



# Milestones in spatial biology\*

## Introduction

“Form follows function” is a famous architecture saying that works exceptionally well for cellular biology. That is to say, the shapes of biological structures, from nucleic acids to large tissue structures, are dictated by the functions they need to carry out. And since cells reside within microenvironments, their functions are influenced by the network of cells surrounding them, sending and receiving messages.

Spatially resolved biology allows researchers to study cells in the context of their tissue microenvironment, enabling a fuller appreciation of cellular function. Recently, *Nature Methods* named spatially resolved transcriptomics its Method of the Year and highlighted the exciting future ahead researchers envision with additional technological improvements<sup>2</sup>.

While spatial transcriptomics and proteomics methods have really blossomed in the last decade, researchers have been trying to understand cellular function in a true morphological context for a long time. In this infographic,\* we journey through time to explore key applications of spatial profiling methods from the early proof-of-principle days to advances in resolution and multiplex detection.

\*This infographic summarizes a non-exhaustive list of academic publications in the spatial biology space. More in-depth reviews of the various spatial methods mentioned have been published by Asp et al<sup>1</sup>.

1. Asp M, et al. *Bioessays* 42: e1900221 (2020).
2. Marx V. *Nat Methods* 18: 9–14 (2021).

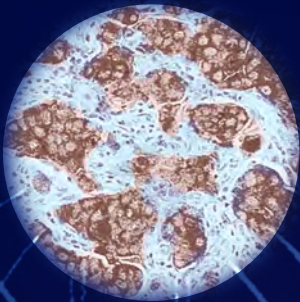
Start ►

# 1941

## Immunohistochemistry (IHC)

Coons et al. demonstrated the use of a fluorescently labeled antibody for visualizing protein targets in tissues<sup>3</sup>

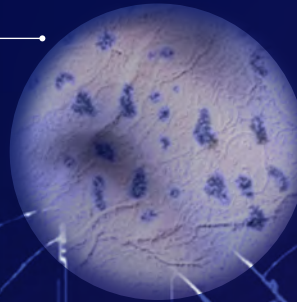
3. Coons AH, et al. *Proc Soc Exp Biol Med* 47: 200–202 (1941).



## RNA ISH

Singer et al. described an in situ hybridization method for mapping mRNA species while maintaining the morphology of analyzed cells<sup>5</sup>

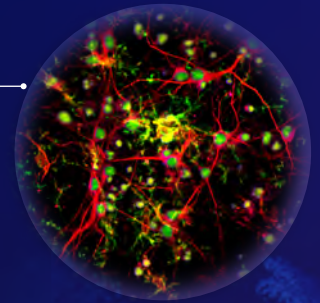
5. Singer RH, et al. *PNAS* 79: 7331–7335, (1992).



## Tyramide signal amplification–based multiplexed IHC

Wang et al. showed the application of TSA to immunofluorescence confocal laser microscopy for visualization of protein localization<sup>7</sup>

7. Wang G, et al. *Methods* 18: 459–64 (1999).



### The Early Days

### The Resolution Revolution

1990–2009

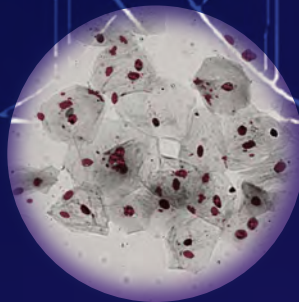
Next

# 1969

## DNA in situ hybridization (ISH)

Pardue et al. reported a DNA-DNA hybridization method using radioactive labeling for detecting the cellular localization of DNA sequences<sup>4</sup>

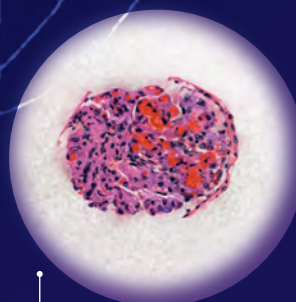
4. Pardue ML, et al. *PNAS* 64: 600–604 (1969).



## Laser capture microdissection

Emmert-Buck et al. published a method for visualizing and dissecting sections of cells microscopically from tissue samples for downstream profiling<sup>6</sup>

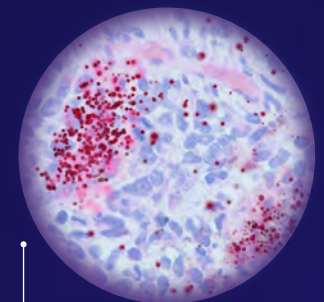
6. Emmert-Buck MR, et al. *Science* 274: 998–1001 (1996).



