These findings were extended by studying human samples from individuals immunized with either BNT162b2 or ChAdOx1. Both vaccines generated T-cell responses to in-frame peptides. However, BNT162b2 elicited T-cell responses to +1 frameshifted peptides. This illustrates that a lack of fidelity in translation can result in unintended consequences arising from a non-uniform protein output from the vaccine, leading to the induction of off-target immune responses and probably affecting product potency. No evidence has been reported linking this phenomenon to safety issues for COVID-19 mRNA vaccines through the many systems that monitor vaccine safety after licensing — including the Vaccine Adverse Event Reporting System, the Vaccine Safety Datalink and the Clinical Immunization Safety Assessment project.

Numerous natural mechanisms can lead to frameshifting[4,5]. The authors examined whether m1Ψ-modified mRNA affects ribosome stalling, a process that can lead to +1 frameshifting. The rate of translation of mRNA is not constant along the length of an mRNA transcript. This is because of differences in the abundance of transfer RNAs that bind to matching mRNA codons and provide specific amino acids during protein production. The ribosome might stall on a 'slippery' mRNA sequence for which few corresponding tRNAs are available, and this could lead to a +1 frameshift to accommodate a more-abundant tRNA instead[6].

Using labelled nucleotides to track translation, the authors observed that the translation rate was slower for m1Ψ-modified mRNA than for unmodified mRNA. If m1Ψ-modified codons lead to ribosome stalling, enlarging the pool of available tRNAs should prevent stalling. Using the drug paromomycin, which enables the binding of non-matching tRNAs, the authors report that the translation rate of m1Ψ- modified mRNA improved with this treatment, which supports the hypothesis that ribosomal stalling is responsible for this phenomenon.

To investigate a possible remedy for ribosomal stalling, the authors used their *in vitro* system. They identified slippery sites in their reporter system and altered the corresponding mRNA sequence so that it had some synonymous substitutions that changed the mRNA sequence but not the encoded amino acid. The goal was to retain the correct sequence of amino acids in frame and limit the effect of the slippery sequence. Substitutions in the sequence reduced +1 frameshifted products *in vitro*, illustrating a method to ameliorate this phenomenon.

This study has implications for the development of modified mRNA products. Further evaluation of the T-cell and antibody response to +1 frameshifted protein products made from vaccine antigens encoded from m1Ψ-modified mRNA vaccines would be informative. Off-target T-cell or antibody responses have the potential to be misdirected against non-relevant target proteins, compromising product performance and resulting in unintended *in vivo* product complexity. Mulroney and colleagues' study highlights a key aspect for future study that might aid our understanding of the design of modified mRNA sequences, thereby enabling further-improved outcomes. Studies to confirm and extend the implications of *in vivo* frameshifting resulting from mRNA modifications, as well as to investigate other methods of amelioration, are warranted.

**Nicholas J. Tursi** and **David B. Weiner** are at the Vaccine and Immunotherapy Center, The Wistar Institute, and at the Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.
e-mail: dweiner@wistar.org

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Genomics

# Gene expression of single cells mapped in tissues

**Patrik L. Ståhl**

A tool that tags individual cells in a tissue with a unique barcode means that the gene-expression profile of each cell can be plotted in its original location. This allows spatial information to be captured at single-cell resolution. **See p.101**

Over the past decade, there have been major advances in techniques that can inform scientists about the properties of single cells, such as their gene-expression profiles[1]. Similar progress has been made with methods that provide spatial information, such as identifying where genes are being expressed in a tissue[2]. On page 101, Russell *et al*.[3] present a tool that they name Slide-tags, which could integrate the best aspects of both of these approaches.

Information about gene expression in a given biological sample can be acquired by sequencing the RNA transcripts present (known as transcriptomics), or by identifying regions of the genome that are accessible to the molecular machinery that controls gene transcription (one example of an epigenomic approach). More broadly referred to as genomics, such techniques can be used to obtain the genetic profiles of single cells, or to spatially map gene expression in whole tissues. Although the two methods have found widespread application in biomedical research, many challenges remain.

