Disruptive Technologies in Haematology for POCT Market

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A blood count is one of the most common blood tests that provides valuable physiological information about the patient's state. The tests are generally performed in specialized laboratories using "Gold standard" high-throughput diagnostic instruments. These automatic haematology analysers are bulky, need qualified operators for sample and data manipulation, and have more maintenance-related requirements. They also must respond to a growing demand for new complex parameters, complexities that are not required for urgent screening at the Point-of-Care. In this article, we present disruptive technologies for sample preparation and cell detection allowing the development of new generic platforms in haematology for a wide range of Point-of-Care applications.

key words
blood count, sample preparation, cell detection

Introduction

Blood count is one of the first prescribed and most ordered tests by doctors as it generally constitutes the first step in the diagnosis and monitoring of many pathologies and treatments. It provides physicians with valuable information on the patient's physiological state, allowing them to make treatment decisions. Nowadays, most automated hematology laboratory analyzers are based on costly, bulky, reagent consuming and complex hardware systems for blood preparation and measurement. Moreover, highly qualified and specialized laboratory technicians are required to handle such instruments.

Today and tomorrow's world and society are shaping up new challenges for the healthcare systems such as the one generated by the Covid 19 pandemic. Therefore, for delocalized and personalized health care applications, there is an increasing need for automated, compact and transportable point-of-care blood cell counters, operable without requiring any training or maintenance. Such solutions would eliminate the need for patients to travel into lab testing facilities and shorten the time needed for doctors to make treatment decisions. Point-of-care devices can be suitably deployed at the bedside, private clinics, research laboratories, rural areas as well as in developing countries.

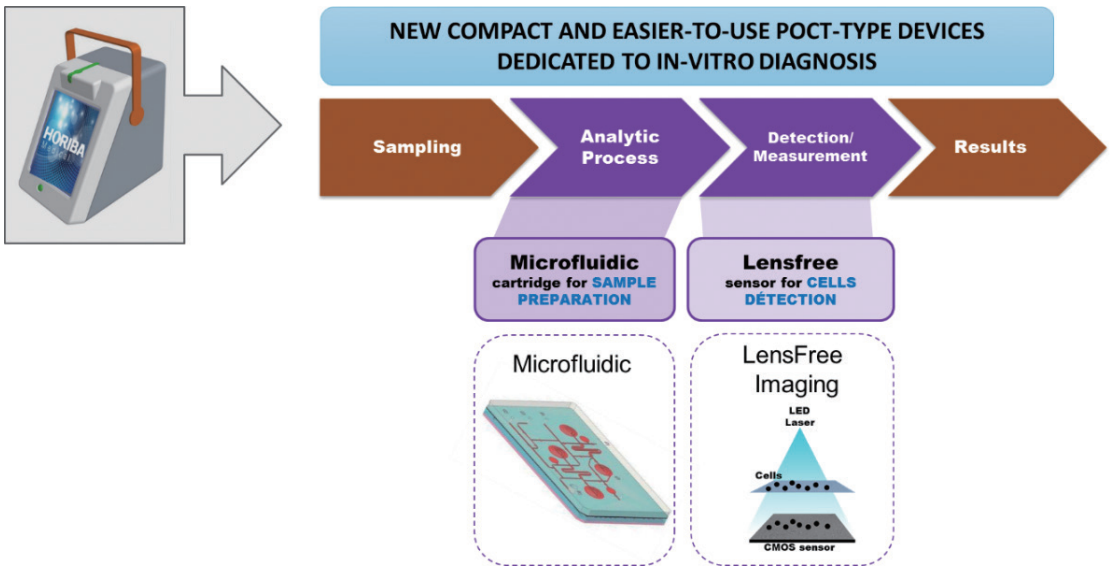

Figure 1 Scheme of the blood analyser principle based on innovative disruptive technologies

Using simple and smart technologies for blood preparation and cell measurement, low power and reagent consumption, these future low cost automated and miniaturized blood cell counters offer a genuine route towards a greater portability of hematology analyzers.

The work presented here is result of an "open innovation" collaborative program held since 2015 with a leading player in technologies development, the French Atomic Energy Commission (CEA), which is the biggest patent applicant in France. By combining their own expertise, both parties can accelerate the delivery of impactful technology solutions (Figure 1).

