



Research review paper

Harnessing spatial transcriptomics to understand host-parasite interactions in plants and animals

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ABSTRACT

Obligate parasites pose a significant threat to animal, human, and plant health by affecting host gene expression through mechanisms that are poorly understood. Spatial transcriptomic technologies are revolutionizing our understanding of animal-parasite interactions, revealing tissue reorganization, cellular responses, and infection dynamics at a microscopic scale. These technologies also accelerate the identification of potential targets for treating animal parasite infections. Despite their potential, the application of spatial transcriptomic technologies to plant-parasite interactions is limited. This review highlights key challenges in applying spatial transcriptomics to plants. By drawing parallels with advances in animal systems, we discuss how spatial transcriptomics could contribute to localize and identify effectors, uncover the molecular mechanisms of plant-parasite infections, and find novel disease control targets. This cross-disciplinary perspective provides a roadmap for future research in plant and animal parasitology.

1. Advancing host-parasite research using spatial transcriptomics

Parasites have a significant impact on humans, animals, and plants, causing disease and economic loss. Each year, more than a billion people are infected with parasites, including helminths such as *Schistosoma mansoni* (schistosomiasis) and hookworms (hookworm disease), as well as protists such as *Plasmodium* (malaria) and *Trypanosoma brucei* (sleeping sickness) (Naveed and Abdullah, 2021). In agriculture, parasitic nematode infections cause severe yield losses, posing a threat to global food production (Nicol et al., 2011). Obligate parasites depend on their host for nutrients, survival, and reproduction, often at the host's expense. To combat infection, host animals and plants initiate a defense response; however, parasites frequently evade or modulate this response to ensure their survival and reproduction (Ezema et al., 2023; Li et al., 2024c). Understanding this complex and dynamic host-parasite interplay at the molecular and cellular level is crucial for developing effective treatments against parasites.

In this review, we discuss the use of spatial transcriptomics to study local patterns in gene expression at the microscale. We focus on the

added value of spatial information in host-parasite interaction studies and illustrate the power of spatial transcriptomics using examples from recent animal-parasite studies. Using these examples as a framework, we elaborate on the applications of spatial transcriptomics to tackle similar biological questions in other host-parasite relationships. Although spatial transcriptomics is a trending technology (Nature method of the year 2021) (Marx, 2021), its development in plants lags that in animals. We thus highlight the technology's potential for plant sciences, bridging animal and plant science fields, to provide inspiration for future studies and advance the field of parasitology.

2. Addressing key questions in animal-parasite interactions through spatial transcriptomics

Researchers initially used microarrays to resolve temporal and dynamic patterns in gene expression during parasite infections in animals and plants across different stages of parasite development (Lovegrove et al., 2006; Xu et al., 2005). However, microarrays relied on the design of transcript-specific probes, requiring prior knowledge, and were unable to detect novel transcripts. The advent of RNA sequencing (RNA-

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Seq), solved these limitations, enabling the untargeted detection of novel transcripts with high specificity and sensitivity in the host, the parasite, or both (Shadab et al., 2019; Siddique et al., 2022). To date, most RNA-Seq studies rely on relatively large, heterogeneously infected tissue samples to meet minimum input requirements for reliably assessing gene expression. These studies average gene expression across all cells present in a sample, thereby masking crucial spatial variation of individual cells within infected host tissues. Single-cell RNA-Seq overcame this limitation. Within the parasitology field, researchers applied single-cell RNA-Seq, for instance, to generate gene expression atlases for several helminths, including *S. mansoni* and *Brugia malayi*, and to identify the cell types involved in host responses to infection, as previously reviewed (Britton et al., 2023). Researchers have also applied single-cell RNA-Seq to protists to reveal gene expression heterogeneity in *Plasmodium berghei* (Bogale et al., 2021), to map the single-cell transcriptome across the *P. berghei* life cycle, now accessible through the interactive Malaria Cell Atlas (Howick et al., 2019), and to identify the host cell types parasitized by *Leishmania donovani* in different tissues (Karagiannis et al., 2023).

Nevertheless, single-cell data, while informative, do not capture the spatial organization of tissues and cells. However, spatial information is crucial for understanding processes such as 1) symmetry breaking, 2) tissue context determination of specific roles, and 3) complex biological dynamics, such as host-parasite interactions. Specifically, resolving the spatial patterns of gene expression during host-parasite interactions is crucial for sedentary parasites that induce permanent feeding structures within host tissues (Wang et al., 2023). These feeding structures break symmetry and function as organs supplying nutrients to the parasite, making their development a unique form of organogenesis. Capturing the tissue and cellular gene expression of these structures is therefore essential for developing effective treatments against parasitic infections. Favorably, advancements in spatial transcriptomics now enable the mRNA quantification across numerous genes or the whole transcriptome while preserving the inherent spatial context (Ebbing et al., 2018; Kruse

et al., 2016; Wang et al., 2022a).

Sedentary parasite infection drastically alters the cellular organization of host tissues by forming immobile and spatially organized feeding structures across multiple host cell types. Additionally, the spatial organization of host tissues can be altered by the induction of local immune responses, such as the infiltration of immune cells, as part of the defense mechanism against the parasite (Hildebrandt et al., 2024; Rao et al., 2021). Spatial organization is crucial for the parasite because it typically initiates infection in a specific cell type near its anterior end. Moreover, biotrophic parasites require unique control mechanisms in and around the feeding structures to avoid or modulate the host's immune response while controlling the host's developmental programs. Recently, several animal-parasite interaction studies employed spatial transcriptomics to investigate the spatial organization in animal hosts during infection with various parasites, including helminths such as *B. malayi*, *Heligmosomoides polygyrus*, and *Fasciola hepatica*, as well as protozoans like *P. berghei* and *T. brucei* (Airs et al., 2022; Gramberg et al., 2024; Hildebrandt et al., 2024; Poveda et al., 2024, BioRxiv preprint; Quintana et al., 2022; Sounart et al., 2023; Williams et al., 2024) (Fig. 1). These studies primarily investigate how parasite infection affects host tissues, covering specific questions including: the contribution of the parasite (multicellular) structural organization to its infectious capacity; how parasite infection affects host gene expression across different tissue regions; the distribution and interaction of cell types within the tissue; the types of immune responses triggered upon infection; and how spatial gene expression informs drug development and identifies novel target treatments. Analyzing the spatial context of infections enables a comprehensive characterization of the dynamic host-parasite interplay.