The main problem for single-cell approaches is that it is difficult to maintain the natural state of cells while attempting to accurately characterize them during analysis. For example, information about the location of a cell in a tissue is lost when cells are dissociated from one another and mixed together during sample preparation. Retaining spatial information is important because it can reveal how cell types are organized in tissues, which is valuable when trying to understand developmental processes or how certain diseases (such as cancer) progress.

Isolating cells from tissues can also be disruptive to the native architecture of the cell itself. Just how disruptive often depends on the composition of the tissue in question. Some cells, such as those in the blood, can be isolated relatively easily, because they are free-floating. By contrast, cells in frozen or fragile tissues are difficult to isolate, because they are prone to breaking apart during tissue dissociation. Thus, when working with tissues of this nature, it is usually only the nuclei of cells that can be isolated[4].

For spatial genomics, resolution is a key concern. When mapping a piece of tissue onto a physical surface, the surface can be divided into a matrix in which each element (referred to as a 'pixel') represents a particular spatial location. The size of the pixels determines the resolution. Although pixel sizes that provide single-cell resolution are technically achievable, spatial pixels usually contain information from a mixture of cells, and it has so far been hard to work out exactly which data belong to which cells in a tissue[2,5].

The possibility of combining single-cell and

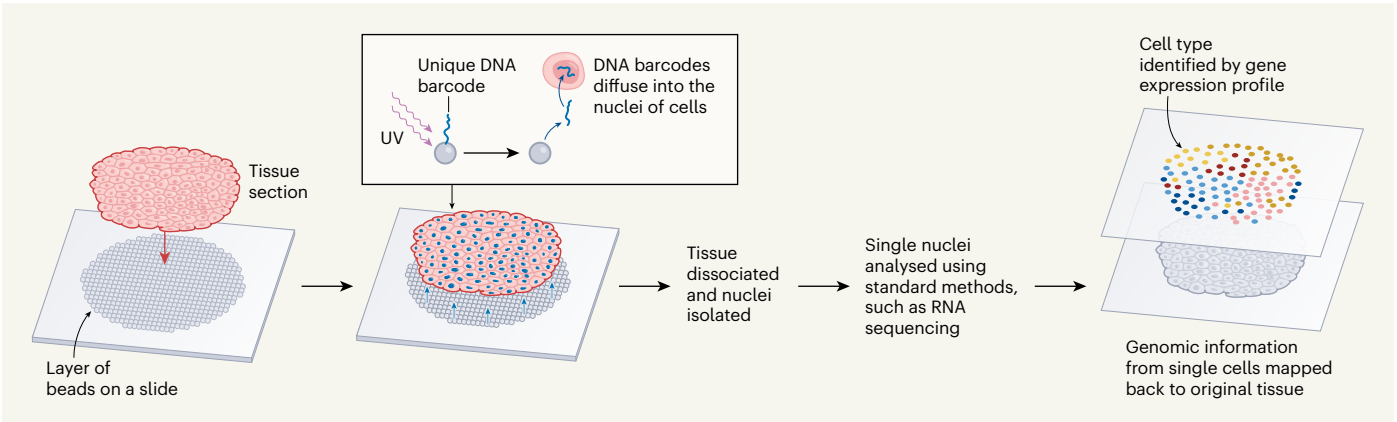


**Figure 1 | Single-cell maps of gene expression using spatial barcoding.** Russell *et al.*[3] have developed a tool called Slide-tags to map the gene expression of single cells in a tissue. A thin section of tissue is applied to a layer of beads on a microscope slide. Each bead has a unique DNA sequence attached to it that acts as a barcode representing a particular spatial location. Ultraviolet light is used to detach the DNA barcode from the bead, and the barcodes diffuse into the cells' nuclei. The tissue is then broken down, and the molecular content of each nucleus is analysed using standard techniques for examining gene expression, such as RNA sequencing. On the basis of the gene-expression profiles and the DNA barcodes of each cell, a representative spatial map of cell types (shown in different colours) can be constructed, which provides information about how cells are organized and how they interact with one another in tissues.

spatial genomics approaches has emerged as a hot topic in biology — but true integration is lacking. Computational approaches have made some headway[5–7], but they are imperfect, because predictions must be made about the cells that are represented in each spatial pixel, and each pixel will never contain the information from exactly one entire cell.

