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Spatial proteomics for protists: perspectives and opportunities from Protistology-UK Autumn meeting 2026

Rachel Foster^{1*}, Clare Bird², Martin Carr³, Alessandra Dupont⁴, Genoveva F. Esteban⁵, Fiona
Henriquez-Mui⁶, David Montagnes⁷, Ellen Nisbet⁸, Ross F. Waller⁹

¹ Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

² Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, UK

³ Department of Physical & Life Sciences, University of Huddersfield, Huddersfield, West Yorkshire,
HD1 3DH

⁴ Protistology-UK Society

⁵ School of Life and Environmental Sciences, Bournemouth University, Poole, Dorset BH12 5BB, UK

⁶ Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, UK

⁷ School of Environmental Sciences, University of Liverpool, Liverpool, L69 7ZX UK

⁸ School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington,
Leicestershire LE12 5RD, UK

⁹ Department of Biochemistry, University of Cambridge, Cambridge, CB2 1QW, UK

*Corresponding author r.foster@nhm.ac.uk

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Abstract

Subcellular spatial proteomics investigates the localisations and associations of proteins within the cell, offering insights into cellular organisation, protein function and biochemical processes. Spatial proteomic approaches are increasingly being applied in protistology to investigate diverse cellular architectures, specialised organelles and symbiotic associations. The Protistology-UK Autumn Meeting 2026, held on the 3-4th September at the Natural History Museum, London, brought together researchers from across the protistology community to attend a dedicated workshop that explored the emerging application of spatial proteomics to protist research. Here, we synthesise examples from the emerging literature on spatial proteomics in protists and highlight current research directions. We also report on the meeting and the outcomes of the workshop, indicating potential applications, challenges and future opportunities for spatial proteomics in protistology. We discuss how these approaches could extend spatially resolved proteomic analyses beyond established model and parasitic protists to address questions in protist cell biology, evolution and ecology.

40 1. Introduction

41 Protistology-UK (P-UK, <https://www.protistology.org.uk/>) is a UK-based learned society that brings
42 together researchers and enthusiasts with interests across the diverse field of protistology, especially
43 encouraging younger researchers. The annual P-UK meeting provides a forum for sharing research,
44 exchanging ideas and discussing emerging approaches and developments in protistology. Typically,
45 the meetings are themed, as well as providing a venue for showcasing the breadth of UK protistology.
46 Previous P-UK meetings have covered a range of themes and have provided a valuable snapshot of
47 current developments and future directions within the field, most recently “Protists and society”
48 (Montagnes, 2025) and the integration of modern and older research methods in protistology (Waller,
49 2023).

50 The 2026 Autumn Meeting, besides covering a range of topics, had a focus of “Introducing Spatial
51 Proteomics for Protists (to a general audience)”. It was held at the Natural History Museum, London
52 (UK), on 3–4 September 2026 and brought together 54 participants from 25 institutions, as well as
53 members from the wider protistology community. Presentations showcased a range of current research
54 on protist biology, as well as the opportunity to explore spatial proteomics for protists in a dedicated
55 workshop.

56 One of the challenges of protist research is that many modern experimental molecular tools, such as
57 transformation technologies and/or inducible expression systems, are lacking for all but a few ‘model’
58 species. This is a barrier to answering scientific questions, particularly those that explore the vast
59 molecular novelty of the genomes of protists. Recently, researchers have embraced the new field of
60 spatial proteomics, which offers cellular context and interpretation of this novelty without the need for
61 genetic modification tools. Specifically, spatial proteomics encompasses approaches that determine
62 the subcellular localisation, distribution and interactions of proteins, providing a spatial dimension to
63 proteomic data. By linking proteins to their cellular context, these approaches can provide insights
64 into protein function, cellular organisation and dynamic biological processes (Lundberg & Borner,
65 2019).

