22. Liu, Y. et al. *Cell* **183**, 1665–1681.e18 (2020).
23. Bergenstråhle, L. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.02.28.963413 (2020).
24. He, B. et al. *Nat. Biomed. Eng.* **4**, 827–834 (2020).
25. Bergenstråhle, J., Larsson, L. & Lundeberg, J. *BMC Genomics* **21**, 482 (2020).
26. Wolf, F. A., Angerer, P. & Theis, F. J. *Genome Biol.* **19**, 15 (2018).
27. Stuart, T. et al. *Cell* **177**, 1888–1902.e21 (2019).
28. Andersson, A. et al. *Commun. Biol.* **3**, 565 (2020).
29. Biancalani, T. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.08.29.272831 (2020).

Author contributions
L.L. and J.L. wrote the manuscript with input from J.F.

Competing interests
J.L., J.F. and L.L. are scientific consultants to 10x Genomics Inc.

Spatially resolved single-cell genomics and transcriptomics by imaging

The recent advent of genome-scale imaging has enabled single-cell omics analysis in a spatially resolved manner in intact cells and tissues. These advances allow gene expression profiling of individual cells, and hence in situ identification and spatial mapping of cell types, in complex tissues. The high spatial resolution of these approaches further allows determination of the spatial organizations of the genome and transcriptome inside cells, both of which are key regulatory mechanisms for gene expression.

Xiaowei Zhuang

Humans, as well as many other organisms, are comprised of different types of cells. Yet the cell type composition and organization are still largely unknown for most organisms. The number of cell types could be vast. For instance, a mammalian brain could contain hundreds to thousands of different types of cells. Single-cell transcriptomic profiling provides a systematic and quantitative approach to identify cell types and determine their composition in tissues. However, when cells are dissociated from tissues for sequencing analysis, their spatial context is lost. Yet the spatial organization of cells is critical for tissue function. Furthermore, inside cells, the genomic DNA is folded into complex three-dimensional (3D) structures, which are vital to gene expression regulation. Likewise, intracellular organization of RNAs provides an important post-transcriptional regulatory mechanism. Thus, high-resolution, spatially resolved single-cell omics analysis is critically needed.

Imaging provides a powerful means for probing molecular organizations inside cells and cellular organizations in tissues, but imaging at the genome scale appeared unreachable even just several years ago. This situation has changed dramatically; genome-scale imaging of individual cells has become a reality. In this Comment, I give my perspective on imaging-based single-cell transcriptomics and genomics methods and their impact on biology. Spatial transcriptomic analysis can also be achieved by spatially resolved RNA capture followed by sequencing, which is described in the Comment by Larsson et al.[1].

Imaging-based single-cell genomics and transcriptomics methods

Two classes of methods have been reported for imaging-based single-cell transcriptomics and genomics, based on in situ sequencing or multiplexed fluorescence in situ hybridization (FISH). Achieving genome-scale imaging with these approaches has both distinct and common challenges.

Multiplexed FISH. FISH has long been used to image genomic loci and RNA transcripts. FISH at the single-molecule level allows highly accurate localization and counting of RNA molecules in cells[2,3]. Single-molecule FISH with combinatorial color schemes has been used for single-cell gene expression profiling — for example, simultaneously imaging more than ten genes, as initially demonstrated[4] in 2002. However, the limited number of distinct color channels presents a major challenge to achieving genome-scale imaging.

To overcome this challenge, we conceived the method of multiplexed error-robust FISH (MERFISH) in early 2013, which identifies genes with a combination of signals from multiple rounds of hybridization (Fig. 1a). Specifically, a *N*-bit binary barcode is assigned to each gene, the "1" or "0" value of each bit corresponding to whether or not the gene is detected in a particular hybridization round (Fig. 1a)[5] — though more complex codes can also be used for MERFISH. This binary coding scheme can distinguish 2^N genes with *N* rounds of hybridization (or 2^{NC} genes, when *C* colors are used for imaging in each of the *N* hybridization rounds to measure a total of *NC* bits). However, a challenge also arises from an approach that identifies a molecule with multiple signals: the finite per-bit error (or, more generally, per-signal error) accumulates over multiple bits (or signals) and amounts to a substantial overall barcode misidentification rate. To overcome this challenge, MERFISH uses coding schemes that allow error detection and/or correction, another key concept of this method[5]. Another noteworthy feature of the above MERFISH coding schemes is the use of the absence of signal in a hybridization round to represent a bit value (for example, "0"), as it allows the fraction of molecules imaged per round to be tuned by adjusting the number of "0" bits per barcode, mitigating the molecular crowding problem associated with imaging a large number of genes. To implement MERFISH, we imprinted the barcodes on cellular RNAs with a library of encoding probes to bring a set of readout sequences to each RNA to define its barcode, and then detected these barcodes by sequential rounds of hybridization with readout probes (Fig. 1a)[5]. This encoding-readout labeling strategy allows genome-scale imaging within a reasonably short experimental duration because hybridization of FISH probes to the exogenously introduced readout sequences

