

Introduction to Two-Cell Analysis Technology

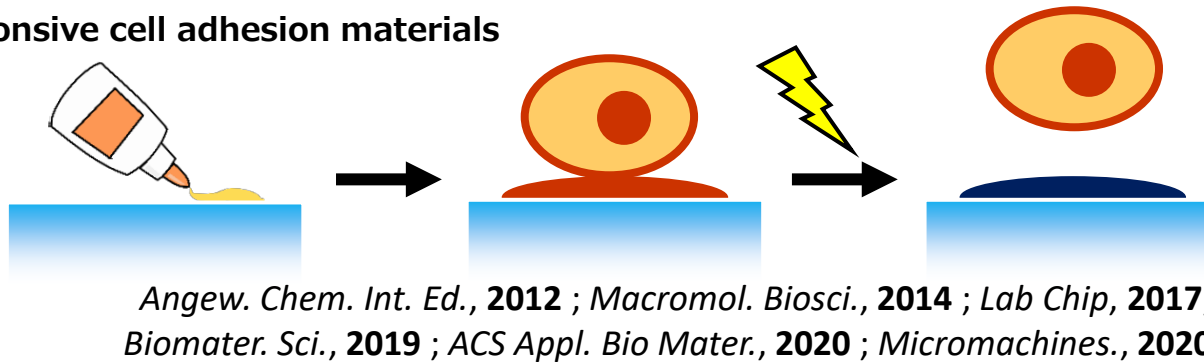
SANKEN
The University of Osaka

Prof. Satoshi Yamaguchi

Light-responsive cell adhesion surface

We have developed a unique light-responsive cell adhesion surface that enables instantaneous manipulation of cell attachment and detachment to substrates.

Light-responsive cell adhesion materials

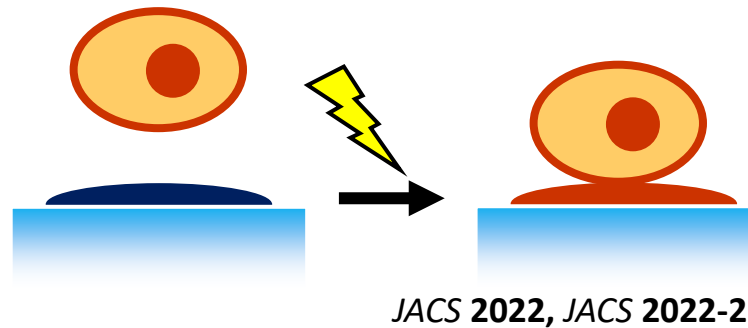


The 1st generation

Light-degradable Cell Adhesion Surface

“光でくっつかない、とれる”

特許登録7205910号
特願2016-061233

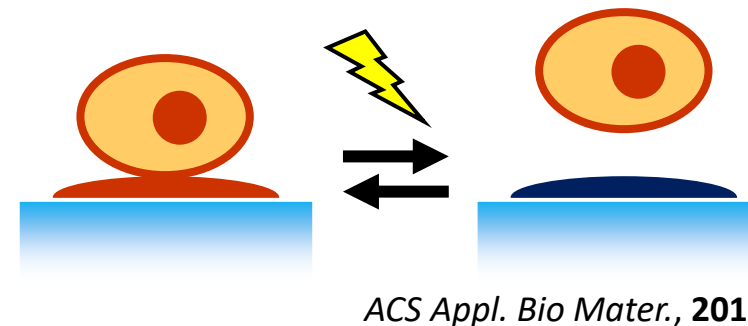


The 2nd generation

Light-activated Cell Adhesion Surface

“光でくっつく”

特許登録6607746号
特願2021-190522
特願2022-76539



The 3rd generation

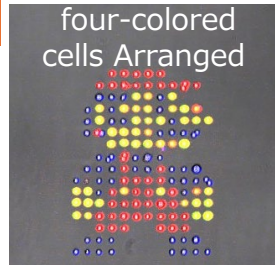
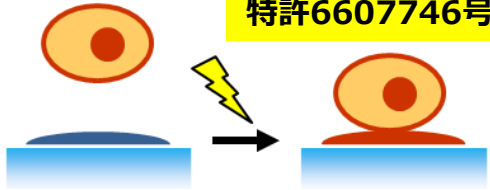
Light Switch-Type Cell Adhesion Surface