66 In this report, we synthesise examples from the emerging literature on spatial proteomics in protists,
67 highlighting current research directions. We also provide an overview of the meeting and its focus on
68 spatial proteomics and consider the application of this technology to protistology research. By
69 bringing together perspectives from researchers working across diverse areas of protistology, we use
70 these discussions to identify common research priorities, appreciate potential applications and
71 challenges, and consider how spatial proteomics might contribute to future developments in
72 protistology research.

2. A brief review of applications of spatial proteomics in protist biology

Protist biology is an outstanding fit for spatial proteomics methodologies: protists are paraphyletic; dominate the tree of eukaryotic life; and encompass a wide range of cellular architectures, organelles and endosymbiotic associations, many of which differ substantially from those characterised in traditional model eukaryotic systems. Furthermore, their genomes and proteomes contain large numbers of lineage-specific and/or uncharacterised proteins for which functional information cannot be reliably inferred from sequence similarity alone.

A main focus of the meeting was to examine the spatial proteomic method LOPIT (Localisation of Organelle Proteins by Isotope Tagging) and evaluate how it can be applied to protists. This method combines gentle mechanical lysis of cells, subcellular fractionation on physical gradients, and quantitative mass spectrometry to infer the localisation of proteins within cells (Dunkley et al., 2004). The essence of the method is ‘guilt by association’, where proteins that co-locate in the cell are assumed to share similar abundance distribution profiles across gradients. Variations on this method include hyperLOPIT (hyperplexed Localisation of Organelle Proteins by Isotope Tagging) that uses additional density-gradient fractionation (shown in Figure 1) and LOPIT-DC (Localisation of Organelle Proteins by Isotope Tagging after Differential ultracentrifugation) that uses differential ultracentrifugation as an alternative fractionation strategy (Geladaki et al., 2019). These approaches all provide a means of simultaneously generating high-resolution maps of thousands of proteins within their different organelles and subcellular compartments.

