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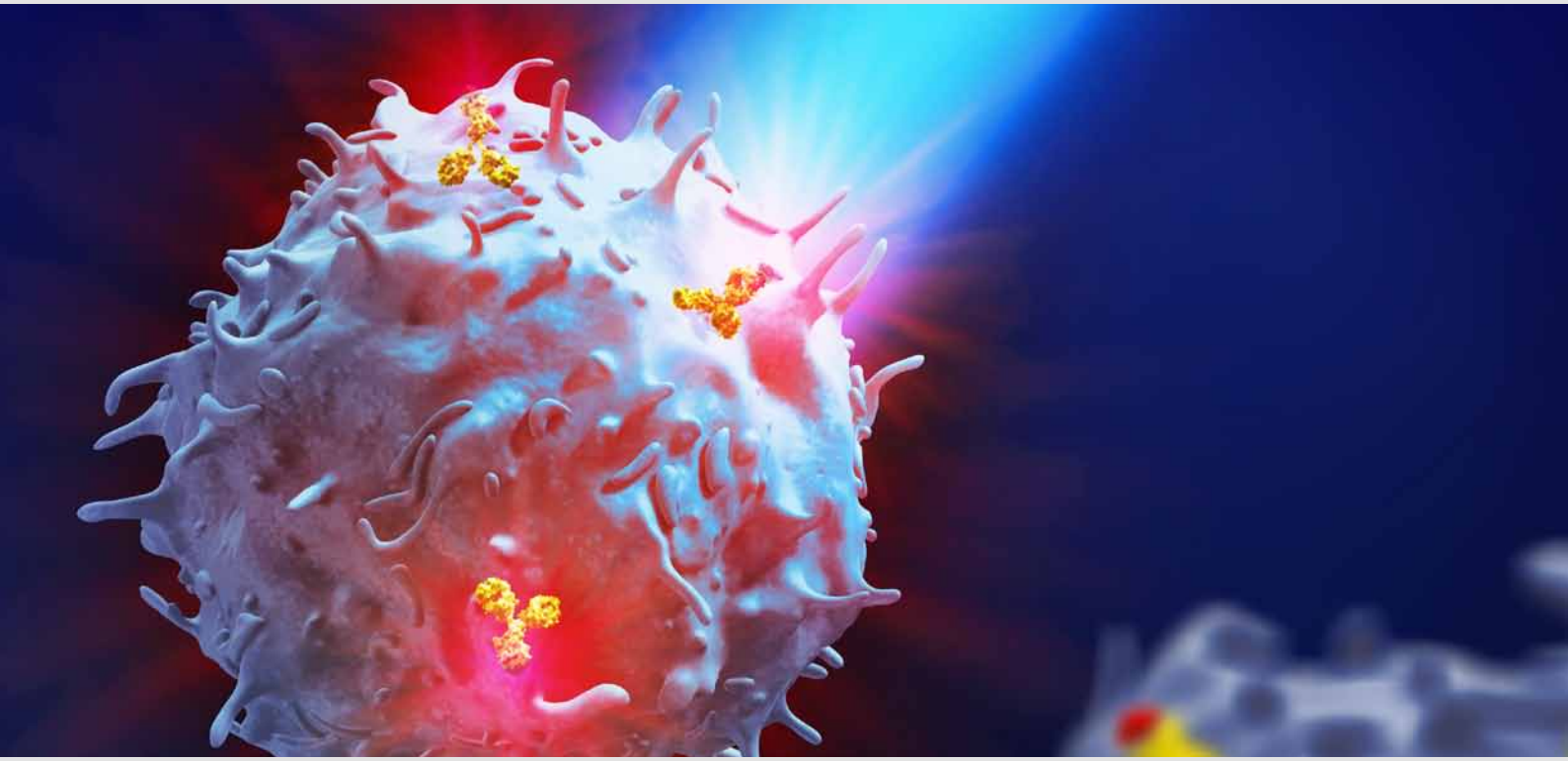

Identification of different *Plasmodium falciparum* life cycle stages in human red blood cells using the MACSQuant® Analyzer

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Introduction

Plasmodium falciparum is the most deadly of the four *Plasmodium* species that can cause malaria and is responsible for almost a million deaths every year. *P. falciparum* is an obligate intracellular parasite that has a complex life cycle with the sexual stages occurring within the mosquito host and the asexual stages occurring within the human host. In the laboratory, we can culture the asexual stages within human red blood cells (RBCs) and these are the stages that are responsible for all the clinical manifestations of the disease. The parasite divides within RBCs via schizogony, whereby each parasite undergoes several rounds of division to give rise to 16 to 32 daughter cells that then burst out of the host cell to trigger a new round of infection. The application of flow cytometry techniques to this field has revolutionized the study of this important pathogen. Given that RBCs have no nuclei, we can follow parasite growth very easily by using fluorescent DNA dyes, e.g., acridine orange (AO). The sensitivity of the MACSQuant® Analyzer even allows us to distinguish between the various asexual stages by detecting the distinct differences in their DNA and RNA content.