3. Distinct features of spatial transcriptomics technologies

Various spatial transcriptomic technologies are available, each with its unique limitations and advantages (Table 1). These technologies can

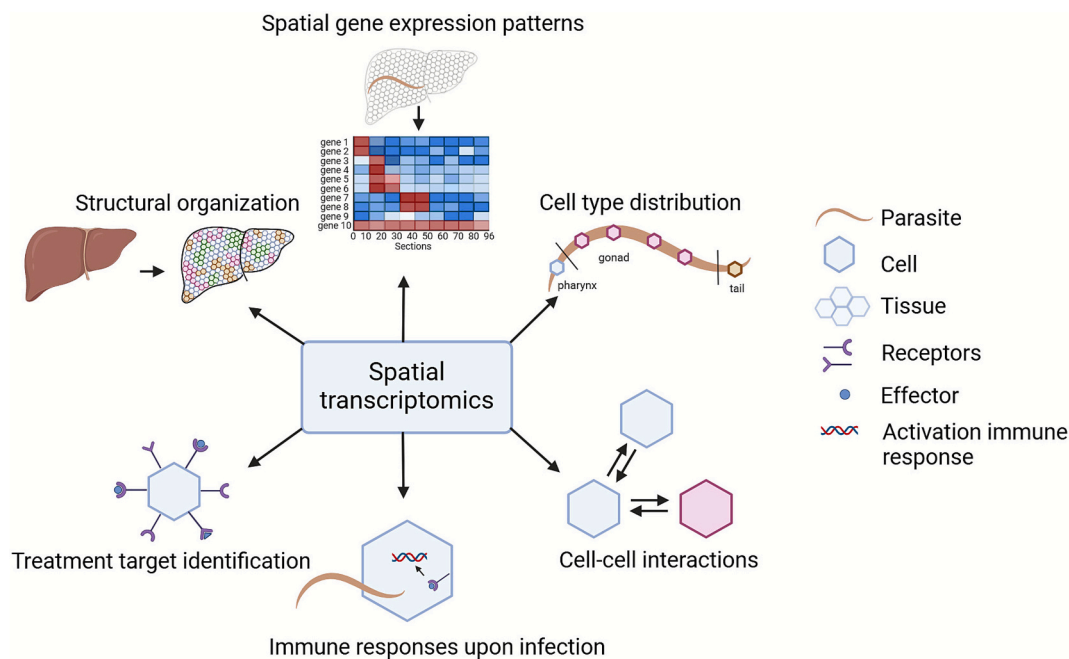


Fig. 1. Applications of spatial transcriptomics in host-parasite interaction studies. Spatial transcriptomics technologies enable to link the structural organization of tissues or organs (e.g., a liver) with gene expression patterns. These technologies can reveal the spatial gene expression patterns across different tissue regions in the host, and how these regions respond to parasite infection. Additionally, spatial transcriptomics provides insights into the cell type distribution within an organism (e.g., pharynx, gonad and tail tissues), how these cells interact with each other and how cell-cell interactions change in response to parasite infection. Furthermore, spatial transcriptomics can reveal localized host immune responses upon infection, identifying which immune cells are activated and where these responses occur within the tissue. By analyzing the changes in spatial gene expression patterns upon parasite infection, genes important in parasite establishment, maintenance, or survival can be identified. These genes are potential targets for treatments. This figure was made using Biorender.

Table 1

Characteristics of spatial transcriptomics technologies applied in plants. Abbreviations: FFPE = formalin-fixed, paraffin-embedded, LCM = laser-capture microdissection, MERFISH = multiplexed error-robust fluorescence in situ hybridization, Stereo-seq = spatially enhanced resolution omics-sequencing.

Method	Tissue treatment	Target	Throughput	Resolution	Capture size	Application in plant(s)
<i>Sequencing-based spatial transcriptomics</i>						
Visium	Fresh frozen or FFPE	Untargeted	High	55 μm	6.5 \times 6.5 mm	Arabidopsis, <i>Picea abies</i> (Giacomello et al., 2017), <i>Populus</i> (Giacomello et al., 2017; Li et al., 2023), maize (Fu et al., 2023), and other species (Du et al., 2023; Li et al., 2024b; Liu et al., 2022a; Peirats-Llobet et al., 2023; Serrano et al., 2024; Song et al., 2023; Zhang et al., 2025)
Stereo-Seq	Fresh frozen	Untargeted	High	0.5-1 μm	Up to 130 \times 130 mm	Arabidopsis (Xia et al., 2022), soybean (Liu et al., 2023), rice (Yao et al., 2024), and other species (Song et al., 2023; Wang et al., 2024; Ye et al., 2024)
Curio Seeker	Fresh frozen	Untargeted	High	10 μm	3 \times 3 mm or 10 \times 10 mm	Arabidopsis (Lee et al., 2025)
RNA tomography	Fresh frozen	Untargeted	High	20 μm (Custom)	Custom	Arabidopsis (Pijnacker et al., 2025)
LCM-Seq	Fresh frozen	Targeted	Low	10-20 μm	Custom (depends on dissection)	Arabidopsis (Berkowitz et al., 2021), lettuce (Chen et al., 2018), cotton (Ando et al., 2021), and other species (Liew et al., 2020; Roux et al., 2014)
In situ sequencing	Fresh frozen or FFPE	Targeted	Low	Single cell (0.5–1 μm)	Custom	Maize (Laureyns et al., 2021)
<i>Imaging-based spatial transcriptomics</i>						
MERFISH	Fresh frozen	Targeted	High	0.1–1 μm	1 mm ²	Arabidopsis (Lee et al., 2025; Nobori et al., 2025)
PHYTOmap	Fresh fixation	Targeted	High	Single cell	Custom	Arabidopsis (Nobori et al., 2023)