In this paper, we introduce first a new disruptive technology for cells detection, counting and differentiation, based on holographic lens-free imaging. Since all the optics and mechanical parts are removed, this low-cost technology, embedded in a device, should lead to ease-of-use with minimal operator training and remove the need for user maintenance. Secondly, we introduce an innovative microfluidic preparation cartridge for automated blood cell counters allowing low consumption and scaled-down integration. The whole blood preparation system embedded in these POCT devices answers different key challenges that need to be addressed regarding microfluidics technology: i) a large range of dilution, ii) dilution accuracy, iii) low cost per test and iv) maintenance free.

These two patented technologies[6, 7, 8] are well adapted to a POCT system, and, in the future, will enable us to propose a different approach to hematology, closer to the patient who needs it.

Lens-Free Imaging

The Lens-Free Technology (LFT) is a simple imaging technique, developed in recent years and under continuing evolution and improvement. Based on in-line holography and numerical processing, LFT gives access to a reconstructed optical field revealing phase and absorption contrasts of the studied samples, which can be cells, particles, crystals, etc... It can generate highly contrasted images of biological objects on a large field-of-view with a micrometer-scale resolution using a very simplified optical setup without magnification. The components of a lens-free imaging system are uncomplicated, small and low-cost. In addition, the wide field of view, together with the high resolution of the sensor, leads to a count (> 10 000 cells) in a single-shot acquisition, consistent with the statistical performances required in haematology. The complexity of the system is transferred to digital processing requiring robust and powerful reconstruction algorithms. Hence, this technology allows the building of an automated, simple, cost-effective, robust, light weight and compact system meeting the requirements for screening point-of-care (POC) tests in human and veterinary markets (Figure 2).

In practice, the sample is simply positioned in a chamber between a light source and a high-resolution optical sensor, for example, a Complementary Metal Oxide Semiconductor (CMOS) (typically a resolution of around 10 MPix and 2 µm pixel size). This sample is illuminated from above by the point-like source of light and the sensor records interferences patterns (holograms) created by a combination of the light diffracted by the objects and the transmitted light. The raw data are then computerized with a numerical reconstruction algorithm, and dedicated

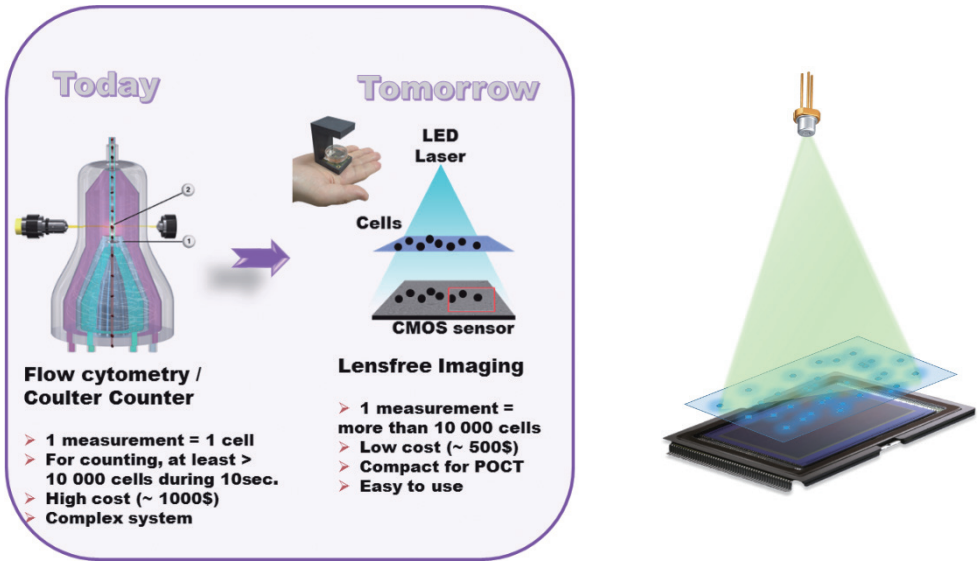

Figure 2 The advantages of the LensFree Imaging system, compared to classical Flow Cytometry setup

