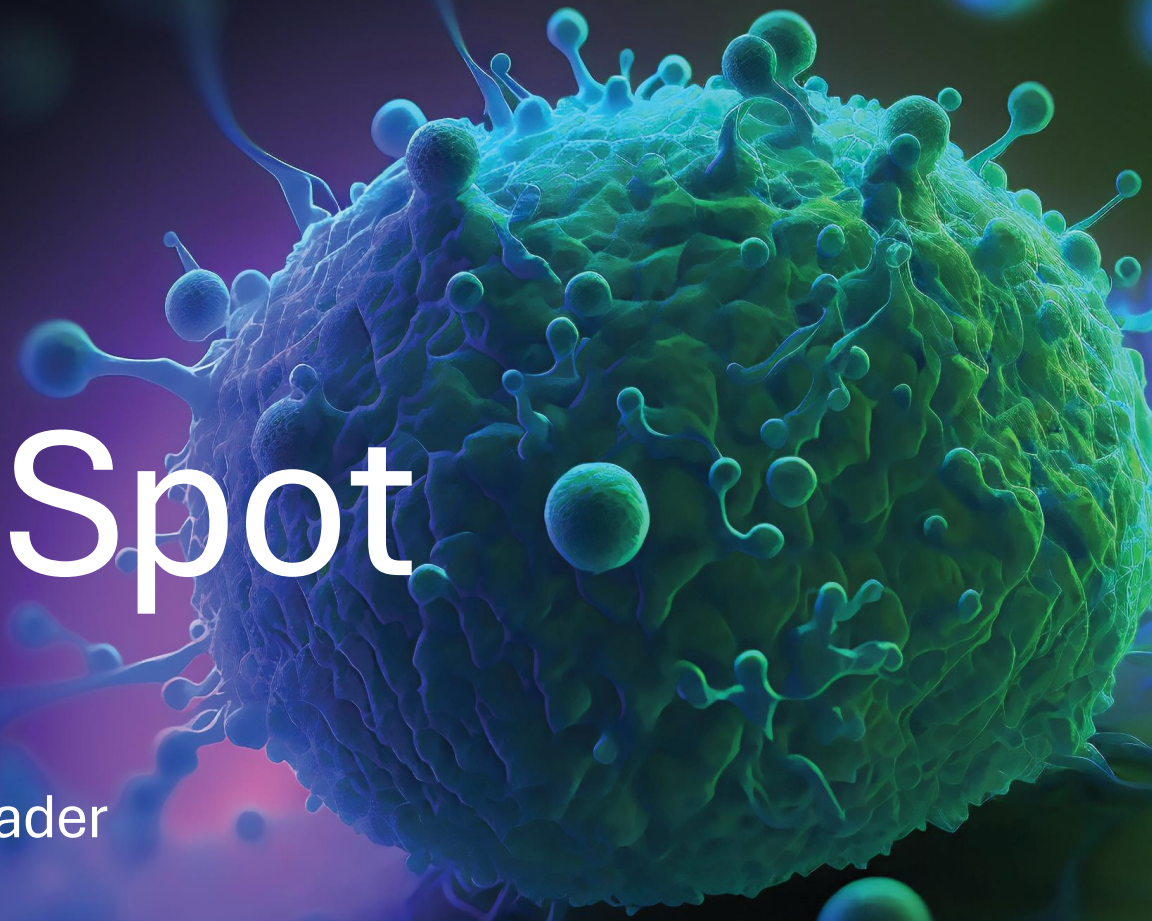
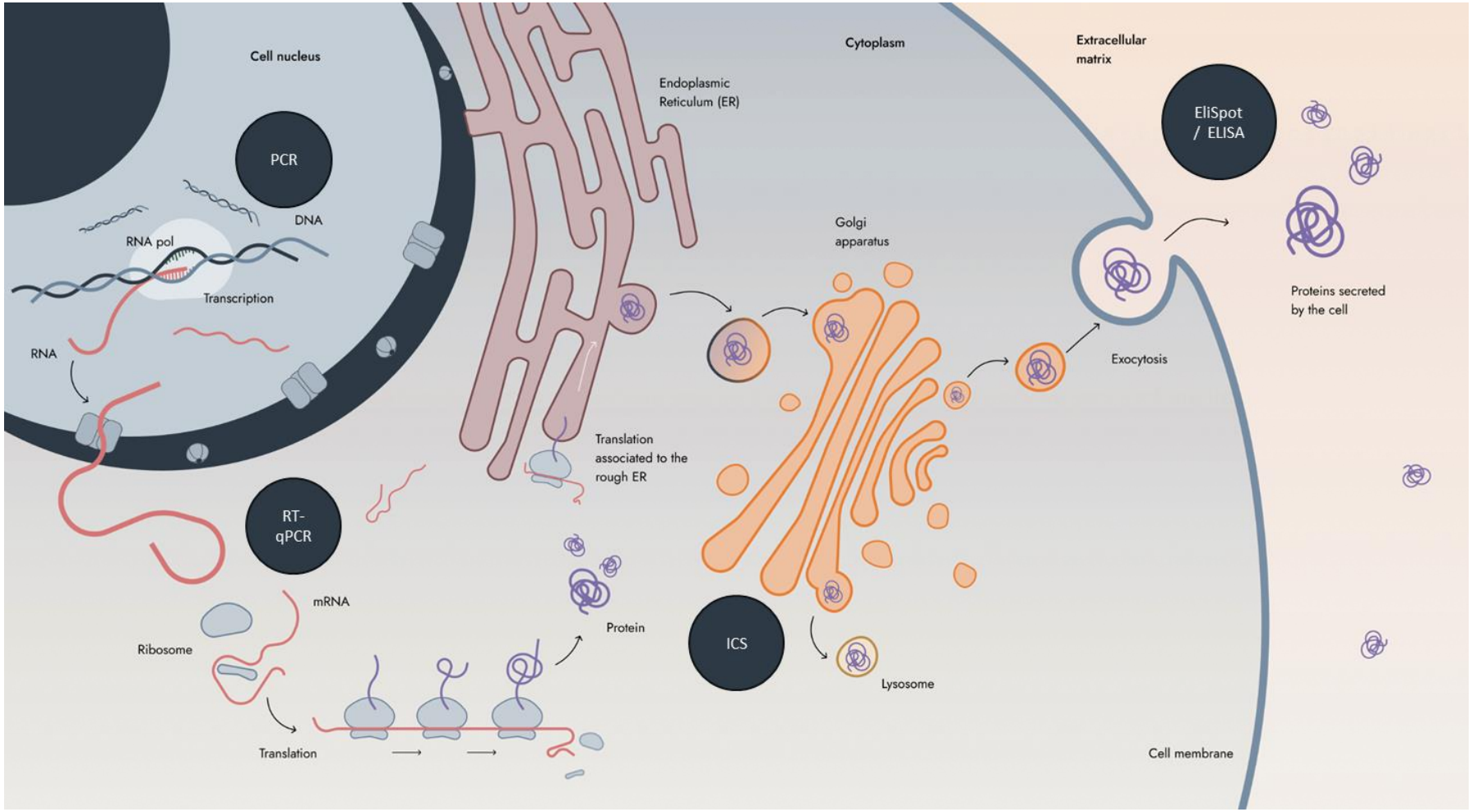
AID | Autoimmun Diagnostika

Insight EliSpot

AID - EliSpot, FluoroSpot and Reader Systems at a glance





PCR — Identifying the molecular characteristics of the cell.

RT-qPCR — Quantification of DNA, mRNA, RNA …

ICS — Frequency of cytokine producing cells.

EliSpot / ELISA — Determination of the functionality of B and T cells. With EliSpot on single cell level.