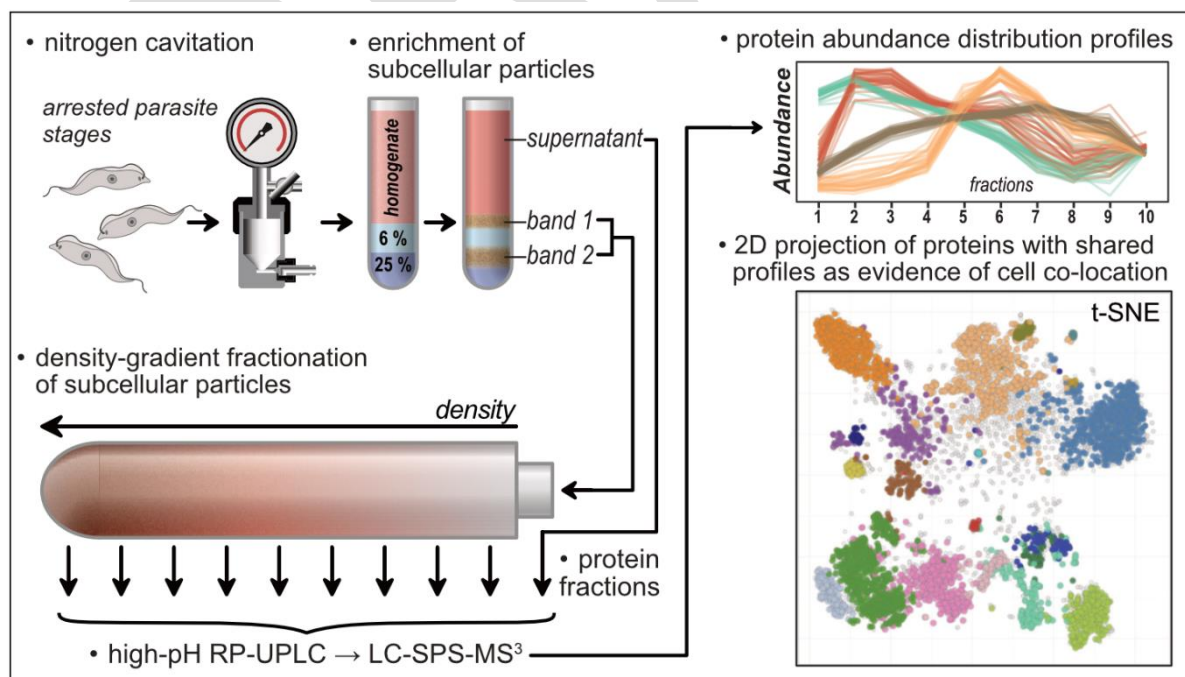


Figure 1: HyperLOPIT workflow. Cells are physically lysed (e.g. nitrogen cavitation) and cellular particles enriched from cytosolic components on density step-gradients. Cell lysates are then further fractionated on a

continuous density gradient, and quantitative mass spectrometry applied to determine the distribution of all proteins across fractions. Machine-learning tools probabilistically correlate the resultant protein profiles with known organelle markers to generate comprehensive maps of protein co- location within the cell including the many otherwise uncharacterised proteins of these divergent eukaryotes. These proteins maps can be visualised with dimension- reducing methods (e.g. t-SNE).

The first application of hyperLOPIT to a protist examined *Toxoplasma gondii*, where Barylyuk et al. (2020) mapped the steady-state subcellular localisation of thousands of proteins in *Toxoplasma gondii* tachyzoites. This work expanded knowledge of specialised apicomplexan structures including micronemes, rhoptries, and dense granules, providing localisation information for many uncharacterised proteins. HyperLOPIT, and the computational modelling that is integral to this method, has also been used to spatially map the proteomes of *Trypanosoma brucei* and *Trypanosoma congolense*, in both the mammalian bloodstream form and the insect midgut procyclic form, enabling comparison of protein localisation across life stages and between species (Moloney et al., 2023). Spatial proteome mapping of *Cryptosporidium* sporozoites using hyperLOPIT has resolved multiple subcellular compartments and identified four distinct secretory organelles involved in interactions with the host cell, including a formally unknown secretory organelle, the ‘small granule’ (Guérin et al., 2023).

Spatial proteomics is also providing insight into the diversity of biochemical processes in protists. LOPIT-DC studies on the free-living, freshwater flagellate *Paratrimastix pyriformis* revealed the highly reduced function of the mitochondria and its role in the cytosolic methionine cycle (Zitek et al., 2022). Subcellular proteomics study of the ciliate *Paramecium tetraurelia* revealed a noncanonical mosaic distribution of glycolytic and gluconeogenic enzymes across the cytosol, mitochondrial matrix and mitochondrial outer membrane, suggesting that *P. tetraurelia* may favour gluconeogenesis over glycolysis (Jirsová et al., 2025).

Recent applications have been extended to less well-characterised protist lineages. For example, Hammond et al. (2025) localised 4,870 proteins to 22 cellular compartments in the diplomonid *Paradiplonema papillatum*, revealing insights into extracellular carbohydrate digestion and the divergent compartmentalisation of metabolism across euglenozoans. As validation of the LOPIT approach, the authors supported several predicted localisations using epitope tagging and fluorescence microscopy, including proteins lacking annotation or homologues outside diplomonids, demonstrating how spatial proteomics can provide functional and cellular context for poorly characterised, lineage-specific proteins (Hammond et al., 2025).

Spatial proteomics can also be used to study endosymbiotic integration. LOPIT-DC studies applied to the trypanosomatid *Angomonas deanei* that contains a bacterial endosymbiont, identified host-

encoded proteins with localisation profiles matching the endosymbiont compartment, providing candidates involved in host control and integration of the endosymbiont (Hammond et al., 2026).