In the current study, Russell and colleagues used existing tricks from spatial and single-cell approaches to develop a technique that almost gives scientists the best of both worlds (Fig. 1). The authors' method is based on one that researchers in the same laboratory had developed previously[8]. They start by covering a glass microscope slide in a layer of beads. Each bead is 10 micrometres wide, resulting in pixels of the same size. Attached to each bead is a string of DNA with a unique sequence, known as a DNA barcode. Because the barcoded beads are randomly distributed on the surface of the slide, the location of each DNA barcode must be identified before a thin section of tissue is added on top of the barcoded beads.

The DNA barcodes are then released from their beads using ultraviolet light, and diffuse up into the tissue. Several barcodes will diffuse into the nucleus of each cell in the tissue, effectively 'tagging' the cell with a spatial identifier. The authors can then map the cell back to its original location in the tissue according to the combination and relative amount of DNA barcodes in it.

In conventional spatially resolved transcriptomic and epigenomic methods, the goal is to get the spatial barcode into the cells, or to get the cellular contents (such as RNA transcripts) out of the cells to meet the spatial barcodes, after which the tissue remains at the surface[2,8]. But the Slide-tags procedure is different. With the barcodes in the cells, the tissue is removed from the surface, the cells are dissociated and single nuclei are isolated — ready for established single-cell analyses[9,10].

The major benefit of Russell and colleagues' method is that genomic information from single nuclei can be analysed with high-throughput workflows that are already optimized and widely used. At the same time, algorithms that the authors developed can pinpoint the exact spatial locations of those nuclei in the original tissue. This work fills a substantial gap in the field: in-depth data about the molecular content of single cells can now be mapped at high resolution.

The ability to link a cell's gene-expression profile with its position in a tissue can provide a multitude of biological insights. For example, Russell *et al.* show that they can map the positions of neuronal and non-neuronal cell types in a section of the brain's cortex, and can even predict cell–cell interactions, such as those taking place between immune cells in lymphoid tissue. Because there is no need to make approximations about what a cell contains or where it is located (as would be the case with computational approaches), Slide-tags can potentially provide a more accurate view of cell-type-specific events in tissues than has previously been possible.

Slide-tags has a few potential limitations. First, only single nuclei can be isolated at present,which means that any molecular information contained in the compartments outside the nucleus — such as RNA in the cytoplasm — is missing. The most obvious goal for the future would be finding a way to retain the entire cell.

Second, several spatial genomics techniques can process tissues preserved in paraffin, whereas Slide-tags is currently limited to fresh tissue that has been frozen. Furthermore, the procedure still requires cells to be dissociated from one another, and this process might not work well on all types of tissue — although the authors do showcase the use of Slide-tags on diverse tissues, such as brain, tonsil and melanoma.

Third, it is currently only possible to identify the barcodes contained in each nucleus for about half of the nuclei isolated, meaning that a substantial amount of data is lost. Both the barcoding and the identification process could be made more effective, so that full transcriptomic and epigenomic data sets can be put into a spatial context.

Despite these drawbacks, Slide-tags is a marked improvement on existing single-cell and spatial genomics techniques, and provides a step towards a more complete integration of the two.

**Patrik L. Ståhl** is in the Science for Life Laboratory, Department of Gene Technology, KTH Royal Institute of Technology, Solna SE-171 65, Sweden.
e-mail: patrik.stahl@scilifelab.se

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