Novel cell-based high-throughput hybridoma screening method using the Celigo image cytometer for antibody discovery

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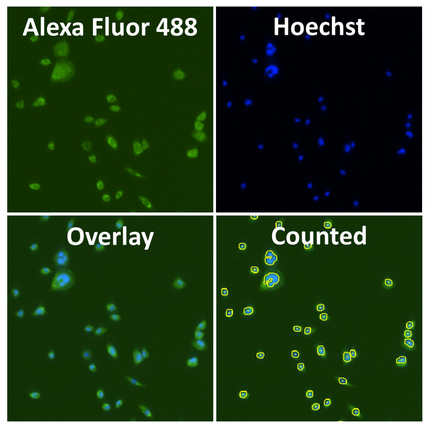
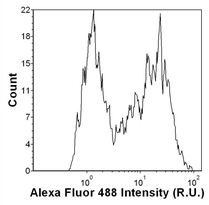
1. ABSTRACT

Hybridoma screening is a highly important process for antibody discovery, which can identify potential clones from hundreds to thousands of hybridoma cultures against the desired target antigen. Traditional screening methods using ELISA and flow cytometry presented technical issues such as limited accuracy, reduced high-throughput capability, increased time and cost. Conventional ELISA screening with coated antigen can also be impractical for difficult to express hydrophobic membrane antigens or multi-chain protein complexes. Here, we demonstrate novel cell-based high-throughput screening methodology using plate-based Celigo Image Cytometer, which avoids nonspecific signals by contrasting antibody binding signals directly on living cells, with and without recombinant antigen expression. The image cytometry-based high-throughput screening method was optimized by detecting the binding of hybridoma supernatants to the recombinant antigen CD39 expressed on Chinese hamster ovary (CHO) cells. Both target CHO and CFSE-stained wild type were co-cultured to simultaneously detect target antibodies (Alexa Fluor 594) and nonspecific binding, which can eliminate the need to produce a separate control sample for each hybridoma supernatant. Next, the image cytometer was used to screen 672 unique hybridoma supernatants to develop the high-throughput screening workflow. The Celigo was used to quickly image and analyze antibody binding of 672 samples, using Hoechst, Alexa Fluor 594 and CFSE fluorescence to identify high, medium, and low binding hits. Finally, the sensitivity of the image cytometer was demonstrated by serial dilution of purified CD39 antibody. Celigo was used to measure antibody affinities of commercial and in-house antibodies to membrane-bound CD39. This cell-based screening procedure can be completely accomplished within one day, significantly improving throughput and efficiency of hybridoma screening. Furthermore, measuring direct antibody binding to living cells eliminated both false positive and false negative hits. The image cytometry method was highly sensitive and versatile, and could detect positive antibody in supernatants at concentrations as low as ~5 ng/mL, with concurrent Kd binding affinity coefficient determination. We propose that this screening method will greatly improve screening technologies and facilitate more efficient antibody discovery.

