POINT-OF-CARE DEVICES

Holographic diagnosis of lymphoma

A holographic approach relying on small-molecule chromogens enables a rapid and inexpensive test for the accurate classification of aggressive lymphoma at the point of care.

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About 70% of cancer-related mortalities occur in low- and middle-income countries (LMICs)[1]. Advanced techniques for cancer diagnosis such as immunohistochemistry, flow cytometry and genetic analysis usually require complicated equipment and highly trained professionals — two assets that are commonly lacking in LMICs[2]. Portable and inexpensive diagnostic tools with 'sample-in, answer-out' ability that can both detect cancer and classify the cancer-positive samples according to malignant phenotypes are critical for the proper recommendation of treatment in LMICs. Writing in *Nature Biomedical Engineering*, Ralph Weissleder, Cesar Castro and co-workers now report a point-of-care (POC) device that uses contrast-enhanced micrography (CEM) and machine learning for the accurate classification of lymphoma[3]. In addition, the researchers tested the device's capabilities in a small prospective trial.

Lymphoma — a cancer of the immune system — targets lymphocytes, which are responsible for fighting infections in the body. Previously, Weissleder and colleagues had developed a smartphone-based system for the detection of cancer cells. The system

used microbeads that, when bound to specific biomarkers on the surface of the cells, caused diffraction[4] and holographic patterns (scattered light from the cell surfaces)[5]. However, diagnosing lymphoid cancer at the POC remains challenging because of the small size of lymphocytes, their neoplasticity and the variety of relevant surface biomarkers.

Weissleder and co-authors now report the development of a device to detect holographic patterns of small-molecule chromogens. These are substances that can be readily converted to a dye when oxidized and used to detect intracellular markers (such as Ki67, a biomarker for proliferation). The device is portable (Fig. 1a), costs about US$180, weighs approximately 1.4 kg, and can be powered by using either a power supply or a battery, thus making it suitable for POC applications. The authors also developed a disposable microfluidic cartridge with functionalized antibody chemistry to selectively capture B lymphocytes from fine-needle-aspirate samples. This cartridge is inserted into the device, which performs immunostaining against target surface markers (including Ki67) on the B cells (Fig. 1b). The authors

recorded a set of holographic images of these signals by using a complementary metal-oxide semiconductor (CMOS) sensor typically used in digital cameras. A machine-learning algorithm used these images to distinguish B cells from sample debris, artefacts and noise, and to determine the number, size and subtype of cancer cells on the basis of specific cell-surface markers that lymphocytes express. The decision algorithms to diagnose lymphoma were based on the World Health Organization guidelines for classifying lymphoid cancers. The holographic CEM images (Fig. 1c) can be recorded and analysed on the device in about 5 minutes, or via a cloud server in about 12 seconds (analysing the results via remote hardware would lower the cost of the device itself, yet it would require a stable Internet connection).

The research team tested the CEM device on 40 patients in the United States. The machine-learning algorithm accurately detected lymphoma with 91% sensitivity and 100% specificity. In addition, the device showed 86% accuracy in distinguishing aggressive and indolent lymphomas. Compared with clinical flow cytometry, the CEM device showed both higher sensitivity
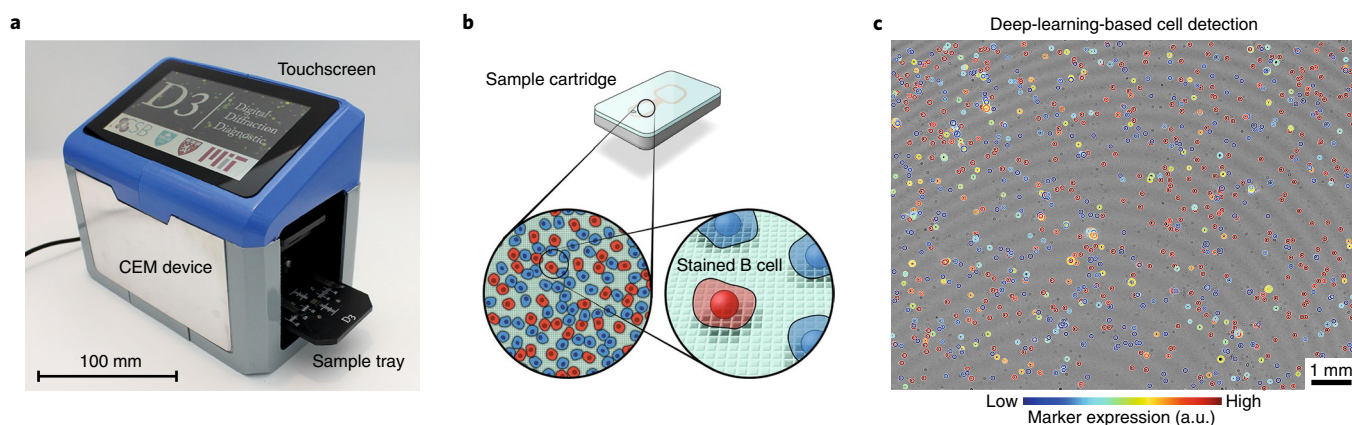
Fig. 1 | Standalone device, based on contrast-enhanced microholography, for the diagnosis of lymphoma. a, The portable device. **b**, Schematic of the microfluidic cartridge, and of on-chip antibody labelling and immunostaining. **c**, A computational-neural-network algorithm trained on holographic cellular images of lymphoma identifies B cells and their locations in samples from fine-needle aspirates. Figure adapted from ref. [3], Springer Nature Ltd.

