nature portfolio

Peer Review File

Hybrid solid-liquid optics enable scalable, multi-immersion light-sheet microscopy

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Parts of this Peer Review File have been redacted as indicated as we could not obtain permission to publish the reports from one of the peer reviewers.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
OVERALL ASSESSMENT
Recommendation: Major Revision Required

This manuscript presents high-quality work that addresses a fundamental limitation in light-sheet microscopy through an elegant optical solution. The HySIL (Hybrid Solid-Liquid Immersion Lens) concept is novel and the experimental validation is rigorous. However, the manuscript requires major revision to adequately address its relationship to closely related prior work (specifically meniscus lens approaches) and to clarify several technical aspects before publication in Nature Biotechnology.

The authors develop SCOPE and Super-SCOPE imaging devices based on a hybrid solid-liquid optical framework that transforms inexpensive, long-working-distance air objectives into high-resolution, aberration-free detection systems for light-sheet microscopy. The approach pairs a solid optical element (plano-convex lens) with precisely refractive index (RI)-matched liquid to create a hemispherical immersion system that: corrects spherical aberrations when imaging through high-RI clearing media, increases effective detection NA by n (SCOPE) or n^2 (Super-SCOPE), maintains compatibility across diverse immersion media (RI 1.33-1.56), and preserves long working distance (~34 mm) and low cost of air objectives.

The authors validate SCOPE through optical simulations, PSF measurements, and demonstrate broad applications including expansion microscopy, tissue clearing, brain organoid screening, and 3D histopathology of human tissue.

The HySIL concept represents a genuine advance beyond classical solid immersion lenses (SILs). Unlike conventional SILs that are limited to surface-proximal imaging, HySIL decouples the solid lens from both the sample and objective, enabling deep volumetric imaging. The solid-liquid hybrid architecture is elegant and generalizable beyond the specific implementations shown. The work tackles a real limitation in the field: achieving submicron resolution over centimeter-scale volumes without expensive, short-working-distance immersion objectives. This constraint has genuinely limited widespread adoption of advanced volumetric imaging, especially with expansion microscopy.

The optical characterization is thorough and the versatility is impressive, as it is demonstrated across many different samples. The use of inexpensive, off-the-shelf components (standard plano-convex lenses, commodity air objectives) combined with modular design that integrates with existing platforms represents a genuine advance in accessibility. The cost reduction is orders of magnitude compared to high-NA immersion objectives.

Major concerns requiring revision:

1. No proper comparison with existing techniques (meniscus lens)

The authors cite Barner et al.'s Solid Immersion Meniscus Lens (SIMlens, Opt. Lett. 2019) but provide only superficial discussion, dismissing it along with other approaches as 'system-specific' or requiring 'custom engineering.' This is

inadequate given the conceptual similarity between SIMlens and SCOPE. Both approaches: Use curved optical elements at the imaging chamber window, aim to correct spherical aberrations for air objectives imaging immersed samples, and position samples in immersion medium while using air objectives for detection.

Critical missing information:

- Why is hemispherical geometry superior to meniscus geometry? Is there a theoretical optical advantage, or is it simply easier to source/manufacture?
- What specific advantage does the solid-liquid hybrid provide over an all-solid meniscus design? The authors claim 'flexible sample positioning' but don't explain why a meniscus lens wouldn't provide this.
- Is the RI-matching liquid the key innovation enabling multi-immersion tolerance? If so, could a meniscus lens be similarly adapted with RI-matched liquid?
- How do resolution, field-of-view, aberration correction, and cost compare directly between approaches?
- Are standard plano-convex lenses truly more accessible than meniscus lenses? What is the actual cost/availability difference?

Suggested revision:

- Add a dedicated comparison section that explicitly contrasts SCOPE with SIMlens and other aberration correction approaches
- Include a comparison table covering: optical geometry, materials, cost, complexity, multi-immersion capability, FOV, resolution, working distance, and appropriate use cases
- Ideally, provide optical simulations comparing meniscus vs. hemispherical geometries under matched conditions
- Acknowledge the conceptual overlap while clearly articulating SCOPE's specific advances beyond existing approaches
- Include the recent paper in Nature Biotech. by Aakhte et al. in the discussion
- If experimental comparison is feasible, direct testing on the same sample would significantly strengthen claims

Without this comparison, readers cannot properly evaluate the novelty and practical advantages of SCOPE relative to existing solutions.

2. Incomplete Characterization of Super-SCOPE

The manuscript introduces Super-SCOPE (Weierstrass super-hemispherical geometry) as providing enhanced resolution with n^2 NA scaling but provides limited experimental validation:

- Figure 1e-g shows simulations, but no experimental PSF data for Super-SCOPE is provided
- Figure 2e demonstrates chromatic alignment for SCOPE but not Super-SCOPE
- The text acknowledges 'tighter optical tolerances and narrower detection spectral bandwidth' but doesn't quantify these limitations
- No biological imaging examples using Super-SCOPE are shown

Suggested addition:
- Provide experimental PSF characterization across wavelengths for Super-SCOPE
- Quantify chromatic aberration limits and acceptable spectral bandwidth
- Include decision criteria for when Super-SCOPE is worth the additional constraints vs. standard SCOPE
- If Super-SCOPE is not practically useful with current implementations, state this clearly

3. Resolution Claims and Expansion Microscopy Validation

The effective pre-expansion resolution claim of ~190 nm (Fig. 3, line 211) is based on measured 0.75 μm PSF and assumed 4× expansion factor. However:

- No direct measurement of expansion factor in the imaged regions is provided
- Expansion non-uniformity is a known issue in ExM and could significantly affect resolution estimates
- For the salamander brain (Fig. 4), expansion factor validation is not mentioned

