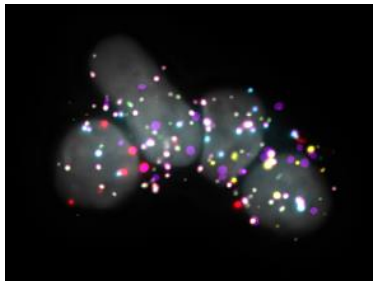
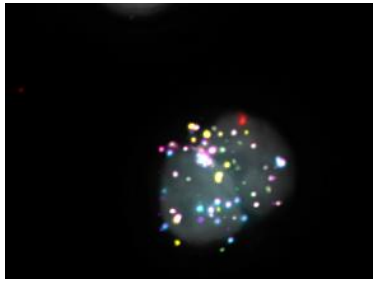
Research Prof. Priv.-Doz. Amin El-Heliebi, PhD
Gottfried Schatz Research Center
Division of Cell Biology, Histology & Embryology
Medical University of Graz, Austria
and CBmed
E-Mail: amin.elheliebi@medunigraz.at

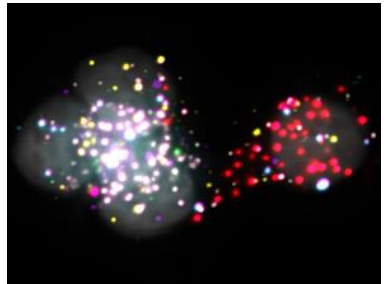
Medical University of Graz

UNIV. COMPREHENSIVE CANCER CENTER Krebszentrum GRAZ
Medizinische Universität & LKH-Univ. Klinikum

In situ RNA expression of CTCs and machine learning approaches for cell classification







Amin El-Heliebi

Karin Pankratz, Lissi Pritz, Lilli Hofmann, Katja Sallinger, Michael Gruber, Sarah Sedik, Christin-Müller, Christina Skofler, Margret Zinnegger, Thomas Kroneis, Amin El-Heliebi