These examples demonstrate the breadth of questions that spatial proteomics can address in protists. However, existing protist applications are heavily concentrated on parasitic protists, particularly apicomplexans and trypanosomatids. The considerable diversity of free-living, symbiotic and environmentally important protists, therefore, represents a largely unexplored opportunity for the application of spatial proteomics.

3. The 2026 Protistology-UK Autumn Meeting

After registration and an informal lunch providing an opportunity for delegates to network, the meeting was opened by the P-UK President, Professor Fiona Henriquez-Mui. The plenary session was delivered jointly by Professor Kathryn Lilley and Professor Ross Waller (both at University of Cambridge). Lilley, who pioneered the development of LOPIT, provided an overview of the historical development of spatial proteomics and the evolution of the technology, while Waller described the application of this technology to his own personal journey in protistology research. This was followed by three invited, 20-minute presentations highlighting recent applications of spatial proteomics to diverse protist systems: Dr Jeremy Wideman (Arizona State University, USA), “Comparative subcellular spatial proteomics”; Dr Lawrence Rudy Cadena (Heinrich Heine University, Germany), “The subcellular proteomics of *Paulinella chromatophora*: A Rhizarian story”; and Dr Michael Hammond (Institute of Parasitology, České Budějovice, Czechia), “Using spatial proteomics to investigate host-endosymbiont integration in *Angomonas deanei*”.

Following a coffee break, delegates then participated in a workshop designed and led by Waller, exploring potential applications of spatial proteomics to protist research. The 54 delegates were divided into six groups of nine and asked to consider a series of questions relating to the biological applications, experimental design and potential challenges of applying spatial proteomics to protist systems (see Section 4). Groups then presented their ideas informally during a reception preceding the conference dinner.

The second day featured contributed talks covering a broad range of topics in protistology and began with three presentations on a diverse range of protistan algae from Arina Maltseva (Uppsala University), Alastair Skeffington (University of Stirling) and Lavender Zhirou Lu (University College, London). The remaining talks in the first session centred on parasitic protists. Paula Zegarra Vidarte (Uppsala University) presented her research on the aquaculture parasite *Spironucleus salmonicida*. This was followed by three presentations featuring spatial proteomics, with Jan Pyrih (Charles University, Czech Republic) and Eden Ferreira (University of York) talking on their research on

discobids and Karolina Maria Kwiatkowska (University of Cambridge) describing her use of LOPIT in *Plasmodium knowlesi*.

The first oral session was followed by a poster session featuring 11 posters on topics as diverse as protist biogeography, nutrient sensing, body-trail decomposition, vaccine development and spatial proteomics. The second session of talks began with Hayley Chesham (University of Huddersfield) presenting her research on codon usage and tRNA evolution across the Tree of Life. Dagmar Jirsova (Arizona State University) then described the use of spatial proteomics to investigate energy metabolic processes across stramenopiles, alveolates and rhizarians. Arthur Taylor (University of Edinburgh) presented research on landscape-scale 18S rRNA metabarcoding and responses of soil protist trophic groups, followed by the final presentation from Sally Warring (Earlham Institute) on single-cell genomics resolving host-specific bacterial associations in a freshwater protist community.

