Introduction
The digital pathology group at CBmed is focused on providing quantitative analysis of biomarkers via a combination of multiplex immunohistochemistry and image analysis in cancer and inflammatory diseases. Through in-situ phenotyping of immune cell populations and analysis of the spatial relationship between cell types, digital pathology brings added value to target discovery and patient stratification projects.

Digitalization
Multiplex IHC enables the detection of several biomarkers on a single section. Digitalization of multispectral images is performed with the Vectra automated quantitative pathology imaging system (PerkinElmer) (Fig. 1). To accurately differentiate the multiple signals, a spectral unmixing process is performed (Fig. 2).

Image Analysis
Tissue segmentation allows for automated separation of tissue compartment such as tumor and stroma (Fig. 3), or recognition of islets (Fig. 4).

Identification of cell components (nucleus, cytoplasm, membrane) facilitates the quantitative analysis of marker intensity on a single-cell level (Fig. 5a-b).

Machine learning-based phenotyping is achieved by detection of biomarker co-expression on a single cell and allows analysis based on cell type rather than single biomarkers (Fig. 6).

Bioinformatics
Custom algorithms are used for spatial analysis of the image analysis data (Fig. 8-9).

- Clustering of cell groups (e.g. tumors, beta cells)
- Distance calculations: cell-to-cell or cell-to-cluster (e.g. immune cells)
- Cell density calculation (e.g. heat maps)
- Visualization of large data sets (e.g. Spotfire)

These useful tools allow the identification of potential correlations in a disease.

Conclusion
Computer-assisted digital pathology enables single-cell level quantification of biomarker expression and the generation of advanced spatial analysis algorithms, which have great potential as prognostic and predictive assays.

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