High Content Imaging for Multidimensional Cell Analysis

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Abstract

Automated high-throughput microscopy has been instrumental for drug discovery and large-scale gene perturbation screens. Advancements in image resolution, microscope speed, and detection sensitivity have greatly aided high content imaging approaches. Here, we describe how high content microscopy can be repurposed for quantitative image-based cytometry, an approach that exploits both the throughput and the resolution of current screening microscopes for multidimensional cell population analyses.

Introduction

Since the beginning of cell biology as a scientific discipline, the performance of microscopes has steadily improved. In particular, better lenses and illumination systems have been developed, which provide more and more detailed pictures of cells and organelles. Nowadays, super-resolution microscopy allows to resolve even the smallest cellular structures beyond the Abbe limit, the resolution limit in classical light microscopy. In parallel to improving optics and resolution, microscopes have been increasingly equipped with automated components. This development paved the way for high-throughput microscopy, which is currently used primarily for high-content screens. However, the power of modern screening microscopes can also be used to precisely map and characterize cellular processes in large cell populations - true to the motto: "the whole is more than the sum of its parts".

From screening microscope to microscopy-based cytometry

The most common application of high-throughput microscopes is in the area of microscopy-based screens. The aim here is to compare large numbers of different biological conditions. This is used, for example, in the development of drugs, when the effects of thousands of chemical compounds are to be compared. Phenotypic screens also play an important role in the characterization of gene functions. In both cases, the automation and performance of microscopes is used to measure as many different conditions as possible within a reasonable time period. But what if high-throughput microscopes were not used to compare as many different conditions as possible, but to examine as many cells as possible for each condition? Using such an approach, a classical immunofluorescence experiment can be transformed into microscopy-based cytometry, which combines the advantages of high-resolution microscopy with the high throughput capabilities known from flow cytometry. By scaling up from a few cells per condition to hundreds or even thousands of cells, one not only improves the informative value, but also gains additional dimensions, for example the position of the analyzed cells in the cell cycle. Based on suitable markers it is thus possible to further subdivide cell populations and to study cellular responses more specifically and in greater detail *(Figure 1)*. The applications are versatile and already helped to foster a better understanding of the cellular processes involved in maintaining genomic stability [1-4].

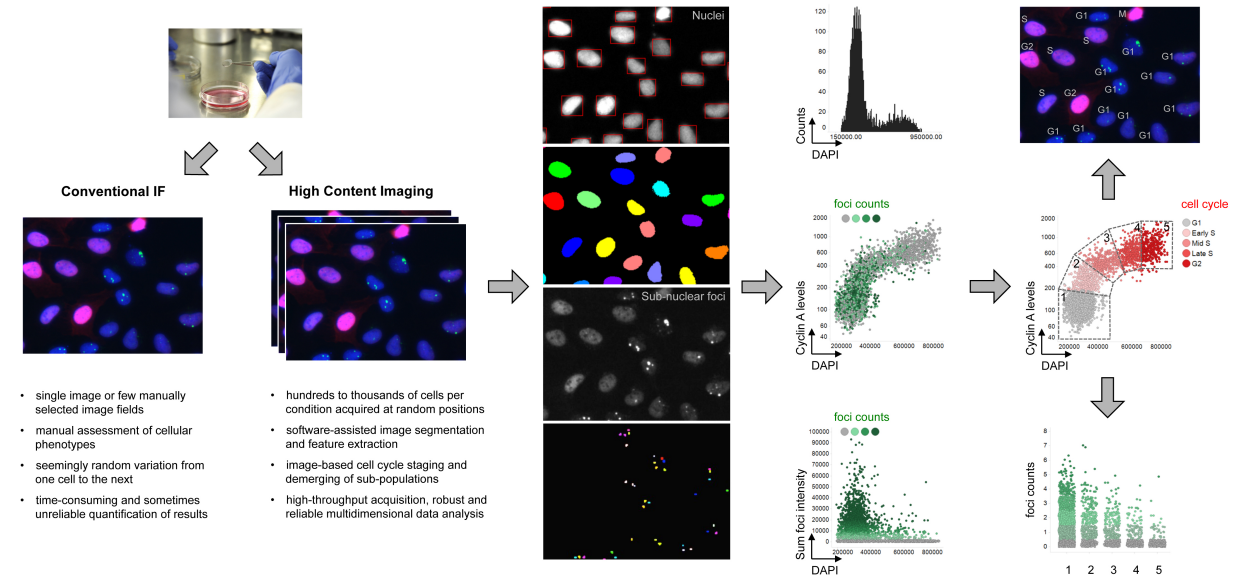

Figure 1: High Content Imaging for multidimensional cell analysis. Starting from conventional immunofluorescent staining (IF), high-throughput microscopy combined with computer-assisted image segmentation (to automatically detect, for example, cell nuclei) and automated image analysis can quantify cellular stress responses in large cell populations and correlate them to the cell cycle. Based on DNA staining (here by DAPI) one-dimensional or, in combination with another cell cycle marker (here Cyclin A), two-dimensional cell cycle profiles can be generated. Cellular phenotypes can thus be quantified in sub-populations and the results compared with the original images.