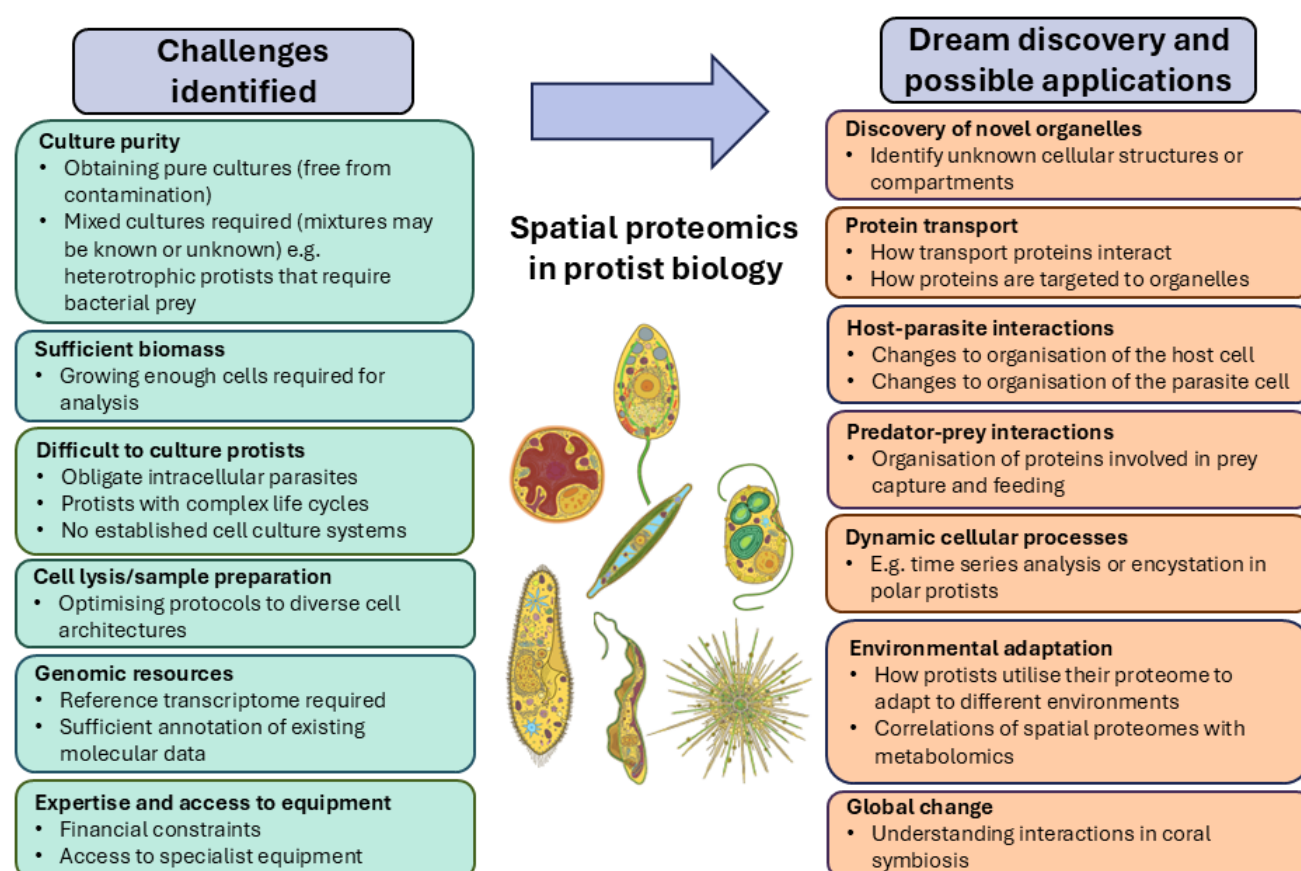
In the closing remarks it was announced that the 2027 P-UK meeting would be a joint meeting with the European Congress of Protistology, hosted by Dr Anastasios Tsousis at the University of Kent from the 2nd to the 7th August 2027 (<https://www.protistology.org.uk/ecop2027>). Also, three prizes were awarded for contributed talks and three for poster presentations, with prizes sponsored by Elsevier. The meeting concluded with lunch and a behind-the-scenes tour of the Natural History Museum's Spirit collection. Protistology-UK is an affiliate of The International Society of Protistologists and the Microbiology Society. The 2026 autumn meeting was sponsored by the Gordon and Betty Moore Foundation.



Figure 2. Photo of conference delegates, taken after the workshop on 3rd September 2026.

