Fraunhofer IIS

MICAIA

Quantitative Analysis of High-Plex Immunofluorescent Whole-Slides

Immunofluorescently stained whole-slide images – especially high-plex ones with five, ten or even more markers – contain a vast density of cell-level information. It is a challenge to decipher this information and derive actionable insights. We have developed a set of tools that help solving this challenge. They are incorporated into in the MICAIA image analysis software .

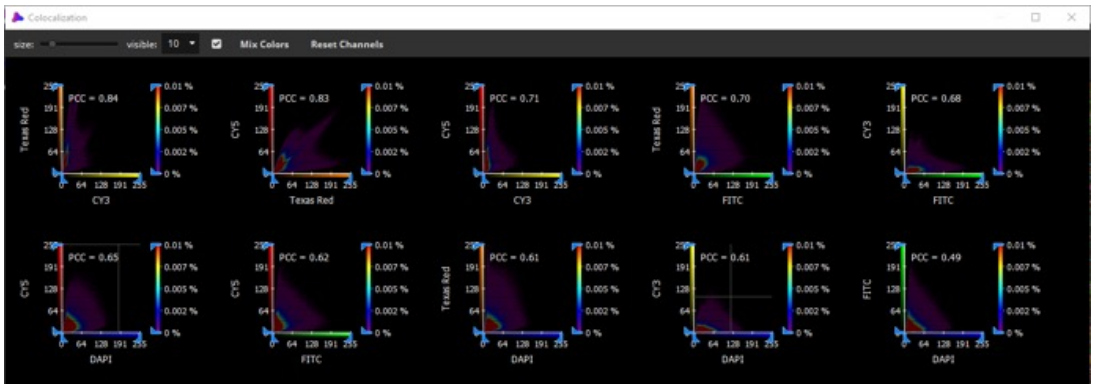
Fast Cross Channel Correlation Analysis

At first, the correlation between any two channels is computed (Pearson Correlation Coefficient, PCC) and displayed in a confusion matric.

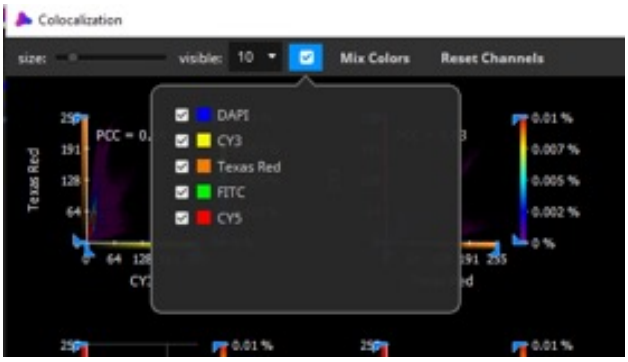
Pearson Correlation Channel Matrix

	DAPI	CY3	Texas Red	FITC	CY5
DAPI		0.61	0.61	0.49	0.65
CY3	0.61		0.84	0.68	0.71
Texas Red	0.61	0.84		0.70	0.83
FITC	0.49	0.68	0.70		0.62
CY5	0.65	0.71	0.83	0.62	

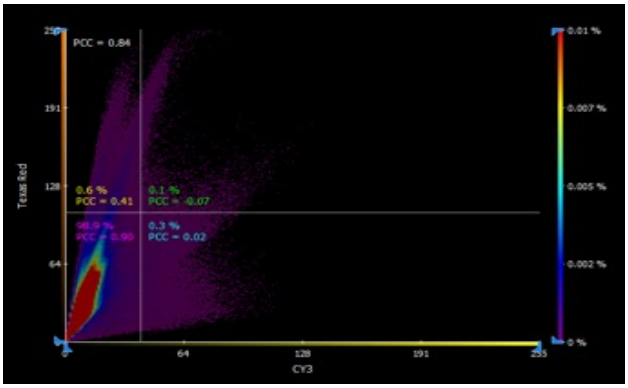
Each such combination is additionally visualized using an interactive scatter plot. The channel combinations are automatically sorted by descending correlation, facilitating fast inspection of the top N correlating combinations. Since the correlation is computed per pixel on a downscaled proxy of the whole-slide and does not involve segmentation of cells, this information is available within seconds.



