Multiple myeloma: Diagnosis, Prognosis and Monitoring by Imaging Flow Cytometry

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Translational Cancer Pathology Laboratory

Disclosures

Patents: “Improvements in or relating to cell analysis” (WO/2019/079851) (PCT/AU2018/051148)

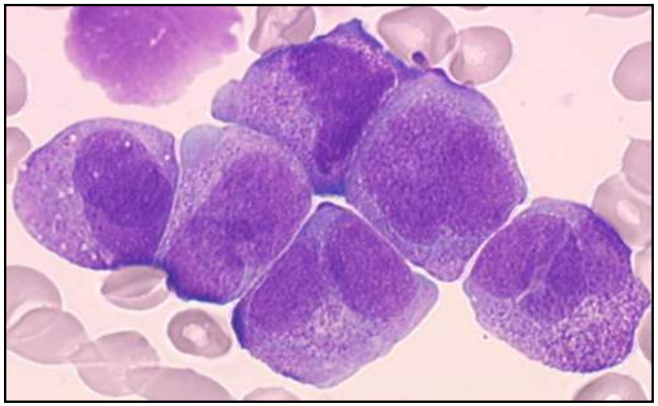
- Australia (Standard) Patent AU2018355889
- Japan (Standard) Patent JP2020524028
- USA (Standard) Patent US20200232019
- Europe (Standard) Patent EP2018870567

Research Collaborations:

- Amnis (since 2019)
  - Agreement – loan instrument and Technology exchange
- Cytek Biosciences
- Luminex Corporation (complete)
- Sysmex Corporation / FAJS (complete)

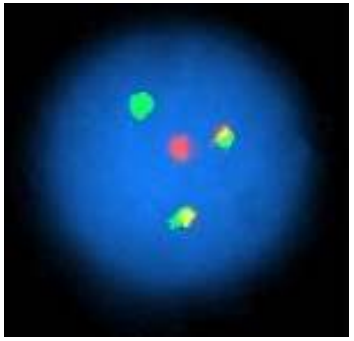
Assessment of blood cancers

Morphology



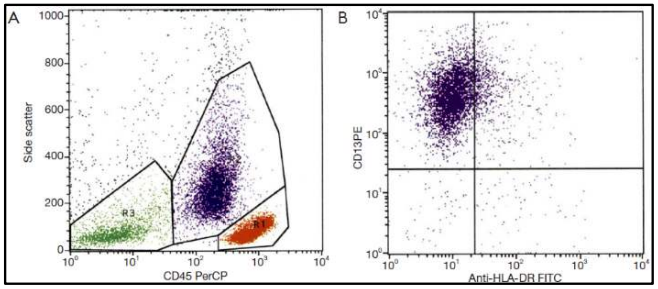
Genetics

t(15;17)(q22;q12)



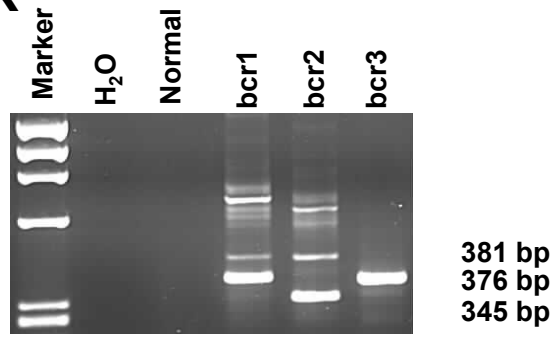
FISH: Dual fusion

Phenotyping



CD9, CD13, CD33, CD117 pos; CD34, HLA-DR neg

RT-PCR

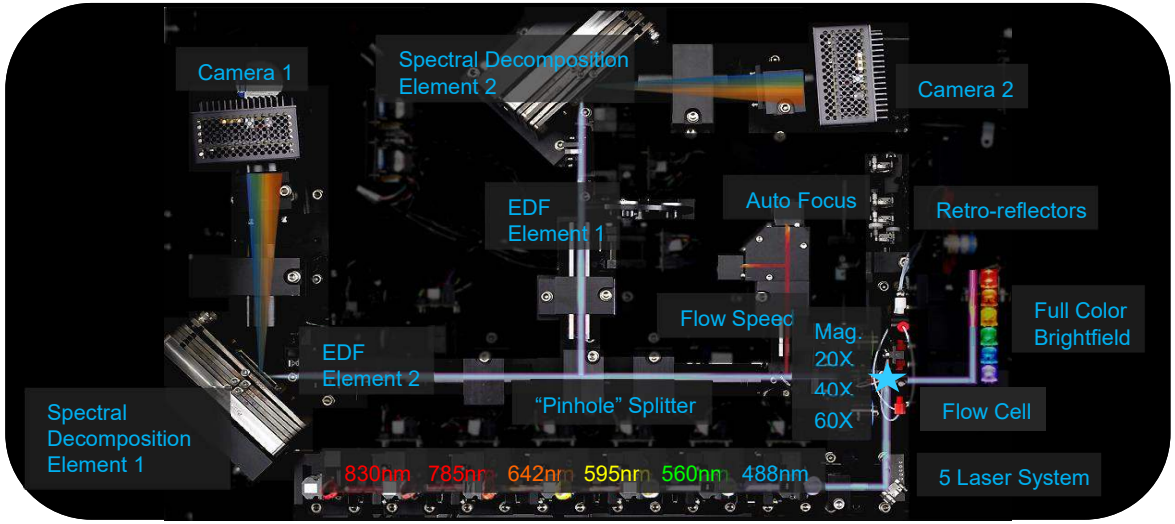


Slide courtesy Prof Wendy Erber

Cytek Amnis ImageStreamX mkII

A technique that combines the high-throughput power of flow cytometry with the cellular localisation information provided by immunofluorescence microscopy.

