Section: Pathology

Meta-Analysis

MERFISH IN SPATIAL TRANSCRIPTOMICS: A COMPREHENSIVE META-ANALYSIS

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ABSTRACT

Background: Spatial transcriptomics (ST) is a molecular technique that helps in gene expression mapping within intact tissue. Among the various approaches, **Multiplexed Error-Robust Fluorescence in Situ Hybridization (MERFISH)** is built on the power of single molecule RNA Fluorescent in situ hybridization which detect individual RNA molecules with high accuracy and sort out thousands of genes at once. Although the technique has been widely adopted across neuroscience, oncology, and developmental biology, its role in spatial transcriptomics is still limited. **Objectives:** This meta-analysis aims to evaluate its applications, accuracy, reproducibility and its comparison with other spatial transcriptomics. We also illustrated how MERFISH integrates with other multi-omics platforms and its role in biomedical research.

Material and Methods: Systematic searches carried out across PubMed, Scopus, Web of Science and EMBASE (2014–2025). Data reporting of MERFISH based spatial transcriptomics carried out with either quantitative or qualitative outcomes. For data extraction PRISMA guidelines are followed. Quantitative analysis was performed on reproducibility measures, gene detection efficiency and spatial resolution while for qualitative synthesis we examined functional and clinical insights from the included studies.

Results: Thirty-four studies (N = 34) met the inclusion criteria covering neuroscience, oncology, developmental biology, and pathology. Across these studies, MERFISH achieved >95% molecular detection accuracy and subcellular spatial precision. On comparing with other techniques like seqFISH+ and Slide-seqV2, MERFISH showed superior spatial resolution (50–100 nm) and maintained a strong multiplexing capacity (>10,000 genes). MERFISH integration with single-cell RNA-seq enhanced tissue-level transcriptome reconstruction and cell-type classification.

Conclusions: MERFISH emerges as a highly reliable, high-throughput and advanced spatial transcriptomics platform with significant translational potential. Integrating MERFISH with computational modelling and multimodal imaging is expected to expand diagnostics, developmental biology and disease mapping applications.

Keywords: MERFISH, spatial transcriptomics, single-cell analysis, multiplexed imaging, gene expression, tissue mapping.

INTRODUCTION

The arrangement of gene expression across spatial domain plays a crucial role in regulating tissue physiology, development, and disease process. The conventional transcriptomic techniques, such as bulk RNA sequencing or single-cell RNA sequencing (scRNA-seq), have provided deep molecular insights into gene regulation but fails to preserve spatial configuration due to tissue disruption. Spatial transcriptomics (ST) therefore bridges this gap and emerged as an innovative approach by preserving the spatial architecture of gene expression.

Among the diverse ST techniques developed in the past decade, Multiplexed Error-Robust Fluorescence in Situ Hybridization (MERFISH) has established itself as one of the highly precise, accurate, robust and scalable modality. MERFISH employs combinatorial labelling and error-robust barcoding schemes to visualize and quantify thousands of RNA species directly in fixed cells and tissues.[1]

In contrast to sequencing-based platforms such as Slide-seq, Visium, or DBiT-seq, which depends on spatially barcoded arrays and subsequent RNA capture, MERFISH attains subcellular spatial resolution through direct optical detection making it powerful tool for analysing transcriptomes in tissues such as tumours, neural tissues and developing embryos.

During the last decade, MERFISH has found extensive applications to across numerous biological and clinical areas, including:

- Detailed mapping of brain regions and neuronal subtype identification
- Spatial profiling of tumour microenvironment.
- Tissue differentiation during development.
- Analysis of intracellular signalling and cell-cell interaction network.

Despite notable technological developments, a comprehensive meta-analysis that systematically assesses the reproducibility, performance and comparative strength of MERFISH relative to other transcriptomic platform is still lacking. This manuscript intends to fill that research voids through an integrated evaluation of existing findings, methodological consistency and potential clinical trajectories.

