

Belgische Vereniging voor Analytische Cytologie vzw.  
Association Belge de Cytologie Analytique asbl.  
Belgian Society for Analytical Cytology.



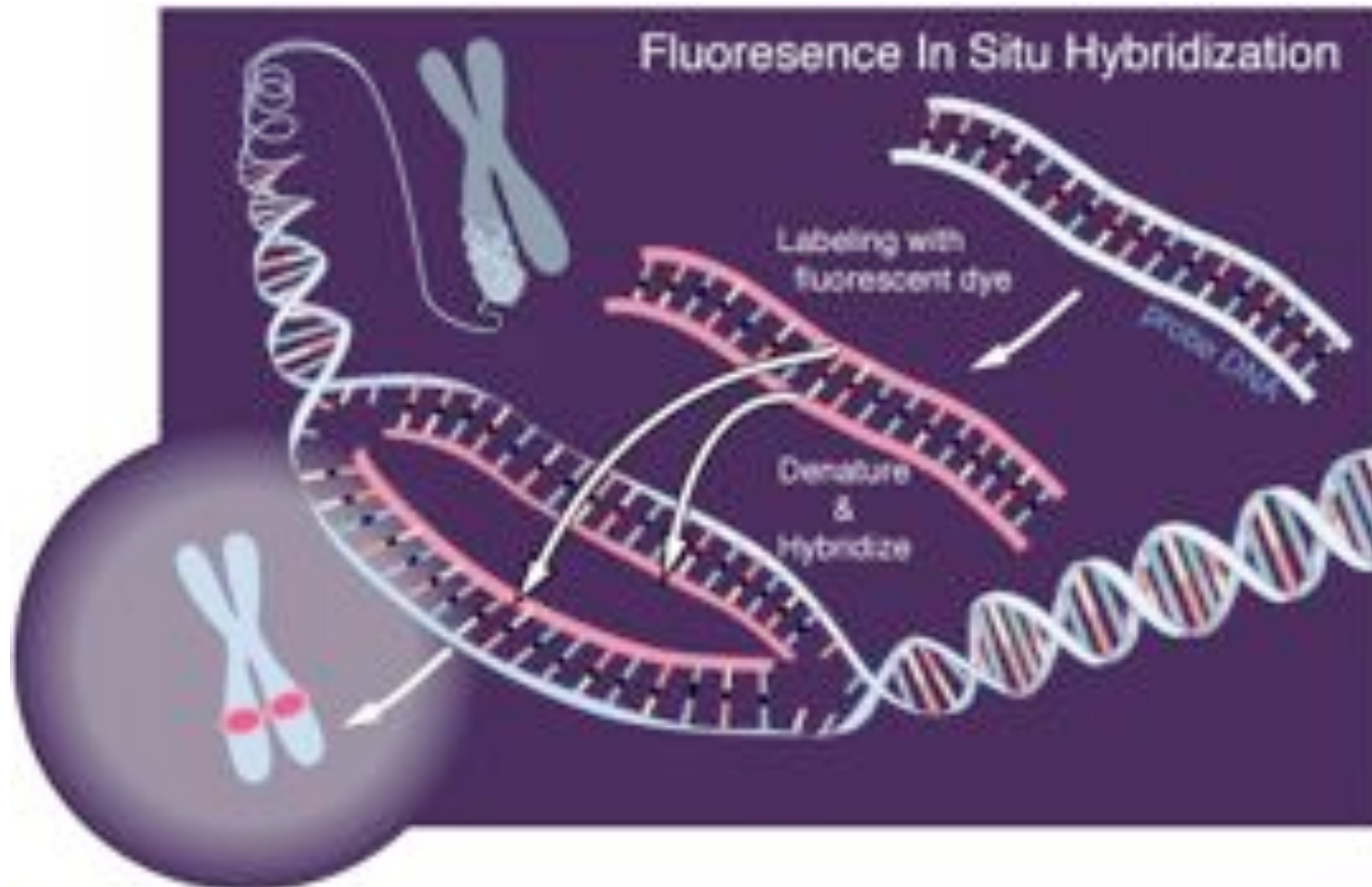
Module n° : 605

**Title : FISH in haematological malignancies**

Lecturer : Femke Hillen

Molecular Biology and Cytometry Course, May 7-8 2009, Mol

# FISH: Fluorescent in-situ hybridisation



# FISH: on what?

- FFPE tissues (breast carcinomas, lymphomas, sarcomas)
- FACS sorted cells
- Bone marrow and blood smears, touch imprint preparation

- Remarks:

fixation for DNA conservation of

\*tissues: neutral buffered formalin

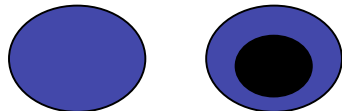
\*cells: paraformaldehyde

\*fresh tissues/cells: air dry, cold methanol/acetic acid

overfixation!; weak signal; increase digestion

stringent washing!; cytoplasmic background

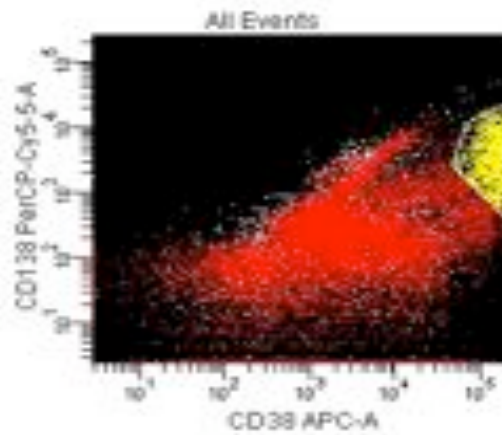
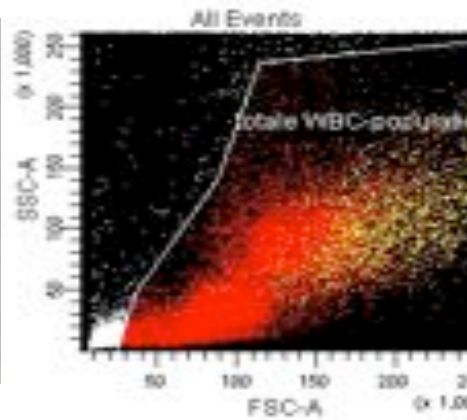
overdigestion!



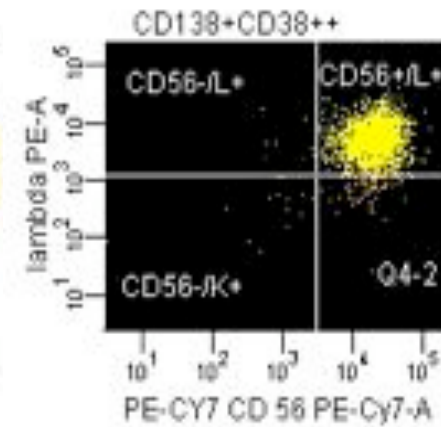
# FACS sorted cells with FACS Aria



Blood or  
bone marrow



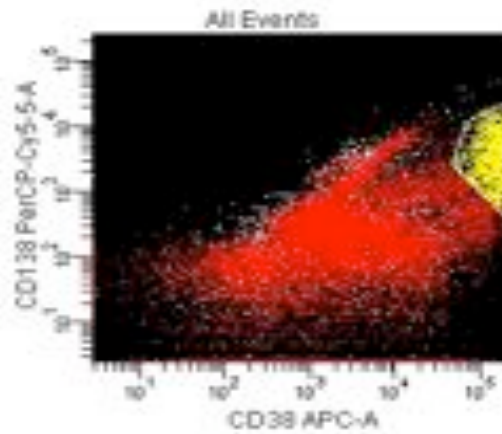
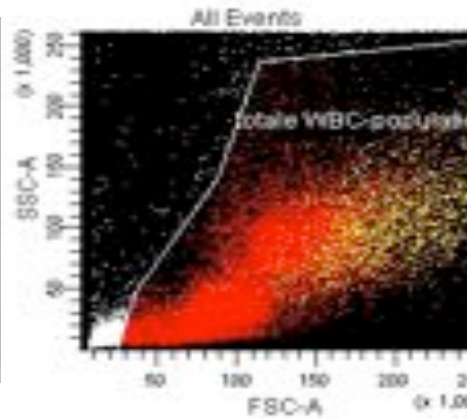
FACS sorting



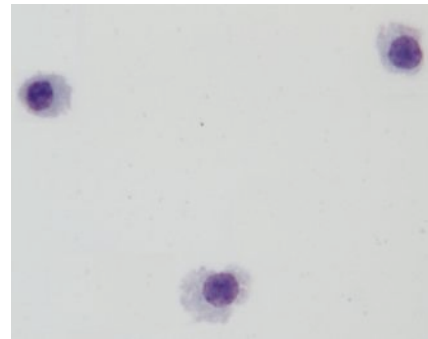
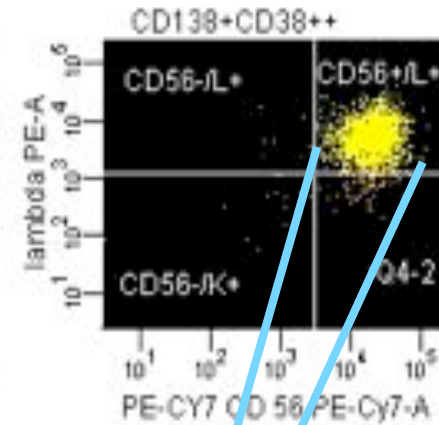
# FACS sorted cells with FACS Aria



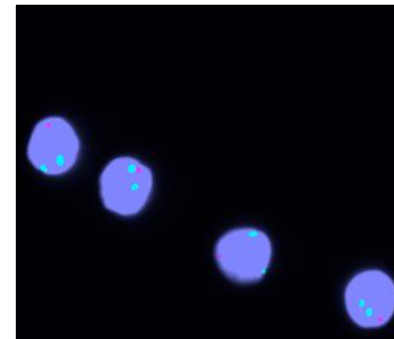
Blood or bone marrow



FACS sorting



H&E



FISH



Advantage:  
Specificity  
Count less cells  
(20)

# Protocols

- *FFPE tissues/sorted cells*
  - \*protocol of pharmDX DAKO (HER2)  
standardised buffers
  - \*protocol of Abbott  
prepare own buffers, longer protocol
- *Fresh tissues/cells*

# Protocols

- **FFPE tissues/sorted cells**
  - Deparaffination
  - Pre-treatment
  - Digestion (Pepsine/Protease)+Dehydratation
  - Fixation (Formalin)
  - Hybridisation (probe)
  - Washing+Dehydration
  - Mounting (DAPI counterstain)
  - Analysation
  