be broadly divided into two categories based on spatial data acquisition, i.e., imaging-based and sequencing-based (Fig. 2). Imaging-based spatial transcriptomic technologies typically rely on in situ hybridization using fluorescently labeled probes to target RNA molecules within a sample (Chen et al., 2015; Femino et al., 1998; Lubeck et al., 2014; Raj et al., 2008). High-resolution microscopy then localizes and quantifies the RNA of interest, often reaching single-cell or subcellular resolution. The number of probes that can be used simultaneously has increased over time to a few hundred or even a few thousand at a time. Nevertheless, imaging-based approaches remain inherently targeted, requiring prior knowledge of the genes of interest and providing only limited coverage of the transcriptome. In contrast, sequencing-based spatial transcriptomics technologies capture mRNA directly from tissue sections using spatial barcodes, followed by cDNA synthesis and high-throughput next-generation sequencing (You et al., 2024). These technologies provide untargeted, transcriptome-wide expression data but often lack single-cell resolution.

Selecting the best-suited spatial transcriptomics technology to address a specific biological question requires careful consideration of relevant factors, including resolution, sensitivity, and tissue type (Fig. 3). For instance, to understand how parasitic infection affects gene expression in host tissues, a high-resolution, transcriptome-wide method is essential for unbiased identification of key molecular players involved in the infection, while capturing tissue-specific responses. Although several technologies offer this, Visium remains the most popular platform, likely due to its commercialization and hence, relative ease of use (Gramberg et al., 2024; Hildebrandt et al., 2024; Poveda et al., 2024, BioRxiv preprint; Quintana et al., 2022). Visium is a sequencing-based platform with four 6.5 mm \times 6.5 mm capture areas. Each capture area contains ~5000 spatially barcoded spots, with a diameter of 55 μm . However, the distance between spots, ~100 μm , exceeds the size of a single cell. Eukaryotic cells typically range from 10 to 30 μm in size, while plant cells can vary from 10 to 100 μm . Therefore, Visium cannot achieve single-cell resolution and, in most biological systems, essentially pools multiple cells (Zhu et al., 2024). Even with a reduced spot size, numerous cells may overlap within one spot, making it impossible to study the gene expression of individual cells. Moreover, due to detection biases and limited sensitivity, capturing the complete transcriptome of a single cell is not guaranteed, even when sequencing-based spatial transcriptomics technologies theoretically provide (sub)cellular resolution (Li and Qiu, 2024; Squair et al., 2021).

The commercialization of Visium by 10 \times Genomics, followed by the

rapid adoption by researchers, led to its application in a variety of experimental settings. This resulted in the development of various optimized protocols for numerous species and tissue types, which can be either fresh or chemically fixed (Antico et al., 2023; Giacomello and Lundeberg, 2018; Gracia Villacampa et al., 2021; Hudson and Sudmeier, 2022; Raghubar et al., 2023). For example, researchers used Visium to reveal clear localized changes in gene expression in small intestinal tissue upon infection with the rodent parasitic nematode *H. polygyrus* (Poveda et al., 2024, BioRxiv preprint). This enabled the mapping of gene expression across different regions of the small intestine, revealing novel insights into the modifications required for granulomata development, the immune cell types involved in the infection process, and the complex host-parasite interactions at the tissue level. However, the limited chip size of Visium forced the researchers to cut the intestine longitudinally and twist the sections onto the slide, thereby distorting the spatial axes. Consequently, the distance and adjacency of the spots on the capture area did not match the tissue's biological structure. Additionally, due to the limited capture area, some tissue sections were excluded, further complicating the analysis of spatial expression patterns (Poveda et al., 2024, BioRxiv preprint). A spatial transcriptomics technology with a larger capture area, or a combination of technologies, may help in cases where convoluted topologies exist. For example, combining Visium and 2 K arrays helped better understand the infection of liver cells by *P. berghei* at different timepoints (Hildebrandt et al., 2024). The advantage of the 2 K arrays is their ability to study large sample sizes (i.e., 38 tissue sections compared to 8 sections in Visium), which enables researchers to capture infected cells at early timepoints when the number of infected cells is low. However, this gain in throughput comes at the expense of spatial resolution (100 μm in 2 K arrays compared to 55 μm in Visium), exemplifying the trade-offs between resolution and throughput among different spatial transcriptomic technologies.

Another critical application of spatial transcriptomics technologies in parasitology focuses on the interactions between cells and molecules during infection, as this provides valuable insights into host-parasite dynamics and immunity. Studying these interactions requires a higher spatial resolution than Visium offers. Slide-seqV2 meets this need, nearly achieving single-cell resolution (10 μm), featuring high RNA capture efficiency across a large capture area of 10 \times 10 mm, enabling the mapping of gene expression with high precision (Stickels et al., 2020). Slide-seqV2 utilizes barcoded beads organized in an array. When researchers place thin tissue slices (10 μm thick) on top of the array,

