Bringing multiplexed immunofluorescence to clinical practice - a proof of concept study

Spatial Proteomics Unit

Katarina Åstrand (KTH, Scilifelab), Carolina Oses (KTH, Scilifelab) , Hans Brunnström (Lund University and Dept of Pathology Region Skåne) Charlotte Stadler (KTH, Scilifelab)

Background and Aims

In histopathology hematoxylin and eosin stains (HE) of tissue biopsies has been the mainstay for diagnosis in clinic. Depending on the tissue origin and suspected cancer type, conventional immunohistochemstry (IHC) is used to stain specific markers that can help in characterizing the tumor and ensure proper diagnosis. In many cases several markers are needed and this can be a time consuming and iterative process. Using immunofluorescence and multiplexed (mIF), all markers can be stained and evaluated in the same section from one single experiment. In this proof of concept study we aim to show the feasibility of mIF for faster diagnosis of lung cancer cases by combing relevant diagnostic markers in one experiment.

Aim 1: Generate a multiplexed panel of relevant markers used in clinic for diagnosis of lung cancer sub types
Aim 2: Use the established panel to diagnose cases of a retrospective cohort
Aim 3: Evaluate the total cost, time and material savings for the mIF approach

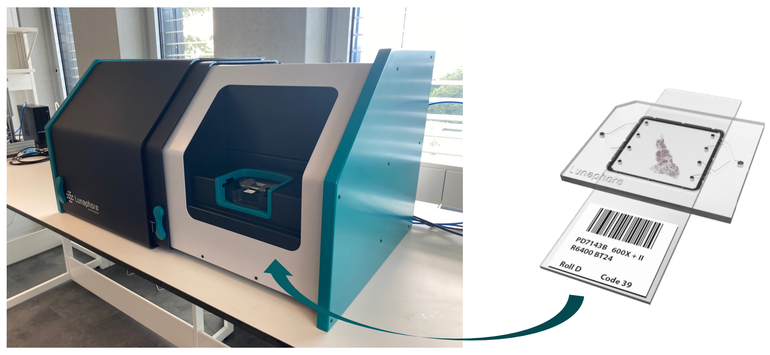
Workflows

The concept of multiplexing with seqenential IF..

Step-by step workflow:

1. Incubate primary abs (n=2)
2. Incubate secondary abs (n=2) (fluorescently labelled)
3. Image
4. Elute ab complex with elution buffer and heat
5. Repeat step 1-4 for x times
6. Automated stacking of images

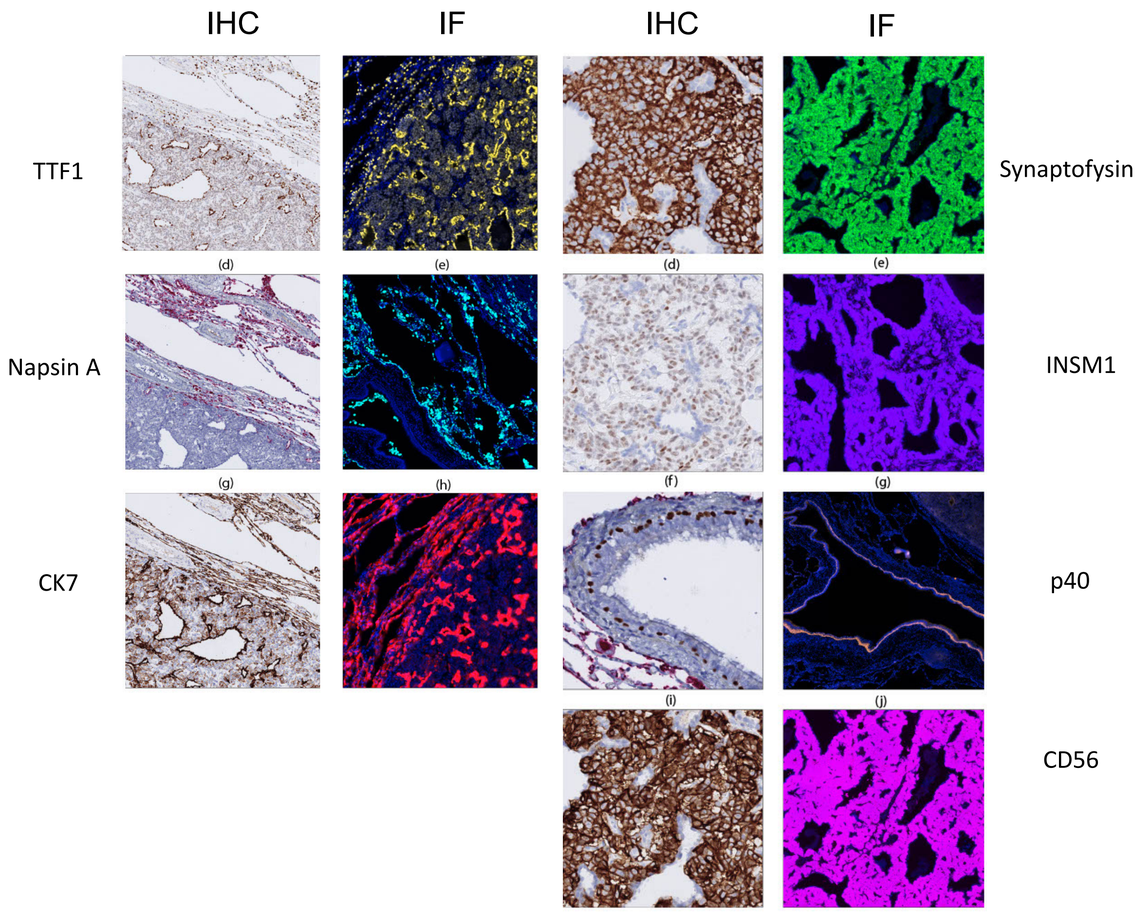
..and doing this in an automated fashion with COMET



