Understanding and Using Biofilm Microenvironments: Multidisciplinary Goals, Gaps, Required Support, and Critical Skills Inventories

OUTCOMES AND RECOMMENDATIONS

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1 KEY OUTCOMES AND RECOMMENDATIONS

A. Outcomes Summary

Defining Microenvironment: Agreed on a working definition emphasizing dynamic, heterogeneous conditions distinct from the bulk environment, shaped by microbial interactions and challenging to measure or control.

Application Goals: Envisioned capabilities such as improved process control, enhanced reliability, and enabling technologies for waste treatment, resource recovery, and bioprocess optimization.

Knowledge Gaps: Identified three major barriers; linking scales and disciplines, managing complexity, and improving methods (including modelling and uncertainty quantification).

Target Activities: Prioritized co-design between modelling and experimentation, skill development, and leveraging existing tools alongside new technologies like microfluidics and spatial omics.

Institutional Support: Called for proactive matchmaking, interdisciplinary funding mechanisms, streamlined administration, and recognition of outputs beyond publications.

Skills Inventory: Revealed strong modelling and experimental expertise but widespread desire for growth in data science, infrastructure, and industry collaboration.

B. Recommendations

i. Enable Interdisciplinary Collaboration

- Proactive matchmaking and cross-institute appointments.
- Funding calls designed for interdisciplinary projects, evaluated by cross-disciplinary panels.
- Small proof-of-concept grants to build capacity and networks.
- Support targeted training workshops and accessible primers for cross-disciplinary skills.

ii. Streamline Administrative Processes

- Simplify grant submission and reporting.
- Increase financial flexibility and standardize systems.
- Facilitate hiring of research software engineers and undergraduate researchers.

iii. Broaden Impact and Recognition

- Provide guidance for industry engagement.
- Recognize non-traditional outputs.

iv. Integrate Modelling and Experimentation

- Promote co-design between modelers and experimentalists.
- Encourage iterative cycles linking *in vivo* and *in silico* approaches.

2 WORKSHOP OUTCOMES

A. Defining 'The Microenvironment'

The first session aimed to establish a shared understanding of "the microenvironment," recognizing that participants approached the term from different perspectives. The prompt was:

"What do you think of when you think about the 'microenvironment'"

While a universal definition remains elusive, the synthesized perspectives suggest a useful working definition:

> ***"A microbial microenvironment is an ecosystem whose chemical and physical characteristics are dynamic and heterogeneous across relatively small spatial scales. It is distinct from, yet interacts with, the bulk environment, often via mass transport processes. Complex emergent behaviours arising from these characteristics present unique challenges in measurement and system control."***

Participants generated forty-three entries, which clustered into four themes. Across these themes, definitions relied on both observable and operational characteristics. A notable common thread was that the microenvironment was often defined by contrast to the bulk environment rather than by intrinsic criteria. One entry stated simply "*different from the bulk*" and received two emphasis dots.

Theme 1, *physical and chemical characteristics*, focused heavily on interactions between the microenvironment and bulk conditions. Representative terms included "*boundary layer*" and "*mass transfer*". Specific properties mentioned were chemical concentrations such as "*EPS* [extracellular polymeric substances*], nutrients, metabolites, O2, pH*". Some entries, including "*steep concentration gradients*" and "*scale of diffusion*" attracted red dots, indicating uncertainty or disagreement. Similarly, physical properties "*density*, [mechanical] *modulus*" as well as "*Product/substrate limitations*" appeared somewhat questionable.

Theme 2, *scale*, addressed both spatial and temporal aspects. Some entries noted absolute scales such as "*Scale range nm->mm*" but many focused also on "*spatial heterogeneity*" and a "*dynamic and Non-static*" temporal nature. Temporal characteristics appeared particularly important, with the words 'dynamic' and 'flux' appearing multiple times, although some related entries drew red dots. Conversely, the idea that the microenvironment itself is not transient and "*maintains identity for a 'significant' time*" appeared questionable with 2 red dots.

Theme 3, *ecology,* centred upon biological interactions. A highly emphasised entry, marked with 5 dots, represented the overall theme that a microenvironment "*emerges through interactions between microbial & environment*" and is defined by its "*spatial/pop*[ulation] *composition*" (also highly emphasised with 3 dots).