MATERIALS AND METHODS

1. Literature Search Strategy

In compliance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines systematic literature searches were conducted across PubMed, Scopus, Web of Science, and EMBASE database from January 2014 to September 2025.The search study incorporates the term:

"MERFISH," "spatial transcriptomics," "multiplexed FISH," "single-molecule imaging," and "gene expression mapping."

Boolean combinations and MeSH terms were applied to maximise coverage of relevant peer-reviewed literature. Reference lists of selected articles were also conducted to identify additional studies meeting inclusion criteria's.

2. Inclusion and Exclusion Criteria

Inclusion parameters were:

1. Studies that applied MERFISH for spatial profiling of gene expression.
2. Article presenting outcome measures such as gene detection efficiency, resolution, reproducibility, or tissue-specific expression.
3. Publication timeframe restricted to 2014–2025.
4. Peer-reviewed original studies in English.

Exclusion parameters were

- Review articles, commentaries, and conference abstracts not presenting original article.
- Studies based on FISH techniques not implementing MERFISH error robust barcoding
- Studies omitting spatial analysis or quantitative performance metrics.

3. Data Extraction and Synthesis

Data extraction was carried out independently by two reviewers using a standardized pre-defined form. Extracted variables included:

- Study design and biological system
- Gene panel size
- Imaging resolution
- Detection efficiency
- Computational reconstruction method
- Validation with scRNA-seq

Any discrepancies during extraction were resolved by consensus. Quantitative findings were combined using DerSimonian–Laird random-effects method and heterogeneity was evaluated using I^2 statistics.

4. Quality Assessment

Study quality of included articles were evaluated via the Newcastle–Ottawa Scale (NOS) for imaging-based molecular studies. Studies achieving score ≥7 were categorised as high-quality. Funnel plots and Egger's regression were used to assess publication bias.

5. Data Presentation

The research literature screening steps were documented using PRISMA-style flow table. Key findings were recorded as mean ± SD values for accuracy and resolution outcomes. Results were stratified by tissue type (neural, tumour, developmental, organoid).

RESULTS

Study Selection and Characteristics

The comprehensive search identified 1,276 records of which 342 duplicates were removed. After title and abstract screening, 156 articles were eligible for full-text assessment and 34 studies met the inclusion criteria for final integration.

The included studies extended over 2015–2025 covering diverse tissues for instance, 15 in

neuroscience, 8 in oncology, 6 in developmental biology and 5 in stem-cell models.

All studies utilized standard MERFISH or MERFISH derived protocols employing combinatorial barcoding with error-robust encoding and sequential hybridization cycles.

Table 1: PRISMA Style Literature Screening

Stage	Description	Number of Records
Identification	Records retrieved from databases	1,276
Screening	After removing duplicates	934
Eligibility	Full-text articles assessed	156
Included	Studies meeting inclusion criteria	34

1. Quantitative Synthesis of Accuracy and Resolution

MERFISH demonstrated >**94–98 % detection accuracy** when compared against smFISH or RNA-seq validation datasets.

Mean subcellular localization precision was **80 ± 15 nm** and average false-positive barcoding errors were below **2 %**.

On comparing MERFISH with other technologies:

- **seqFISH +** method showed around 10 % lower detection efficiency than MERFISH ($p < 0.05$).
- **Slide-seq V2** and **Visium** achieved lower spatial resolution (10–55 µm vs. < 0.1 µm for MERFISH).

MERFISH's molecular counting correlated strongly with scRNA-seq ($r = 0.91$, $p < 0.001$) confirming quantitative reliability.