Alternatively, the set of displayed plots can be constrained to contain only combinations with participation of a given marker type.

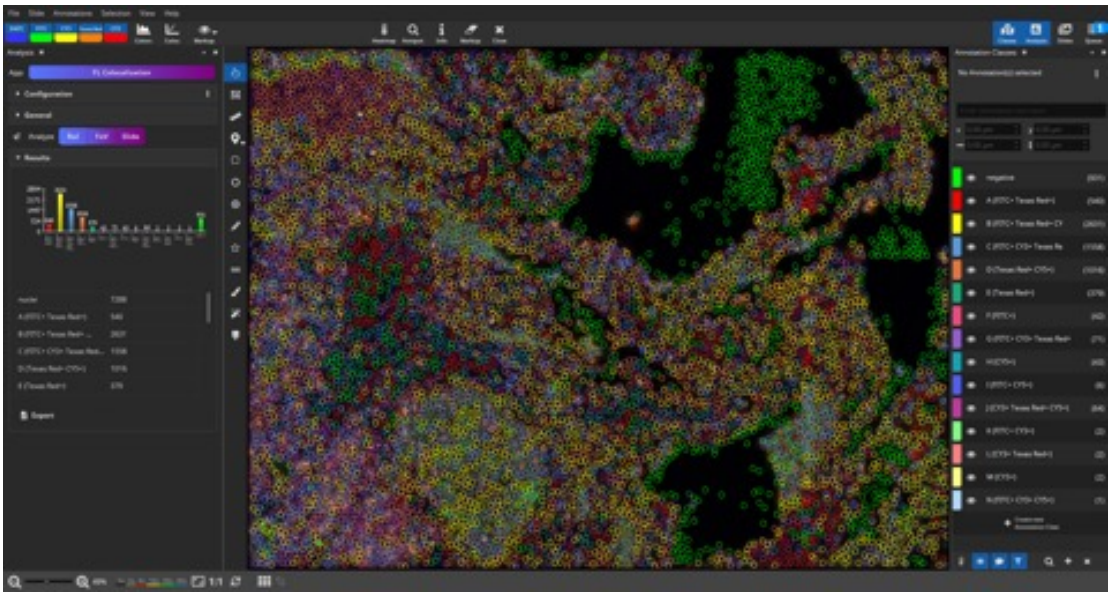


A single scatter plot allows defining quadrants or even a custom rectangular value range and will compute the correlation of each such value range. The axes can be interactively constrained and the heatmap sensitivity adjusted.



Cell-based Colocalization Analysis

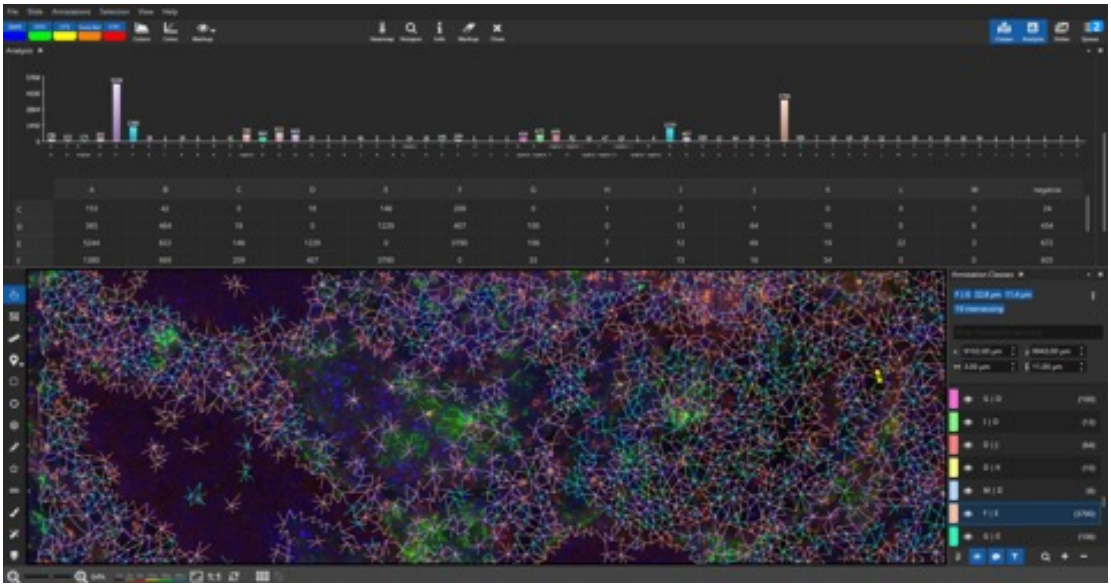
For a cell-based colocalization analysis, an image analysis App has been created. The user selects which fluorescent channel marks all cells, e.g. typically the DAPI marker. This channel is then analyzed first and cells are identified. At each cell center, a circle of a user-defined diameter in µm is created. Then, each additional marker is evaluated inside the circle according to one or two criteria: 1) whether the mean intensity exceeds a user-defined threshold and optionally also 2) whether the ratio of pixels in the circle exceed another user-defined threshold. This way, it is decided for each marker whether it is expressed or not by the cell. Each occurring combination yields a cell phenotype, which is visualized as a markup class and assigned a custom color. Classes are denoted, for example, "A FITC+, Cy3+)", "B (Texas Red+, Cy5+)", … The user will know what specific immune cell is referred to by this class name and may optionally rename the class.