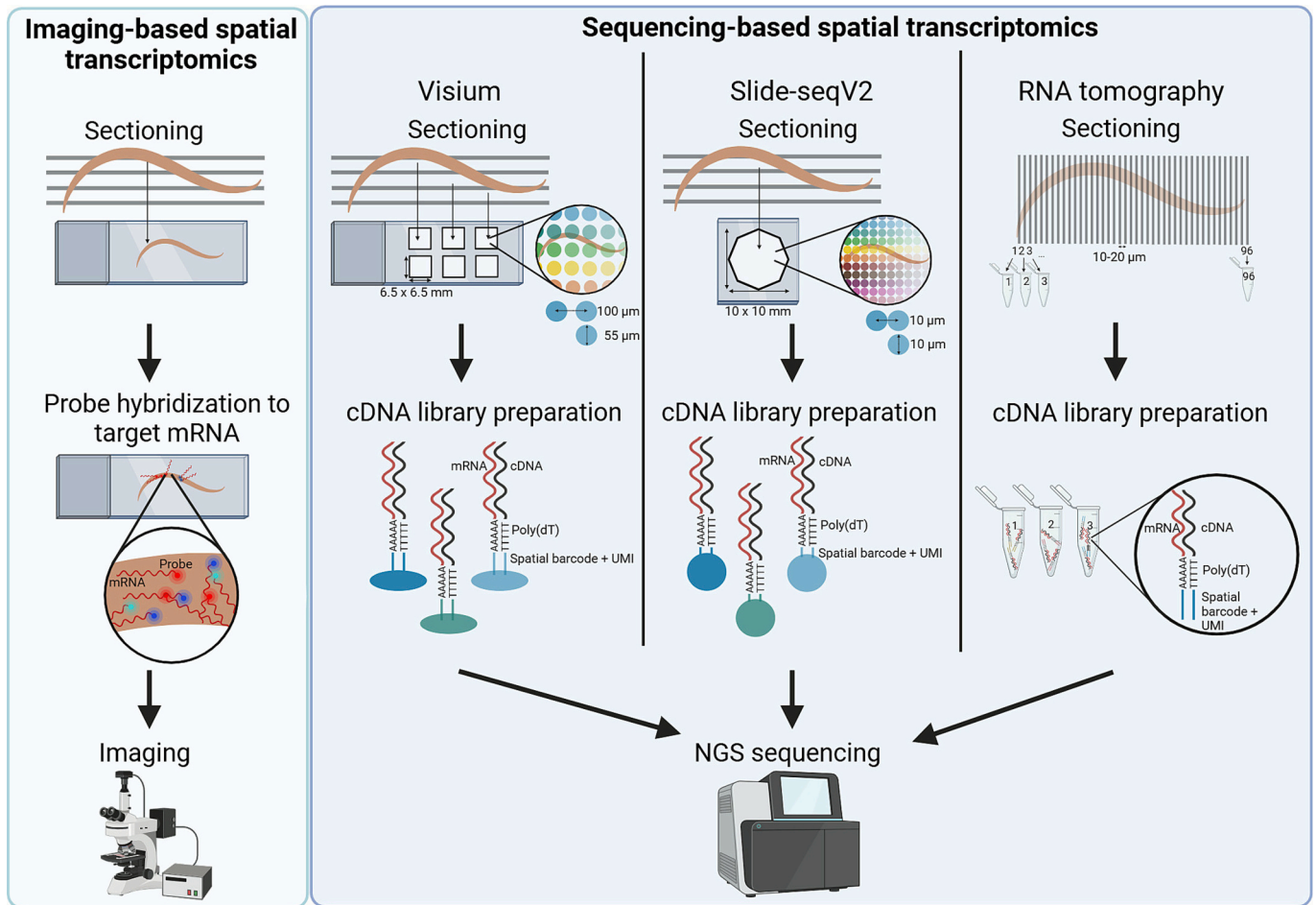


Fig. 2. Schematic overview of imaging- and sequencing-based spatial transcriptomic technologies used in animal-parasite studies. Spatial transcriptomics technologies can be divided in imaging-based and sequencing-based spatial transcriptomics. Imaging-based technologies use in situ hybridization, where tissue sections are hybridized with fluorescently labeled probes targeting specific mRNAs. These probes allow for subcellular localization of target mRNA using microscopy. On the other hand, sequencing-based spatial transcriptomics technologies involve tissue sectioning and cDNA library preparation using spatial barcodes for next-generation sequencing (NGS). The spatial barcodes consist of a unique barcode per spot/section to capture the spatial position of the RNA, a poly(dT) tail, to hybridize with the poly(dA) tail of mRNA molecules, and a Unique Molecular Identifier (UMI) to distinguish between molecules. The main differences between the illustrated sequencing-based technologies are the resolution and capture area size. Visium uses glass slides with four 6.5×6.5 mm capture areas, each containing ~5000 spatially barcoded spots with a diameter of $55 \mu\text{m}$ and $\sim 100 \mu\text{m}$ distance between spots. Slide-seqV2 has a capture area of 10×10 mm and uses barcoded beads to capture mRNA from tissue sections, reaching $10 \mu\text{m}$ resolution. With RNA tomography, up to 96 sections of $10\text{--}20 \mu\text{m}$ can be collected per batch in tubes containing spatially barcoded primers, capturing $960\text{--}1920 \mu\text{m}$ of tissue along a specific axis. Of each section, RNA is isolated, and cDNA is prepared. For all, Visium, Slide-SeqV2, and RNA tomography, the prepared cDNA libraries are sequenced using NGS. This figure was made using Biorender.

mRNA released from cells in the tissue binds to the beads. The approach reverse transcribes mRNA to cDNA, followed by sequencing to capture gene expression from specific tissue locations (Stickels et al., 2020). Researchers applied Slide-seqV2 to mouse spleens with and without the parasitic protist *Plasmodium* infection (Williams et al., 2024). In the spleen, naïve CD4⁺ T cells differentiate into various effector T cell subtypes, essential for an effective immune response against *Plasmodium*. Slide-seqV2 contributed to mapping these T cell subtypes and identifying the molecular and unique cellular interactions that influence cell differentiation during *Plasmodium* infection (Williams et al., 2024). Although the technology provided valuable insights into host tissue responses, it detected very few *Plasmodium* genes per bead, indicating its unsuitability for studying the parasite itself (Williams et al., 2024). Thus, the high spatial resolution of Slide-seqV2 comes at the cost of sensitivity and capturing efficiency of low-abundance transcripts. Furthermore, the high costs of Slide-seqV2 restrict the number of tissue sections, replicates, and time points in the experimental design, thereby limiting a dynamic view of molecular and cellular interactions (Williams et al., 2024).