“光でくっついたり
とれたり”

特許登録7236126号
特願2019-222051

Light-responsive cell adhesion surface

特許6607746号

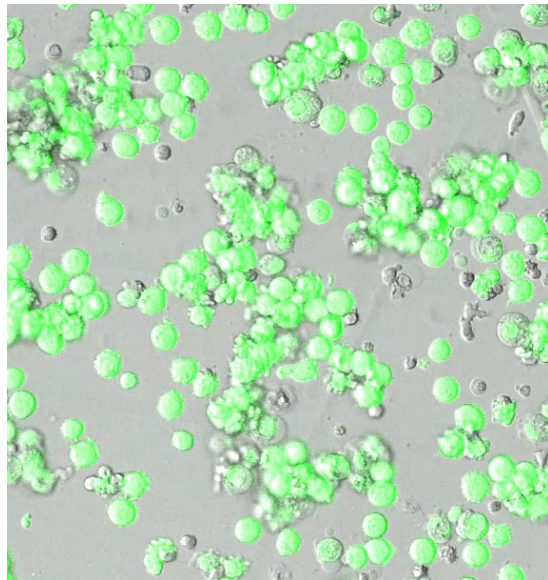


ヒト白血病細胞株
K562細胞

Calcein染色

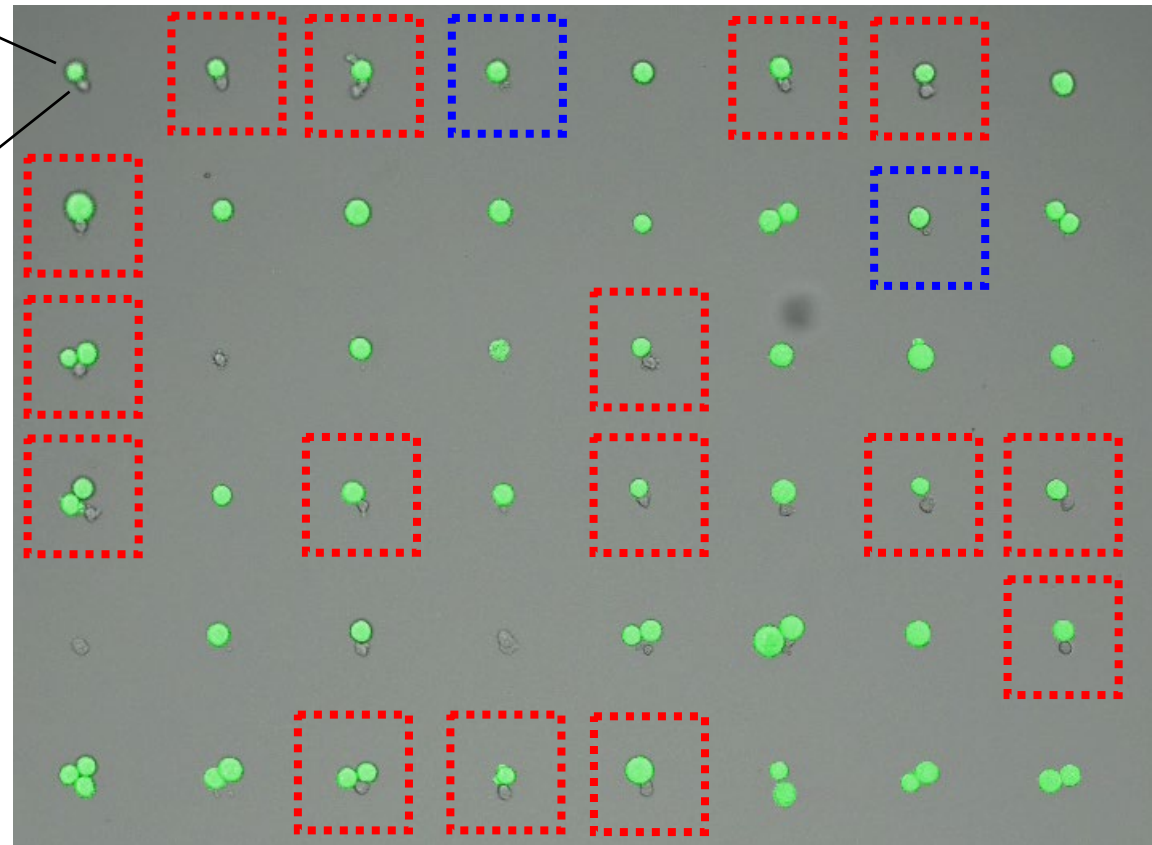
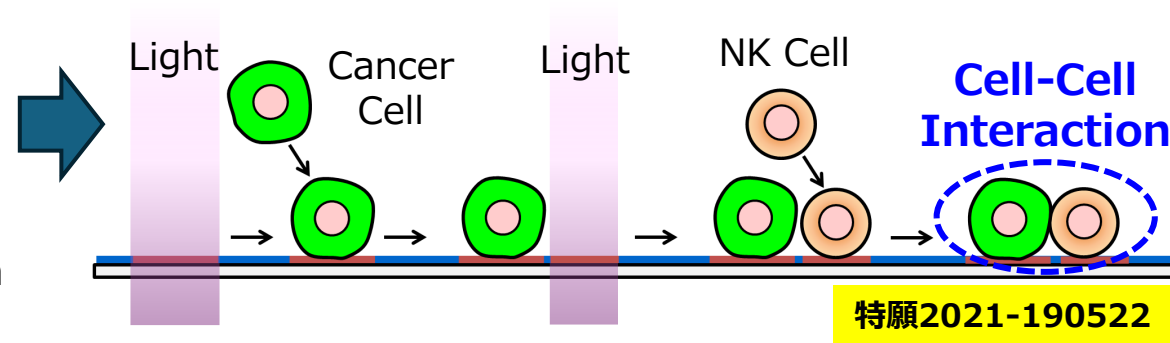
NK細胞

