Split-signal FISH for detection of chromosome aberrations in lymphoid malignancies

The EuroFISH project

Instituto Português de Oncologia Francisco Gentil
Centro Regional de Oncologia de Lisboa, S. A.

José Cabeçadas
Serviço de Anatomia Patológica

Diagnostics in hemato-oncology

1. Making the diagnosis
   Normal ↔ reactive ↔ malignant

2. Classification of hematopoietic malignancies
   - relation with prognosis
   - relevance of risk-group definition in treatment protocols

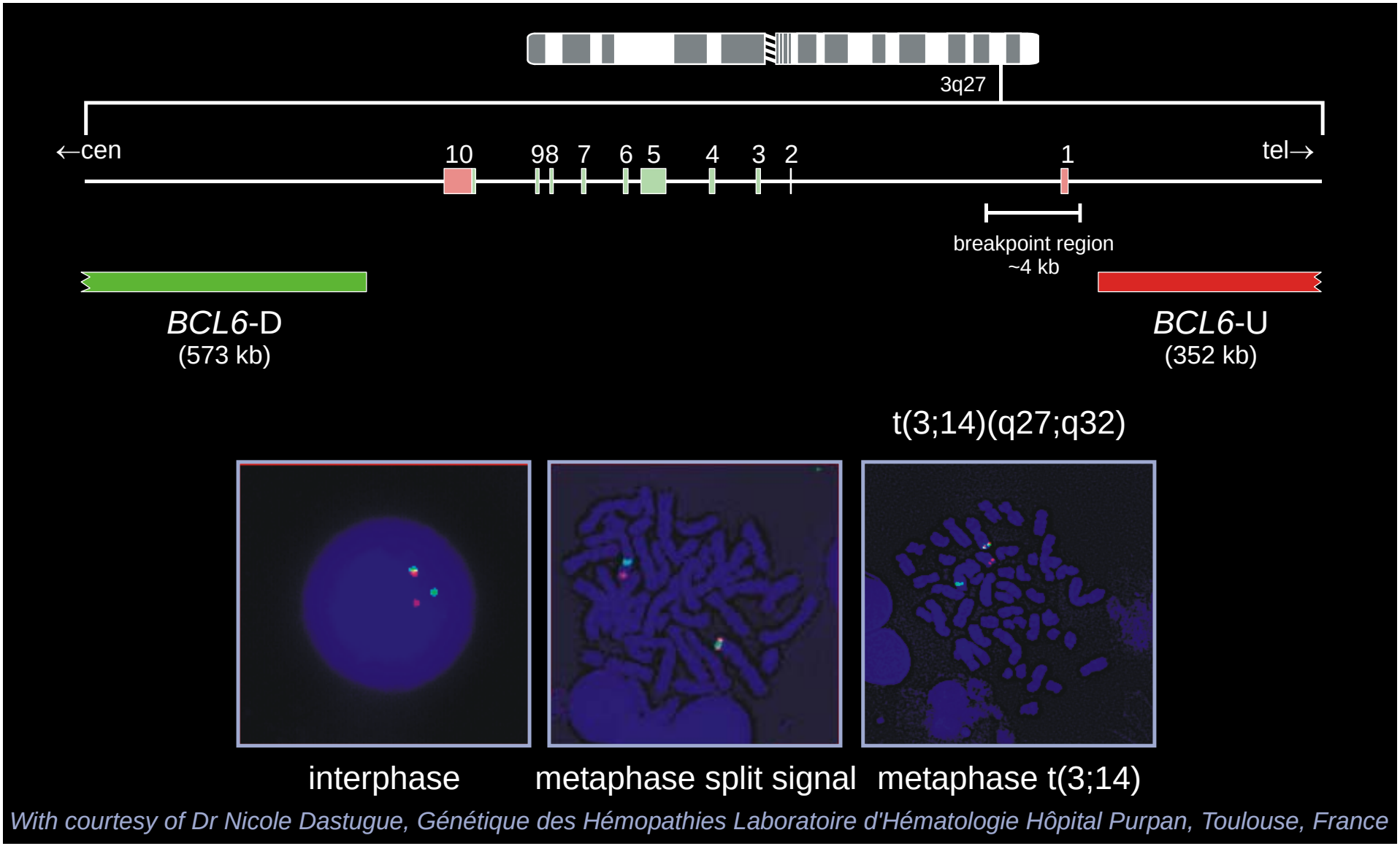
   → Particularly based on chromosome aberrations, resulting in fusion gene transcripts or aberrantly (over) expressed genes

