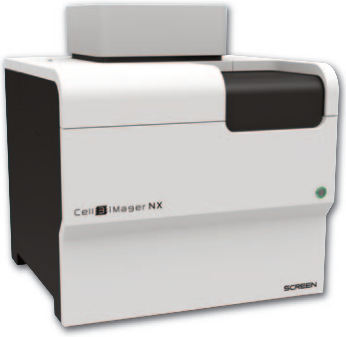
Application Note vol. 3

Single-cell cloning of CHO-K1 cell

Introduction



Single-cell cloning is a very important technique in the R&D process to establish cell lines for various purposes, such as for developing anibody drugs. The gene-transfected/edited cells are seeded onto a well plate by limiting dilution method. Monoclonality of colonies derived from single cells is usually evaluated by microscope, which is subjective, irreproducible and labor-intensive.

Cell3iMager NX is equipped with functions to evaluate the monoclonality and proliferative of colonies, enabling simple and rapid single-cell cloning. With Cell3iMager NX, a complete dataset can be obtained without inadvertently changing the data, further ensuring the data integrity. This article demonstrates a few application examples of Cell3iMager NX on single-cell cloning analysis. CHO-K1 cell line is used in these examples because of its wide utilization in the production of antibody drugs. Cells were imaged over time and traced after-seeding stage to colony formation stage to assess monoclonality.

Materials & Methods

Product Used:	Cell3iMager NX	**Samples & Reagents:**	CHO-K1 cell (ATCC) F-12K medium (ATCC)
Cell dispenser:	NichiMartCUBE (Nichiryo)		FBS (Biosera) Penicillin/streptomycin (Nacalai Tesque) 96 well plate flat bottom (CORNING)

Methods

CHO-K1 cells cultured in 10 cm dish were de-tached with Trypsin/EDTA, adjusted to 0.5, 1, 2 cells/well by limiting dilution and transferred onto 96 well plate using cell dispenser. Bright-field imaging was performed after seeding (day 0) and periodically with the 10x objective lens of Cell3iMager NX. (Fig.1)

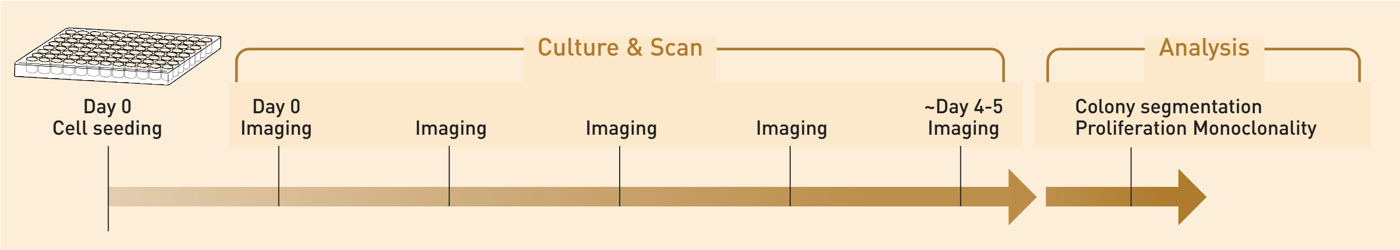


***Fig. 1:** Imaging/Analysis flow*

By applying deep learning-strengthened image analysis function of Cell3iMager NX, CHO-K1 cell colonies can be detected in the acquired images. The cell colonies labelled in Fig.2 were selected as the training data for the deep learning function. Then a deep-learning model was created by performing training for 1 hour using the training dataset. By applying the model to the acquired images, it became possible to accurately segment the cell colonies without being affected by brightness and debris. Further analysis values can be obtained via segmented colonies, including object area and position in the well. In addition, a deep-learning model can be used repeatedly once it is created, shortening the required time to set up the experiment and the subsequent analysis parameters.