Studying gene expression patterns of low-abundant transcripts

requires a spatial transcriptomics technology with higher capture efficiency, e.g., RNA tomography (Junker et al., 2014). This method relies on sequential cryo-sectioning and sequencing, where the thickness of the sections determines the resolution. Slicing samples across different planes enables the generation of transcriptome-wide 3D models (Junker et al., 2014; Mayeur et al., 2021). RNA tomography requires low-input RNA, allowing gene expression analysis within a single organism or tissue. For instance, RNA tomography provided a detailed analysis of the *B. malayi* nematode head region, a small but functionally important region for host-parasite communication (Airs et al., 2022). This study utilized $20 \mu\text{m}$ -thick sections of the head region along the anterior-posterior axis, revealing that genes express differently across various tissues. However, the complexity and distinct expression in tissues like the pharynx and intestine, meant that the resolution of RNA tomography alone was insufficient to distinguish between gene expression patterns of these tissues (Airs et al., 2022). Although in this example the researchers focused solely on the nematode, RNA tomography performed on plant roots infected with nematodes mapped the parasite and the host mRNA simultaneously (Pijnacker et al., 2025).

Given that sequencing-based spatial transcriptomics technologies

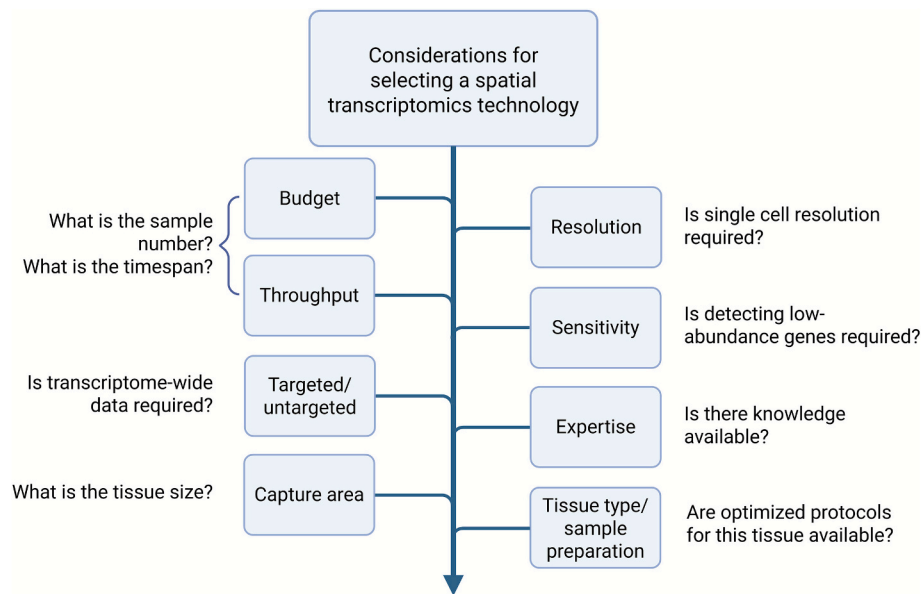


Fig. 3. The factors that should be considered when selecting a spatial transcriptomics technology for a specific biological question.

typically lack cellular resolution, they are often complemented by single-cell RNA-sequencing (Li et al., 2024a; Wan et al., 2023). Single-cell resolution data can be obtained at high throughput using platforms such as 10× Chromium, while the spatial dimension, which is lost during single-cell sequencing, can be reintroduced using spatial transcriptomics technologies. An example of such an integrative approach involved single-cell RNA-sequencing to identify candidate cell types in the murine central nervous system that respond to the parasitic protist *T. brucei* infection, followed by Visium spatial transcriptomics to study the spatial distribution of these cell types (Quintana et al., 2022). This approach uncovered novel interactions between innate and adaptive immunity in mouse hosts during parasitic infection.

Taken together, each spatial transcriptomics technology has its advantages and disadvantages in terms of resolution, sensitivity, and throughput. When selecting a spatial transcriptomics technology for a specific biological question, several factors should be considered, including the number of genes studied, tissue type, spatial resolution, capture size, budget, and expertise, to determine the most suitable technique.

4. Applications of spatial transcriptomics in host-parasite interaction research

Inspired by animal-parasite studies, we discuss the application of spatial transcriptomics to parasitic systems across diverse hosts, including plants, to address three outstanding questions in various parasitic systems. To highlight how spatial transcriptomics offers unique opportunities to dissect evolutionary convergences and divergences in parasitism, we first describe the fundamental biological differences in host immunity, tissue organization, accessibility, and infection dynamics between animal and plant systems.

Both plants and animals have sophisticated immune systems to combat pathogen attacks. Although there are similarities in their innate sensing mechanisms of infections (Nürnberg et al., 2004; Pradeu et al., 2024; Spoel and Dong, 2012), such as the use of specialized pattern-recognition receptors to detect pathogen-associated molecular patterns and damage-associated molecular patterns (Bentham et al., 2017; Zipfel and Felix, 2005), key differences exist. For instance, animals rely on a mobile, compartmentalized, and hierarchical immune system, while plants employ a cell-autonomous, two-tiered defense system (Bentham et al., 2017; Nürnberg et al., 2004).

Animals possess both innate and adaptive immunity with circulating lymphocytes in the blood and lymphatic systems. These lymphocytes undergo clonal expansion to generate a long-lasting adaptive immunological response (Alugupalli et al., 2004). Interestingly, parasitic helminths are master modulators of adaptive immunity, driving a Type 2 immune response characterized by increased levels of cytokines IL-5, IL-5, and IL-13, expansion and activation of regulatory T cells, and alternatively activated macrophages (Maizels et al., 2004). Spatial transcriptomics enabled the mapping and characterization of diverse immune cell populations across various tissues using marker gene expression (Engblom et al., 2023; Oliveira et al., 2025). However, tracking lymphocyte migration requires high-resolution spatial transcriptomics combined with time-series data.