image processing workflows are specifically designed for each new application. Briefly, the reconstruction step allows, starting from the raw diffraction patterns acquisition, the reconstruction of an in-focus absorption map. From the acquired defocused hologram, classical gradient autofocus algorithms were applied to compute the position of the object plane (i.e. the focus plane). In this selected plane, an iterative reconstruction algorithm from a single image based on specific norm minimization is used. On this reconstructed image, grey-level thresholding is performed to detect the more contrasted objects. A binary mask is created: a 0 value is associated to the background, and a 1 value is assigned to the detected components. At this stage, there are between hundreds and thousands of components, depending on the sample. The detected components are sorted out based on morphometric characteristics or finer supplementary criteria using grey-level. This leads to the identification and characterization of most of the blood cells, depending on the considered application (Figure 3).

This new technique for measuring cells requires increased computation capacities. We implemented algorithms on an Nvidia Jetson Nano Graphical Processing Unit (GPU)

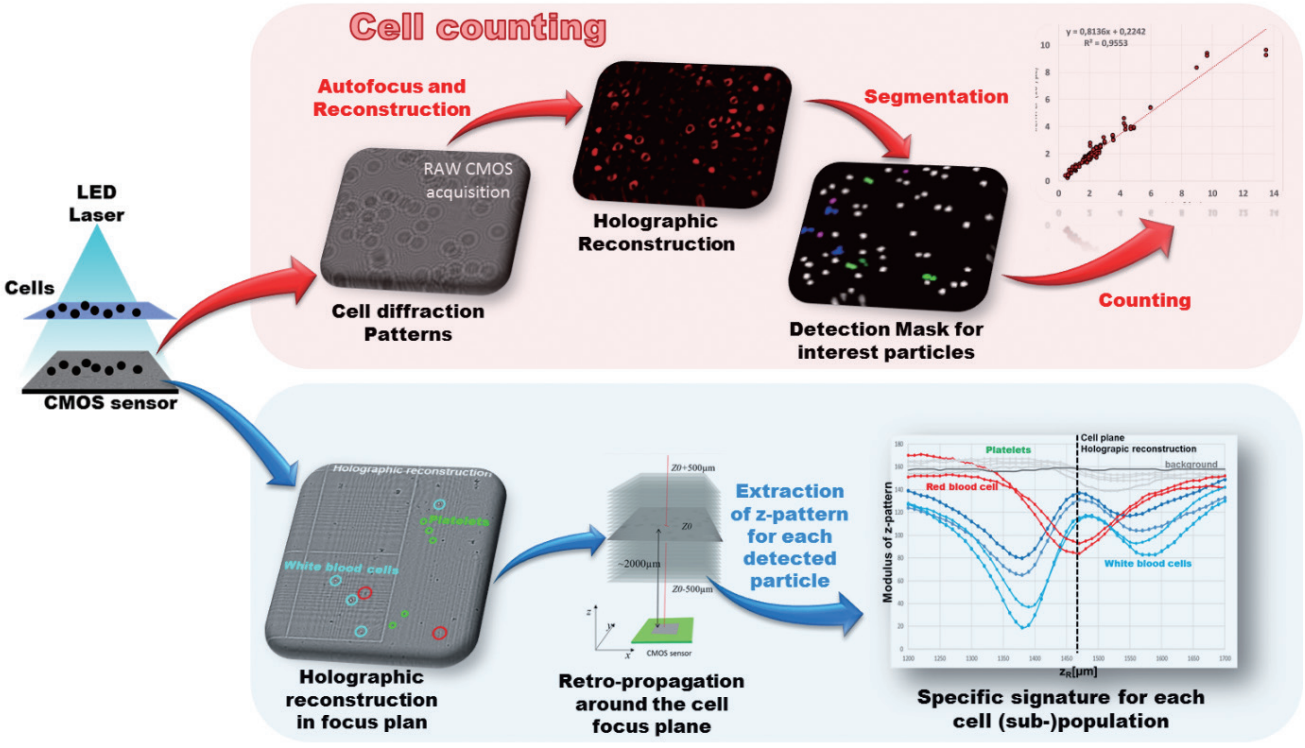

Figure 3 The steps of the algorithm for image reconstruction and cells detection and identification.

