Supporting Information

Spatial Transcriptomics:
Emerging Technologies in Tissue Gene Expression Profiling

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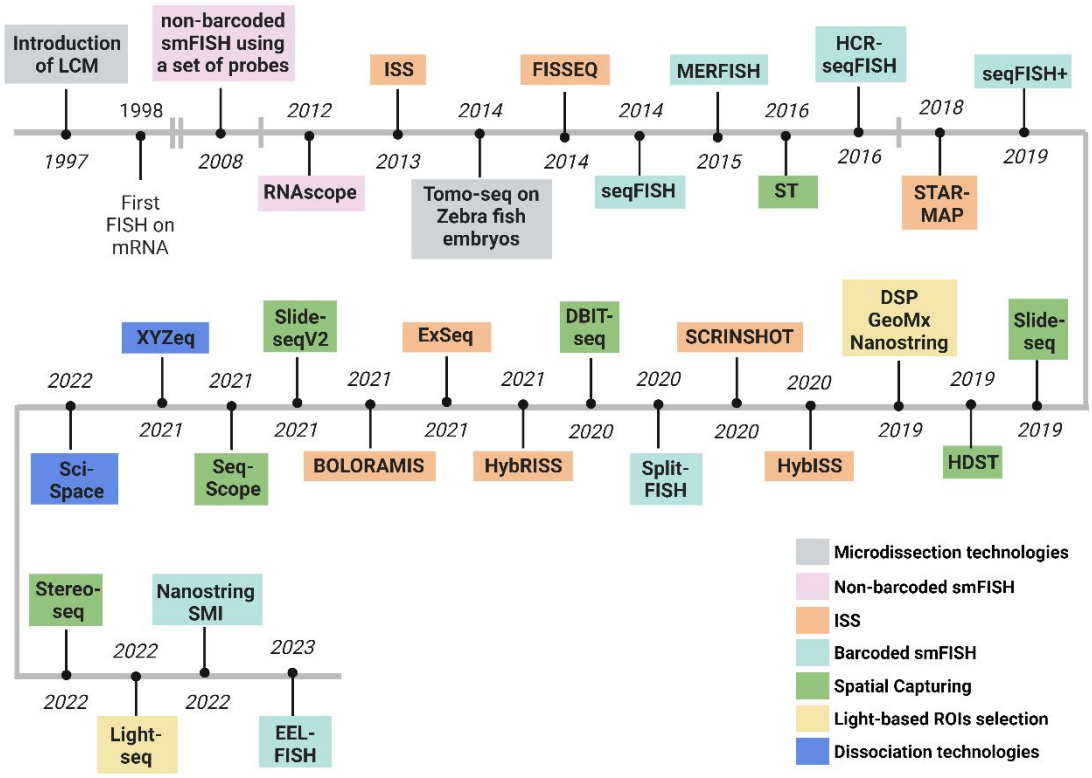

Figure S1. Spatial Transcriptomics Timeline. Scheme of Spatial Transcriptomics technologies classified by types and the date they were first published.

Method	Efficiency	Number of gene transcripts	Size resolution	Barcoding Strategy (*in situ*)	Type of Tissue	Commercialized	Advantages	Limitations
ISS-Based methods								
ISS	5-30 %	222	Single-cell	SBL	Fresh Frozen	Yes, as Xenium	-Robust detection -High SBR	-Image crowding -Moderate multiplexing capacity
HybISS	Similar to ISS	120	Single-cell	SBH	Fresh Frozen	No	-Enhanced SBR compared to ISS	-Lower number of transcript gene demonstrated than ISS
HybRISS	Increased 5-fold compared to ISS	50	Single-cell	SBH	Fresh Frozen	No	-Enhanced efficiency -Possibility to study more targets than mRNA species	-Lower number of transcript gene demonstrated than ISS
SCRINSHOT	Improved efficiency compared to ISS	29	Single-cell	SBL	Fresh Frozen	No	-Enhanced efficiency -Possibility to study more targets than mRNA species	-Lower number of transcript gene demonstrated than ISS
FISSEQ	(<0,0001%)	Transcriptome wide	Single-cell	SBL	Fresh Frozen	No	-Possibility to conduct *de novo* analyses	-Remarkable low efficiency
ExSeq	60 %	297	Single-cell	SBL/NGS	Fresh Frozen	No	-Enhanced efficiency than FISSEQ -Possibility for *Ex Situ* Analyses	-Increased imaging time
BOLORAMIS	10 % - 35 %	96	Single-cell	SBL	Fresh Frozen	No	-Possibility to study more targets than mRNA species	-Lower number of transcript gene demonstrated than ISS
STAR-MAP	Similar to scRNA-seq	~1000-2700	Single-cell	SEDAL	Fresh Frozen	No	-Possibility to study 3D sections (150 µm thick)	-3D sections limited to 28 genes
smFISH-Based methods								
MERFISH	80-95 %	~10,000	Single-cell	N-binary word code decodification	Fresh Frozen FFPE	Yes, as MER-Scope	-High multiplexity capacity -High efficiency -Include Error Correction Scheme	-Low SBR -High number of probes
HCR-seqFISH	~80 %	249	Single-cell	Color-codes associated to mRNA species	Fresh Frozen	No	-High efficiency -Include Error Correction Scheme	-Low SBR -Need for specialized equipment -Moderate multiplexing capacity
SeqFISH+	49 %	~10,000	Single-cell	Color-codes associated to mRNA species	Fresh Frozen	No	-High multiplexity capacity -High efficiency -Include Error Correction Scheme	-High number of probes -Need for specialized equipment -Methodology difficult to handle
Split-FISH	71 %	317	Single-cell	N-binary word code decodification	Fresh Frozen	No	-Enhanced SBR ratios -Include Error Correction Scheme	-Moderate multiplexing capacity
SMI-Nanostring	NA	980	Single-cell	N-binary word code decodification	Fresh Frozen and FFPE	Yes	-Automated system -Possibility to protein co-detection -Include Error Correction Scheme	-Moderate multiplexing capacity
EEL-FISH	~13 %	~2000	Single-cell	N-binary word code decodification	Fresh Frozen	No	-High SBR ratios -Reduced optical crowding	-Lower efficiency compared to other smFISH methods