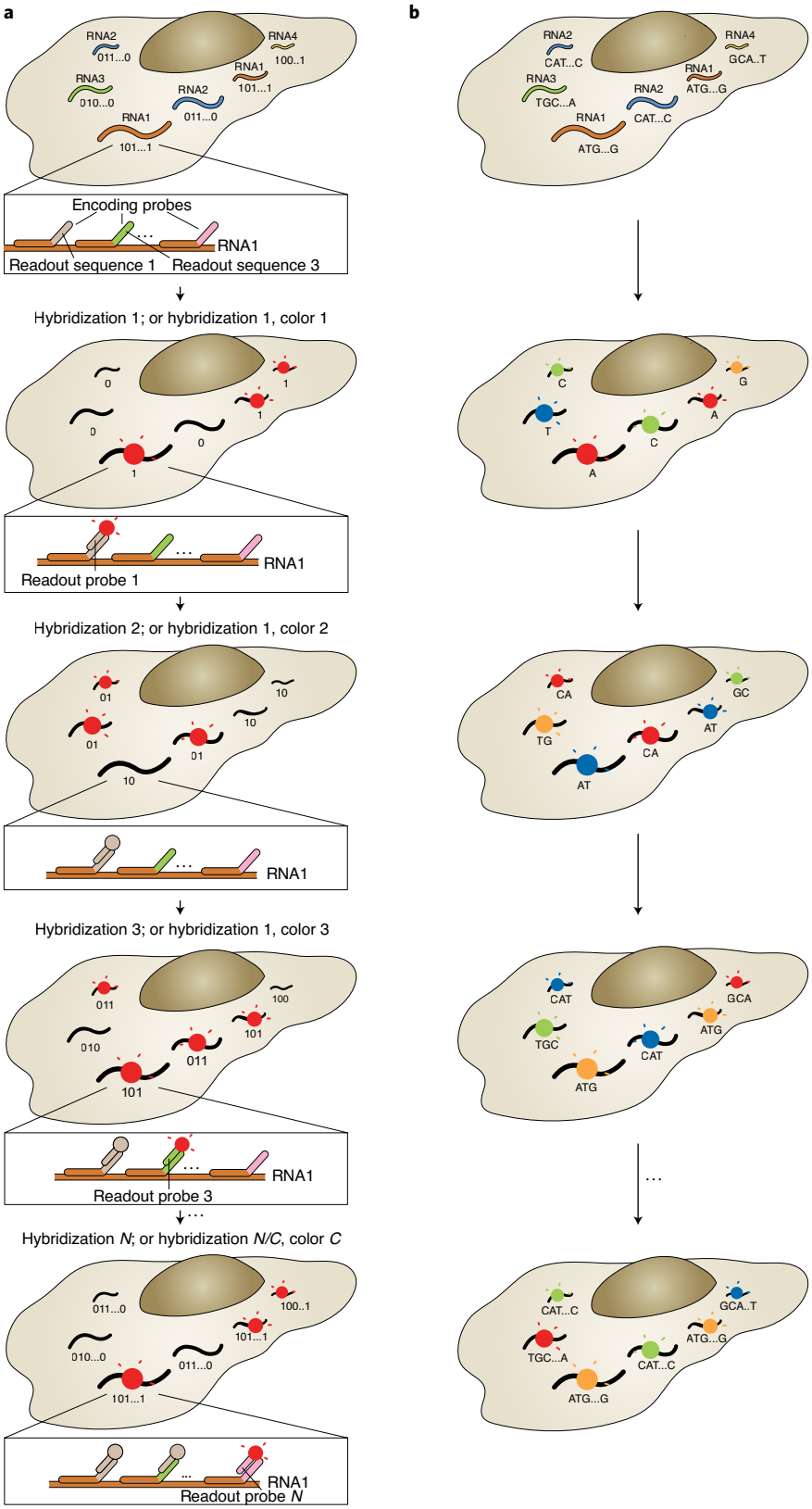

Fig. 1 | Imaging-based single cell genomics and transcriptomics approaches. a, A multiplex FISH scheme. For simplicity, a MERFISH scheme is used as an illustrative example. A binary barcoding scheme is shown together with how the barcodes are imprinted on the RNAs with a library of encoding probes and detected bit-by-bit with distinct readout probes. **b**, A simplified scheme for in situ sequencing. The nucleic acid sequence could either be the endogenous sequences of the genes or barcode sequences assigned to the genes, which are detected nucleotide-by-nucleotide via sequencing. Depending on the different sequencing chemistries used (not depicted here), the colors of the circles may indicate the identity of individual nucleotide or adjacent dinucleotide pairs.

is much faster than hybridization directly to cellular RNAs[5].

In 2015, a couple of years after the conception of this method, MERFISH imaging of ~1,000 genes in individual cells was reported, providing the first experimental demonstration of transcriptome-scale multiplexed FISH[5]. Using the same MERFISH design, imaging of >10,000 genes in individual cells was subsequently demonstrated using 23 hybridization rounds and three-color imaging[6], leveraging expansion microscopy[7] to help further reduce molecular crowding associated with imaging high-density RNA libraries[8]. MERFISH imaging of complex tissues has also been demonstrated using hydrogel-based clearing to reduce tissue background[9,10]. Recently, MERFISH has been extended to DNA imaging, enabling simultaneous imaging of the 3D organization and transcriptional activity of the chromatin at the genome scale[11].

In parallel to the development of MERFISH, a different multiplexed FISH method, seqFISH, was developed that distinguishes RNAs by a sequential color code (for example, red-red-red, red-red-green, red-red-blue, …)[12]. In seqFISH, different sets of genes are assigned different colors in each hybridization round, with a new set of FISH probes hybridized to cellular RNAs to change the color assignment in each round[12]. With C colors and N hybridization rounds, C^N different genes can