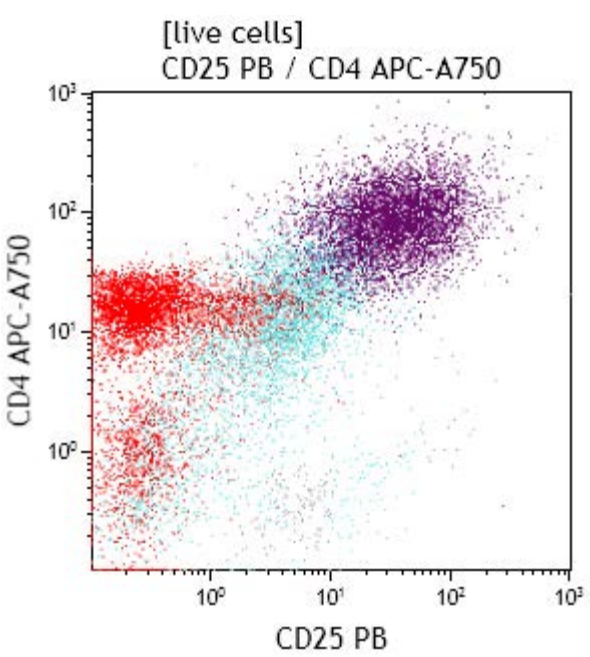

[live cells] CD45 K0 / SS INT
grans : 0.01%
lymphocytes : 90.49%
monocytes : 0.01%
SS INT
CD45 K0

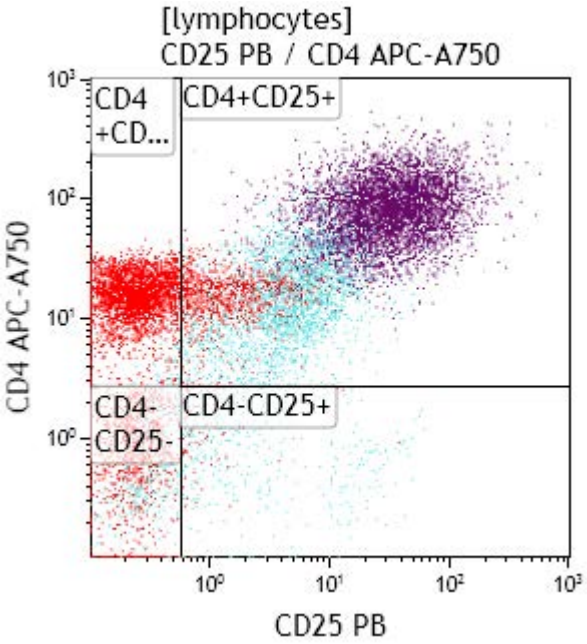

[live cells] CD3 PC7 / SS INT
CD3 : 33.39%
SS INT
CD3 PC7


[live cells] CD30 FITC / CD3 PC7
CD3+ CD30-
CD30+CD3+
CD3-CD30-
CD3-CD30+
CD3 PC7
CD30 FITC


[CD3] TCR gd PC5.5 / TCR ab PE
TCRab +gd- : 92.63%
TCRab+gd+ : 0.23%
TCRab-gd- : 3.62%
TCRab-gd+ : 3.52%
TCR ab PE
TCR gd PC5.5


[live cells] CD3 PC7 / CD1a APC
CD1a+CD3-
CD3+CD1a+
CD3-CD1a-
CD1a-CD3+
CD1a APC
CD3 PC7


[live cells]
CD25 PB / CD4 APC-A750
CD4 APC-A750
CD25 PB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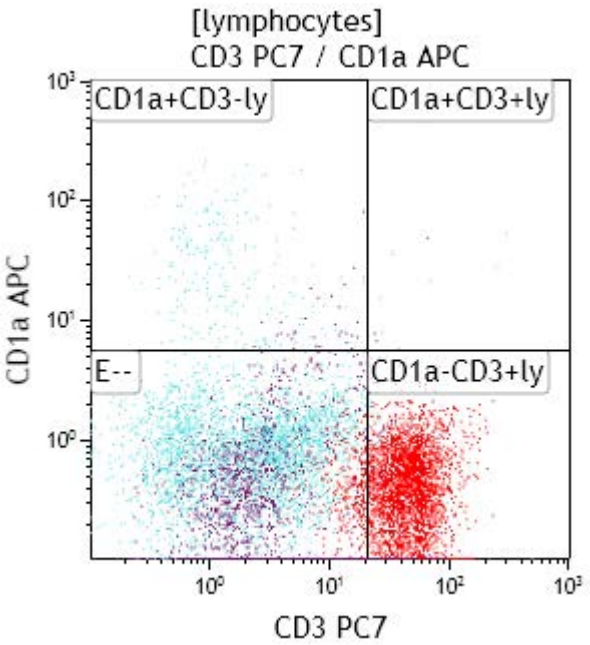
CD4 +CD...
CD4+CD25+
CD4-CD25-
CD4-CD25+
CD4 APC-A750
CD25 PB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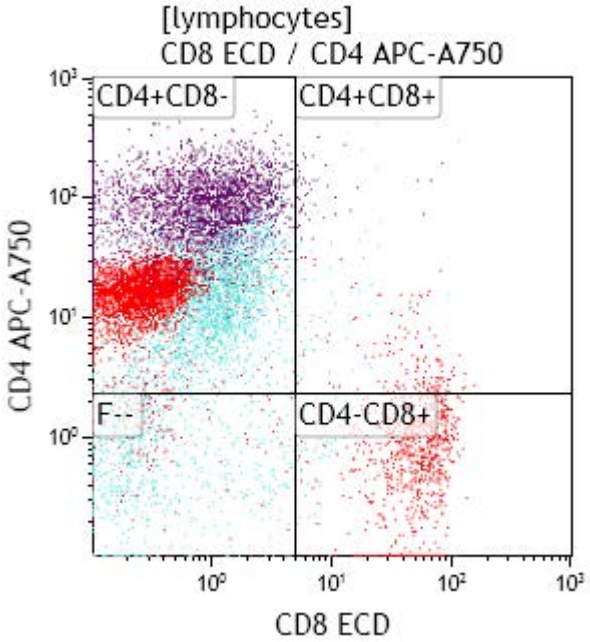
CD3 PC7 / CD1a APC
CD1a+CD3-ly
CD1a+CD3+ly
E--
CD1a-CD3+ly
CD1a APC
CD3 PC7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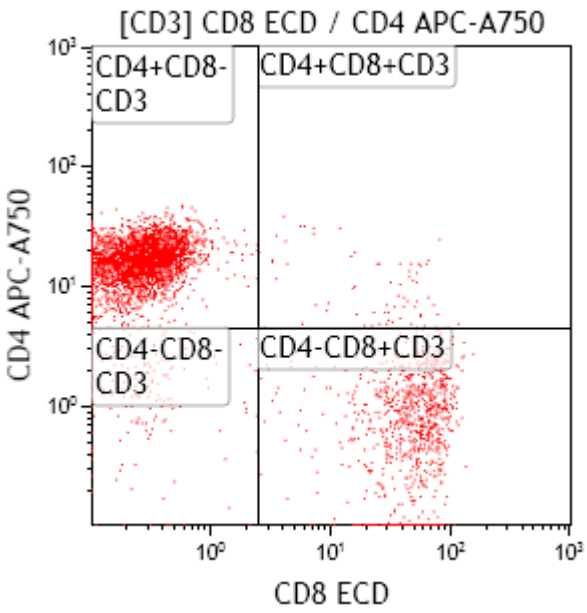
CD4+CD8-
CD4+CD8+
F--
CD4-CD8+
CD4 APC-A750
CD8 ECD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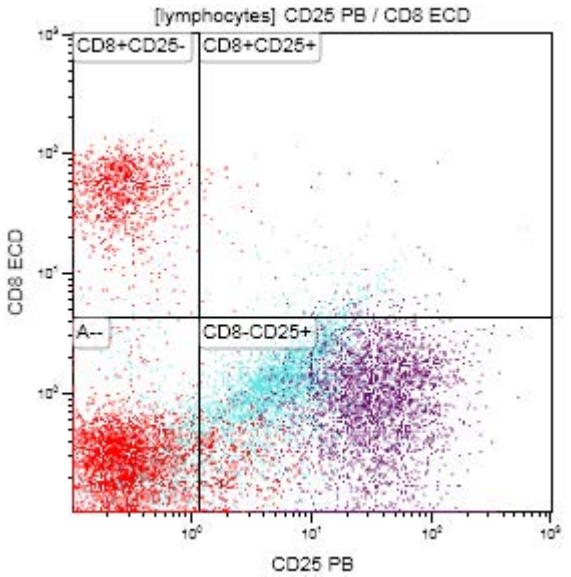
CD4+CD8-CD3
CD4+CD8+CD3
CD4-CD8-CD3
CD4-CD8+CD3
CD4 APC-A750
CD8 ECD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CD8+CD25-
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CD8-CD25+
CD8 ECD
CD25 PB

ORIGINAL ARTICLE

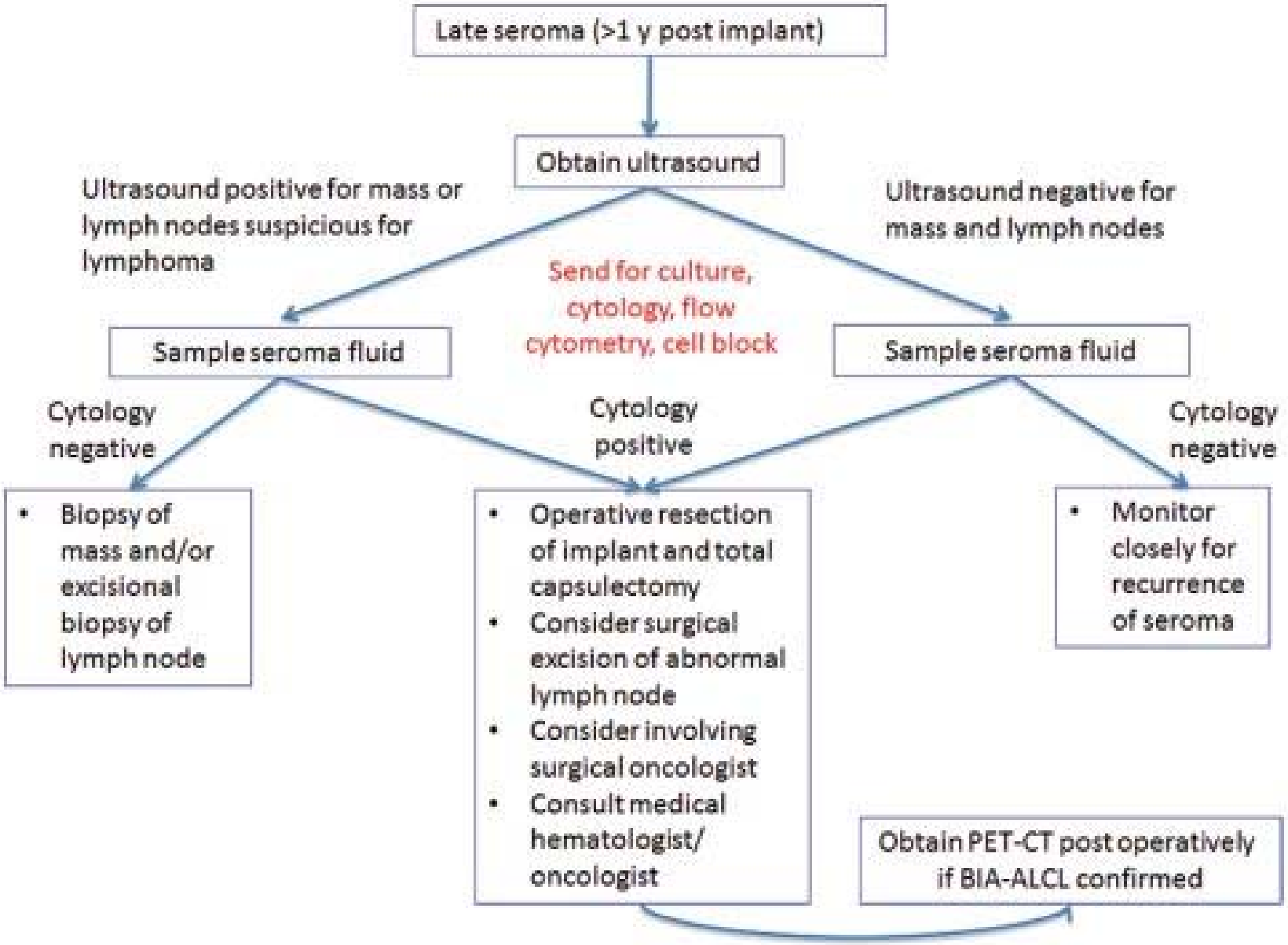
Breast

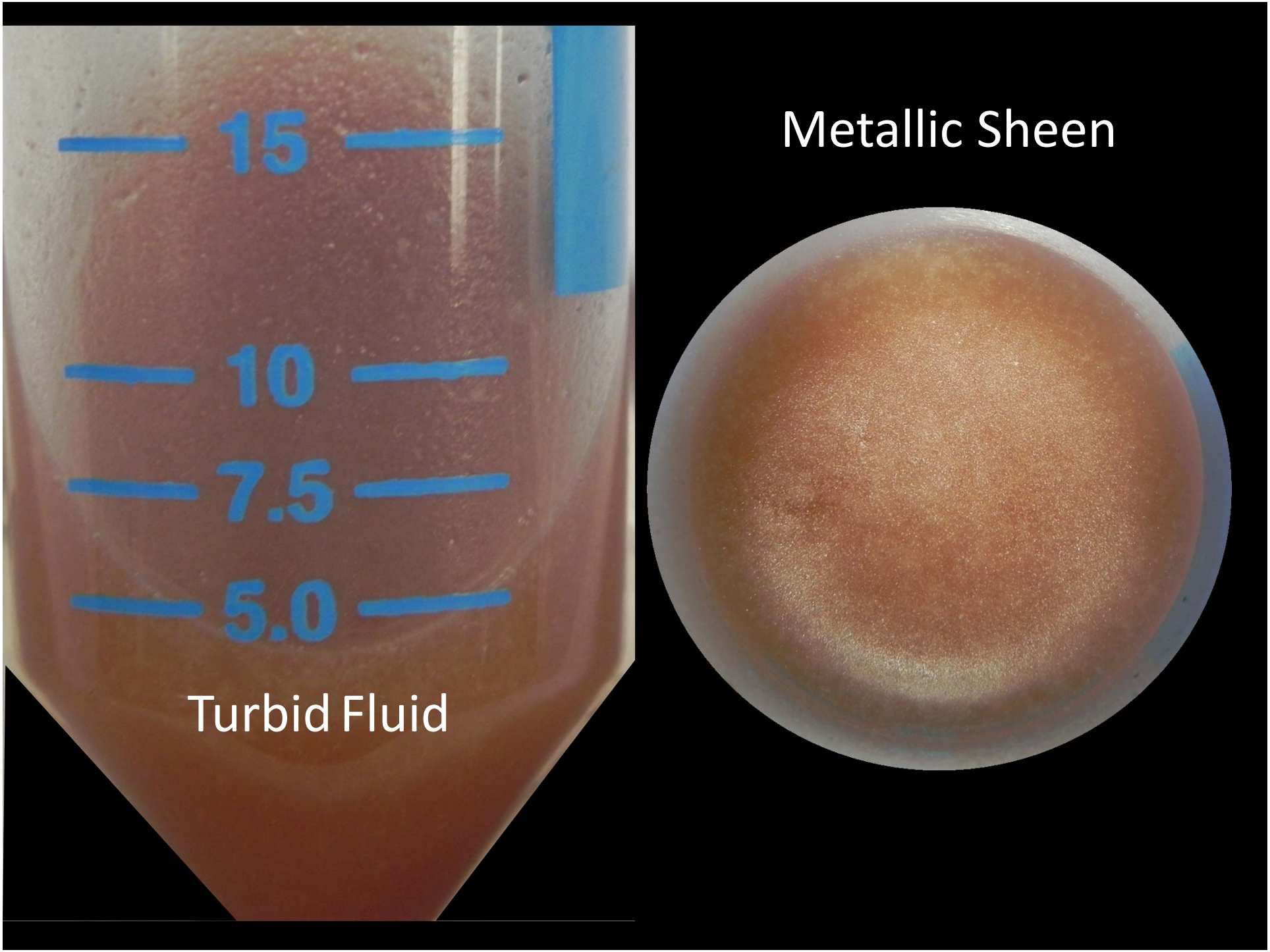
Breast Implant–associated Anaplastic Large Cell Lymphoma: Updated Results from a Structured Expert Consultation Process