amin.elheliebi@medunigraz.at

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09.02.2022

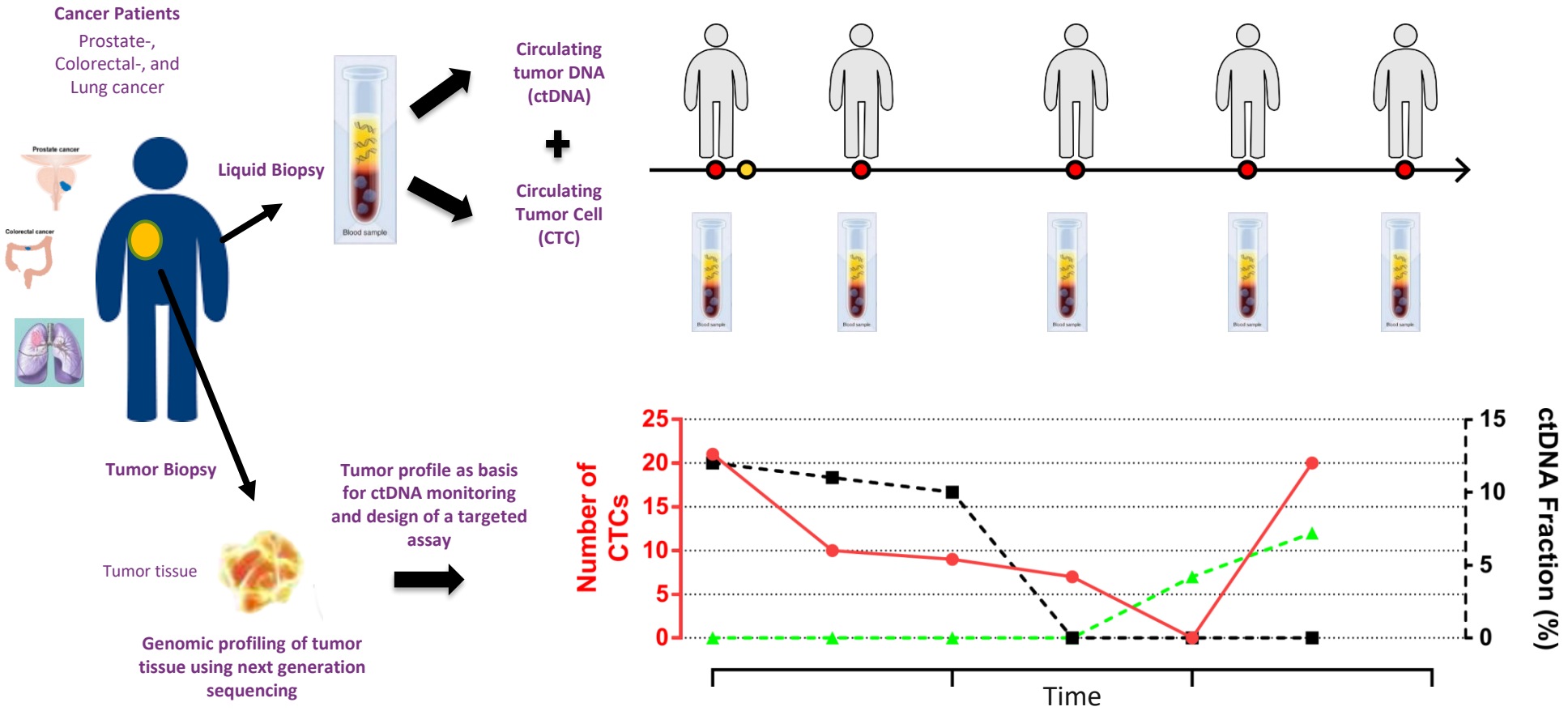
Overarching focus of our research group

- **We hypothesize that** the appearance of CTCs and ctDNA in blood is depending on
  - the histological architecture including

- **and the** architecture differs in shedding and non-shedding areas of the tumor

- **Our overall objective is to** elucidate which factors contribute to the dissemination of CTCs and ctDNA into blood

1-Page Graphical Abstract „Liquid biopsy in cancer“



- **Implementation of European (CEN)- and International (ISO)- standardized workflows for liquid biospies (CTC + ctDNA)**
- **We thereby aim to implement CTCs and ctDNA in the clinical management of**
  - Prostate Cancer (PC)-
  - Colorectal Cancer (CRC) and
  - Non-small Cell Lung Cancer- (NSCLC) Patients
- **with sampling at multiple time points**

Adapted from Lee et al., Nat Genet. 2018
Adapted from Haber et al., Cancer Discov 2014
https://www.spidia.eu/