- **Fresh tissues/cells**
  - Fixation with methanol/acetic acid+ Washing with SSC
  - Hybridisation (probe)
  - Washing with SSC/NP-40+Dehydratation
  - Mounting (DAPI counterstain)
  - Analysation

# Analysis

- FFPE/Inprints/smears

100 cells

- XY-FISH

500 cells

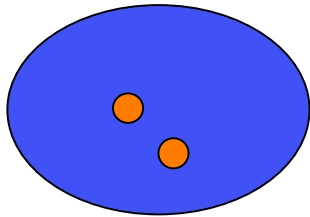
- Sorted cells

20 cells

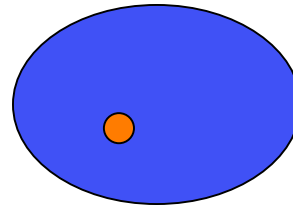


# Analysis

- Numeric aberrations



Normal cell: 2O

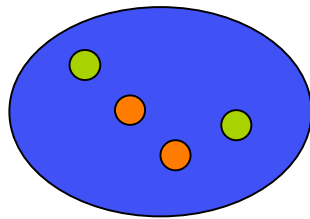


Abberant cell: 1R

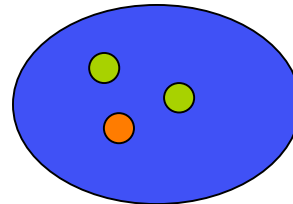
- Chromosomal aberrations (translocations)

# Analysis

- Numeric aberrations



Normal cell: 2O2G



Abberant cell: 1O2G

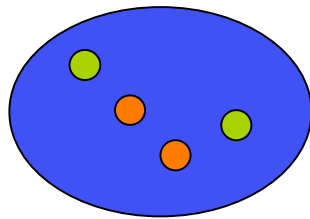
● INTERNAL CONTROL

same chromosome  
centromere probe

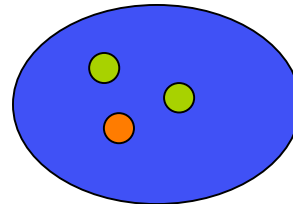
- Chromosomal aberrations (translocations)

# Analysis

- Numeric aberrations



Normal cell: 2O2G



Abberant cell: 1O2G

● INTERNAL CONTROL

same chromosome  
centromere probe

- Chromosomal aberrations (translocations)

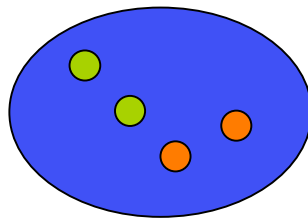
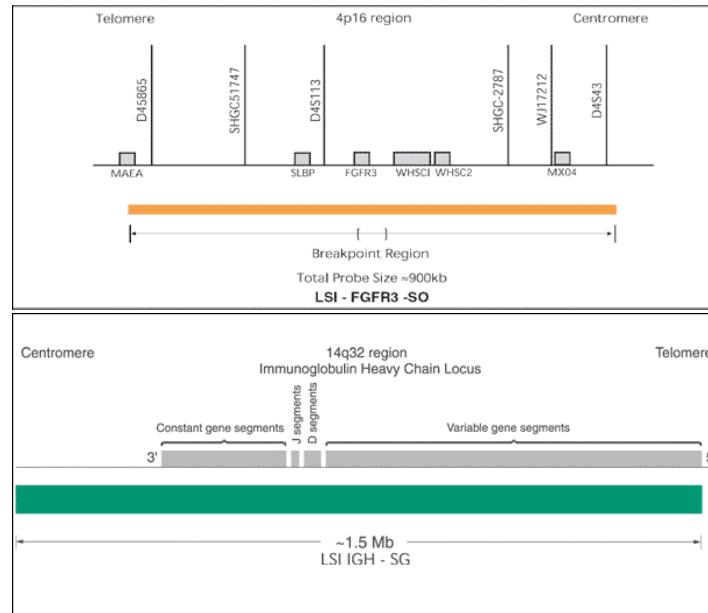
- \* Dual Fusion Translocation Probe

- \* Dual Color, Break Apart probes

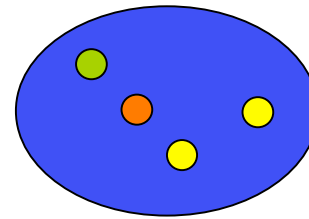
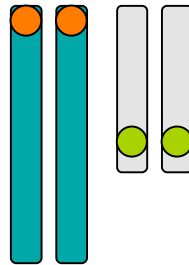
# Probe types

- Dual Fusion Translocation Probe

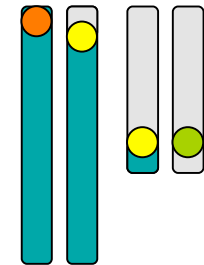
t(4;14)



Normal cell: 20G

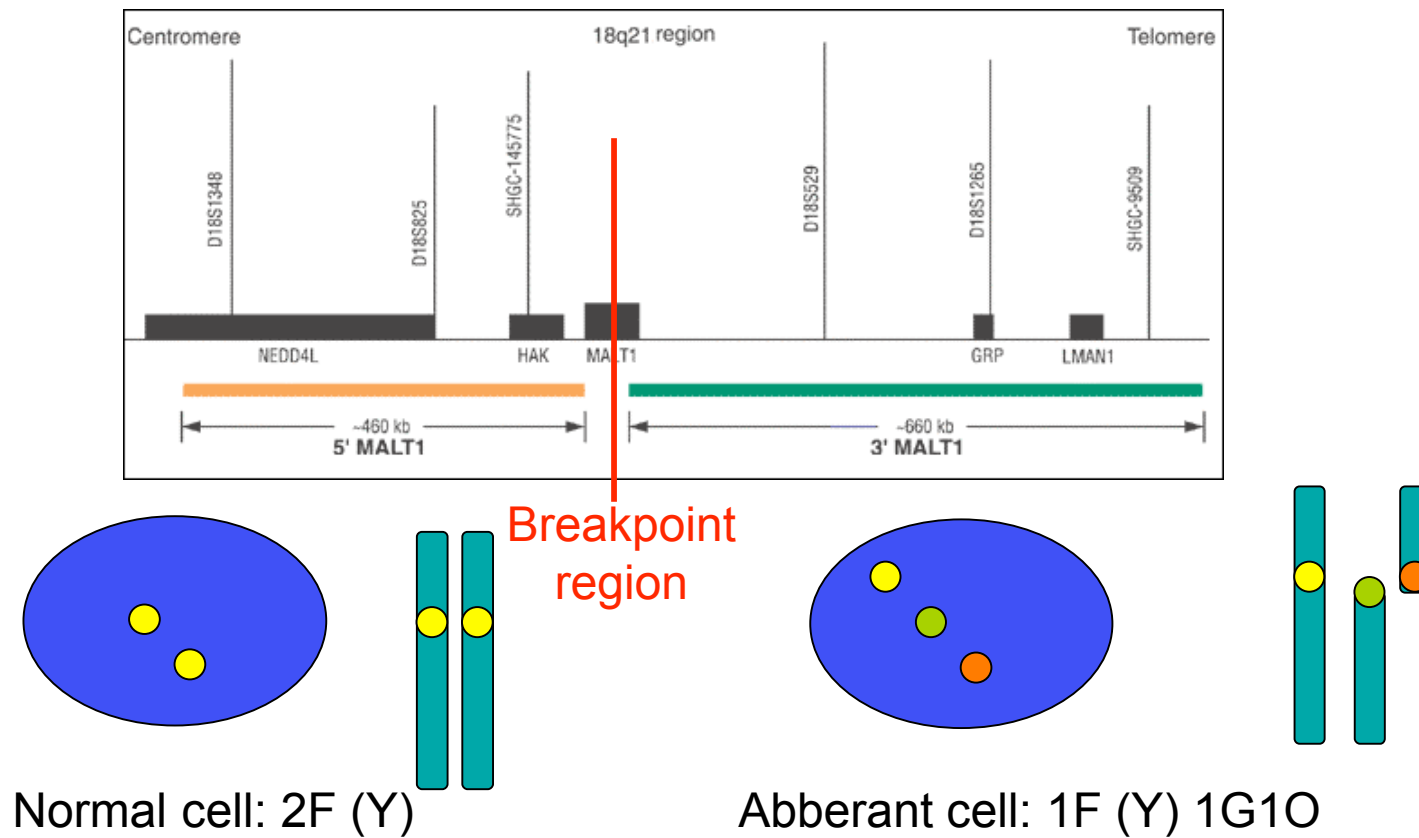


Abberant cell: 2F (Y) 1G10



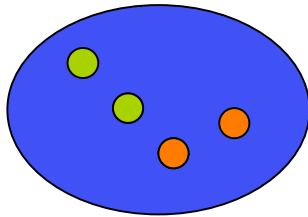
# Probe types

- Dual Color, Break Apart probes

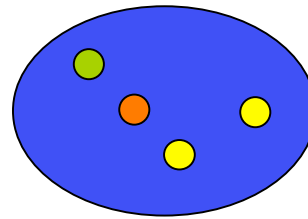


# Probe types

- Dual Fusion Translocation Probe

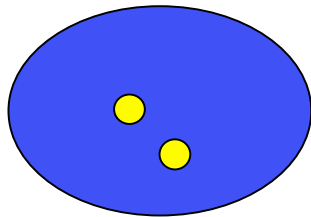


Normal cell: 2O2G

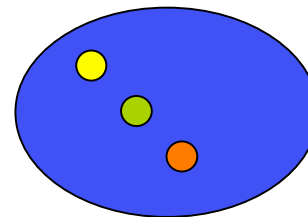


Abberant cell: 2F (Y) 1G1O

- Dual Color, Break Apart probes



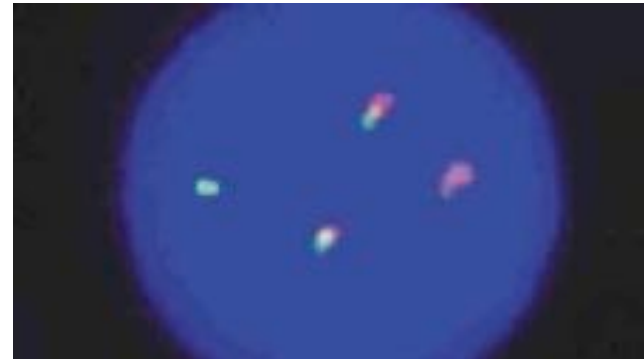
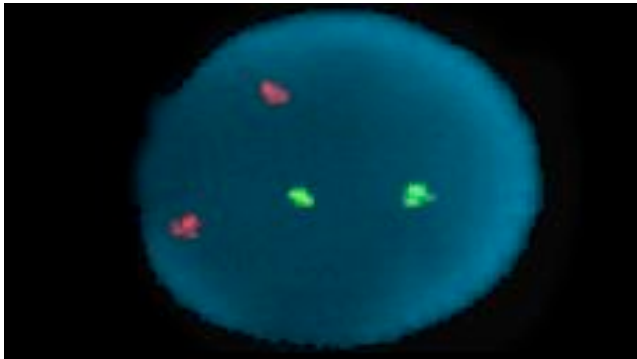
Normal cell: 2F (Y)