AMNIS ImageStreamX mkII



- 5 excitation lasers, 10 fluorescent parameters (including scatter) and brightfield
- Imaging at 20x, 40x, 60x magnification with extended depth of field (EDF)

ISXmkII: magnification and EDF

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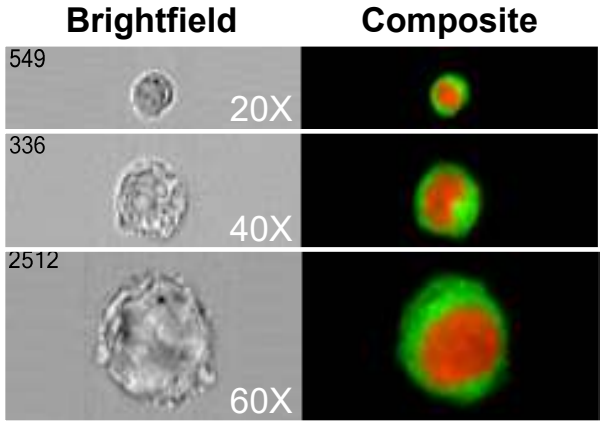
“Multimag”

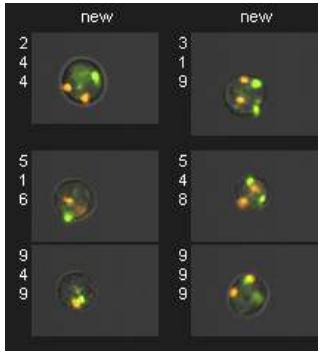
Select the magnification in relation to the size of the cell of interest and the level of resolution required for image analysis

- 20x: 120 µm wide, 8 µm depth of field
- 40x: 60 µm wide, 4 µm depth of field
- 60x: 40 µm wide, 2.5 µm depth of field

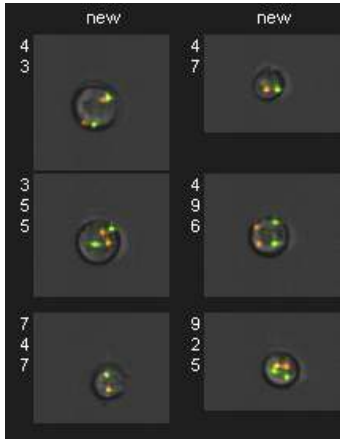


Extended Depth of Field (EDF)

Enables accurate spot count analysis



No EDF

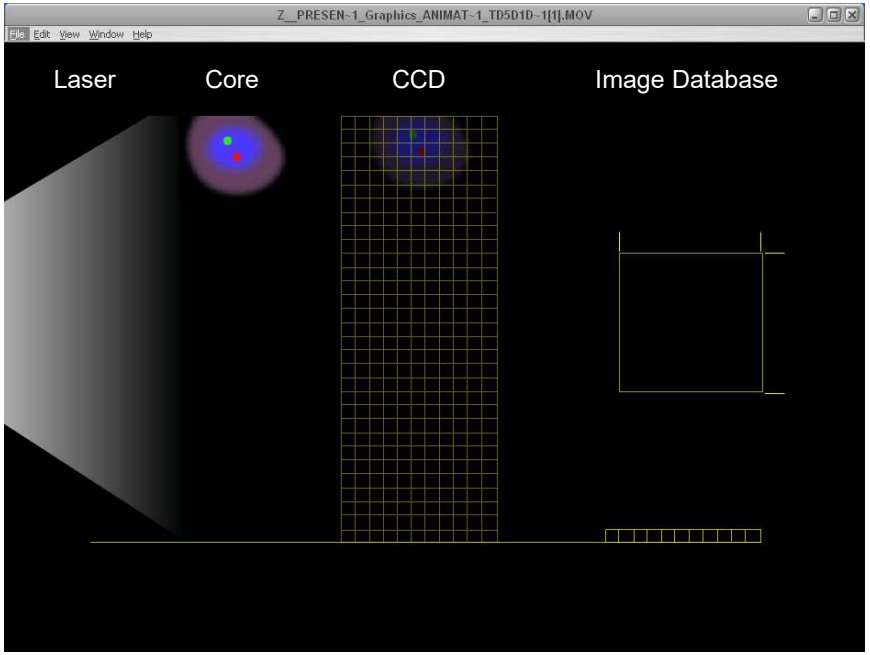


With EDF

Time delay integration (TDI)

TDI CCD

- Excite fluorescence over the entire height of the detector
- Light is detected in the first pixel row and transferred to the pixel below in exact synchrony with the velocity of the cell as it goes streaming by
- Light is integrated over the entire height of the detector to achieve high photonic sensitivity
- Images don’t streak or blur and maintain 0.5um per pixel resolution