3. Detection of minimal residual disease (MRD)
   - evaluation of treatment effectiveness
   - MRD-based risk-group stratification
     (treatment reduction / treatment intensification)

Classification via oncogenetic events in lymphomas

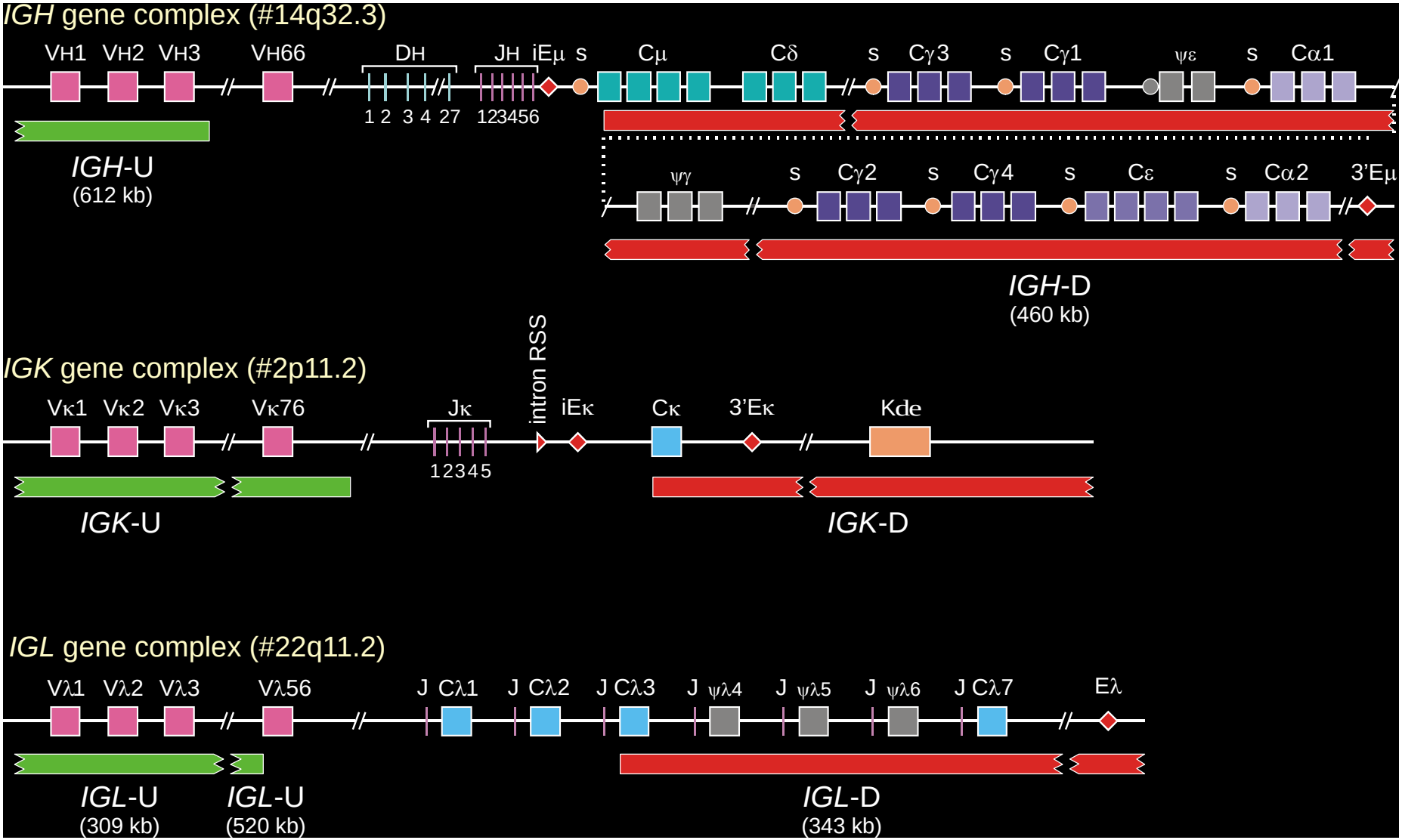
Affected gene	FISH (split-signal)	Southern blotting	(RT) PCR	Antibodies
BCL1 CCND1	++	±	± (30%)	++
BCL2	++	±	+ (70%)	++
MYC	++	±	±	–
BCL6	++	±	±	–
BCL10	++	±	?	–
PAX5	++	±	?	–
MALT	++	±	?	–
ALK	++	±	+ (>90%)	++