Table 2: MERFISH performance metrics (2015–2025)

Parameter	MERFISH (mean ± SD)	Comparative Method	p-value
Detection accuracy	96.7 ± 2.5 %	seqFISH + = 87.4 ± 3.1 %	< 0.05
Spatial resolution	0.08 ± 0.02 µm	Slide-seq V2 = 15 µm	< 0.001
Gene panel size	5,000–10,000 genes	Visium = 1,500 genes	—
Multiplexing cycles	15–25	seqFISH + = 30–40	—
Quantitative correlation (r)	0.91 ± 0.04	RNA-seq validation	< 0.001

3. Biological Applications

Neuroscience

Fifteen studies implemented MERFISH to map neuronal subtypes across mouse cortex and hippocampus.

Through MERFISH analysis scientists discovered over 60 neuronal and glial subpopulations which showed spatial gradients associated with synaptic plasticity, metabolism, and neurotransmitter identity (Chen et al., 2015; Xia et al., 2019). The combination of calcium imaging and gene expression analysis through MERFISH revealed connections between functional electrophysiological domains and gene expression domains.

Oncology

Eight studies showed MERFISH power in tumour microenvironment mapping especially in breast and colorectal cancers.

Researchers who combined MERFISH with immunofluorescence multiplexing detected immune-checkpoint gene clusters which localized near invasive margins and hypoxic niches (Huang et al., 2021).

MERFISH outlined stromal tumour interactions and spatial heterogeneity in PD-L1, VEGFA and CXCL12 expression.

Developmental and Organoid Systems

MERFISH enabled researchers to construct spatially organised temporal transcriptomic of embryonic tissues and organoids (Guo et al., 2022). In gastruloid models, it showed patterning of signalling pathways (FGF, WNT, and BMP) during morphogenesis, linking gene gradients with lineage specification.

Computational Integration

Researchers integrated MERFISH with single cell RNAseq and machine learning pipelines to improve cell type identification and tissue reconstruction accuracy. Graph based spatial clustering (SpaGCN, Giotto) applied to MERFISH data enhanced neighbourhood level gene network identification (Zhang et al., 2023).

4. Heterogeneity and Quality Assessment

The combined detection accuracy heterogeneity was $I^2 = 34$ %, indicating low to moderate deviation among studies.

Quality appraisal using NOS revealed 28 studies (82 %) scored ≥ 7, denoting high methodological reliability.

No significant publication bias was observed (Egger's test $p = 0.31$).

DISCUSSION

MERFISH as a High Resolution Spatial Platform

MERFISH shows greater efficacy than conventional transcriptomic assays by allowing direct spatial quantification of thousands of RNA molecules without needing to dissociate the tissue. This method achieves high reliability against optical and hybridisation noise through its combinatorial barcoding strategy and robust error correction techniques which typically uses a Hamming distance of at least.[4]

This level of accuracy has enabled researchers to map cell type specific transcriptomes within intact tissue microenvironments and illuminate fine grained

spatial relationships that shape both normal function and disease. As this thechnique can localize transcripts at the subcellular level it provides unique insight into processes such as RNA transport, local translation and synaptic signaling.

For example, MERFISH assessment in neuronal dendrites have shown that mRNAs encoding synaptic receptors and signaling enzymes occupy distinct spatial domains, pointing toward subcellular functional compartmentalization (Xia et al., 2019).

Comparison with Other Spatial Transcriptomic Techniques

On Comparing with seqFISH +, MERFISH gives slightly reduced multiplexing cycles for higher signal to noise ratio and lower error rates.

On Comparing with Slide-seq, HDST, or Visium, which rely on spatially barcoded beads or arrays, MERFISH achieved orders of magnitude higher spatial precision (< 100 nm vs. 10 µm).

Sequencing based methods remain favourable for whole transcriptome coverage, whereas MERFISH focuses on targeted panels of several thousand genes. Hence, hybrid workflows combining MERFISH and scRNA-seq are becoming increasingly prevalent (Wang et al., 2022).

Integration with Multi-Omics and Imaging Platforms

Recent advances lead to the combination of MERFISH with immunofluorescence, ATAC-seq and proteomics generating multimodal spatial atlases.

The MERFISH-Plus and MEx-FISH variants embed higher photon budget fluorophores and sequential barcoding to extend detection beyond 10 000 genes per assay (Zhang et al., 2023).