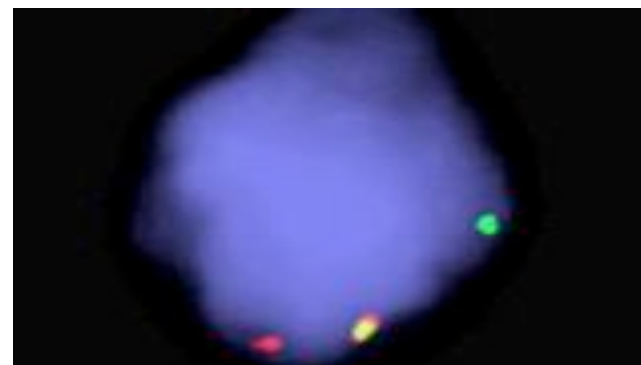
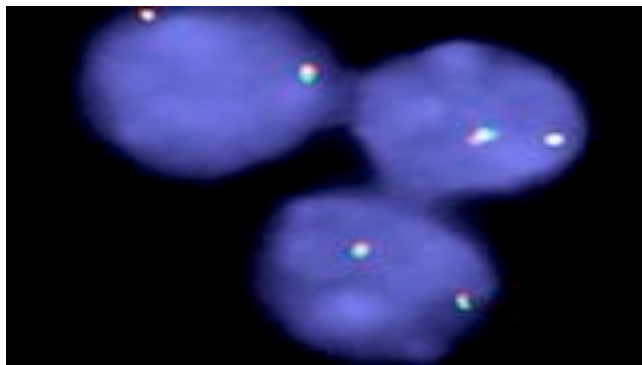
Abberant cell: 1F (Y) 1G1O

# Probe types

- Dual Fusion Translocation Probe



- Dual Color, Break Apart probes



# Panels of probes

- CLL  
tri 12 P neutral  
del 13 P positive  
del 11 P negative  
del 17 p53 P negative
- Kahler/MM  
t(4;14) P negative  
t(11;14) P neutral  
t(14;16) P negative  
del RB1 P discussion  
del 17 p53 P negative
- Mantel cell lymphoma  
t(11;14) D
- Follicular lymphoma  
t(14;18) D
- Burkitt lymphoma  
c-myc D
- MALT lymphoma  
MALT t(11;18) D
- ALK lymphoma ALCL  
ALK D, P positive

.....