Split-signal FISH of *BCL6* gene



With courtesy of Dr Nicole Dastugue, Génétique des Hémopathies Laboratoire d'Hématologie Hôpital Purpan, Toulouse, France

Split-signal FISH for human Ig genes



Dept. of Immunology, Erasmus MC, Rotterdam

Detection of illegitimate recombinations in the *IGH* locus in B-cell lymphomas

Chromosome aberration	*IGH* partner gene	Type of lymphoma	Frequency
t (11;14) (q13;q32)	*BCL1* (*CCND1*)	Mantle cell lymphoma	>95%
t (14;18) (q32;q21)	*BCL2*	Follicular lymphoma	>90%
		Diffuse large B cell lymphoma	~25%
t (8;14) (q24;q32)	*MYC*	Burkitt's lymphoma	>95%
t (1;14) (p22;q32)	*BCL10*	MALT lymphoma	~5-10%
t (9;14) (p13;q32)	*PAX5*	Lymphoplasmacytic lymphoma	~50%

Conclusions concerning FISH in hemato-oncology

1. Excellent diagnostic tool for detection of well-defined chromosome aberrations
2. Can also be used on tissue sections (including paraffin-embedded material)
   → evaluation at single cell level in histological context
   ATTENTION: FISH signals might be lost if nuclei in the section are cut (particularly problematic in fusion-signal FISH, much less in split-signal FISH)
3. Requires limited handlings
   e.g. combined blocking/hybridization step
   e.g. usage of directly labeled probes
4. Split-signal FISH has several major advantages
   – detection of aberrations, independent of partner gene
   – minimization of false-positive
   – identification of partner gene or affected chromosome region, if metaphase spreads are available
5. Combination with other techniques, particularly immunofluorescence staining

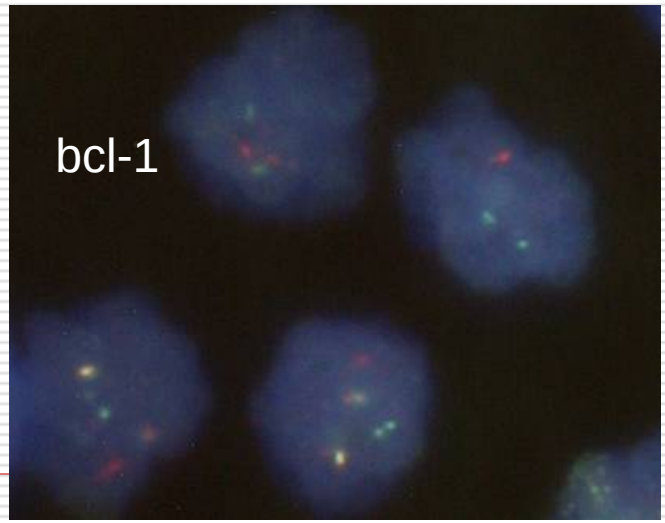
EuroFISH

- Goal: Test a standardized protocol throughout Europe and validate the diagnostic approach on selected entities of lymphomas

  - Stage 1: Test the standardized protocol
  - Stage 2: Can the FISH protocol be used throughout Europe?
  - Stage 3: Is the diagnostic approach reliable?

EuroFISH - preliminary evaluation

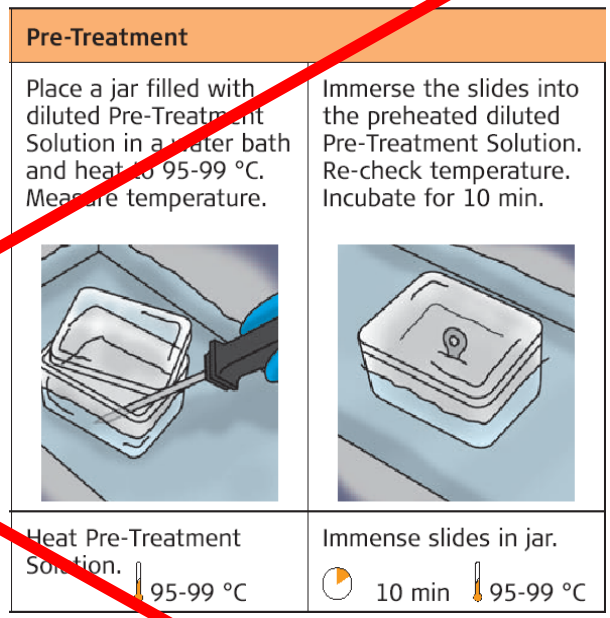
- 5 labs (DK, IT, NL, PT, UK)
- 3 entities (FL, MCL, Burkitt) x 5
- 3 probes (bcl-1, bcl-2, c-myc)
- Background
- Signal intensity
- Nuclear morphology