4. A summary of the spatial proteomics workshop

186 The workshop described above presented each group with the following questions/directions: 1. What
 187 type of protists are the group working on? 2. What research questions could LOPIT address in each
 188 organism? 3. What are potential challenges/considerations for such experiments? 4. Develop and



189 explore experimental plan. 5. What existing data types are available for this protist (or its relatives)
 190 that could be more fully interpreted with spatial proteomics information? 6. What is the dream
 191 discovery that LOPIT could make in your protist? In Figure 3 we provide a summary of the
 192 challenges identified and the dream discovery/possible applications discussed during the workshop.

193 **Figure 3.** An overview of identified challenges and dream discovery and possible applications of spatial
 194 proteomics in protist biology highlighted by workshop participants. Protist illustrations reproduced from
 195 (Keeling and Eglit, 2023).

196

197 A significant theme of the challenges discussed centred on the need to grow protist cells in culture to
 198 obtain sufficient biomass. Even protists that can be readily cultured, such as many free-living species,
 199 can be heterotrophic and, therefore, require bacterial or other microbial prey, which complicates the
 200 generation of spatial proteomic data. However, polyxenic cultures, in which the associated
 201 microorganisms are defined and balanced in relative abundance (Clark & Diamond, 2002), may not
 202 necessarily preclude these approaches, as demonstrated by the application of LOPIT-DC to
 203 *Paratrimastix pyriformis* that was maintained in a polyxenic culture (Zítek et al., 2022). In contrast,
 204 many parasitic protists, particularly obligate intracellular parasites, cannot currently be cultured

independently of their hosts because suitable host–parasite culture systems have not been established; this represents a fundamental barrier for spatial proteomic analyses of these organisms.

Cellular heterogeneity and complex life cycles may provide both challenges and opportunities. Protein localisation may change in response to developmental stages or environmental conditions, suggesting that spatial proteomics could be used to investigate processes such as encystation, host invasion or environmental adaptation. However, obtaining homogeneous populations for spatial proteomic analysis can be challenging. A related consideration is that the conditions required to maintain a protist in culture may not reflect its natural cellular state. Culture conditions, nutritional status and interactions with prey may influence protein abundance and localisation, highlighting the importance of considering experimental conditions when interpreting spatial proteomic data.

Participants also highlighted financial constraints and limited access to instrumentation and the specialist expertise required to undertake spatial proteomic analyses, particularly smaller research groups working on non-parasitic protists. In addition, the availability of suitable genomic or transcriptomic resources for poorly characterised protists poses a barrier to interpreting proteomic data. However, these limitations may also provide opportunities for new collaborations between protistologists and groups with established spatial proteomics facilities and expertise.

When asked to consider their “dream discovery” using spatial proteomics, workshop participants proposed a range of potential applications reflecting the breadth of protist biology. These included the discovery and characterisation of unknown organelles or cellular structures, resolving the molecular basis of host–parasite and predator–prey interactions, and investigating the integration and function of endosymbionts within host cells. Other suggestions focused on dynamic processes, such as changes in protein localisation during encystation or across different life-cycle stages, and on understanding how protists remodel their cellular machinery in response to environmental conditions. Another emerging discussion point was the discovery-driven nature of spatial proteomics, in which unexpected discoveries may emerge from extensive spatial mapping rather than from testing a predefined hypothesis.

5. Future directions and conclusion

This P-UK autumn meeting 2026 highlighted that spatial proteomics has many potential applications in protistology research, from the characterisation of novel organelles and protein-trafficking pathways, to investigating endosymbiosis, host–parasite interactions, and the evolution of eukaryotic cellular complexity. The workshop discussions demonstrated that significant practical and technical challenges remain when applying this technology to protists, including obtaining sufficient culture biomass and purity, difficulties in sample preparation and the challenges of data interpretation from organisms with limited genomic data. Addressing these will be important for extending spatial

proteomics beyond established model systems and realising its potential value across the field of protistology.

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RF – Conceptualisation, Writing – original draft, Writing – review and editing; EN, RFW - Writing – original draft, Writing – review and editing; CB, MC, AD, GFE, FHM, DM - Writing – review and editing

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