D=diagnosis  
P=prognosis

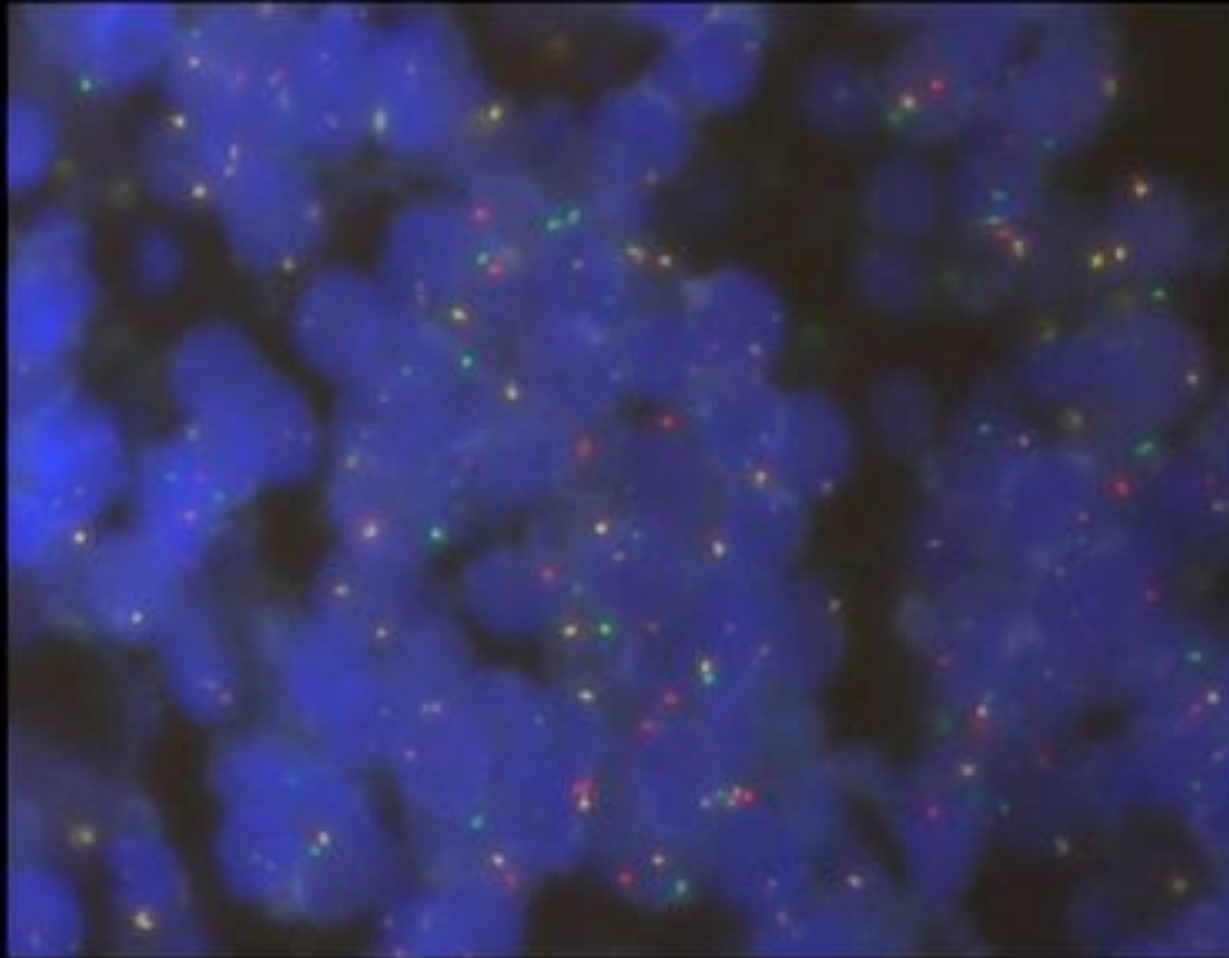


# Analysis of patients slides

- Name and type of probe
- Image (triple filter)
- Result: POS or NEG

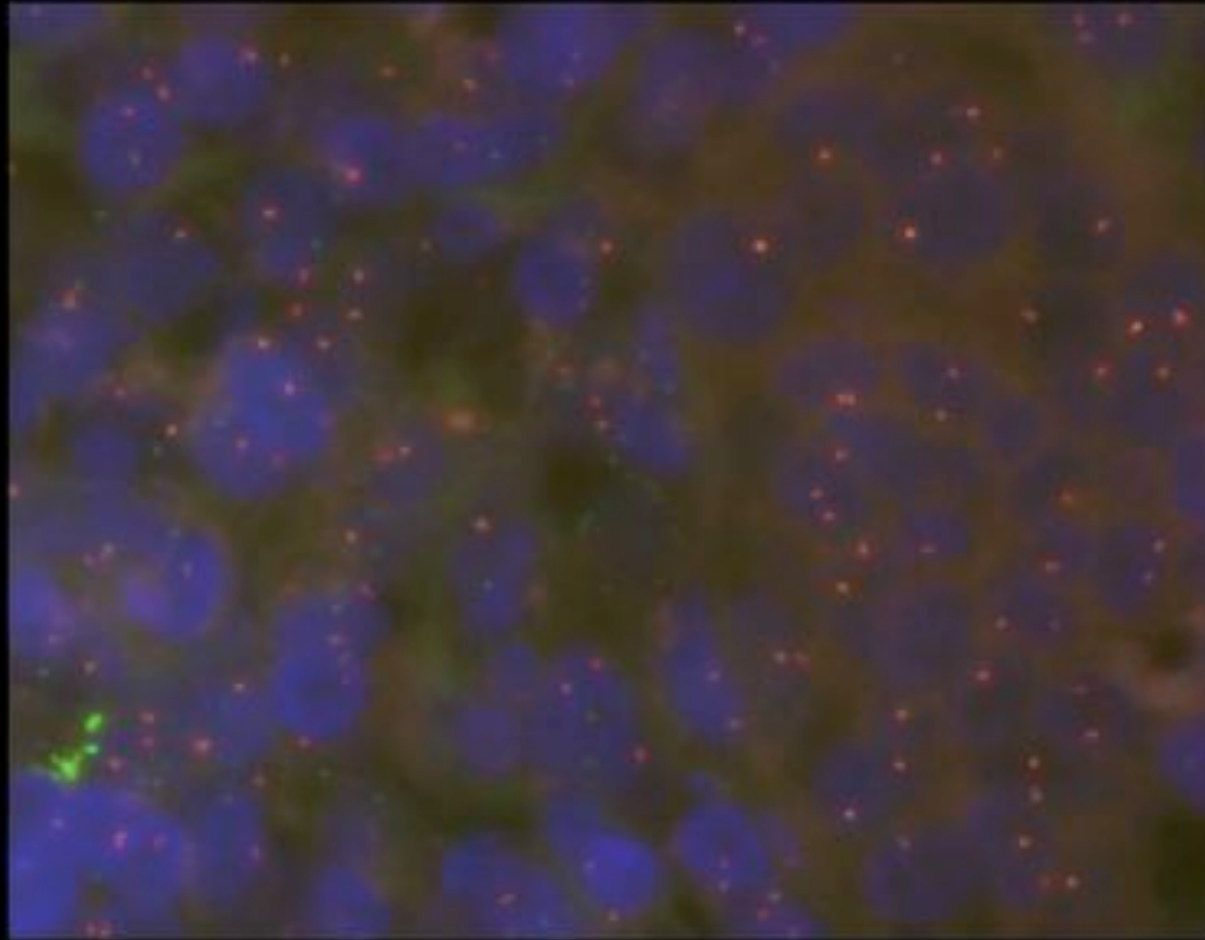
1

# TMALT, MALT lymphoma: Dual color, break apart probe



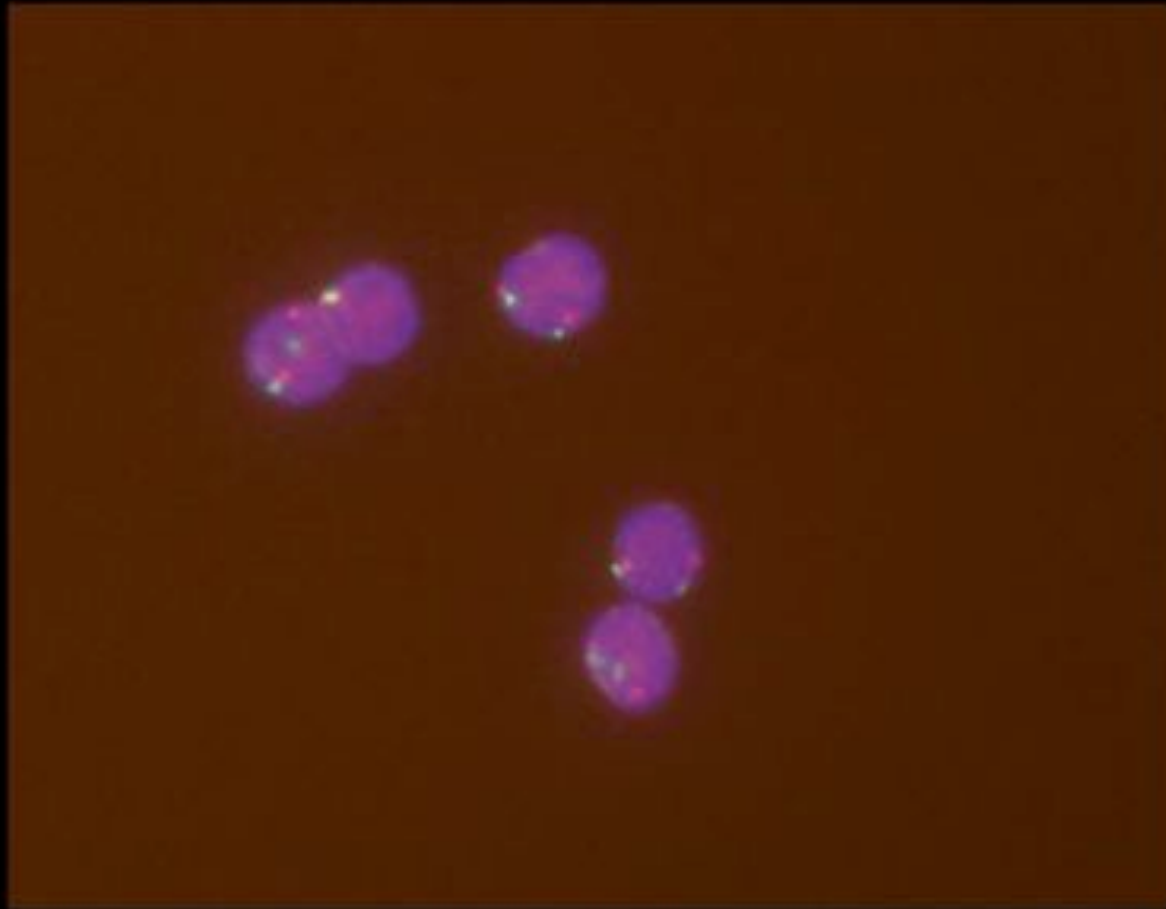
2

# TMALT, MALT lymphoma: Dual color, break apart probe



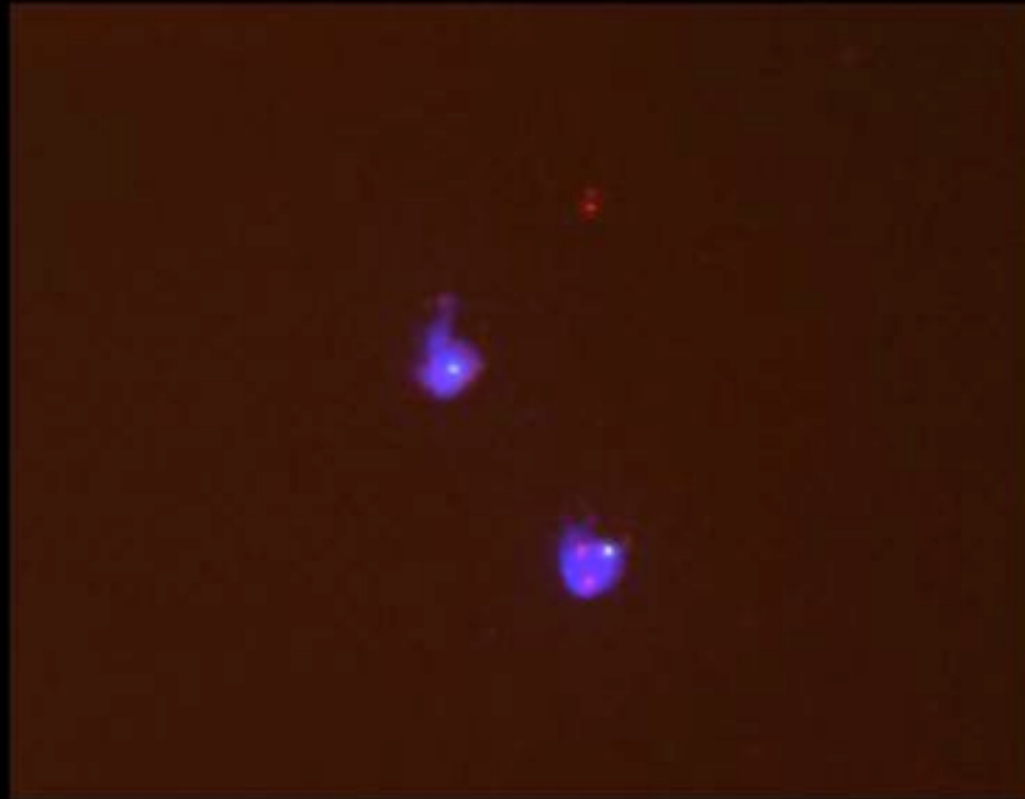
