

Tecnologia multispettrale per la caratterizzazione dell'immunofenotipo in situ

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mIHC vs FACS, RNA-seq and standard IHC

FACS:

Immunophenotyping
Quantification

! Tissue context is lost

RNA-seq

Immune signature
Prognostic classifiers

! Tissue context is lost

IHC:

Tissue context

! 2 markers

! No immunophenotyping

Multiplex IHC

Technological viability

Challenges

- Limited samples

Simultaneous identification
up to 6 antigens

- Antibody cross reactivity

- Autofluorescence in FFPE

Autofluorescence subtraction

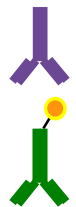
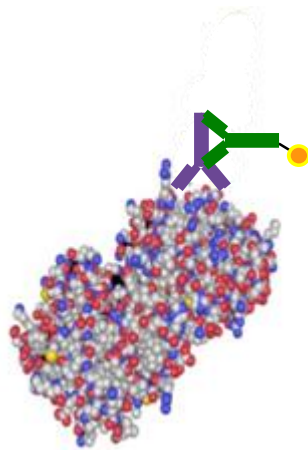
- Immunophenotyping

Tissue contextualization

Quantification

Technological reliability

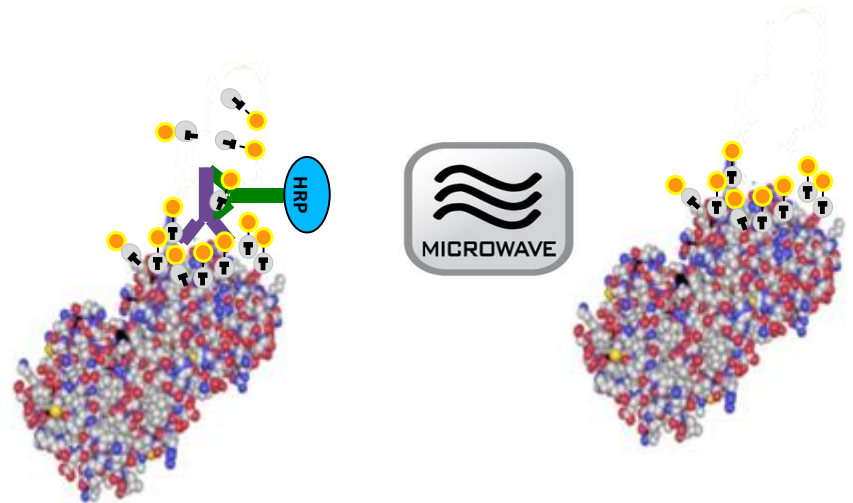
Standard Detection



Anticorpo primario

Anticorpo secondario
Coniugato a fluorocromo

Tyramide Signaling Amplification Detection

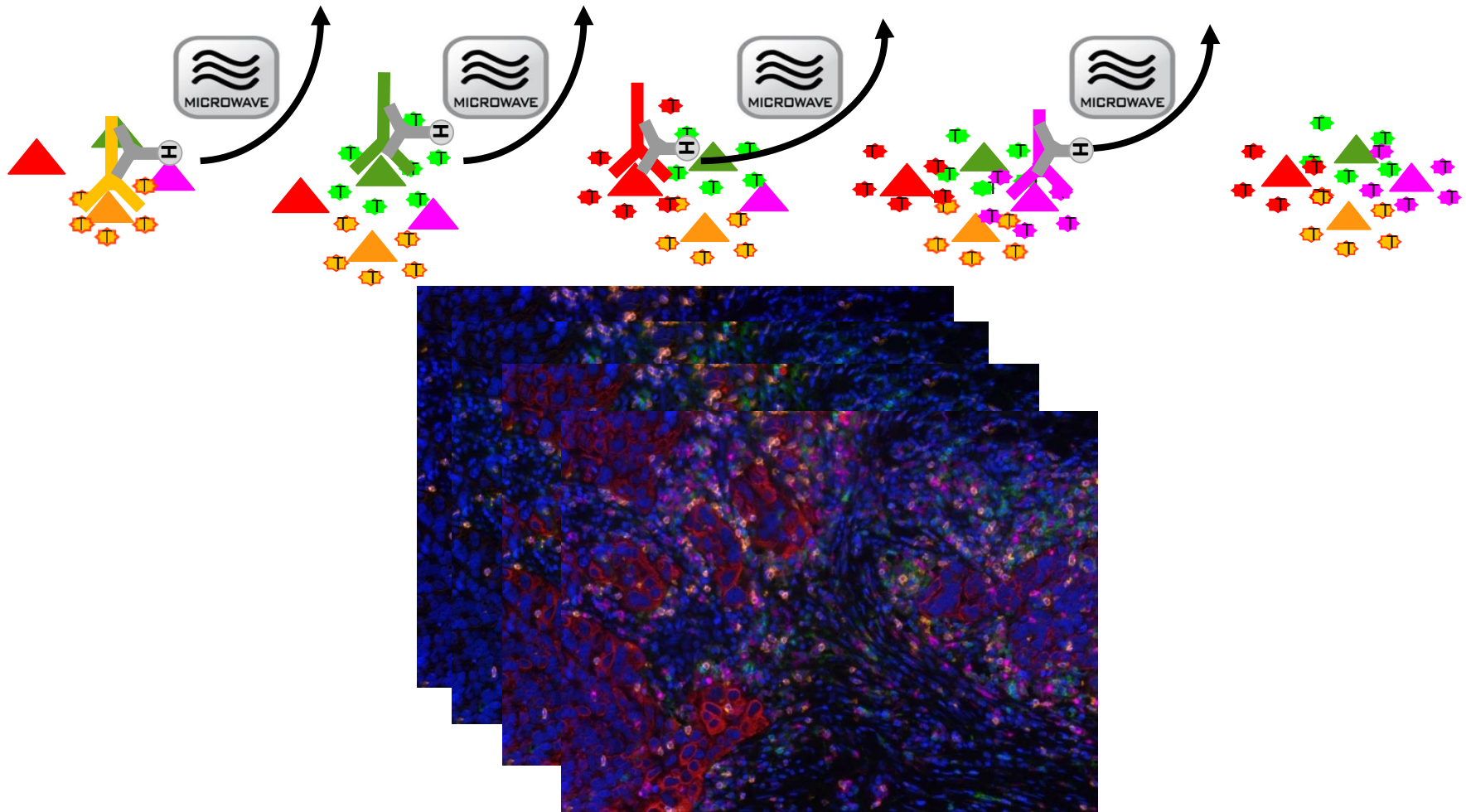


Anticorpo secondario
Coniugato all'enzima

Tiramide coniugata
a fluorocromo



Technological reliability



Technological reliability

Multispectral Quantitative Pathology System

Filters

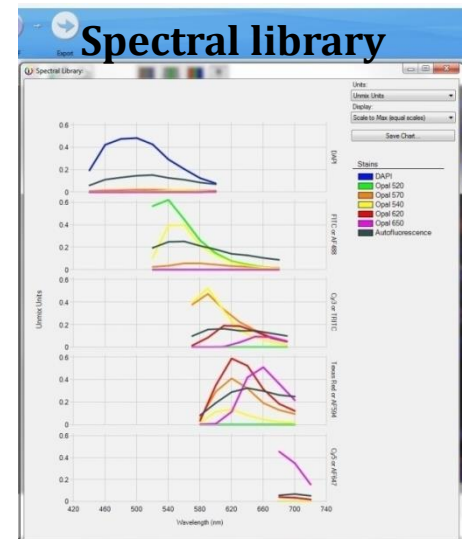


Multispectral
camera

720nm



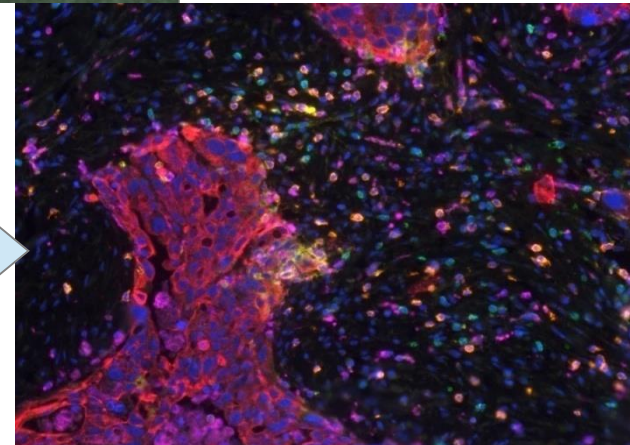
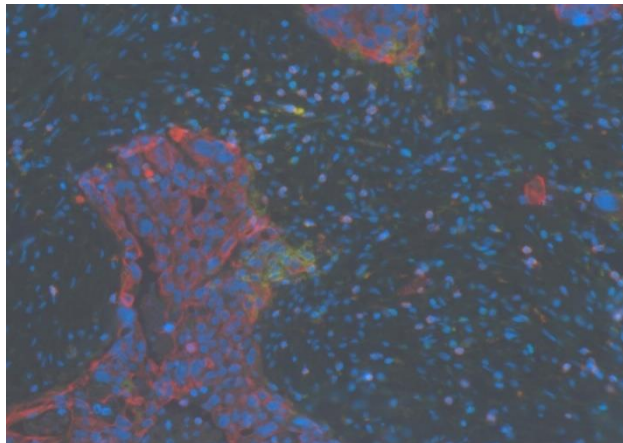
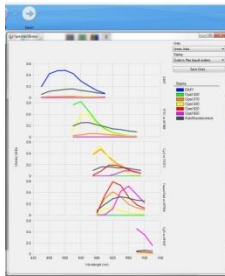
440nm