be distinguished. The original seqFISH paper demonstrated imaging of 12 genes in individual cells[12]. Subsequently, seqFISH adopted the concept of using coding schemes capable of error correction and demonstrated imaging of ~250 genes, with the help of hybridization chain reaction (HCR)-based signal amplification to facilitate tissue imaging[13]. In seqFISH, every targeted gene is detected in each hybridization round in one of the color channels; thus the fraction of molecules detected per color channel per round is fixed by the number of color channels (for example, ~25% for four-color imaging). Hence, the molecular crowding problem presents a challenge to scaling up the number of genes imaged in seqFISH. Recently, 10,000-gene imaging was reported

using a multiplexed FISH scheme akin to MERFISH that uses the binary codes that allow the fraction of molecules imaged per round to be adjusted, the error-correction concept to increase gene identification accuracy, and the encoding-readout labeling strategy to eliminate the slow step of direct hybridization to cellular RNAs in each round. Using three sets of 80-bit binary codes with four "1" bits, this work imaged 10,000 genes with 80 rounds of hybridization and three-color imaging without using expansion microscopy[14]. In principle, when molecular density is limiting, achieving the same molecular density reduction by either sample expansion or more hybridization rounds should incur a similar amount of increase in imaging time[6].

Spatially resolved single-cell profiling has also been achieved using sequential hybridizations that detect a single gene in each color channel per hybridization, as in osmFISH[15]. This approach provides excellent signal quality in tissue imaging, albeit at a lower level of multiplexity, and it does not necessarily require single-molecule imaging, hence providing a means to image genes with expression levels too high or sequences too short for reliable single-molecule detection and barcode identification. This approach can also be combined with the approaches described above to cover both a large number of genes and genes that are difficult for single-molecule detection.