How replication stress is inherited to the next generation of cells

Like us humans, our cells are not immune to stress. For example, stress often occurs during DNA replication if the genetic material is to be copied correctly within just a few hours. This is usually done with astonishing precision, but sometimes it comes to mistakes and certain sections of the DNA are not completely or not correctly replicated before the cell enters the next cell cycle phase. The more stress a cell is exposed to during DNA replication, the more often such mistakes occur. Interestingly, these errors do not automatically mean that the cell stops in its cell cycle or even dies. Some of the DNA lesions originating from the S phase of the cell cycle persist until mitosis and are even inherited in the next cell generation [5,6]. But what effect does this have on the daughter cells? Using high-content imaging, we were able to quantify both the inherited replication stress-induced lesions in the daughter cell generation and measure the impact on the next G1 phase [7]. Combined with time-lapse microscopy experiments using suitable markers to determine G1 length, this revealed that replication stress and its associated burden on genome stability lengthened the G1 phase and consequently delayed entry into the next S phase *(Figure 2)*.

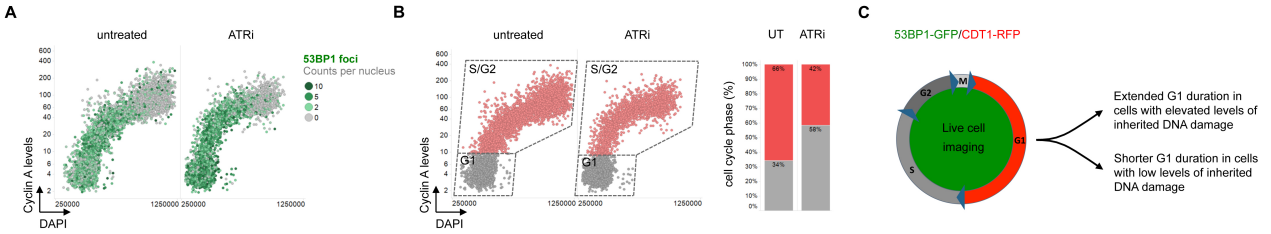

Figure 2: Replication stress affects the duration of the G1 phase in daughter cells. (A) Replication stress, here caused by inhibition of the checkpoint kinase ATR (ATRi), increases the number of DNA lesions (marked by accumulation of the protein 53BP1 in sub-nuclear foci). **(B)** Inherited DNA lesions prolong the G1 phase and lead to an accumulation of damaged cells in this phase of the cell cycle. **(C)** By performing live cell imaging of cells expressing both a DNA damage marker (53BP1-GFP) and a marker for the G1 phase (BacMam CDT1-RFP), inherited DNA lesions and G1 duration can be correlated directly at single cell level.

Interestingly, the delayed entry into the next S phase was dependent on the tumor suppressor protein p53, which is frequently mutated in cancer cells. Thus, it is conceivable that inheritance of DNA lesions may synergize with p53 dysfunction during carcinogenesis, to promote an accumulation of mutations when cells prematurely re-enter S phase *(Figure 3)*. Together with recent data from other groups [8-11], these findings may explain why proliferating cells do not transition synchronously from G1 to S phase, but remain in G1 for varying time depending on the stress level of the previous generation [12].

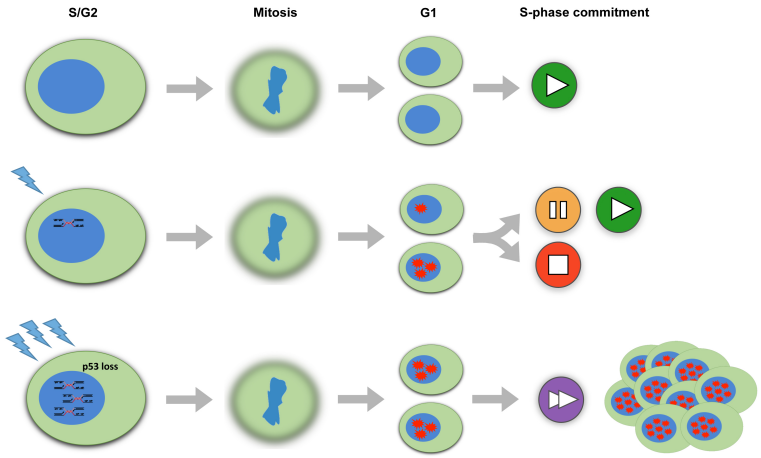

Figure 3: Transmission of replication stress and DNA damage from one generation of cells to the next. Depending on the severity of inherited DNA damage following replication stress, the cell has the ability to rapidly enter the next S phase (play), prolong G1 phase (pause and resume), or prevent re-entry into the cell cycle (stop). These possibilities are largely regulated by the tumor suppressor protein p53. If p53 is missing or mutated, cells prematurely enter the next S phase, further aggravating replication stress and resulting in a build-up of mutations (cancer risk, fast forward).

Acknowledgments

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