Establishing of the multiplexed panel

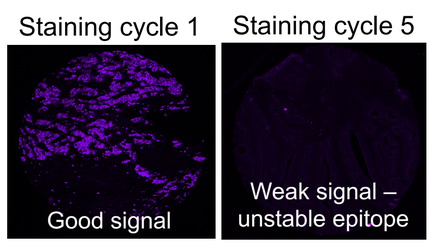
Aim 1

In a **first step** every individual marker has to be validated and optimized with IF readout. The staining pattern of the single plex IF is compared to the conventional IHC images.

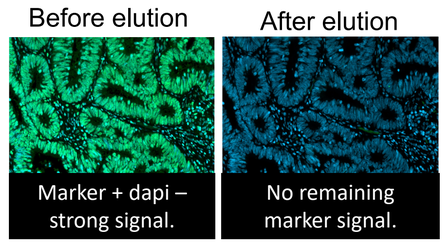


In a **second step**, the epitope stability and staining intensity of every marker is evaluated across different cycles. Also the elution efficiency is evaluated. Together these parameters decide the order of antibody stainings in the multiplexed experiment.

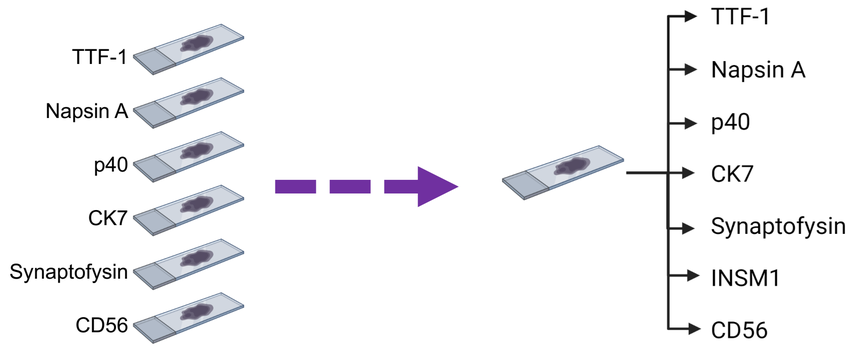
Ex epitope stability



Ex elution efficiency.



After successful optimization of step 1 and 2 the full 7 plex panel was established as below:



Conclusions and Future Steps

In this work we have developed a 7 plex panel of routinely used markers for diagnosing lung cancer biopsies. This panel will now be expanded with more markers (PAX8, Gata 3 and CDX2) to also distinguish origin of metastases ending up in lung. Eventually, a cohort of lung cancer patients with various tumor types will be analyzed using the multiplexed IF images to evaluate the accuracy and usefulness of mIF in lung cancer diagnosis compared to conventional IHC.