Time Lapse Micro-Culture AI Analysis

Introduction

Microscopy techniques have played an important role in providing information about a specific cell's biology (2). For example, through microscopy, we have been able to further understand cell behavior, cellular processes, and disease, and through live cell imaging, we can monitor these changes in cell behavior/processes over time. However, taking these images and forming quantitative meaningful observations has proven to be more challenging.

The status quo has mainly to look at cell counts or cell confluency to from a quantitative readout. However, this is often done completely by the human eye segmenting certain characteristics (1). Although this can be good enough to form conclusions, it can make it difficult to compare data between groups (1). As even the most trained eyes may see things slightly differently, and therefore, there is no standard way of analyzing live-cell imaging (1). Not to mention going through each image by hand can be quite time consuming. One to make this process more streamlined and efficient is in using AI.

At Enrich Biosystems we have developed the TROVO; a live cell imager that can track cell to cell behaviors overtime within our microwell system. These microwells sequester cells that allow microenvironments to ensue between cancer cells and CAR-T/TIL/TCR-T cells.

In addition to live cell imaging, the TROVO can also retrieve cells of interest for further downstream applications. In order to consistently retrieve the best killing T cells we have developed a standard way of using the AI grouping feature within our software. This feature allows for thousands of images to be quickly analyzed based on confluency, cell shape, and/or cell behavior, and then grouped based on their similarities. Users are able to easily identify which groups that have the best killing T cells and/or best proliferating T cells to then retrieve.

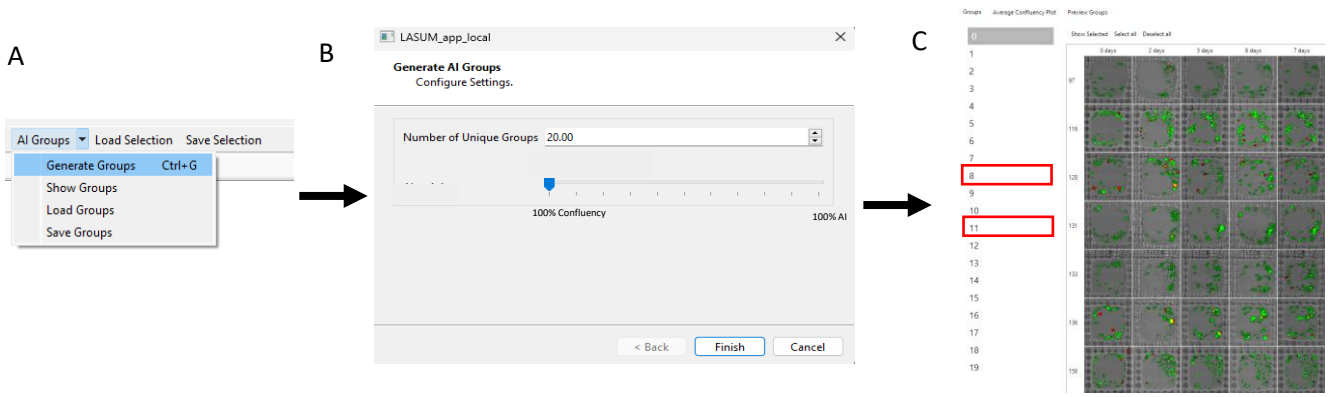
In this paper, we aim to describe a standard method of using AI to analyze the co-culture kinetics of thousands of images in an easy, user-friendly manner.

Approach and Methods

One of the novel features of our TROVO system is its ability to retrieve cells in these microwells of interest for downstream applications. To effectively select for these microwells we have developed a feature in our LASUM software using confluency and AI to choose groups of microwells to retrieve with little human influence.

Using a two-round selection process described below in Figure 1 and Figure 2, we can correctly identify the best proliferating and killing T cells.