Now, the amount, location and density (in #/µm2) is known. Individual classes as well as the whole-slide-image channels can be hidden or shown. The markup layer can be displayed in a semi-transparent way and optionally, one or multiple classes can be instead visualized by a heatmap:

Cell Cell Interactions

In a next step, it may be worthwhile to gain insights on possible spatial interactions between phenotypes. The "Cell-cell interactions" App interprets cells as nodes in a graph and connects each cell with its nearest neighbors. A connection (edge) between a cell of type A with a cell of type B is denoted an A-B connection. Each occurring combination is again visualized as a markup class in the UI.

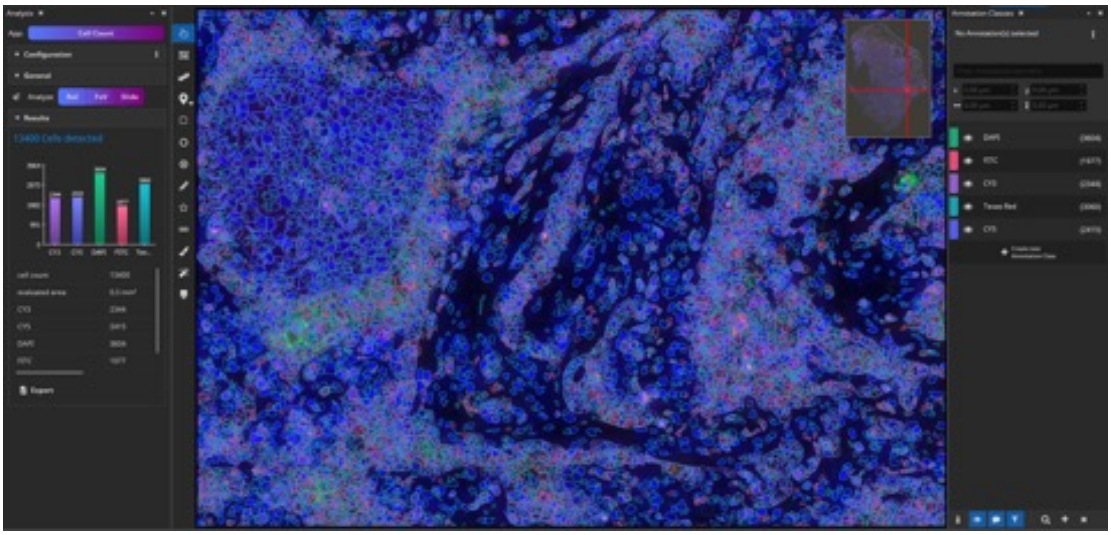


By default, connections between cells of the same phenotype will not be created, though this behavior can be changed. Optionally, long connections can also be omitted, either based on the standard deviation (default: edges with length outside of the 1σ interval are omitted) or by defining an absolute threshold in µm.