3

# Deletion 11 (ATM/CEP11): numerical probes



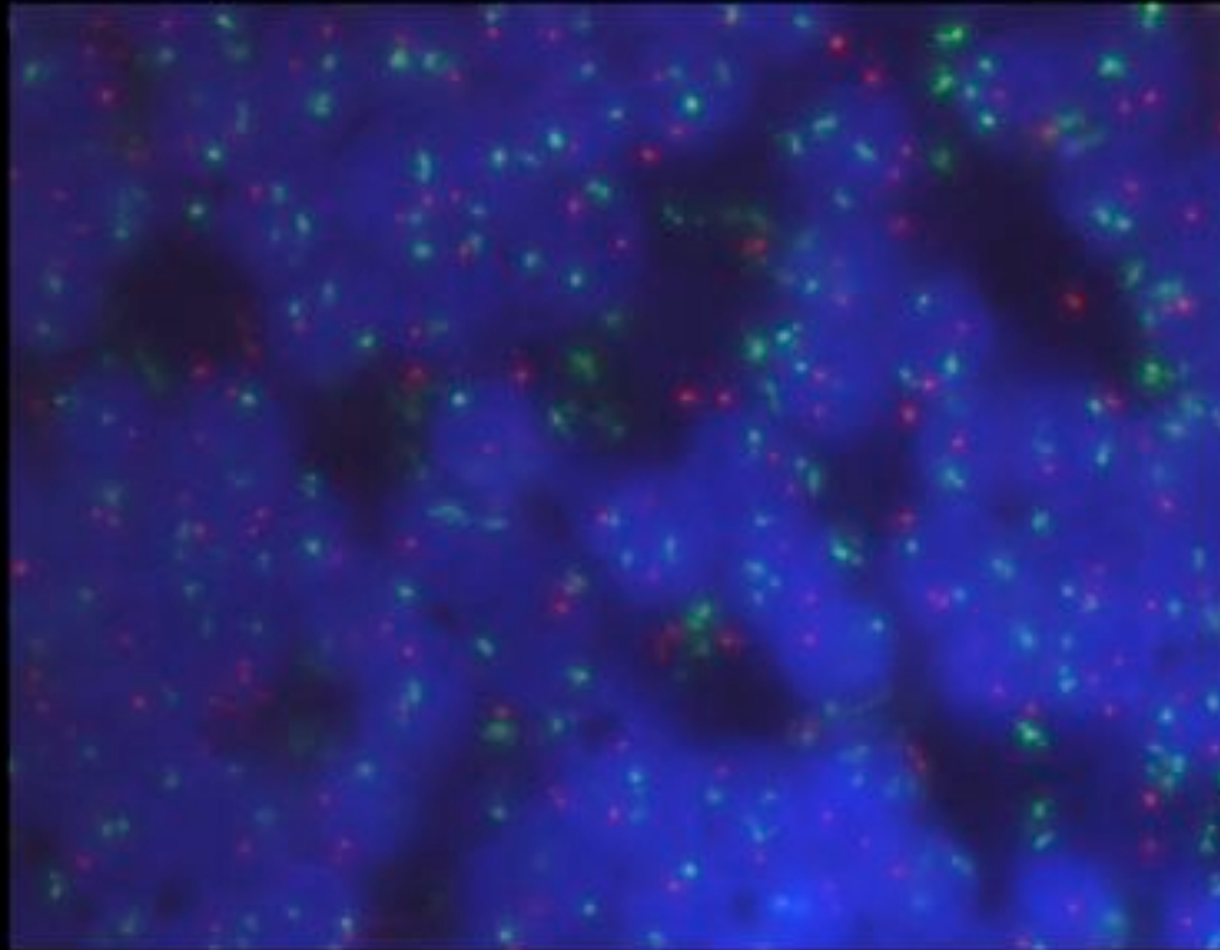
4

# Delete RB1: numerical probe



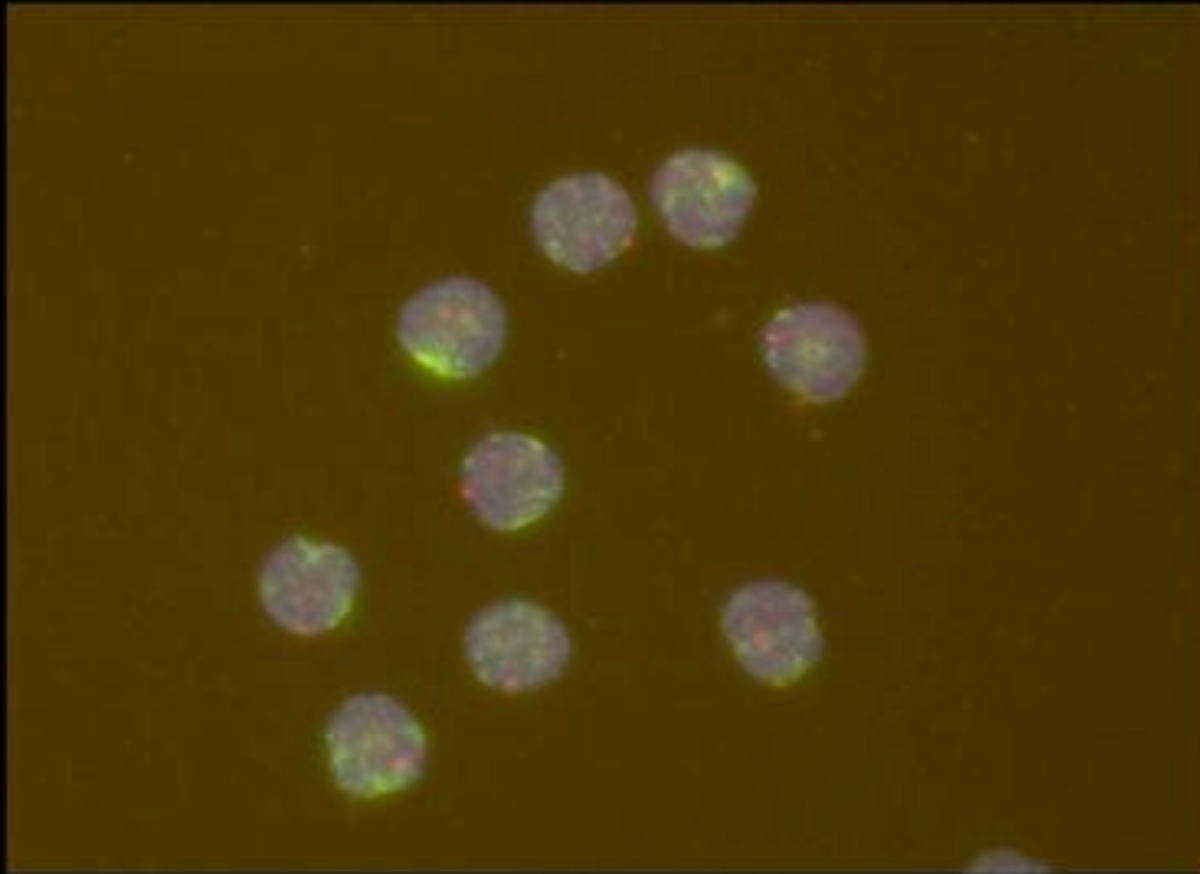
5

# Trisomie 12/deletie 13: numerical probes



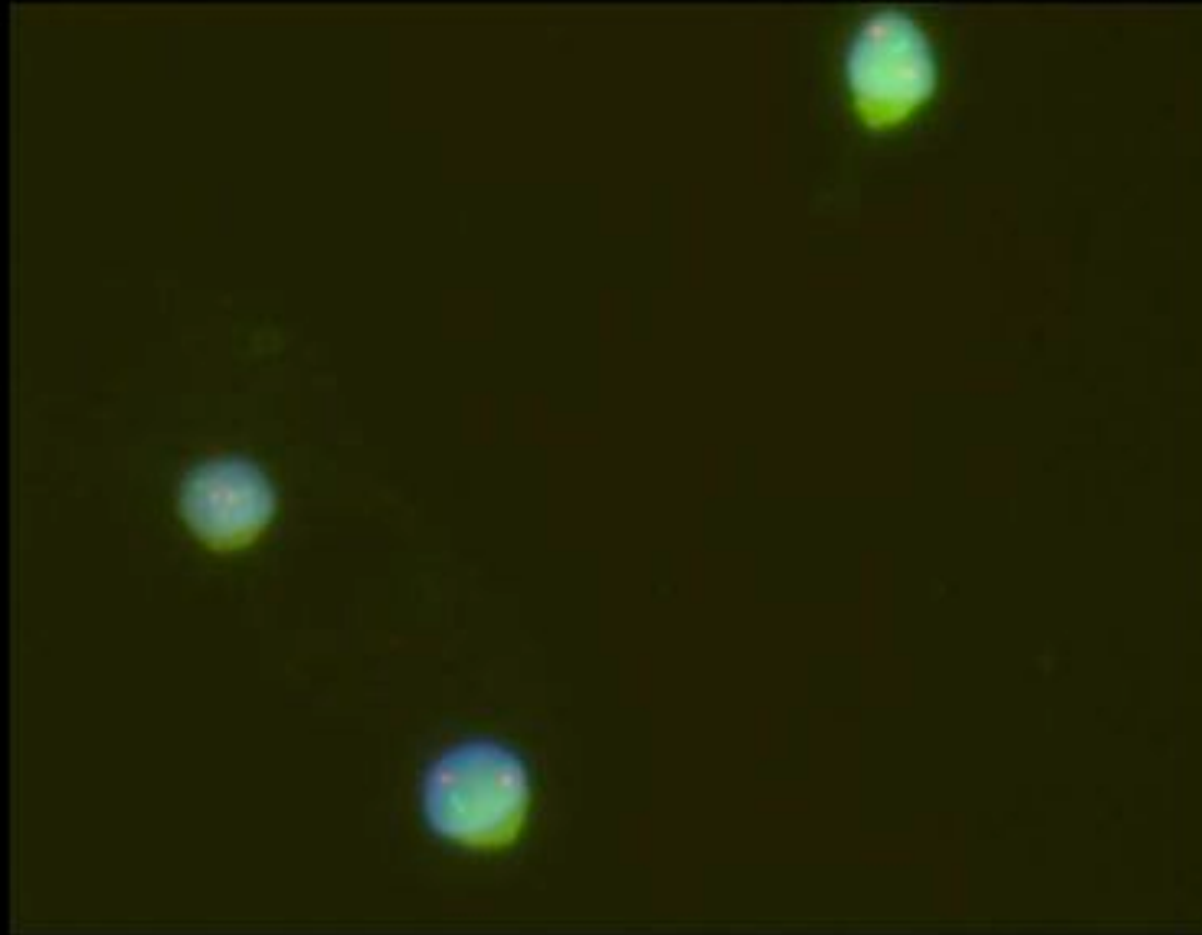
6

# Trisomie 12/deletie 13: numerical probes



7

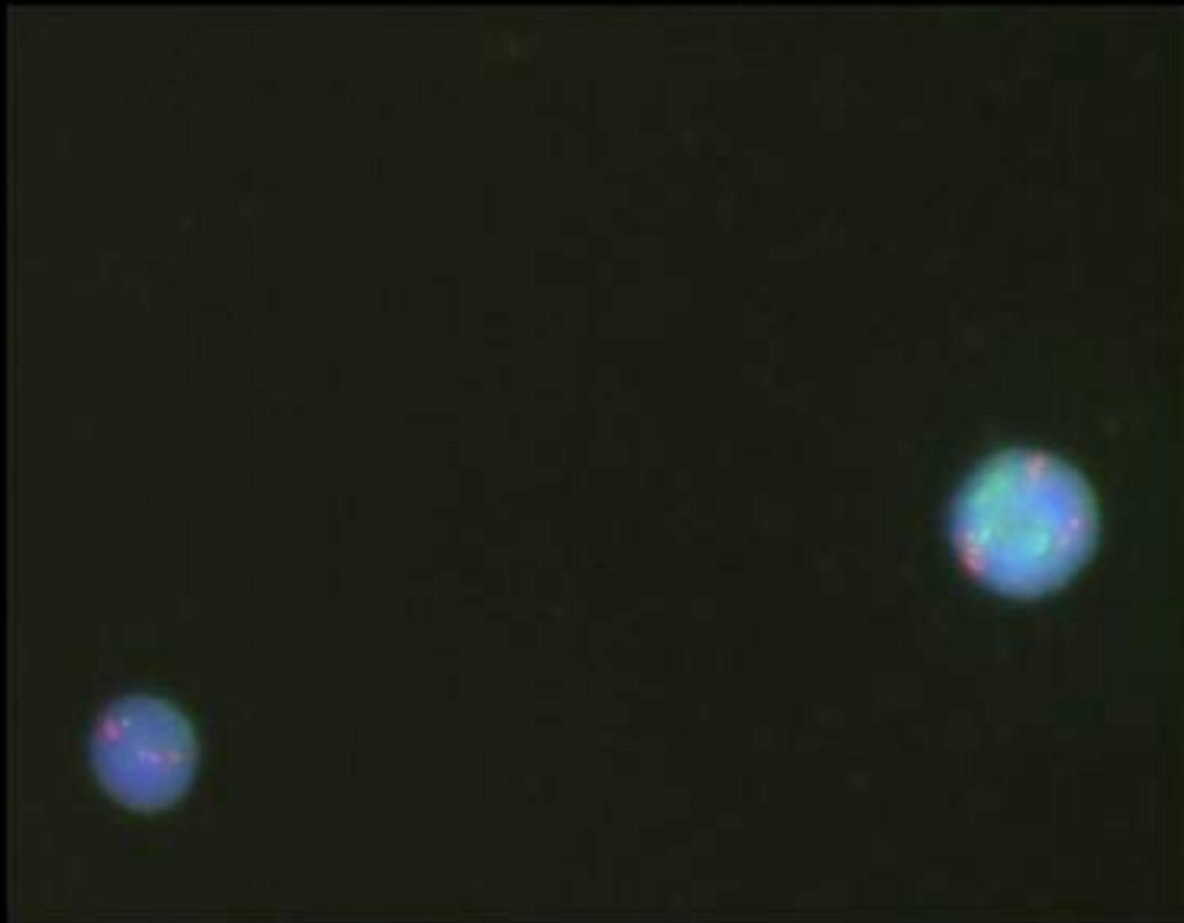
# T (11,14) Kahler: dual fusion translocation probe