Theme 4, *control and measurement*, concerned either practical abilities to influence the microenvironment or to discern empirical features raised in the other themes. One entry proposed that the difficulty in measuring the microenvironment is a defining characteristic. Although the concept of 'engineering' microenvironments appeared in multiple entries, some highly emphasized, the idea that the microenvironment performs a desired function was strongly questioned (4 red dots).

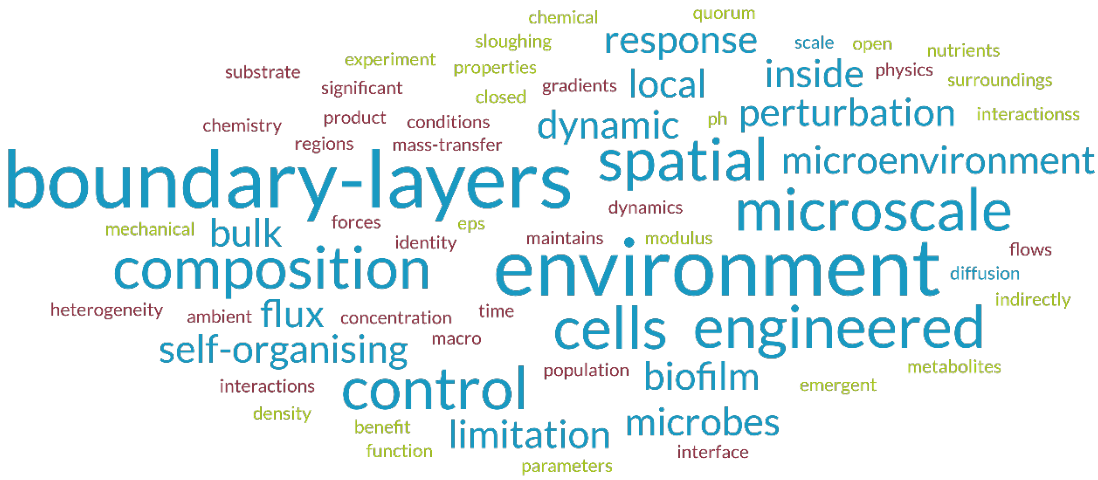

Figure 1: Word cloud generated from normalised terms in Session A responses

B. Desired Application Goals and Knowledge Advances

The second session encouraged participants to "begin with the end in mind" and envision a scenario where resources, expertise, and methodology were not limiting. The prompt was:

"What would be possible with sufficient understanding/control of the microenvironment?"

From a synthesis of thirty-seven responses, the goal of better understanding the microenvironment includes both **high aspiration targets** as well as **well-defined near-term functional capabilities**. Both depend heavily on **better control and reliability** of both engineered systems and managed natural environments derived from **improved system insight**. Individual entries fell into relevant themes.

Theme 1, *insight* concerns an improved ability to better observe and understand the microenvironment. Highly emphasized entries included "*identifying key/limiting substrates & other parameters*", and "*cross platform understanding (water <-> soil)*". The idea of "*better in silico for experimental screening, hypothesis generation*" was contentious and "*Transferring between scale*" received 3 red dots, indicating notable uncertainty.

Theme 2, *control,* was the dominant focus, accounting for forty percent of all entries. This theme included abilities arising from better insight, such as "*faster reactions*", "*more controllable outputs*" and "*better yields*". Reliability and resilience were recurring sub-themes, although one entry of that type "*Maximise reproducibility (Batch 2 Batch integration)*", was contentious.

Theme 3, *function,* related to specific technologies enabled by better insight and control. Lignocellulosic degradation was mentioned in multiple highly emphasized entries. Other technologies included pharmaceutical breakdown, nitrogen fixation, and alginate recovery. No entries in this theme were contentious and only one, regarding methylotrophic methane consumption, received a red dot.

Theme 4, *aspiration* revolved around general and often self-described "bold claims" that built upon the other three themes. Illustrative entries include "*Designer microenvironments*" and "*Bold claim 1 Speed up innovation by 1000x*". One contentious entry was "*Exotic structure* *engineering*" and the idea of "*Challenging evolutionary stable states*" was strongly questioned, receiving 5 red dots.