In contrast to animals, plants lack both mobile immune cells and a circulatory system and instead rely solely on innate immunity, which includes localized responses and systemic signaling. Immune activation in plants is often restricted to specific cell layers near the infection sites (Ross, 1961; Zhu et al., 2023). Consequently, spatial transcriptomics in plants is uniquely suited to resolve small clusters of responding cells at the interface of feeding cell complexes (e.g., giant or syncytial cells) in sedentary plant-parasitic nematode infections.

Beyond the differences in animal and plant immunity, cellular structural differences affect tissue accessibility. Animal tissues are generally softer as cells lack a cell wall, which simplifies tissue sectioning and the penetration of probes to detect transcripts of interest (Panstruga et al., 2023). Nevertheless, helminth larvae often follow complex, multi-stage, and migratory life cycles involving movement through the liver, lungs, or bloodstream, which may complicate sample processing (Ferrer-Font et al., 2020). Despite this, a chronic helminth infection frequently induced profound tissue remodeling, including fibrosis, muscle hypertrophy, or granuloma encapsulation. Spatial transcriptomics allows functional contextualization of these structural changes at high resolution, identifying specific host cells driving pathology.

Unlike animal tissues, plants have rigid cell walls and fixed layers, which provide a substantial technical advantage for reproducible spatial transcriptomics sectioning. However, secondary metabolites, may complicate tissue permeabilization and RNA capture (Giacomello et al., 2017; Koonjul et al., 1999; Leh et al., 2019). Ultimately, while spatial transcriptomics is a powerful tool in both systems, implementation must account for these biological and structural differences.

4.1. Where are parasite effectors localized pre- and post-infection, and can we identify novel putative effectors?

Many parasites secrete effector molecules into their host during infection to support parasitism, for example, by inducing the formation of feeding structures or by suppressing host immunity. Yet, the function and the (sub-)cellular localization of many effectors within host cells remain unknown.

Spatial transcriptomics allows the mapping of parasite gene expression along its anterior-to-posterior axis. Researchers applied this approach to the head region of the human parasitic nematode *B. malayi* (Airs et al., 2022). This region is critical for feeding, effector secretion, chemo- and mechanosensing, as well as reproduction. The authors combined RNA tomography with microscopy to study gene expression patterns in the parasite's head region and determine the subcellular localization of effector transcripts. Furthermore, the spatial and tissue-specific transcriptomics data helped map the distribution of current anthelmintic drugs and identify potential new drug targets or vaccine candidates (Airs et al., 2022).

Similarly, in *Arabidopsis* roots infected with the cyst nematode *Heterodera schachtii*, RNA tomography mapped the location of the nematode's pharyngeal glands—which secrete effector genes crucial for feeding structure formation—by analyzing well-annotated gland gene expression profiles. The analysis identified multiple *H. schachtii* genes with similar gland expression patterns, which suggests these uncharacterized genes may be putative effectors or involved in effector ontogenesis or gland functioning (Pijnacker et al., 2025).

Building on the *B. malayi* and *H. schachtii* studies, researchers can apply spatial transcriptomics to other parasites (Airs et al., 2022; Pijnacker et al., 2025). Because parasites express effectors in a spatio-temporal manner, future work must collect samples across both pre-parasitic and parasitic life stages. As parasite tissues and cells become better defined, researchers can identify potential effector-producing glands or organs, which, in turn, allows for the investigation of novel effector roles in host parasitism.

4.2. What are the molecular mechanisms underlying the development of nematode feeding structures in plants?

Similar to the nematodes *Trichinella spiralis*, which forms specialized nurse cells within carnivorous and omnivorous animal muscles, and *H. polygyrus*, which establishes niches in the intestine of rodents, plant-parasitic root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.) form permanent feeding structures within their host roots. However, the infection mechanisms of animal and plant parasites differ.

T. spiralis infectious larvae are released in the stomach, enter the epithelium of the small intestine, and mature into adult worms through four developmental molts (Mitreva and Jasmer, 2006). Within two to three weeks, each female produces hundreds of larvae. These larvae migrate via the lymphatic and blood circulatory system, eventually invading muscle fibers, where they transform muscle cells into multinucleated, collagen-encapsulated nurse cells for nutrient supply. Though *T. spiralis*-infected muscle cells attempt regeneration and repair, they fail, likely due to conflicting signals produced by larvae and host cells (Wu et al., 2013). The mechanisms *T. spiralis* uses to modulate host immunity remain largely unknown (Park et al., 2022; Wang et al., 2020).

H. polygyrus employs a different infection strategy. Infective *H. polygyrus* larvae penetrate the small intestine submucosa, where they undergo two developmental molts and become adults. The adults migrate back to the lumen, where they feed on host intestinal tissue, mate, and lay eggs, which are secreted in the feces (Reynolds et al., 2012). The host responds to *H. polygyrus* infection by accumulating innate and adaptive immune cells around the invading larvae, called granuloma. Each *H. polygyrus* worm induces its own granuloma,

resulting in multiple granulomata in the host's intestinal tissue. Although the function of the granulomata requires further study, innate immune cells in the granulomata immobilize and kill *H. polygyrus* larvae and contribute to tissue repair (Ariyaratne and Finney, 2019; Esser-von Bieren et al., 2015).

Plant-parasitic nematodes, in contrast to animal parasites, form feeding structures through local host cell reprogramming rather than immune cell recruitment. Root-knot nematodes induce giant cells by repeated nuclear division without cytokinesis (Bird, 1961; Jones and Payne, 1978), while cyst nematodes create a large syncytium through cell wall dissolution in between neighboring host cells (Golinowski et al., 1996; Grundler et al., 1994). These feeding structures consist of multinucleate, hypertrophied cells with dense cytoplasm, numerous proliferating organelles, and fragmented vacuoles. Although transforming root cells into feeding structures requires extensive gene expression reprogramming, the underlying molecular mechanisms remain poorly understood.