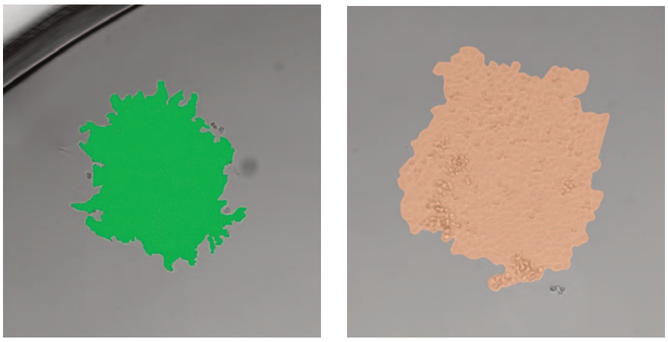


***Fig. 2:** Labeled image of day 5 colony (Left) and segmentation image (Right)*

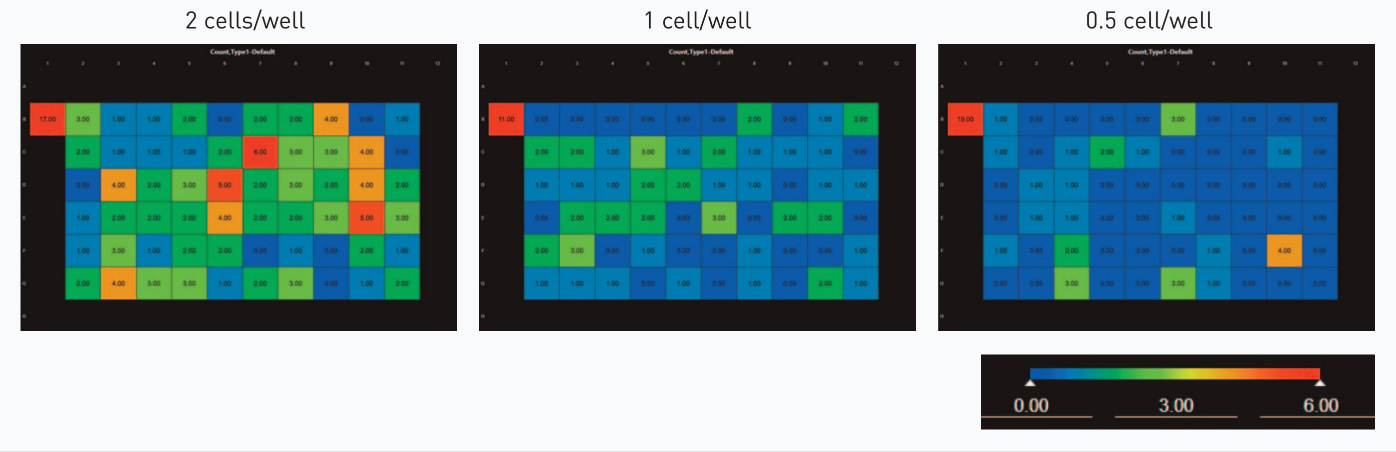
Results

Segmentation of colonies

CHO-K1 cells were seeded at various cell densities in 96 well plates and imaged over time (Day 0,1,4,5). Imaging focus position was determined using a reference well seeded at 200 cells/well. Clear images were obtained by imaging ±5µm from the focus position and stacking those images.

On day 5, the number of colonies contained in each well was quantified for the groups with various seeding cell densities. (Fig.3) It is easy to evaluate monoclonality by using the heatmap which shows the number of colonies in the each well.

By using the Crop Object function, it is possible to automatically crop images based on colony positions, further enabling easy confirmations for the colony position in the well plate. (Fig.4) Time Course function enables us to check the monoclonality by tracking the same position in the same well. Tracing the day 5 colony back to day 0 (after seeding) showed the colony to be a single cell origin. (Fig.5)



2 cells/well

Count,Type1-Default

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	17.00	3.00	1.00	1.00	2.00	0.00	2.00	2.00	4.00	0.00	1.00	
C		2.00	1.00	1.00	1.00	2.00	6.00	3.00	3.00	4.00	0.00	
D		0.00	4.00	2.00	3.00	5.00	2.00	3.00	2.00	4.00	2.00	
E		1.00	2.00	2.00	2.00	4.00	2.00	2.00	3.00	5.00	3.00	
F		1.00	3.00	1.00	2.00	2.00	0.00	1.00	0.00	2.00	1.00	
G		2.00	4.00	3.00	3.00	1.00	2.00	3.00	0.00	1.00	2.00	
H												

1 cell/well

Count,Type1-Default

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	11.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00	1.00	2.00	
C		2.00	2.00	1.00	3.00	1.00	2.00	1.00	1.00	1.00	0.00	
D		1.00	1.00	1.00	2.00	2.00	1.00	1.00	0.00	1.00	1.00	
E		0.00	2.00	2.00	2.00	0.00	3.00	0.00	2.00	2.00	0.00	
F		2.00	3.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	
G		1.00	1.00	1.00	0.00	1.00	0.00	1.00	0.00	2.00	1.00	
H												

0.5 cell/well

Count,Type1-Default

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	19.00	1.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	
C		1.00	0.00	1.00	2.00	1.00	0.00	0.00	0.00	1.00	0.00	
D		0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
E		0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	
F		1.00	0.00	2.00	0.00	0.00	0.00	1.00	0.00	4.00	0.00	
G		0.00	0.00	3.00	0.00	0.00	3.00	1.00	0.00	0.00	0.00	
H												