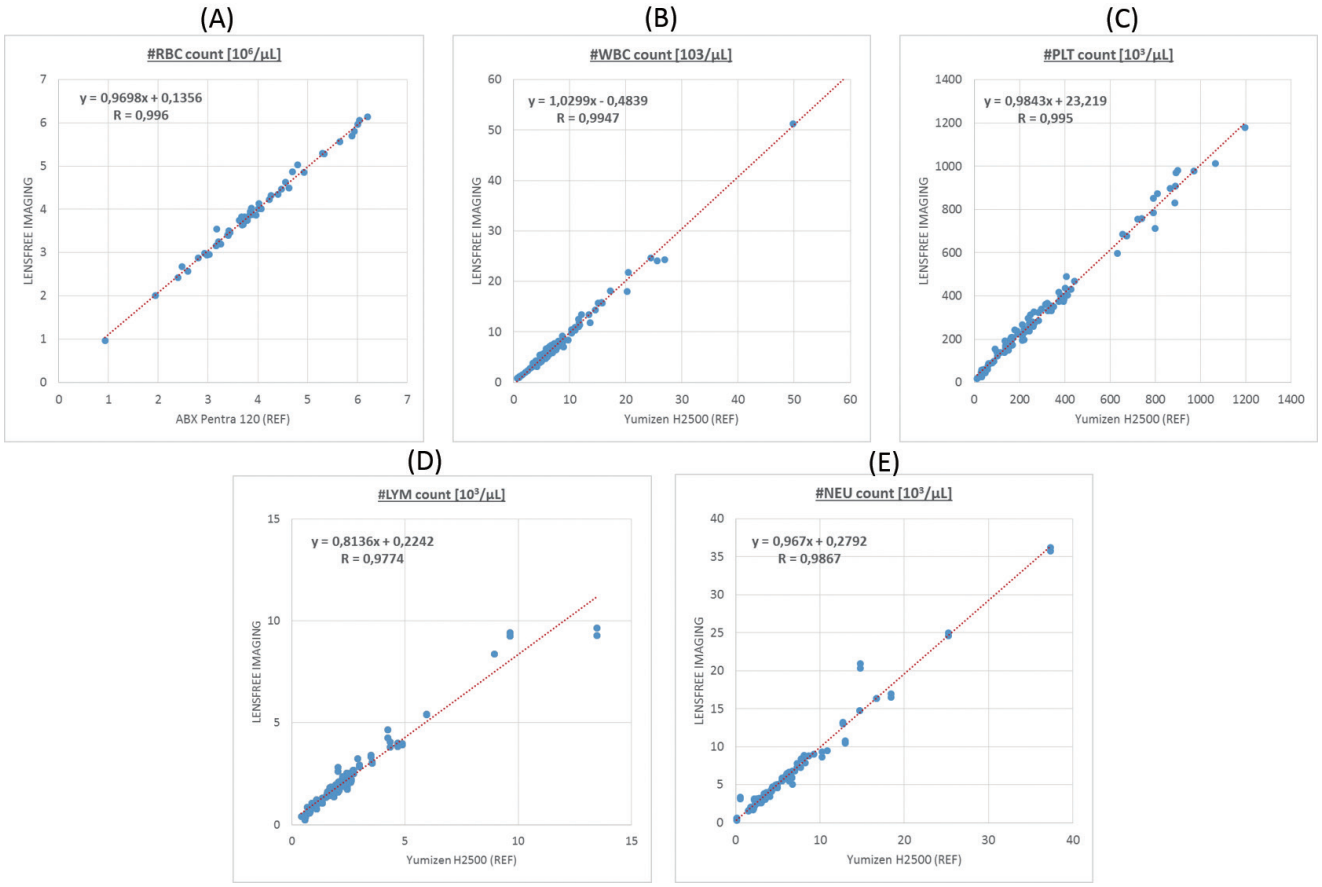

Figure 4 Summary of haematological analytical performances obtained with LFT in comparison with classical measurement systems embedded on current HORIBA Medical routine analysers

for massive parallelization. Increasing connectivity and the versatility of this sensor allow us to consider various and remote application fields.[2]

As a proof-of-concept, several applications have already been investigated with LFT, in the human and veterinary haematology field. We display, in Figure 4, performances obtained for red blood cells (RBC), white blood cells (WBC) and platelets (PLT) counting, as well as for lymphocyte (LYM) and neutrophil (NEU) identification, in comparison with results obtained with our reference HORIBA Medical analyzers (ABX Pentra 120 and Yumizen H2500).