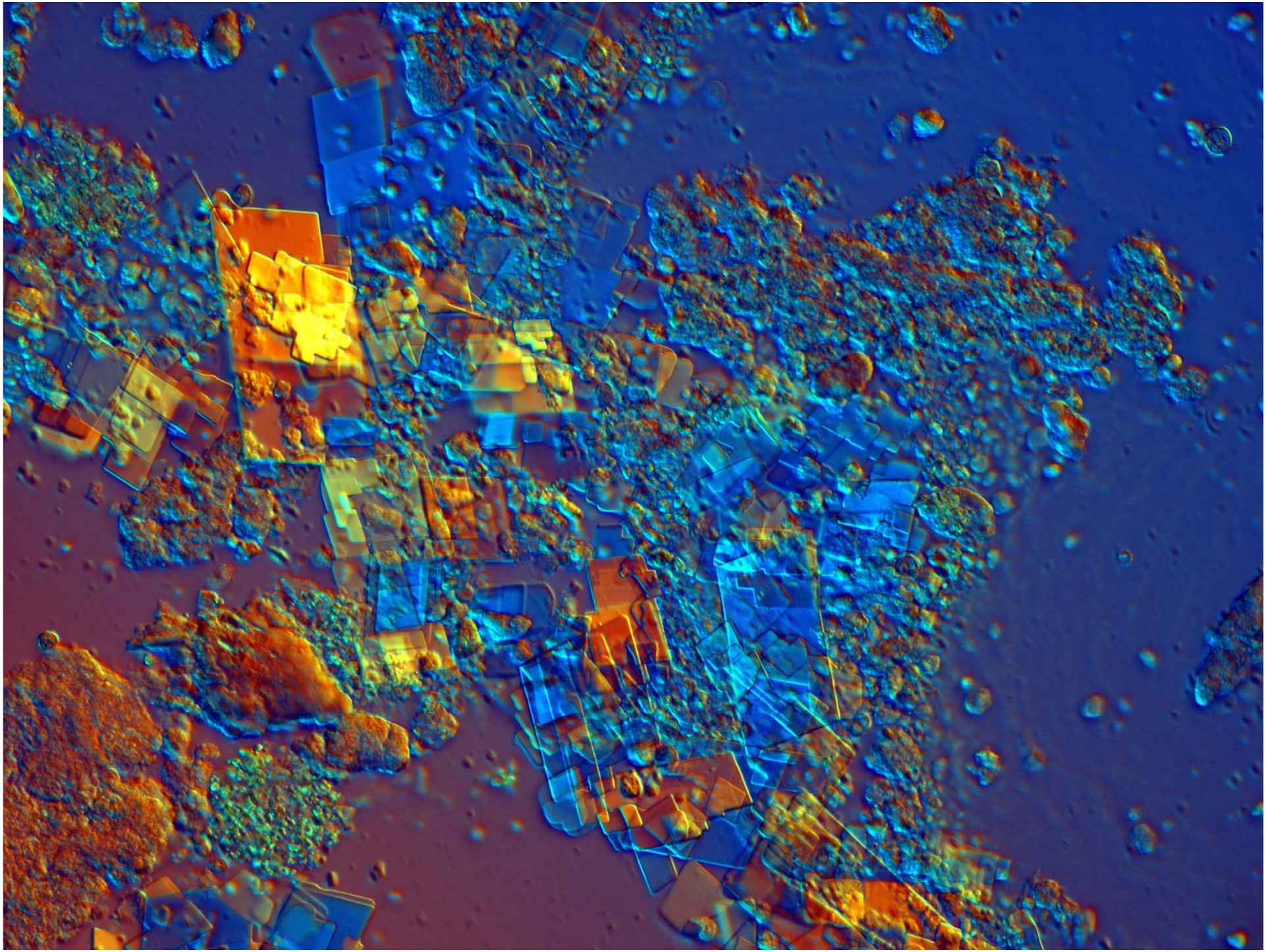
Benjamin Kim, MD, MPhil*†
Zachary S. Predmore, BA*
Soeren Mattke, MD, DSc*
Kristin van Busum, MPA*
Courtney A. Gidengil, MD, MPH*‡

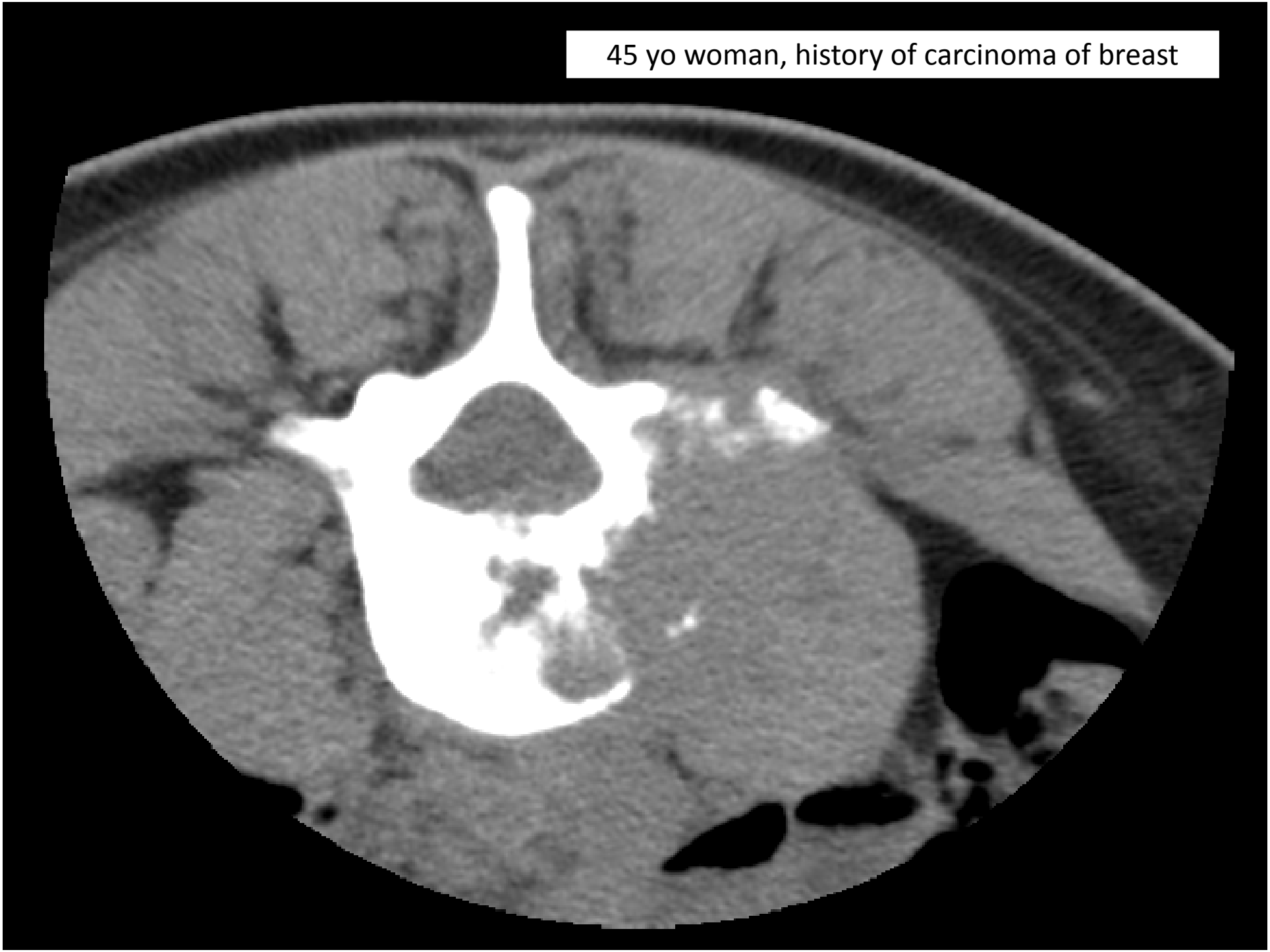
Workup

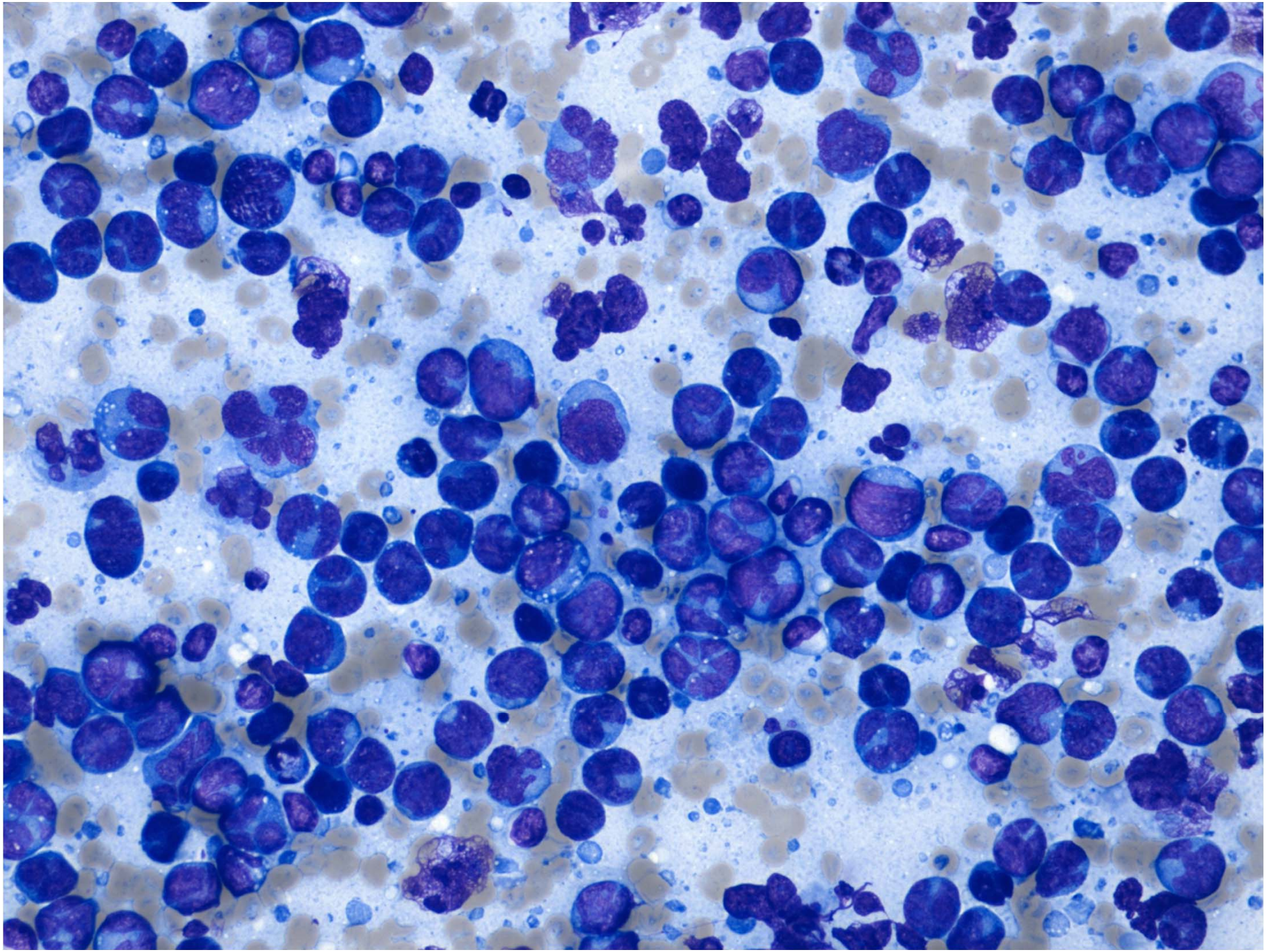
Panelists universally agreed that chronic or unexplained seromas occurring more than 1 year after breast implantation should be considered for a breast ultrasound (9, 0.00) and seroma fluid sampling, with the fluid sent for culture, cytology, flow cytometry, and cell block (9, 0.00). There was also consistent agreement that seroma fluid specimens should be sent for cytology and flow cytometry (9, 0.08), tissue specimens should be sent for immunohistochemical analysis (including for CD30 and ALK; 9, 0.17), and specimens should be sent to a hematopathologist with experience in diagnosing ALCL (9, 0.92), with less consistent agreement that T-cell receptor gene rearrangement should also be performed (8, 1.36).

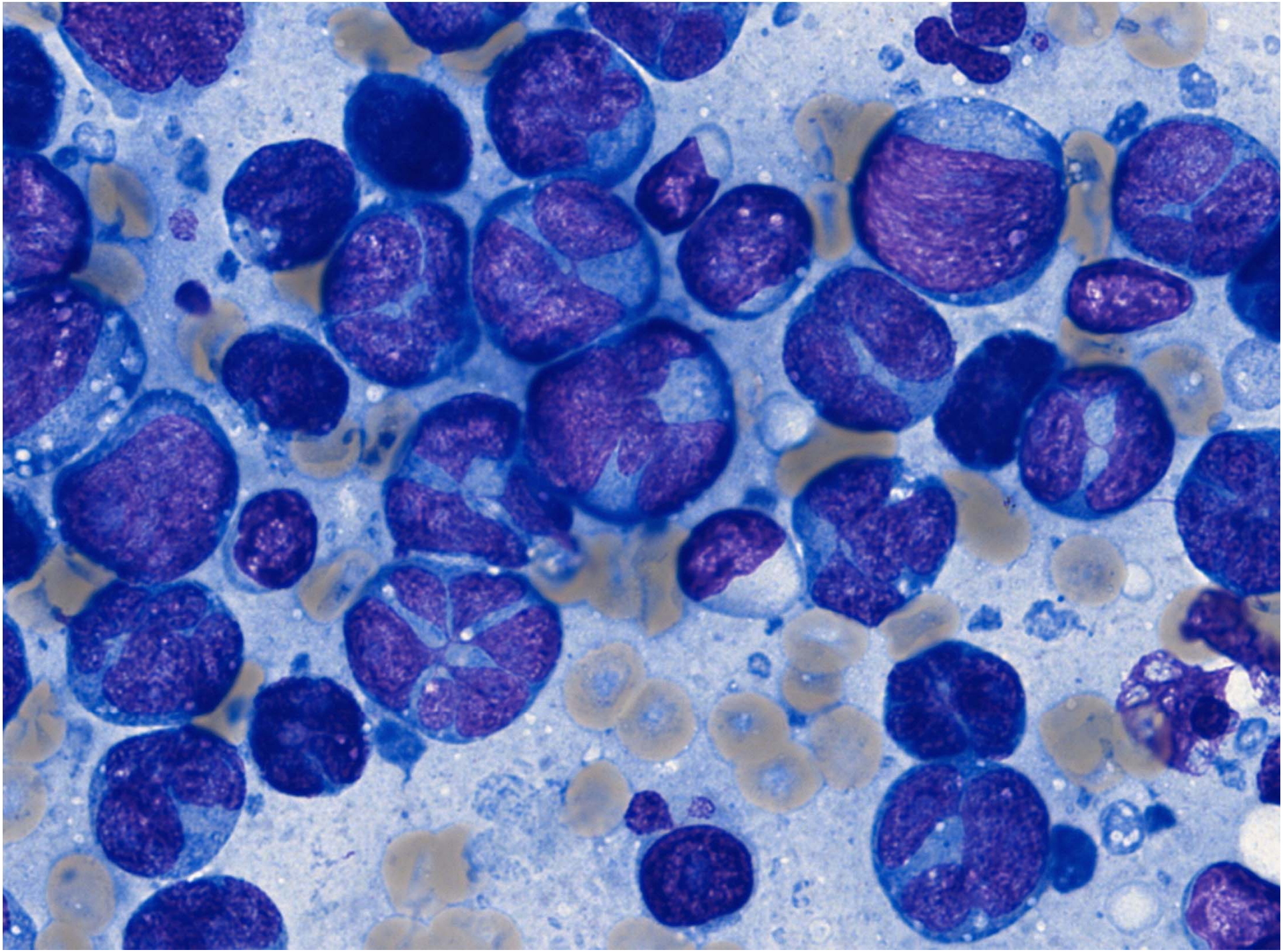

Late seroma (>1 y post implant)
Obtain ultrasound
Ultrasound positive for mass or lymph nodes suspicious for lymphoma
Ultrasound negative for mass and lymph nodes
Send for culture, cytology, flow cytometry, cell block
Sample seroma fluid
Sample seroma fluid
Cytology negative
Cytology positive
Cytology negative
• Biopsy of mass and/or excisional biopsy of lymph node
• Operative resection of implant and total capsulectomy
• Consider surgical excision of abnormal lymph node
• Consider involving surgical oncologist
• Consult medical hematologist/ oncologist
• Monitor closely for recurrence of seroma
Obtain PET-CT post operatively if BIA-ALCL confirmed