## Branched DNA ISH

Player et al. presented the use of branched DNA signal amplification for detecting low-copy human papillomavirus DNA in subcellular compartments<sup>8</sup>

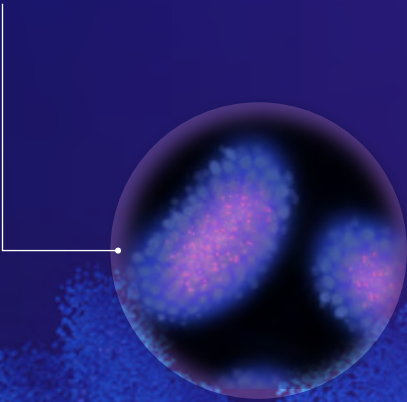
8. Player AN, et al. *J Histochem Cytochem* 49: 603–12 (2001).



## Single molecule ISH (smISH)

Raj et al. demonstrated a method for multiplex gene expression profiling using singly labeled oligonucleotide probes<sup>10</sup>

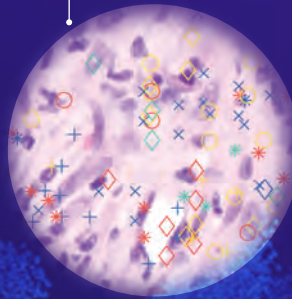
10. Raj A, et al. *Nat Methods* 5: 877–879 (2008).



## In situ sequencing (ISS)

Ke et al. reported the application of sequencing for the multiplex detection of single mRNA molecules<sup>11</sup>

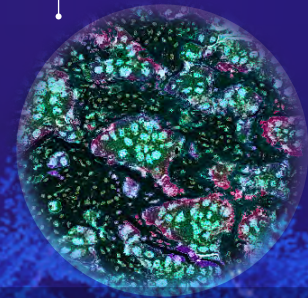
11. Ke R, et al. *Nat Methods* 10: 857–60 (2013).



## Multiplexed ion beam imaging

Angelo et al. published a multiplexed IHC method to image proteins targets bound to metal-tagged antibodies via secondary ion mass spectrometry<sup>13</sup>

13. Angelo M, et al. *Nat Med* 20: 436–42 (2014).



## The Resolution Revolution

1990–2009

## The Multiplex Expansion

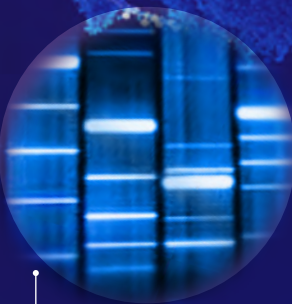
2010–2021

Next

## Hybridization chain reaction

Dirks et al. described the use of nucleic acid probes as signal amplifiers for biosensing applications<sup>9</sup>

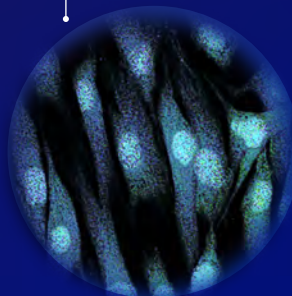
9. Dirks RM and Pierce NA. *PNAS* 101: 15275–15278 (2004).



## Fluorescence ISS (FISSEQ)

Lee et al. described the use of fluorescence in situ RNA sequencing for highly multiplexed subcellular RNA analysis<sup>12</sup>

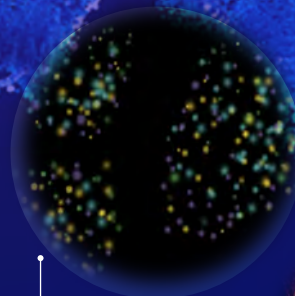
12. Lee JH, et al. *Science* 343: 1360–1363 (2014).



## seqFISH

Lubeck et al. showed a multiplexed mRNA detection method employing a sequencing barcoding strategy<sup>14</sup>

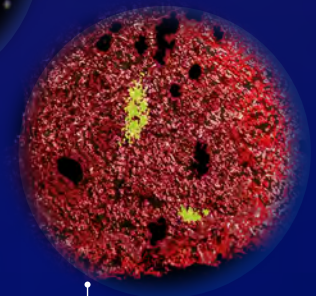
14. Lubeck E, et al. *Nat Methods* 11: 360–361 (2014).



## Spatially encoded assays

Chee et al. demonstrated highly multiplexed spatial addressing of mRNA in fixed tissue samples<sup>15</sup>

15. Chee MS, et al. Poster #1682T. ASHG Annual Meeting (2014).



## Spatial transcriptomics (ST)

Stahl et al. demonstrated the visualization of tissue sections and quantification of their transcriptomes using unique positional barcodes<sup>17</sup>

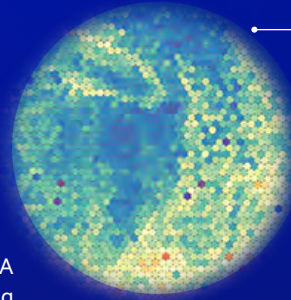
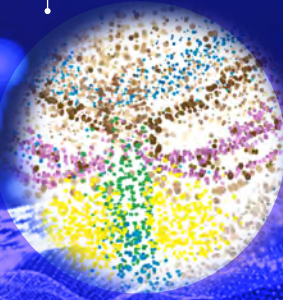
17. Stahl PL, et al. *Science* 353: 78–82 (2016).