Those preliminary results show good agreement between the LFT and conventional hematology analyzers on this set of parameters. No major discrepancies were observed among the different sample database evaluated (more than 100 normal and pathological samples). R correlation parameters are all located above 0.95.[1, 3, 4]

Microfluidic Preparation

In this project, the main objective is to develop microfluidic cartridges allowing precise preparation of small samples with great accuracy and repeatability, and scaled-down integration for the associated analyzer. However, developing such a system with a small footprint comes with several challenges regarding microfluidics technology such as: sample introduction, reagent management, efficient fluid sample mixing, low cost-per-test and more importantly, large yet precise dilutions (Figure 6a).

To reach the desired high dilution ratios of the small blood samples and still achieve high precisions, we opted for a technology based on a hyper-elastic membrane to create collapsible chambers with large volumes and high aspect ratios (Figure 6b). Optimal configuration of these chambers and channels was reached through extensive Computational Fluid Dynamic (CFD) simulation studies using the YALES2BIO software (Figure 5). This technology offers a high-volume precision as the amount of used liquids is directly controlled by the chamber size. Futhermore, the microfluidic cartridge can be easily replaced by a “Plug & Play” system, leading to a maintenance free system.

For a first application case as a proof-of concept, the cartridge was designed to handle two precise dilutions simultaneously, one at 1/1000 ratio and another one at 1/10 ratio. The first dilution of the whole blood is performed for counting the RBCs and the second dilution for

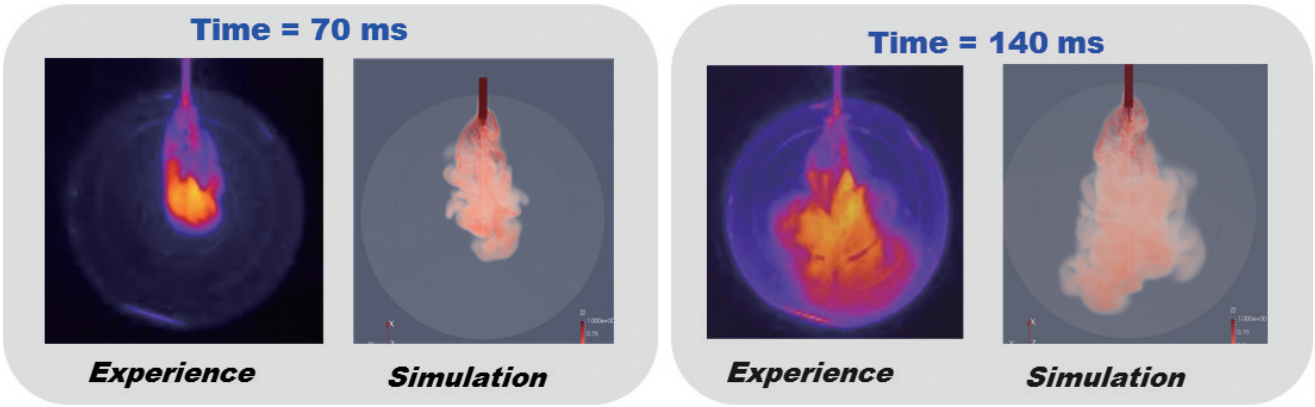

Figure 5 Comparison of sample injection on a dilution chamber. Experience confirms optimal results obtained by CFD computing.