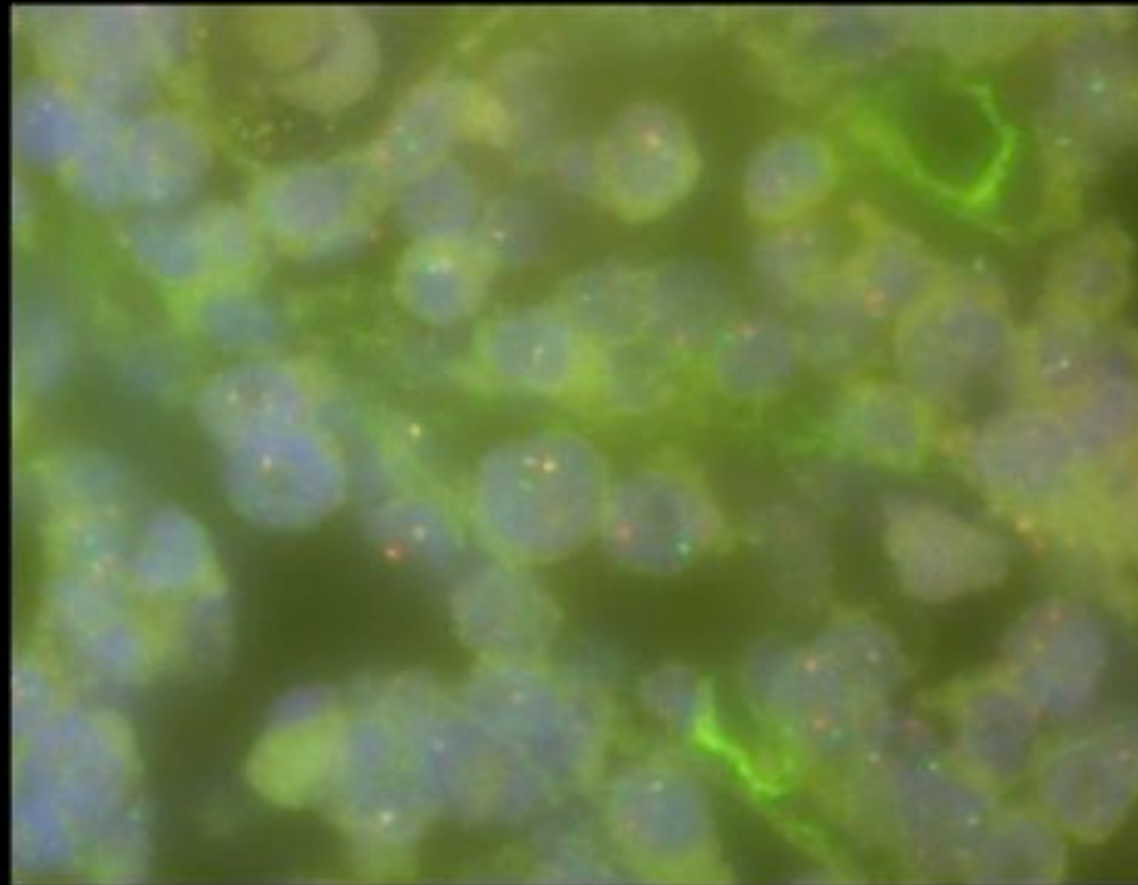
8

# T (11,14) Kahler: dual fusion translocation probe



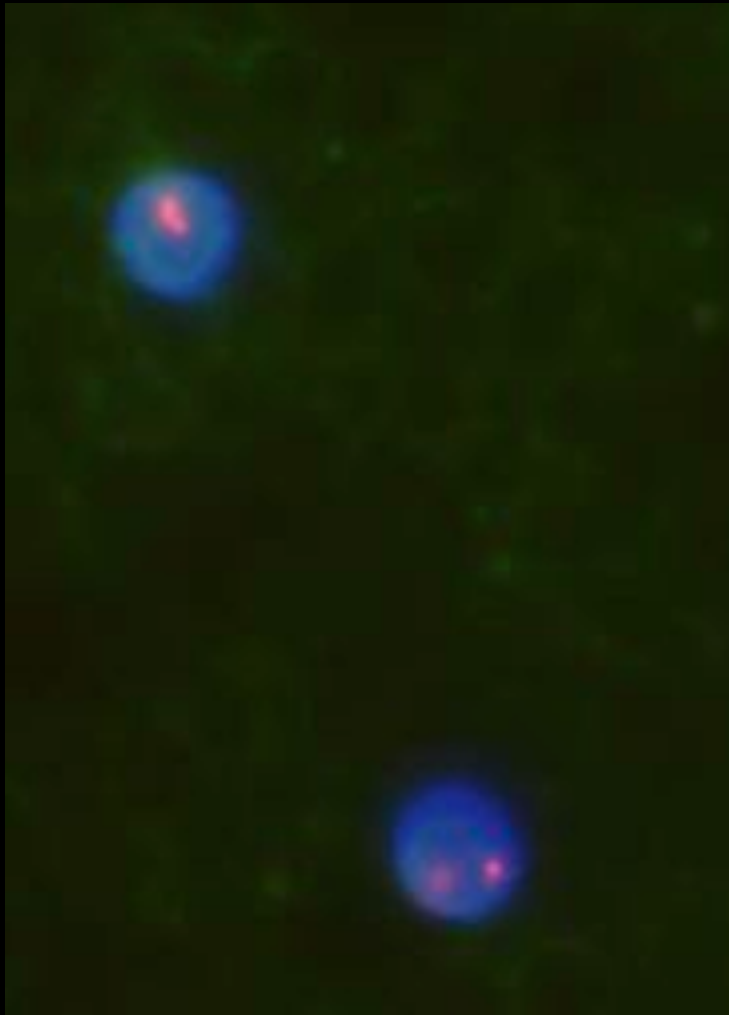
9

# C-myc Burkitt lymphoma: dual color, break apart probe



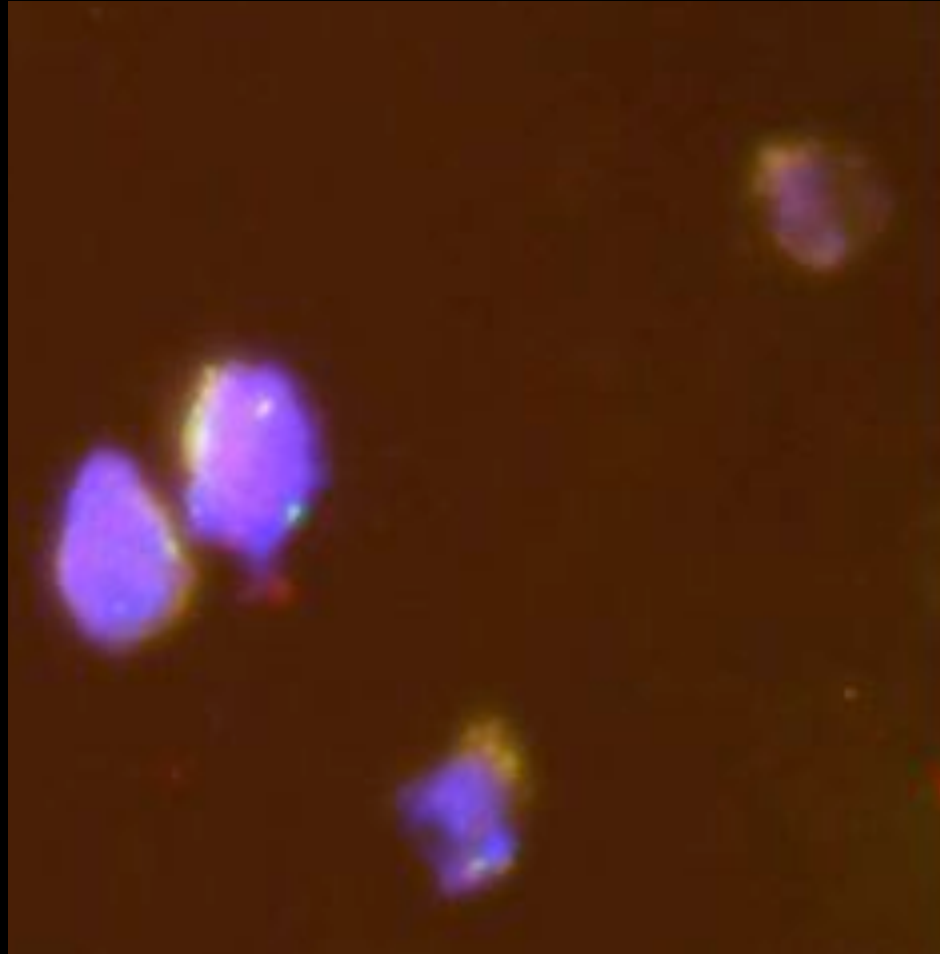
10

# XY FISH



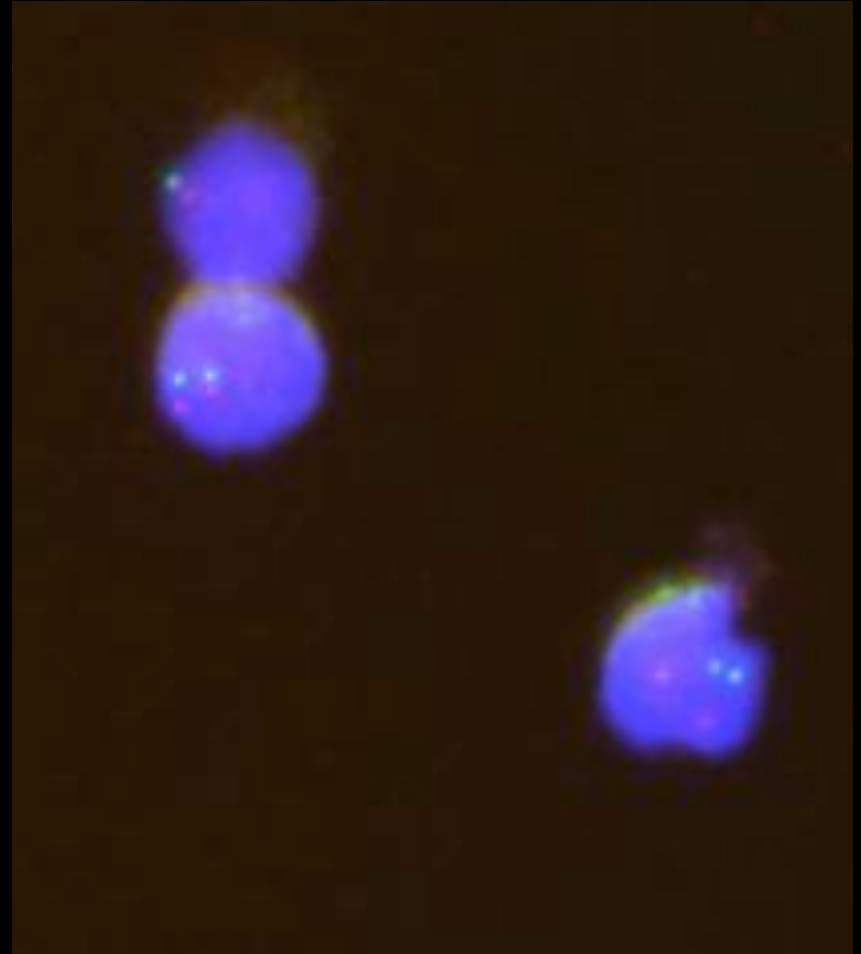
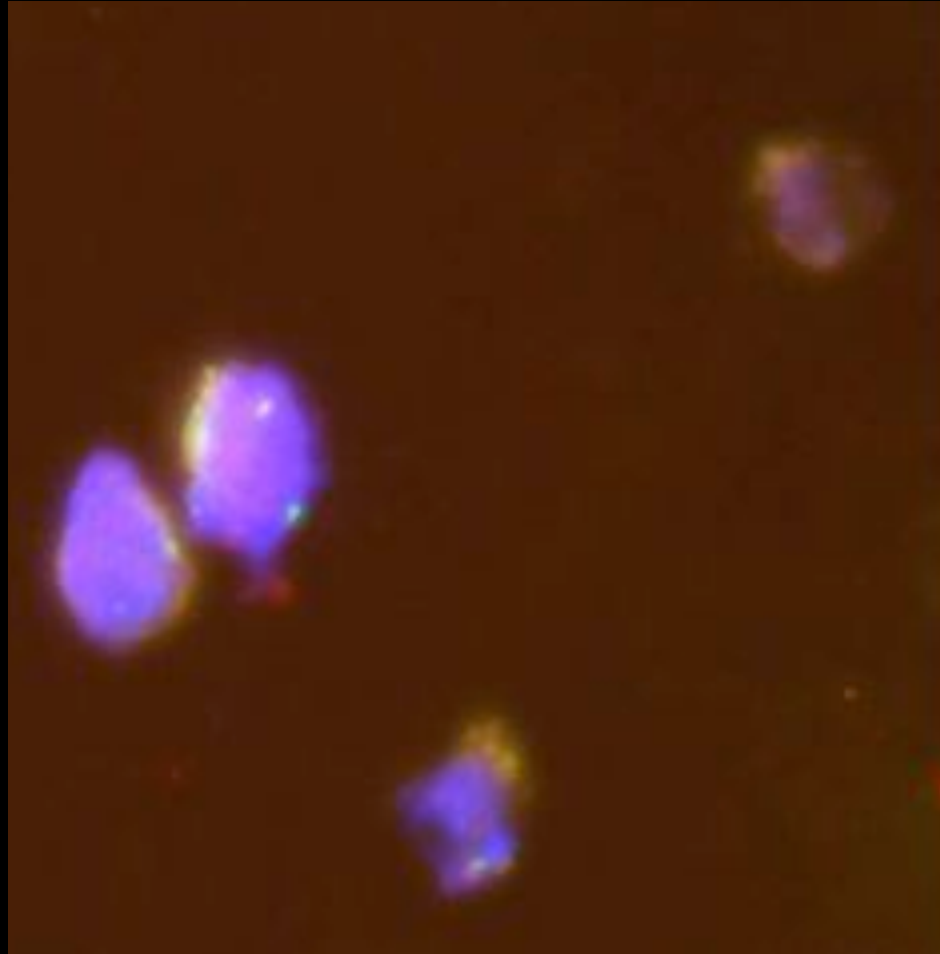
11