Critical Steps in FISH Procedure: Pre-Treatment

DakoCytomation

- Pre-heat the water bath and the jar with diluted Pre-treatment Solution to 95-99 °C **before** immersing the cradle with the slides into the jar.
- The temperature in the jar will fall when immersing the slides. Use plastic or metal cradle (NOT glass) to minimize fall in temp.
- Alternatively, transfer slides one by one. Temperature at 95 °C or higher is critical.
- **Re-check the temperature** and heat if necessary to at least 95 °C or higher **before** starting the alarm clock set at 10 min.
- The higher temperature the better signals!



Critical Steps in FISH Procedure
Pre-Treatment

DakoCytomation

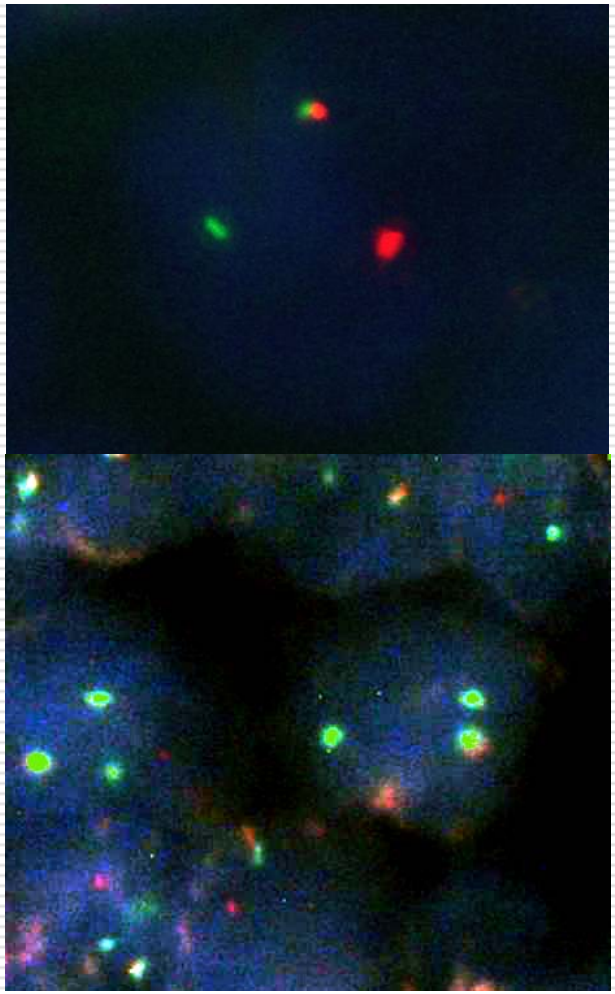
Microwave:

- Use the small plastic container (with holes in the lid), the metal cradle and 250 ml Pre-Treatment Solution. The slides must be covered.

- The metal cradle should be used all way through the FISH procedure

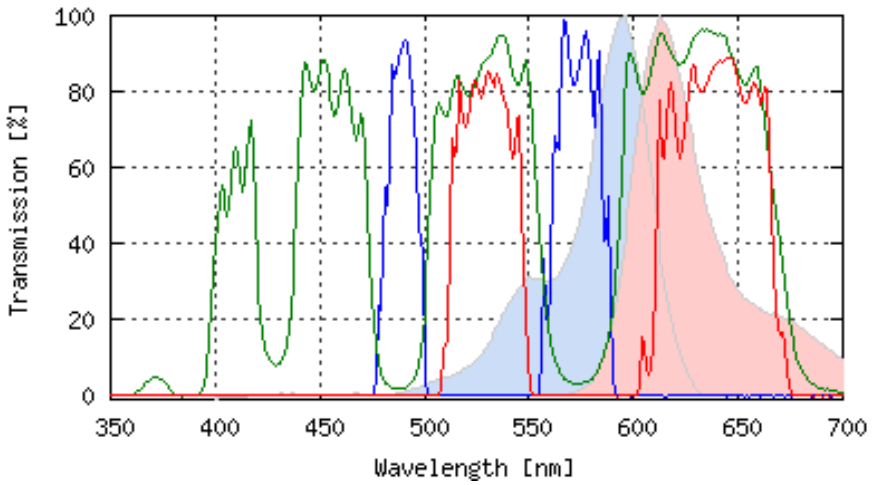
Dako split-signal

- Use the correct filter!!