Table S1. Comparison of ISS and smFISH-based technologies.

Method	Efficiency	Number of mRNA species	-Spot size -Spot-to-spot distance	Covered area	Barcoding Strategy (ex situ)	Type of Tissue	Commercialized	Advantages	Limitations
Microdissection Technologies									
LCM	NA	Transcriptome wide	Single-cell resolution	NA	NGS	Fresh Frozen and FFPE	Yes	-Easy implementation (Microdissection microscope) -Available for FFPE	-Limited number of single cells analyzed per tissue
Tomo-seq	NA	Transcriptome wide	Up to 8 µm	NA	NGS	Fresh Frozen	No	-Easy implementation (Sections from a biological system)	-Resolution limited to the area sectioned (lack single-cell resolution)
Spatial Capturing Technologies									
ST/Visium	~30.000 UMIs per 100 µm² area	Transcriptome wide	-100 µm / 55 µm -200 µm / 100 µm	42.25 mm²	NGS	Fresh Frozen and FFPE	Yes	-Most available commercial option -Available for FFPE	-Lack single-cell resolution
Slide-seqV2	~550 UMIs per 10 µm² area	Transcriptome wide	-10 µm -10 µm	7 mm²	NGS	Fresh Frozen	No	-High spot size resolution	-Analyses require grouping areas
HDST	~10 UMIs per 2 µm² area	Transcriptome wide	-2 µm -2 µm	13.68 mm²	NGS	Fresh Frozen	No	-High spot size resolution	-Low efficiency -Analyses require grouping areas
DBIT-Seq	~5000 UMIs per 10 µm² area	Transcriptome wide	-10 µm or 50 µm -10 µm or 50 µm	1 mm² or 25 mm²	NGS	Fresh Frozen and FFPE	No	-Adapted as a microfluidic system	-Uncovered spaces between squares -Analyses require grouping areas
Seq-Scope	5-25 UMIs per HDMI cluster ~1000 UMIs per 10 µm² area	Transcriptome wide	-Submicrometric HDMI cluster -600 nm	0. 2 mm²	NGS	Fresh Frozen	No	-High spot size resolution -Submicrometric HDMI clusters	-Reduced capturing area -Analyses require grouping areas
Stereo-seq	62 UMIs per DNB cluster (2 µm² area) ~1000 UMIs per 10 µm² area	Transcriptome wide	-220 nm -600 nm	50 – 200 mm²	NGS	Fresh Frozen	No	-High spot size resolution -Submicrometric DNB clusters -High capture area (up to 200 mm²)	-Analyses require grouping areas
Pixel-seq	~1000 UMIs per 10 µm² area	Transcriptome wide	~1 µm -Continuous spot-to-spot distance	5-15 mm² areas	NGS	Fresh Frozen	No	-High spot size resolution -Developed to be scalable	-Analyses require grouping areas
Light-based ROI selection technologies									
GeoMx DSP-Nanostring	~1000 gene transcripts in 400 µm diameter ROIs	Transcriptome wide	ROIs from 10-600 µm	NA	NGS or nCounter	Fresh Frozen and FFPE	Yes	-Easy implementation (Automated system) -Available for FFPE -Possibility for protein codetection	-For whole tissue transcriptomics analyses, laborious manual selection of a limited number of ROIs
Light-seq	~1,000–10,000 UMIs per 10 µm² area	Transcriptome wide	Minimum ROI: 2 µm	NA	NGS	Fresh Frozen	No	-ROIs resolution up to 2 µm -Option for sample reutilization	-For whole tissue transcriptomics analyses, laborious manual selection of a limited number of ROIs
Spatial Cell/Nuclei Dissociation Technologies									
XYZeq	~1000 UMIs per 500 µm diameter wells	Transcriptome wide	-500 µm wells -500 µm distance	NA	NGS	Fresh Frozen	No	Cells dissociated as barcoded spots	Limited resolution to areas of 500 µm
Sci-space	~2000 UMIs estimated per cell	Transcriptome wide	-73.2 µm -222 µm	NA	NGS	Fresh Frozen	No	Nuclei dissociated as barcoded spots	Limited to nuclear transcriptomics analyses

Table S2. Comparison of microdissection, spatial capture, light-based ROIs selection, and dissociation-based technologies.