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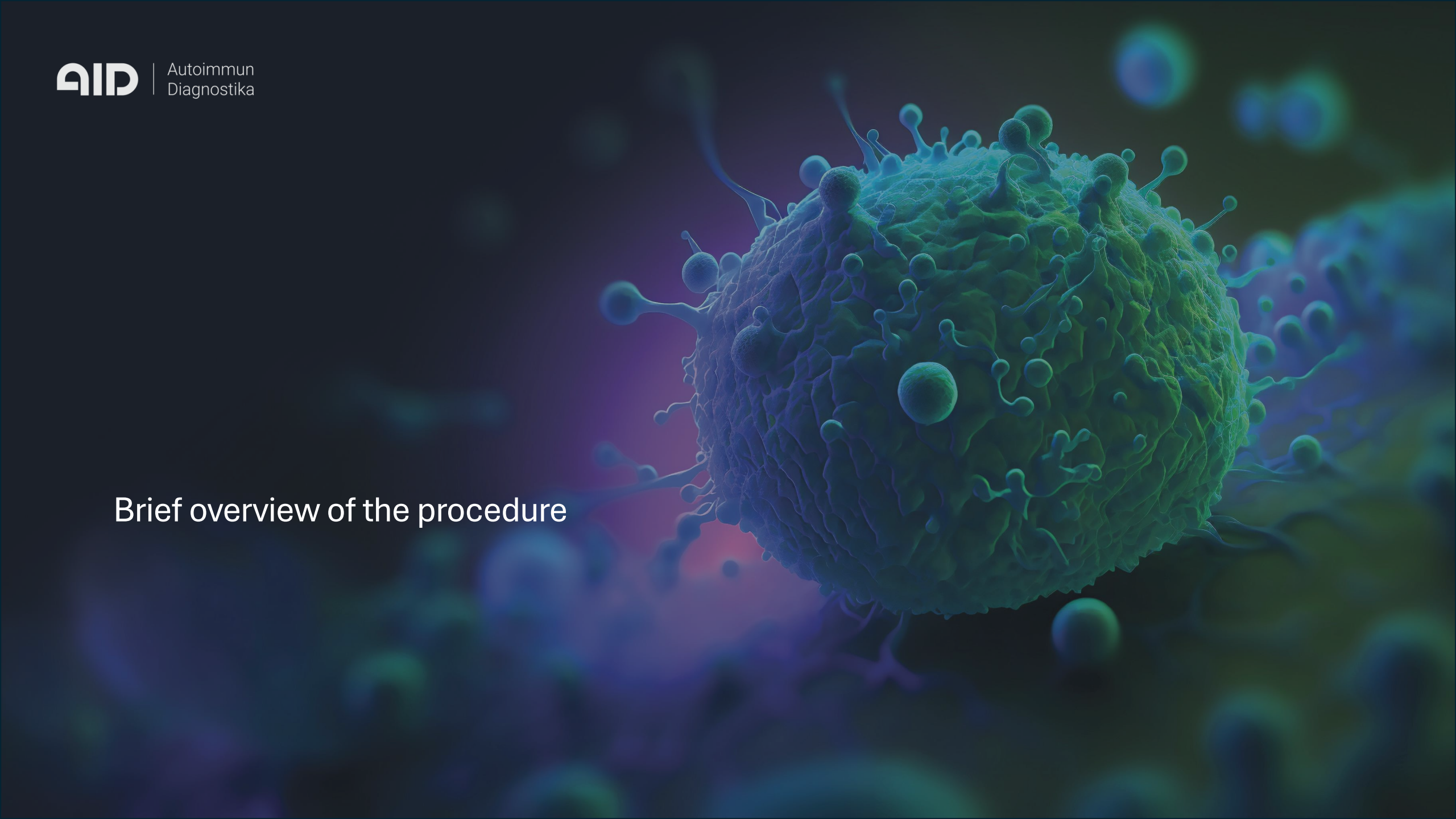
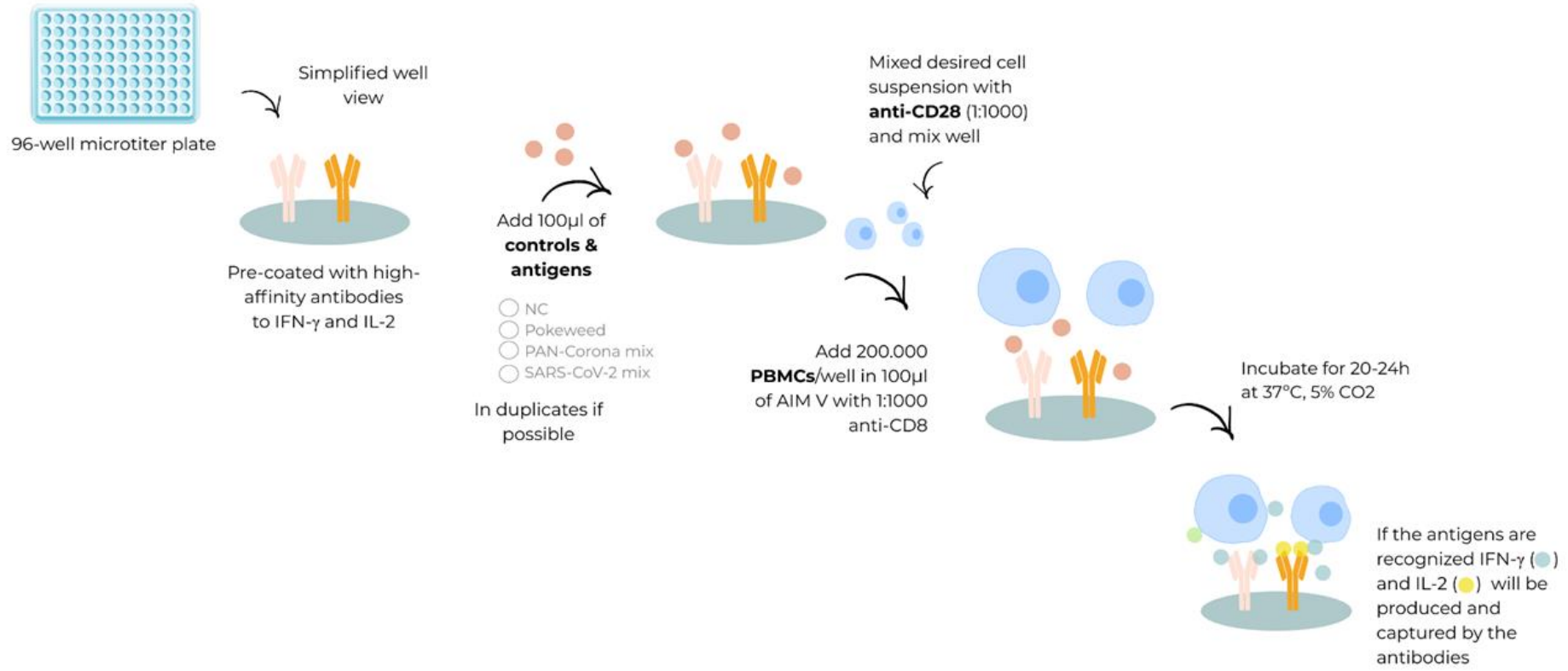


Brief overview of the procedure

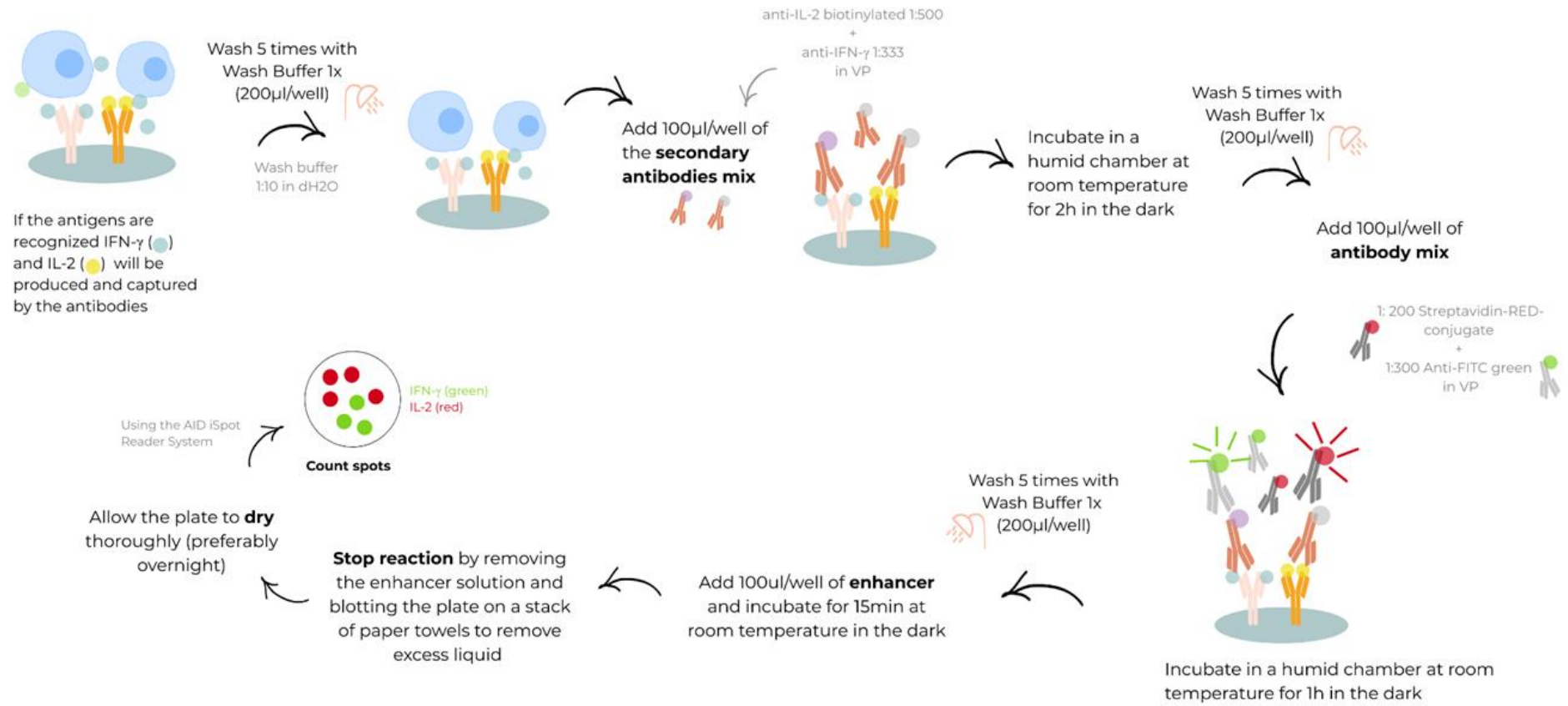
Overnight stimulation

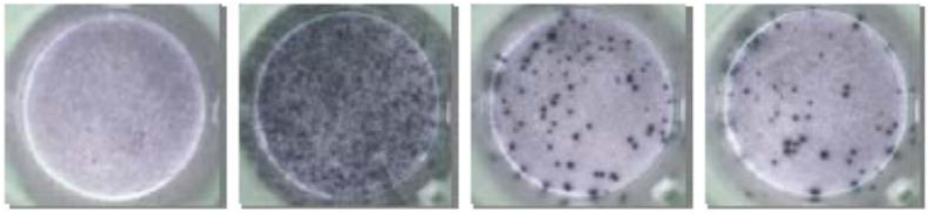
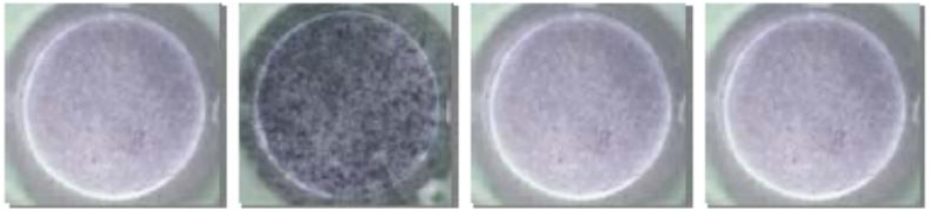
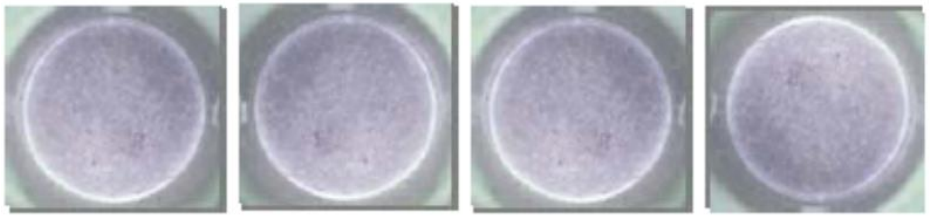
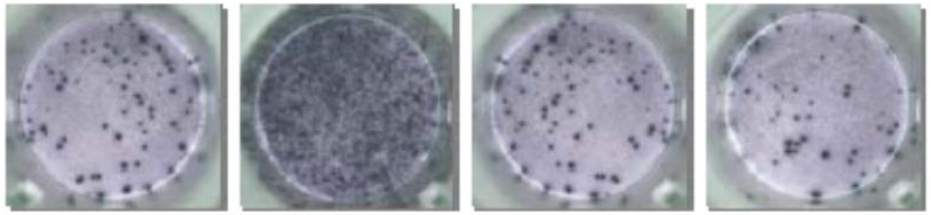
Day 1