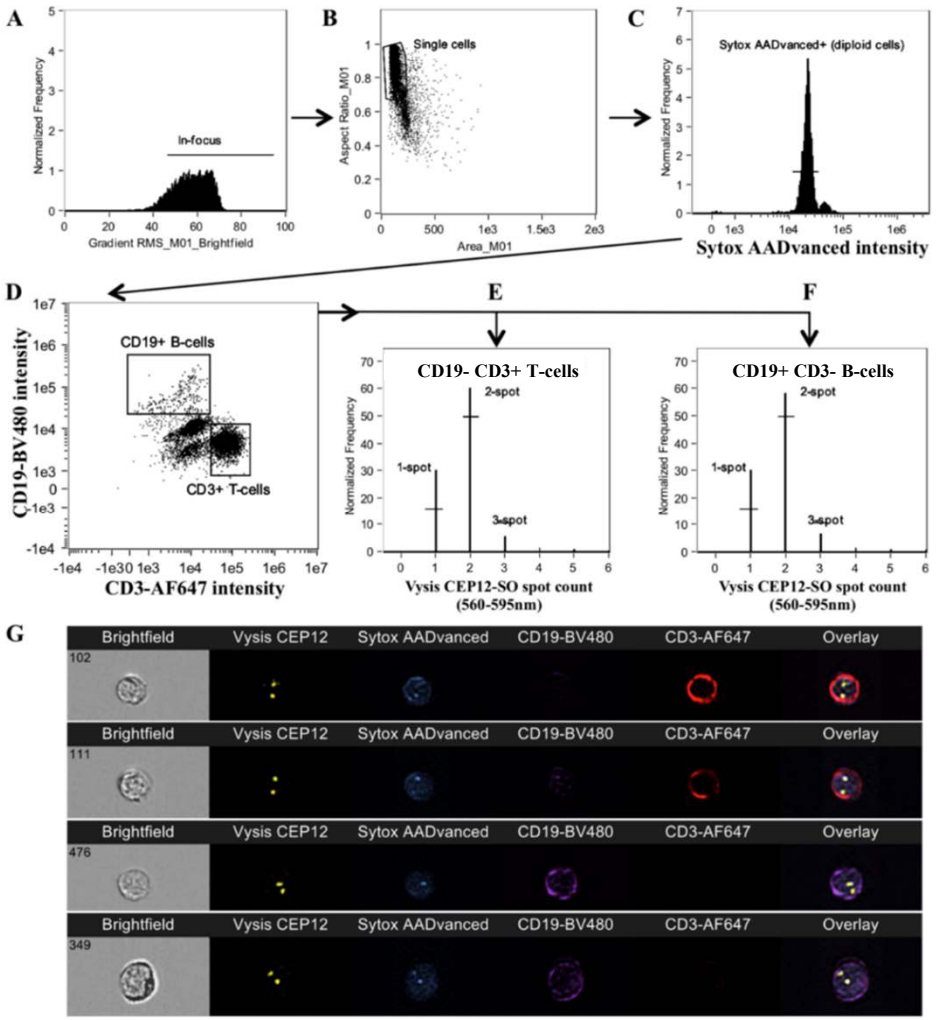


IDEAS analysis – the best of both worlds

Image analysis using masks

Flow cytometry gate strategy

Feature calculations



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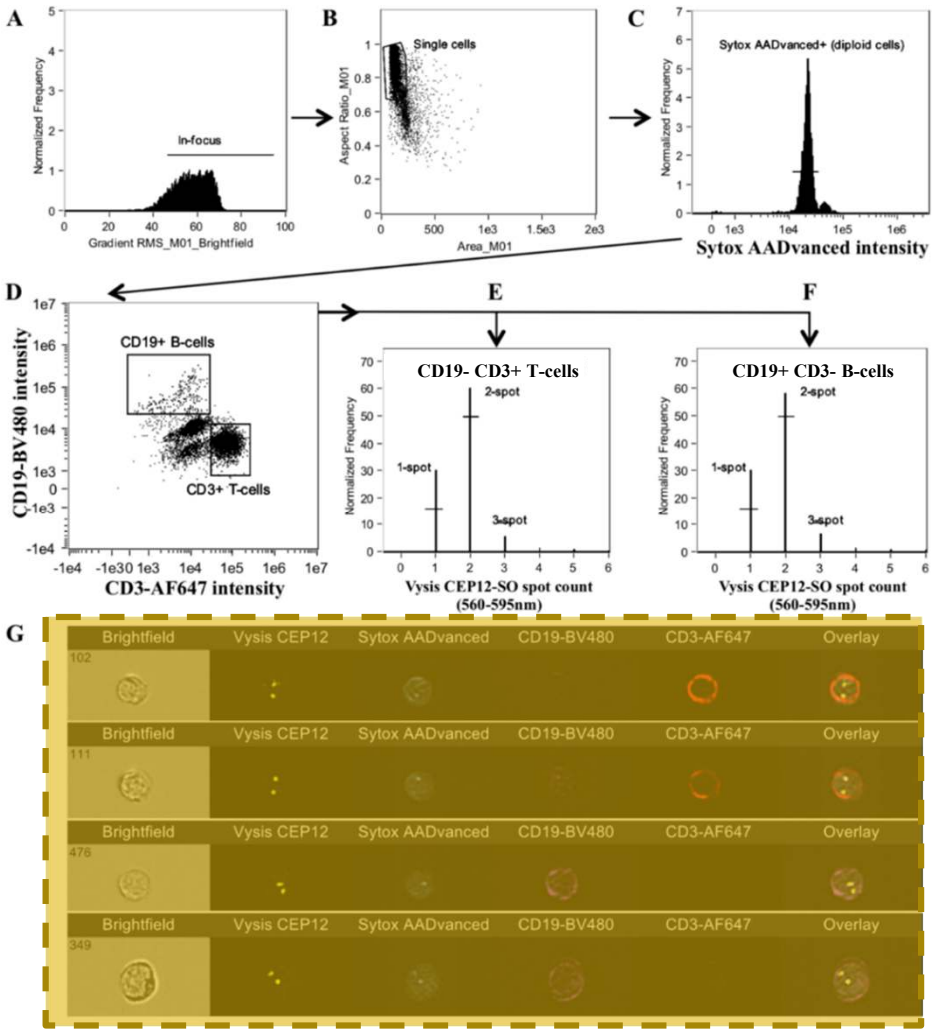

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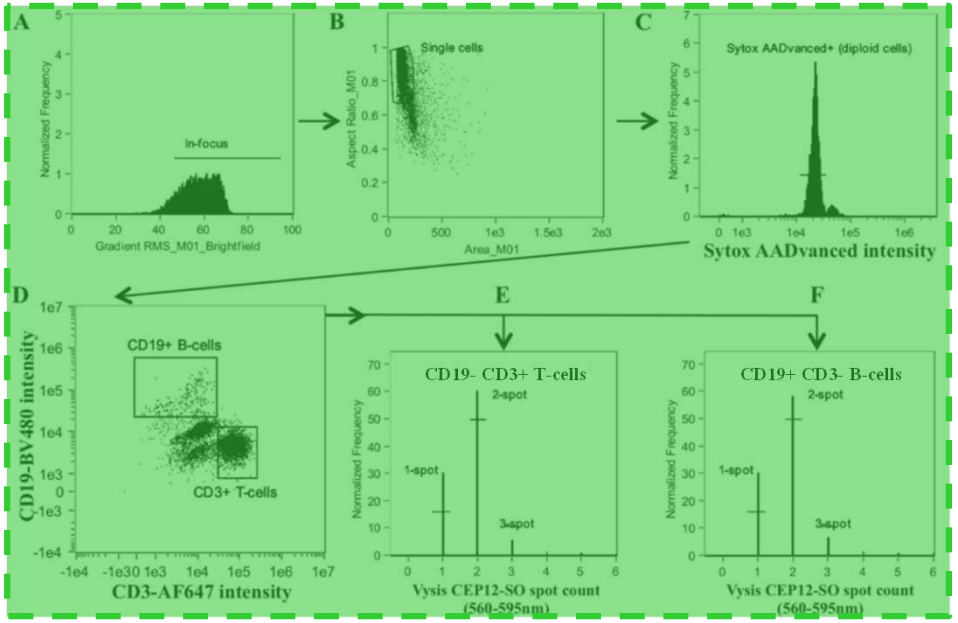