## Slide-seq

Rodrigues et al. reported a method for inferring RNA localization via sequencing of RNA transferred from tissue sections<sup>19</sup>

19. Rodrigues SG, et al. *Science* 363: 1463–1467 (2019).



## Visium Spatial Gene Expression

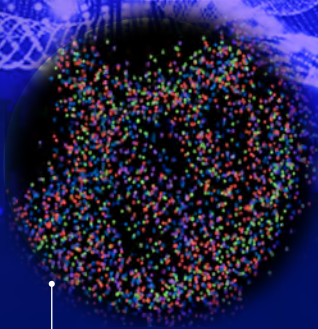
Ji et al. showed the application of Visium spatial whole transcriptome profiling to detail the spatial organization of tumor cell populations<sup>21</sup>

21. Ji AL, et al. *Cell* 182: 497–514.e22 (2020).

## The Multiplex Expansion

2010–2021

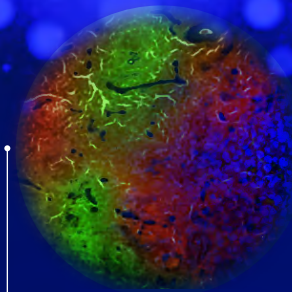
Next



## MERFISH

Chen et al. presented a specific error-tolerant approach to multiplexed single-molecule counting and mapping of mRNAs in single cells<sup>16</sup>

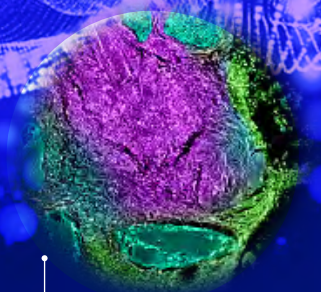
16. Chen HK, et al. *Science* 348: aaa6090 (2015).



## Cyclic multiplex immunofluorescence

Goltsev et al. published an antigen staining method for cytometric multiplexed imaging of protein targets in single cells and tissue sections<sup>18</sup>

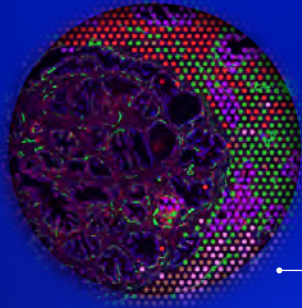
18. Goltsev Y, et al. *Cell* 174: 968–981 (2018).



## Digital spatial profiling

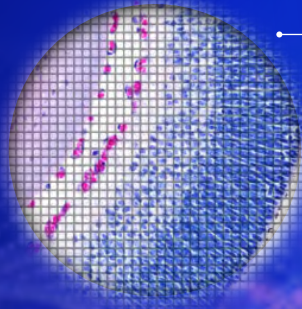
Amaria et al. published the use of digital spatial profiling for multiplexed IHC of immune markers<sup>20</sup>

20. Amaria RN, et al. *Nat Med* 24: 1649–1654 (2018).  
Erratum in: *Nat Med*. 2018 Oct 25.



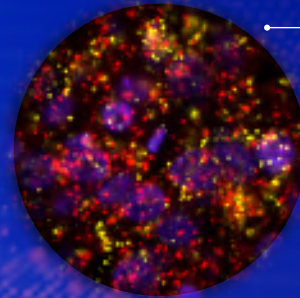
### Visium Spatial Gene and Protein Expression

Will add highly multiplexed protein detection to Visium whole transcriptome profiling of tissue sections



### Visium HD

Will bring single cell scale resolution to Visium spatial profiling



### Xenium In Situ

Will allow precise spatial mapping of RNA and protein with a subcellular readout

## The Next Frontier

2022 and beyond

## Trailblazing the future of spatial biology

Spatially resolved biology, including whole transcriptomic and targeted in situ methods, allows scientists to build a more complete view of cellular function in the tissue context. Visium from 10x Genomics is a spatial discovery platform that allows whole transcriptome profiling of fresh and formalin-fixed paraffin-embedded (FFPE) tissues. And Xenium, our new in situ platform, provides the highest spatial resolution with targeted gene and protein detection, enabling translational and, ultimately, clinical applications. See biology in new ways with the most comprehensive spatial resolution and scale.

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