at 600x Speed



The status of individual cells remains unknown.

Two Cell Analysis

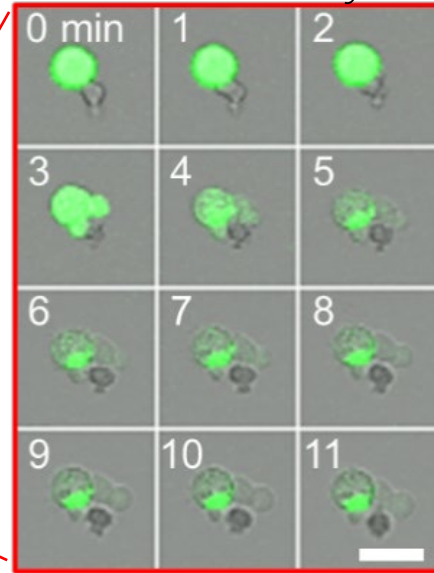


Visualizing the heterogeneity of individual interactions

By partially immobilizing cells on a substrate for analysis, we achieved observation of interactions with resolution down to individual cells and acquired spatiotemporal image data.

By combining AI analysis, which is well-suited for image analysis, it has become possible to perform preemptive analysis on cells destined to die.

Two cell analysis



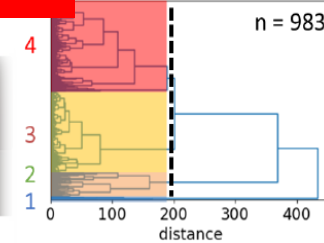
① Image Data



② Detection of Target Cells



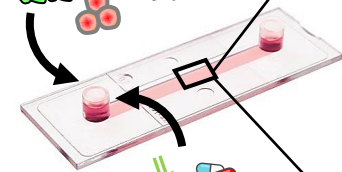
Auto Analysis



大量データ処理・解析システム

③ 標的細胞位置情報

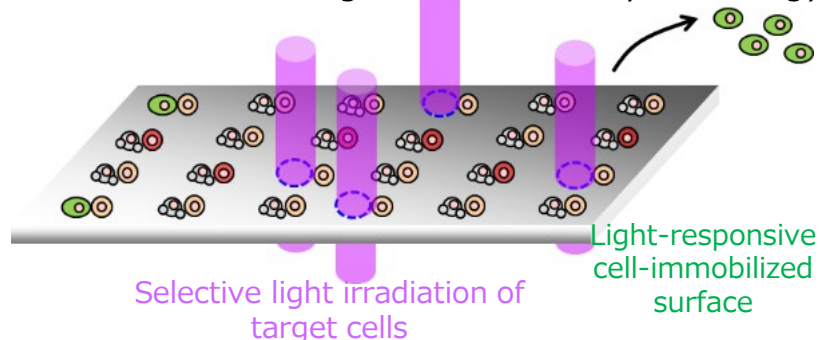
がん細胞
免疫細胞



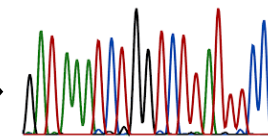
様々な条件下（薬剤等共存下）で解析

High-speed optical recovery

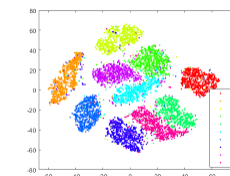
Cells exhibiting interesting behavior can be recovered using light-based recovery technology.



Sequence analysis of Receptor



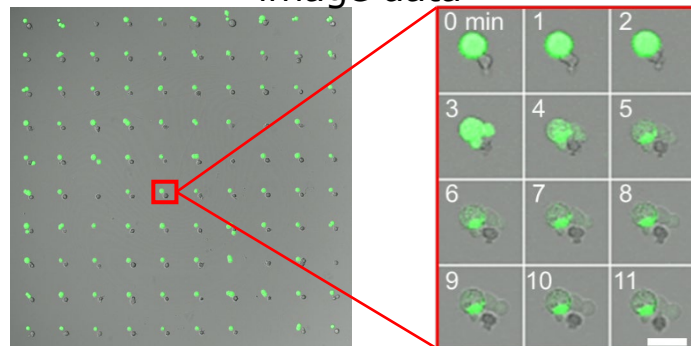
scRNA-seq



AI-Based Automatic Classification

It can automatically classify not only the presence or absence of cell death induction but also clinically important cell death induction mechanisms.