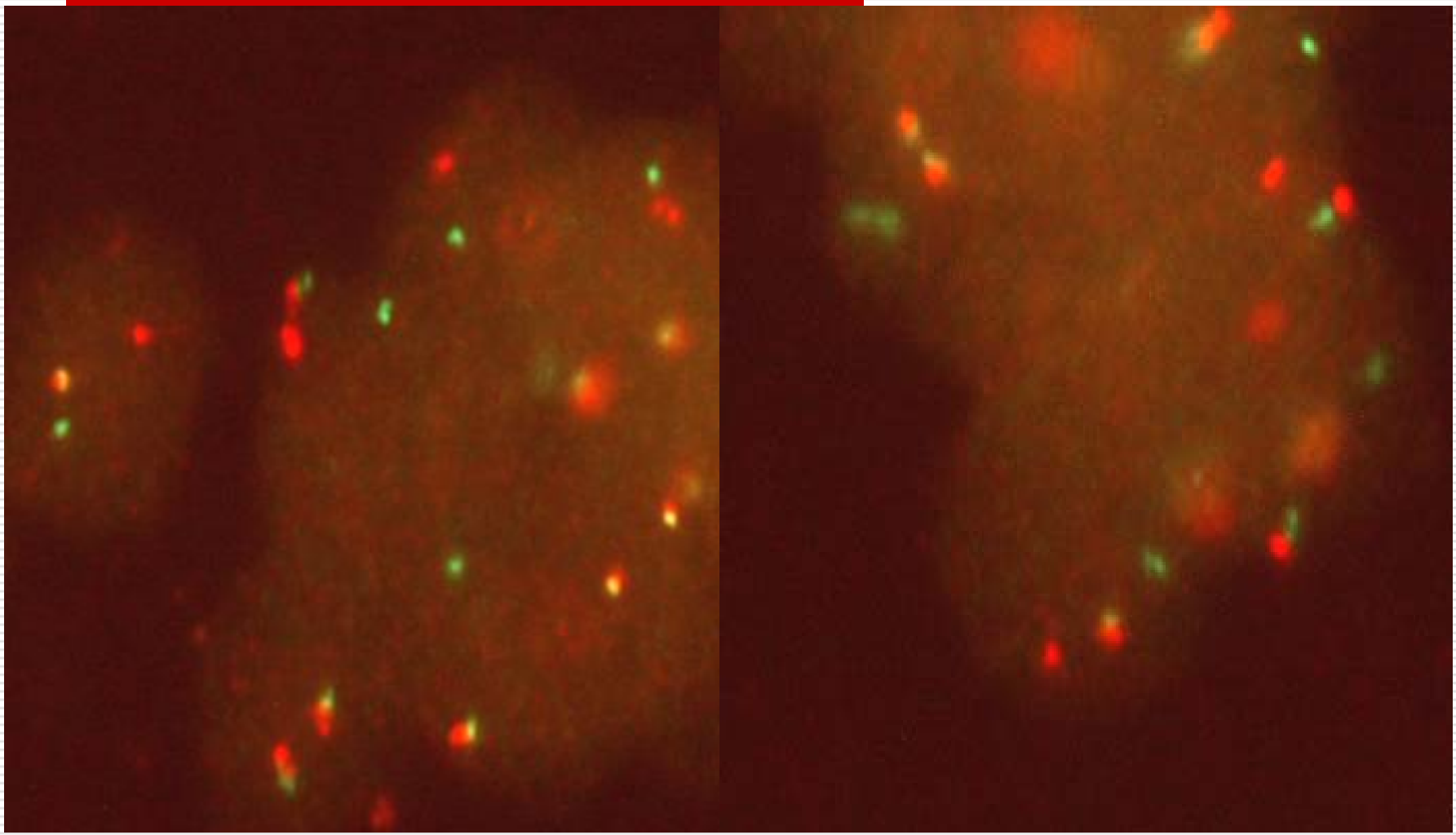


Filter for Dako split-signal probes

- DAPI
- Texas Red/FTIC
  - FITC
    495nm 520nm
  - Texas Red
    596nm 615nm



New filter



EuroFISH

- Structure:
  - 8 labs (DK, ES, FR, GE, IT, NL, PT, UK)
- Management
  - J Han van Krieken
  - Anke van Rijk ankevanrijk@pathol.umcn.nl
- Partnership
  - Dako A/S

