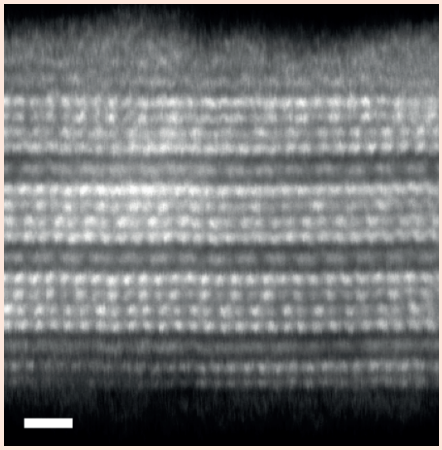
VAN DER WAALS HETEROSTRUCTURES

The natural way

Franckeite is a naturally occurring mineral discovered in Bolivia at the end of the eighteenth century. It belongs to the sulfosalt family and is composed of an alternating sequence of weakly bound, incommensurately stacked PbS and SnS_2 layers separated by van der Waals gaps. The strong compositional segregation for Pb and Sn atoms leads to markedly different electronic band structures for the different layers, whose overall properties may be further complicated by Pb/Sb and Sn/Fe partial substitutions.

Now, writing in *Nature Communications*, two independent research groups report the exfoliation of franckeite into few-layer-thick heterostructures that can be thought of as natural counterparts of an artificial van der Waals heterostructure. Both A. J. Molina-Mendoza *et al.* (*Nat. Commun.* **8,** 14409; 2017) and M. Velický *et al.* (*Nat. Commun.* **8,** 14410; 2017) exploit mechanical and liquid-phase exfoliation processes to obtain ultrathin nanoflakes. The cross-section of a typical sample is shown (from the study of Velický *et al.*; scale bar, 1 nm), visualized by means of high-angle annular dark-field scanning transmission electron microscopy. Remarkably, Velický *et al.* even succeed in exfoliating single-unit-cell-thick heterostructures.



MACMILLAN PUBLISHERS LTD

In comparison with their artificial analogues, franckeite-based van der Waals heterostructures show advantageous properties because the mutual orientation of the different layers is preserved across the nanoflakes and, simultaneously, the occurrences of spurious interlayer adsorbates are kept at a minimal level. Also, the franckeite nanoflakes are stable even after exposure to air for several months — even though Velický *et al.* observe a marked degradation of the thinnest samples and, in general, surface deterioration effects. This stability is reflected in the preservation of the p-type, narrow-bandgap semiconducting behaviour also observed for the bulk material, as confirmed by means of transport measurements in back-gated devices and by scanning tunnelling spectroscopy. This behaviour is in stark contrast to phosphorene, also a two-dimensional p-type, narrow-gap semiconductor possessing comparable electronic properties — yet, not air-stable.

The two groups also demonstrate possible technological applications for the considered nanoflakes. Molina-Mendoza *et al.* focus on the moderate bandgap values (~0.7 eV) to investigate the potential of few-layer franckeite as a near-infrared photodetector. Moreover, exploiting the p-type semiconducting character, they fabricate proof-of-principle p–n photodiodes made of few-layer franckeite and n-doped few-layer MoS_2. Velický *et al.* focus instead on the electrochemical properties of exfoliated franckeite samples and, in particular, show good electric double-layer capacitance and high electron-transfer rate values. These aspects suggest the possible exploitation of few-layer franckeite for applications in energy-storage and conversion applications.

GIACOMO PRANDO

NANOMEDICINE

Catching tumour cells in the zone

A microfluidic chip with progressively stronger magnetic field gradients along its length can sort and classify circulating tumour cells based on the expression of cell surface markers.

Susan E. Leggett and Ian Y. Wong

Circulating tumour cells (CTCs) disseminating through the bloodstream represent a missing link between the initiating tumour and its metastatic colonies[1]. The isolation and analysis of CTCs from patient blood samples could enable noninvasive 'liquid biopsies' for the early detection, diagnosis and prognosis of cancer[2]. However, CTCs are highly heterogeneous and extremely rare (1–10 CTCs per billion blood cells), making them difficult to detect and isolate. CTCs have been separated using size-based filtration or other microfluidic techniques as they tend to be larger than blood cells[3]. Nevertheless, these physical approaches may overlook some subset of CTCs that are comparable in size to blood cells. Alternatively, CTCs may be labelled or captured using antibody-conjugated magnetic nanoparticles, which can be highly effective since unlabelled cells exhibit minimal magnetic susceptibility. Recently, Ozkumur *et al.* used a magnetic field gradient to deflect magnetically labelled CTCs from an inertially focused stream of cells[4]. Issadore *et al.* interrogated magnetically labelled CTCs using miniaturized Hall-effect sensors, which transduce magnetic fields into a change in output voltage[5]. Together, these approaches have primarily been used to either enrich or detect CTCs.