Round 1 Selection:



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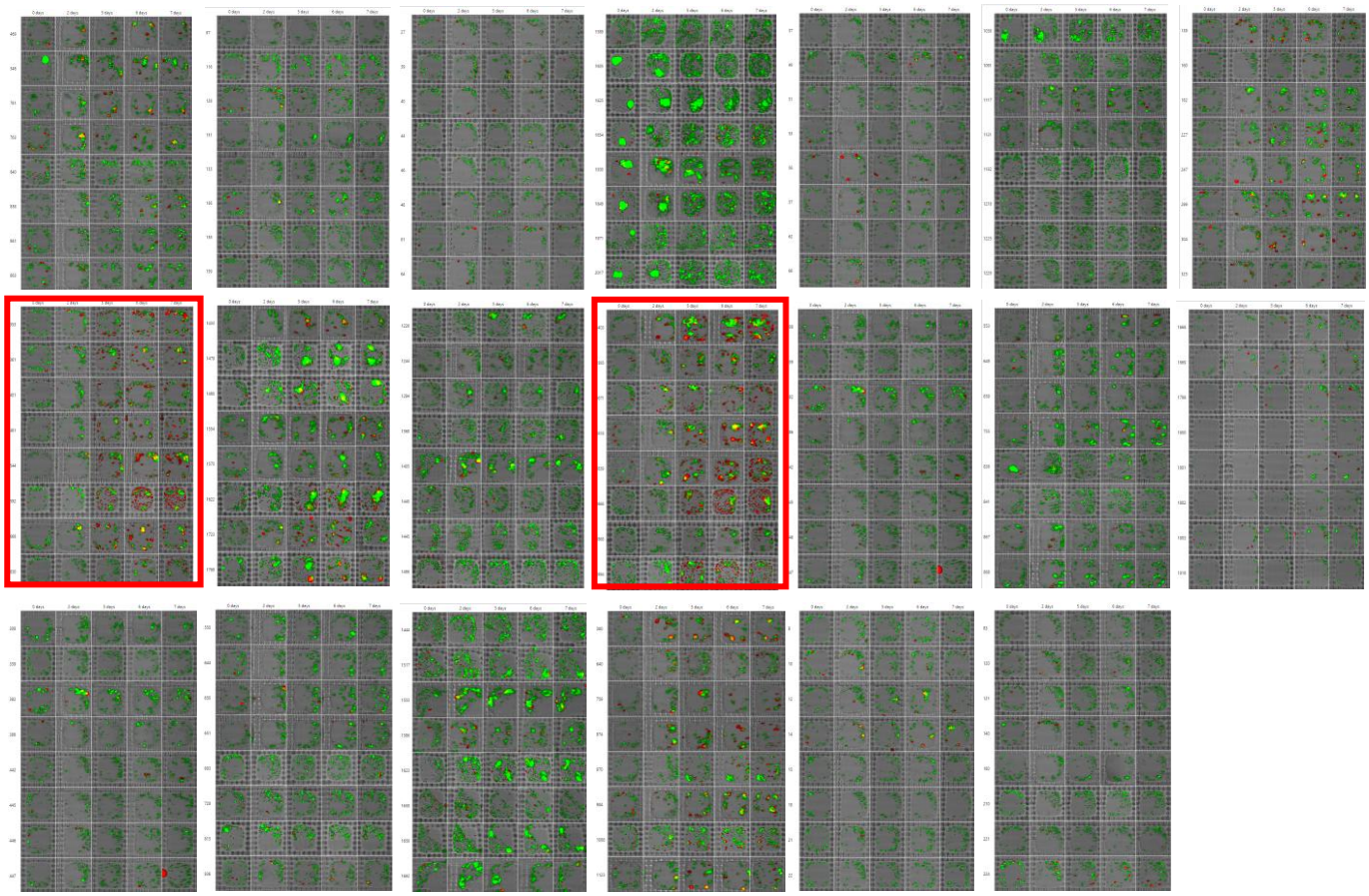
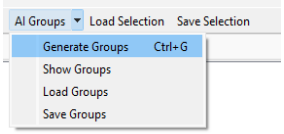


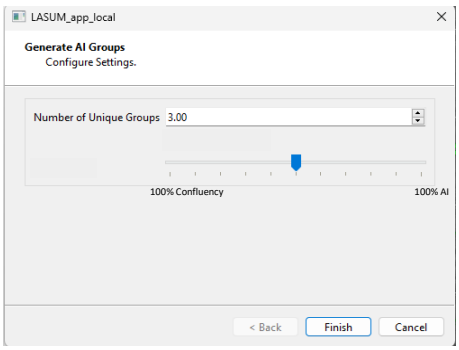
Figure 1. To select microwells of interest clink into a well and select AI groups and then generate groups (A). This will bring you to the configure settings screen. Here you can select the number of groups you'd like the microwells to be grouped into, along with a scale that will use confluency, cell shape, or a combination of both, to determine these groups. Using a real dataset we have consistently found that choosing 20 groups and using 100% confluency in round 1 results in 2-3 groups being of interest to move forward with. In this particular data set this is highlighted in red (B-C) and each group set can be viewed in D.

