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IMAGING

Lighting up tumours

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Since immunohistochemistry (IHC) was developed over half a century ago, it has become an indispensable technique in cancer research. The principle of this technique relies on the ability of antibodies to bind specifically to the target antigens detecting their location within the cells and revealing the presence of cancer biomarkers. IHC has, however, one important disadvantage: it can only detect up to four antigens simultaneously. Now, two reports published in *Nature Methods* and *Nature Medicine* have described a novel IHC approach that allows the simultaneous detection and visualization of up to 100 antigens.

In the first study, two research groups joined forces to develop an imaging approach to enhance the capabilities of CyTOF mass cytometry. On the one hand, the group led by Bernd Bodenmiller works on mass cytometry—which measures the abundance of metal isotopes tagged to antibodies—to study signalling networks in cancer cells. On the other hand, the group led by Detlef Günther has developed a novel laser ablation chamber, which enables imaging of simultaneous isotope distribution in tissues at subcellular resolution. "Therefore, for us, combining mass cytometry and laser ablation was a logical step that would address the need for highly multiplexed measurements with high spatial resolution to study the tumour microenvironment," explains Bodenmiller. The authors used standard IHC methods to prepare tissue sections and then stained these sections with antibodies "each labelled with a pure, defined metal isotope" describes Bodenmiller, "currently, we can stain the tissue with 32 antibodies simultaneously." Subsequently a laser system that ablates the tissue spot-by-spot was used. "As a result we determine for every spot on the tissue the metal isotope content and—by inference via the antibodies—which markers were present. Assembling these spots (pixels) into an image generates a highly multiplexed tissue image," says Bodenmiller. These results helped to delineate a highly dimensional and comprehensive map of cancer cell heterogeneity in different breast cancer subtypes and, hopefully, will provide insights into the influence of the microenvironment in tumour cell phenotypes.

In the second study, Garry Nolan and colleagues developed a method called multiplexed ion beam imaging (MIBI) that uses secondary ion mass spectrometry to image metal isotope-carrying antibodies. "MIBI has resolution of 200–300 nm compared with 1–2 µm for laser ablation," states Nolan. With this approach, the authors imaged breast tumour tissue sections stained with clinically relevant metal-conjugated antibodies. They also quantitatively validated the method by comparing it with an FDA approved clinical IHC platform demonstrating that the technique may be useful in a diagnosis setting.

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Original articles Angelo, M. *et al.* Multiplexed ion beam imaging of human breast tumors. *Nat. Med.* doi:10.1038/nm.3488 | Giesen, C. *et al.* Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat. Methods* doi:10.1038/nmeth.2869