Suggested addition:
- Provide local expansion factor measurements in imaged tissue regions (e.g., using fiducial markers or anatomical landmarks)
- Discuss expansion uniformity and its impact on resolution estimates
- If expansion varies significantly, provide resolution ranges rather than single values

ADDITIONAL TECHNICAL CONCERNS

4. Limited Field-of-View Discussion

SCOPE achieves excellent performance over limited FOV (1.2 mm for 10×, 2.7 mm for 5×). For whole-organ imaging (e.g., centimeter-scale mouse brain), extensive tiling is required. The manuscript should address:

- Stitching accuracy and reliability across large tile arrays
- Throughput implications - approximate imaging time for representative samples
- Data management challenges for large stitched datasets
- Whether FOV limitations could be addressed with different lens geometries within the HySIL framework

5. Direct Performance Comparison to Immersion Objectives

While PSF measurements are excellent, direct image quality comparisons to conventional immersion objectives are limited. A side-by-side comparison on the same sample (even if limited to the immersion objective's working distance) would strengthen claims about comparable performance at orders-of-magnitude lower cost.

Suggested addition:
- Add supplementary figure comparing SCOPE vs. high-NA immersion objective on identical tissue region
- Include quantitative metrics: SNR, contrast, resolution in biological tissue

6. Practical Implementation Details

Several practical considerations need clarification:

Sample alignment sensitivity: How critical is precise positioning of the sample relative to the hemispherical surface? What are acceptable tolerances?

Temperature stability: RI matching at ~1.46 likely requires temperature control. What temperature variations are acceptable? How is this managed?

Interface maintenance: How is the solid-liquid interface cleaned between samples? How often does the immersion oil need replacement?

Live imaging compatibility: Can SCOPE be used for live specimens or only fixed/cleared samples? If live imaging is possible, what are the constraints?

A practical troubleshooting guide in supplementary materials would significantly enhance utility.

MINOR ISSUES AND SUGGESTIONS

7. Figure Organization and Clarity

- Figure 1 is dense and covers both theoretical foundations (c-g) and practical implementation (h). Consider splitting into two figures: (1) optical theory and simulations, (2) practical device design and integration.

8. Biological Impact and Novelty

The biological examples are largely proof-of-concept demonstrations rather than hypothesis-driven studies. While appropriate for a methods paper, the Discussion could be strengthened by:

- Identifying specific biological/clinical problems where SCOPE's unique capabilities (large volume + high resolution + multi-immersion) are essential
- Discussing what types of biological questions become newly addressable

9. Technical Clarifications

- Line 82: 'effective detection NA (scaled by n and n^2 for SCOPE and Super-SCOPE, respectively)' - This key claim deserves more physical explanation in the main text
- Provide estimated imaging times for representative samples to help readers assess throughput
- Include cost breakdown comparing SCOPE-enabled system to traditional high-NA immersion objective approaches

Reviewer #2

(Remarks to the Author)

Gong et al. present a solid immersion lens (SIL)-based optical design for high-resolution microscopic imaging through low-NA air objectives. They first characterize optical performance for two lens geometries (SCOPE and Super-SCOPE) using Zemax simulations, confirming reduced spherical aberrations and enhanced resolution on-axis. They emphasize the tolerance of the SCOPE approach with respect to lens-medium refractive index (RI) mismatches, effectively allowing an RI range between water (1.33) and oils (up to 1.54). They then experimentally test the SCOPE approach using two 34-mm working distance air objectives (5x and 10x) to demonstrate sub-micron resolution imaging within large imaging volumes (several mm to cm side length). In combination with a light-sheet microscopy platform they apply the SCOPE approach for imaging demonstrations using various samples, including expanded tissue sections, samples cleared with different protocols, and histological samples from human breast cancer. They put forward their approach as 'practical and cost-effective foundation for next-generation volumetric microscopy'.

The SIL-based optical method for advancing high-resolution, large-volume imaging using light-sheet microscopy is an interesting approach that indeed may offer low-cost improvements when using low-NA air objectives. The example SCOPE applications presented in the manuscript provide nice imaging data demonstrating this potential for improvements. The main issue with the manuscript is, however, that the novelty of the presented approach remains unclear, as very similar approaches have already been published. In addition, the system characterization is incomplete, with off-axis evaluation in the simulations as well as full PSF quantification for the light-sheet imaging data missing. It also remains vague, without any specific further investigation, in how far the authors see their approach as going beyond previous approaches and establishing a 'general optical framework' (line 68). In the following we elaborate on several of these issues in our specific comments.

1. Variations of the SIL technique have been known in light-sheet microscopy field (e.g., refs 13, 19 both from 2019), and their use was NOT limited to surface imaging. For example, [ref 13] described the SIL concept (SIMlens) and applied it to long-WD air objectives (see their Figs. 2 and 3). Given these previous demonstrations, the following statement seems unsubstantiated (line 107): "However, unlike conventional SILs, which are inherently limited to surface-proximal imaging (Fig. 1c), HySIL decouples the solid lens from both the sample and the objective, enabling aberration-free imaging throughout large, cleared specimens". It remains the question: What is exactly the difference between SIMlens [ref 13] and HySIL?

2. Figure 1e,f and Suppl. Fig 1a show Huygens PSF on-axis. This can depart, however, substantially from the PSF off-axis for a reasonable field-of-view (FOV) size, which therefore needs to be assessed. In own simulations that we performed in Zemax for the 5x air objective (NA 0.28), we found that the Strehl ratio falls below the diffraction limited performance criterion (Strehl 0.8) already at field position 700 µm (Strehl 0.67) and that diffraction-limited performance is limited to 0.5 mm field point, i.e., 1 mm total FOV. The authors should add PSF off-axis characterization for both the 5x and 10x objective and report the diffraction-limited FOV sizes.