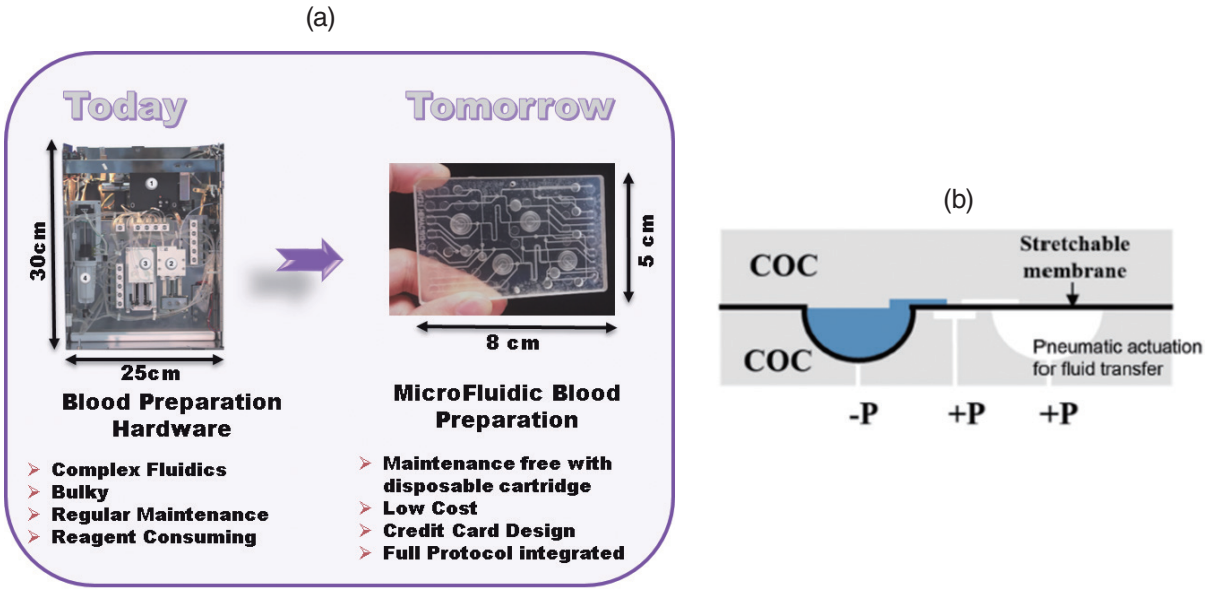

Figure 6 (a) The advantages of the Microfluidic preparation, compared to classical technology, (b) Principle of FlowStretch Technology. Chambers and valves are actuated using pneumatic pressure (COC : Cyclic Olefin Copolymer)

counting the WBC and the PLTs, and for the Hemoglobin measurement. During this second dilution, a lysis of RBCs is performed. This lysis step is necessary for two reasons: first, RBCs are numerous and can hinder the proper visualization of WBCs. Second, the lysis of RBCs allows the release of hemoglobin in the solution, and in this way the measurement of the hemoglobin concentration can be performed.[5]

To achieve the dilution protocol, a specific architecture and an associated protocol is designed. The several zones for different preparations are visible on Figure 7(a) one sample preparation for RBC counting (Zone I), and another one for WBC/PLT counting and Hemoglobin measurement (Zone II). The whole blood is first sampled in two successive cali-brated meandershaped channels (Figure 7(b)). Each meander channel is connected to two stretchable chambers that can be filled with reagents. By successive actions of the two opposed chambers, the calibrated blood sample is efficiently and quickly diluted and mixed with calibrated reagent (Figure 7(b)). The precise and repeatable dilution ratio is simply provided by the ratio volume of the meander channel and collapsible chamber. Thus, the resulting dilution ratio is generated solely by the geometry of the cartridge and does not depend on fluid properties. Repeatability performances obtained for blood parameters with microfluidic preparation are shown in Figure 8 (Left). In Figure 8 (Right), the correlation of Hemoglobin measurement obtained with mock-up coupling Microfluific sample preparation and LensFree Imaging sensor and the one obtained with classical routine analyser is depicted.

Figure 8 (Left) shows a good agreement between the expected statistical performances and the actual Coefficient of Variation (CV) measured during repeatability standard operating protocols. For each parameter, the actual value is slightly higher than the target. This is a normal effect of the variability introduced by the hardware such as the pneumatic driving of the actuated