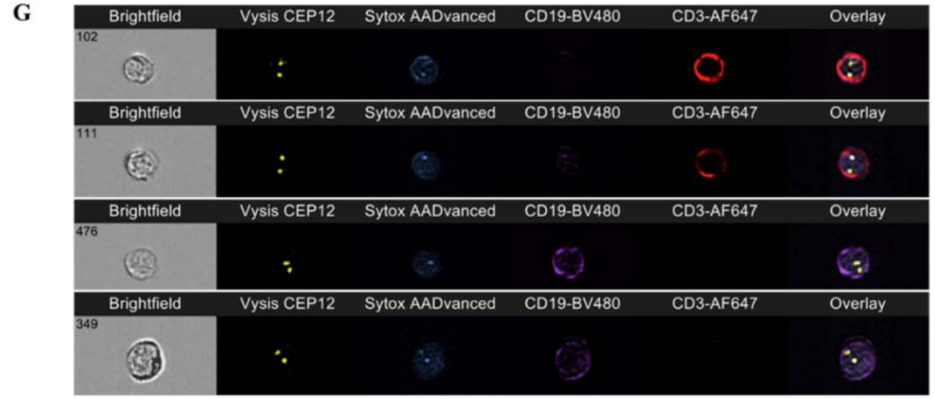

IDEAS analysis – the best of both worlds

Image analysis using masks

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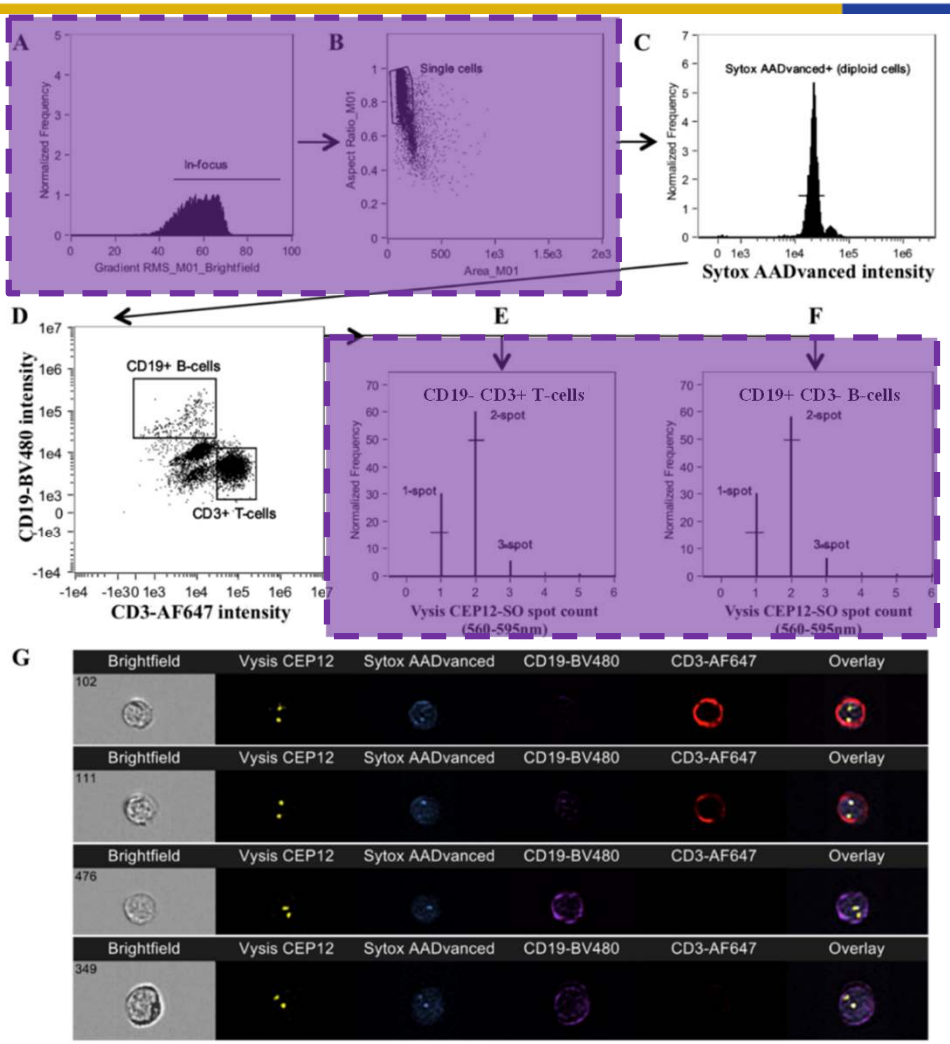

Immuno-flowFISH

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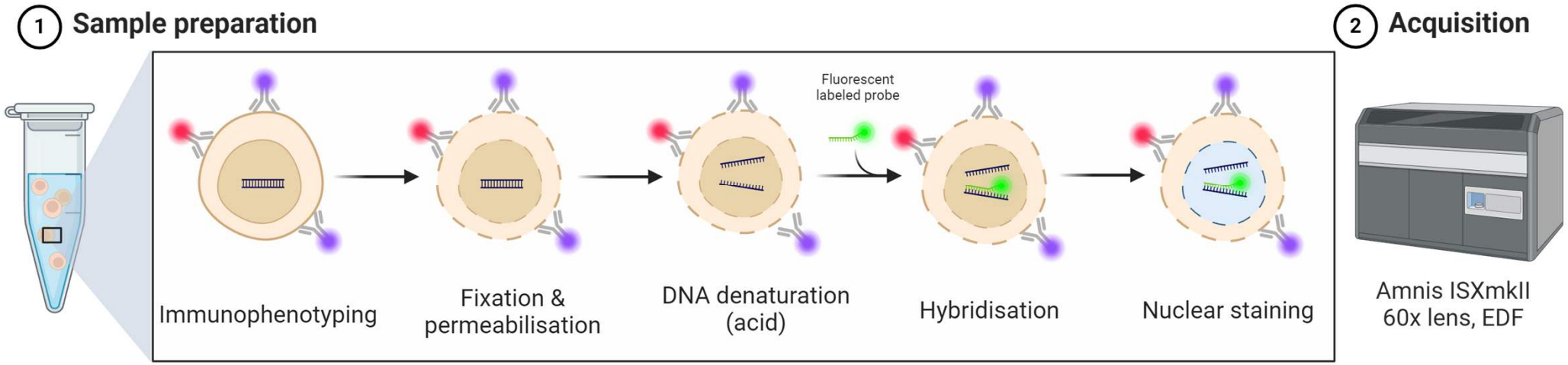