3. For the experimental validation, a FOV of 1 mm is shown in Figure 2c for the Mitutoyo 10x/0.28 objective. That is quite small and one would rather expect assessment of a 2-mm FOV for this magnification to efficiently use modern sCMOS cameras (sensor width around 20 mm). A FOV of 2.6 mm is mentioned in Suppl. Fig. 1d but not quantified.

4. The quantification of PSFs for the light-sheet application in Figure 2 is incomplete as the quantification of the lateral PSF width (on-axis and off-axis) is missing. It may have been skipped because relatively large (1 micron-sized) fluorescent beads apparently were used. A full PSF characterization with sub-resolution fluorescent beads should be presented, not least because all subsequent imaging demonstrations were done with the light-sheet microscope system.

5. Overall, based on the presented data and our own simulation, we find that HySIL offers limited functionality for imaging cleared tissues with modern sensors (diagonal of 22-30 mm) and low-magnification objectives (5x-10x), although it may be useful with higher magnifications (20-50x) if the working distance of the respective objectives is sufficient.

6. Minor: For Figs. 3 and 4 the specification of the depth-color coding range is missing.

Reviewer #3

(Remarks to the Author)

A. Summary of the key results

The manuscript addresses one of the challenges in modern light microscopy: the difficulty of imaging large, cleared samples with inexpensive air objectives while still achieving aberration-free, subcellular or submicron resolution. The authors show that this limitation can be overcome through a hybrid solid–liquid optical framework (HySIL), implemented as SCOPE and Super-SCOPE. These devices enable submicron-resolution, aberration-free light-sheet microscopy using low-cost, long-working-distance air objectives, substantially enhancing effective numerical aperture and correcting spherical aberrations across a wide range of refractive indices. Their approach supports centimeter-scale imaging and is validated through optical simulations, PSF measurements, and diverse biological demonstrations, including expansion microscopy, multiple tissue clearing methods, organoid imaging, and 3D histopathology. Overall, the work offers a compelling and accessible solution for scalable, high resolution volumetric imaging at the centimetre scale, and in my view the imaging community would benefit greatly from learning about it.

B. Originality and significance:
This work presents an original and impactful contribution to the field of volumetric microscopy. The HySIL framework introduces a hybrid solid–liquid optical strategy that differs fundamentally from classical solid immersion lenses and from existing approaches to refractive-index-matched imaging. By enabling submicron resolution, aberration free imaging with inexpensive long working distance air objectives, the work opens a new design space for accessible high NA light sheet microscopy. The ability to maintain high image quality across a wide range of refractive indices and sample preparation methods further underscores the practical significance of the approach. The combination of conceptual novelty, technical elegance, and broad applicability positions this work to have a meaningful and lasting impact on the imaging community.

C. Data & methodology: validity of approach, quality of data, quality of presentation

The methodological framework is sound and well aligned with the goals of the study. The combination of optical simulations, PSF measurements, and biological imaging provides a coherent and logically structured validation pipeline. The optical modeling is appropriate for the questions being addressed, and the experimental PSF data are of high quality and clearly demonstrate the expected performance improvements. The biological datasets—including expanded tissues, cleared samples across multiple refractive indices, organoids, and human specimens—are well chosen and effectively illustrate the versatility of the system.
The quality of data presentation is strong: figures are clear, well annotated, and appropriately scaled to highlight both optical performance and biological detail. The manuscript communicates the methodology in a transparent and accessible manner, enabling readers to understand the experimental workflow and the rationale behind key design choices. A few additional details such as alignment tolerances and robustness of the hybrid solid–liquid interface would further enhance clarity, but overall the data and methodology are presented at a high standard.

D. The manuscript makes appropriate use of quantitative metrics for evaluating optical performance, particularly in the PSF characterization and resolution analysis. The reported PSFs appear representative, though the treatment of measurement variability and uncertainty could be described in more detail. For the biological imaging results, the figures effectively illustrate qualitative performance, but the manuscript would benefit from clearer reporting of sample-to-sample variability or quantitative measures of consistency across preparations. While extensive statistical analysis is not essential for this type of methodological paper, a brief discussion of uncertainties, both in optical measurements and in biological demonstrations, would strengthen the rigor and transparency of the work.

E. The conclusions drawn in the manuscript are generally robust and well supported by the presented data. The combination of optical simulations, PSF measurements, and diverse biological demonstrations provides a solid foundation for the authors' claims regarding the performance and versatility of the HySIL framework. The system appears reliable across a wide range of refractive indices and sample preparation methods, and the biological imaging results consistently reflect the expected improvements in resolution and aberration correction. While the overall validity of the approach is strong, the robustness of the Super-SCOPE configuration would be further reinforced by including direct experimental PSF measurements. Nonetheless, the conclusions are coherent, appropriately framed, and aligned with the evidence provided.

F. Suggested improvements: experiments, data for possible revision
1) Direct PSF measurements for Super-SCOPE would substantially reinforce the performance claims of this configuration. The current manuscript includes experimental PSFs only for SCOPE, leaving the Super-SCOPE characterization supported primarily by simulations. A measured PSF would provide a more complete and balanced validation. Generally validation of Super-SCOPE is much less present in the paper comparing to SCOPE and might be further improved, or alternatively - Super-SCOPE might be skipped in order to simplify the paper for readers.
2) Although the distinction between SCOPE and Super-SCOPE is described, a short summary table or schematic might help readers grasp the differences more quickly - supposing that paper still wants to talk about both SCOPE and Super-SCOPE.
3)Additional benchmarking against a commercial high-NA immersion objective might help contextualize the practical performance gains of the HySIL approach, especially for readers less familiar with the limitations of long working distance air objectives.
4) A brief comparison between HySIL and classical solid immersion lenses would help to introduce the novelty of the approach.
5) Additional information on alignment tolerances, chamber assembly, and the robustness of the hybrid solid–liquid interface would benefit users aiming to adopt the method.
6) Benchmarking against commercial high-NA immersion objectives could further clarify the performance gains.
7) some image are missing scale bars and this should be improved

G. References: appropriate credit to previous work?
The manuscript cites relevant and up-to-date literature across light sheet microscopy, tissue clearing, expansion microscopy, and solid immersion lens design. Foundational work is appropriately acknowledged, and the authors position their contribution within the existing landscape in a balanced way.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
The abstract and summary are clear, well structured, and accurately reflect the scope and contributions of the work. They effectively communicate the motivation, the core innovation of the HySIL framework, and the main experimental findings without overstating the claims. The introduction provides appropriate context, clearly articulating the limitations of existing approaches and the need for a method that enables high resolution imaging across diverse refractive indices and large

sample volumes. It situates the work within the broader landscape of light sheet microscopy and solid immersion lens design in a way that is accessible to both optical engineers and biological users.