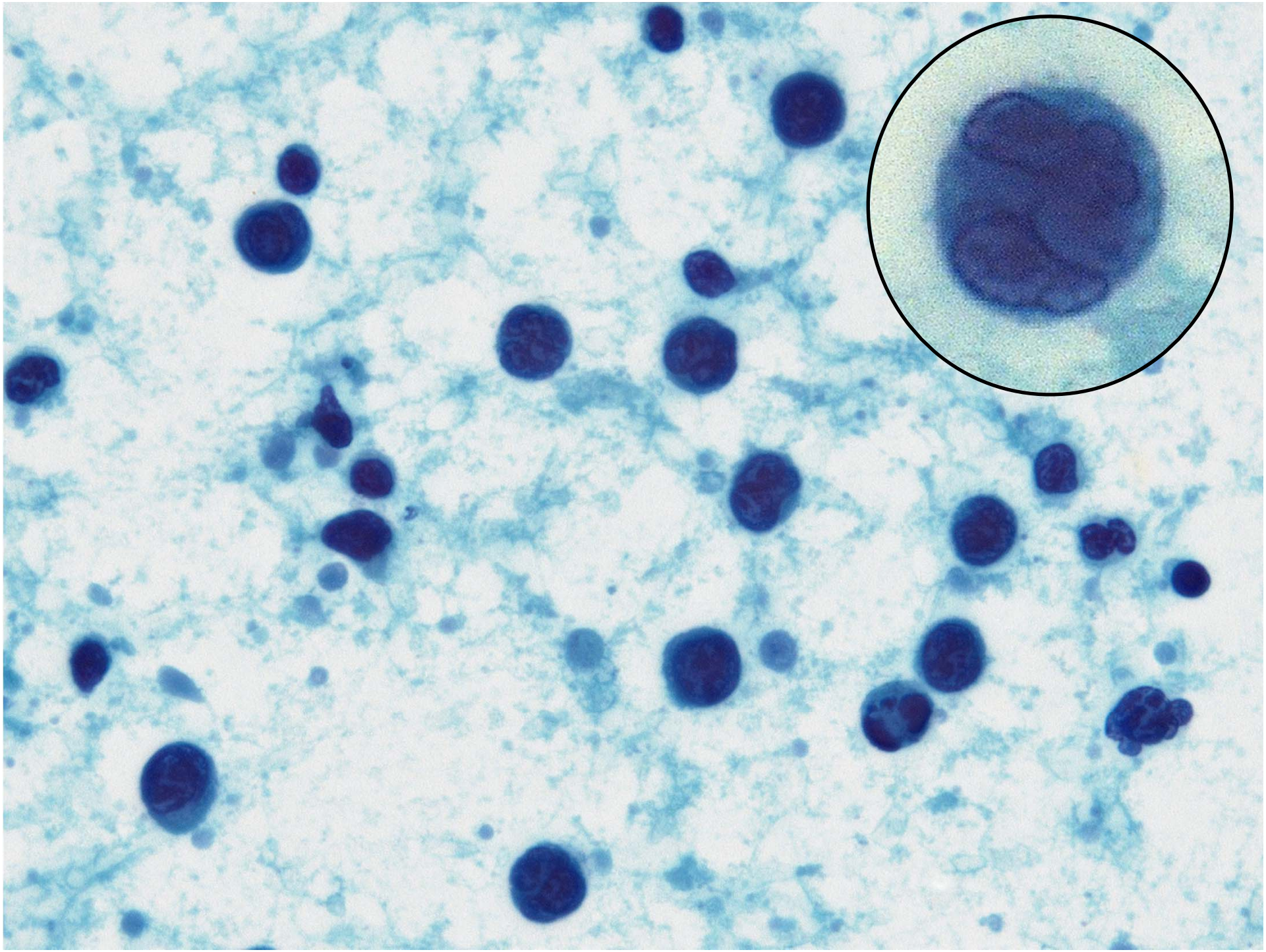

15
10
7.5
5.0
Turbid Fluid
Metallic Sheen




45 yo woman, history of carcinoma of breast







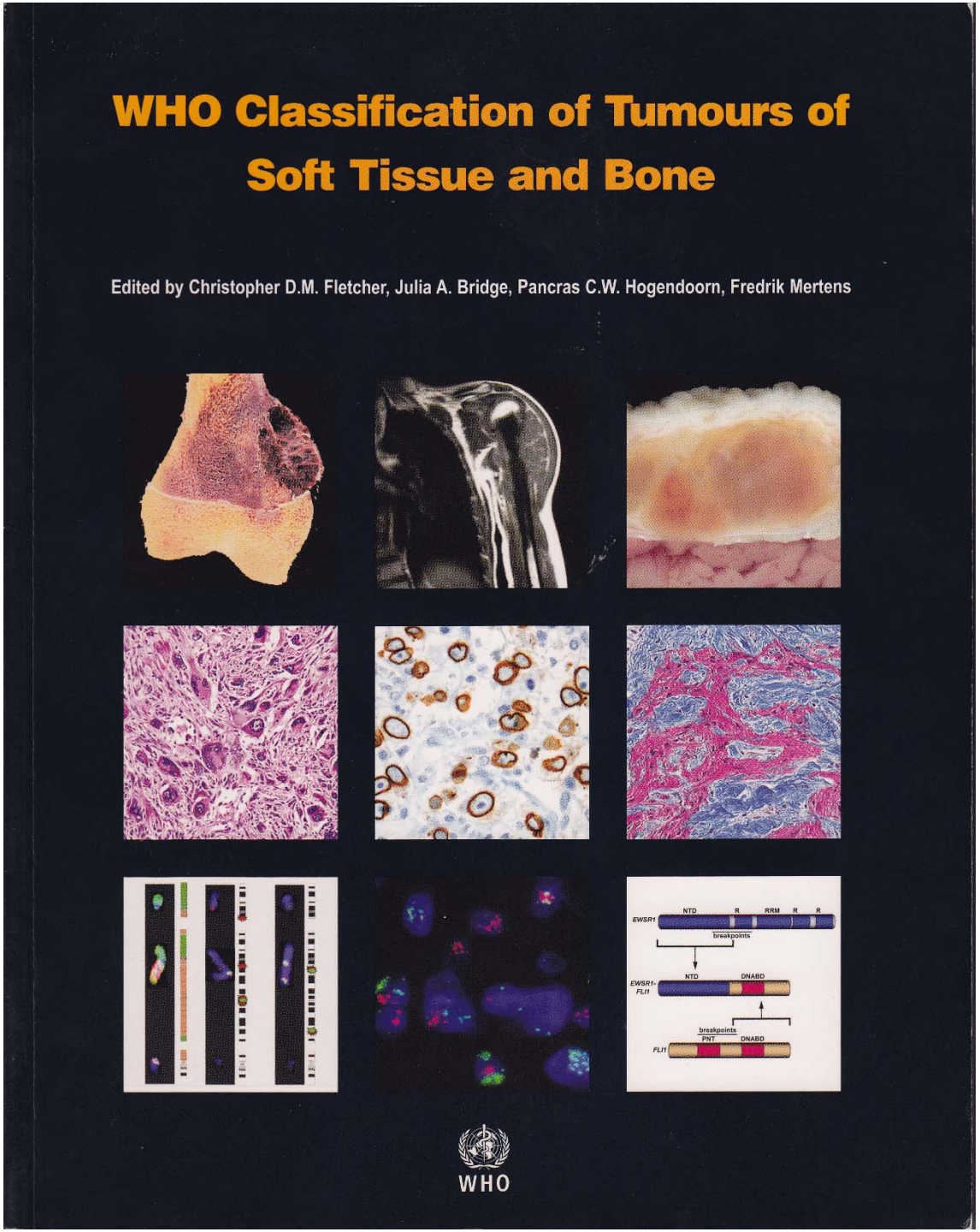
The American Journal of Surgical Pathology 14(4): 329–334, 1990

Primary Lymphoma of Bone

A B-Cell Neoplasm with a High Frequency of Multilobated Cells

Carolyn K. Pettit, M.D., Lawrence R. Zukerberg, M.D., Mark H. Gray, M.D., Judith A. Ferry, M.D., Andrew E. Rosenberg, M.D., David C. Harmon, M.D., and Nancy L. Harris, M.D.

12 patients, average age 41 years
-Mixed lytic and sclerotic pattern of bone destruction
-Dissociated cells without follicle formation
-9/12 prominent nuclear clefts
-4/12 majority of cells had prominent multilobation
-4 additional cases occasional multilobated cells
-Classified as DLBCL, NOS (Working Formulation)



Limited data on FISH in PB-DLBCL.

BCL2/18q21 breakpoints were found in ~25%

MYC/8q24 breakpoints in 10% of the cases

"One major diagnostic problem is that the cells tend to be crushed, in particular in trephine biopsies of the long bones. If a biopsy shows such crush artifact, a diagnosis of malignant lymphoma should be suspected and immunohistochemistry should always be performed."
BCL6/3q27 reported variably

CHOP-R - >90% overall survival

Objectives

- To review morphologic features of some benign lymph node cell types that can be mistaken for alien cells in cytology preparations
- To review features of some benign lymphoid proliferations that can be mistaken for lymphoma
- To review features of lymphomas that can be mistaken for epithelial neoplasms
- To suggest possible lab routines or additional procedures that can help avoid errors

Lab Routines

- Encourage ROSE and prepare smears by “one-step” method
- Whenever possible use a Romanovsky-Giemsa type stain in conjunction with Papanicolaou
  - Try preparing Field’s stain or Marshall’s stain
- Consider using “RALT” (Rapid Assessment for Laboratory Triage) for all samples submitted with request for flow cytometry, history of known lymphoma or clinical presentation suspicious for lymphoma
  - Mass lesions in transplant patients (?PTLD)
  - Fluid collections associated with breast implants
  - Evaluate Giemsa stained cytospin, consider flow, FISH, molecular and IHC