Prostate cancer - The aim

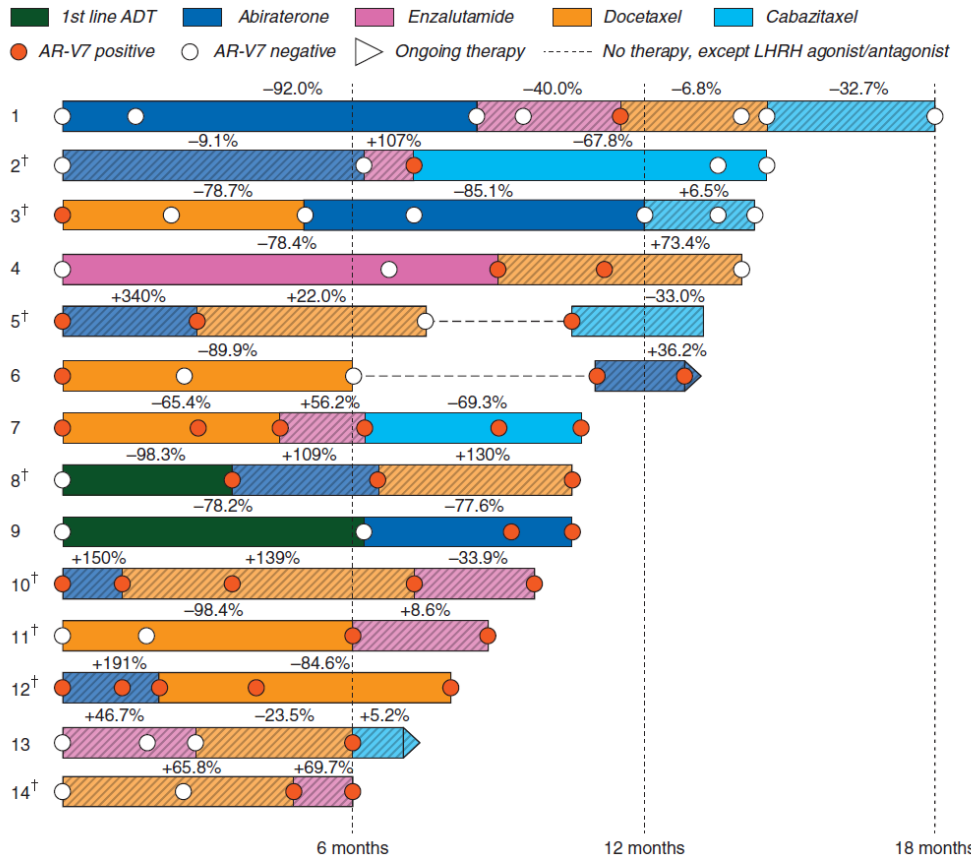


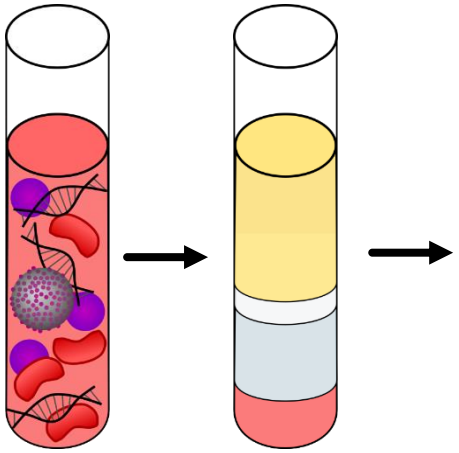
Figure 1. Swimmer plot indicating treatments that patients received, along with timing (and AR-V7 status) of CTC sampling, and whether or not PSA responses occurred during each therapy. Shaded boxes indicate failure to achieve PSA response; unshaded boxes indicate achievement of 50% PSA reduction on therapy. Percentage values indicate best PSA response. Daggers indicate deceased patient. Thirteen of 14 patients have previously been included in our prior publications, but only in the context of a single therapy.

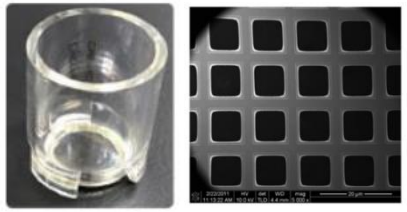
1st line ADT Abiraterone Enzalutamide Docetaxel Cabazitaxel
AR-V7 positive AR-V7 negative Ongoing therapy No therapy, except LHRH agonist/antagonist

Investigation of resistance markers by liquid biopsies

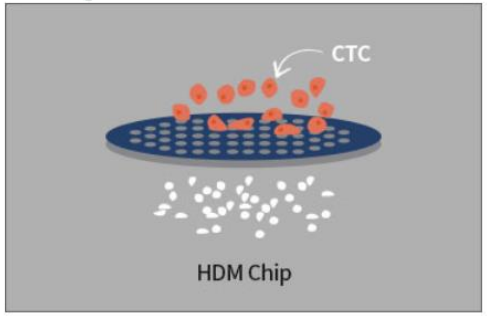
Adapted from Nakazawa et al., Ann Oncol 2015

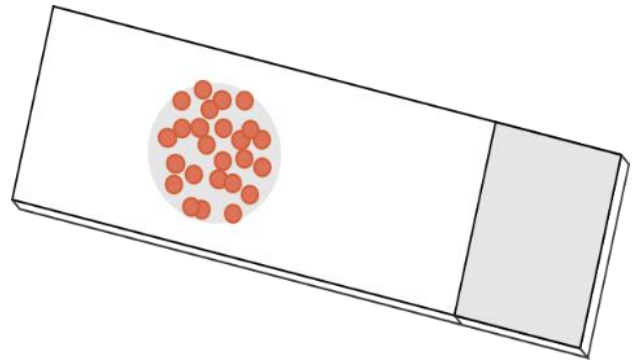
Smart Biopsy CTC enrichment





More than
500,000 pores/chip





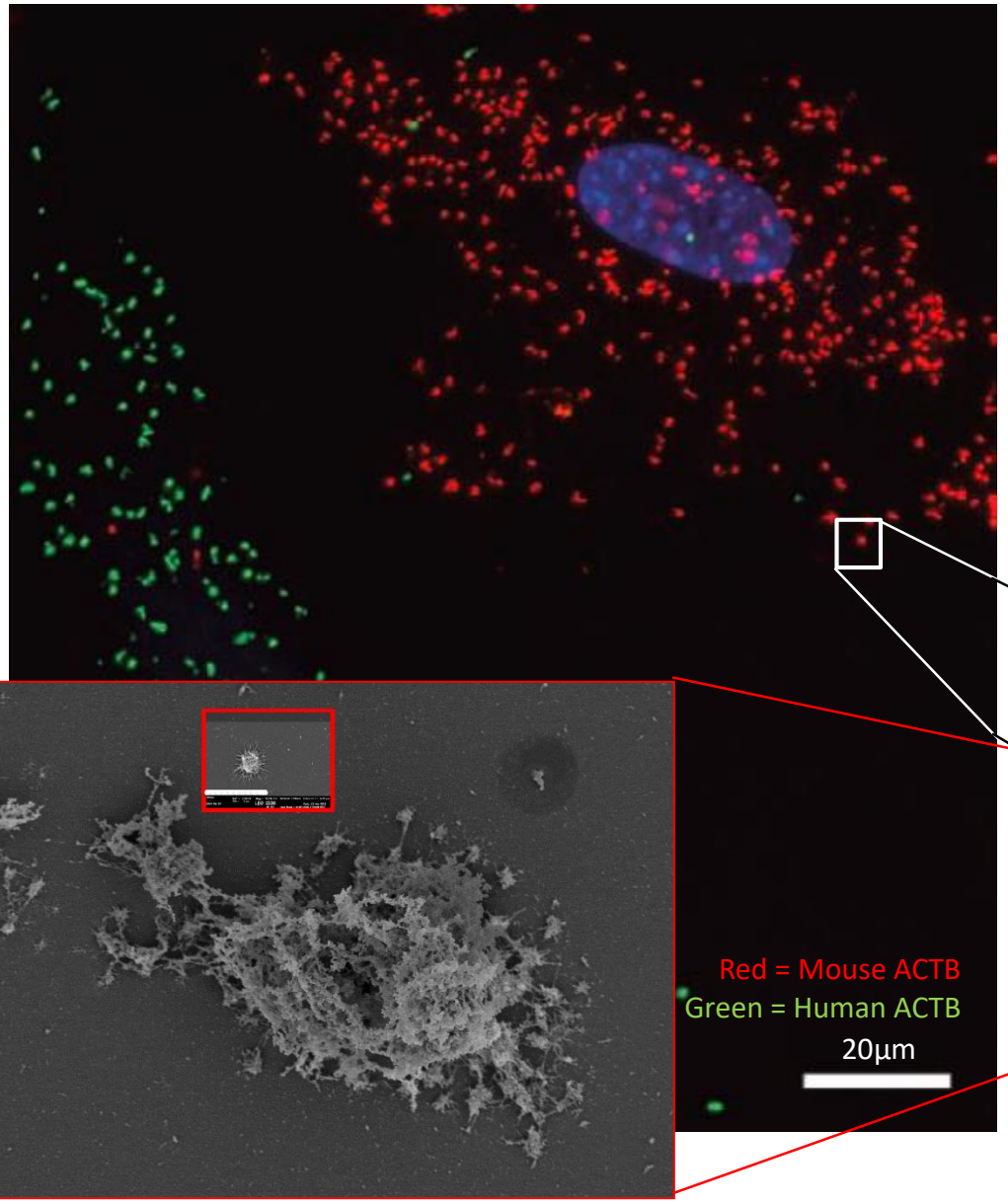
1) Double negative selection
   - Depletion of blood cells
   - Density gradient centrifugation