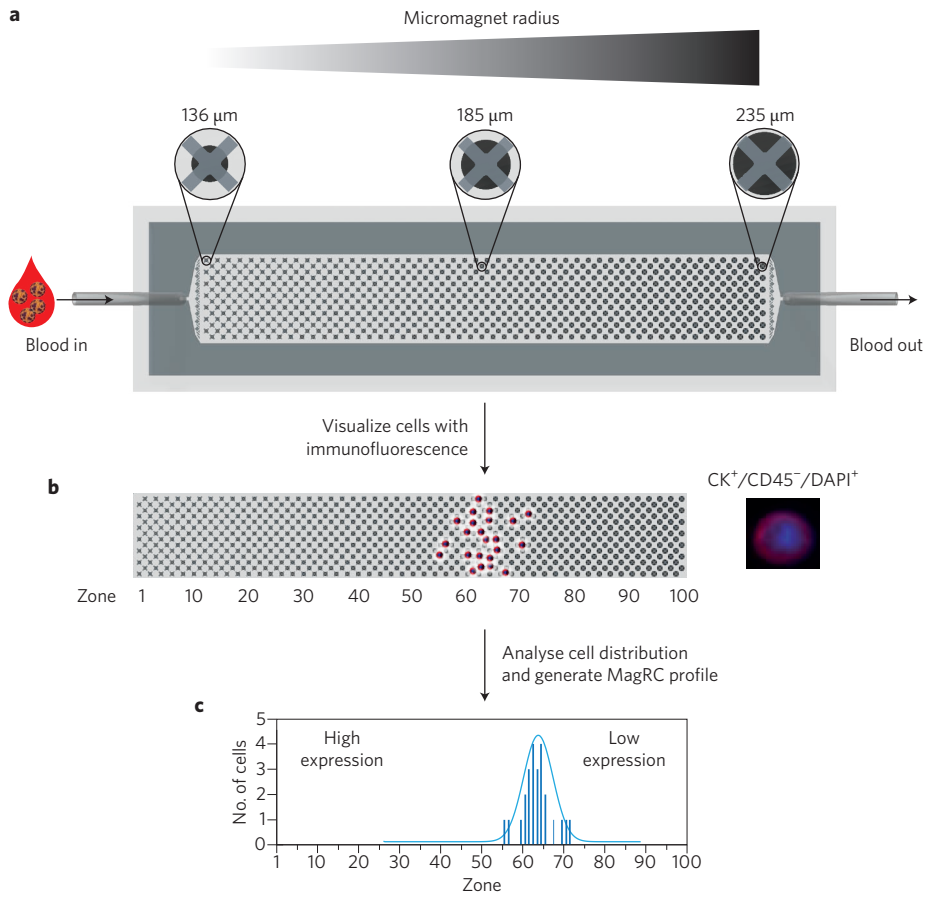


Figure 1 | Microfluidic separation of CTCs using magnetic ranking cytometry. **a**, Blood samples with magnetically labelled CTCs are flowed through arrays of circular micromagnets of increasing radius capped by X-shaped microstructures. The vertical magnetic field gradient increases along the length of the device, deflecting CTCs downwards into slower flows for capture. **b**, CTCs within 100 distinct capture zones are immunofluorescently stained for surface marker expression. **c**, Cell distribution in each zone is analysed and the capture zone is correlated with the expression levels of the cell surface marker. Figure adapted from ref. 6, Nature Publishing Group.

Writing in *Nature Nanotechnology*, Shana Kelley and colleagues from the University of Toronto now report simultaneous magnetic sorting and classification of highly heterogeneous CTCs in a microfluidic device, an approach called magnetic ranking cytometry[6].