16 Dako A/S FISH probes

- CCND1
- BCL2
- BCL3
- BCL6
- BCL10
- MYC
- PAX5
- MLT1
- ALK
- TCL1
- IGH
- IGK
- IGL
- TCRAD
- TCRB
- TCRG

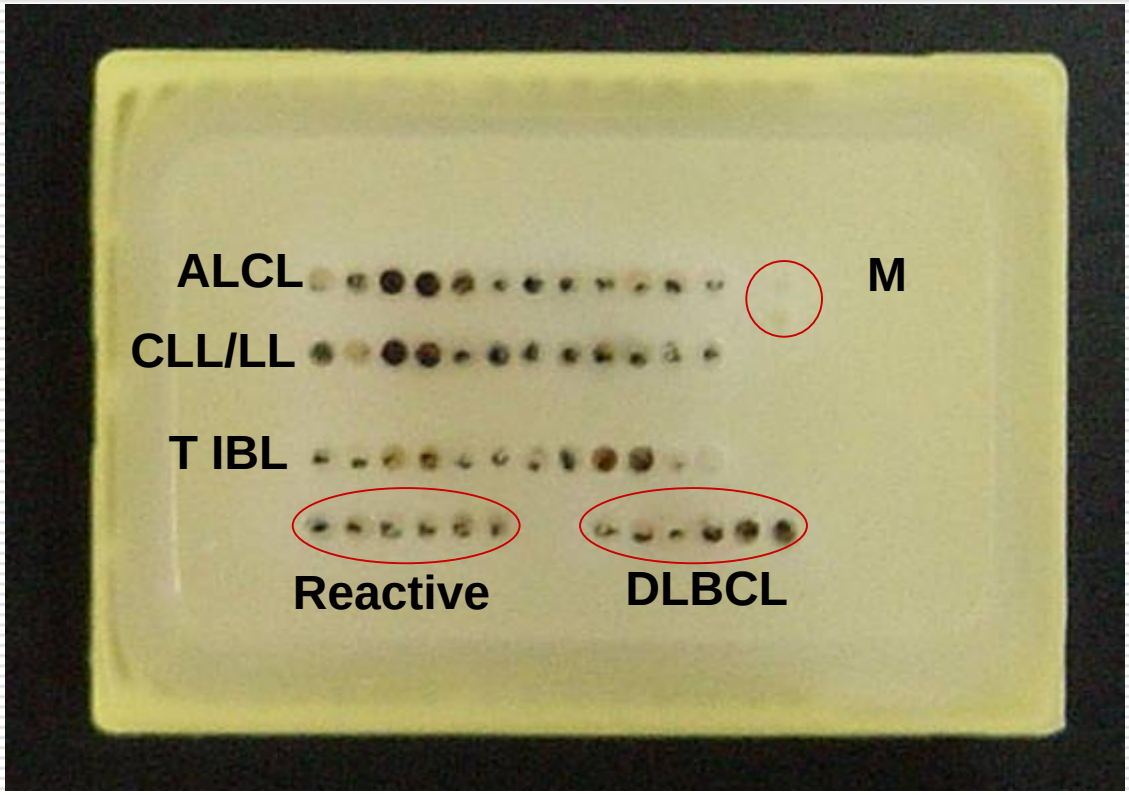
EuroFISH - aims

- Stage 1: local confirmation of the usefulness of the standardized protocol in all laboratories on their own tissue samples.
- Stage 2: clarify the interlaboratory performance, and also whether the protocol has robustness for tissue blocks from a variety of sources.
- Stage 3: learn the reliability and specificity of the approach, both aimed to be above 90%.

EuroFISH - methods

- Stage 1:
- Each of the 8 laboratories will provide 24 to 30 tissue blocks, including, 3 tissue blocks from DLBCL patients, 3 tissue blocks from “Reactive” patients, and 6 tissue blocks from each of 3 or 4 of the other 9 lymphoma categories included in the study. (TMA)
- Each of the 4 probes selected for the particular laboratory will be tested on slides cut from the laboratory’s own TMA-block.