Overall, multiplexed FISH approaches provide in situ single-cell transcriptomics and 3D genome analysis with high spatial resolution. Another advantage of these methods is their high detection efficiency. For example, MERFISH has demonstrated ~80% detection efficiency of RNA molecules for >10,000-gene imaging[6]. Such high detection efficiency allows quantification of genes with low expression levels. Advances in high-diversity oligonucleotide library generation facilitate renewable and cost-effective probe production for multiplexed FISH[5,16]. Imaging more genes is clearly feasible — for example with more hybridization rounds — and in fact increasing the genome coverage is not limited by how many genes can be imaged, but by how short a sequence can be reliably detected. In our experience, sequences as short as ~200 nt can be accessible to MERFISH with branched-DNA-based signal amplification in hydrogel-cleared samples[17]. Combination with proximity-based probes[18,19] may allow even shorter sequences to be imaged. Development in this direction will benefit the detection of short transcripts, short gene regulatory elements, and splice isoforms with small sequence differences.

In situ sequencing. In parallel, imaging-based in situ sequencing approaches have also been developed for single-cell transcriptomic analysis with high spatial resolution (Fig. 1b). In situ sequencing can be performed in either a targeted or untargeted manner.

In targeted in situ sequencing, a set of genes are preselected and unique nucleotide sequences are delivered to these genes as barcodes by hybridization of padlock probes, which are then circularized and amplified by rolling circle amplification and detected by in situ sequencing, as originally introduced in ref. [20]. Using a gap-filling strategy to copy the endogenous sequences to the padlock probes, in situ sequencing also allows single-nucleotide variations in genes to be detected[20]. Recently, methods have been developed to improve the multiplexity or detection efficiency of in situ sequencing by eliminating the inefficient RNA-to-cDNA conversion step, improving sequencing accuracy, using hydrogel-base clearing or sample expansion, combining with FISH, or using more efficient gap-filling enzymes[21–25]. In particular, the STARmap method uses two-component padlock probes to allow direct binding to RNA and circularization, hydrogel chemistry to allow tissue clearing, and an improved sequencing method to reduce error, which together allowed imaging of 1,020 targeted genes with a detection efficiency that is comparable to that of single-cell RNA sequencing[21].

In untargeted in situ sequencing, as introduced in FISSEQ[26], cellular RNAs are converted to cDNA, amplified, and sequenced without any preselection. The advantage of this approach is its genome-wide coverage, and >8,000 genes have been imaged in the same samples by FISSEQ[26]. On the other hand, because all RNA species are sequenced, to avoid the molecular crowding problem and allow detection of individual molecules, FISSEQ uses sequencing primers to allow only a small fraction of the amplicons to be sequenced, which leads to a low detection efficiency. Reported estimates[21,23] of this detection efficiency range from 0.2% to <0.01%. Combination with expansion microscopy, as in expansion sequencing[24], helps mitigate the molecular crowding problem to some extent and increases the spatial resolution, but the untargeted expansion sequencing still has a much lower detection efficiency than targeted expansion sequencing of tens to hundreds of genes[24].

Further approaches could be considered to mitigate the molecular crowding problem. For untargeted in situ sequencing, specific removal of abundant housekeeping RNAs may help increase the detection efficiency of other genes. For targeted sequencing, a possible solution may lie in a strategy used in multiplexed FISH: using the absence of signal to adjust the molecular density. The fraction of RNAs detected per color channel in each sequencing step is ~25% when the four nucleotides are each assigned a distinct color. If, instead, the absence of signal (rather than a color) is assigned to one of the nucleotides or some dinucleotide pairs (depending on the sequencing chemistry used), the RNA density per image can then be tuned by adjusting the enrichment of such nucleotide or dinucleotide pairs in the barcode sequences. This may allow a large number of genes to be detected with high detection efficiency. Achieving a substantial reduction in molecular density would require a relatively long barcode, and it remains to be seen whether the sequencing read-length limit would pose a challenge.

For all targeted approaches, including both in situ sequencing and multiplexed FISH, a conceptually simple approach — dividing the targeted genes into multiple groups and imaging them sequentially one group at a time (as also suggested in ref. [21]) — could also be used to overcome the molecular crowding problem. For example, 10,000 or 20,000 genes could be divided into 10 or 20 groups of 1,000 genes, and with an efficient method to image 1,000 genes (typically achievable with no more than ~10 rounds of multicolor imaging), imaging 10,000 or 20,000 genes would require no conceptual change in the method but simply 10 or 20 times as many imaging rounds. When molecular density (crowding) is the limiting factor, this divide-and-conquer approach would not require more imaging rounds than barcoding all genes together because proportionally longer barcodes are needed in the latter case to achieve the same density dilution.