and classification accuracy, and was able to successfully analyse a higher proportion of clinical samples because it required fewer cells for the diagnosis.

For widespread use in POC settings, affordability and adaptability of a molecular diagnostic test are crucial. Weissleder and co-authors plan to develop an inexpensive 'self-contained cartridge' that uses lyophilized reagents to enable the use of the device in LMICs where the cold chain cannot always be maintained. The complete assay currently takes 3 hours, meaning that sample processing needs to be simplified, also because POC devices are intended to be used by individuals with minimal technical expertise. A large field trial with a robust device in the hands of local healthcare providers would aid in determining the suitability of the device for LMICs.

Although other research groups have developed microfluidic devices with magnetic arrays for sorting B cells, and have also diagnosed lymphoma by using high-resolution confocal three-dimensional images[6], these devices are at present less appropriate for use in LMICs because they require expensive microscopes for analysing the results. Alternatives to cell-based biomarkers, such as circulating microRNAs (miRNAs), are currently under investigation for cancer diagnosis[7]. For example, elevated miRNA levels have been reported in patients with B-cell lymphoma[8]. Commercial systems such as the PanelChip Analysis System (Quark Biosciences) are available for quantifying miRNA expression levels and were recently used to distinguish healthy subjects from oral-cancer patients[9] via their miRNA expression patterns. Nevertheless, miRNA technology may not be suitable for LMICs, as it requires complex sample preparation and expensive equipment. Compared with alternatives such as flow cytometry, confocal microscopy and miRNA analysis, the holographic approach that Weissleder and co-workers developed has advantages for its potential use in the diagnosis of lymphoma in LMICs. The diagnostic device may be able to decrease the time to treatment for lymphoma in limited-resource settings because it can serve as a screening tool when trained pathologists are not available. Another advantage of the device is that it can distinguish between benign and aggressive lymphoma, which could help clinicians to make better-informed decisions about treatment. The high accuracy of the device compared with flow cytometry also means that CEM may be broadly useful in the field of cancer diagnostics. ❐

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References

1. Cancer factsheet go.nature.com/2MuHccB (World Health Organization, 2018).
2. Yager, P., Domingo, G. J. & Gerdes, J. *Annu. Rev. Biomed. Eng.* **10**, 107–144 (2008).
3. Im, H. et al. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-018-0265-3 (2018).
4. Im, H. et al. *Proc. Natl Acad. Sci. USA* **112**, 5613–5618 (2015).
5. Pathania, D. et al. *Theranostics* **6**, 1603–1610 (2016).
6. Saliba, A.-E. et al. *Proc. Natl Acad. Sci. USA* **107**, 14524–14529 (2010).
7. Mitchell, P. S. et al. *Proc. Natl Acad. Sci. USA* **105**, 10513–10518 (2008).
8. Lawrie, C. H. et al. *Br. J. Haematol.* **141**, 672–675 (2008).
9. Hsieh, C.-H. et al. *Sci. Rep.* **8**, 10684 (2018).