Author Rebuttal letter:

Response to Reviewers
We thank the reviewers for the careful evaluation, highly constructive feedback, and their valuable volunteer time. Guided by the reviewers' feedback, we have substantially revised the manuscript to improve its clarity, rigor, and positioning. The main additions and revisions include:
Expanded comparison with prior approaches (SIMlens and related methods), including substantial text updates, a conceptual schematic (Supplementary Fig. 2a,b), and a detailed comparison table (Supplementary Table 1)
Additional simulations and analyses, including comparisons across multiple lens geometries (plano-convex, biconvex, and meniscus lenses) within the HySIL framework and prior approaches, as well as off-axis (Strehl ratio-based) field-of-view characterization (Supplementary Fig. 2c–f)
New experimental validations, including PSF measurements for Super-SCOPE (Supplementary Fig. 3), extended light-sheet PSF characterization (updated Fig. 2d), and benchmarking against high-NA immersion objectives on the same sample (Supplementary Fig. 4)
Improved discussion of practical implementation, including alignment tolerance, temperature stability (Supplementary Fig. 2e), and system integration
Quantitative estimation of expansion factor in salamander brain expansion microscopy (Supplementary Fig. 5)
Expanded discussion of biological relevance and applications in Discussion

All substantive changes are highlighted in dark red font in the revised manuscript.

Reviewer #1 (Remarks to the Author):
OVERALL ASSESSMENT
Recommendation: Major Revision Required
This manuscript presents high-quality work that addresses a fundamental limitation in light-sheet microscopy through an elegant optical solution.
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major revision to adequately address its relationship to closely related prior work (specifically meniscus lens approaches) and to clarify several
technical aspects before publication in Nature Biotechnology.
The authors develop SCOPE and Super-SCOPE imaging devices based on a hybrid solid-liquid optical framework that transforms inexpensive,
long-working-distance air objectives into high-resolution, aberration-free detection systems for light-sheet microscopy. The approach pairs a solid
optical element (plano-convex lens) with precisely refractive index (RI)-matched liquid to create a hemispherical immersion system that: corrects
spherical aberrations when imaging through high-RI clearing media, increases effective detection NA by n (SCOPE) or n^2 (Super-SCOPE),
maintains compatibility across diverse immersion media (RI 1.33-1.56), and preserves long working distance (~34 mm) and low cost of air
objectives.
The authors validate SCOPE through optical simulations, PSF measurements, and demonstrate broad applications including expansion microscopy,
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The HySIL concept represents a genuine advance beyond classical solid immersion lenses (SILs). Unlike conventional SILs that are limited to
surface-proximal imaging, HySIL decouples the solid lens from both the sample and objective, enabling deep volumetric imaging. The solid-liquid
hybrid architecture is elegant and generalizable beyond the specific implementations shown. The work tackles a real limitation in the field: achieving
submicron resolution over centimeter-scale volumes without expensive, short-working-distance immersion objectives. This constraint has
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inexpensive, off-the-shelf components (standard plano-convex lenses, commodity air objectives) combined with modular design that integrates
with existing platforms represents a genuine advance in accessibility. The cost reduction is orders of magnitude compared to

high-NA immersion
objectives.
Major concerns requiring revision:
1. No proper comparison with existing techniques (meniscus lens)
The authors cite Barner et al.'s Solid Immersion Meniscus Lens (SIMlens, Opt. Lett. 2019) but provide only superficial discussion, dismissing it
along with other approaches as 'system-specific' or requiring 'custom engineering.' This is inadequate given the conceptual similarity between
SIMlens and SCOPE. Both approaches: Use curved optical elements at the imaging chamber window, aim to correct spherical aberrations for air
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Critical missing information:
- Why is hemispherical geometry superior to meniscus geometry? Is there a theoretical optical advantage, or is it simply easier to
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matched liquid?
- How do resolution, field-of-view, aberration correction, and cost compare directly between approaches?
- Are standard plano-convex lenses truly more accessible than meniscus lenses? What is the actual cost/availability difference?
Suggested revision:
- Add a dedicated comparison section that explicitly contrasts SCOPE with SIMlens and other aberration correction approaches
- Include a comparison table covering: optical geometry, materials, cost, complexity, multi-immersion capability, FOV, resolution, working distance,
and appropriate use cases
- Ideally, provide optical simulations comparing meniscus vs. hemispherical geometries under matched conditions
- Acknowledge the conceptual overlap while clearly articulating SCOPE's specific advances beyond existing approaches
- Include the recent paper in Nature Biotech. by Aakhte et al. in the discussion
- If experimental comparison is feasible, direct testing on the same sample would significantly strengthen claims
Without this comparison, readers cannot properly evaluate the novelty and practical advantages of SCOPE relative to existing solutions.

