**DIAPath** has developed a wide range of computer tools for the quantification of immunostaining in human and animal tissue samples by means of digital image analysis. This approach provides morphological controls, taking into account antigen location at histological and cell levels, and thus avoids problems due to cell and tissue heterogeneity. It also furnishes objective measurements of antigen expression in contrast to visual scoring systems. This constitutes a promising approach for the characterization and validation of tissue-based biomarkers useful for diagnostic, prognostic and/or therapeutic purposes.

**EXTRA-CELLULAR MATRIX**

The quantification of the extra-cellular matrix components in histological tissues provides a strong clinical indicator in various diseases such as chronic hepatitis, cirrhosis, lung fibrosis or osseocartilaginous disorders.

**CELL PROLIFERATION INDEX**

The proliferation index represents the proportion of cells that are dividing. Its quantification in the some tumor types provides a prognostic information.

**INFLAMMATORY MICROENVIRONMENT**

The quantification of the labelling index of inflammatory cells (number or area of inflammatory cells per mm² of tissue) is mainly used to characterize the inflammatory reaction status.

**CANCER BIOMARKERS**

Cancer biomarkers aid in the classification of neoplasms as well as in the choice of therapeutic decisions.

**Figure 1:** Automated segmentation of collagen fibers in a mouse liver tissue stained with Goldner’s trichrome. (A) Goldner’s trichrome staining highlighting collagen fibers in green and liver cells in purple. (B) Image showing the detection of collagen fibers (green) and liver cells (pink).

**Figure 2:** Automated segmentation of collagen fibers in a mouse liver tissue stained with picrosirius. (A) Picrosirius staining highlighting collagen fibers in red and liver cells in purple. (B) Image obtained after the automated detection of collagen fibers (red) and liver cells (yellow).

**Figure 3:** Automated segmentation of basal lamina in a mouse skin tissue. (A) Immunolabelling of collagen fibers type VII (brown), (B) Image obtained after the automated detection of collagen fibers (Burgundy).

**Figure 4:** Automated segmentation of nuclei. (A) Nuclei of proliferating tumor cells are immunolabeled using an antibody targeting Ki67 (brown) while nuclei of non proliferating cells are counterstained with hematoxylin (blue). (B) Image showing the detection of the nuclei of proliferating cells (burgundy), the non proliferating cell nuclei (dark blue) and the other tissue components (light blue).

**Figure 5:** Automated segmentation of inflammatory cells expressing Foxp3 (A) Immunolabelling of Foxp3-positive T lymphocytes (brown). (B) Computer-assisted detection of Foxp3-positive T lymphocytes (burgundy).

**Figure 6:** Automated segmentation of TTF-1, a marker used to aid in the classifications of neoplasms. (A) Nuclear TTF-1 immunolabelling in a case of renal adenocarcinoma sample (brown). (B) Image obtained after the automated detection of TTF-1 positive nuclei (burgundy).
PROXIMITY LIGATION ASSAY (PLA)
PLA enables to detect, quantify and determine cell localization of complexes of proteins (with potential interaction) in histological tissue sections.

Figure 7: Automated segmentation of spots corresponding to protein complexes after z-stacking (i.e. merging an image stack). (A) Proteins highlighted using the proximity ligation assay procedure (brown spots). (B) Image showing the spot s (burgundy), the cell nuclei (dark blue) and the other tissue components (light blue).

VIRTUAL NORMALIZATION OF IHC STAINING
A method for image normalization has been developed by DIAPath for providing coherent quantitative characterization of IHC biomarkers across different batches of IHC.

Figure 9: Illustration of image normalization. (A and B) Images of CD3 IHC slides acquired from 2 IHC batches using different antibody and reagent lots. (C) Normalization of image B taking into account the first IHC batch (image A) as reference.

IN SITU HYBRIDIZATION (ISH)
ISH enables to detect, quantify and determine cell localization of single-stranded DNA or RNA in histological tissue sections.

Figure 8: Automated segmentation of spots corresponding to mRNA after z-stacking (i.e. merging an image stack. (A) The presence of mRNA is highlighted using the in situ hybridization procedure (brown spots). (B) Binary image showing the detection of brown spots (burgundy), the nuclei non proliferating cells (dark blue) and both the cytoplasm of cells and the extracellular matrix (light blue).

COLOCALIZATION OF PROTEIN EXPRESSION
DIAPath has developed specific techniques to colocalize different IHC or (special) stains carried out on the same tissue slide and/or on serial tissue slides.

Figure 10: IHC staining colocalization on serial TMA slides, showing (A) IGF-I and (B) IGFBP2 IHC expression in colonic tissues, to compute staining (C) overlap map and index ($r_o$). In the map blue indicates no overlap whereas yellow to red show increasing overlap levels.