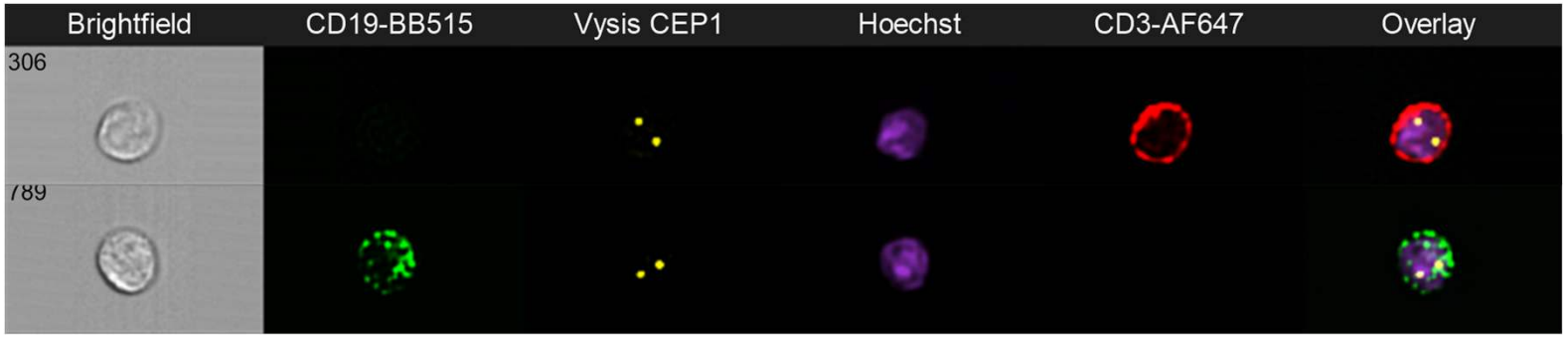

Hui et al. *Methods* 2018; Hui et al. *Cytometry A* 2019

Australian patent filed October 2017; International patents filed April 2020

Chronic lymphocytic leukaemia (CLL) +12

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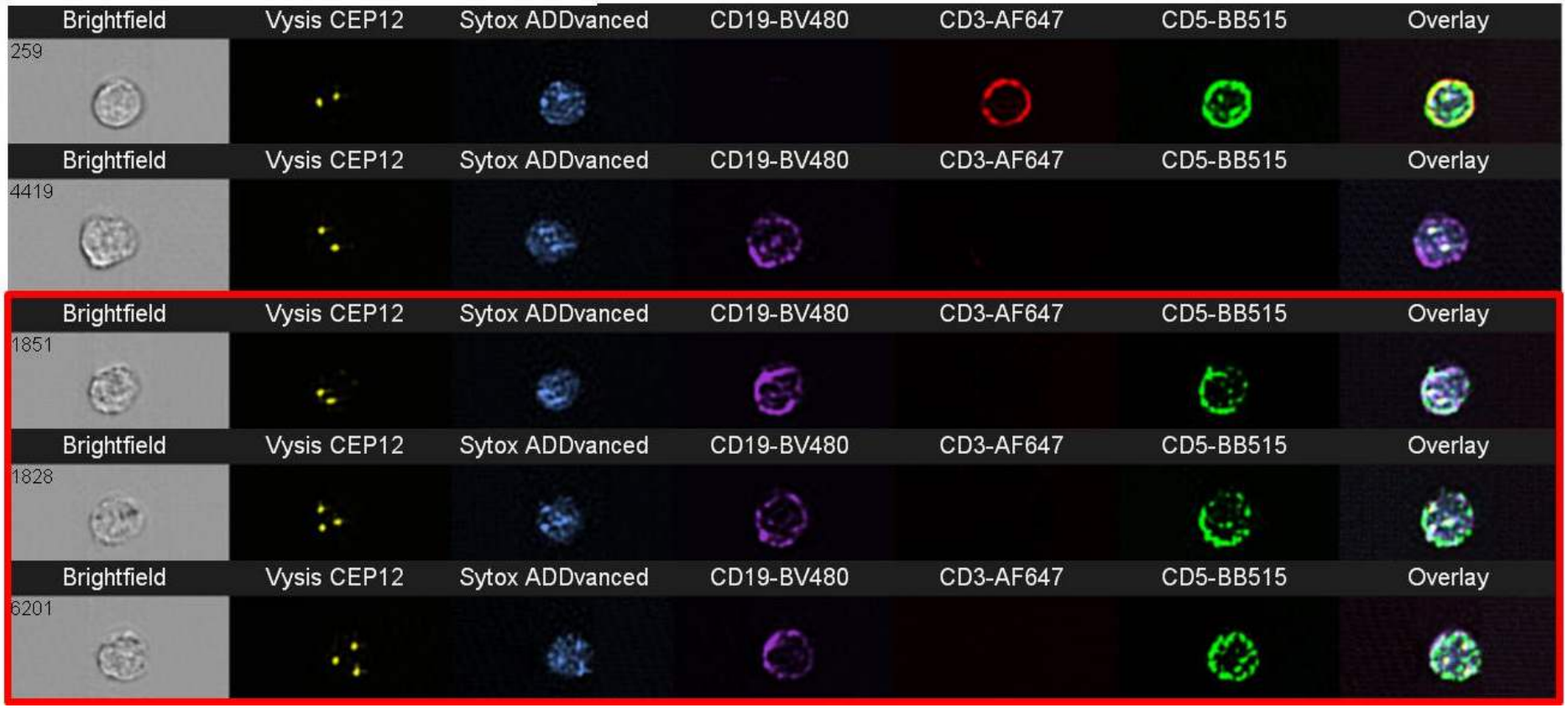
CD3 **CD5** **CD19** antibodies, **CEP12** FISH probe