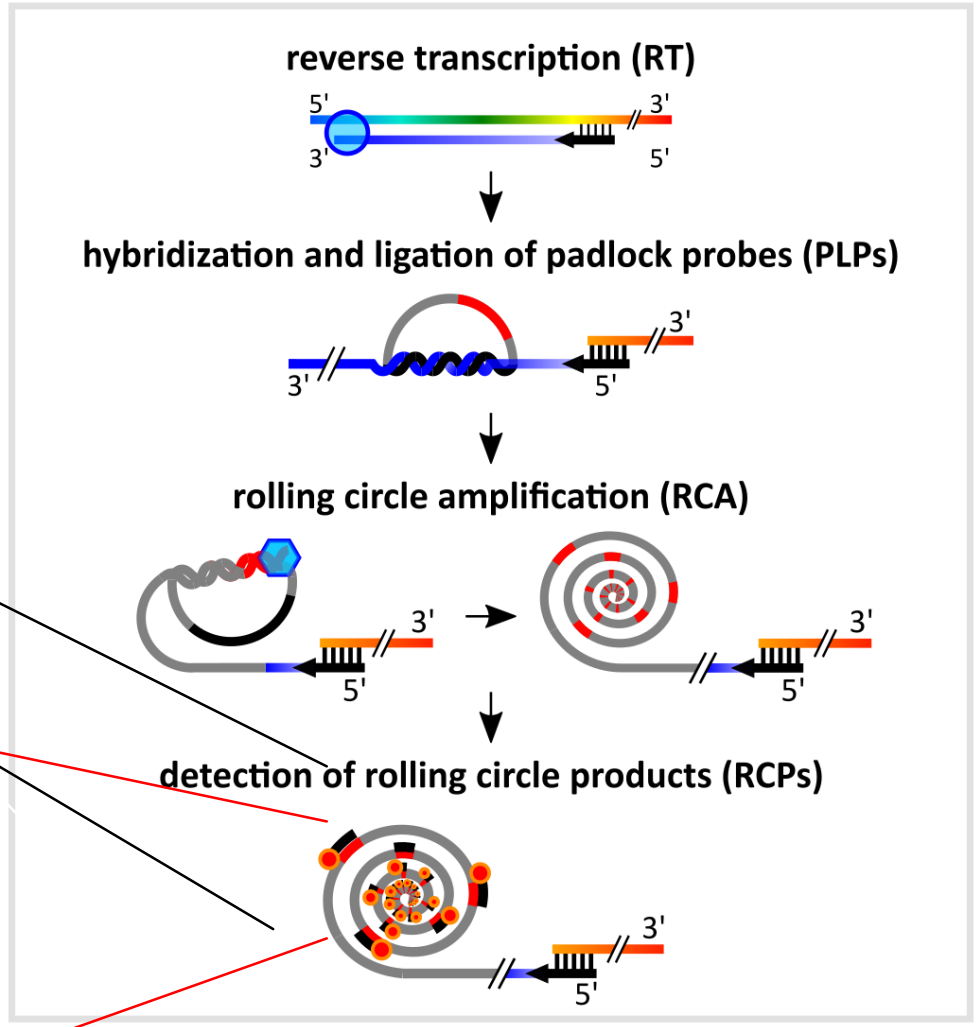
2) Size based gravity flow filtration

3) Cytospin of cell fraction for in situ padlock probe approach

Kim et al., Analytical Biochemistry 2013
Lee et al., Oncology Letters 2017
http://www.cytogenlab.com/