To better understand feeding structure formation, it is crucial to identify the gene expression changes that support nematode establishment and the host immune responses that prevent it. Spatial transcriptomics enables the study of individual nematode feeding structures in plant roots, while most other technologies require pooling infection structures of similar age, place, and orientation within the host. Finding such biological replicates is challenging because nematodes infect at different times and locations within the root, resulting in a lack of synchronization and creating a heterogeneous mix of infection structures. Furthermore, the difficulty in identifying feeding structures at the onset of parasitism keeps the research focus mainly on later stages of infection, hampering time-series analysis. Unlike animals, whose tissues and cells organize structurally and functionally into conserved modular units, plant root architecture is highly variable. This root architecture, including, e.g., the number, position, and shape of lateral roots and root hairs, depends on developmental stage, environmental conditions, and genotype. This high variability makes spatial mapping and alignment of plant samples particularly challenging. Nevertheless, understanding the nematode-induced genetic reprogramming of the host during the initiation and expansion of feeding structures is essential for developing control measures against sedentary plant-parasitic nematodes in crop plants.

4.3. How are host immune responses to parasite infection spatially confined?

Hosts typically induce localized immune responses upon infection with parasites. This host response is crucial for controlling the infection and minimizing disease severity. The spatial extent of the restricted immune response remains unclear, but it could be studied using spatial transcriptomics. In the context of the parasitic protist *Plasmodium* infection, mouse splenic microanatomy, cellular co-localizations, and molecular interactions were studied (Williams et al., 2024). Specific T cell differentiation occurred near the site of infection, showing the local immune response of the host. This observation could not have been possible without the spatial context.

Like the use in *Plasmodium* infections, spatial transcriptomics can be utilized to study animal tissues infected by other sedentary parasites to identify the specific immune cell types attracted to the infection site and analyze how these immune cells respond to the parasite's presence (Williams et al., 2024). In plants, spatial transcriptomics enables researchers to identify local changes in gene expression during infection, providing insights into how plants recognize and respond to parasite infection. If single cells were isolated from the affected tissue, the local immune response would be lost in the total cellular input and become undetectable. Moreover, by testing various timepoints after infection, it could be tested how the local immune site and response change over time. With a similar approach, spatial transcriptomics can identify genes and tissues crucial for parasite survival, enabling the prioritization of

these genes for (targeted) drug development (Airs et al., 2022; Gramberg et al., 2024). The response to such a treatment could also be studied using spatial transcriptomics. For instance, miniature spatial transcriptomics applied to *B. malayi* nematodes, and their endosymbiotic bacteria *Wolbachia* revealed spatially resolved gene expression changes in response to antibiotic doxycycline treatment against *Wolbachia* (Sounart et al., 2023). The antibiotic reduced *Wolbachia* abundance and induced distinct gene expression changes compared to non-treated worms, including the downregulation of genes linked to heme metabolism, a pathway critical for symbiosis. Although the direct effects of antibiotic treatment could not be separated entirely from *Wolbachia* reduction, this study demonstrates how spatial transcriptomics can reveal tissue-specific responses to anti-helminthic drug treatments (Sounart et al., 2023). Such an approach could also be applied to study the effect of biocides on parasite infection (Airs et al., 2022; Gramberg et al., 2024).

5. The opportunities and challenges of applying spatial transcriptomics in plants

Despite the need for spatial transcriptomics in plant research, its application lags behind that in animal research. Nonetheless, researchers successfully applied various spatial transcriptomics technologies, including Visium and spatially enhanced resolution omics sequencing (Stereo-Seq), in plants (Table 1). Stereo-Seq, which combines in situ RNA capturing with DNA nanoball-patterned assays to achieve single-cell resolution, has yet to be applied to parasite studies. In plants, spatial transcriptomics showed promising applications to reveal cell types involved in shoot regeneration of tomato callus (Song et al., 2023), leaf cell (sub)types in Arabidopsis (Xia et al., 2022), cell type-specific and spatial gene expression profiles of the peanut peg (Liu et al., 2022b), to demonstrate co-expression of two distinct photosynthesis pathways within the same cell (Moreno-Villena et al., 2022), and to provide insights in the spatial gene expression during flower organogenesis in *Phalaenopsis* Big Chili (Liu et al., 2022a). However, fewer spatial transcriptomics technologies are available for plant tissues compared to those for animal tissues, due to both biological and technical challenges. Compared to animal cells, plant cells have distinct features, such as cell walls, chloroplasts, and vacuoles, which present specific challenges for sample preparation.

The presence of complex polysaccharide cell walls hinders efficient capture of cellular mRNA for spatial transcriptomic analysis. Because the composition of cell walls varies across species and tissues, researchers must optimize the preparation procedure for each plant sample. Tissues with thick or highly lignified cell walls are hard to degrade and prone to cracking during cryo-sectioning (Chen et al., 2021; Giacomello and Lundeberg, 2018). To prevent cracking, researchers can pre-cool tissue blocks before sectioning or embed fresh samples in compounds such as Tissue-Tek or Optimal Cutting Temperature (OCT) (Giacomello and Lundeberg, 2018). Reducing section thickness (typically 10 to 20 μm) can improve tissue permeabilization, which in turn enhances mRNA release. Particularly for tissues with thick cell walls, it may be necessary to use a hydrolytic enzyme mix, consisting of e.g., cellulases, hemicellulases, and pectinases, to (partially) degrade the cell wall and improve mRNA accessibility (Giacomello et al., 2017). Conversely, the plant cell wall stains easily, providing a clear and identifiable outline of single cells for downstream analysis. Many spatial transcriptomics protocols complement well with histological treatments used for preserving and visualizing morphological structures. For instance, common cell wall stains such as Calcofluor White, for cellulose (Nobori et al., 2023), Safranin O, for lignified tissues (Peirats-Llobet et al., 2023), and Toluidine Blue, to distinguish between lignified and non-lignified tissues (Giacomello et al., 2017; Song et al., 2023), provide valuable cellular context before sequencing. More general stains, e.g., Trypan Blue, which stains dead plant cells (Liu et al., 2022a), and Hematoxylin & Eosin, which typically stains the nuclei, extracellular

matrix, and cytoplasm (Moreno-Villena et al., 2022), require further exploration in the context of these spatial technologies. However, as with tissue preparation, staining applicability depends on the tissue type; starchy maize endosperm cells, for example, cannot be stained with Hematoxylin & Eosin (Fu et al., 2023).