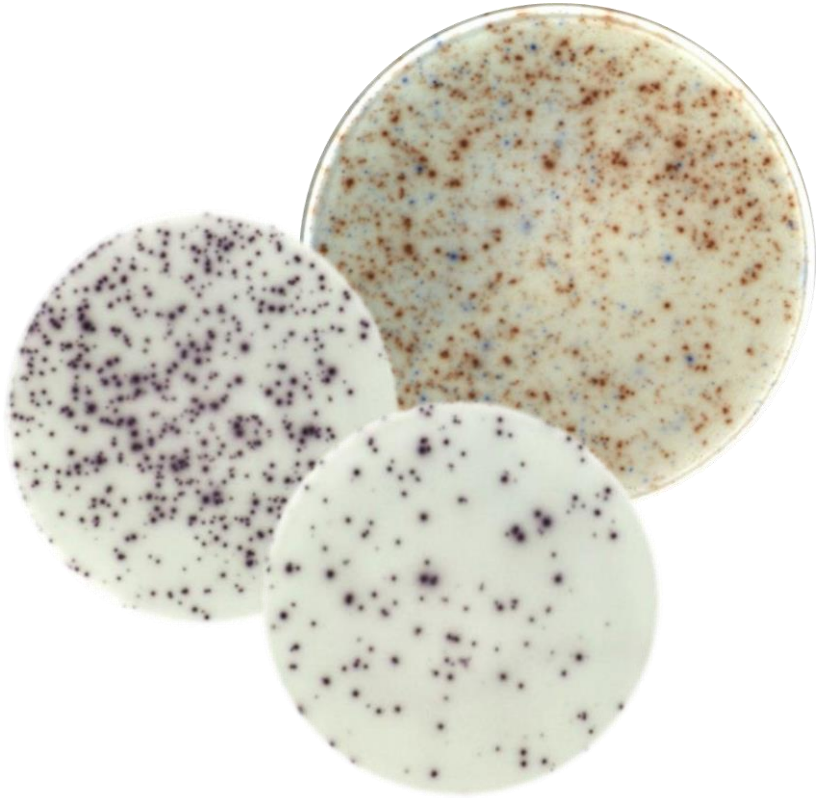


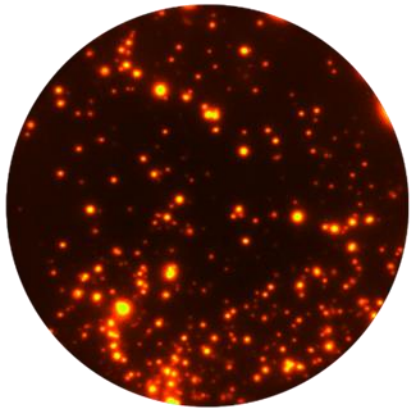
PBMCs: Peripheral Blood Mononuclear Cells

Day 2

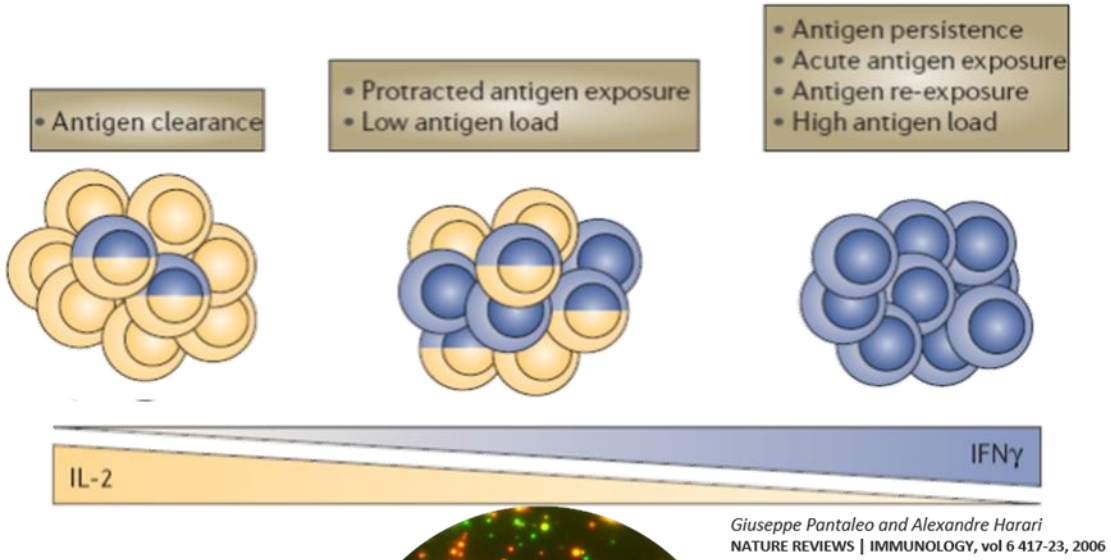

If the antigens are recognized IFN-γ and IL-2 will be produced and captured by the antibodies
Wash 5 times with Wash Buffer 1x (200µl/well)
Wash buffer 1:10 in dH2O
Add 100µl/well of the **secondary antibodies mix**
anti-IL-2 biotinylated 1:500 + anti-IFN-γ 1:333 in VP
Incubate in a humid chamber at room temperature for 2h in the dark
Wash 5 times with Wash Buffer 1x (200µl/well)
Add 100µl/well of **antibody mix**
1: 200 Streptavidin-RED-conjugate + 1:300 Anti-FITC green in VP
Incubate in a humid chamber at room temperature for 1h in the dark
Wash 5 times with Wash Buffer 1x (200µl/well)
Add 100ul/well of **enhancer** and incubate for 15min at room temperature in the dark
Stop reaction by removing the enhancer solution and blotting the plate on a stack of paper towels to remove excess liquid
Allow the plate to **dry** thoroughly (preferably overnight)
Using the AID iSpot Reader System
IFN-γ (green)
IL-2 (red)
Count spots

	Negative Control	Positive Control	Antigen 1	Antigen 2	
Donor 1					Reactive
Donor 2					Not reactive
Donor 3					Not valid
Donor 4					Not valid

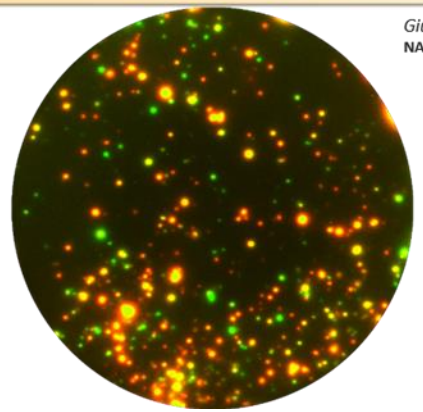




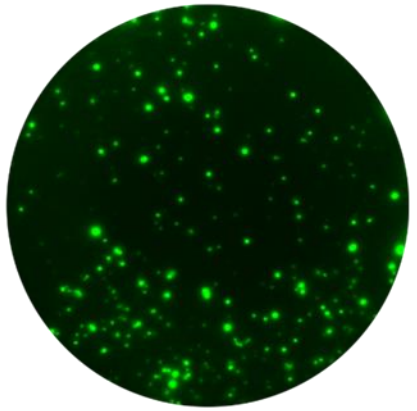
Memory T cells
Interleukin-2 EliSpot
Red fluorescence



Giuseppe Pantaleo and Alexandre Harari
NATURE REVIEWS | IMMUNOLOGY, vol 6 417-23, 2006