2. CELIGO IMAGING CYTOMETRY FOR ANTIBODY BINDING DETECTION

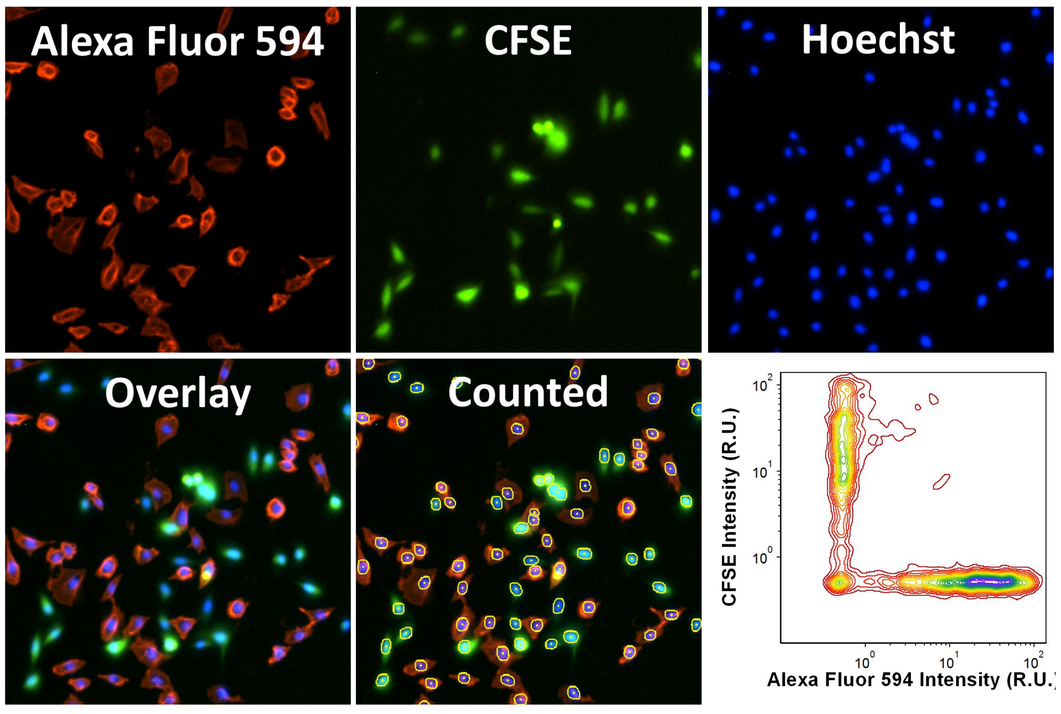




1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and capture bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation end-point and kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and antibody binding signal

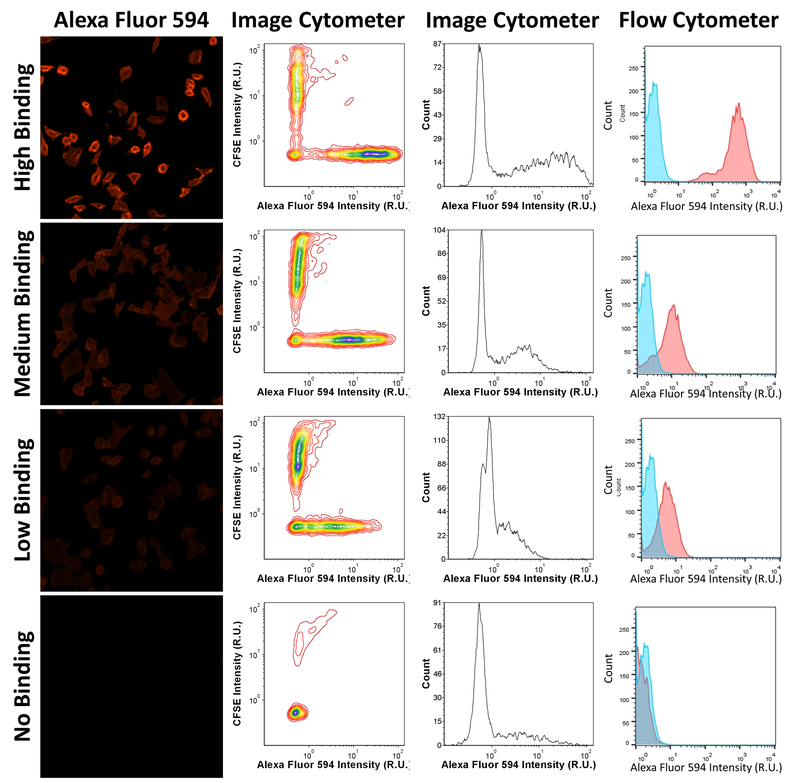
3. HYBRIDOMA SCREENING METHOD

Seed co-culture of CHO-CD39 and wild type CHO (CFSE), incubate overnight → Pipette in hybridoma supernatant and incubate for 1 h, wash 2X → Fix the cells and stain with secondary Donkey anti-mouse IgG (H+L)-AF594 → Image and analyze using Celigo image cytometer



- Co-culture of CHO-CD39 and wild type CHO cells allow simultaneous detection of target antibody binding to the former, as well as non-specific binding to the latter
- All of the cells are identified by Hoechst staining in Celigo, then the fluorescent intensities of CFSE and AF594 are measured and plotted in FCS Express
- The scatter plot shows antibody in supernatant binding to the CHO-CD39 cells and not the wild type CHO

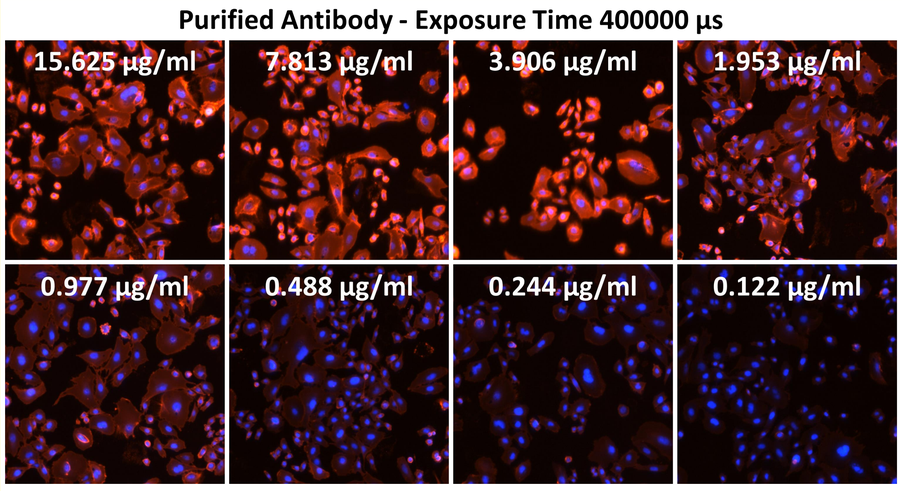
4. HIGH-THROUGHPUT HYBRIDOMA SCREENING EXPERIMENT