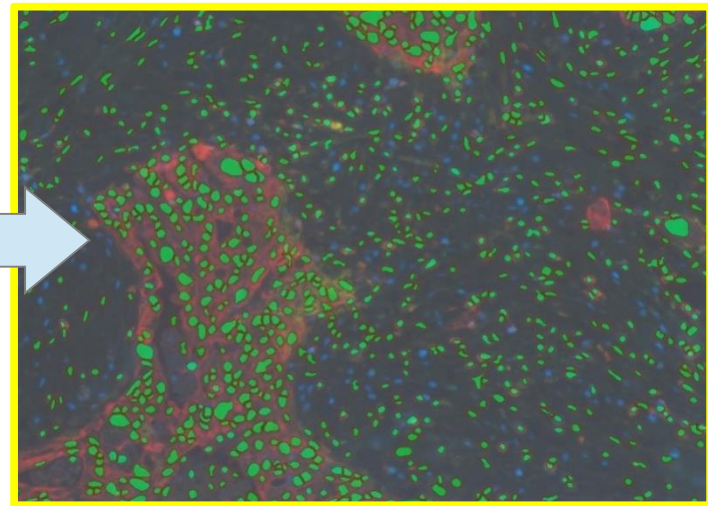
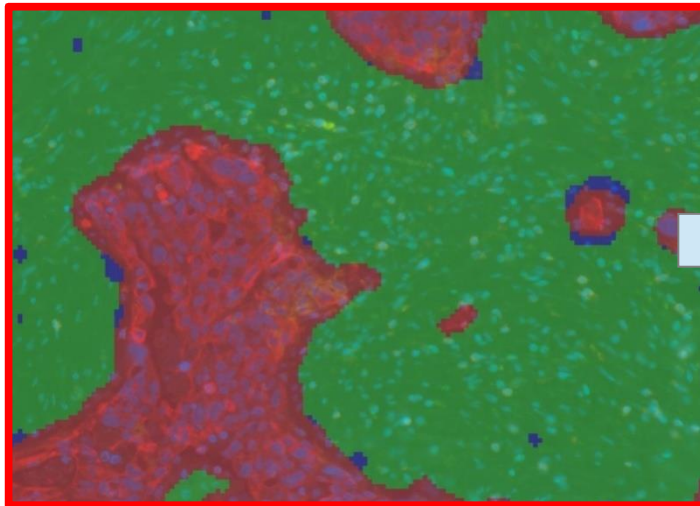
**CRO
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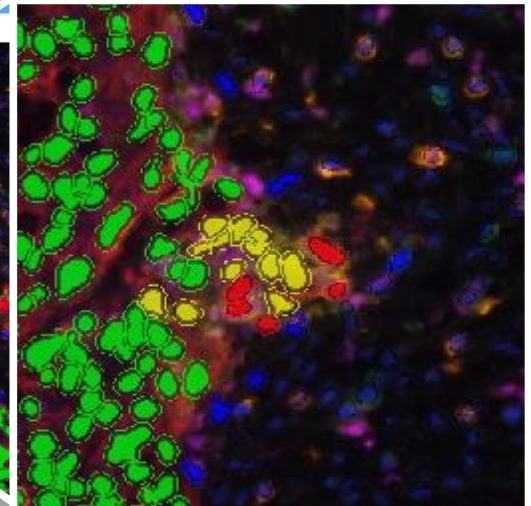
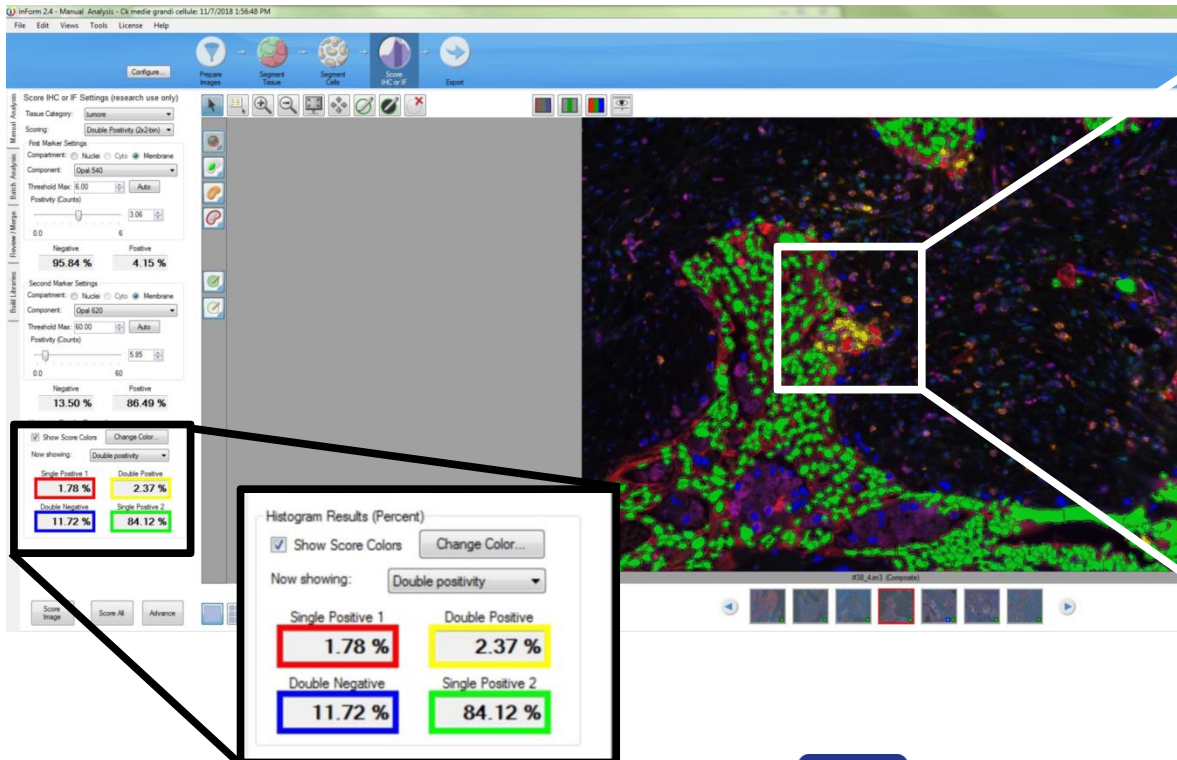
Technological reliability