Double-stained
multifunctional T cells
Yellow



Activated T cells
Interferon-γ EliSpot
Green fluoresence

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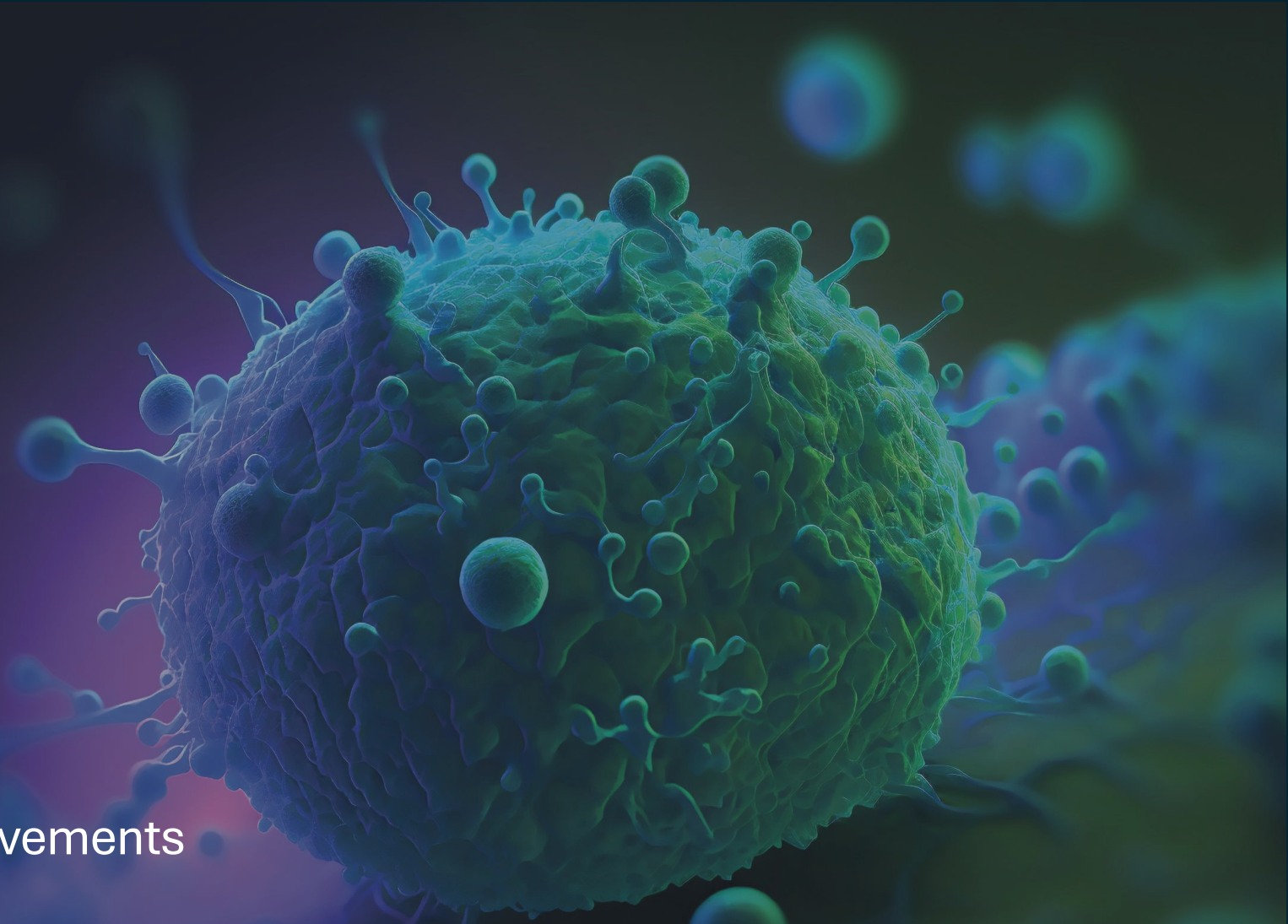
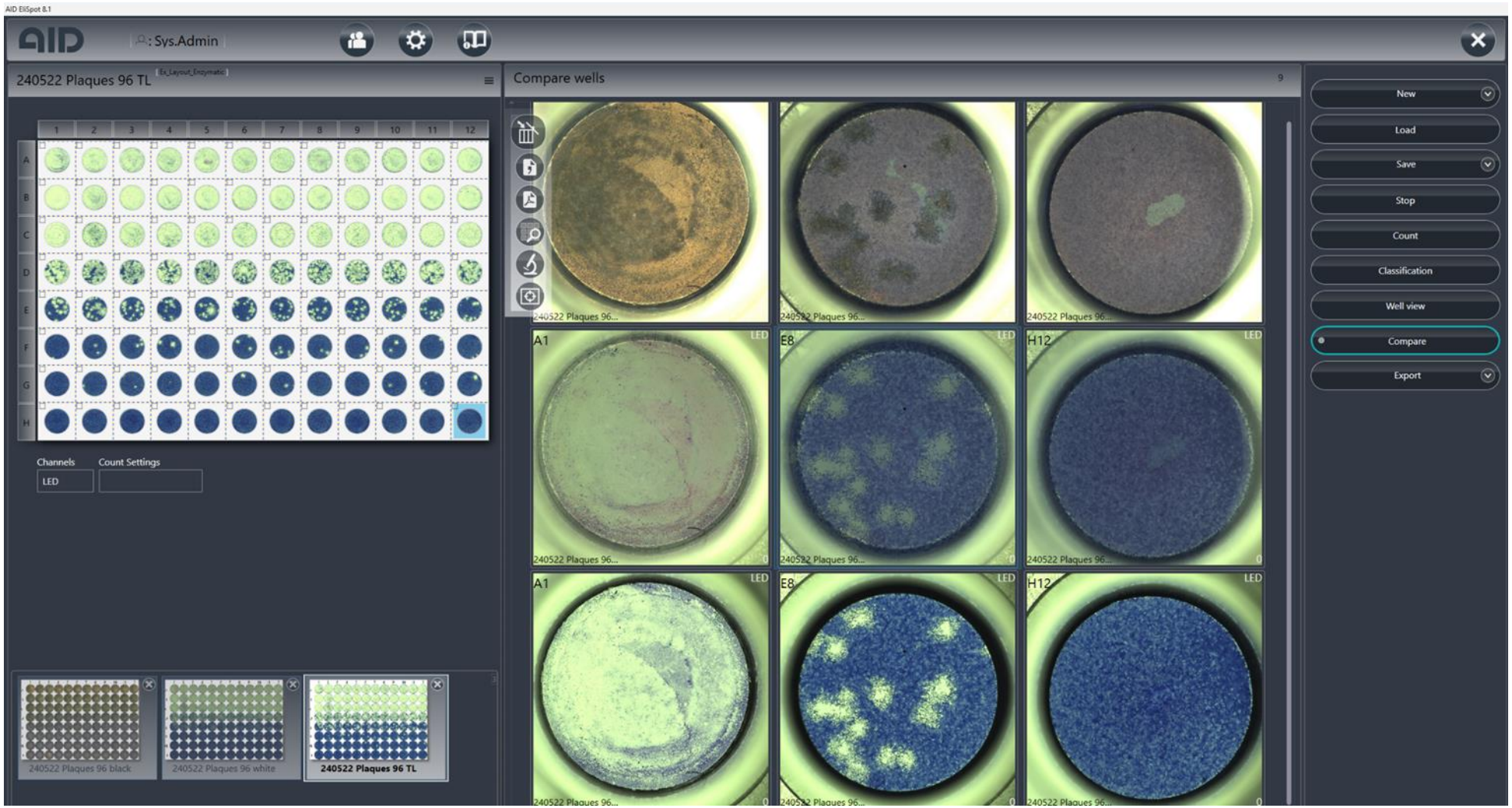
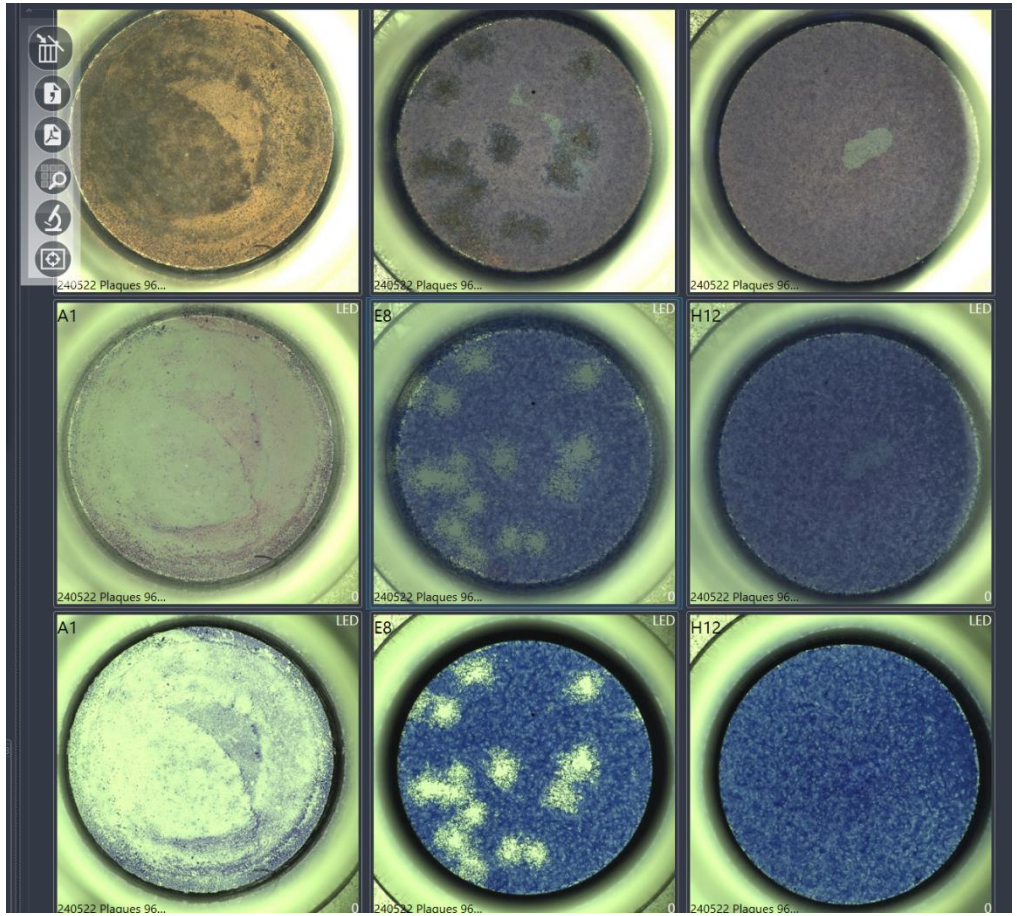