We thank the reviewer for this important and detailed critique. We agree that a clear, quantitative comparison with meniscus lens-based approaches is essential, and have revised the manuscript accordingly.
In summary, we now include:
- expanded discussion in the Introduction, Results, and Discussion sections (in dark red font) and a conceptual schematic comparison (Supplementary Fig. 2a–b)
- new results (Supplementary Fig. 2) demonstrating HySIL as a generalizable optical framework, with compatibility across multiple lens types (plano-convex, biconvex, and meniscus, among others)
- implementation and experimental PSF validation of the Super-SCOPE configuration (Supplementary Fig. 3), explicit clarification of current limitations, and positioning as an example of the flexibility of the HySIL framework in uniquely enabling Weierstrass super-hemispherical detection
- a comprehensive quantitative comparison with meniscus-based approaches (Supplementary Table 1).

In detail:
1. Conceptual distinction from meniscus-based approaches
In meniscus-based systems (e.g., SIMlens and Aakhte et al., 2025), the meniscus lens acts as a wavefront-matching element placed between the objective and the immersion medium. Its two curved surfaces are designed (or selected, for off-the-shelf lens) to minimize refraction at refractive-index boundaries. This approach requires precise refractive-index matching among the sample, holder, and immersion medium, while the lens material (typically BK7) differs from the surrounding medium.
Such systems have been adapted to different sample refractive indices by tuning the holder and immersion medium to match the sample RI. For example, Barner et al. (2019, 2020) and Glaser et al. (2022) used custom-designed SIMlens elements with curvature centers aligned to the focal point, whereas Aakhte et al. (2025) demonstrated an off-the-shelf meniscus lens combined with a concave mirror to generate light sheets as thin as ~0.85 μm using an air objective.
In contrast, the HySIL framework adopts a fundamentally different and more generalizable refractive architecture. In HySIL, the solid optical element (which can take various lens forms with at least one curved surface) is refractive-index–matched to the chamber liquid, forming a single, continuous hybrid optical medium with only one remaining refractive interface - the curved surface facing the air objective. Samples spanning a wide range of refractive indices are mounted in cuvettes, with the cuvette glass matched to the

chamber medium. This design effectively decouples the sample and its immersion medium from the chamber optics, enabling greater flexibility across imaging conditions.
This architecture provides key advantages over meniscus-based approaches:
- substantially greater design flexibility as it is not restricted to a specific lens geometry (like meniscus) and performs equivalently with plano-convex, biconvex, and meniscus lenses (Supplementary Fig. 2) - indeed, any lens with a curved surface of appropriate radius.
- improved resolution, field of view, and aberration correction compared to meniscus (Supplementary Table 1)
- enables innovations beyond conventional SIL-like configurations, such as super-hemispherical geometries (Super-SCOPE; Supplementary Fig. 3) and more.

Overall, HySIL represents a flexible refractive design framework, enabling improved aberration correction, increased resolution and signal collection, while preserving the advantages of air objectives (long working distance, low cost, and robust chromatic correction).
To clarify this distinction, we now include a schematic comparison (Supplementary Fig. 2a–b, reproduced below) and a quantitative comparison of performance (Supplementary Table 1).

2. Demonstration of generalizability of the HySIL framework
HySIL is not restricted to a specific lens geometry. We demonstrate this flexibility through simulations using multiple off-the-shelf geometries (plano-convex, biconvex, and meniscus lenses), all achieving similarly high performance within the HySIL framework (Supplementary Fig. 2c-d).
Importantly, this general framework enables systematic exploration of advanced geometries beyond conventional SIL-like implementations. As an example, we introduced the design and implementation of Super-SCOPE configuration based on super-hemispherical (Weierstrass) geometry. Although not fully optimized in this work, both simulations and new experimental PSF measurements (Supplementary Fig. 3) support the feasibility and enhance performance (in some parameters) of such extensions.

3. Improved resolution, field of view, and aberration correction relative to meniscus-based approaches
We have included a detailed comparison of the performance of HySIL with prior meniscus-based studies (Supplementary Table 1). These comparisons show that, under comparable conditions, HySIL approach provides improved resolution, field of view, and enhanced aberration corrections. For example:
- Resolution & FOV: Barner et al. reported ~1.09 µm (x), 0.68 µm (y) (anisotropic in x and y) lateral resolutions using a 20x/0.40 NA detection air objective. In contrast, HySIL achieves ~0.75 µm lateral resolution using a much lower-NA 10x/0.28 objective (and ~0.56 µm, if using Super-SCOPE config with the same objective). The FOV is also larger in HySIL, 0.74 mm (with Strehl ratio > 0.8), compared to 0.4 mm in SIMlens for those objectives. The contrast becomes even more striking if we assess useful FOV to be exceeding the expected air-equivalent diffraction limited FWHM, yielding about 1.2 mm. For 5x/0.15NA detection objectives, Barner et al. report 2.6 microns (x) and 1.7 microns (y), which is worse than HySIL performance of ~1.2 µm using a lower NA (5x/0.14NA) objective.
Note that, these previous SIMlens studies did not characterize wide-field axial FWHM to allow a comparison with this work.
- Spherical and chromatic aberrations: Prior SIMlens studies do not report quantitative assessments of spherical aberration or chromatic alignment. In contrast, HySIL provides detailed characterization (e.g., Supplementary Fig. 2d reports W040 aberration coefficients), along with explicit demonstrations of chromatic alignment. In the absence of such analyses in prior work, the reported lower resolution may indicate less effective aberration correction compared to the HySIL approach.
- Off-the-shelf meniscus comparison: Aakhte et al. demonstrated the use of an off-the-shelf meniscus lens for light-sheet illumination, achieving submicron thickness through the use of a concave mirror in the illumination path. We performed Zemax simulations comparing detection with the same off-the-shelf lens used conventionally versus within the HySIL framework. As shown in Supplementary Fig. 2c-d, HySIL configurations achieve higher Strehl ratios over a larger field of view, consistent with observations in Aakhte et al.