Supplementary Material

Smear Preparation and Romanovsky Giemsa Stains

Smear Preparation

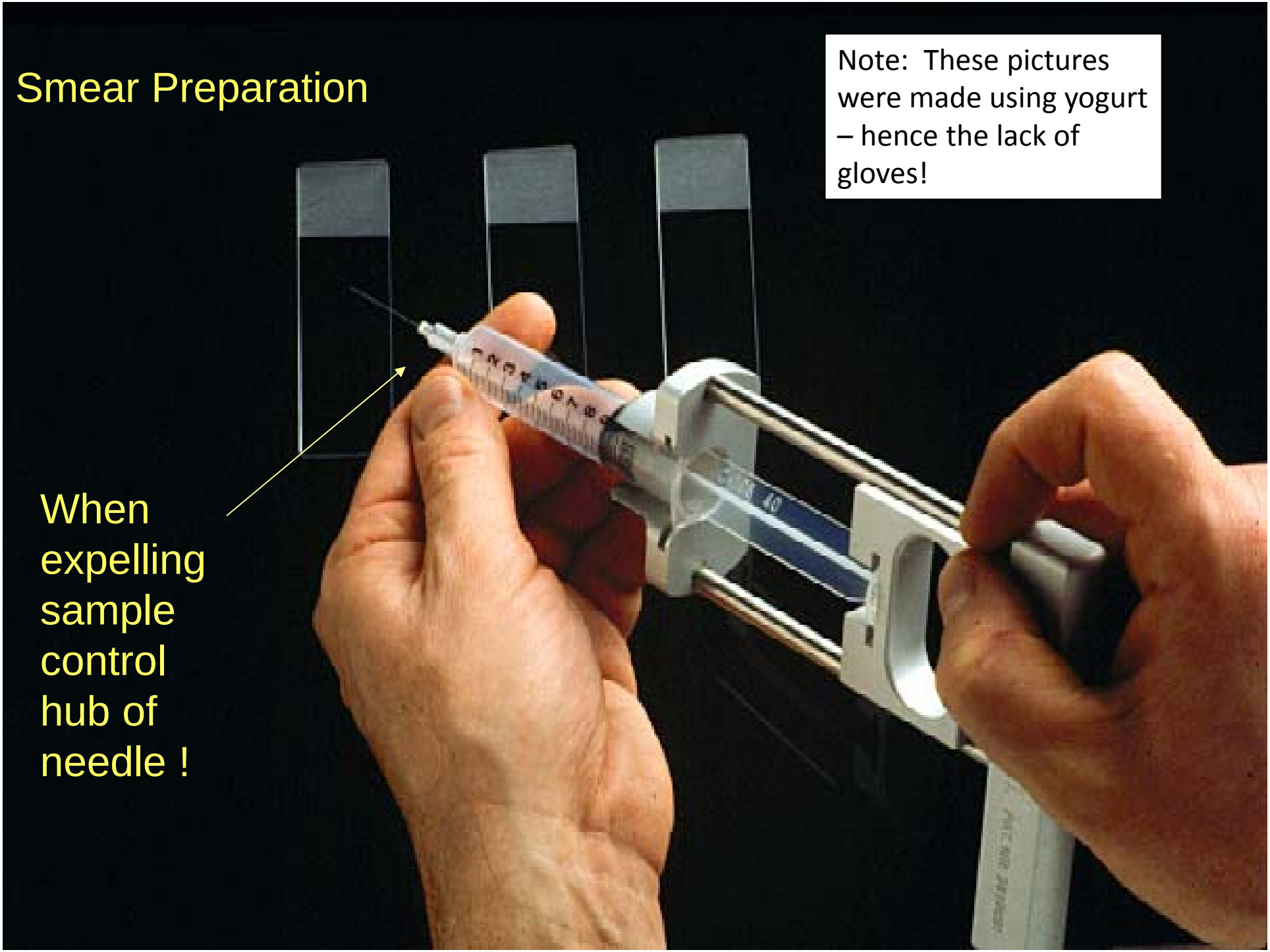
The “One-Step” Method

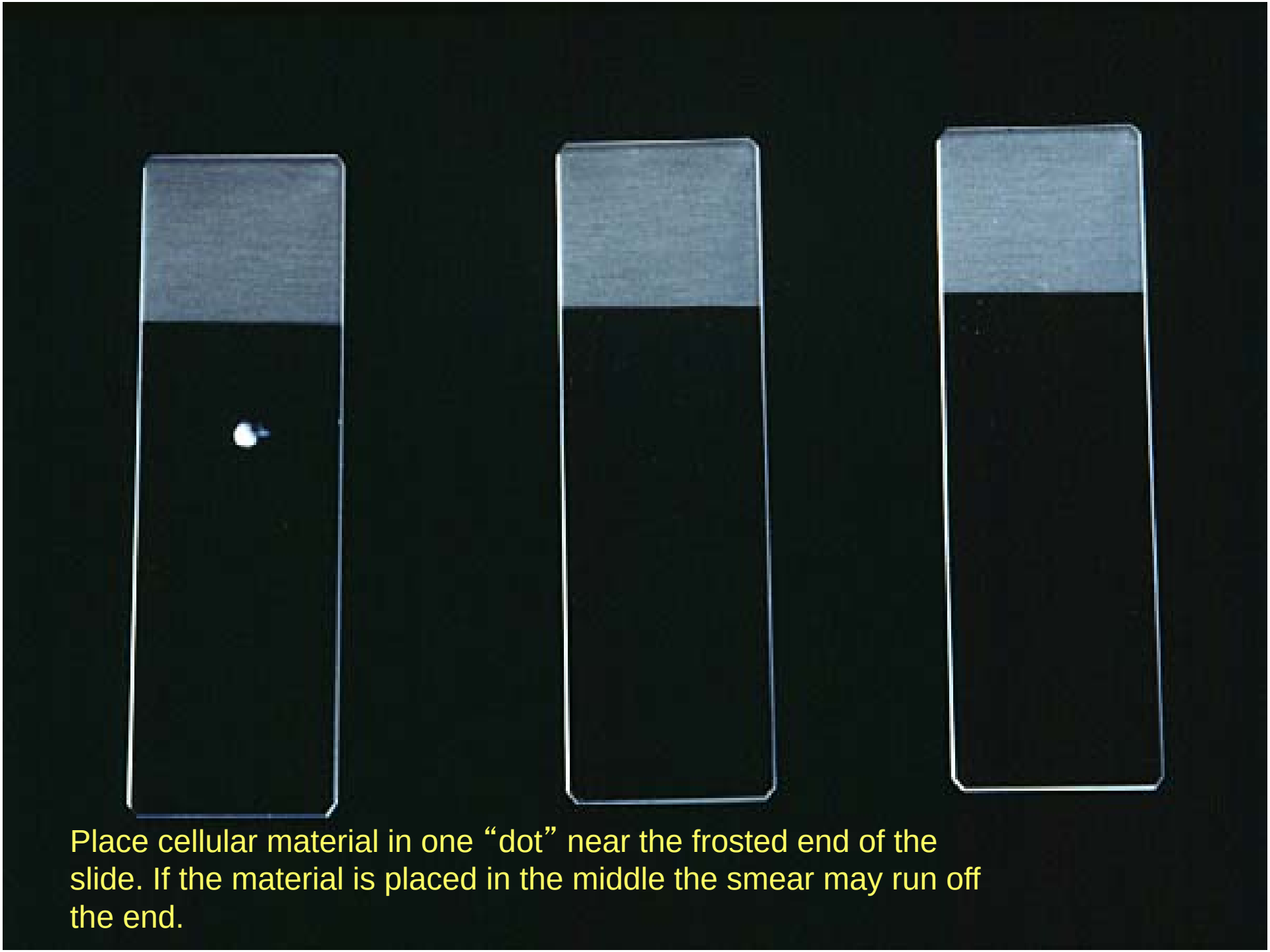
Dividing Material for Multiple Smears

Triaging Material for IHC and Molecular

Smear Preparation

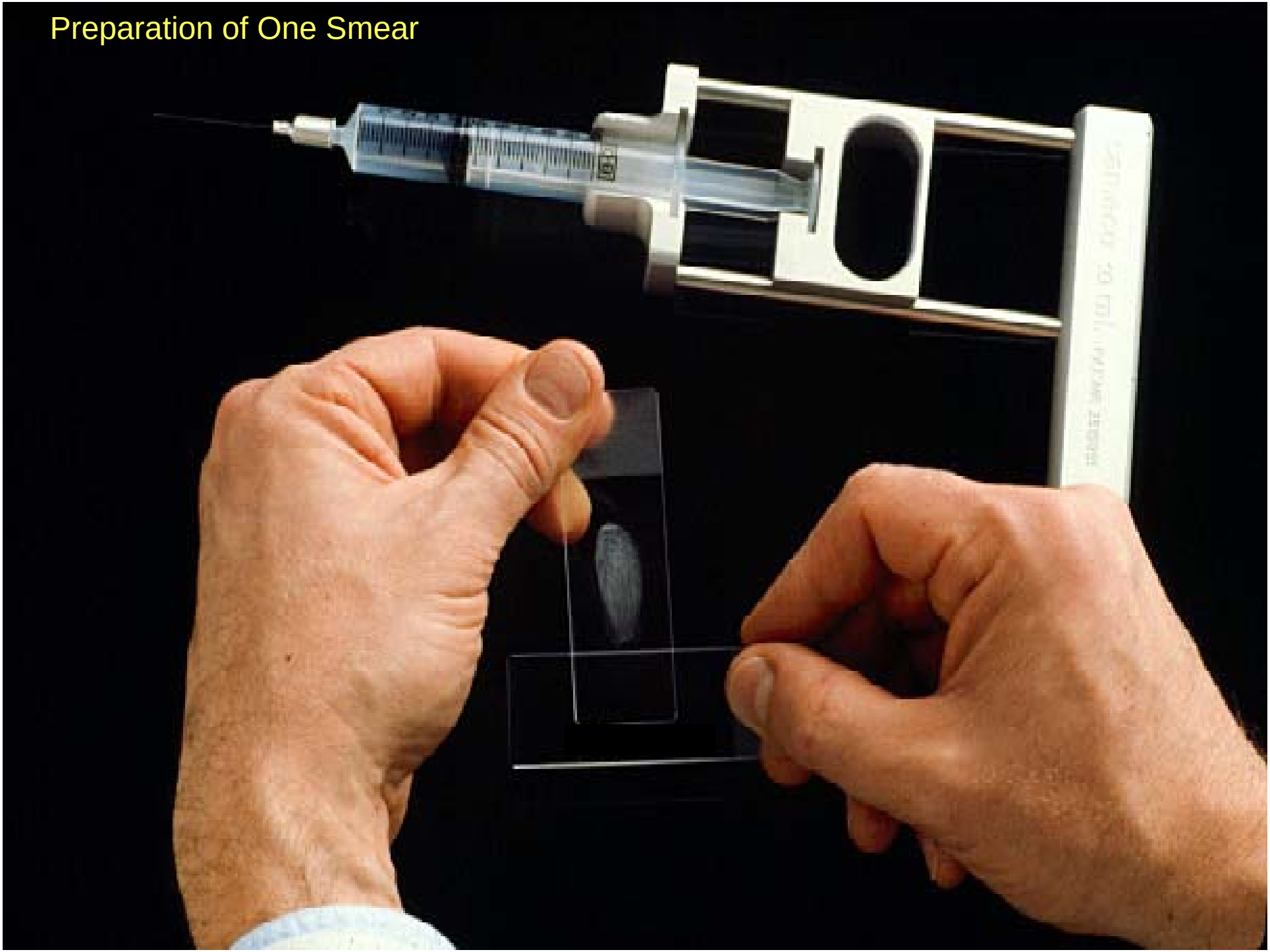
Note: These pictures were made using yogurt – hence the lack of gloves!



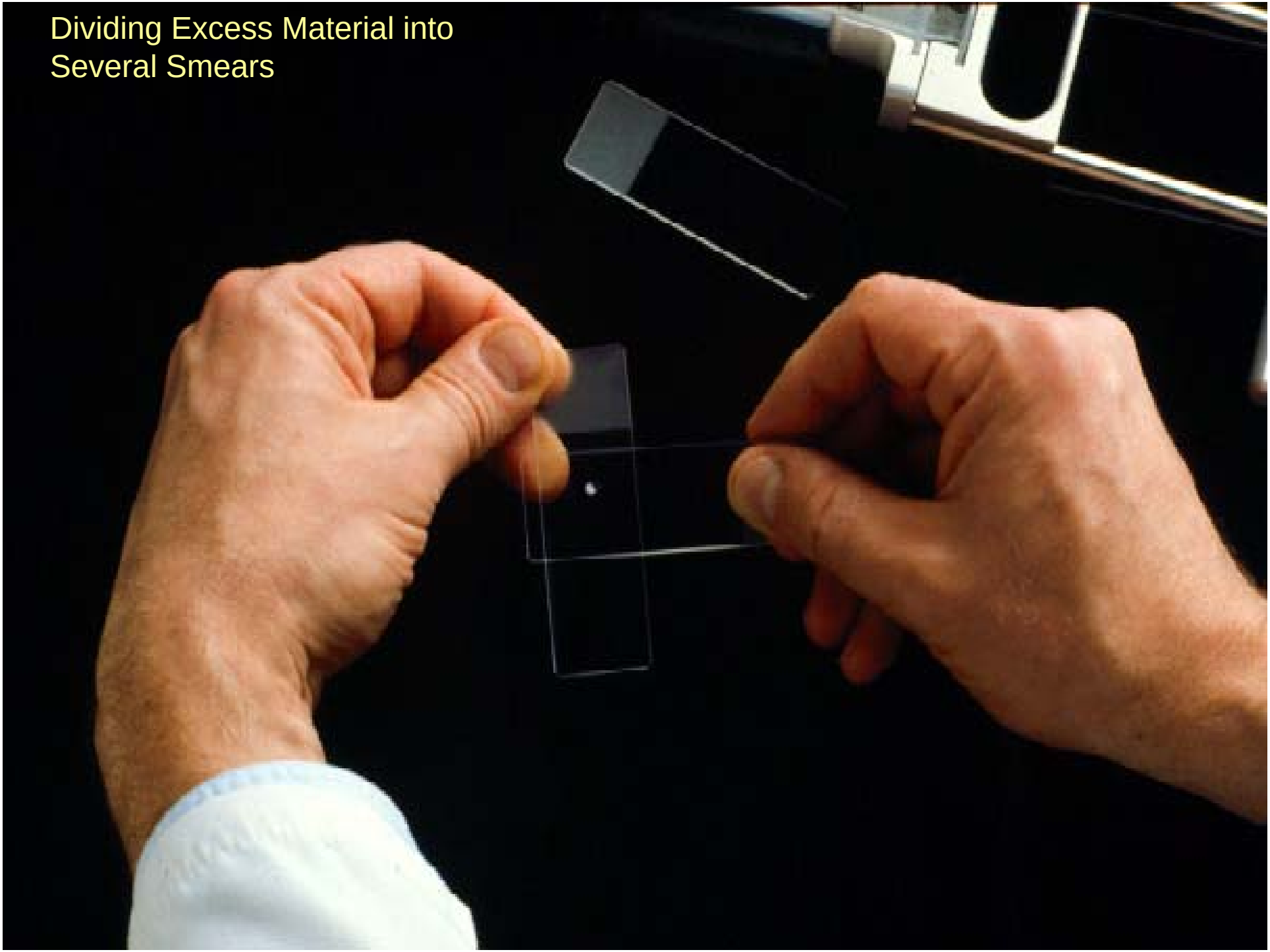


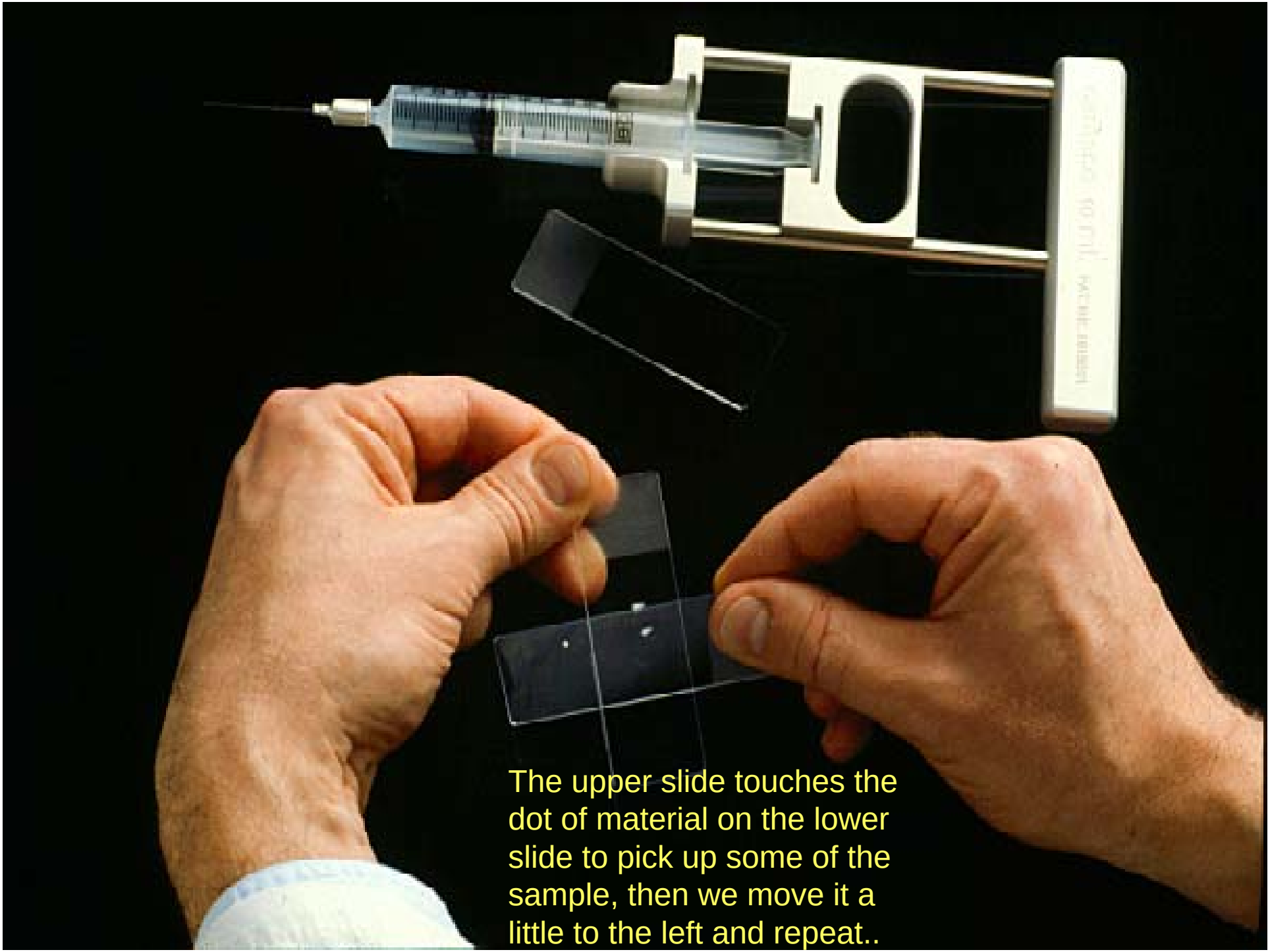
Place cellular material in one “dot” near the frosted end of the slide. If the material is placed in the middle the smear may run off the end.