Technological reliability



Technological reliability



Clinical relevance

Understanding of the tumor immune landscape



Contents lists available at ScienceDirect

Journal of Immunological Methods

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2019

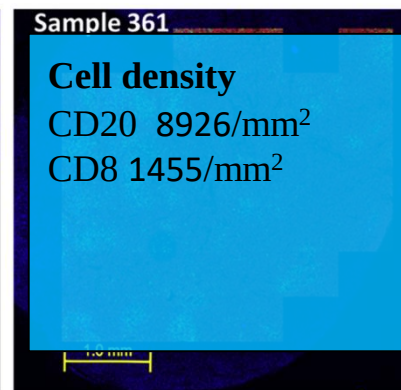
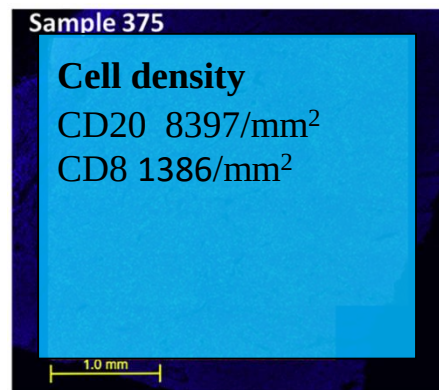


Multiplex immunofluorescence staining and image analysis assay for diffuse large B cell lymphoma

Chung-Wein Lee*, Yan J. Ren¹, Mathieu Marella¹, Maria Wang, James Hartke, Suzana S. Couto

High cell density

6-plex X 5 panels → 16 biomarkers



CD20
CD8

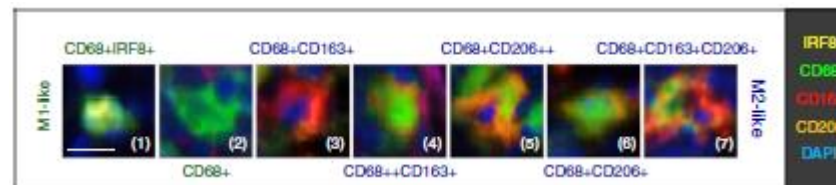
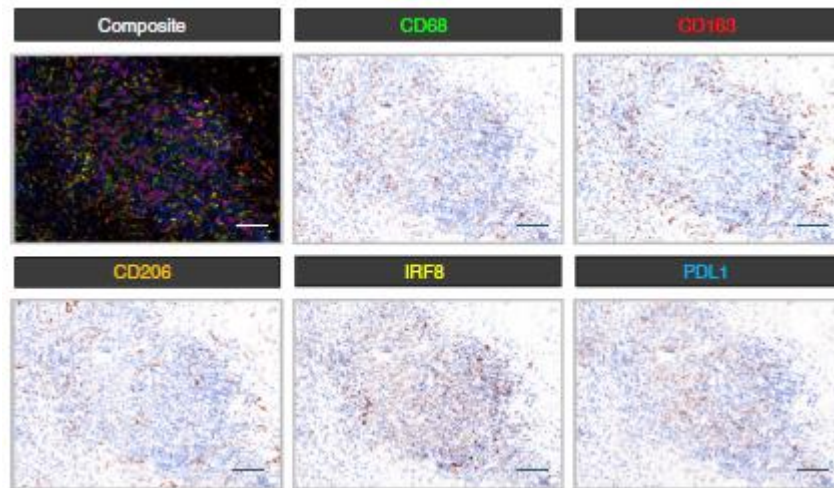


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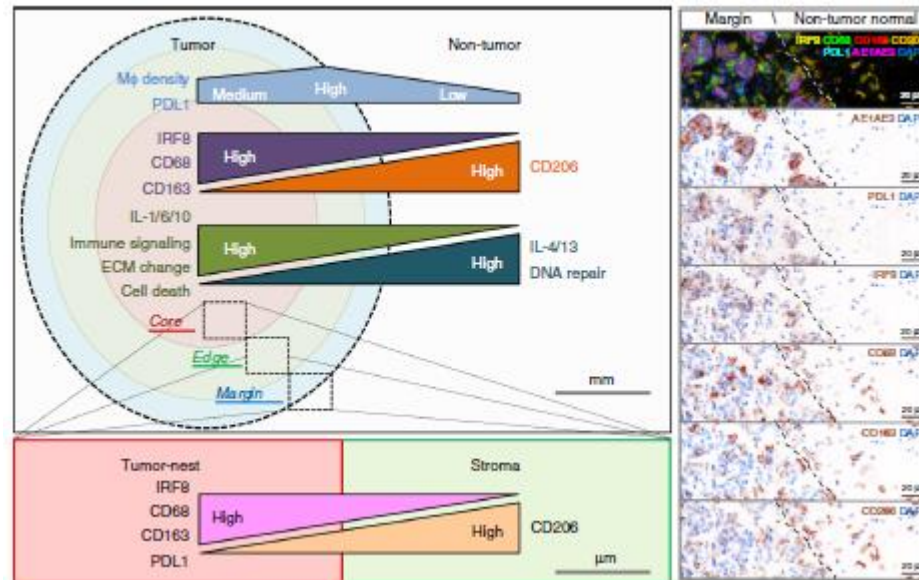
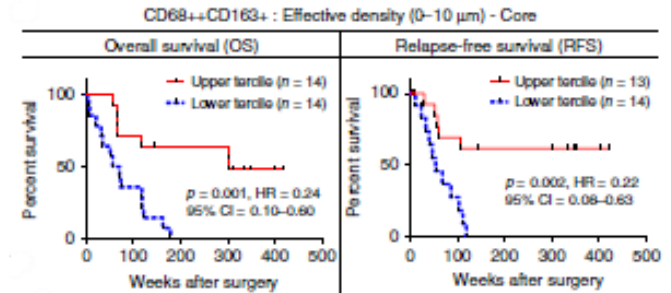
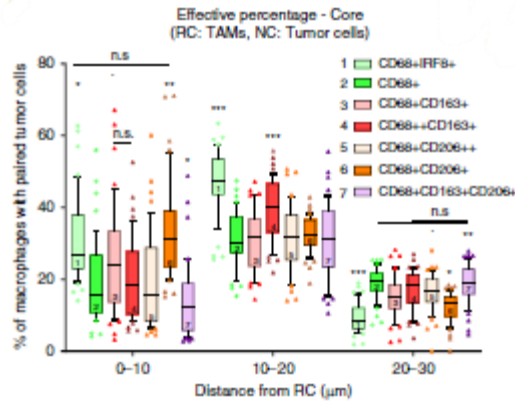
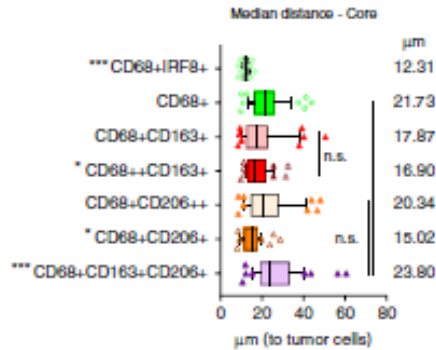
Clinical relevance