It is, however, important to note that increasing the number of genes imaged will, in general, increase the overall measurement time and/or degrade the measurement accuracy for multiplexed FISH and in situ sequencing. For many applications, the ability of a targeted approach to make a judicious choice of genes to image is a more efficient way to reach the goal. Moreover, computational approaches are emerging to impute genome-wide expression of cells profiled with a targeted gene set by integration with single-cell RNA-sequencing data, providing another potential means to help fill the gap (for example, refs. [27,28]).

Biological applications

Spatial atlases of cell types in tissues. Spatially resolved single-cell transcriptomic analyses have allowed the identification and

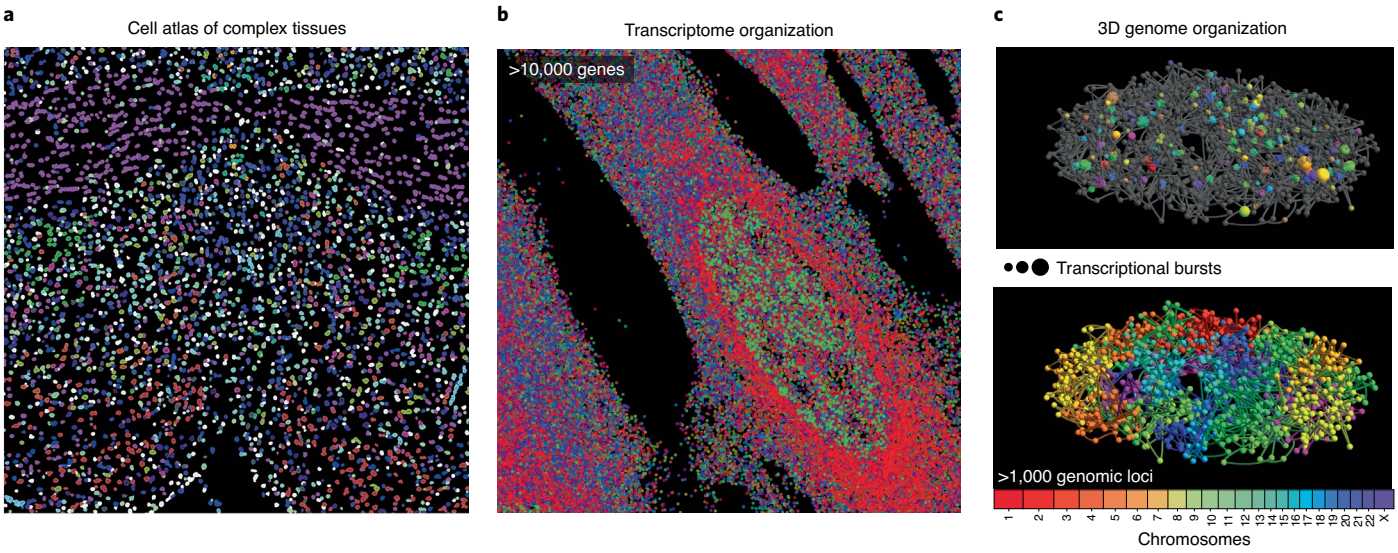

Fig. 2 | Three example application areas of imaging-based single-cell genomics and transcriptomics. a, Cell atlas of complex tissues. A cell-type map of a region in the mouse hypothalamus[10] is shown as an example, with different types of cells shown in different colors. **b**, Intracellular organization of the transcriptome. MERFISH image of >10,000 genes in individual cells[6] is shown as an example, with RNA molecules from different genes shown in different colors. **c**, 3D organization of the genome. MERFISH image of >1,000 genomic loci (bottom) and transcription bursts of >1,000 genes (top) in a single nucleus[11] is shown as an example.