Another challenge unique to plant cells is the presence of large vacuoles, which typically contain high water content and can cause tissue hardening or ice crystal formation upon freezing. Such hard tissues may rupture and lose morphology during cryo-slicing (Giacomello and Lundeberg, 2018; Sang and Kong, 2024). To counter this problem, a thin layer of cryo-embedding media, such as Tissue-Tek or OCT, can be used to preserve tissue morphology and support optimal cutting. Additionally, tissues with high water content may require paraformaldehyde tissue fixation to maintain structural integrity and prevent degradation (Giacomello and Lundeberg, 2018).

Furthermore, secondary metabolites complicate library preparation by inhibiting reverse transcription and affecting RNA quality and quantity (Koonjul et al., 1999; Leh et al., 2019). A possible solution is the addition of polyvinylpyrrolidone 40 (PVP40) during cDNA synthesis and permeabilization (Giacomello et al., 2017). PVP40 is often used in DNA/RNA extraction protocols because it binds to polyphenols, thereby removing these secondary metabolites and increasing the efficiency of transcript capture and reverse transcription (Porebski et al., 1997).

Since no standardized protocol exists for tissue preparation in spatial transcriptomics, optimization is required for each plant tissue type. Consequently, the applicability of spatial transcriptomics in plant sciences presents considerable room for improvement.

6. Concluding remarks and future perspectives

Spatial transcriptomics technologies capture gene expression while preserving the spatial context of transcripts within tissue sections. This approach provides unprecedented insights into host cell responses, tissue organization, and infection dynamics in animal-parasite interactions. Inspired by these findings, plant researchers should use similar approaches to unravel the gene expression patterns during parasite infection in plants. This necessity demands optimizing the spatial transcriptomics workflow, particularly the cryo-sectioning and RNA capturing, because plant tissues fundamentally differ from animal tissues. Moreover, the highly variable architecture of plant tissues and variations in infection timing and location complicate the spatial mapping, alignment of biological replicates, and time-series analysis. A potential solution is using the spatial location of the nematode's anterior end as a reference for aligning biological replicates or generating 3D models of infected tissues.

The spatial transcriptomics field is rapidly advancing, enabling larger capture areas, higher resolution, greater sensitivity, and broader applicability across kingdoms. However, comparing spatial transcriptomic technologies to select the most appropriate for a particular study remains difficult due to the lack of standardized evaluation criteria and reference tissues with well-defined morphology and expression profiles (You et al., 2024; see also Outstanding questions). Variations in measurement units and spot sizes, ranging from 50 μm (multiple cells), to 0.5-1 μm (subcellular spots), further complicate comparison. For spatial transcriptomic technologies that lack single-cell resolution, identifying single cells and their spatial distribution is challenging. This is because transcripts from a specific spatial location may comprise a heterogeneous mix of cells and multiple cell types (Andersson et al., 2020). To overcome this resolution limit, researchers can integrate spatial transcriptomics with single-cell RNA-Seq data, using deconvolution on reference gene expression profiles from single-cell RNA-Seq datasets to help resolve cell type distribution and interactions within spatial transcriptomics data (Longo et al., 2021).

Beyond comparison, spatial transcriptomics datasets are typically noisy and sparse because each spatial spot only captures a small fraction of the total RNA present in the sample (Abrar et al., 2023; Heydari and

Sindi, 2023; Wang et al., 2022c). Noise, whether biological or technological, can lead to misinterpretation and bias (Gurazada et al., 2021). There are several ways to deal with noise. For instance, researchers clean datasets using thresholds to filter out low-quality spots or sections (Shang and Zhou, 2022; You et al., 2024). Additionally, they normalize read counts to minimize differences across spots or sections (Holler and Junker, 2019; Wang et al., 2022b), and employ recently developed denoising algorithms like SproD (Wang et al., 2022c), SpotGF (Du et al., 2024), and NoVaTeST (Abrar et al., 2023). However, data normalization and denoising are not without risks, as this may result in signal loss and affect downstream analysis and biological interpretation (Atta et al., 2024; Saiselet et al., 2020). Thus, it is crucial to perform additional quality checks on each dataset to ensure accurate data handling and robust biological interpretation.

While spatial transcriptomics-derived therapeutics are absent on the market, this is likely temporary, as spatial and tissue-resolved data from parasite-infected animal tissues already reveal potential targets for drug and vaccine development (Airs et al., 2022; Gramberg et al., 2024; Sounart et al., 2023). Furthermore, spatial transcriptomics can map treatment responses with tissue or cellular resolution (Sounart et al., 2023). We can extend these methods to plants to study the molecular mechanisms underlying feeding structure formation and host responses, enabling the identification novel targets for parasite management.

Ultimately, selecting the right spatial transcriptomics technology for a specific biological question is challenging. No single platform is universally optimal, requiring careful consideration of trade-offs among resolution, throughput, capture area, and costs.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve language/readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

CRediT authorship contribution statement

Anna Pijnacker: Conceptualization, Writing – original draft, Visualization. **Christine W. Bruggeman:** Conceptualization, Writing – original draft. **Hendrik C. Korswagen:** Writing – review & editing. **Geert Smant:** Conceptualization, Writing – review & editing. **José L. Lozano-Torres:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare no competing interests.

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Data availability

No data was used for the research described in the article.

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