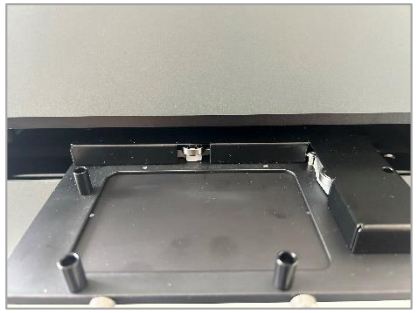
For more flexibility

New generation Hardware improvements

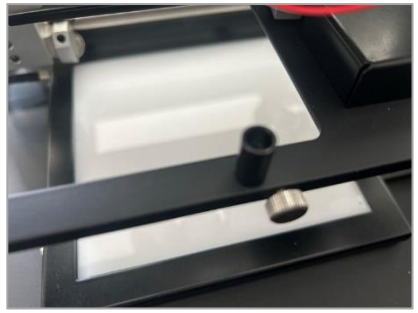


Compare different plates and read outs to show the difference



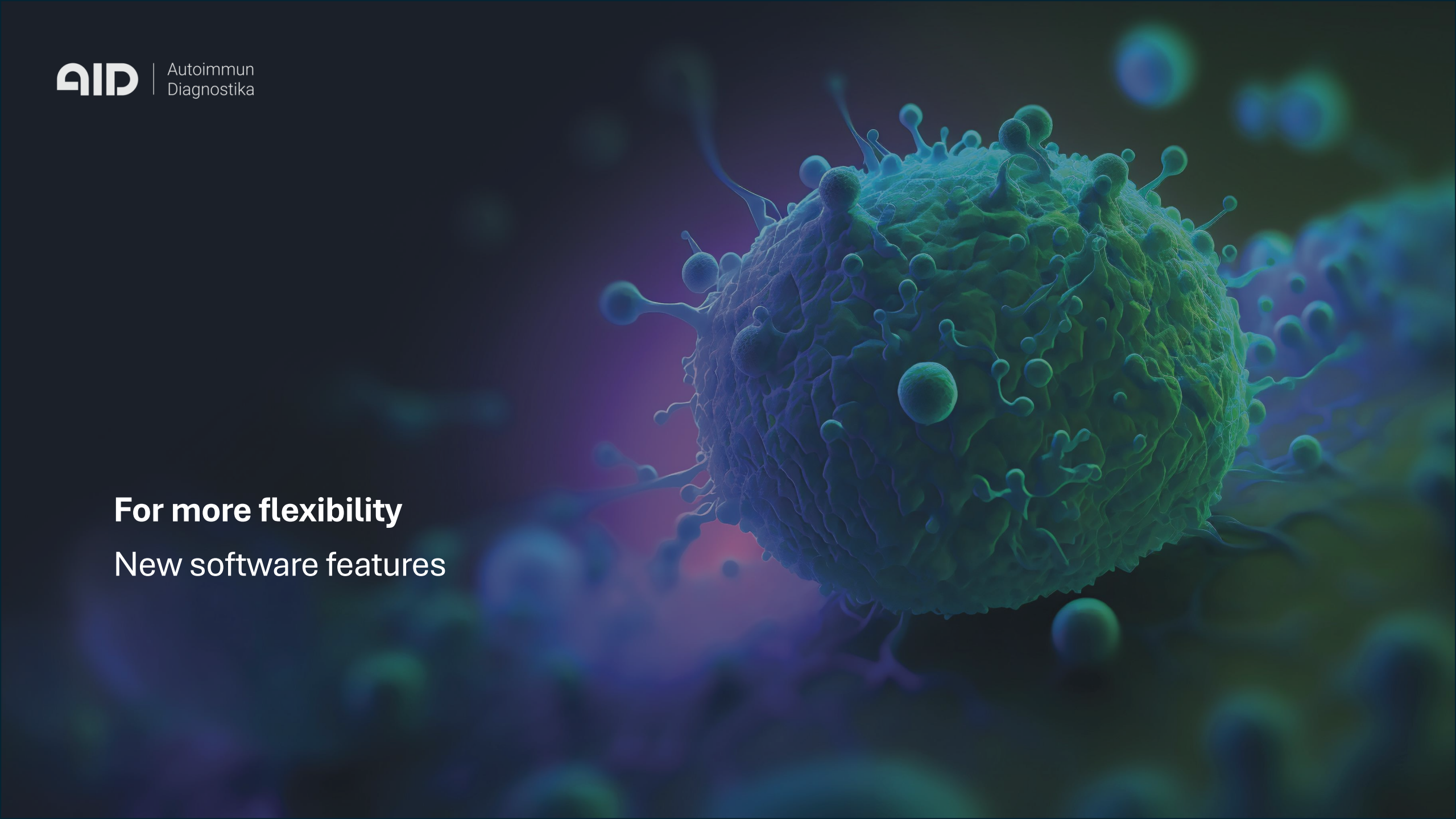


Light from above



Light from below

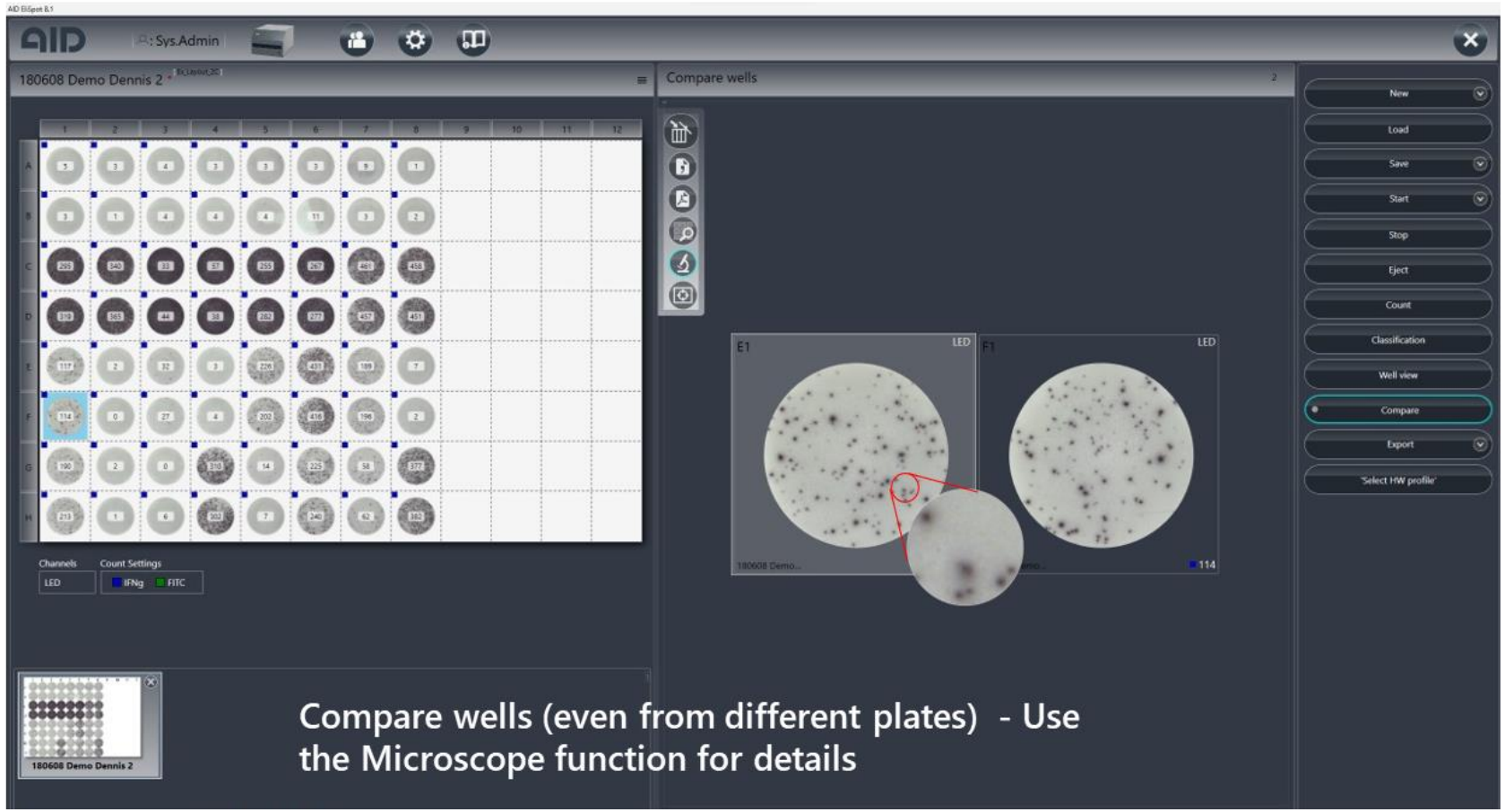
Autoimmun Diagnostika

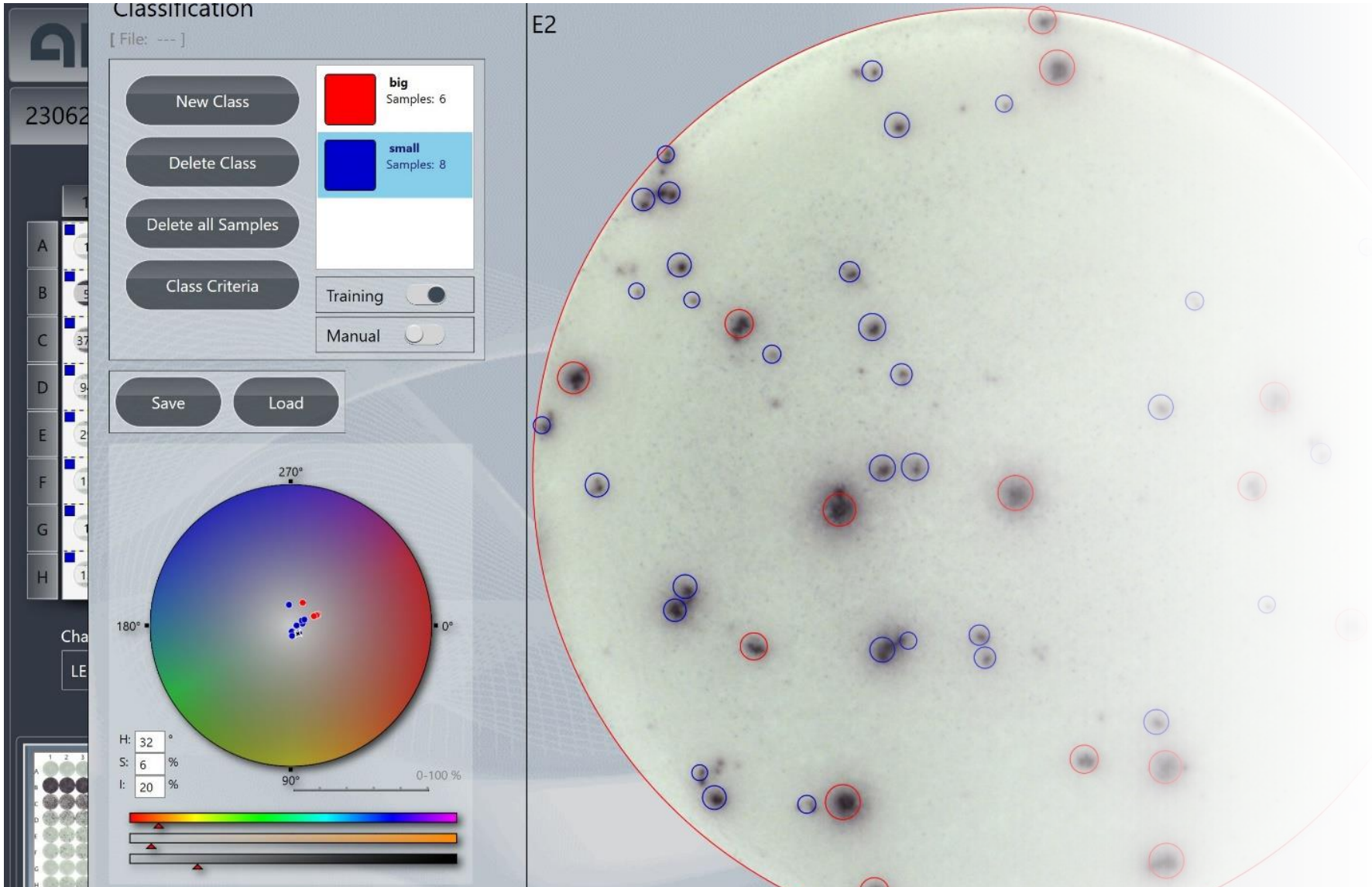


For more flexibility

New software features

Improved analysis of your enzymatic EliSpot





Classify spots according to their:

Size	Saturation
Hue	Intensity

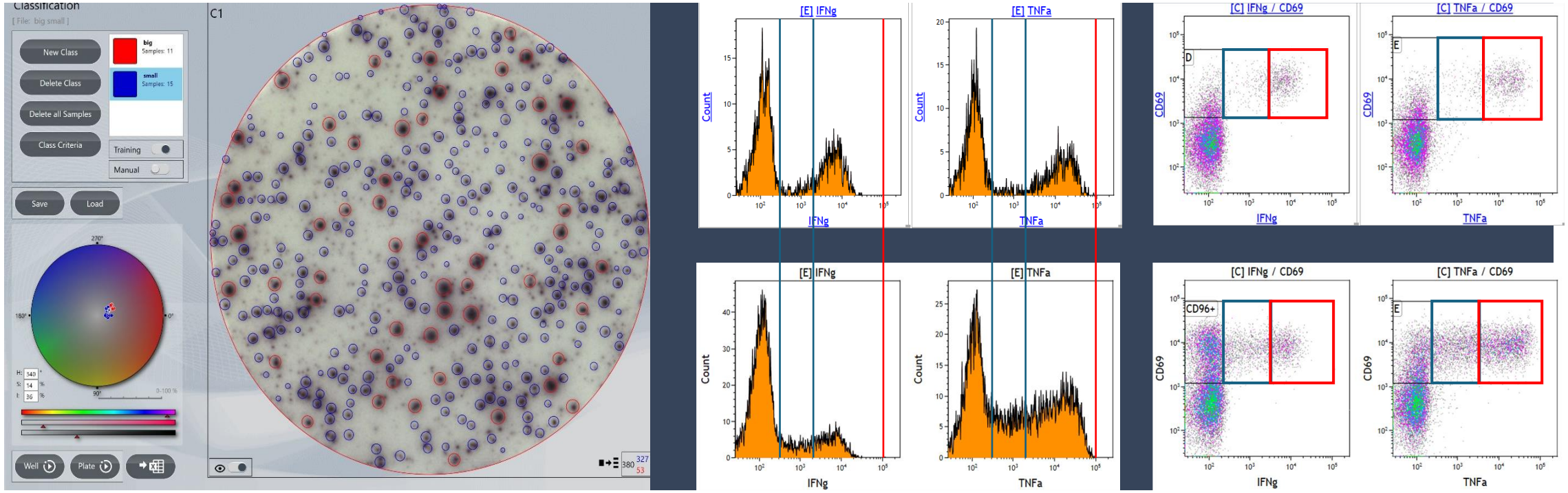
The emphasis of each parameter can be selected in a range of 0-100%.

The Software can be trained to find your spots of interest.

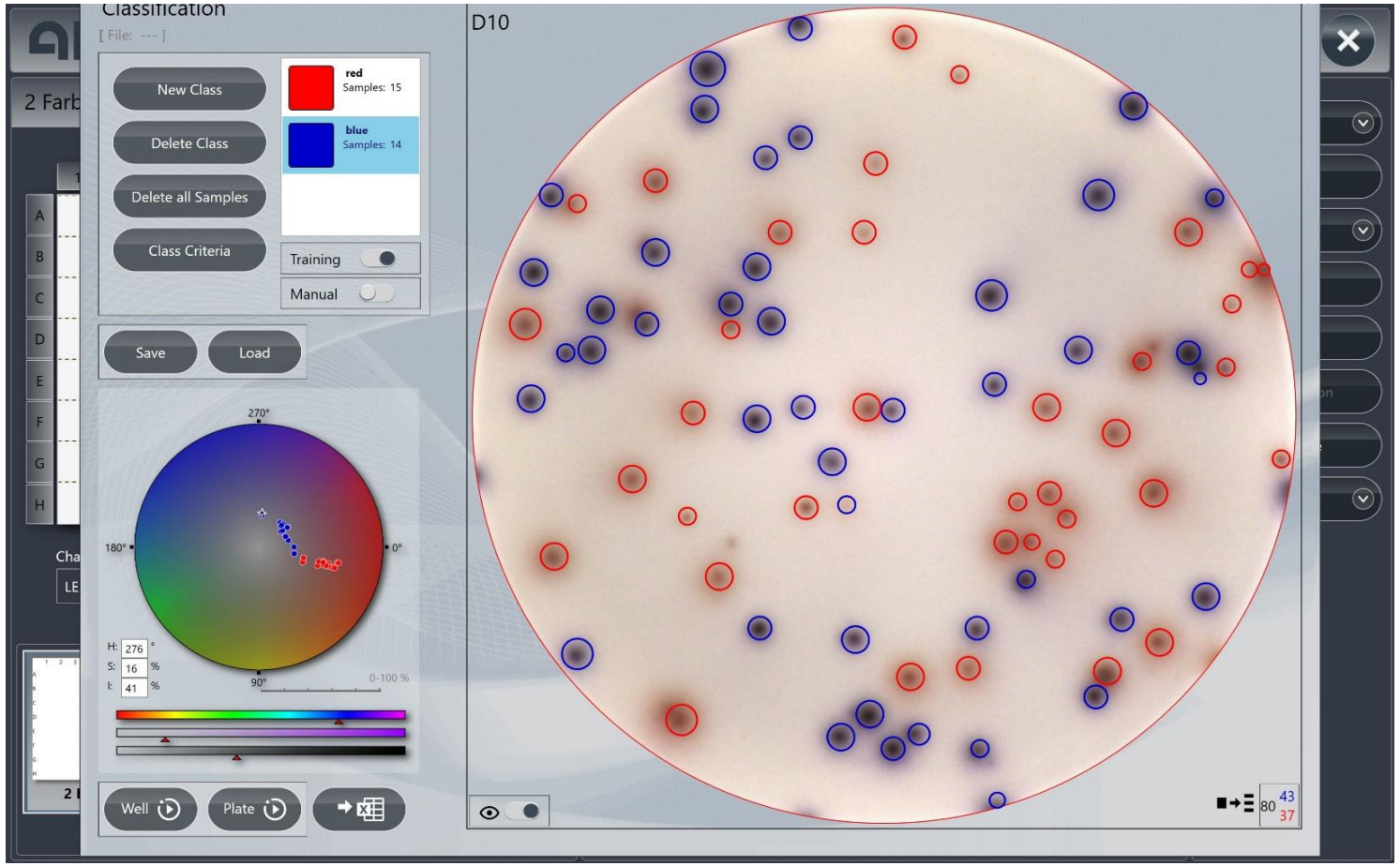
Just set up a new group and teach the software which spots should be included.

Catching up with flow cytometry

For all our customers who are used to ICS, the new Classification tool additionally allows to distinguish between big and small spots in the style of highs and dims.