Macrophage spatial heterogeneity in gastric cancer defined by multiplex immunohistochemistry

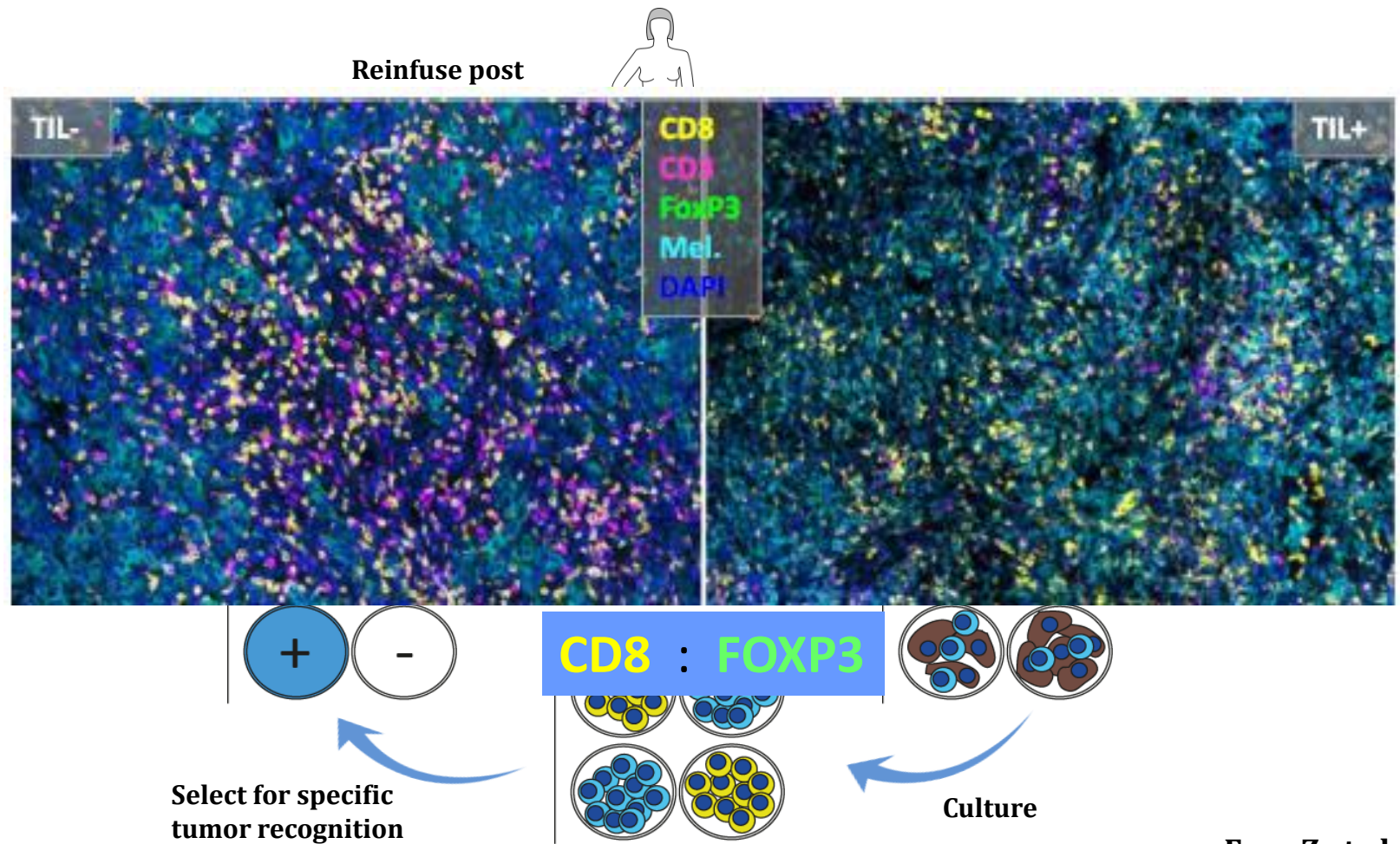
Yu-Kuan Huang^{1,2,3}, Minyu Wang^{1,2,3}, Yu Sun^{2,3}, Natasha Di Costanzo¹, Catherine Mitchell⁴, Adrian Achuthan³, John A. Hamilton^{3,5}, Rita A. Busuttil^{1,2,3} & Alex Boussios^{1,2,3}