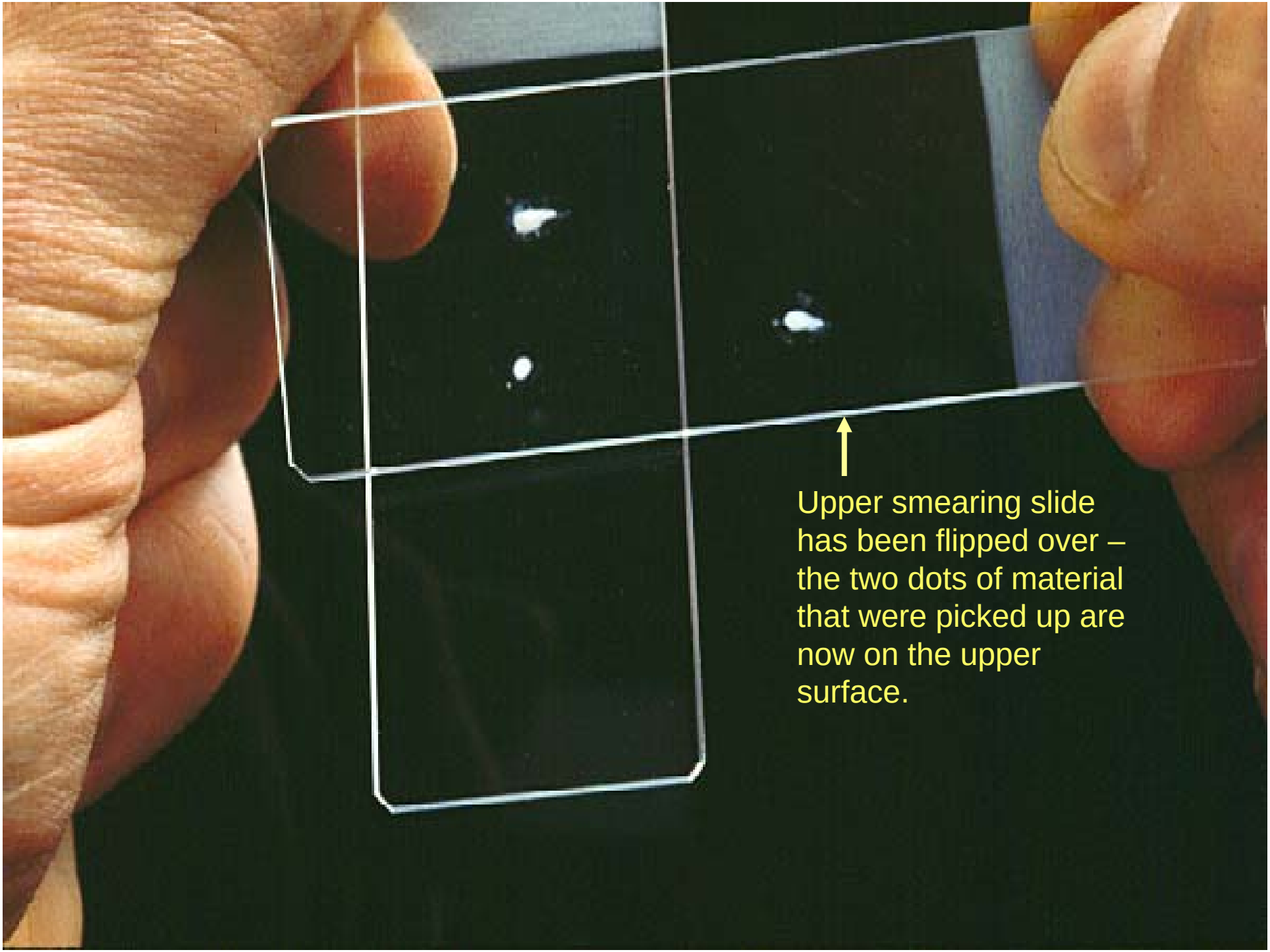
Preparation of One Smear

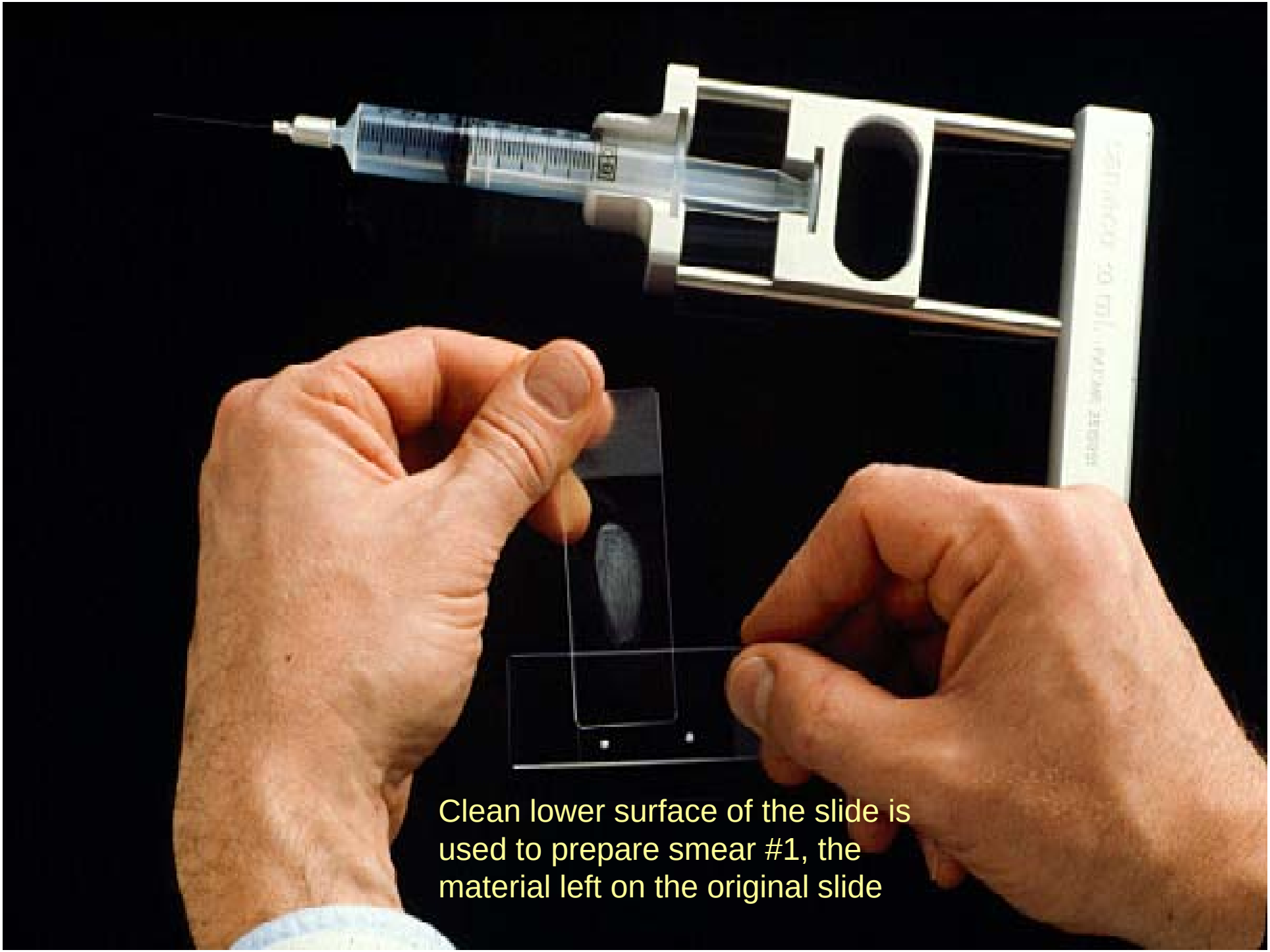


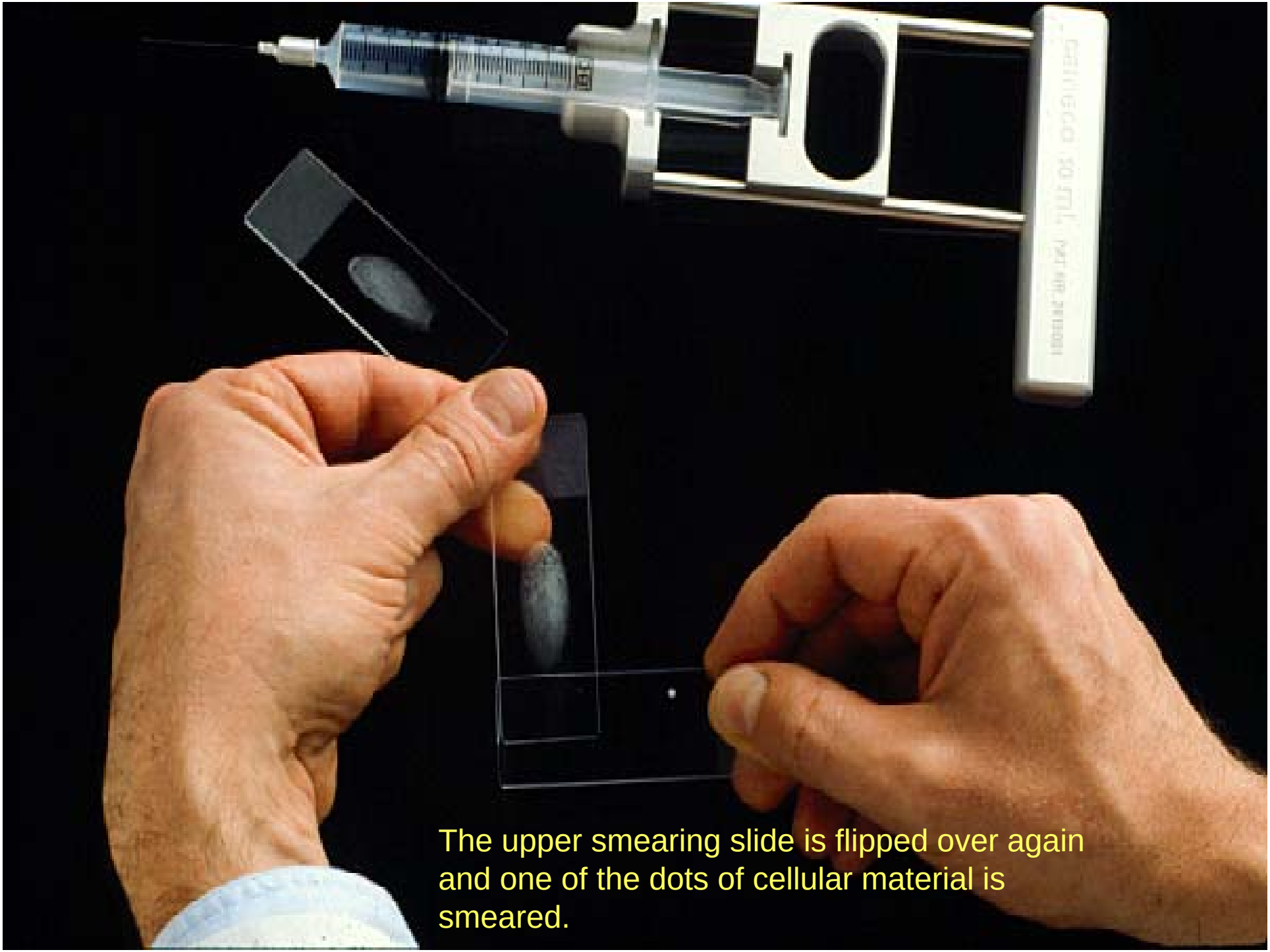
Dividing Excess Material into Several Smears



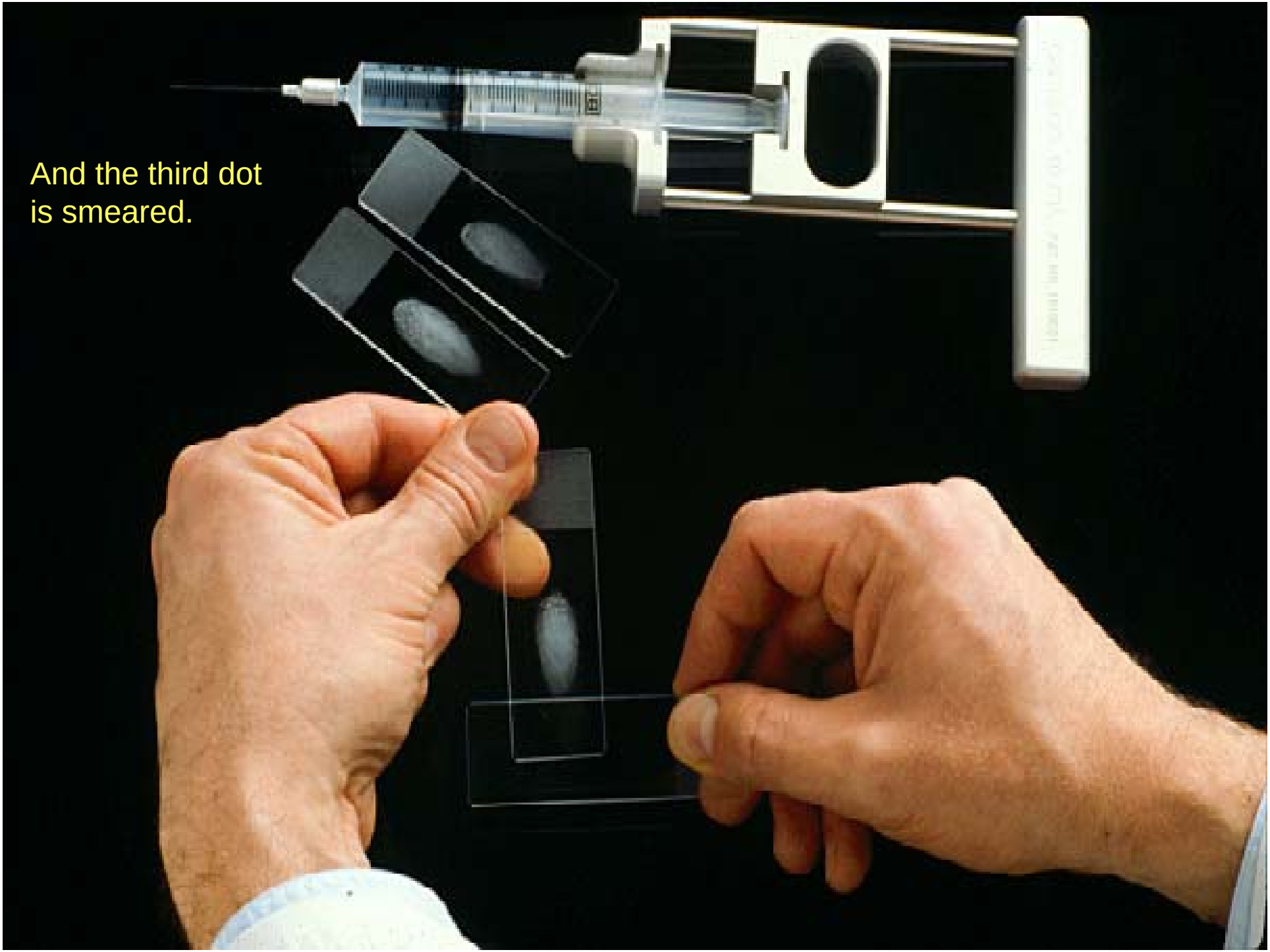

The upper slide touches the dot of material on the lower slide to pick up some of the sample, then we move it a little to the left and repeat..