Complex, multicomponent mixtures can be separated through the competition of orthogonal physical fields and fluid flows, a technique known as field-flow fractionation[7]. Kelley and colleagues demonstrate an elegant variation on this technique by gradually strengthening a vertical magnetic field gradient relative to an axial fluid flow, resulting in one hundred 'capture zones' along the length of their microfluidic device (Fig. 1a). The capture zones — created using semiconductor fabrication techniques — consisted of an array of circular nickel micromagnets capped by polymeric X-shaped microstructures that locally impeded fluid flow. When a blood sample was introduced, magnetically labelled CTCs were vertically deflected towards slower flows near the base of the 'X-structure'. The diameter of these nickel micromagnets increased along the length of the device, which corresponded to progressively increasing magnetic field gradients. Consequently, CTCs with varying magnetic labelling could be captured in different regions. For example, densely labelled CTCs (resulting from greater expression of cell surface markers) might be localized by the weaker micromagnets near the entrance, while sparsely labelled CTCs were likely to lodge further downstream near the stronger micromagnets. Through immunofluorescent staining, the spatial distribution of CTCs (Fig. 1b) was then correlated with the expression of cell surface markers (Fig. 1c). As a proof-of-concept, four cancer cell lines with varying levels of epithelial cell adhesion molecule expression were processed using this device. The resulting spatial capture profiles were well separated and were qualitatively consistent with measurements using conventional flow cytometry. Nevertheless, appreciable non-specific capture of blood cells was observed, which were subsequently excluded from the analysis due to the absence of epithelial surface markers.

CTC isolation and analysis represents a powerful technique to track tumour progression and heterogeneity over time. Recent evidence suggests that tumour cells may dynamically undergo an epithelial-to-mesenchymal transition (EMT)[8], resulting in diminished epithelial cell surface markers with a corresponding increase in invasiveness and drug resistance. To demonstrate the device is capable of capturing the changing profiles of CTCs, Kelley and colleagues used a mouse model implanted with an oestrogen-sensitive human breast cancer cell line (MCF-7). Oestrogen stimulation in the treatment group increased the numbers of CTCs in the mouse blood and formation of micrometastases in the lung. Over time, the spatial CTC capture profile dramatically shifted towards distant capture zones, indicating a decrease in epithelial surface marker expression and suggestive of an EMT. In comparison, the untreated control group displayed fewer CTCs, no lung metastases and a smaller shift in spatial capture profile over time.

Next, blood samples were analysed from human patients with localized or metastatic prostate cancer. Such tumour biopsies are typically graded with a Gleason score of 6–10, where higher numbers correlate with greater malignancy. Using the device, it was shown that localized tumours with a Gleason score of 6 disseminated CTCs with relatively high epithelial surface marker expression levels. In contrast, localized and metastatic tumours with increasing Gleason scores disseminated CTCs with decreased epithelial surface marker expression, again suggestive of an EMT. Interestingly, the higher resolution of the magnetic ranking cytometry device revealed that the statistical distribution of surface markers was more heterogeneous for an intermediate Gleason score of 7, but less heterogeneous for low or high Gleason scores. These intriguing clinical observations will need corroboration from a larger cohort of patients and with explicit

measurements of mesenchymal markers associated with EMT.

Overall, this work represents an innovative proof-of-concept for isolation and analysis of CTCs from human patient samples. This technology could be augmented by integration of upstream sample preparation (for example, premixing blood samples with magnetic beads) and downstream molecular analyses (single cell genomics, transcriptomics, proteomics, etc.). Biologically, it is becoming appreciated that tumours may shed a wide variety of circulating biomarkers beyond individual CTCs. Future work could assess the feasibility of isolating smaller extracellular vesicles[9] or larger multicellular clusters[10], which can express a complex mosaic of cell surface markers. However, it should be noted that antibody-based approaches can be ineffective for tumours that lack established surface markers (for example, from melanomas), which has been addressed elsewhere by finding CTCs that remain after the removal of blood cells with known surface markers[4]. Ultimately, the use of complementary technologies that measure distinct classes of circulating biomarkers may enable more predictive and comprehensive patient diagnostics as well as new fundamental insights into tumour heterogeneity. ❐

Susan E. Leggett and Ian Y. Wong are in the School of Engineering, Center for Biomedical Engineering and Pathobiology Graduate Program, Brown University, Providence, Rhode Island 02912, USA.
e-mail: ian_wong@brown.edu

References

1. Massague, J. & Obenauf, A. C. *Nature* **529,** 298–306 (2016).
2. Alix-Panabières, C & Pantel, K. *Cancer Discov.* **6,** 479–491 (2016).
3. Gossett, D. R. *et al. Anal. Bioanal. Chem.* **397,** 3249–3267 (2010).
4. Ozkumur, E. *et al. Science Trans. Med.* **5,** 179ra47 (2013).
5. Issadore, D. *et al. Science Trans. Med.* **4,** 141ra92 (2012).
6. Poudineh, M. *et al. Nat. Nanotech.* **12,** 274–281 (2017).
7. Giddings, J. C. *Science* **260,** 1456–1465 (1993).
8. Yu, M. *et al. Science* **339,** 580–584 (2013).
9. Kalluri, R. *J. Clin. Invest.* **126,** 1208–1215 (2016).
10. Aceto, N. *et al. Trends. Cancer.* **1,** 44–52 (2015).

Published online: 21 November 2016