Stage 1 - PT



EuroFISH - methods

- Stage 2:

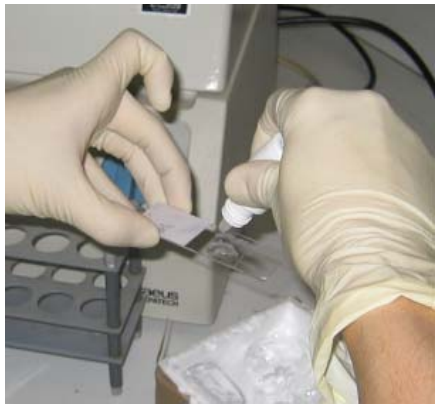
- Four (4) new TMA's will be constructed in Nijmegen, such that every laboratory and every relevant entity will be represented on all 4 TMA's (35 tissue cones each). Slides containing sections from these TMA's will be provided by the coordinator to each laboratory.

- Each laboratory will do FISH analysis on 16 TMA slides (using their 4 individual probes on each of the 4 TMA's).

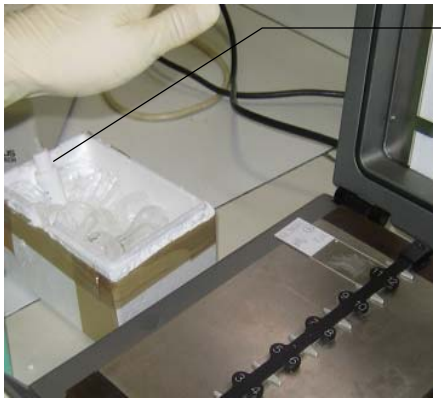
Critical Steps in FISH Procedure:
Pepsin Digestion

DakoCytomation

Add Pepsin & Place on Hybridizer - One Slide at a Time



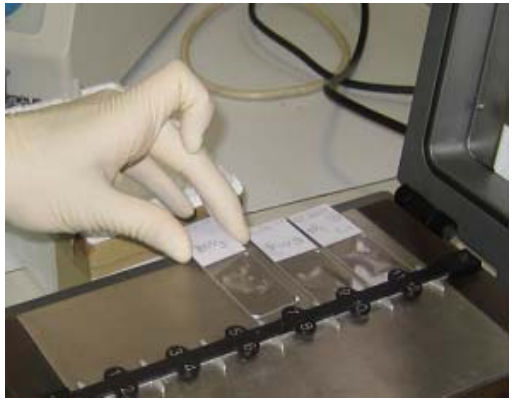
Make slides "ready".
Add Pepsin on slide 1



Place slide 1 on Hybridizer.
Start program. Timer: 0

Keep Pepsin on ice between applications.

Add pepsin & place slide 2.
Timer: 20 sec



Add Pepsin & place slide 3.
Timer: 40 sec

Add Pepsin & place slide 4.
Timer: 60 sec

Incubate until Timer shows e.g. 6 min

EuroFISH - stage 3

- Reactive
- DLBCL
- CLL/LL
- MCL
- FCL
- Gastric MZL
- Splenic MZL
- BL
- LPL
- ALCL
- T-LBL

Tissues provide by each individual laboratory

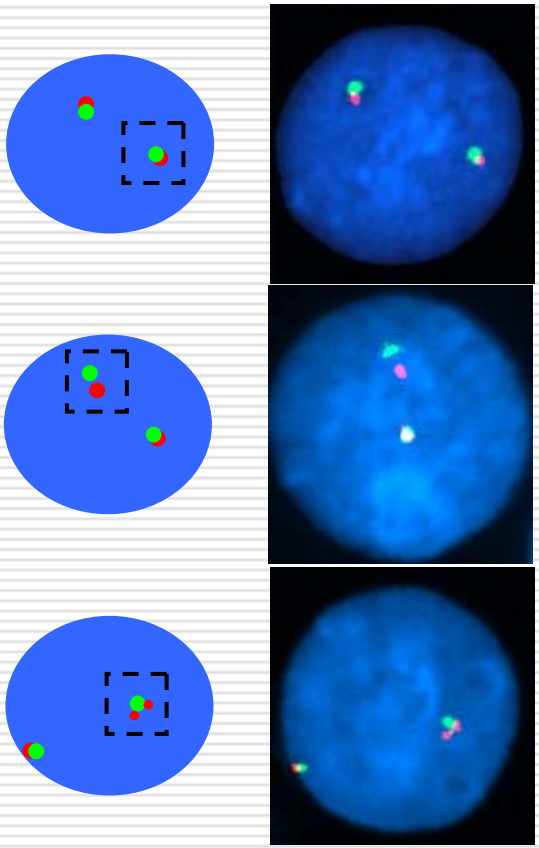
	country code							
	DE	DK	ES	FR	IT	NL	PT	UK3
No of cases								
MCL		5	10	10				5
B-CLL/SLL		10				10	10	
FCL	10		10					10
Gastric Malt	10			10	5	5		
Splenic MZL			10	10	5	5		
BL-African					20			
BL-non African	10	5	5	5			5	
LPL	4	4	4	4	4	4	4	4
ALCL	5	5	5	3	5	5	5	
T-LBL	10	5				10	5	
DLBCL 1								
Reactive Patients 2								