Clinical relevance



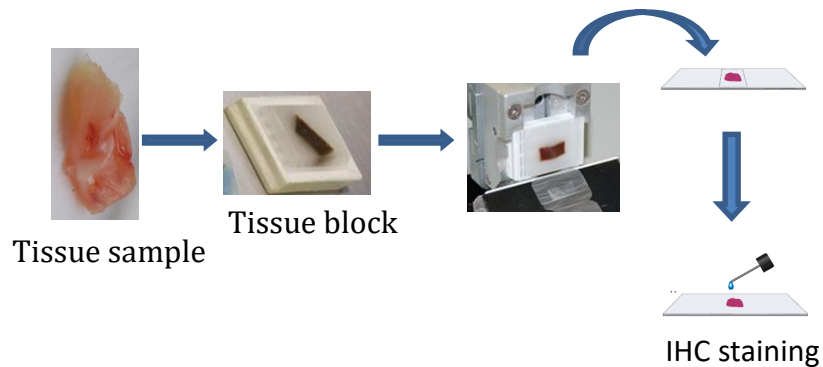
Clinical utility



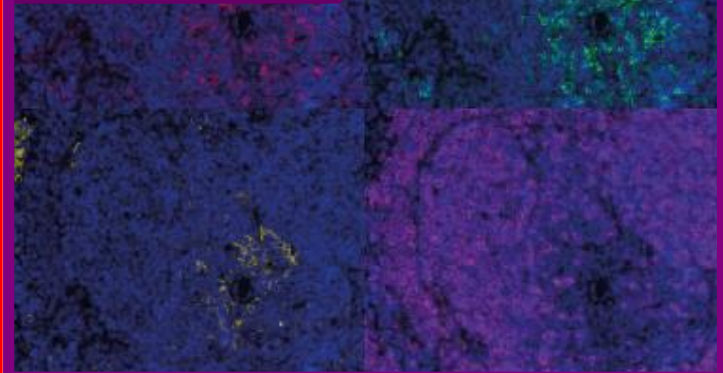
Feng Z et al. 2015
Journal of Immunotherapy of Cancer

Technological validation

1. Sample processing

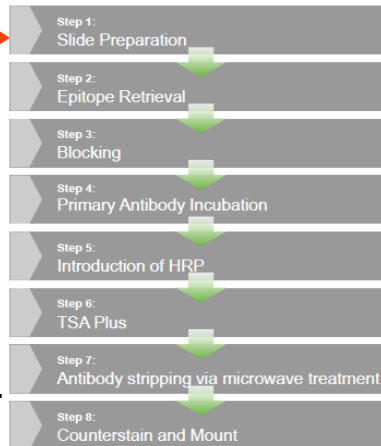


2. Protocol Optimization and validation



3. mIHC protocol development

CD4 (OPAL52)
CD8 (OPAL57)
PD-L1 (OPAL54)
pan Cks (OPAL650)
CD68 (OPAL650)

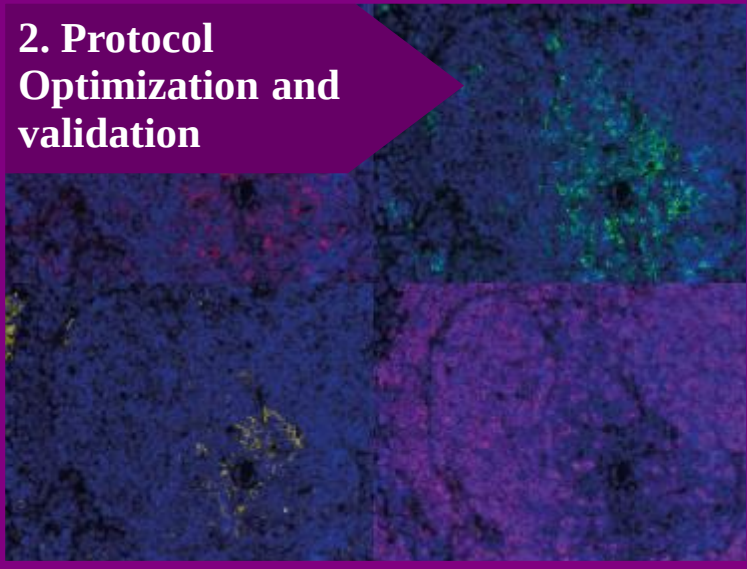


4. Image Analysis



Considerations

2. Protocol Optimization and validation

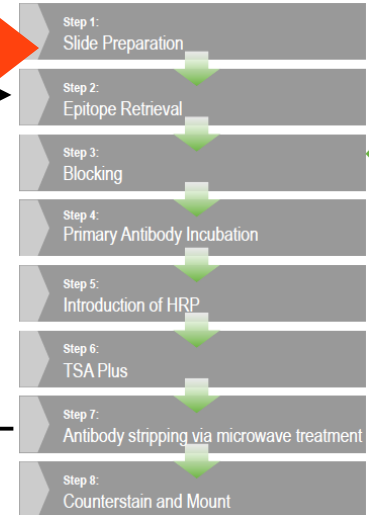


Which/how many markers?