Round 2 Selection:

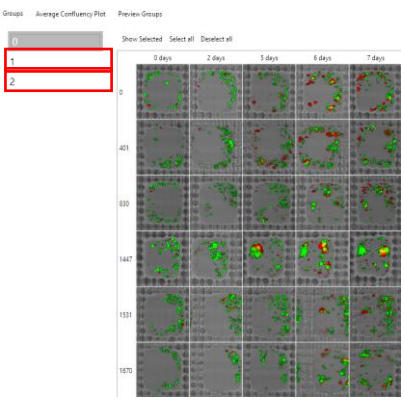
A



B



C



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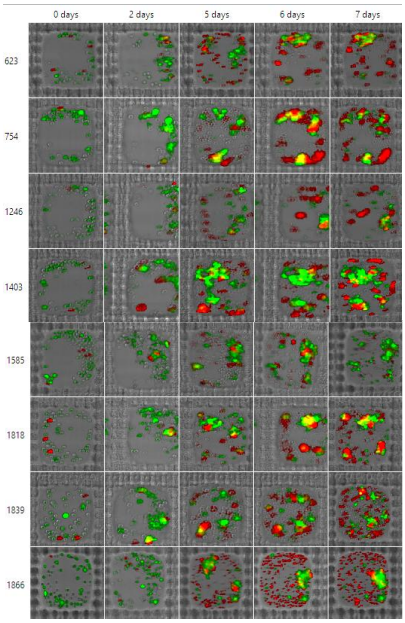

E

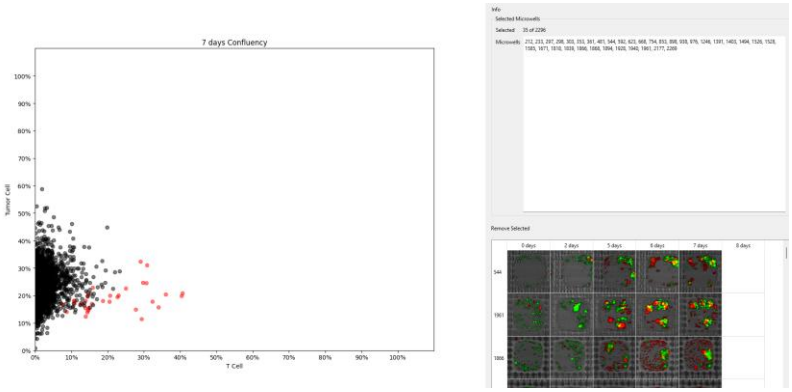

Figure 2. To remove any unwanted microwells, a second round of selection is performed on the selected microwells from the first round. In this round, the microwells are divided further into three groups, and 50% confluency and 50% AI are used(A-B). Out of the three groups generated, the best two groups are selected for retrieval ©. Examples of the final microwells in this dataset that were selected for retrieval are shown in (D), and their location on the plot stat graph is in (E). Out of 2,296 microwells, the final number selected for cell retrieval is 35: top 1.5%.

Conclusions

Using our LASUM software, the TROVO's AI grouping features allow for data to be quickly analyzed and effectively grouped by either confluency, cell shape, or both with very little human input. This multidimensional approach allows users to get a more in-depth analysis of their cells and their spatial arrangements with one another. This could lead to more consistent results between experiments and cell lines. In the above example, we were able to generate groups using confluency and AI as a way to select microwells to be retrieved. We were able to successfully apply this method to three other examples, yielding similar results.

In Development

- Using larger wells with >5000 microwells
- Different size libraries
- Single-color fluorescence
- Three-color fluorescence

References

1. Cuny AP, Schlottmann FP, Ewald JC, Pelet S, Schmoller KM. Live cell microscopy: From image to insight. Biophys Rev (Melville). 2022 Apr 21;3(2):021302. doi: 10.1063/5.0082799. PMID: 38505412; PMCID: PMC10903399.

2. Thomas A. Nketia, Heba Sailem, Gustavo Rohde, Raghu Machiraju, Jens Rittscher, Analysis of live cell images: Methods, tools and opportunities, Methods, Volume 115, 2017, Pages 65-79, ISSN 1046-2023, https://doi.org/10.1016/j.ymeth.2017.02.007.

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