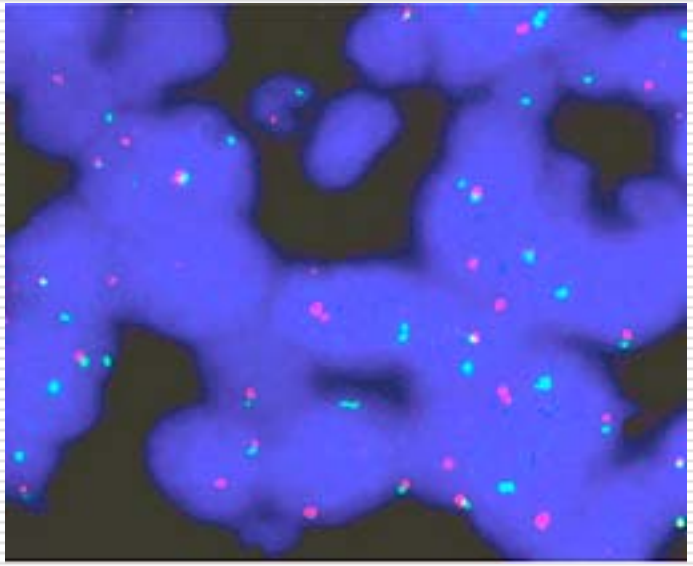
DE= Germany IT=Italy
DK= Denmark NL= The Netherlands
ES= Spain PT= Portugal
FR= France UK= United Kingdom
1) DLBCL, each lab will provide 13/14 cases

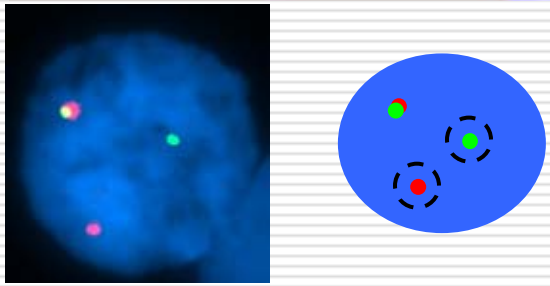
Overview of Entities, Tissue Blocks and Probes for Stage 3

Original Tissue blocks	DLBCL (n=100)	Reactive (n=50)	MCL (n=30)	SLL / B-CLL (n=30)	FCL (n=30)	Gastric MALT (n=30)	Splenic / Nodal MZL (n=30)	BL (n=30)	LPL (n=30)	ALCL (n=30)	T-LBL (n=30)
Final blocks	(n=8) TMA's		(n=30) single	(n=30) single	(n=30) single	(n=30) single	(n=30) single	(n=30) single	(n=30) single	(n=30) single	(n=30) single
CCND1	+	+	+	+							
BCL2	+	+			+						
BCL3	+	+		+						+	
BCL6	+	+			+						
BCL10	+	+				+					
MYC	+	+			+			+			
PAX5	+	+							+		
MLT1	+	+				+					
ALK	+	+								+	
TCL1	+	+									+
IGH	+	+	+	+	+	+	+	+	+	+	
IGK	+	+	?	?	?	?	?	+	?		
IGL	+	+	?	?	?	?	?	+	?		
TCRAD	+	+								+	+
TCRB	+	+								+	+
TCRG	+	+								+	+

Split-signal evaluation







Co-localization

EuroFISH

- Han van Krieken
- David Mason
- S Hamilton-Dutoit
- Lorenzo Leoncini
- José Cabeçadas
- Juan Garcia
- C. Copie-Begrman
- Martin Hansman
- Tim Poulsen
- Steen Matthiesen
- Henrik Aspe
- Niels Foged
- Jacques van Dongen

Euro-FISH

Participants of the Euro-FISH start-up meeting, November 2005

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