# Deletion RB1: numerical probes



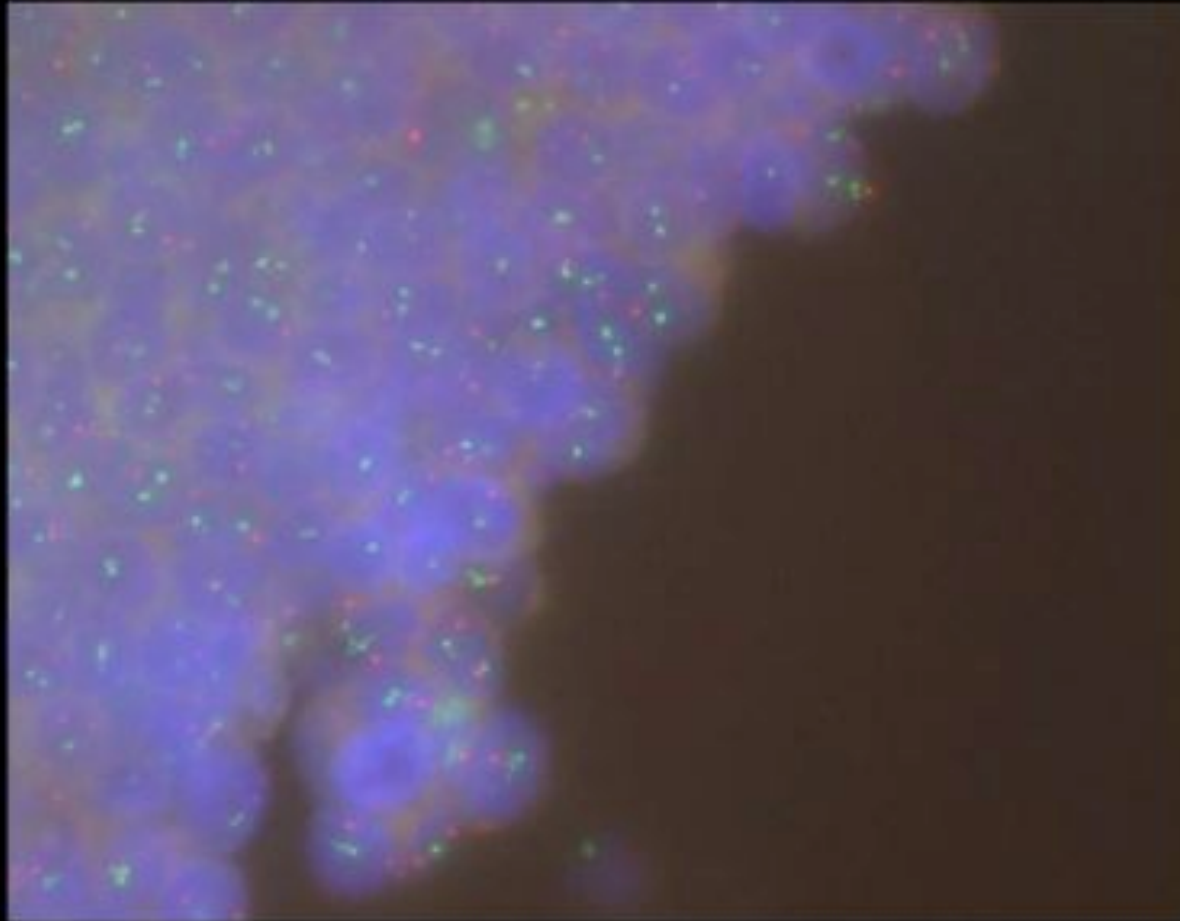
11

# Deletion RB1: numerical probes



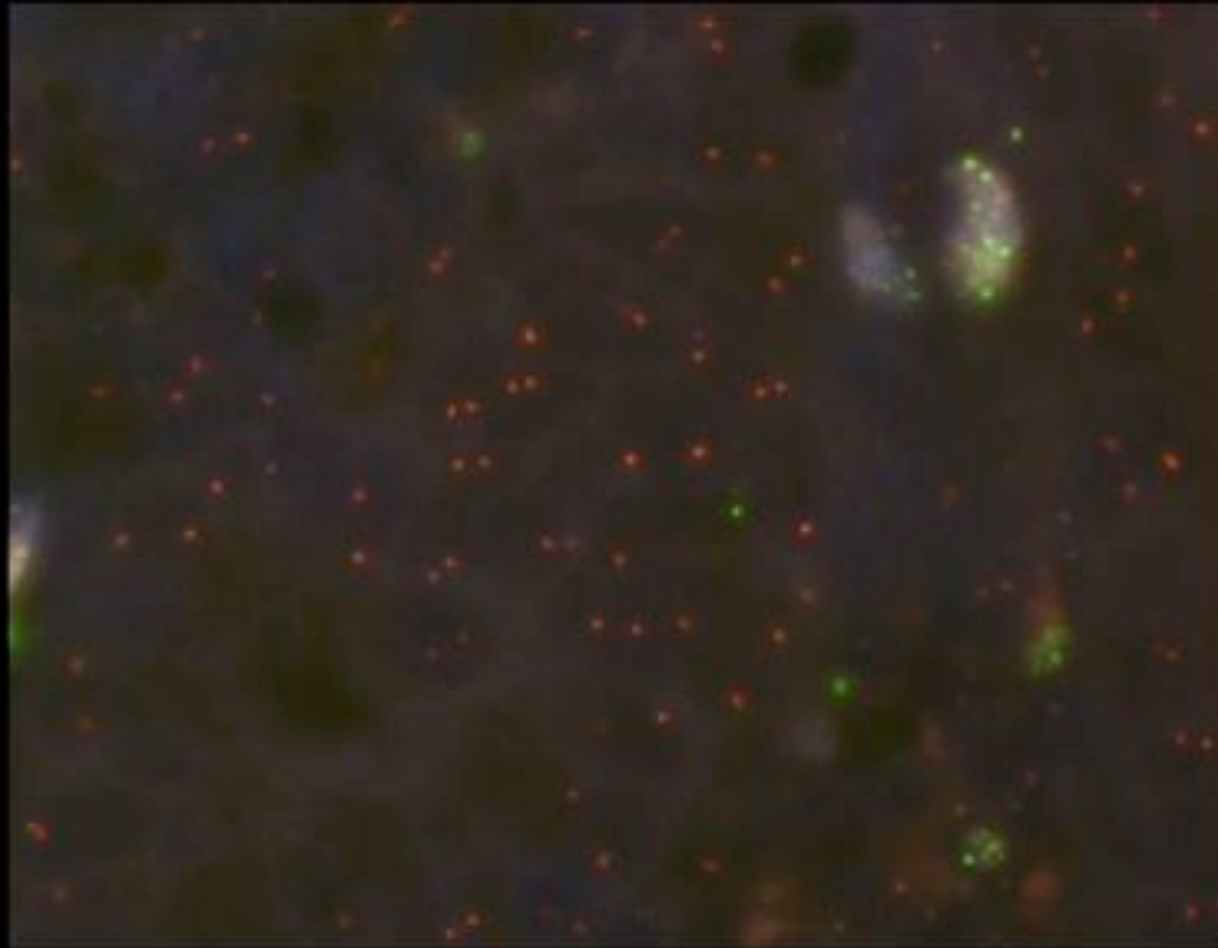
12

T (14;18) Follicular lymphoma:  
dual fusion translocation probe



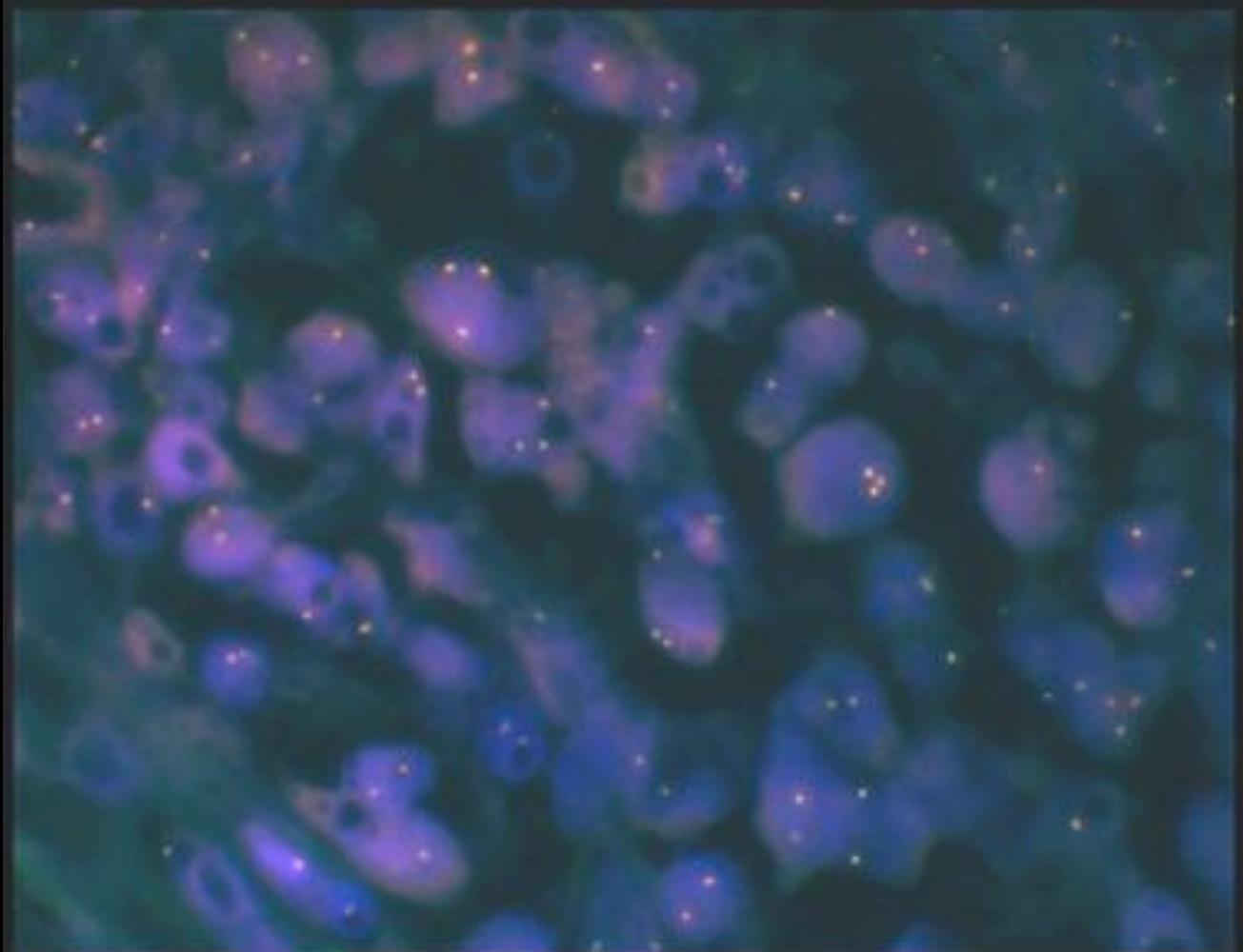
13

# T MALT lymphoma: dual color, break apart probe



14

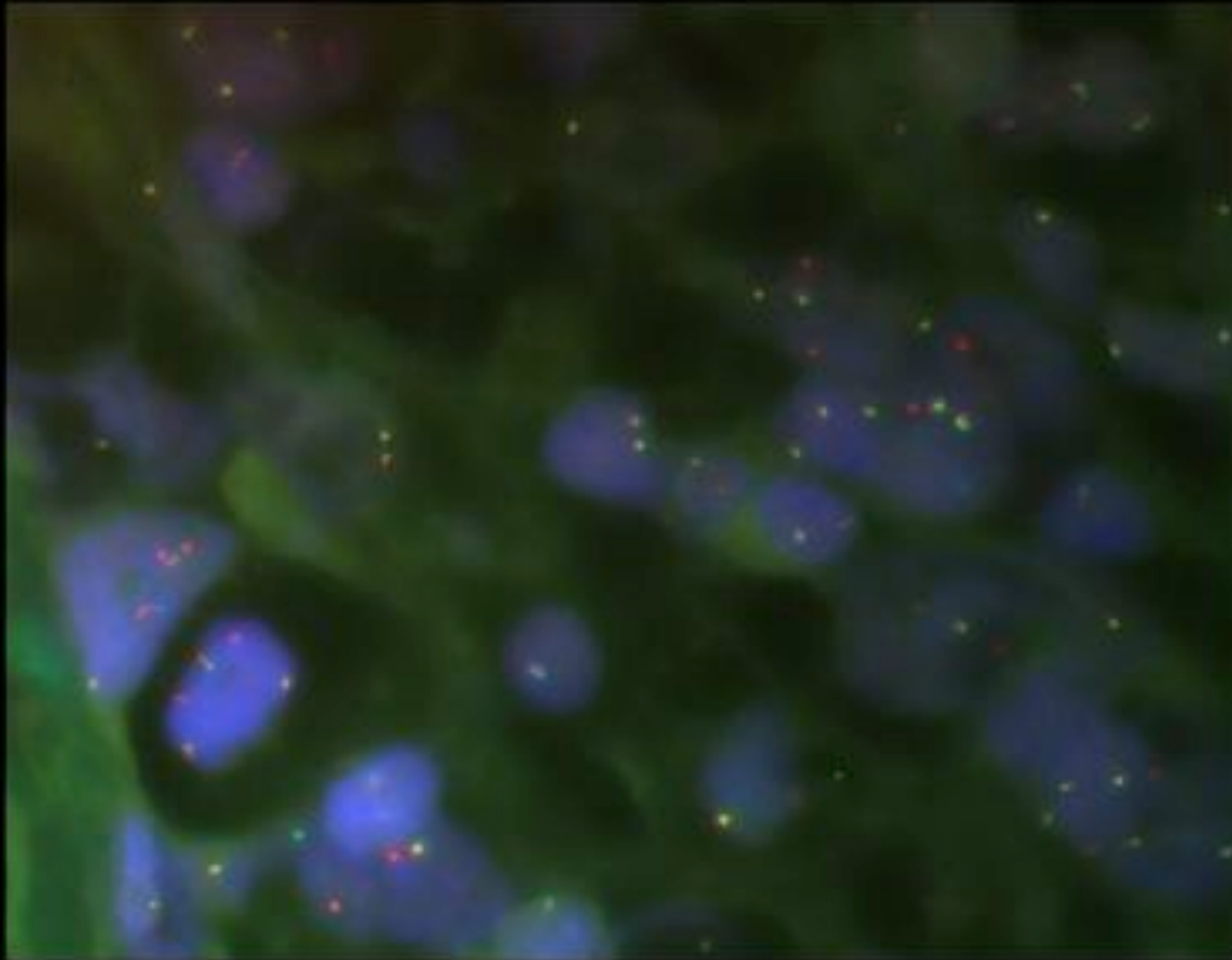
# ALK, ALK lymphoma dual color, break apart probe





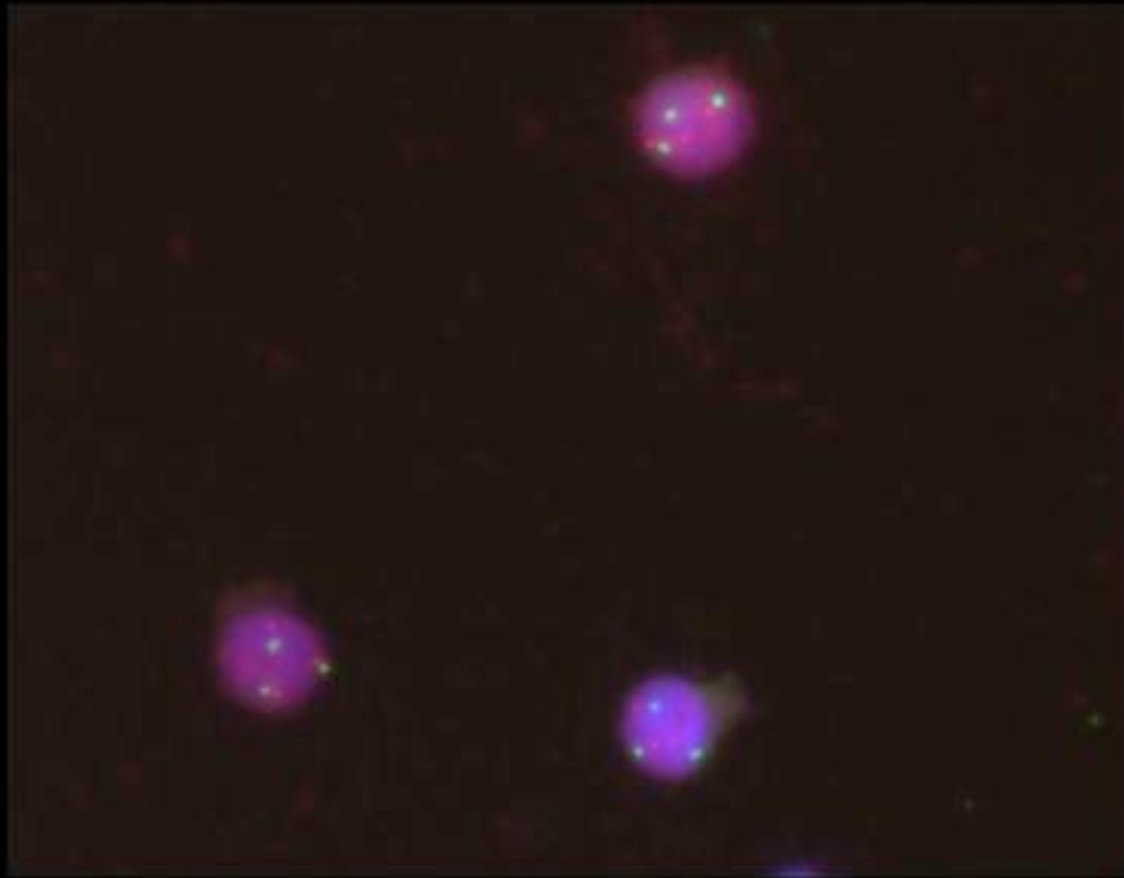
14

# ALK, ALK lymphoma dual color, break apart probe



15

Deletion 17:  
dual color, break apart



The background of the slide is a microscopic image of numerous purple-stained cells, likely bacteria or yeast, showing various shapes and internal structures. The cells are densely packed and fill the entire frame.

? Questions ?

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