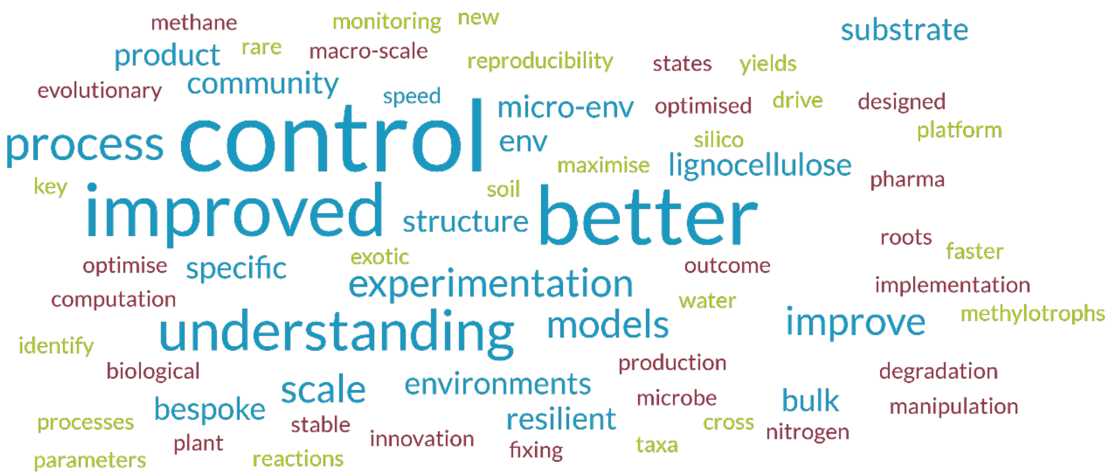

Figure 2: Word cloud generated from normalised terms in Session B responses

C. Identifying Knowledge Gaps and Technical Barriers

The third session was designed to uncover why the activities and capabilities envisioned in the previous session are not currently feasible. The prompt was:

"What don't we sufficiently understand?"

Participants were asked to explicitly **not** yet propose solutions towards closing those gaps.

An overarching issue identified was that **integrating knowledge across scales and disciplines remains highly challenging**. The difficulty lies in the **inherent complexity** of the systems and **the need for more robust methods** to address that complexity.

Across all groups, forty-four individual entries were noted, which fell into three major themes.

Theme 1, *linkages and knowledge integration,* can be summarised as: "*We lack frameworks and empirical data to connect micro-scale phenomena to macro-scale system behaviour, and to integrate physical and biological processes, including between real-world systems and models."* The inability to relate across scales, from micro to macro, and to bridge models with experiments was mentioned repeatedly and often received multiple emphasis dots. The difficulty in linking theory with practice was also noted.

Theme 2, *complexity*, focused on the fundamental lack of strategies to predict or manage complexity. At a systems level, this gap hinders process control and reliability. Biological heterogeneity and stochasticity were identified as underlying causes. Participants noted we lack understanding of how diversity, evolution, and phenotypic variability shape function and reproducibility in microbial systems.

Theme 3, *methods*, centred on tools and approaches required to manage complexity. At a high level, participants noted that increased training in rigorous experimental design would improve research quality. Wider adoption of specific tools, such as spatial transcriptomics, and developing new capabilities, such as novel *in situ* metabolite quantification, were also suggested. Modelling issues formed a large subset within this theme. Models often lack validated parameters and robust uncertainty quantification. Identifiability and observability remain unresolved, limiting predictive power. There were also calls for better dialog between modellers and experimentalists, particularly with regards to co-designing for better parameterization and validation.