Method: In Situ combined with Padlock Probes



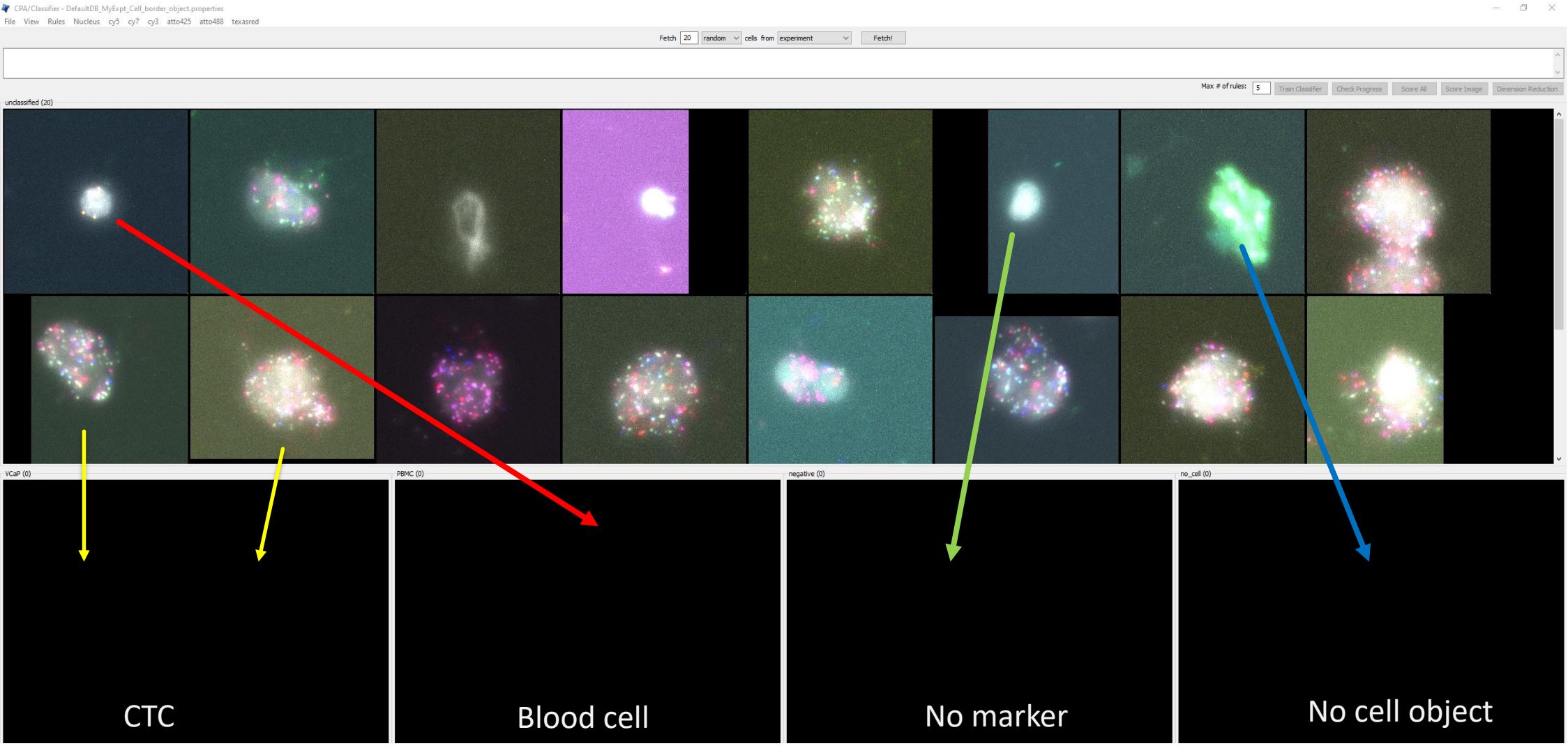


Modified from Larsson et al., 2010, Nature Methods
Modified from Hofmann et al., MIMB 2019
Modified from Landegren., personal communication