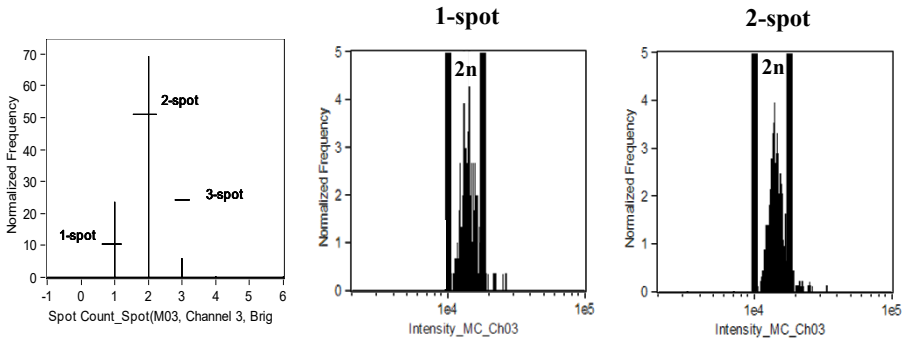
45% CD19+CD5+ CLL cells with +12

Hui et al. *Methods* 2018

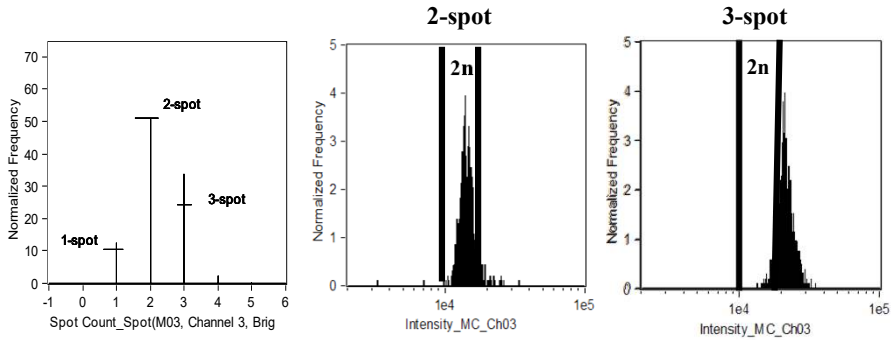
FISH probe “spot count” accuracy

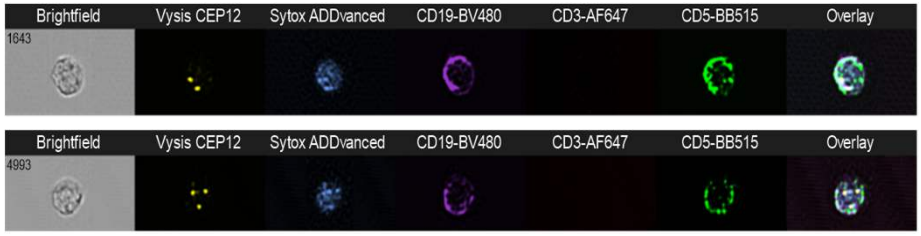
Analysis: Peak mask (Bright 17.5) for Vysis CEP12-SpectrumOrange probe fluorescence and Spot Count feature calculation

CD3+CD5+ T cells



CD19+CD5+ CLL





Hui et al. *Methods* 2018

Multiple myeloma

Plasma cell neoplasm in the bone marrow

Clonal expansion of terminally differentiated B-cells

Disseminated with circulating disease

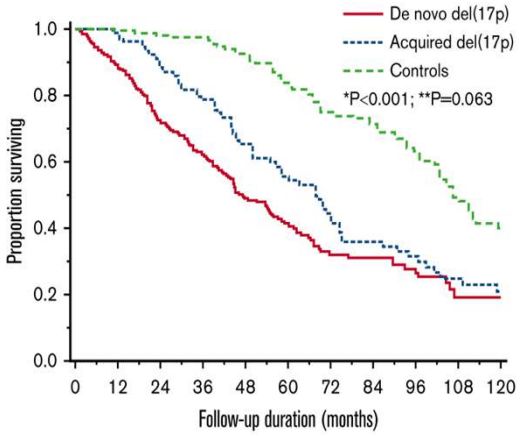
Primary abnormalities

- Trisomies (odd numbered chromosomes)
- Translocations of the *IgH* locus (14q32)

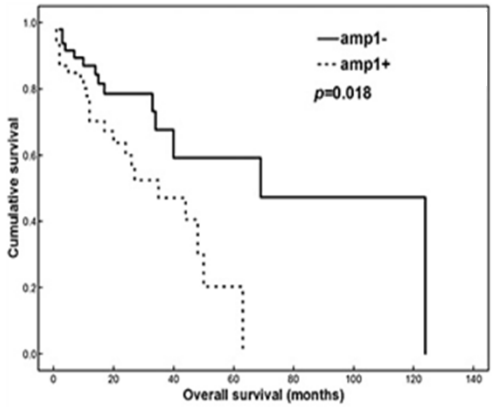
Secondary abnormalities

- Amplification and deletions

Risk Category	Cytogenetic Abnormalities
Standard Risk	Trisomy 3, 5, 7, 9, 11, 15 ,19, 21 t(6;14); t(11;14)
High Risk	t(4;14); t(14;16); t(14;20) del(17p); amp(1q21)