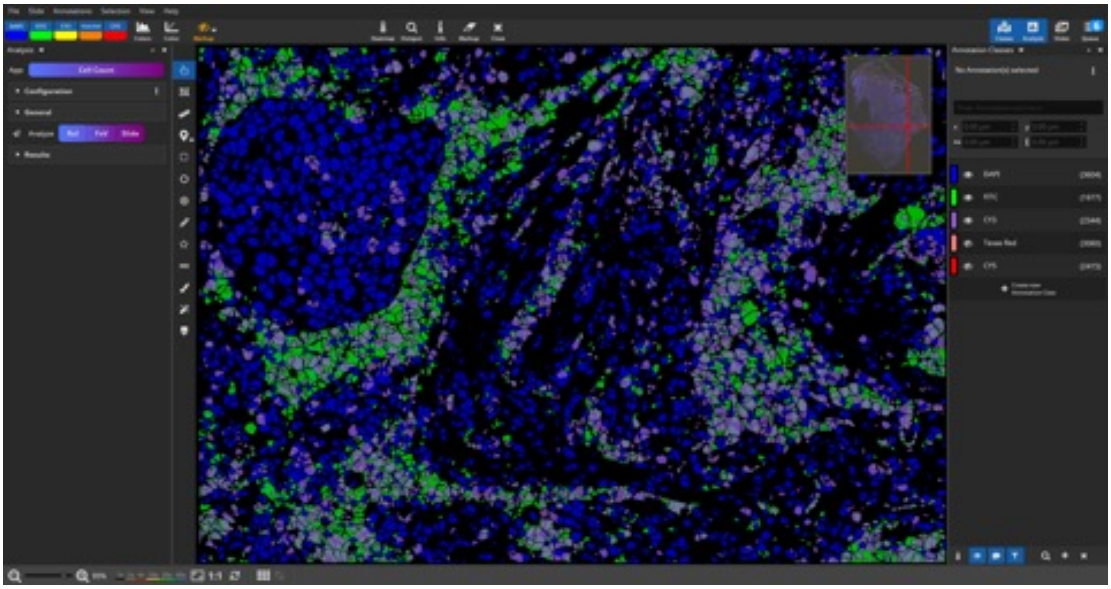
A bar chart displays, which connections occur most frequently. Again, the heatmap visualization can be used to display regions where connections of certain type occur most frequently. Connection frequencies are additionally displayed in a matrix.

Cell Shapes

So far, the cell shape has been ignored. If the shape is of interest, another app can be used which segments cells accurately. This app analyzes each channel independently.



By default, only the cell outlines are displayed, so that the image shines through. Optionally, markup layers can be set up to use a solid fill:



Each of the above Apps allows exporting the markup into GeoJson or Aperio XML, which can be opened by QuPath or Leica ImageScope. Additionally, they support exporting quantitative results to CSV. E.g. the Cell Segmentation App will create one row per cell and include metrics such as the location, area, compactness, elongation or mean intensity:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	slide_id	entity	object_id	roi	class	count	center_x_un	center_y_un	box_width_u	box_height_	area_um2	perimeter_u	circularity	convexity	intensity
2	LuCa-7color_Scan1.	AnalyzedRoi		AnalyzedRoi		43490	6358,7	3435	1341,3	997,4	1,34E+06				
3	LuCa-7color_Scan1.	class		AnalyzedRoi		43490									
4	LuCa-7color_Scan1.	SubRoi		all		43490	0	0	0	0	1,34E+06				
5	LuCa-7color_Scan1.	cell	1		CY5	1	7028,2	3859,2	0	0,5	18,35	30,5	0,25	0,98	54
6	LuCa-7color_Scan1.	cell	2		DAPI	1	7028,2	3691,4	0	0,5	19,34	32,49	0,23	0,96	4
7	LuCa-7color_Scan1.	cell	3		FITC	1	7028,2	3594,8	0	0,5	18,6	23,94	0,41	0,96	1
8	LuCa-7color_Scan1.	cell	4		Texas Red	1	7027,9	3916,2	1,5	8	11,66	16,47	0,54	0,96	100
9	LuCa-7color_Scan1.	cell	5		Texas Red	1	7027,9	3877,1	1,5	7,5	11,41	17,17	0,49	0,94	29
10	LuCa-7color_Scan1.	cell	6		CY5	1	7027.9	3703.3	1.5	6.5	10.42	14.68	0.61	1	1

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