spatial mapping of cell types directly in intact tissues (Fig. 2a). For example, MERFISH has been used to profile >1 million cells in the hypothalamic preoptic region and ~300,000 cells in the primary motor cortex of the mouse brain, identifying nearly 100 cell types in each region and generating highly granular spatial atlases of cell types of these regions[10,29]. STARmap[21] and osmFISH[15] have been used to map and provided new insights into spatial organizations of cell types in the visual, prefrontal and somatosensory cortices. seqFISH[13] and probabilistic cell typing by in situ sequencing (pciSeq)[22] have provided new insights into cell type organization in the hippocampus. Spatially resolved single-cell analyses have also revealed potential cell–cell interactions mediated by gap junctions between inhibitory neurons[21], by ligand–receptor pairs[14] or by paracrine signaling[10]. Furthermore, the combination of STARmap and MERFISH with neuronal activity imaging has identified neuronal cell types activated by visual stimuli, drugs and social behaviors, providing functional annotations to the cell atlas[10,21]. Combination of MERFISH with retrograde labeling allows projection targets of molecularly defined neuronal cell types to be mapped[29], helping to decipher neural circuits. In addition to research on healthy brains, spatially resolved transcriptomic analyses have also provided interesting insights into neurodegenerative disease[30].

In addition to the brain, spatially resolved single cell transcriptomics approaches have also begun to illuminate the cell type organization of other tissue types — for example, combination of in situ sequencing with other spatial and single-cell transcriptomic analyses has provided spatiotemporal mapping of the embryonic heart[31] and neural crest[32]. One may anticipate a rapid increase in the number of spatially resolved cell atlases of different organs and organisms, which will advance fundamental understandings of the cellular basis of tissue function. Application of these approaches to pathological tissues will also advance our understanding of diseases and may eventually help improve therapeutics.

The importance of this application area is underscored by a number of large-scale initiatives, such as the Human Cell Atlas (HCA), BRAIN Initiative Cell Census Network (BICCN) and Human Biomolecular Atlas (HubMAP) projects. These large-scale efforts place a great demand on the volume of spatially resolved data. Although some imaging methods already have a relatively high throughput (for example, MERFISH imaging of tens of thousands of cells per overnight experiment has been reported[33]), extending cell atlas efforts from small animals to human still requires a substantial increase in the imaging throughput, which can be anticipated through a variety of means. With the rapid increase in the data volume, concurrent development of computation methods for scalable data handling and analyses are also greatly needed.

Intracellular organization of the transcriptome and 3D organization of the genome. Spatial distributions of RNAs inside cells is an important mechanism of post-transcriptional regulation, which is vital for a variety of cellular processes, ranging from cell motility to embryo development to synaptic plasticity in neurons. The high spatial resolution of imaging-based single-cell transcriptomics allows the intracellular spatial organization of RNAs to be mapped at the genome scale (Fig. 2b), providing insights into distinct transcriptome compositions in different subcellular regions and compartments[5,6,14,24,34], such as distinct transcriptome compositions and organizations in soma, axons and dendrites of neurons[24,34]. Despite its importance, intracellular organization of transcriptomes is underexplored for most cell types. Single-cell transcriptome imaging will substantially enrich our knowledge of this important area of cell biology.

The 3D organization of genomic DNA is critical to many genomic functions, ranging from gene expression regulation to genome replication. Genome-scale chromatin imaging can provide direct visualization of the 3D genome organization in individual cells in the context of nuclear structures and functions. Multiplexed FISH has allowed the 3D organization of chromatin to be

traced in single cells, initially tracing tens of genomic loci by sequential hybridization, with one locus imaged per color channel per hybridization round[35]. Recent extension of MERFISH to DNA has enabled imaging of >1,000 genomic loci, together with the nascent RNAs of >1,000 genes and protein markers for multiple nuclear bodies, allowing the 3D organization of chromatin to be traced at the genome scale and its relationship with transcription and nuclear stuctures to be interrogated in individual cells (Fig. 2c)[11]. In situ sequencing has also been recently extended to chromatin tracing, demonstrating imaging of tens of loci in single cells, with a potential to increase the multiplexity to the genome scale[36]. These and other, similar approaches have provided insights into chromatin organizations and chromatin interactions with other nuclear structures, as well as their relationships with transcription in both cultured cells and tissues[11,35–43]. The ability to directly visualize the genome in 3D will greatly advance the understanding of how genome organization influences gene regulation and other genome functions.