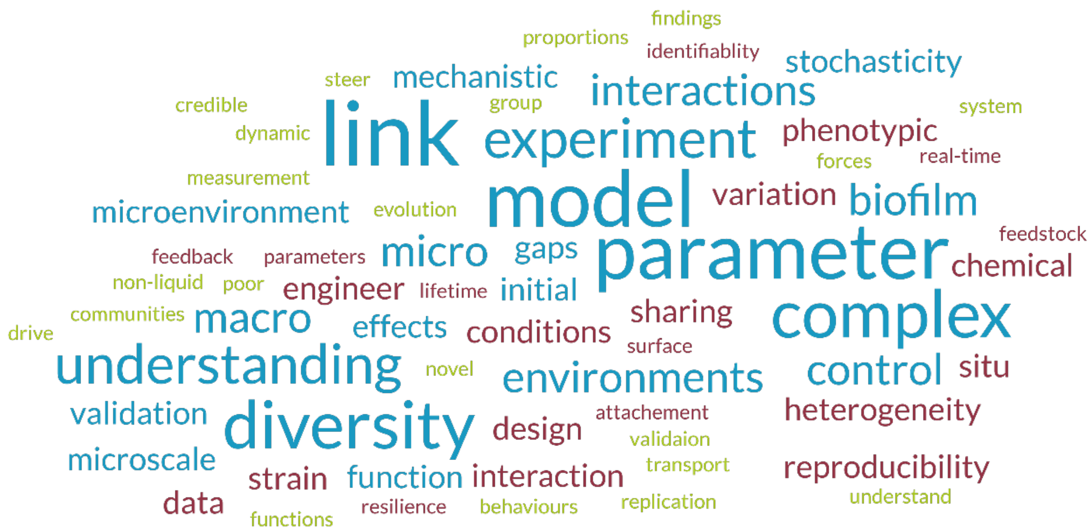

Figure 3: Word cloud generated from normalised terms in Session C responses

D. Target Activities for Advancing the State of the Art

The goal of this activity was to identify actions that could close the gaps highlighted in the previous session. The prompt was:

"What do we need to do to close the gaps?"

Additional guidance included "types of experiments?", "advances in methods or equipment", and "other issues".

While some responses concerned specific technological and methodological advances, nearly two-thirds instead emphasized cultivating professional skills in existing technologies and fostering interdisciplinary collaboration as a means of accessing additional capabilities. It is evident that **while new technologies are always welcomed, enhancing our ability to use and access existing methods is at least equally important**.

Theme 1, *improved tools and methods*, encompassed technological developments aimed at enhancing our ability to sense or influence the microenvironment. Specific advances included developing microfluidic platforms, single cell culturing, and label-free imaging techniques. Broader goals included improving identifying cell phenotypes and observing metabolic switching. Some also reflected on experimental philosophy, calling for greater focus on application-based experiments while also advocating for more precise targeting and rapid execution of exploratory experiments.

Theme 2, skill development and collaboration, largely focused on enhancing our ability to take advantage of existing methods. Participants called for lowering skill acquisition barriers through: targeted workshops, supporting "*short, simple 'primers'*" to guide learning, including cross-discipline jargon "Rosetta stones". Suggestions included matchmaking via an interdisciplinary "*buddy system*" to encourage cross-domain skill transfer. There were also calls for participants to act as "*discipline ambassadors*", using language accessible between domains and by seeking interdisciplinary collaborators earlier in project development.

Theme 3, *integrating modelling and experimentation,* was best captured by one entry illustrating a cyclical diagram connecting the words *in vivo* and *in silico* and containing the word 'efficient'. Participants viewed modelling not as an isolated activity but as a tool to make experiments more informative and efficient, reflecting a repeated theme of the workshop highlighting a desire for more interaction between disciplines. Entries emphasized how models can support experiments, particularly by aiding design and interpretation, and highlighted the need for co-development between modelers and experimentalists.