Materials and methods

P. falciparum was cultured in human RBCs as described[1]. For the routine analysis of parasitemia, RBCs were incubated with AO. AO binds to *Plasmodium* DNA and RNA within infected RBCs, which can then be detected flow cytometrically.

5 µL of the parasite culture were diluted in 100 µL of AO (1.5 µg/mL in PBS) and incubated for 20 minutes at 37 °C. Cells were then applied to the MACSQuant Analyzer at the medium-flow setting. Green and red AO fluorescence signals were detected by FITC and PerCP/Cy™5 channels respectively.

Results

Total RBCs were detected by their forward and side scatter properties (fig. 1A).

Infected, AO-stained RBCs were analyzed via FITC and PerCP/Cy5 channels (fig. 1B and C). The fraction of infected RBCs amounted to 6.27%. The MACSQuant Analyzer's high sensitivity even allowed us to discriminate between RBCs carrying different *Plasmodium* life cycle stages. The AO-positive cells can be differentiated into ring stage parasites (fig. 1C, light green box), trophozoite stage parasites (light blue box) and schizont stage parasites (yellow box).

Discussion

The possibility of following the different life cycle stages with the MACSQuant Analyzer allows us to rapidly estimate drug IC_{50}s and do chemical screens for malaria drug discovery.

In the past, we used a hemocytometer to

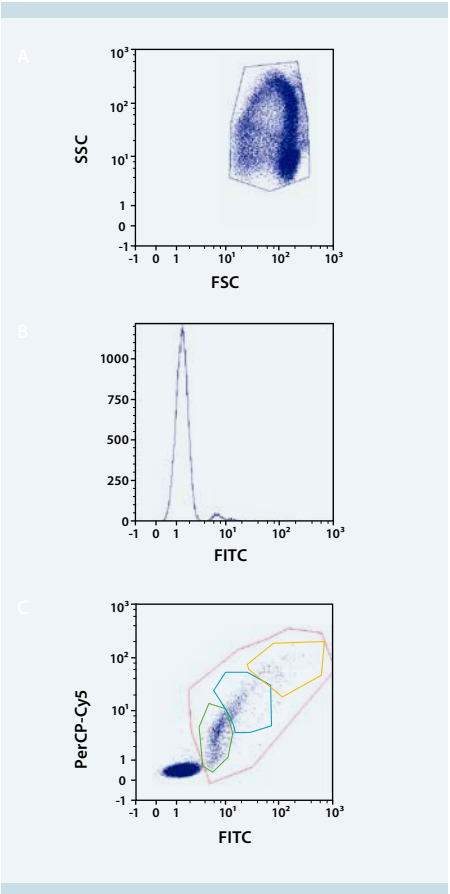

Figure 1 Flow cytometric analysis of RBCs infected with *P. falciparum*. Total (A) and infected RBCs (B, C) were detected with the MACSQuant Analyzer.

estimate absolute parasite numbers for various applications. The ability of the MACSQuant Analyzer to provide absolute cell counts has now opened up the possibility of doing high-throughput experiments that were previously impossible using a hemocytometer.

One important and routine aspect of our research involves isolation of clones via limiting dilution. We used to estimate the appearance of clones via change in media color. However, the sensitivity of the MACSQuant Analyzer now allows us to confirm the appearance of clones much earlier and with more confidence. This has considerably sped up our experimental timeline.

We often study protein function by tagging the gene with GFP. In the past, we used to screen our clones manually using a fluorescence microscope, which was both time-consuming and labor-intensive. Since the MACSQuant Analyzer has three lasers, we can use orthologous DNA stains that allow us to screen our clones rapidly for GFP fluorescence.

The malaria parasite digests the hemoglobin within the RBC to utilize it as a source of amino acids. The free heme moiety is crystallized within a special vacuole. The heme crystal, known as hemozoin, is magnetic, and is only present in certain life cycle stages of the parasite. The MACSQuant Analyzer with the built-in MACSQuant Column and MACSQuant Cell Enrichment Unit now allows us to isolate and study these life cycle stages in particular.

Conclusion

The MACSQuant Analyzer has changed our research for the better. It provides us with the ability to perform high-throughput experiments, changed the way we do several routine experiments, and allows us to achieve results in a much shorter time frame. It is an important tool that has improved our research on a terrible but difficult-to-study human pathogen.

Reference

1. Drew, M.E. *et al.* (2008) J. Biol. Chem. 283: 12870–12876.

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MACSQuant Analyzer	130-092-197