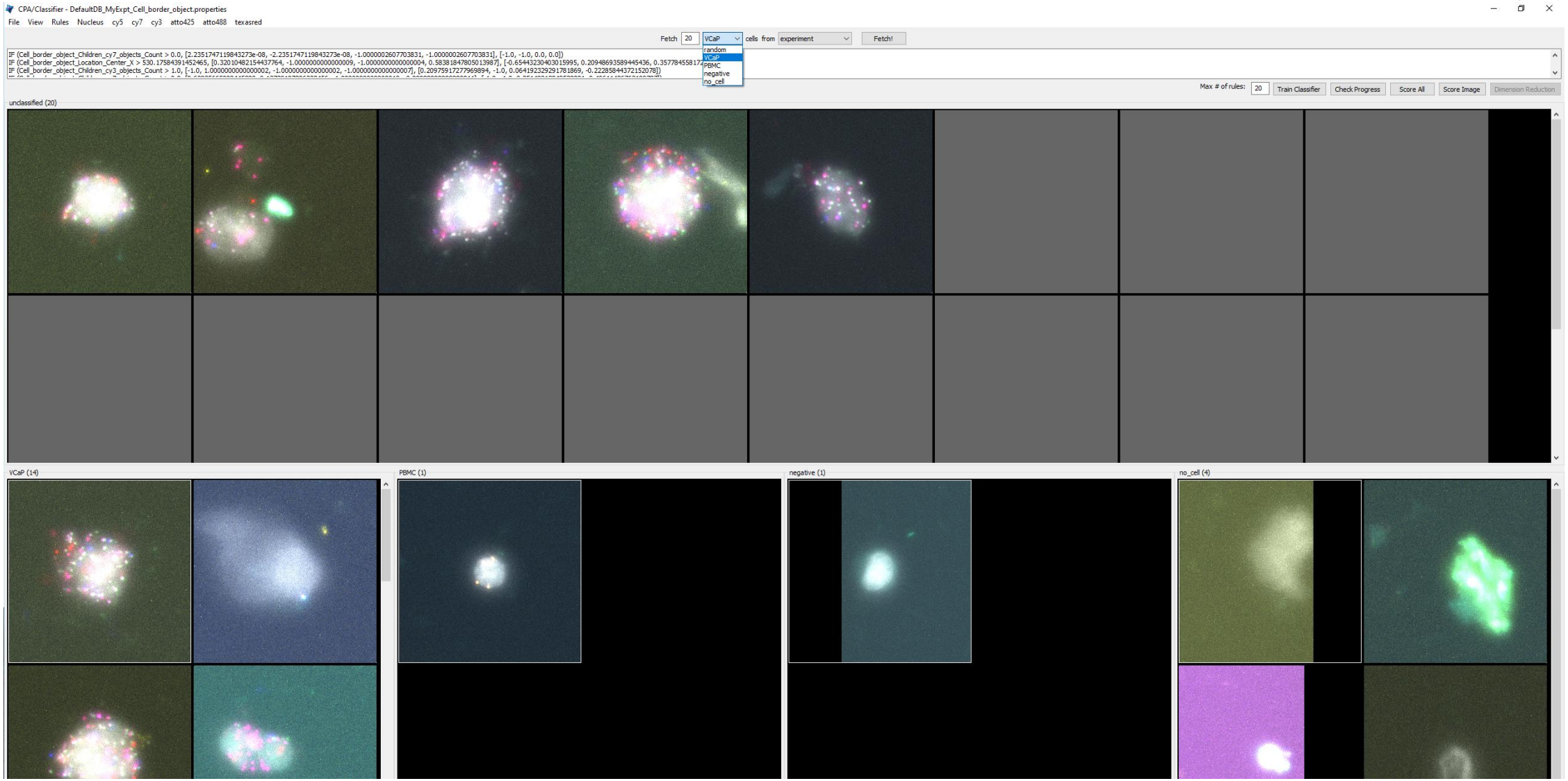
In situ padlock technology = Visualisation of RNA transcripts