Validated antibodies/protocols?

3. mIHC protocol development

CD4
(OPAL52)
CD8
(OPAL57)
PD-L1
(OPAL54)
pan Cks
(OPAL650)
CD68
(OPAL650)



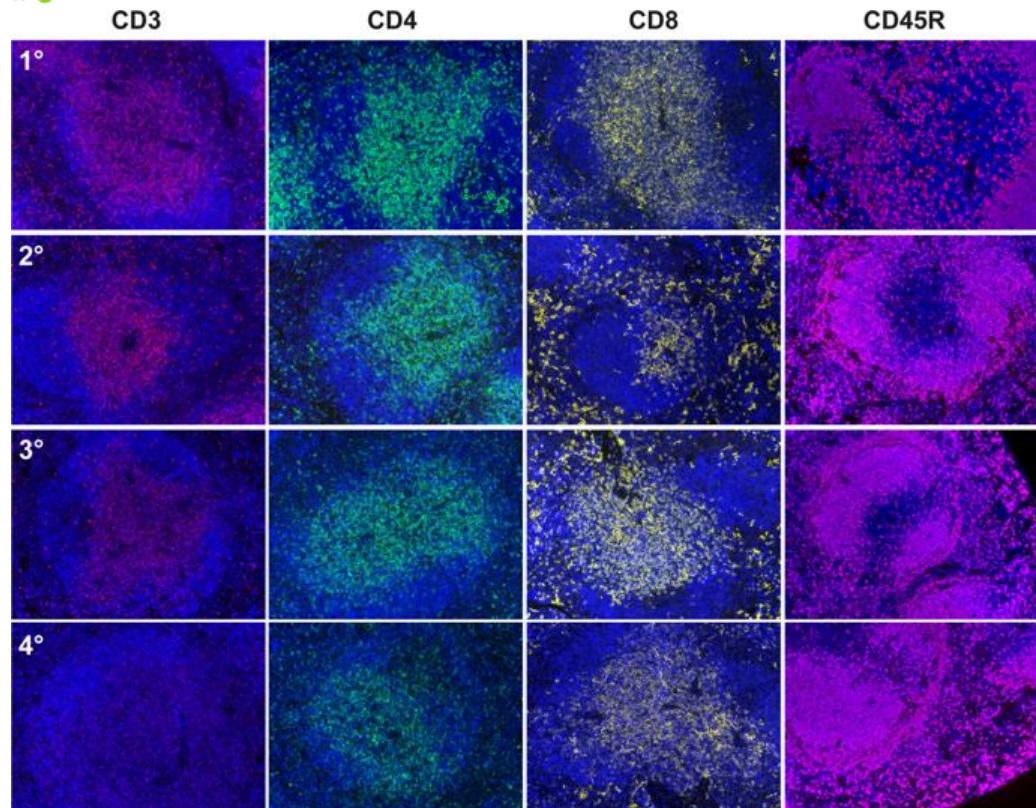
Multiplex protocol

Validated protocols?

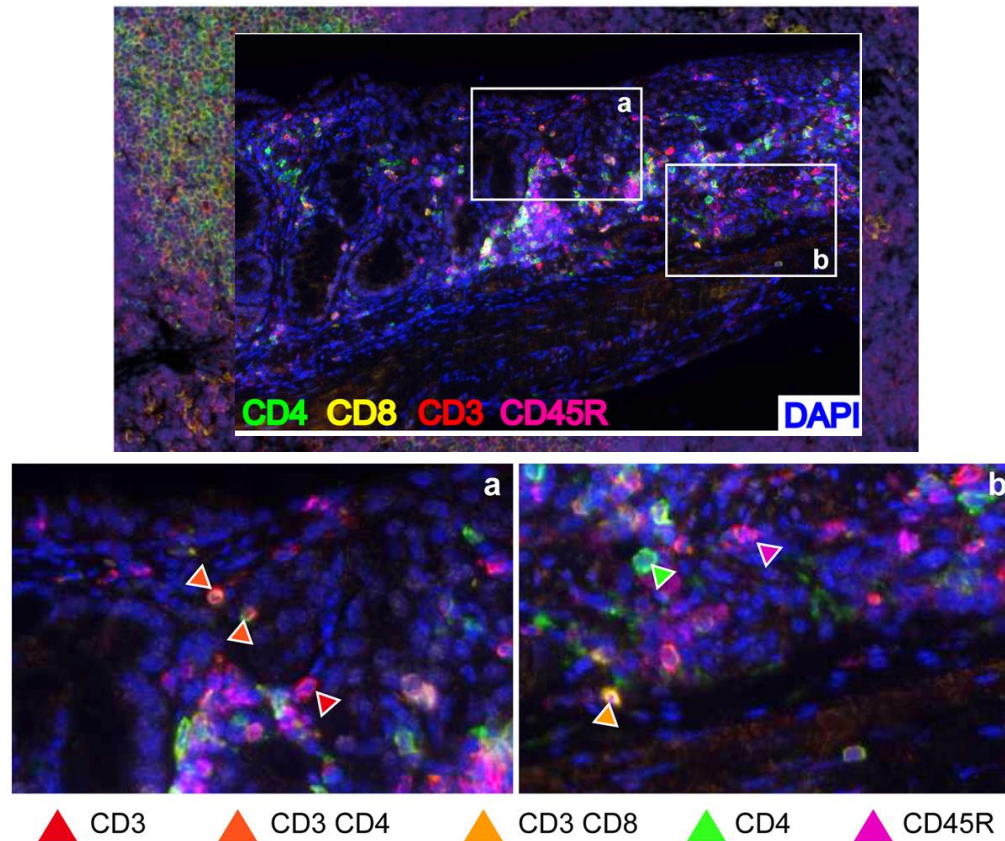
Technological validation

Multiplex staining depicts the immune infiltrate in colitis-induced colon cancer model

