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HETEROGENEITY

# A multidimensional overview

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Times have changed: gone are the days when flow cytometry involved just a few markers for analysis. With the advent of mass cytometry that takes advantage of isotope-conjugated antibodies, dozens of parameters can be mapped for each individual cell, but how does one 'visualize' such data? Dana Pe'er and colleagues have designed a new algorithm to help with this.

Although several algorithms already exist to allow an assessment of mass cytometry data, most result in either a loss of individual cell data or the loss of nonlinear relationships. To overcome these limitations, Pe'er and colleagues developed viSNE, which enables the visualization of the data based on an existing algorithm called t-distributed nearest neighbour embedding (t-SNE). Essentially, viSNE allows the best visualization of single-cell data while preserving local and global geometry (non-linear relationships), and these are viewed using viSNE map.

Why is this important for cancer biologists? Using previously generated mass cytometry data of normal bone marrow samples the authors first verified that viSNE could independently map the different cell types present in the bone marrow on the basis of the expression of a number of different cell membrane-expressed proteins. Having passed this test, the authors then tried viSNE out on two human samples of acute lymphoblastic leukaemia (ALL) that were labelled with 29 different antibodies. Unlike the maps generated from the two normal bone marrow samples, which overlapped, the two ALL samples were more different than they were similar. In contrast to the distinct and related subpopulations evident in the normal marrow samples, the ALL samples mostly mapped as a large, irregular and related mass of abnormal cells, clearly distinct from the few remaining healthy cells (~5%) that were present in the ALL samples. Similar results were evident in two acute myeloid leukaemia (AML) samples. The ability of viSNE to allow the visualization of individual leukaemia cells in the context of 29 different markers as opposed to the four to eight markers currently used in the clinic, allows the identification of additional structure within the population, new abnormal marker combinations and new leukaemic subpopulations. Interestingly, each individual leukaemia sample formed a distinct viSNE map, indicating that viSNE provides another method with which to study the heterogeneity both within and between leukaemia samples.

viSNE was also used to analyse disease relapse, based on two AML samples taken from the same patient before treatment and at relapse. The viSNE map clearly showed that specific subpopulations of cells were lost as a result of therapy and that specific subpopulations arose after therapy. One of the signs of potential relapse in patients with leukaemia is the presence of minimal residual disease (MRD; small numbers of leukaemic cells still present in the blood or bone marrow) after treatment. Current approaches to detect MRD require an expert pathologist and often the detection of cell phenotypes present in the original leukaemia, meaning that leukaemic cells with new phenotypes that arise after therapy could be missed. Can viSNE help? The authors spiked a healthy bone marrow sample with 0.25% ALL cells that contained a metal barcode. The resulting viSNE map that compared a healthy bone marrow sample with the spiked one identified a small group of cells that were distinct from the subpopulations of healthy cells. Unblinding revealed that all the cells in this small subpopulation contained the metal barcode, indicating that the viSNE algorithm might be useful in the detection of MRD.

As viSNE can be used on data generated by any multidimensional, single-cell technology, its uses in cancer biology could be extensive. Indeed, the capacity to identify small, distinct subpopulations could help to detect rare, drug-resistant cells.

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**ORIGINAL RESEARCH PAPER** Amir, E. D. *et al.* viSNE enables visualization of high-dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nature Biotech.* 19 May 2013 (doi:10.1038/nbt.2594)

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