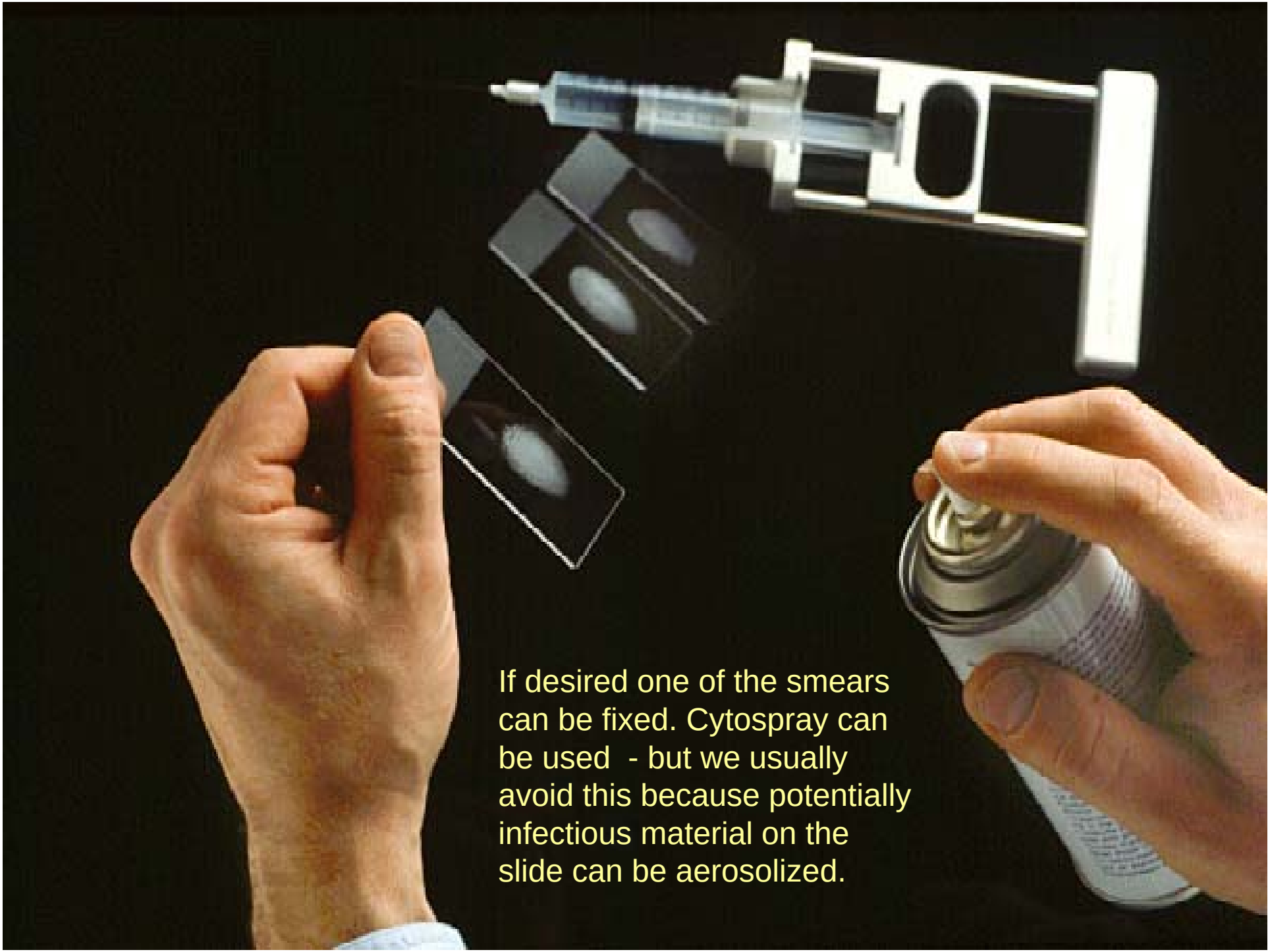

Upper smearing slide has been flipped over – the two dots of material that were picked up are now on the upper surface.

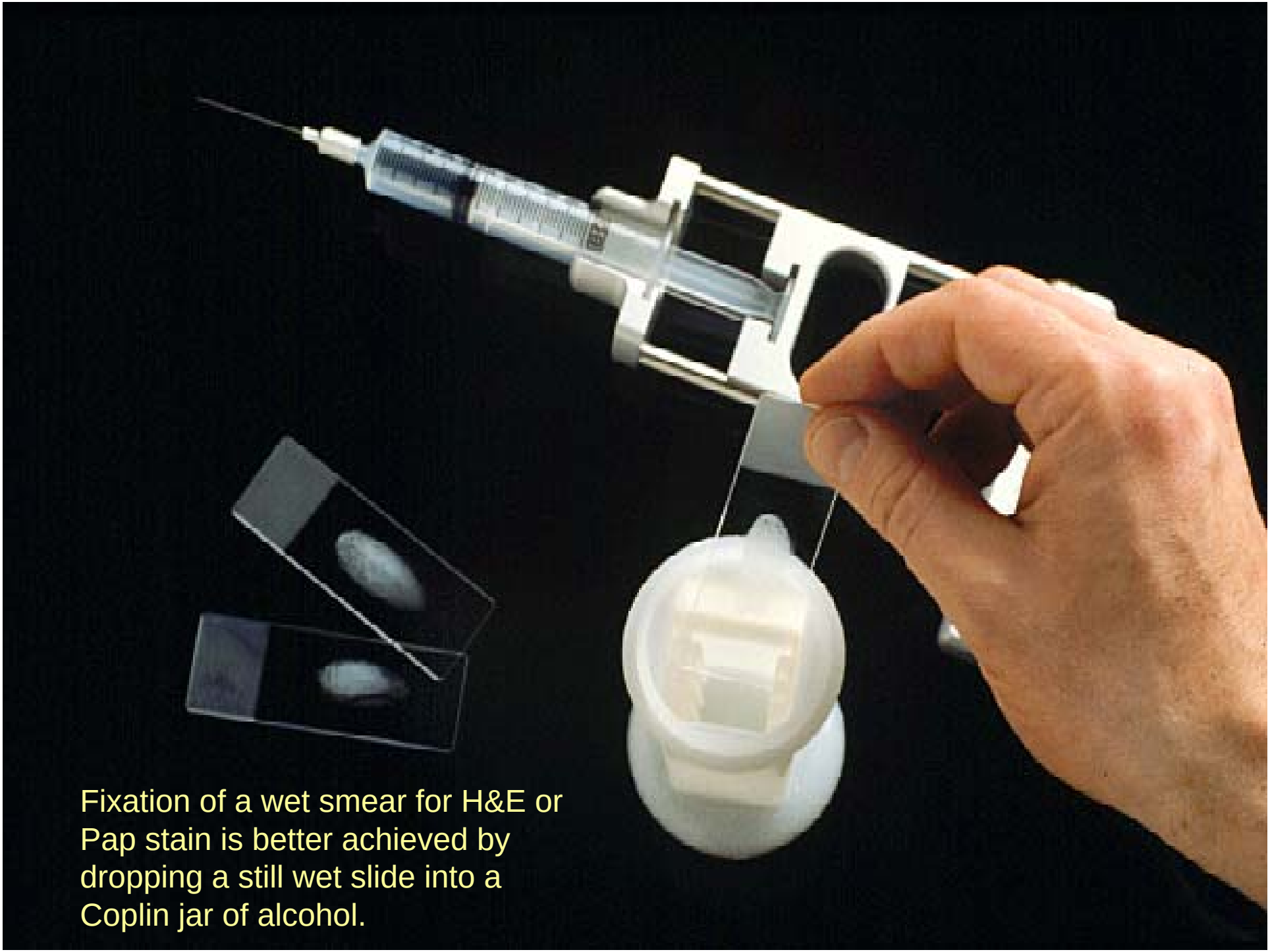

Clean lower surface of the slide is used to prepare smear #1, the material left on the original slide



The upper smearing slide is flipped over again and one of the dots of cellular material is smeared.

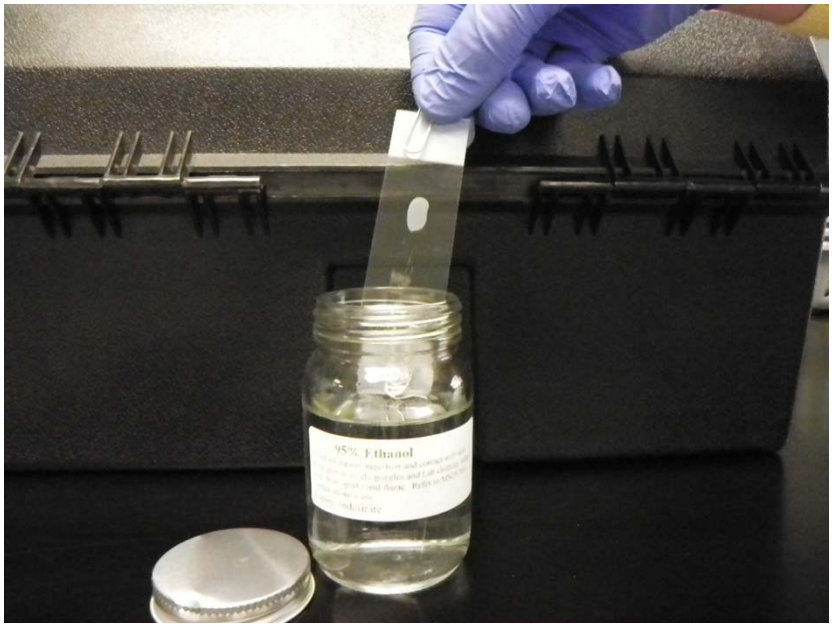

And the third dot
is smeared.


If desired one of the smears can be fixed. Cytospray can be used - but we usually avoid this because potentially infectious material on the slide can be aerosolized.



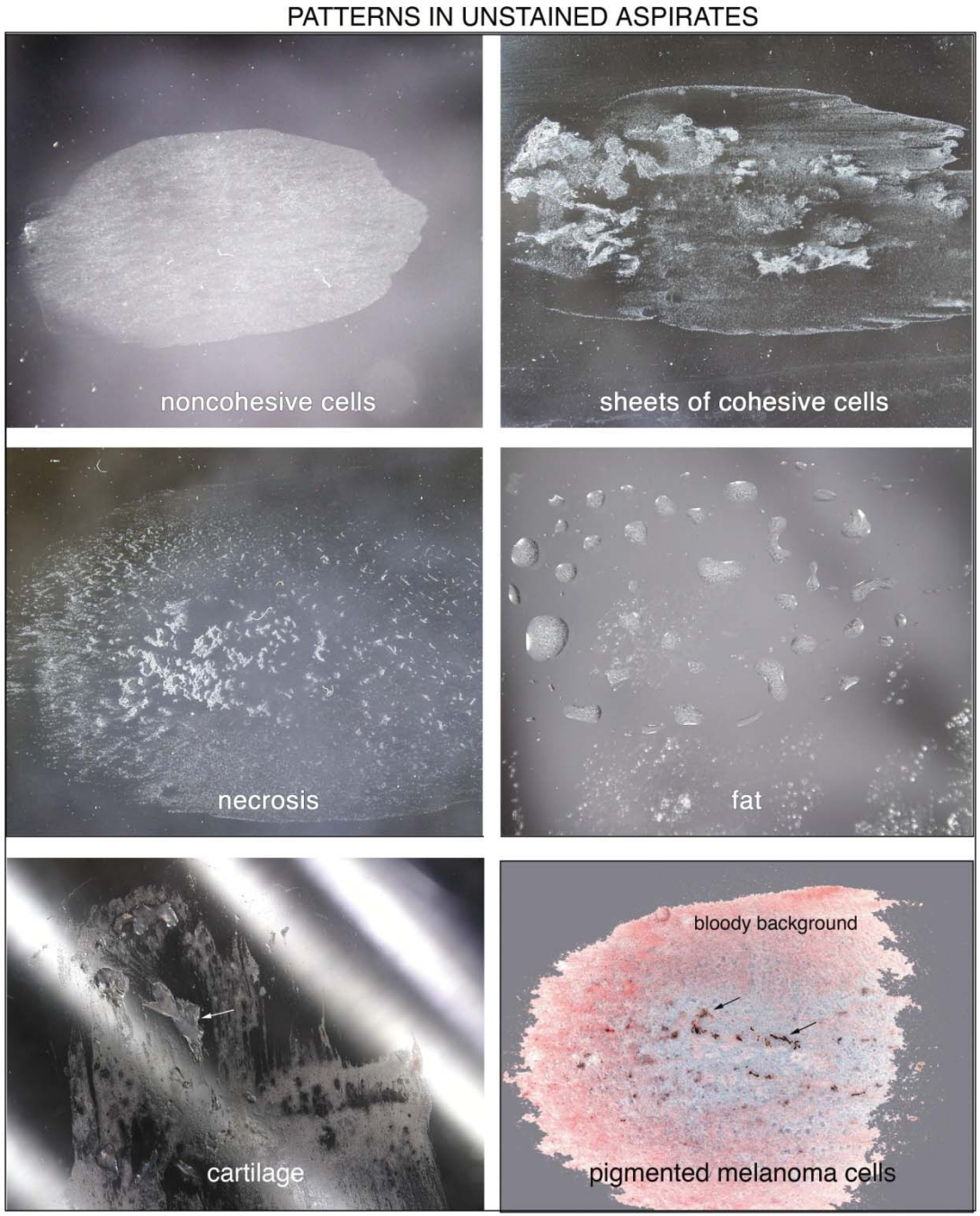
Fixation of a wet smear for H&E or Pap stain is better achieved by dropping a still wet slide into a Coplin jar of alcohol.

Fixation



Small fixative bottles with a tight fitting lid are easily carried to the bedside

Several slides can be placed in the same bottle if a paperclip is placed on the labelled end of the slide.



Gross

Preparation of smears facilitates immediate visual "gross" inspection of the aspirate.

It is usually possible to tell the type of tissue present and its quality

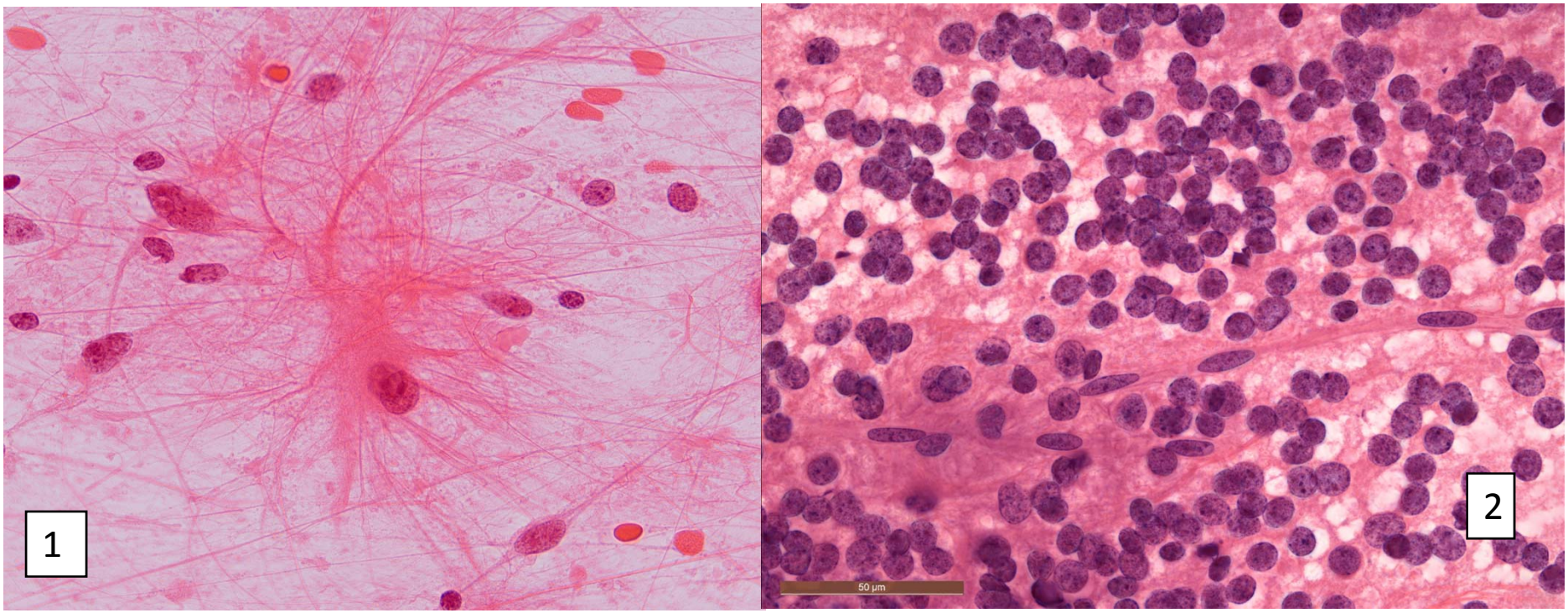
Mayall FG et al. The gross appearances of fine needle aspiration cytology samples. J Clin Pathol. Jan 2009;62(1):57-59.