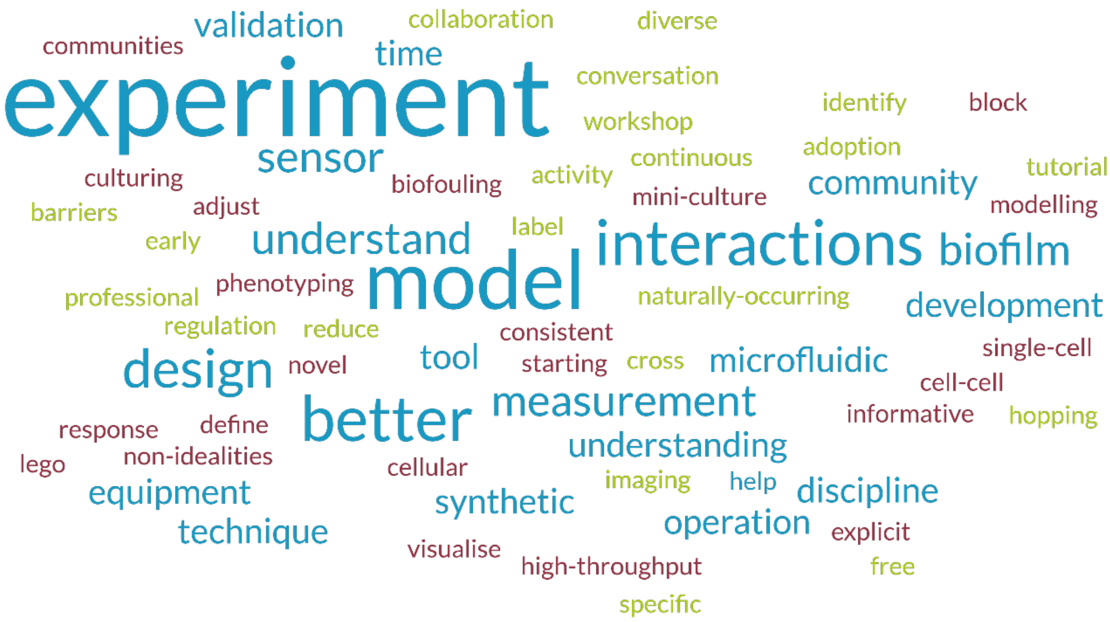

Figure 4: Word cloud generated from normalised terms in Session D responses

E. Institutional Support Reflection and Requests

After envisioning their goals, identifying barriers, and considering ways to overcome them, participants were asked to reflect on the support needed to close those gaps. The guiding question was:

"How are/can we be supported in closing gaps?"

The prompt encouraged attention to both institutional strengths and weaknesses, included "soft issues" such as recruitment barriers, and left "institution" broadly defined.

Most responses centred on **interdisciplinary work**, specifically on **reducing barriers** and **aligning structural support with the recognized need** for collaboration. Other prominent themes included improving impact, enhancing recruitment, and reducing administrative burden. While funding was mentioned, concerns focused on allocation and evaluation rather than increasing overall amounts.

Theme 1, *supporting interdisciplinary work,* included three specific issues. First, participants called for stronger cross-institute appointments and fellowships, as well as active matchmaking services to facilitate collaboration rather than relying on passive networks. Second, while it was recognised that funding bodies acknowledge the importance of interdisciplinary work, execution was seen as lacking. A recurrent frustration was the rigid division of remits in practice, such as "*Biologists to BBSRC Engineers to EPSRC STOP THIS APPROACH*". Participants requested truly cross-disciplinary calls preventing 'bouncing' between bodies when working at "60% NERC, 40% EPSRC" and further requested assurances that those calls were evaluated by cross-disciplinary reviewers. Finally, a poll (3-0) indicated strong preferences for small proof-of-concept opportunities to build capacity and connections, rather than large-scale grand initiatives which presuppose them.

Theme 2, *reducing administrative burden,* focused on streamlining processes and providing training. Suggestions included continued or improved grant writing and submission support, financial flexibility, and connected-together efforts to cut 'red tape' and standardise systems.

Theme 3, *recruitment*, echoed similar concerns about process efficiency, particularly for hiring students and researchers. Participants noted that hiring undergraduate researchers and research software engineers specifically could be streamlined.

Theme 4, *enhancing impact,* was largely industry focused, with calls for better guidance on identifying, engaging and collaborating with industrial partners. One entry, unrelated to industry, called for better formal recognition of non-traditional research outputs such as software maintenance and primer design.