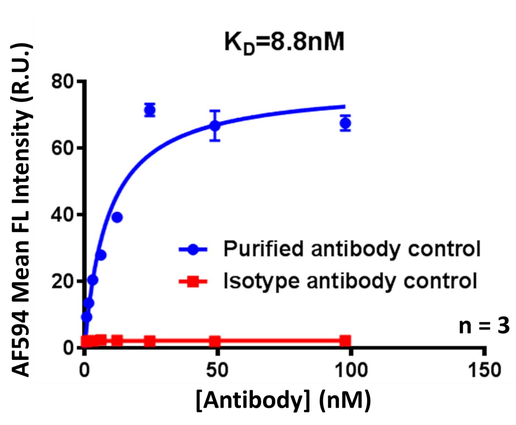


P1	1	2	3	4	5	6	7	8	9	10	11	12	P4	1	2	3	4	5	6	7	8	9	10	11	12
A	0.57	0.60	0.59	0.54	0.61	0.57	0.53	0.59	0.62	0.65	0.58	0.63	A	0.66	5.89	0.64	0.69	0.68	0.65	0.52	0.57	0.66	0.58	0.65	0.70
B	0.70	7.45	0.56	0.55	0.59	0.51	0.59	0.60	0.57	0.62	0.57	0.64	B	0.56	0.59	0.55	0.59	7.19	0.61	0.57	0.68	0.67	0.61	0.72	0.67
C	18.99	0.57	0.56	0.57	0.61	0.53	0.57	0.59	0.57	0.57	0.56	0.59	C	0.62	0.59	7.20	0.56	5.14	0.60	0.66	0.67	0.70	0.65	0.66	0.66
D	0.58	0.55	0.54	0.55	0.57	0.64	0.62	0.58	0.56	0.61	0.57	0.64	D	0.65	0.57	6.92	0.61	0.60	0.65	0.60	0.64	0.60	0.67	0.63	5.84
E	0.63	0.61	0.58	0.61	0.61	0.63	0.67	0.57	0.60	0.55	0.61	0.65	E	0.66	0.55	0.67	0.64	0.58	0.61	0.63	0.65	0.61	0.61	0.64	0.57
F	0.59	0.55	0.54	0.60	0.54	0.51	0.60	0.57	0.53	0.57	0.52	0.63	F	0.63	0.55	0.70	0.58	0.57	0.65	0.55	0.54	0.53	0.66	0.60	0.54
G	0.58	0.58	0.54	0.57	0.63	0.62	0.56	0.54	0.57	0.57	0.61	0.59	G	0.64	0.55	0.61	0.55	0.63	0.62	0.61	0.64	0.66	0.67	0.65	0.60
H	0.61	0.60	0.57	0.62	0.53	0.64	0.58	0.63	0.58	0.57	0.60	0.61	H	0.63	0.69	0.57	0.63	0.65	0.69	0.68	0.61	0.57	0.62	0.65	7.45
P2	1	2	3	4	5	6	7	8	9	10	11	12	P5	1	2	3	4	5	6	7	8	9	10	11	12
A	0.58	0.58	0.62	0.60	0.58	0.58	0.59	0.56	0.59	0.59	0.59	0.65	A	1.55	0.56	0.73	0.63	0.66	0.60	0.69	0.56	0.66	0.64	0.61	0.62
B	0.60	0.60	0.57	0.55	0.56	0.57	0.57	0.54	0.59	0.59	0.51	0.60	B	0.66	0.57	0.64	0.61	0.62	0.60	0.66	0.58	0.57	0.65	0.52	0.56
C	0.60	0.54	0.54	0.52	0.59	0.55	0.52	0.53	0.53	0.52	0.55	0.64	C	0.55	0.58	0.58	0.55	0.61	0.66	0.54	12.31	0.71	0.55	0.59	0.66
D	0.58	0.58	0.54	0.59	0.93	0.58	0.53	0.55	0.56	0.53	0.55	0.56	D	0.60	0.60	0.68	0.62	0.57	0.67	0.59	0.59	0.73	0.57	0.65	0.59
E	0.52	0.51	0.56	0.54	17.79	0.54	0.54	0.52	0.53	0.56	0.54	0.62	E	0.67	0.61	0.60	0.55	0.56	0.62	0.66	0.57	0.63	0.57	0.58	0.62
F	0.61	0.52	0.52	0.54	0.56	0.53	0.61	0.53	0.53	0.54	0.53	0.63	F	0.61	0.62	0.55	0.60	0.60	0.58	0.64	0.61	0.58	0.59	0.66	0.60
G	0.57	0.59	0.60	0.54	0.61	0.53	0.54	0.54	0.51	0.55	0.54	0.60	G	0.59	0.60	0.61	0.57	0.64	0.65	0.63	0.53	0.55	0.60	0.58	0.64
H	0.60	0.60	0.59	0.53	0.55	0.59	0.56	0.57	0.57	0.54	0.98	10.96	H	0.57	0.55	0.63	0.64	0.61	0.65	0.57	0.60	0.60	0.66	0.58	0.58
P3	1	2	3	4	5	6	7	8	9	10	11	12	P6	1	2	3	4	5	6	7	8	9	10	11	12
A	0.54	0.62	0.67	0.61	0.63	0.60	24.17	0.58	0.68	0.66	0.62	0.61	A	0.63	0.61	0.58	0.58	0.59	0.61	0.57	0.60	0.59	0.63	0.62	0.64
B	0.56	0.66	0.55	0.59	0.62	0.60	0.69	0.60	0.60	0.60	0.59	0.61	B	0.61	0.59	0.59	0.56	6.64	7.12	0.59	0.64	0.59	0.61	0.61	0.66
C	0.58	0.65	0.56	0.61	0.57	0.67	0.62	0.66	0.67	0.59	0.63	C	0.55	0.58	0.54	0.59	0.57	8.39	0.55	0.60	0.59	0.54	0.66	0.60	
D	0.57	0.66	0.63	0.62	0.62	0.63	0.60	0.60	0.62	0.63	0.60	0.62	D	0.56	0.54	0.56	0.54	0.55	0.59	0.61	0.58	0.53	0.54	0.57	0.61
E	0.51	0.66	0.58	0.57	0.61	0.62	0.62	0.57	0.61	0.60	0.57	0.61	E	0.59	0.53	0.56	0.57	0.59	0.59	0.56	0.64	0.63	0.59	0.52	0.58
F	0.66	0.55	0.63	0.57	0.60	0.66	0.64	0.65	0.67	0.58	0.62	0.62	F	0.59	0.53	0.58	0.55	0.57	0.57	0.60	0.56	0.55	0.61	0.62	0.57
G	0.64	0.58	0.60	0.57	0.63	0.61	0.68	0.65	0.66	0.66	0.64	0.58	G	0.57	0.57	0.63	0.61	0.52	0.63	0.58	0.62	0.57	0.67	0.56	0.56
H	0.62	0.59	0.58	0.59	0.59	0.67	0.64	0.65	0.67	0.60	0.67	0.64	H	0.51	0.57	0.60	0.57	0.60	0.56	0.54	0.56	0.58	0.60	0.59	0.57