Fig. 3: *Number of colonies per well at each seeding cell density*

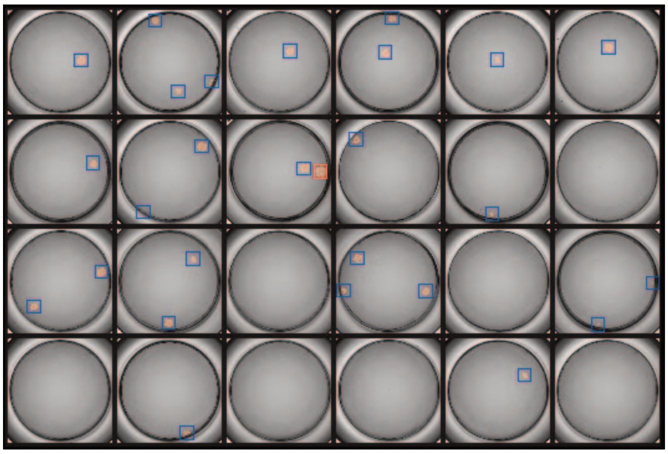


Fig. 4: *Colonies position in well plate*

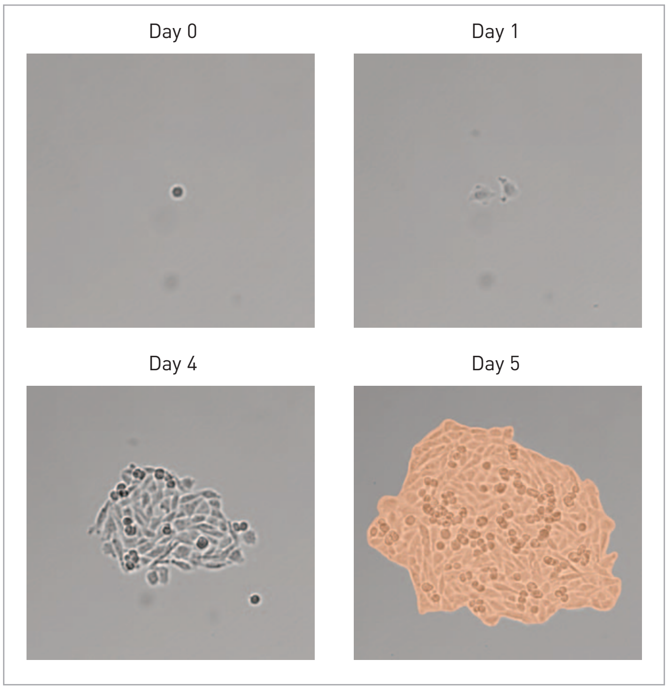


Fig. 5: *colony segmentation (Day 5) and evaluation of single cell origin*

Quantitative evaluation of colonies

Next, we evaluated the colony proliferation using the area value from the colony features. The area of colonies contained in the well plate (Day 5) seeded at the cell density of 1 cell/well was plotted with the histogram. (Fig.6) The average colony area was 0.118mm² and maxim colony derived from single cell was 0.266mm². We focused on the colonies on day 5 and evaluated the area distribution at this time point, but it is also possible to segment on each day and evaluate cell proliferation over time.

Conclusion

In this article, monoclonality and proliferation of CHO-K1 cells were evaluated by using Cell3iMager NX. Monoclonality can be quickly determined by comparing the images acquired in chronological order without analysis. In addition, we also revealed that one cell per well is an ideal cell density for single-cell cloning, yet the final colony numbers may vary per well. Cell3iMager NX's colony segmentation and colony counting function can immediately evaluate the monoclonality and proliferation of the colony, which is expected to contribute to the work efficiency in biomedical research and development.

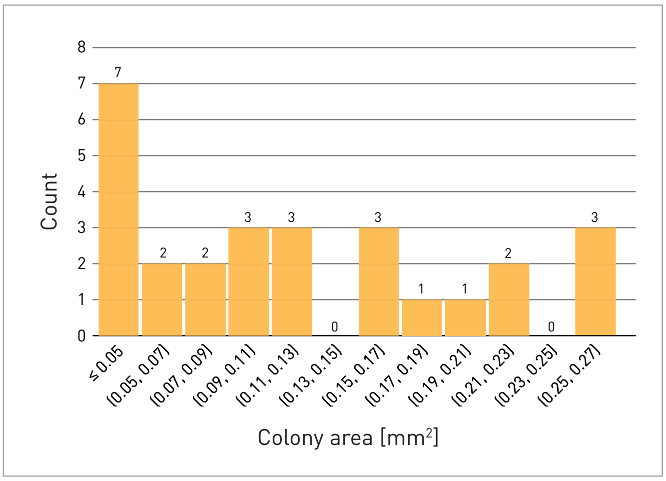


Fig. 6: *Distribution of colony area*