Supervised machine learning – create training set

- Use drag and drop to sort cells into the classes



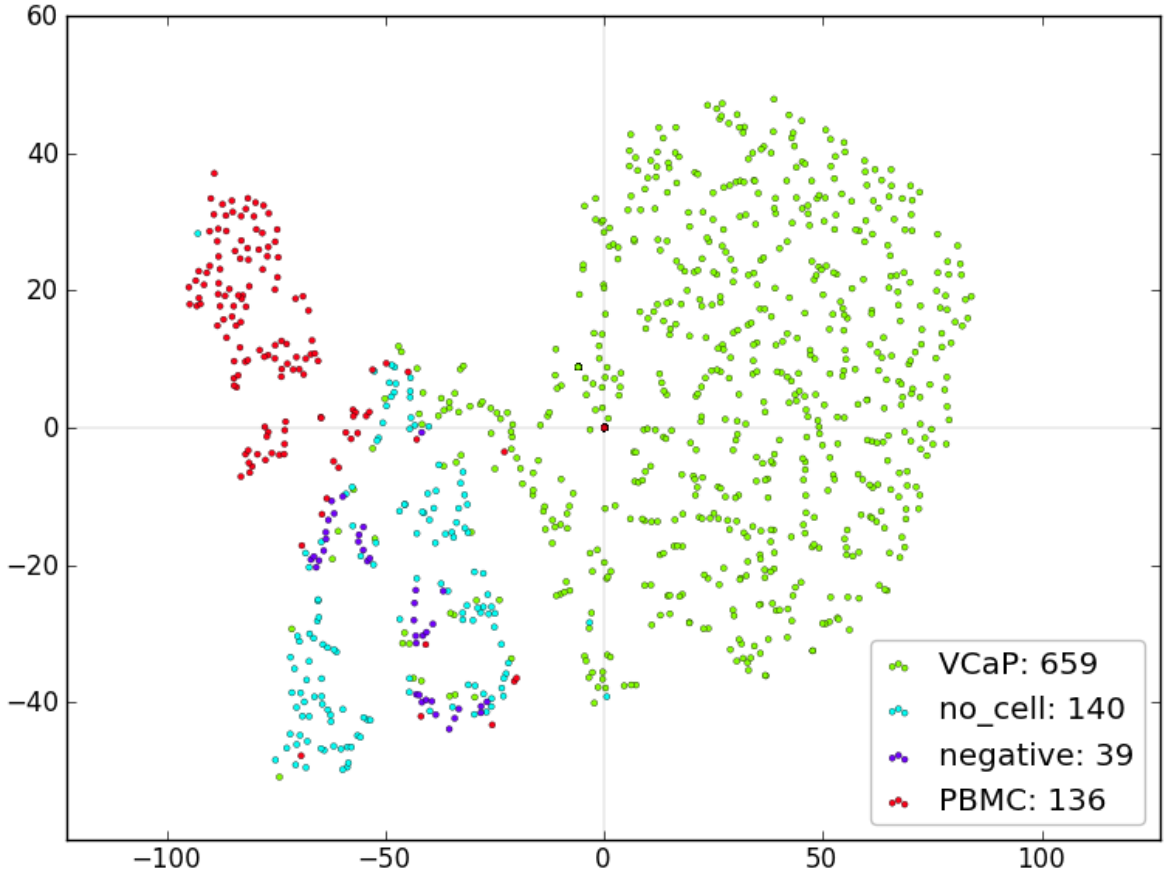
Supervised machine learning – create training set



6) Data visualization

- Dimension reduction – t-SNE plot

Supervised machine learning count



Manual count

Comparison to manual analysis:

- cy3 negative = VCaP cell
- cy3 positive = PBMC

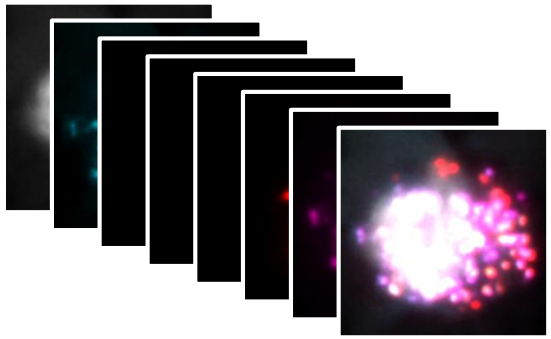
Cell count

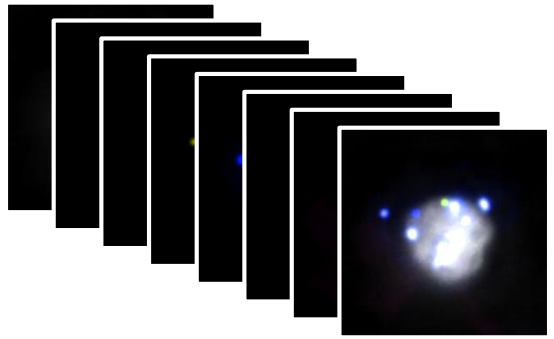
VCaP:	661 (cy3-)
No Cell:	Not counted manualy
Negative= No RCP	118
PBMC:	138 (cy3+ only)

Workflow for *in situ* padlock probe (PLP) analysis + machine learning classification

Multiplex *in situ* padlock probe assay

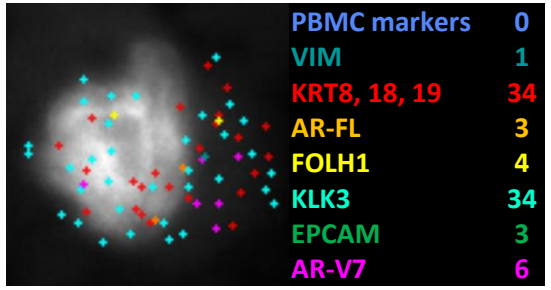
- CTC enriched patient samples (SmartBiopsy Cell Isolator)
- Visualize mRNA markers as fluorescent spots
- Panel of PBMC- and CTC-markers and *VIM*