- CD39 antibody binding signals are measured using Celigo to identify positive supernatant hits
- The AF594 FL signals for high, medium, low, and no binding on CHO-CD39 cells are shown on the right
- The measured fluorescent intensities are compared directly between Celigo and flow cytometer that show similar results
- All of the positive hits showed binding to CHO-CD39 cells and not the CHO wild type cells, which indicated no non-specific binding
- The flow cytometry method required additional control sample for each tested supernatant, which increased time and material
- Co-culturing both cell types allowed reduction in material used for the screening assay

5. MEASUREMENT





- Celigo can also be used to determine antibody binding affinity for the target Mab and Isotype antibody
- The CHO-CD39 cells were subjected to different antibody concentrations in order to measure the fluorescent intensities
- The fluorescent signals were used to plot the binding curve in Graphpad Prism and then calculate the Kd value for binding
- For this target antibody, the Kd was calculated to be 8.8 nM
- The lowest detectable signal 3 x STD above the background was measured to be 4.88 ng/mL

6. CONCLUSION

- The Celigo can significantly increase hybridoma screening speed, which is advantageous because hybridoma cells are genetically unstable that will require quick identification of positive supernatant, and subsequently subclone and expand
- By using this method, high-throughput hybridoma screening can be performed using direct cell counting in fluorescent images
- The method allowed the co-culture of target CHO-CD39 and wild type CHO cells that can measure binding as well as non-specific binding, which can eliminate the need of extra samples for control, thus reducing the time and material of a screening assay
- Finally, images are strong evidence for any positive hits, where using ELISA or flow cytometry do not offer