Other applications. In addition to imaging endogenous cellular RNAs and DNAs, the ability of these multiplexed imaging methods to identify a large number of artificial nucleic acid barcodes introduced into cells has also led to other important applications. These include imaging-based pooled genetic screening, which enables screening based on phenotypes difficult to access by non-imaging means, such as cellular structure and dynamics[44–48]; high-throughput lineage tracing, which provides insights into the roles of clonal history and spatial locations in cell fate determination[49,50]; and high-throughput neuronal projection mapping, which allows simultaneous mapping of the projection targets and spatial locations of many neurons[51].

There are more applications of, and more insights generated by, imaged-based single-cell genomics than space allows in this short Comment. It is exciting to see that, in such a short time since the field was born, so much has already been learned, with much more to come.

Looking beyond the transcriptome and genome: spatially resolved multimodal omics

The phenotype and function of a cell are determined by many different properties, and multimodal imaging allows different properties to be probed in the same cells. Combination of genome and transcriptome imaging with imaging of other properties, such as cell morphology, signaling and function, will provide not only an improved classification of cell types and states, but also a better understanding of the molecular basis underlying these cellular properties. Transcriptome and 3D genome analyses are the two modalities that have already reached the genome scale by imaging. Other omics modalities may also be accessible to imaging. Multiplexed protein imaging, although having yet to reach the genome scale, has already made an impact in wide range of areas. Its combination with transcriptome and 3D genome imaging can enhance our ability to discover cell types and states and accelerate our understanding of the molecular mechanisms underlying gene regulation. One may also envision imaging-based epigenomics and epitranscriptomics, given the availability of probes that can recognize DNA and histone modifications, DNA accessibility and RNA modifications, along with their compatibility with multiplexed FISH and in situ sequencing. It is exciting to anticipate the new waves of biological discoveries that spatially resolved, multimodal omics will bring.

Concluding remarks

Imaging-based single-cell genomics combines the power of high-throughput genome-scale analysis with the high spatial resolution afforded by imaging, and opens a new path to tackle problems that were previously intractable. These approaches have already begun to advance many areas of biology, addressing a wide range of problems, from the regulation of gene expression to the spatial and functional organization of distinct types of cells in tissues. This list will rapidly expand with time, transforming not only fundamental biological research but also our ability to diagnose, understand and treat diseases. ❐

Xiaowei Zhuang [1,2,3] ✉

[1]*Howard Hughes Medical Institute, Cambridge, MA, USA.* [2]*Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA.* [3]*Department of Physics, Harvard University, Cambridge, MA, USA.*