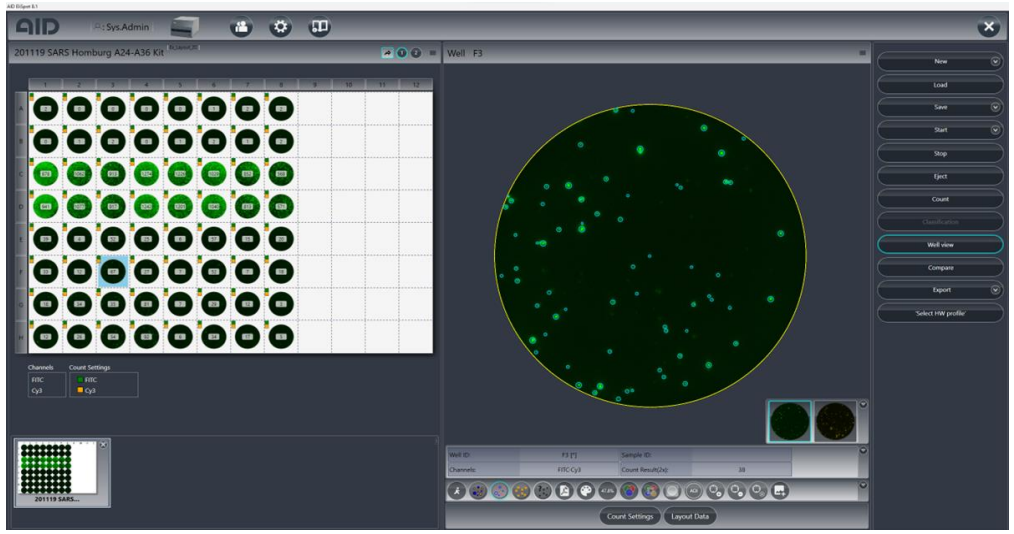
Classify spots according to their colour



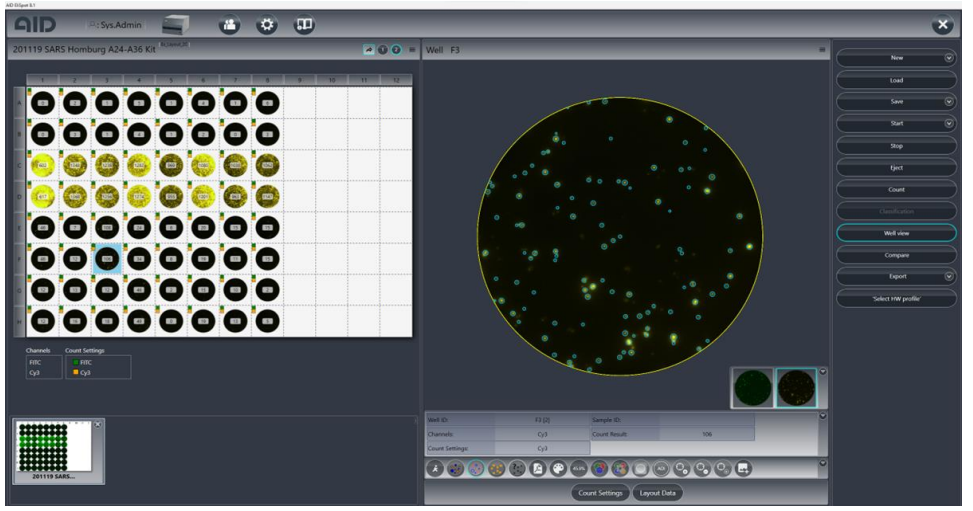
For multi cytokine analysis we generally recommend the use of fluorescent labels.

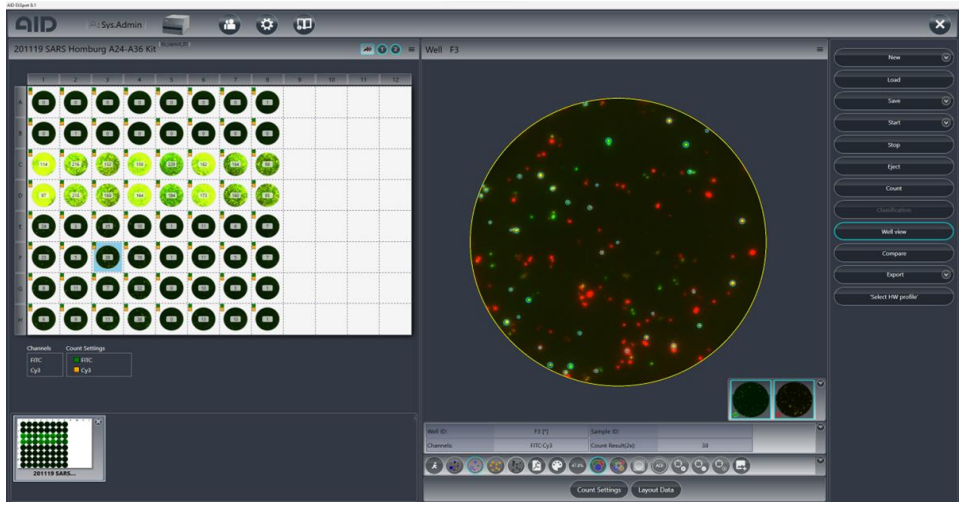
However, despite the inherent drawbacks of enzymatic two colour EliSpot Assays the AID EliSpot Reader Software v8.1 is capable of distinguishing between spots of different colours.

Green channel



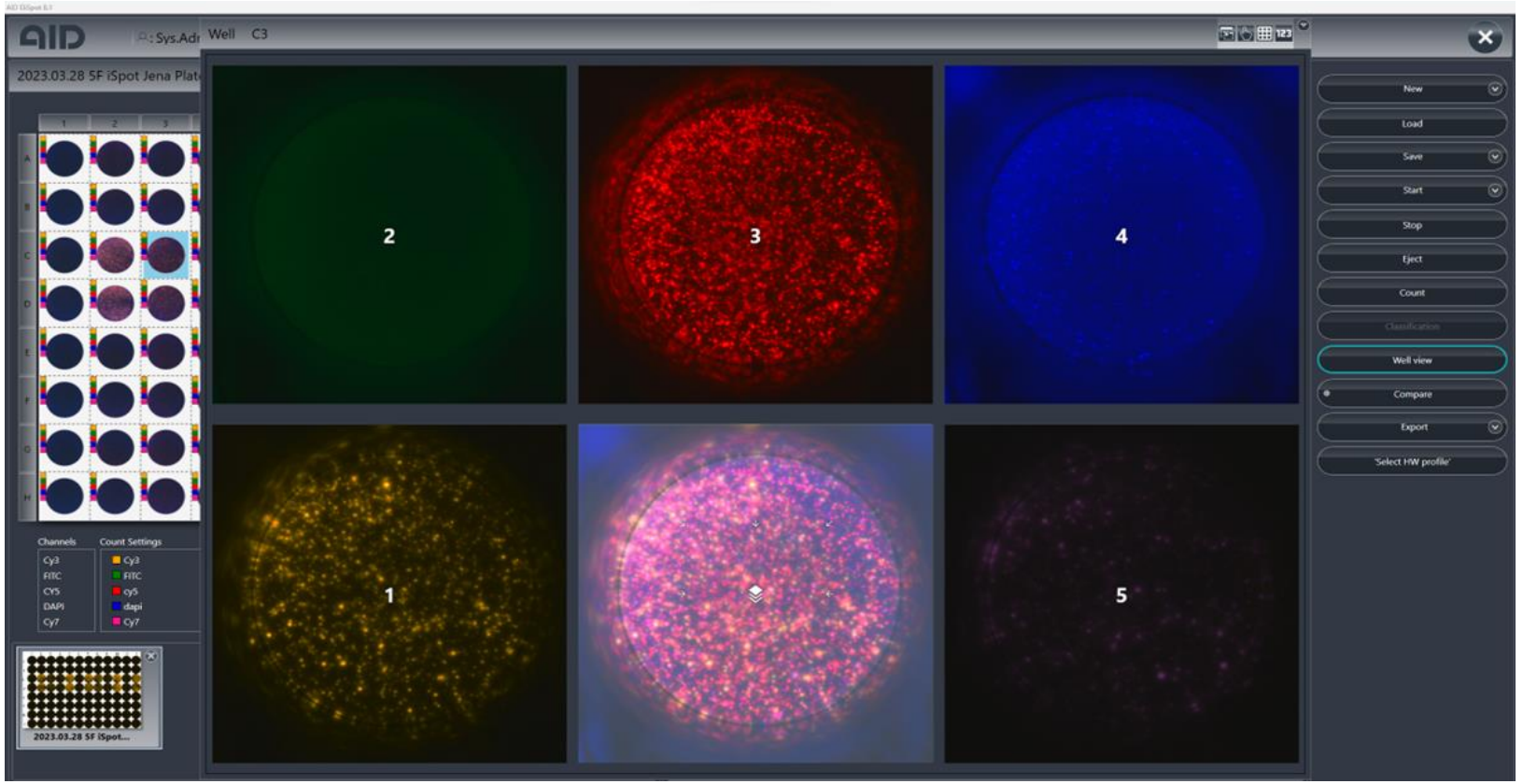
Red channel





Overlay of green and red channel

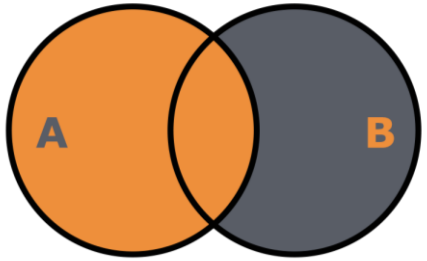
View and compare different channels at once