4. Broad sample compatibility
HySIL enables broad compatibility across sample preparation conditions without requiring any changes in optical hardware or chamber or immersion oil, as demonstrated through technical characterizations and imaging of a wide range of biological samples prepared with different methods.

5. Advantages of off-the-shelf implementation
We also highlight that HySIL is compatible with a wide range of off-the-shelf lenses of varying shapes and types. This flexibility:
- enables simpler system integration and broader accessibility;

substantially reduces cost, supporting scalable adoption (e.g., integration with compact, ultra-low-cost pLSM platforms);
facilitates flexible exploration and optimization of optical designs to achieve specific performance goals (e.g., Super-SCOPE).

In summary, we clarified that HySIL is best understood as a flexible optical design framework, rather than a single implementation. It:
supports multiple lens geometries (plano-convex, biconvex, meniscus, and others);
enables effective NA enhancement and aberration reduction with long-working-distance air objectives;
allows extension to higher-performance configurations (e.g., Super-SCOPE); and
decouples optical performance from system-specific constraints.

2. Incomplete Characterization of Super-SCOPE
The manuscript introduces Super-SCOPE (Weierstrass super-hemispherical geometry) as providing enhanced resolution with n^2 NA scaling but
provides limited experimental validation:
- Figure 1e-g shows simulations, but no experimental PSF data for Super-SCOPE is provided
- Figure 2e demonstrates chromatic alignment for SCOPE but not Super-SCOPE
- The text acknowledges 'tighter optical tolerances and narrower detection spectral bandwidth' but doesn't quantify these limitations
- No biological imaging examples using Super-SCOPE are shown
Suggested addition:
- Provide experimental PSF characterization across wavelengths for Super-SCOPE
- Quantify chromatic aberration limits and acceptable spectral bandwidth
- Include decision criteria for when Super-SCOPE is worth the additional constraints vs. standard SCOPE
- If Super-SCOPE is not practically useful with current implementations, state this clearly

We thank the reviewer for this important and constructive critique and agree on the need for improved validation of the Super-SCOPE configuration, as well as clearer acknowledgment of its current limitations. We performed further experimental PSF measurements for the Super-SCOPE configuration using the 10x/0.28 NA, 34 mm WD air objective and gold nanoparticles (prepared identically to the SCOPE wide-field PSF measurements; Supplementary Fig. 3). These results confirm the expected resolution enhancement of the Weierstrass super-hemispherical geometry, achieving ~0.56 μm lateral resolution compared to ~0.75 μm with the standard SCOPE configuration using the same objective, consistent with the expected n^2 scaling of the effective numerical aperture.
We have also clarified the current limitations of this implementation in the manuscript text. Current design of Super-SCOPE is more sensitive to misalignment and less tolerant to variations in optical properties, which limits its robustness and spectral bandwidth in practice. Accordingly, further development will be required to improve stability and multi-color performance. At present, Super-SCOPE is best suited for applications that prioritize maximum spatial resolution under well-controlled, single-color conditions, whereas standard SCOPE remains a more robust and broadly applicable solution for multi-color, large-volume imaging across diverse sample conditions.

3. Resolution Claims and Expansion Microscopy Validation
The effective pre-expansion resolution claim of ~190 nm (Fig. 3, line 211) is based on measured 0.75 μm PSF and assumed 4× expansion factor.
However:
- No direct measurement of expansion factor in the imaged regions is provided
- Expansion non-uniformity is a known issue in ExM and could significantly affect resolution estimates
- For the salamander brain (Fig. 4), expansion factor validation is not mentioned
Suggested addition:
- Provide local expansion factor measurements in imaged tissue regions (e.g., using fiducial markers or anatomical landmarks)
- Discuss expansion uniformity and its impact on resolution estimates
- If expansion varies significantly, provide resolution ranges rather than single values
We thank the reviewer for raising this important point. We agree that expansion microscopy presents often overlooked challenges in accurately estimating expansion factors and their uniformity across tissues. Our primary goal in this study was to demonstrate compatibility of the SCOPE imaging platform with expansion microscopy workflows, rather than to make precise claims about absolute resolution. To avoid overinterpretation, we have revised the manuscript to remove the pre-expansion resolution estimate for the mouse brain data that relied on an assumed expansion factor.
At the same time, since this is the first application of expansion microscopy to salamander brain tissue, we performed quantitative measurements to estimate both the effective expansion factor and its uniformity under our experimental conditions (Supplementary Fig. 5). Specifically, we compared neuronal soma morphology across multiple brain regions between expanded and non-expanded tissues, showing ~2.5-fold uniform expansion and preservation of cellular morphology, as indicated by consistent soma dimensions

and ellipticity. The manuscript has been updated accordingly.

ADDITIONAL TECHNICAL CONCERNS

4. Limited Field-of-View Discussion
SCOPE achieves excellent performance over limited FOV (1.2 mm for 10×, 2.7 mm for 5×). For whole-organ imaging (e.g., centimeter-scale mouse
brain), extensive tiling is required. The manuscript should address:
- Stitching accuracy and reliability across large tile arrays
- Throughput implications - approximate imaging time for representative samples
- Data management challenges for large stitched datasets
- Whether FOV limitations could be addressed with different lens geometries within the HySIL framework

We thank the reviewer for raising these important points of practical relevance. We have now expanded the Discussion section to address these aspects.
In addition, since HySIL is implemented as an imaging add-on within the previously published pLSM framework (Chen et al, 2024, Nature Biomed. Engg.), our preference would be to refer the reader to that work for a more detailed discussion of stitching, data throughput, and data management considerations, in order to avoid redundancy. Importantly, the SCOPE/HySIL framework is agnostic to the underlying imaging system and therefore inherits the performance characteristics and scalability of the platform with which it is integrated.