Lakshman et al. Blood 2018



Grzasko et al. Haematol Oncol 2013

Immuno-flowFISH myeloma study

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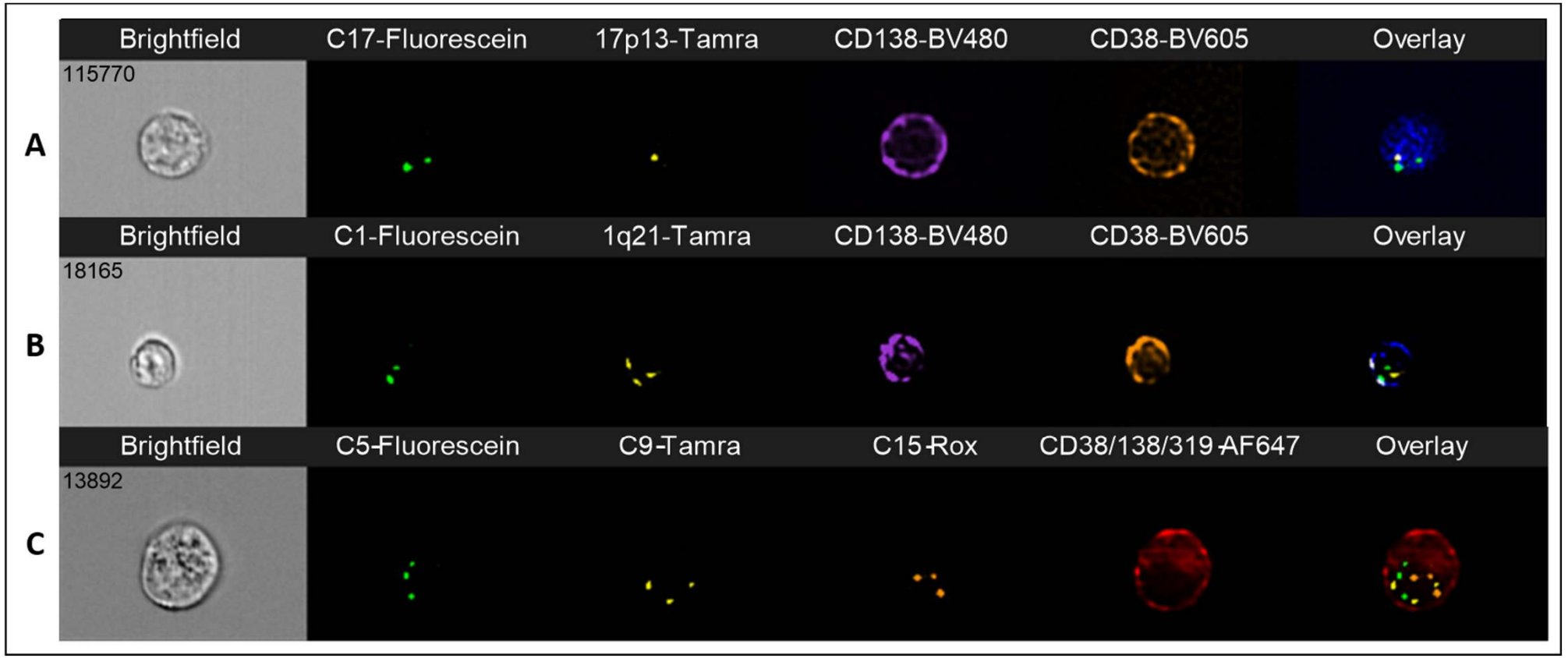
Aim of this study was to determine whether primary and secondary chromosomal abnormalities could be detected by “immuno-flowFISH”

- Primary and secondary abnormalities in bone marrow
- Secondary alterations (gain(1q) and del(17p)) could be detected in rare circulating plasma cells

Method

- 100 bone marrow (BM) or blood (PB) samples from 67 myeloma patients
- Red cell lysis (BM) or Ficoll mononuclear cell separation (PB)
- Immunophenotyped with CD3-BV510, CD19-BV480, CD38-AF647, CD138-AF647, CD319-AF647
- Nuclei stained with SytoxAADvanced

Bone marrow: primary and secondary abnormalities



Erber at al. *Journal of Human Genetics* 2023

Bone marrow: t(11;14) *IGH::MYEOV* translocation

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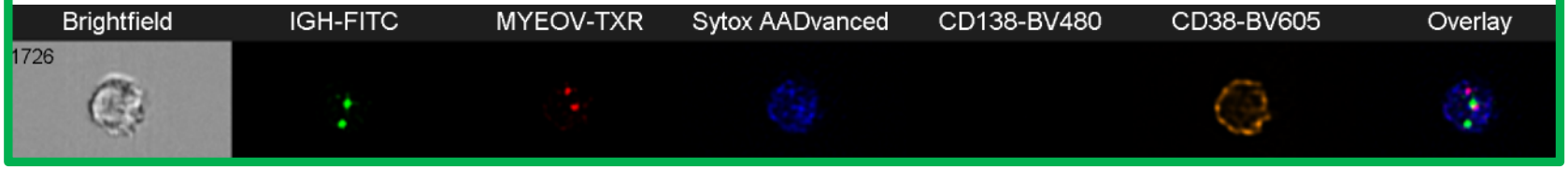
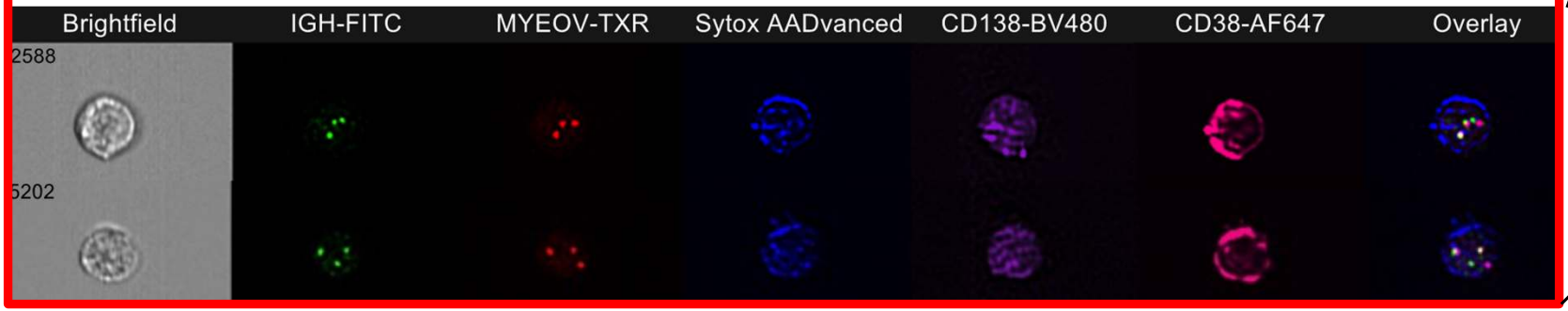
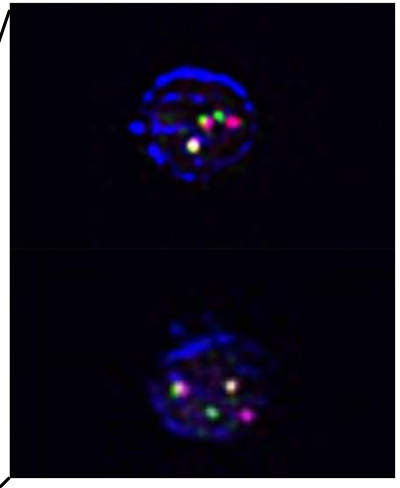
CD138 CD38 antibodies, t(11;14)(q13;q32) *IGH-MYEOV* dual fusion probe