Mayall F et al The utility of assessing the gross appearances of FNA specimens. Cytopathology. Dec 2010;21(6):395-397.

Geddie: Wintrobe's Atlas of Hematology

Stains

Rapid H&E, as used for frozen sections, can also be used for rapid assessment.
Commonly used for neuropathology smears.

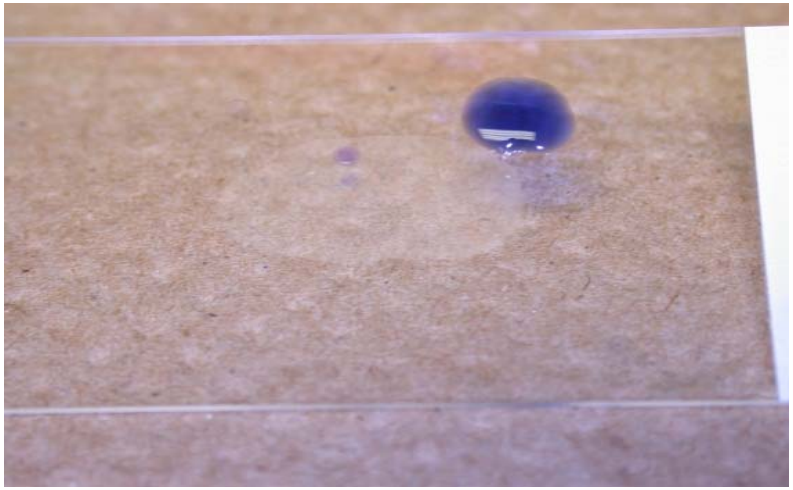


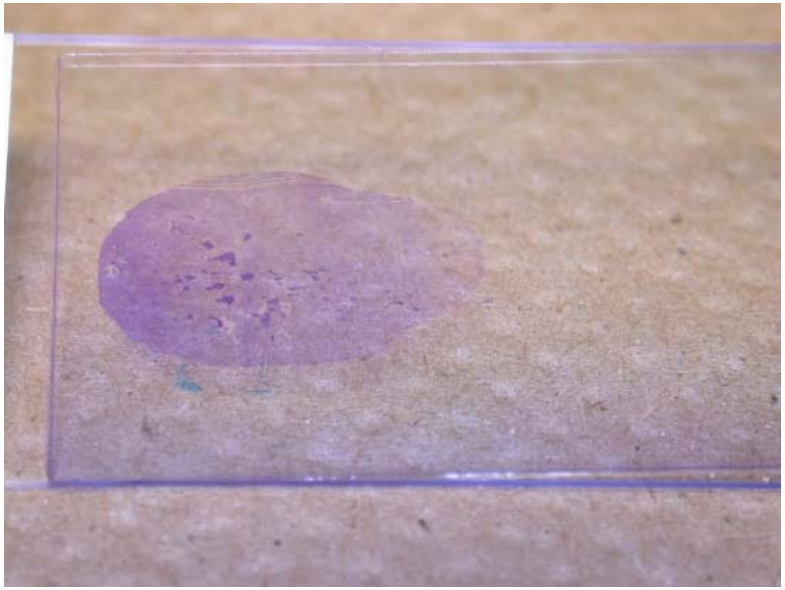
H&E Alcohol fixed smears - Reactive Astrocytes adjacent to Pilocytic Astrocytoma (1)
Central neurocytoma (2)

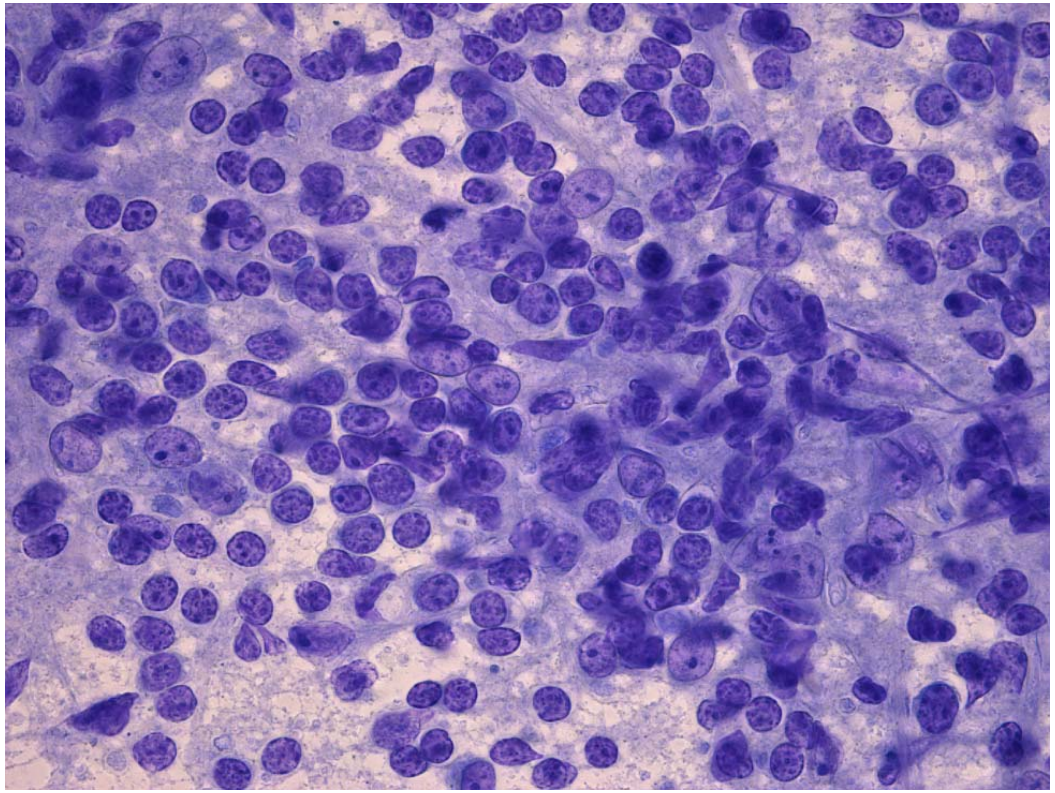
Toluidine Blue on Alcohol Fixed Smears

- An alternate stain for rapid assessment
- Wet fix a smear in alcohol
- Place a single drop of 1% Toluidine Blue on the smear and then drop a coverslip on top
- Examine the smear then slide the coverslip off and place the smear in alcohol (or just drop in alcohol)
- Stain routinely with Papanicolaou

Rapid Stains for ROSE







"Tol blue" rapid stain – essentially just coverslip the alcohol fixed smear with a drop of toluidine blue. The smear can be examined immediately

Romanowsky-Giemsa Stains

- All RG stains are based on a combination of Methylene blue, Eosin and the oxidation products of Methylene blue, called the Azure dyes – RG stains euchromatin and heterochromatin equally
- The “RG effect” refers to metachromatic purple staining of DNA, while RNA is blue.
- The RG effect is almost entirely dependent on the presence of Azure B
- Common Rapid Romanowsky stains like Diff-Quik usually heavier on Azure C

Journal of Molecular Diagnostics Vol12 No 6
November 2010

Technical Advance

Archival Fine-Needle Aspiration Cytopathology (FNAC) Samples

Untapped Resource for Clinical Molecular Profiling

Striking difference in DNA integrity of iso-aspirate smears prepared as either DQ or Pap.

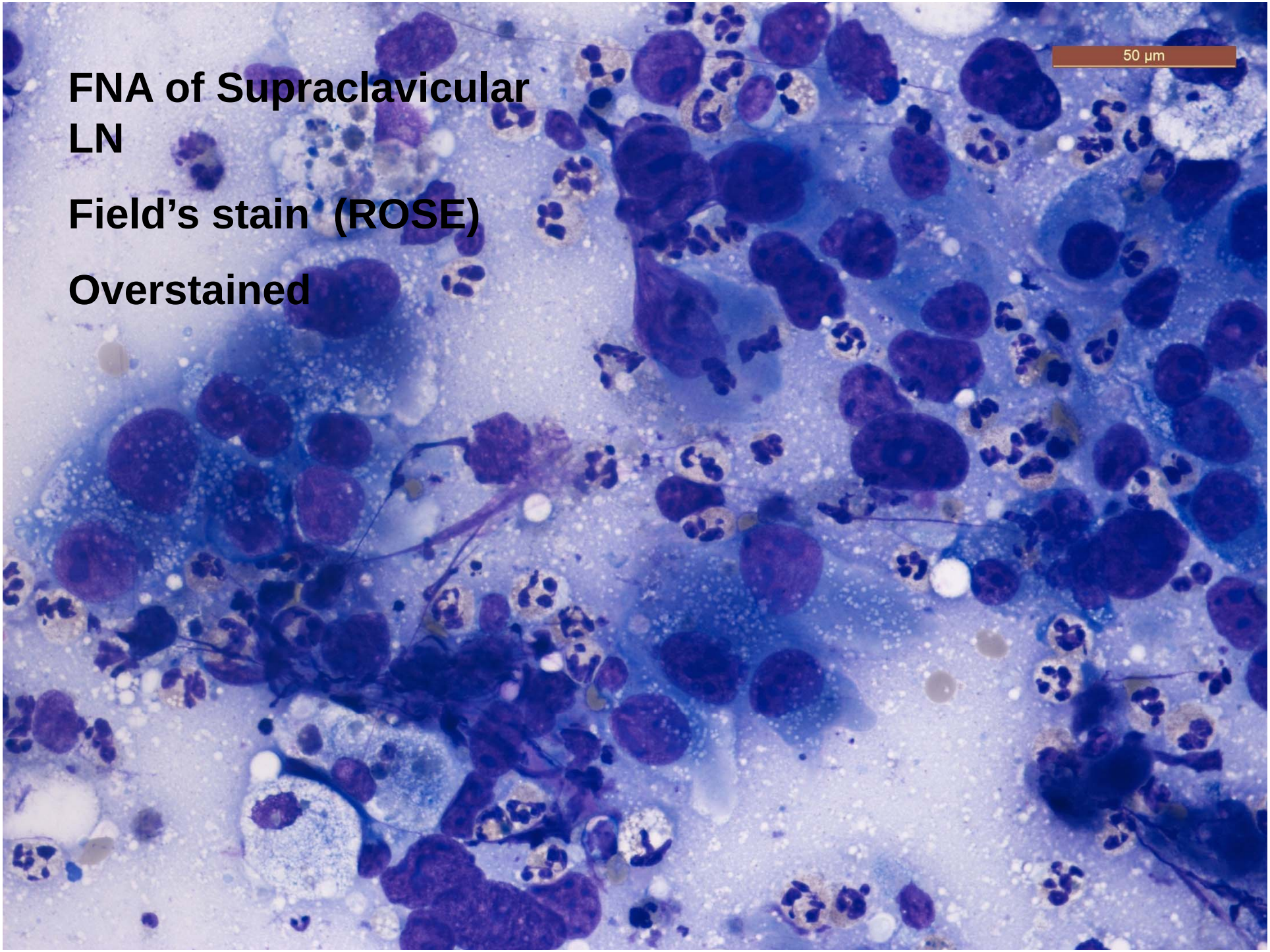
J. Keith Killian,* Robert L. Walker,*
Miia Suuriniemi,* Laura Jones,* Stephanie Scurci,*
Parvati Singh,* Robert Cornelison,*
Shannon Harmon,* Nichole Boisvert,* Jack Zhu,*
Yonghong Wang,* Sven Bilke,* Sean Davis,*
Giuseppe Giaccone,† William I. Smith, Jr.,‡
and Paul S. Meltzer*

From the Genetics Branch, and Medical Oncology Branch,† Center for Cancer Research, National Cancer Institute, Bethesda; and the Department of Pathology,‡ Suburban Hospital, Bethesda, Maryland*

In making air dried Giemsa stained slides you are creating a "tissue bank" of material that can be used for future molecular testing either for patient care or research (subject to patient consent and institutional approval.)

Rapid Romanowsky Stains

- Diff-Quik, Hemacolor, Field's Stain – increased amounts of Methylene Blue
- Stain rapidly, may look more blue than purple and tend to overstain – danger of "blastic" chromatin, normally pale, appearing mature
- May see "pseudo nucleoli" especially if smear is not completely dry
- Wright's stain – less Azure B – somewhat paler bluish-purple



FNA of Supraclavicular LN

Field's stain (ROSE)

Overstained

Diff Quik® Stain

- A rapid RG type stain – a modification of "Field's" stain for malarial thick films
- Field's - Solution A = Azure B + phosphate salts, Solution B = Eosin + phosphate salts
- Diff-Quik - Solution A = Eosin substitute xanthene S Solution B = Azure A/C + buffers

Field, J.W. Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol. XXXV. No.1. July, 1941.

FURTHER NOTE ON A METHOD OF STAINING MALARIAL PARASITES IN THICK BLOOD FILMS.

BY

J. W. FIELD, M.D.*

From the Institute for Medical Research, Federated Malay States.