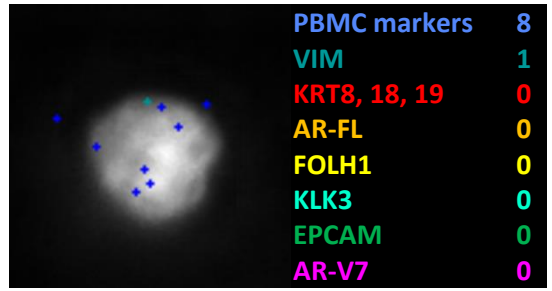




CellProfiler: image analysis

- Signal detection, decoding, and feature extraction





CellProfiler Analyst:

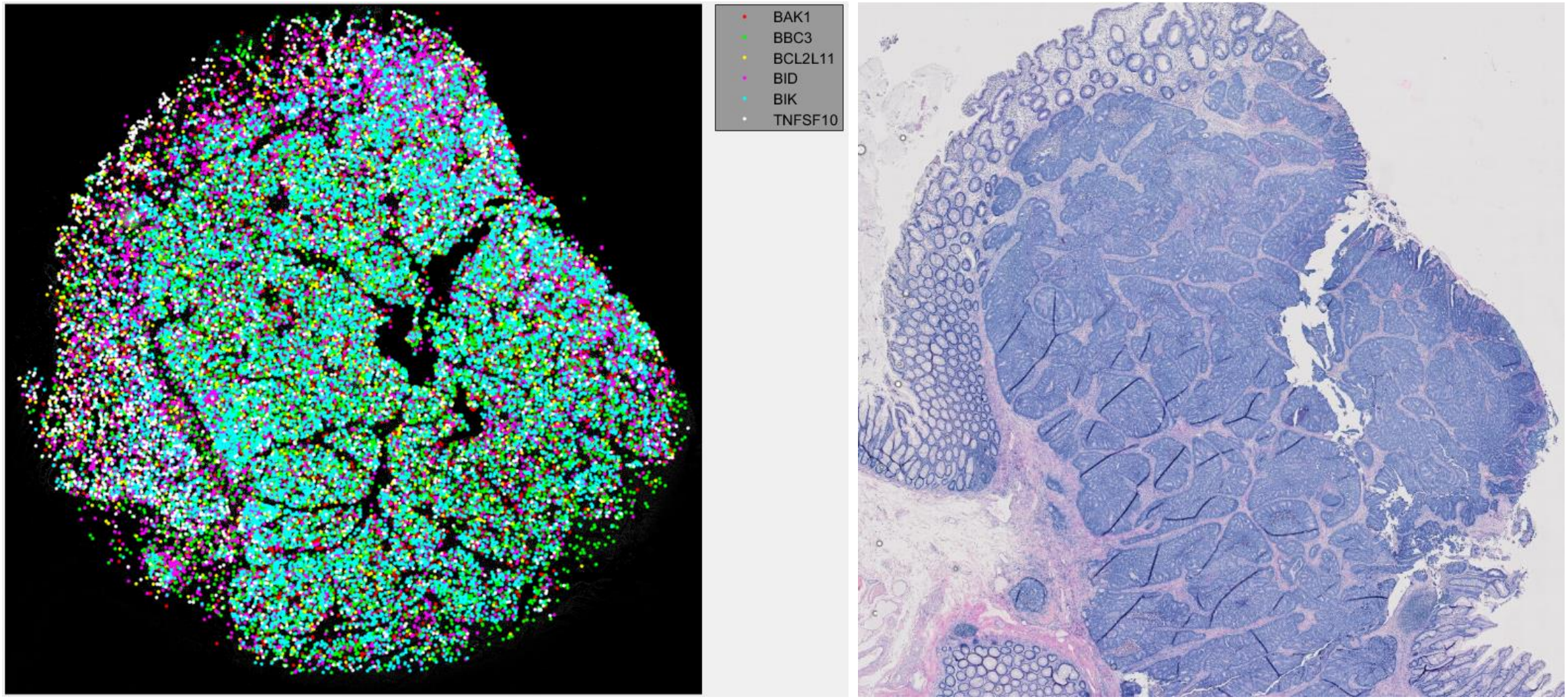
- supervised machine learning-based classification

CTC

PBMC

Expert revision of candidate cells

Pro-apoptotic



El-Heliebi Amin
Do not post, unpublished data

Adapted from Sallinger, El-Heliebi et al., poster presentation ACTC 2021
Gyllborg et al., Nucleic Acid Research 2020

Summary

- + In situ padlock probes can be used to detect any expressed transcript
  - = visualization of RNA
- + Can be quantified
- + Can be used instead of unspecific antibodies
- - Very low expressed transcripts are challenging

Research Group El-Heliebi

Collaboration partners