Eliana Pivetta¹, Alessandra Capuano¹, Eugenio Scanziani^{2,3}, Lucia Minoli^{2,3}, Eva Andreuzzi¹, Maurizio Mongiat¹, Gustavo Baldassarre¹, Roberto Doliana¹ & Paola Spessotto¹



Technological validation

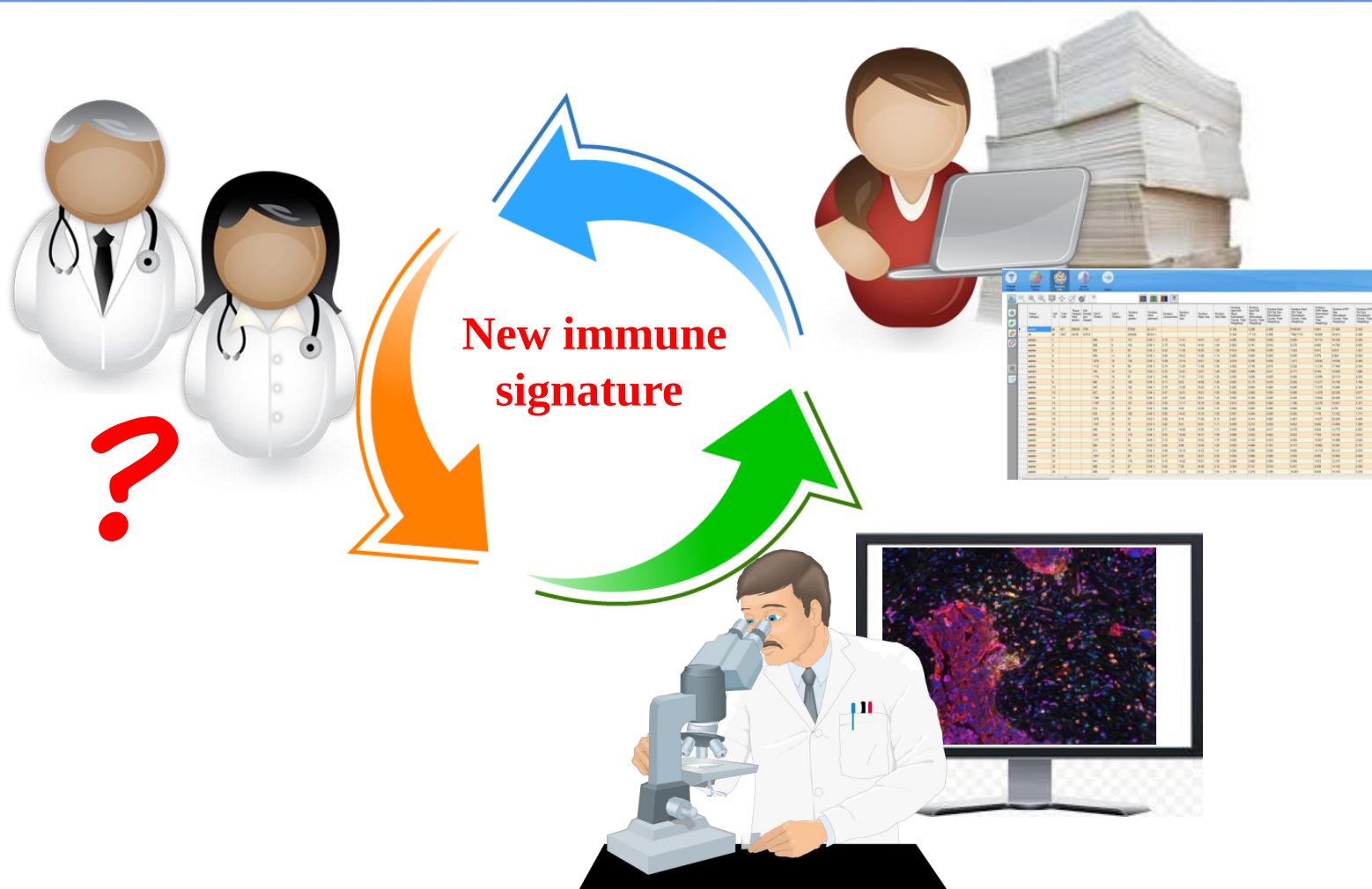


Pivetta *et al.* Scientific Report 2019



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Considerations



Acknowledgements

Dott.ssa Spessotto Paola

Responsabile piattaforma IvIvI

- Capuano Alessandra

Dr Baldassarre Gustavo

Direttore dell'Oncologia Molecolare e Modelli Preclinici di
Progressione tumorale



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02/03/2020

Technological viability