In an earlier communication I described a simple, rapid and apparently reliable method of staining thick blood films for malaria (FIELD, 1940). The method, developed originally on lines suggested by the work of SIMONS (1938), and PAMPANA (1938), finally introduced what was believed to be a new idea in blood staining—the use of the haemoglobin to provide colour contrast and, in effect, to serve as a counter-stain. SIMONS had drawn attention to the

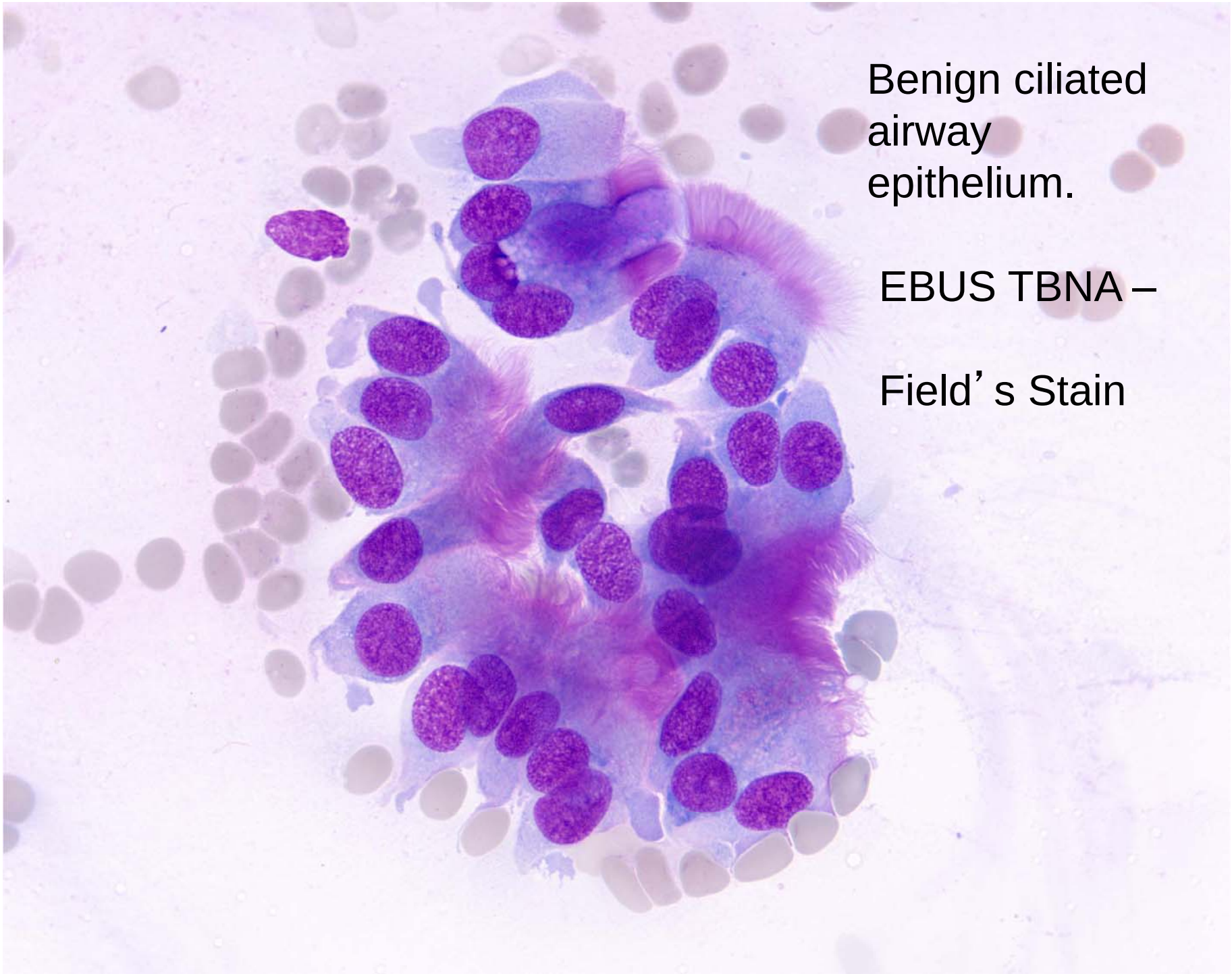
* My assistant, Mr. YAW WAH CHEW, has tested the effects of a variety of stains on dried blood and the final working out of a practical thick-film staining method for rapid malarial diagnosis was much facilitated by his observations.

My thanks are also due to Mr. R. A. WRIGHT, Veterinary Officer, Malacca, for the supply of trypanosome-infected blood.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.
Vol. XXXV. No. 1. July, 1941.

Field's Stain for Rapid Assessment of Fine Needle Biopsies

- Air dry and fix smear in alcohol
- Smear goes into Eosin first (as for Diff-Quik) although this is Field's solution #2 - about 3-4 dips depending on thickness
- Smear then goes into Giemsa (as for Diff-Quik) which is Field's solution #1 – about 4-5 dips
- Rinse in tap water. Re-stain in Giemsa if too pale



Benign ciliated airway epithelium.

EBUS TBNA –

Field’s Stain

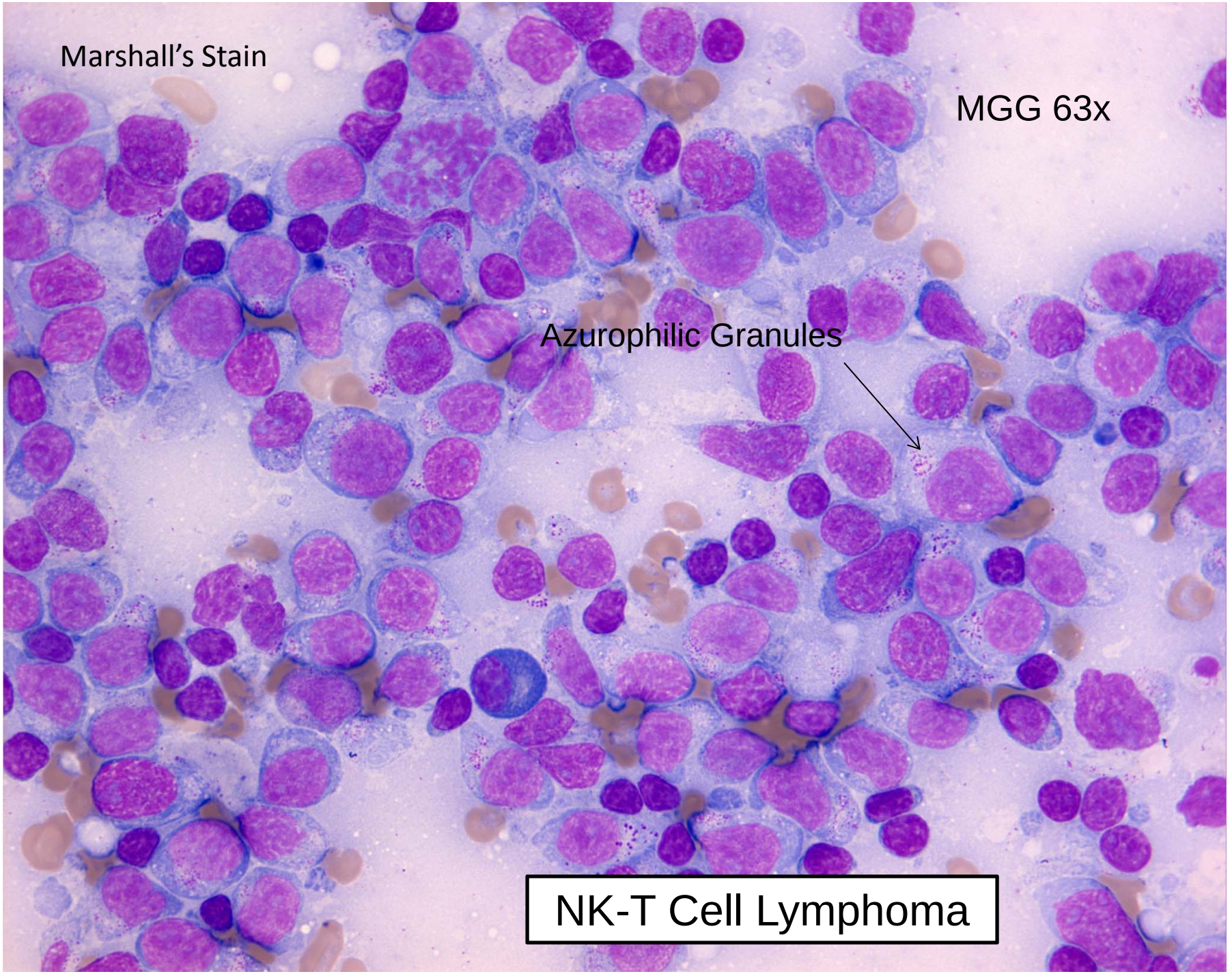
Traditional Romanowsky stains

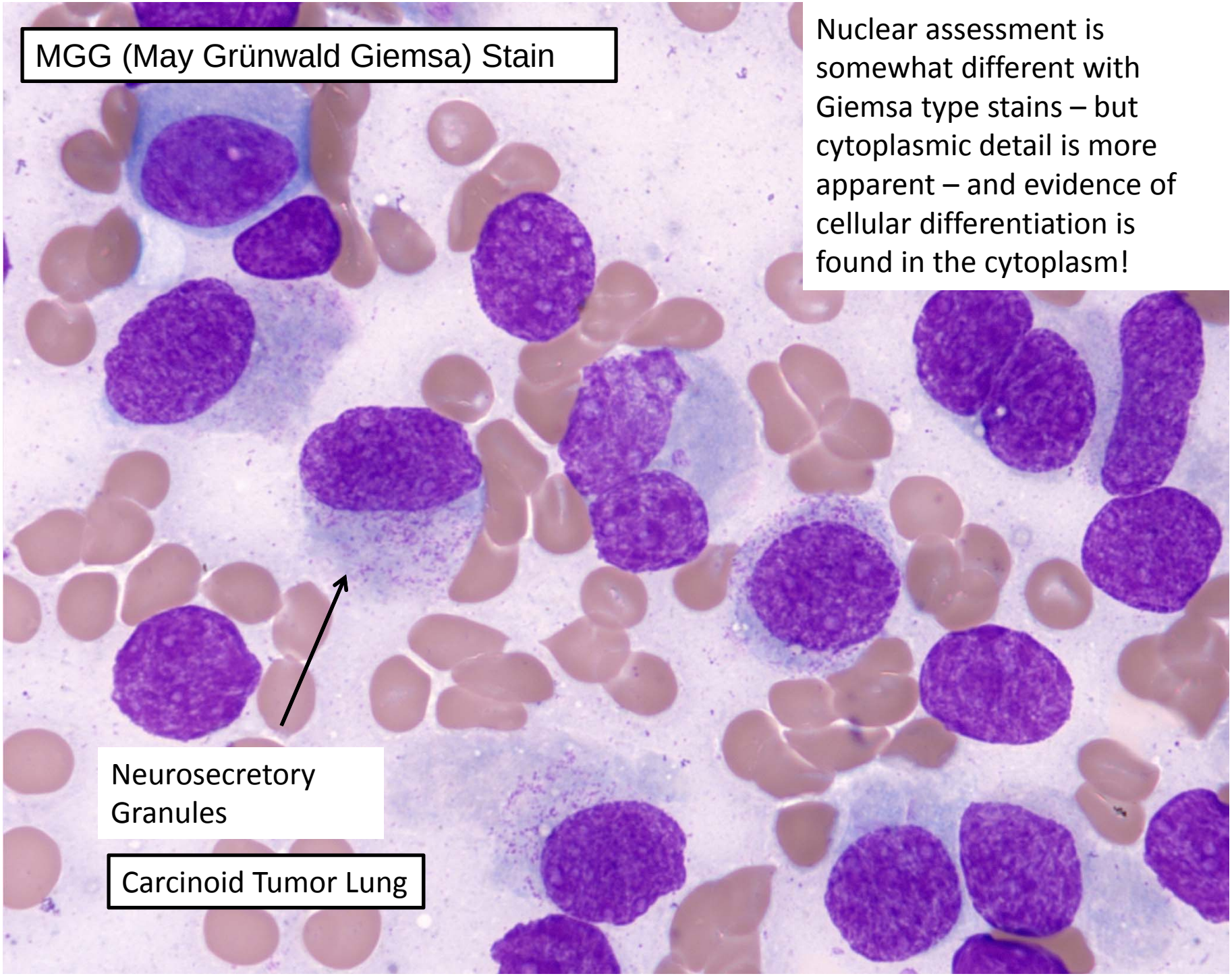
- May-Grünwald-Giemsa, Leischman's etc. although they take longer, provide more subtle gradations of colour and better distinction of purple DNA and pale blue RNA, and cytoplasmic granules
- Wright's stain is a traditional hematology stain but it tends to understain FNB smears
- The main problem with these stains is that they are critically sensitive to alterations in pH - working solutions need to be continually changed.

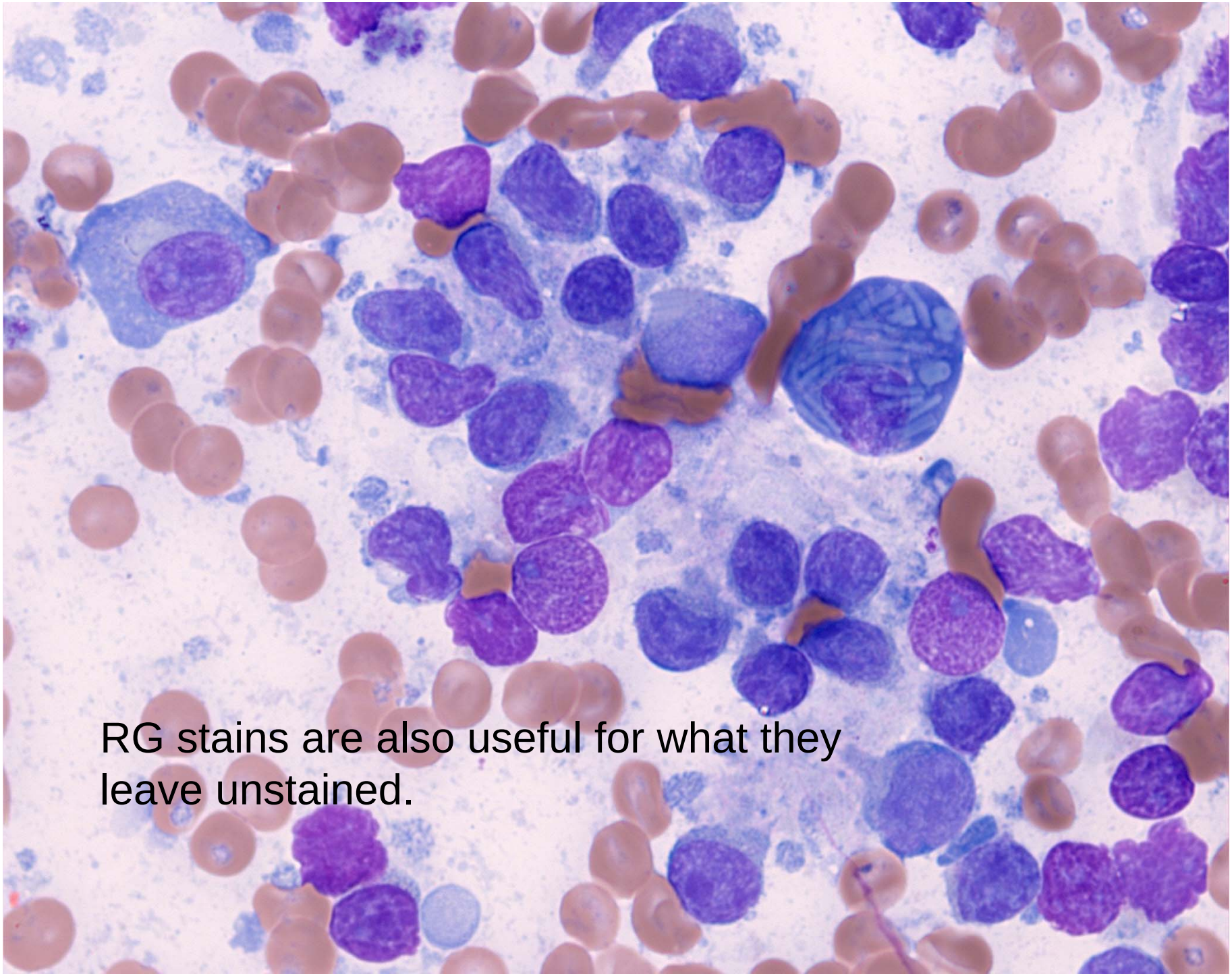
Marshall's Stain

- To simplify staining and standardize results Marshall advocated a one part stain consisting of Methylene blue, Azure B, Eosin
- Working stain is prepared from a stock solution - smear is placed in working stain for 10 minutes
- Marshall used buffer but de-ionized water works well
- Inexpensive, simple and very consistent

Marshall et al. Staining properties and stability of a standardized Romanowsky stain. J. Clin Pathol. 31.3 (1978): 280-82


Marshall's Stain
MGG 63x
Azurophilic Granules
NK-T Cell Lymphoma


MGG (May Grünwald Giemsa) Stain
Nuclear assessment is somewhat different with Giemsa type stains – but cytoplasmic detail is more apparent – and evidence of cellular differentiation is found in the cytoplasm!
Neurosecretory Granules
Carcinoid Tumor Lung


RG stains are also useful for what they
leave unstained.