A vast amount of time-series image data

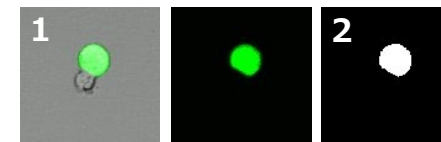


簡易自動解析システム

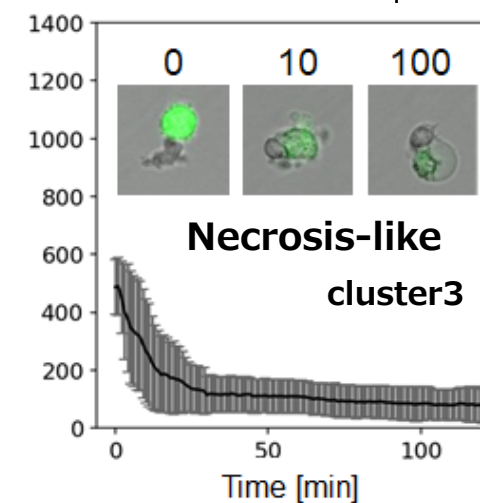
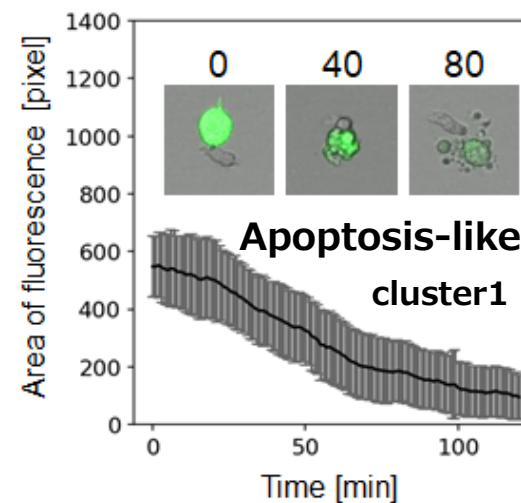
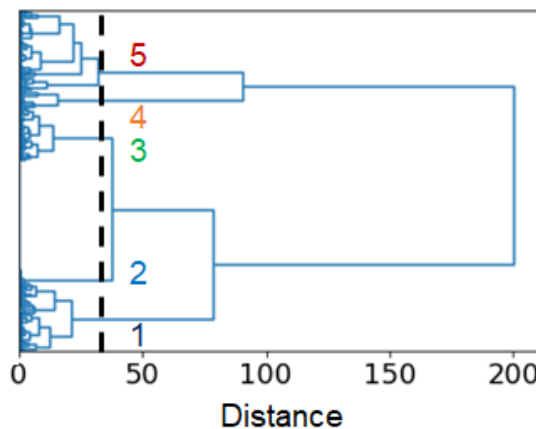
1. 細胞ペアの経時画像の切り出し
2. 蛍光画像の二値化 (**Image J**)
3. 輝度が閾値以上のピクセルのカウント (**Python**)
4. 蛍光の時間変化データを階層的クラスタリング解析

(**Scipy.cluster.hierarchy**モジュール, **Python**)

教師無し機械学習 (ワード法)



	Time [min]			
	0	1	2	...
Cell 1	0	0	0	
Cell 2	409	430	421	...
Cell 3	339	348	385	
...				



Yamahira et al., *J. Am. Chem. Soc.* **2022**, *44*, 13154

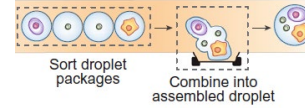
Upon automatic classification based on cytotoxicity, two of them were found to involve distinct cell death mechanisms.

Comparison with Other Analytical Methods

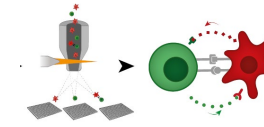
特願2021-190522



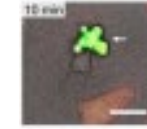
PNAS, **113**, E3599 (2016)



PNAS, **113**, e2110867119 (2022)



Nat. Biotechnol. **38**, 629 (2020)



J. Biol. Chem. **293**, 16348 (2018)

	Two cell Analysis	Microwell Method	Droplet Method	Flowcytometry	Conventional Interaction Analysis
High Throughput	○	○ (一度に数万ペア)	◎ (一度に数100万ペア)	◎ (一度に数千万ペア)	× (一度に1～数ペア)
Acquisition of Time-Series Images	○	△ (細孔に押し込められた状態で)	×	×	○
Observation of Morphological Characteristics	◎	×	○ (浮遊細胞のみ)	×	◎ (制限がない)
Observation of Adherent Cells (Solid Tumors) in an Adherent and Spread State	○	×	×	×	○
Single-cell picking	◎	△ (浮遊細胞のみ可能)	×	×	△ (浮遊細胞のみ、難しいが)
High-speed single-cell sorting	◎	×	×	×	×

Why not utilize this technology for your company's exploratory research and evaluation?

By arranging diverse cell populations on a plate for observation, we can:

- Analyze interactions between two cells at high resolution at the cellular level
- Accumulate data over time to enable analysis along a temporal axis
- Recover and analyze cells of interest by irradiating them with a laser
- Apply AI to image analysis for automated cell classification and early-stage reaction analysis through cell recovery



- Discovery and research of novel immune cells, progressively subdivided and functionally characterized: T cells → Th1 cells → Th1-Treg cells
- Exploration using cells expressing fusion proteins with GFP, etc., enabling analysis based on intracellular signaling pathways
- Time-series analysis allows evaluation of cell migration capabilities
- By applying drugs to the surrounding area, assessment of drug effects on cell-cell interactions is also possible.