Normal pattern



Myeloma: CD38/CD138-positive plasma cells with 3 split *IGH* and *MYEOV* spots





1R1G2F balanced translocation

Hui at al. *Journal of Clinical Pathology* 2023

Bone marrow: t(4;14) *IGH::FGFR3* translocation

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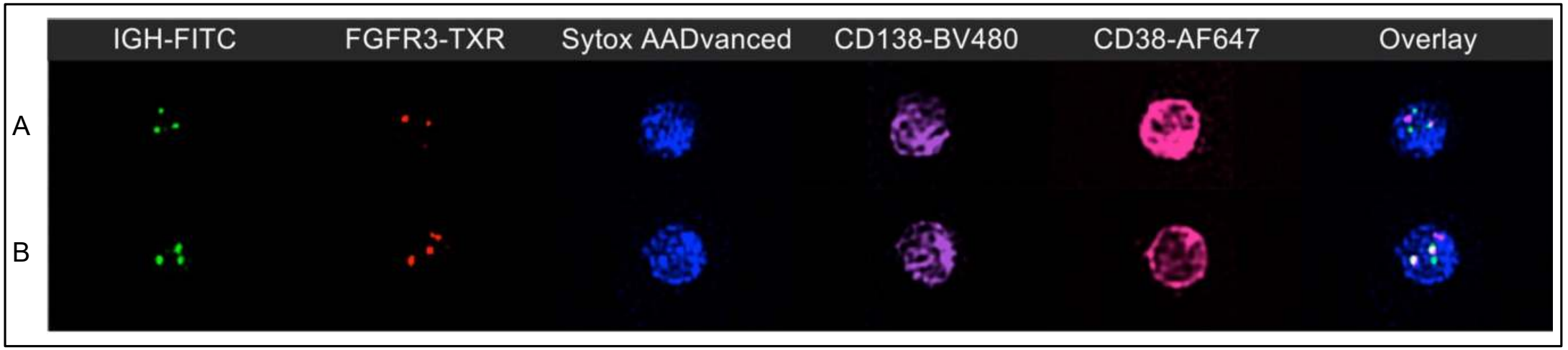
CD138 CD38 antibodies, t(4;14)(q13;q32) *IGH-FGFR3* dual fusion probe



A **2R3G0F** trisomy 14
B **1R1G2F** balanced translocation

Hui at al. *Journal of Clinical Pathology* 2023

Bone marrow: del(17p)

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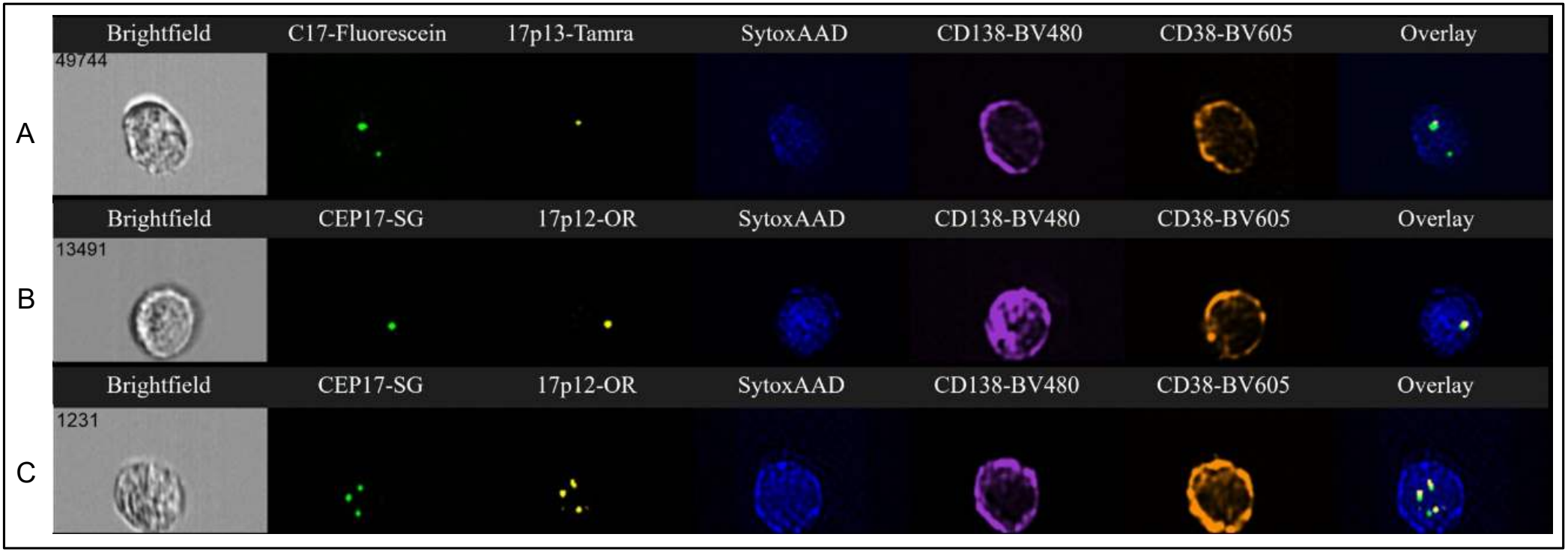
CD138 CD38 antibodies, **C17**, **17p12** probes



A 14 del(17p)
B monosomy 17
C trisomy 17

Mincherton at al. *International Journal of Laboratory Hematology* 2024

Summary

Bone marrow

- Primary and secondary abnormalities were detectable in BM of multiple myeloma
- Hyperdiploidy: most common abnormality was trisomy of chromosomes 5, 9, and 15
- *IGH::FGFR3* and *IGH::MYEOV* translocations were detected: balanced and unbalanced
- Chromosome 1: most common abnormality was 3 FISH spots with the 1q21 locus probe, indicating trisomy 1 or gain(1q)
- Chromosome 17: one 17p FISH signal and two for C17 or del(17p); one FISH spot for both 17p and C17 probes (monosomy 17); three for both 17p13 and C17 (trisomy 17); and 1 or 2 copies of 17p and 4 for C17 (possible tetrasomy 17 with concurrent del(17p))

Blood

- Circulating plasma cells were present in all samples tested
- Plasma cells comprised 0.01- 0.55% of cells
- Secondary abnormalities detected included “dual hit” with gain(1q21) and del(17p) in separate cells and in 1 case within the same cell

Acknowledgements

Multiple myeloma patients

Prof Wendy Erber, Dr Henry Hui, Dr Stephanie Lam, Dr Hun Chuah, Dr Hasib Sidiqi, Dr Jacques Malherbe, Dr Jason Stanley, Tom Mincherton, Sarah Clarke, Matthew Harms, James McQuillan

Haematologists at Sir Charles Gairdner Hospital, Royal Perth Hospital, Fiona Stanley Hospital, Hollywood Private Hospital and PathWest Laboratory Medicine