

Flow-cytometric diagnosis of malaria

Malaria is still one of the most important parasitic diseases, killing around one million people each year. The reliable detection of the parasites is crucial for diagnosis as well as research. This includes detection and differentiation of asexual and sexual forms, the determination of the different maturation stages, identification of the species, reliable quantification of parasites as well as assessing viability and metabolic state. Considering the size of the parasites and their intra-erythrocytic localization, flow cytometry, which was developed for high-speed single cell analysis, appears to be an ideal method. Notably, over the last decade instrumentation has seen many changes, including the use of cheaper solid state lasers, which led to much lower prices. Small, robust, “portable” multi-parameter instruments are now available which often allow the user to assess and modify the optical bench easily, even between different measurements. Additionally, most have now an optional autosampler for 96 or 384 micro-well plates. Flow cytometry is also part of modern haematology analyzers, usually to analyze leukocytes, reticulocytes and platelets. Today, several nucleic acid stains, including DNA and RNA specific stains, are available to detect the parasite inside erythrocytes. Green-Fluorescent-Protein transfected strains have become common in research applications. Availability of diverse metabolic and viability probes, especially membrane potential probes, make it possible to assess the metabolic activity, interesting in applications to measure drug effects. Finally, the simple detection of the malaria pigment (haemozoin), both in granulocytes/monocytes or in parasitized erythrocytes, has been shown to be very useful for several new applications. Importantly, it allows diagnosis of malaria with automated haematology counters during routine FBC analyses. However, using simple measurement of depolarized Side-Scatter, easily installed on almost any flow cytometer, it allows the detection of parasite maturation which is useful as sensitivity test, and it may allow to distinguish gametocytes from asexual forms for research applications, like investigation of transmission blocking agents.