5. Direct Performance Comparison to Immersion Objectives
While PSF measurements are excellent, direct image quality comparisons to conventional immersion objectives are limited. A side-by-side
comparison on the same sample (even if limited to the immersion objective's working distance) would strengthen claims about comparable
performance at orders-of-magnitude lower cost.
Suggested addition:
- Add supplementary figure comparing SCOPE vs. high-NA immersion objective on identical tissue region
- Include quantitative metrics: SNR, contrast, resolution in biological tissue

We thank the reviewer for this important suggestion. We agree that direct comparison with a high-NA objective is valuable for contextualizing performance.
We have therefore included a side-by-side comparison of the same sample imaged using SCOPE and a conventional high-NA immersion detection system (Supplementary Fig. 4). Specifically, we compared CLARITY-cleared Thy1-GFP mouse brain tissue imaged with the SCOPE-enabled pLSM system (10x/0.28 NA air objective) and a previously established CLARITY-optimized light-sheet microscope (COLM) equipped with a cleared-tissue-optimized 10x/0.6 NA, 8 mm WD immersion objective.
This comparison shows that SCOPE achieves comparable signal levels, signal-to-noise ratios, and resolution of fine neuronal structures relative to the high-NA immersion objective (Supplementary Fig. 4), while also providing substantially greater (~4-fold) working distance, simpler system integration, and significant cost advantages.

6. Practical Implementation Details
Several practical considerations need clarification:
Sample alignment sensitivity: How critical is precise positioning of the sample relative to the hemispherical surface? What are acceptable
tolerances?
Temperature stability: RI matching at ~1.46 likely requires temperature control. What temperature variations are acceptable? How is this managed?
Interface maintenance: How is the solid-liquid interface cleaned between samples? How often does the immersion oil need replacement?
Live imaging compatibility: Can SCOPE be used for live specimens or only fixed/cleared samples? If live imaging is possible, what are the
constraints?
A practical troubleshooting guide in supplementary materials would significantly enhance utility.

We thank the reviewer for highlighting these important practical considerations. We agree that implementation details are critical for broader adoption and have further expanded the manuscript text accordingly. We also aim to establish a dedicated GitHub repository with step-by-step protocols, design files, troubleshooting guidance, and updates to support community use and further development of the

SCOPE/HySIL framework.
We have clarified the following points in the manuscript:
Optimal positioning: Best performance is achieved when the imaging region is located near the designed optical condition (approximately one radius of curvature from the curved interface). In practice, the system tolerates small deviations (on the order of a few hundred micrometers) without noticeable loss of image quality.
Temperature stability: Although refractive index matching is temperature-dependent, typical laboratory variations (~20-25 °C) result in only minor changes ($dn/dT \approx -0.000365$ over 15-35 °C for the oil used), with negligible impact on imaging performance, as supported by simulations (Supplementary Fig. 2e).
Maintenance: The solid–liquid interface is maintained using standard optical handling procedures. The chamber and lens are cleaned with lens tissue and ethanol after use, and immersion oil is replaced only if visibly contaminated. No specialized maintenance is required beyond standard microscopy practice.

Although demonstrated here with fixed, cleared, and expanded samples, the HySIL framework can be adapted for live imaging due to its tolerance to refractive-index variation. This could be further optimized using lower-refractive-index materials (e.g., MgF_2, RI ≈ 1.38; Thorlabs Cat# LA6004) together with matched immersion media from Cargille labs.

MINOR ISSUES AND SUGGESTIONS
7. Figure Organization and Clarity
- Figure 1 is dense and covers both theoretical foundations (c-g) and practical implementation (h). Consider splitting into two figures: (1) optical
theory and simulations, (2) practical device design and integration.
8. Biological Impact and Novelty
The biological examples are largely proof-of-concept demonstrations rather than hypothesis-driven studies. While appropriate for a methods paper,
the Discussion could be strengthened by:
- Identifying specific biological/clinical problems where SCOPE's unique capabilities (large volume + high resolution + multi-immersion) are
essential
- Discussing what types of biological questions become newly addressable
9. Technical Clarifications
- Line 82: 'effective detection NA (scaled by n and n^2 for SCOPE and Super-SCOPE, respectively)' - This key claim deserves more physical
explanation in the main text
- Provide estimated imaging times for representative samples to help readers assess throughput
- Include cost breakdown comparing SCOPE-enabled system to traditional high-NA immersion objective approaches

We thank the reviewer for these helpful suggestions and have revised the manuscript to improve clarity, contextual impact, and technical presentation.
We agree that Figure 1 combines both theoretical and implementation aspects. We chose to present a unified figure to provide a continuous narrative from optical principles to practical realization, which we believe improves accessibility for a broad readership. However, we would be happy to separate the figure if preferred by the reviewer or editor.
We have expanded the Discussion to better highlight biological and clinical applications where SCOPE's combined capabilities (large imaging volume, submicron resolution, and multi-immersion compatibility) offer clear advantages.
We have further clarified the effective NA scaling in the main text, including its physical basis in refractive index–mediated angular compression and the additional scaling introduced by the super-hemispherical (Weierstrass) geometry. We also refer to Fig. 1 (c-e), which schematically illustrates these concepts, along with citations to foundational and review literature.
Regarding cost, SCOPE itself requires only an off-the-shelf lens and is implemented within the previously published pLSM framework. To avoid redundancy, we refer readers to that work for detailed system-level cost analysis. As SCOPE is system-agnostic and readily integrates with different platforms, the overall cost is primarily determined by the underlying imaging system.