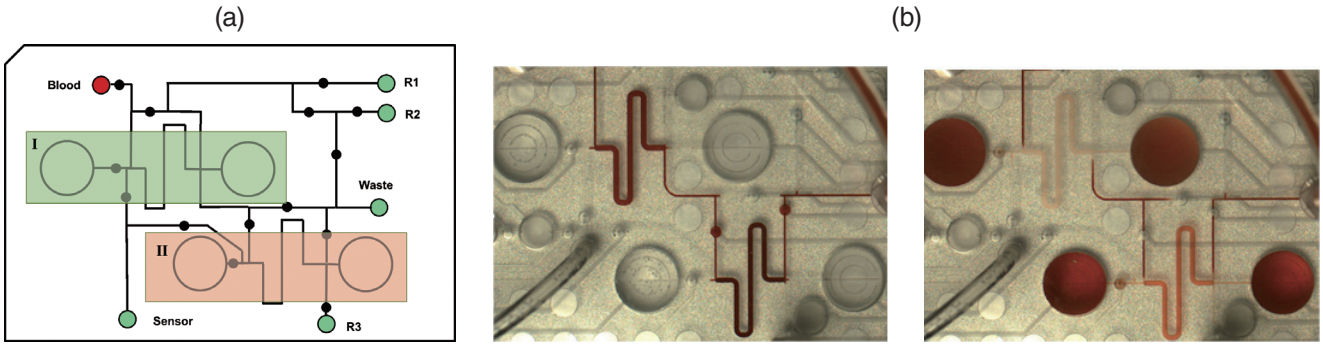

Figure 7 a) The architecture of the microfluidic cartridge with the two regions corresponding to the two performed dilutions. b) Representative images of the sampling and mixing steps in the meander shaped channel with the dilution chambers

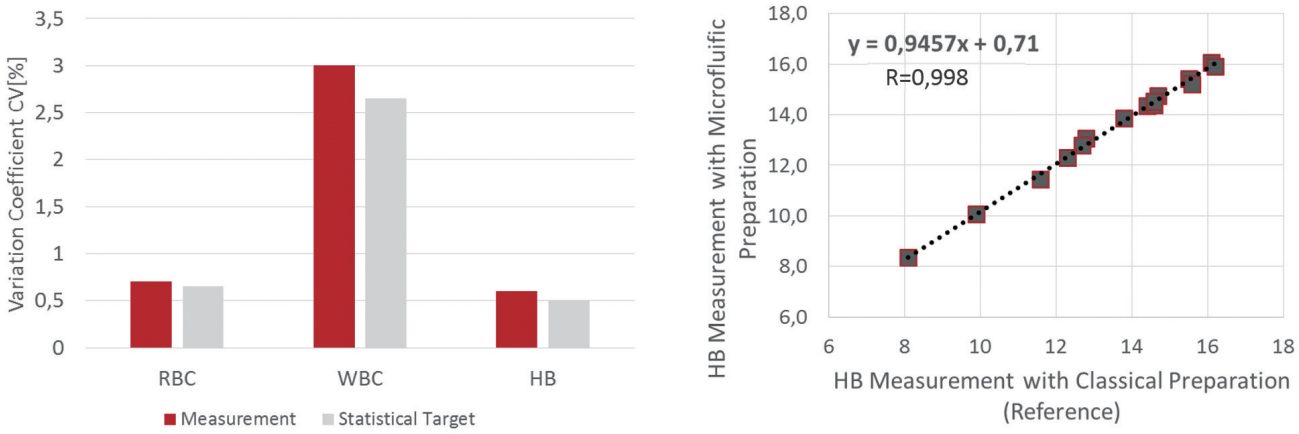

Figure 8 (Left) Repetability performances for blood parameters with microfluidic preparation - (Right) Performances on Hemoglobin Measurement with mock-up coupling Microfluific sample preparation and LensFree Imaging sensor

membrane. Likewise, the correlation on the hemoglobin parameter (Figure 8 Right) shows a good agreement with our qualified internal reference HORIBA Medical Yumizen H2500 It suggests an accurate preparation of the blood sample, from its sampling to the lysis, the dilution and the measurement of concentration.

Conclusion

These innovative technologies pave the way to the development of new generic platforms for a wide range of POCT applications. The technology leads that we are following are opening a new way of performing diagnostics. By reducing the hardware complexity and the footprint of the instruments, we may now envision smaller, more reliable and more versatile devices. Whether it is at the doctor's office, closer the patient's bed at the hospital, or somewhere remote in the field, we propose two key assets for building more efficient ways of delivering the patient the care he/she needs. We have demonstrated a proof-of-concept feasibility on essential parameters. We now need to work on the enhancement of the algorithms involved in the computation of the LFT results. Indeed, since our primary goal is to deliver impactful insights to the physicians, we need to ensure now that we can address pathological conditions as well as healthy ones.

* Editorial note: This content is based on HORIBA's investigation at the year of issue unless otherwise stated.

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