Reasearchers have correlated MERFISH data with spatial metabolomics overlays from MALDI imaging to study in situ correlations between transcriptomic profiles and metabolic changes (Li et al., 2024).

Clinical and Translational Implications

The potential of MERFISH technique proves beneficial for clinical pathology applications in the fields of cancer diagnostics, neurodegenerative disorders and infectious diseases. By generating detailed spatial RNA maps, it allows pathologists to pinpoint disease specific microenvironment and identify rare cell populations that may drive therapy endurance.

In oncology, MERFISH based molecular cartography is emerging as an important tool in precision medicine, helping researchers disclose transcriptional heterogeneity within tumors. In neurology, its use in Alzheimer's and Parkinson's models has shed light on region specific transcriptomic remodeling (Zhao et al., 2024).

This technique is also advancing through its coupling with AI-based image analysis pipelines, which supports automated diagnostic scoring and moves the field closer to true computational histopathology.

Several research groups have already proposed clinical grade workflows that combine MERFISH outputs with digital pathology images to meet modulatory standards for molecular diagnostics (Lee et al., 2025).

Limitations and Challenges

However, MERFISH has multiple strengths it still faces practical and analytical issues:

1. Targeted gene panels which is limited to pre-selected genes; novel transcript discovery requires complementary sequencing.
2. Optical throughput, as it has long imaging cycles and photobleaching which can restrict tissue area coverage.
3. Computational demand as multi-terabyte image datasets require high-performance computing for decoding and registration.
4. Sample preparation variability as tissue fixation, sectioning, and autofluorescence can impact reproducibility.

Standardization of experimental pipelines and open-source analytical tools (e.g., MERlin, Starfish) are reducing these issues, promoting data harmonization across laboratories.

Future Directions

Future spatial transcriptomics may extend MERFISH to whole transcriptome capabilities and 3D volumetric visualization and in vivo applications.

Evolving hybrid methods such as DNA coded imaging probes and optical clearing techniques can allow thicker tissue sections and multiplexing beyond 100 000 genes (Wei et al., 2025).

Incorporation with artificial intelligence will automate transcript decoding and spatial clustering, facilitating real-time molecular diagnostics.

Furthermore, clinical translation will benefit from standardized reporting frameworks similar to MIAME for transcriptomics providing reproducibility, transparency and regulatory acceptance.

CONCLUSION

This comprehensive meta-analysis demonstrates how MERFISH is advancing spatial transcriptomics. MERFISH integrates error proof molecular barcoding with single-molecule imaging and spatial mapping which gives power to researchers to study gene expression directly within intact tissues.

Our analysis of 34 studies showed that MERFISH consistently shows exceptional performance, with detection accuracy above 95%, subcellular resolution under 100 nm, and strong reliability across a wide range of tissue types. On combining with single cell RNA sequencing, advanced imaging and computational deconvolution tools, it greatly enhances transcriptome reconstruction and tissue level interpretation.

From a translational view, MERFISH gives striking spatial insights into areas such as neurodevelopment, tumor heterogeneity and regenerative biology. It helps unveil molecular interactions that shape both normal physiology and disease. Most importantly, it bridges a critical gap between traditional histology

and molecular profiling by linking morphological features with gene expression patterns which offers a potential shift in how precision pathology is being approached.

This said, the technology is not without challenges. Current limitations include the size of gene panels, imaging speed and the considerable sunstantial demands. However, the field is moving very fast towards whole transcriptome spatial profiling, 3D tissue mapping and AI-driven image decoding advances that will further increase its diagnostic and research utility.

In summary, MERFISH has become a cornerstone technology for spatial transcriptomics by combining strong quantitative performance with high spatial accuracy and meaningful biological interpretation. As ongoing improvements will continue to refine the method, its integration into clinical workflows and multi-omics platforms is primed to redefine spatial biology and precision medicine in the years ahead.

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