✉*e-mail: zhuang@chemistry.harvard.edu*

Published online: 6 January 2021
https://doi.org/10.1038/s41592-020-01037-8

References

1. Larsson, L., Frisén, J. & Lundeberg, J. *Nat. Methods* https://doi.org/10.1038/s41592-020-01038-7 (2021).
2. Femino, A. M., Fay, F. S., Fogarty, K. & Singer, R. H. *Science* **280**, 585–590 (1998).
3. Raj, A., van den Bogaard, P., Rifkin, S. A., van Oudenaarden, A. & Tyagi, S. *Nat. Methods* **5**, 877–879 (2008).
4. Levsky, J. M., Shenoy, S. M., Pezo, R. C. & Singer, R. H. *Science* **297**, 836–840 (2002).
5. Chen, K. H., Boettiger, A. N., Moffitt, J. R., Wang, S. & Zhuang, X. *Science* **348**, aaa6090 (2015).
6. Xia, C., Fan, J., Emanuel, G., Hao, J. & Zhuang, X. *Proc. Natl Acad. Sci. USA* **116**, 19490–19499 (2019).
7. Chen, F., Tillberg, P. W. & Boyden, E. S. *Science* **347**, 543–548 (2015).
8. Wang, G., Moffitt, J. R. & Zhuang, X. *Sci. Rep.* **8**, 4847 (2018).
9. Moffitt, J. R. et al. *Proc. Natl Acad. Sci. USA* **113**, 14456–14461 (2016).
10. Moffitt, J. R. et al. *Science* **362**, eaau5324 (2018).
11. Su, J. H., Zheng, P., Kinrot, S. S., Bintu, B. & Zhuang, X. *Cell* **182**, 1641–1659.e26 (2020).
12. Lubeck, E., Coskun, A. F., Zhiyentayev, T., Ahmad, M. & Cai, L. *Nat. Methods* **11**, 360–361 (2014).
13. Shah, S., Lubeck, E., Zhou, W. & Cai, L. *Neuron* **92**, 342–357 (2016).
14. Eng, C. L. et al. *Nature* **568**, 235–239 (2019).
15. Codeluppi, S. et al. *Nat. Methods* **15**, 932–935 (2018).
16. Beliveau, B. J. et al. *Proc. Natl Acad. Sci. USA* **109**, 21301–21306 (2012).
17. Xia, C., Babcock, H. P., Moffitt, J. R. & Zhuang, X. *Sci. Rep.* **9**, 7721 (2019).
18. Landegren, U. *BioEssays* **15**, 761–765 (1993).
19. Goh, J. J. L. et al. *Nat. Methods* **17**, 689–693 (2020).
20. Ke, R. et al. *Nat. Methods* **10**, 857–860 (2013).
21. Wang, X. et al. *Science* **361**, eaat5691 (2018).
22. Qian, X. et al. *Nat. Methods* **17**, 101–106 (2020).
23. Iyer, E.P.R. et al. Preprint at *bioRxiv* https://doi.org/10.1101/281121 (2018).
24. Alon, S. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.05.13.094268 (2020).
25. Chen, X., Sun, Y. C., Church, G. M., Lee, J. H. & Zador, A. M. *Nucleic Acids Res.* **46**, e22 (2018).
26. Lee, J. H. et al. *Science* **343**, 1360–1363 (2014).
27. Stuart, T. et al. *Cell* **177**, 1888–1902.e21 (2019).
28. Biancalani, T. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.08.29.272831 (2020).
29. Zhang, M. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.06.04.105700 (2020).
30. Chen, W. T. et al. *Cell* **182**, 976–991.e19 (2020).
31. Asp, M. et al. *Cell* **179**, 1647–1660.e19 (2019).
32. Soldatov, R. et al. *Science* **364**, eaas9536 (2019).
33. Moffitt, J. R. et al. *Proc. Natl Acad. Sci. USA* **113**, 11046–11051 (2016).
34. Wang, G. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.12.07.414060 (2020).
35. Wang, S. et al. *Science* **353**, 598–602 (2016).
36. Nguyen, H. Q. et al. *Nat. Methods* **17**, 822–832 (2020).
37. Bintu, B. et al. *Science* **362**, eaau1783 (2018).
38. Nir, G. et al. *PLoS Genet.* **14**, e1007872 (2018).
39. Mateo, L. J. et al. *Nature* **568**, 49–54 (2019).
40. Cardozo Gizzi, A. M. et al. *Mol. Cell* **74**, 212–222.e5 (2019).
41. Sawh, A. N. et al. *Mol. Cell* **78**, 96–111.e6 (2020).
42. Liu, M. et al. *Nat. Commun.* **11**, 2907 (2020).
43. Takei, Y. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.11.29.403055 (2020).
44. Lawson, M. J. et al. *Mol. Syst. Biol.* **13**, 947 (2017).
45. Emanuel, G., Moffitt, J. R. & Zhuang, X. *Nat. Methods* **14**, 1159–1162 (2017).
46. Wang, C., Lu, T., Emanuel, G., Babcock, H. P. & Zhuang, X. *Proc. Natl Acad. Sci. USA* **116**, 10842–10851 (2019).
47. Feldman, D. et al. *Cell* **179**, 787–799.e17 (2019).
48. Camsund, D. et al. *Nat. Methods* **17**, 86–92 (2020).
49. Frieda, K. L. et al. *Nature* **541**, 107–111 (2017).
50. Chow, K.-H. K. et al. Preprint at *bioRxiv* https://doi.org/10.1101/2020.02.21.958678 (2020).
51. Chen, X. et al. *Cell* **179**, 772–786.e19 (2019).

Acknowledgements

I am grateful to J. Moffitt for suggestions and comments on the paper, and to J. Moffitt, B. Bintu and J. Fan for help with figures. This work is supported in part by the National Institutes of Health. X.Z. is a Howard Hughes Medical Institute investigator.

Competing interests

X.Z. is a cofounder and consultant of Vizgen and an inventor on patents applied for by Harvard University related to MERFISH.