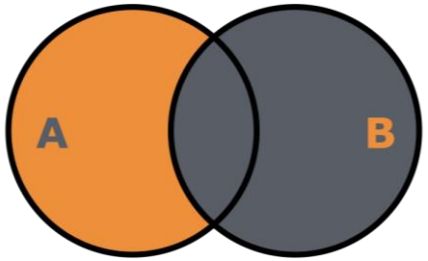


Combine different channels and count your spots

INCLUSIVE

Double stained spots





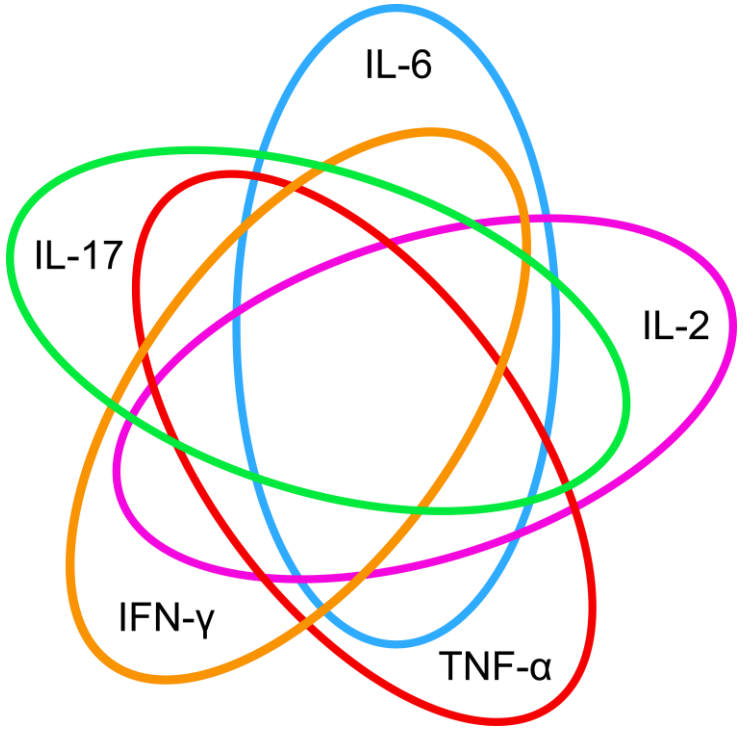
EXCLUSIVE

Single cytokine

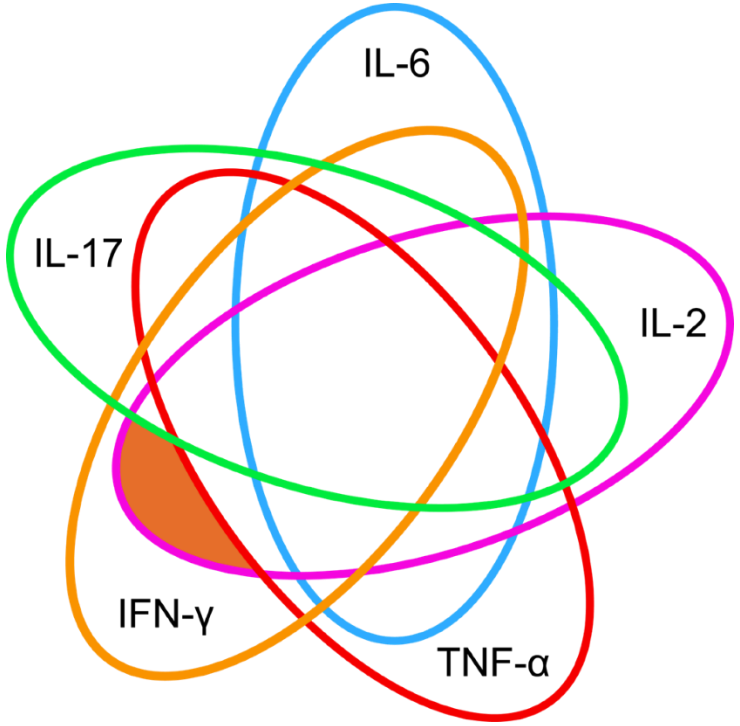
Number of possible combinations

Combination	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
DAPI	■					■	■	■	■							■	■	■	■	■	■						■	■	■	■	■
FITC		■				■				■	■	■				■	■	■				■	■	■		■		■	■	■	■
Cy3			■				■			■			■	■		■			■	■			■	■	■	■	■		■	■	■
Cy5				■				■			■		■		■		■		■		■	■		■	■	■	■	■		■	■
Cy7					■				■			■		■	■			■		■	■	■	■	■		■	■	■	■		■

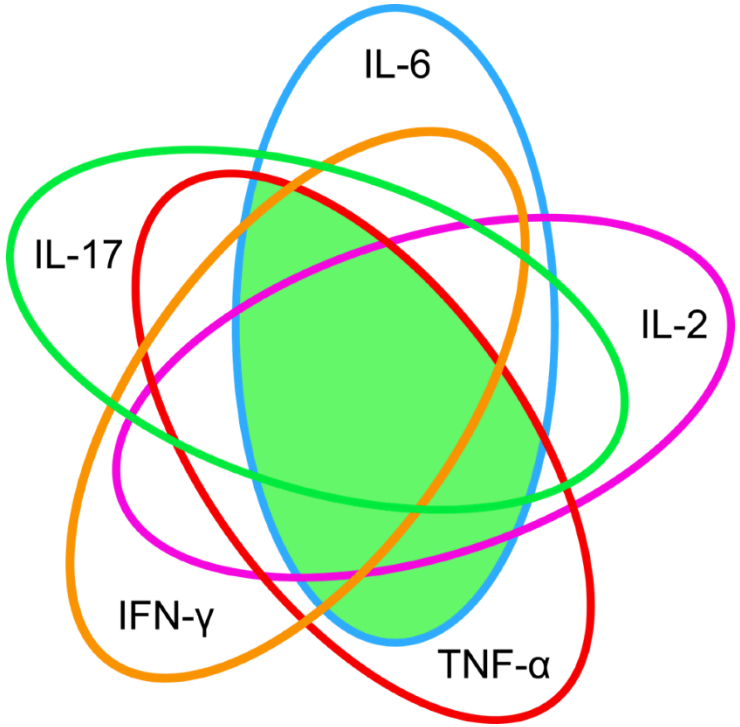
Logical operators can be used to define your cell population of interest.



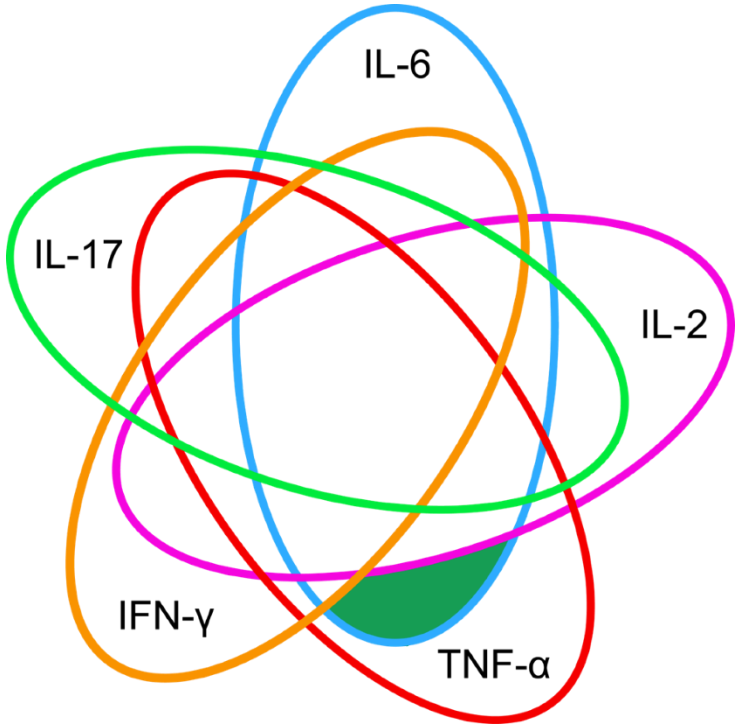
IFN-γ + IL-2 positive cells only



IL-6 + TNF-α positive cells including all spots with additional cytokines



IL-6 + TNF-α positive cells excluding all additional cytokines



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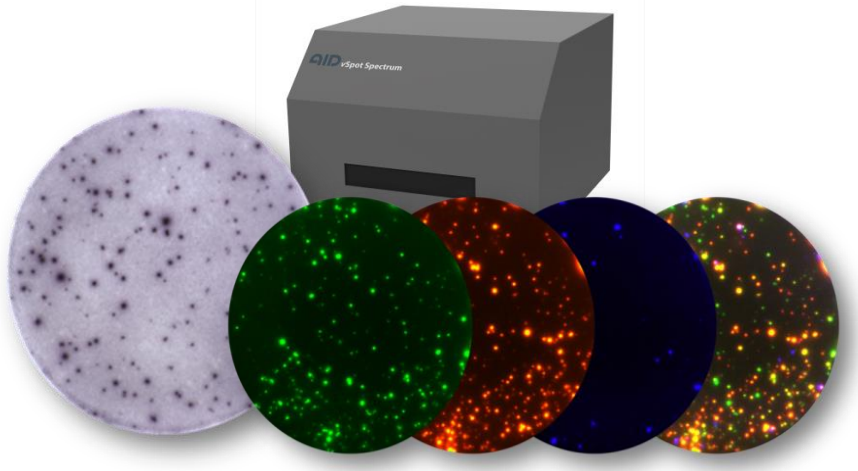
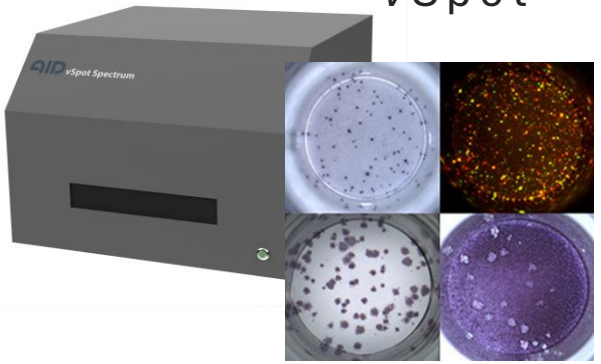
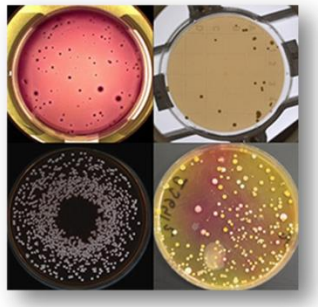
EliSpot Robot

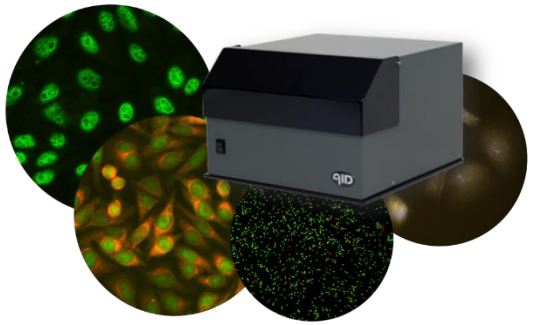
EliSpot / *i*Spot



*v*Spot





Colony Counter

*multi*Spot