Figure 5: Word cloud generated from normalised terms in Session E responses

F. Practitioner Skills Inventory and Matchmaking

The final activity differed from previous sessions and aimed to produce a skills inventory and potential collaborations. Group boards were split into columns labelled '*Have*', '*Grow*', and '*Collab*'. Each group member selected a row and listed relevant skills, techniques, or equipment they currently possessed, wished to develop, or hoped to access through collaboration. The prompt was:

*"Three sets of sticky-notes with specific **tools, methods, techniques, knowledge**:*
- *you currently use*
- *you would acquire if you had time/budget*
- *you would like from collaborators"*

Across all groups, 159 entries were collected and clustered into 10 categories:

- **Modelling & Simulation:** Included computational fluid dynamics, agent-based models and process level models, with goals ranging from process optimisation through multi-scale extension, uncertainty estimation, and improving parameter identification.
- **Wetlab Measurement & Techniques:** Spanned molecular methods, culturing technologies, synthetic biology, and integrating spatial data.
- **Microbial Ecology & Evolution:** Focused on microbial interactions and evolutionary dynamics in both real-world systems and theoretical contexts.
- **Process Engineering & Startup:** Emphasized applied technologies such as algal systems, bioelectrical processes, and material recovery, with frequent mention of scale-up challenges.
- **Infrastructure & Hardware:** Highlighted enabling equipment, particularly for high-throughput automation and micro-scale observation/manipulation (*e.g.,* microfluidics, micro-probes).
- **Omics & Bioinformatics:** Covered informatics for molecular methods, including 16S and metagenomic surveys, genome analysis, and multi-omics approaches.
- **Data Science & Machine Learning:** Included traditional data analysis and visualisation alongside integration of machine learning.

- **Communication, Policy, & Training:** Encompassed outreach, teaching, and engagement with policymakers.
- **Experimental Design:** Focused on improving practices for defining experimental parameters, often through model-driven approaches.
- **Collaboration & Industry:** Notably absent from participants' current skill sets but listed as a desired area for growth and collaboration, suggesting discomfort or unfamiliarity with this domain.

The absolute tallies (***Table 1***) do not account for either varying participant verbosity or focus; some listed over 20 items, others fewer than 10. Similarly, emphasis varied: some participants focused on equipment while others implied equipment access without listing it. Overall, individual participants provided balanced amounts of *Have*, *Grow*, and *Collab* items. Individual profiles spanned a spectrum from modelling to experimental work and from theoretical to applied approaches. No participant claimed expertise in *Collaboration & Industry* or *Communication, Policy, & Training*, though some expressed interest in these areas, indicated a perceived gap in broadening impacts.

Table 1 Categorized Skills and Capabilities Inventory of Participants

CATEGORY	HAVE	GROW	COLLAB	TOTAL
Modelling & Simulation	24	5	11	*40*
Wetlab Measurement & Techniques	13	5	11	*29*
Microbial Ecology & Evolution	8	8	7	*23*
Process Engineering & Startup	8	4	8	*20*
Infrastructure & Hardware	7	4	3	*14*
Omics & Bioinformatics	6	3	1	*10*
Data Science & Machine Learning	4	3	2	*9*
Communication, Policy, & Training	0	4	3	*7*
Experimental Design	1	3	0	*4*
Collaboration & Industry	0	1	2	*3*
TOTAL	*71*	*40*	*48*	**159**

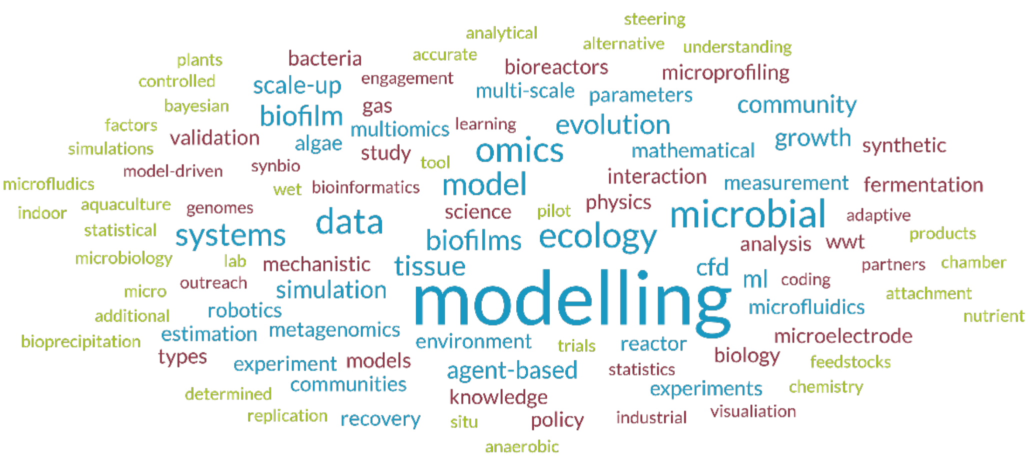

Figure 6: Word cloud generated from normalised terms in Session F responses

When normalized for 'verbosity', several correlations emerged when grouped into "related measures" (have-have) and "development measures" (have-grow, have-collab). Full correlations are provided as a data file, but caution is warranted given small sample sizes. For example, a strong positive correlation between *Omics* and *Bioinformatics* largely reflects one participant's interests rather than an inherent link. We focus instead on broad trends:

i. Have-Have Correlations

Few categories showed strong positive correlation. Moderate negative correlations (-0.2 to -0.5) were more common, suggesting little conceptual overlap and high specialisation among participants.

ii. Have-Grow Correlations

Participants generally wished to deepen expertise in areas they already possessed, reflected in moderate to high positive correlations (>0.3). Exceptions included *Experimental Design*, which was listed as "had" by only one participant. Many also sought to broaden capabilities selectively, with *Data Science & Machine Learning*, *Infrastructure & Hardware*, and *Process Engineering* emerging as popular growth areas. These choices suggest interest in staying current, expanding technical capacity, and pursuing real-world applications. Negative correlations indicate selective expansion rather than indiscriminate growth

iii. Have-Collab Correlations

Participants typically sought collaborators outside their own skill sets, shown by mild to moderate negative correlations between "*Have*" and "*Collab*" within categories. *Microbial Ecology & Evolution* was an exception, as expertise in this area often overlapped with complementary specializations. Desired collaborations frequently bridged wet-dry and theoretical-applied divides: modellers sought wetlab partners, while wetlab specialists rarely sought additional wetlab collaborators. *Collaboration & Industry* was widely desired across domains, reflecting a perceived gap. Interestingly, no participant listed *Experimental Design* as a desired collaborator skill. *Process Engineering* was the most requested collaboration area. No single "*Have*" category dominated collaboration-seeking.

Transitivity of collaboration preferences, for the subset where one positive correlation exceeded 0.20 in at least one direction, varied (Table 2). When positive in both directions, correlations were moderately to highly positive; when a negative correlation existed in one direction, it was generally small. A notable exception was *Infrastructure & Hardware*: participants with experimental infrastructure showed mild positive correlation with modellers, but modellers were strongly negatively correlated with infrastructure. However, modellers strongly favoured wetlab collaborators (0.83), suggesting they prioritize broad experimental expertise over specific infrastructure. Similar dynamics may explain mild negative correlations for *Process Engineering & Startup* expertise.

Table 2: Correlations between *Have/Collab* pairs where at least one member has a positive correlation above 0.20.

Category X	**Category Y**	***Have* X, *Collab* Y**	***Have* Y, *Collab* X**
Both Positive			
Omics & Bioinformatics	Infrastructure & Hardware	0.94	0.76
Modelling & Simulation	Wetlab Measurement	0.83	0.15
Process Engineering & Startup	Microbial Ecology & Evolution	0.22	0.33
One Negative			
Omics & Bioinformatics	Data Science & Machine Learning	0.87	-0.14
Data Science & Machine Learning	Process Engineering & Startup	0.42	-0.10
Infrastructure & Hardware	Process Engineering & Startup	0.29	-0.16
Infrastructure & Hardware	Modelling & Simulation	0.28	-0.49
Wetlab Measurement & Techniques	Process Engineering & Startup	0.21	-0.20
No Pair			
Data Science & Machine Learning	Collaboration & Industry	0.62	NA
Wetlab Measurement & Techniques	Collaboration & Industry	0.47	NA
Microbial Ecology & Evolution	Communication, Policy & Training	0.21	NA
Process Engineering & Startup	Communication, Policy & Training	0.91	NA

Despite the small participant population, one of the clearest findings is that participants overwhelmingly sought collaborators with complementary expertise rather than additional specialists within their own domains. This suggests that future networking, funding, and community-building activities may be most effective when focused on creating links between capability clusters, rather than reinforcing existing disciplinary groupings