Reviewer #2 (Remarks to the Author):
Gong et al. present a solid immersion lens (SIL)-based optical design for high-resolution microscopic imaging through low-NA air objectives. They
first characterize optical performance for two lens geometries (SCOPE and Super-SCOPE) using Zemax simulations, confirming reduced spherical
aberrations and enhanced resolution on-axis. They emphasize the tolerance of the SCOPE approach with respect to lens-medium refractive index
(RI) mismatches, effectively allowing an RI range between water (1.33) and oils (up to 1.54). They then experimentally test the SCOPE approach
using two 34-mm working distance air objectives (5x and 10x) to demonstrate sub-micron resolution imaging within large imaging volumes (several

mm to cm side length). In combination with a light-sheet microscopy platform they apply the SCOPE approach for imaging demonstrations using
various samples, including expanded tissue sections, samples cleared with different protocols, and histological samples from human breast cancer.
They put forward their approach as ‘practical and cost-effective foundation for next-generation volumetric microscopy’.
The SIL-based optical method for advancing high-resolution, large-volume imaging using light-sheet microscopy is an interesting approach that
indeed may offer low-cost improvements when using low-NA air objectives. The example SCOPE applications presented in the manuscript provide
nice imaging data demonstrating this potential for improvements. The main issue with the manuscript is, however, that the novelty of the presented
approach remains unclear, as very similar approaches have already been published. In addition, the system characterization is incomplete, with off-
axis evaluation in the simulations as well as full PSF quantification for the light-sheet imaging data missing. It also remains vague, without any
specific further investigation, in how far the authors see their approach as going beyond previous approaches and establishing a ‘general optical
framework’ (line 68). In the following we elaborate on several of these issues in our specific comments.
1. Variations of the SIL technique have been known in light-sheet microscopy field (e.g., refs 13, 19 both from 2019), and their use was NOT limited
to surface imaging. For example, [ref 13] described the SIL concept (SIMlens) and applied it to long-WD air objectives (see their Figs. 2 and 3).
Given these previous demonstrations, the following statement seems unsubstantiated (line 107): “However, unlike conventional SILs, which are
inherently limited to surface-proximal imaging (Fig. 1c), HySIL decouples the solid lens from both the sample and the objective, enabling
aberration-free imaging throughout large, cleared specimens”. It remains the question: What is exactly the difference between SIMlens [ref 13] and
HySIL?
We thank the reviewer for the careful evaluation of our manuscript and for the constructive and detailed feedback. We appreciate the recognition of the potential of this approach and agree that clarifying novelty, strengthening system characterization, and improving comparisons to prior work are important. The manuscript has been substantially revised accordingly. To avoid redundancy, we refer to our detailed response to a similar comment from Reviewer #1 above.
In brief, we have substantially expanded the Introduction, Results, and Discussion to provide explicit comparisons between HySIL and SIMlens-related approaches. We also include a conceptual schematic (Supplementary Fig. 2a,b) and a detailed comparison table (Supplementary Table 1).
We further clarify that, whereas SIMlens uses a curved optical interface as a wavefront-matching element, HySIL relies on refractive-index matching between the solid optical element and the immersion medium to create a continuous optical interface, fundamentally altering the optical architecture. This enables improved robustness to refractive-index mismatch, greater flexibility in lens geometry, enhanced resolution, field of view, and aberration correction, and the ability to extend to advanced configurations such as Super-SCOPE.

2. Figure 1e,f and Suppl. Fig 1a show Huygens PSF on-axis. This can depart, however, substantially from the PSF off-axis for a reasonable field-
of-view (FOV) size, which therefore needs to be assessed. In own simulations that we performed in Zemax for the 5x air objective (NA 0.28), we
found that the Strehl ratio falls below the diffraction limited performance criterion (Strehl 0.8) already at field position 700 µm (Strehl 0.67) and
that diffraction-limited performance is limited to 0.5 mm field point, i.e., 1 mm total FOV. The authors should add PSF off-axis characterization for
both the 5x and 10x objective and report the diffraction-limited FOV sizes.
3. For the experimental validation, a FOV of 1 mm is shown in Figure 2c for the Mitutoyo 10x/0.28 objective. That is quite small and one would
rather expect assessment of a 2-mm FOV for this magnification to efficiently use modern sCMOS cameras (sensor width around 20 mm). A FOV
of 2.6 mm is mentioned in Suppl. Fig. 1d but not quantified.
4. The quantification of PSFs for the light-sheet application in Figure 2 is incomplete as the quantification of the lateral PSF width (on-axis and off-
axis) is missing. It may have been skipped because relatively large (1 micron-sized) fluorescent beads apparently were used. A full PSF
characterization with sub-resolution fluorescent beads should be presented, not least because all subsequent imaging demonstrations were done
with the light-sheet microscope system.
5. Overall, based on the presented data and our own simulation, we find that HySIL offers limited functionality for imaging cleared tissues with
modern sensors (diagonal of 22-30 mm) and low-magnification objectives (5x-10x), although it may be useful with higher magnifications (20-50x)

if the working distance of the respective objectives is sufficient.
6. Minor: For Figs. 3 and 4 the specification of the depth-color coding range is missing.

We agree that off-axis performance is an important consideration. We have now included Strehl ratio–based analysis across the field of view (Supplementary Fig. 2), demonstrating diffraction-limited performance (Strehl ≥ 0.8) over a 1.7 mm FOV for the 5x/0.14 NA objective (with ~1.2 µm lateral resolution) and 0.74 mm for the 10x/0.28 NA objective (with <0.75 µm lateral resolution). These values are broadly consistent with the reviewer's simulations. (The reviewer reports a somewhat larger diffraction-limited FOV for the 0.28 NA case. This may potentially be because 0.28 NA is the nominal NA for detection in air, and either needs to be scaled by n (1.46) or the aperture stop diameter corresponding to air should be used in the SCOPE configuration.)
Experimental FWHM measurements (Fig. 2c) further show that lateral FWHM remains better than the air-based diffraction limit over ~2.5 mm (5x) and ~1.2 mm (10x) fields. We now clarify in the Results that this represents the usable FOV with maintained image quality, whereas the diffraction-limited FOV is defined separately using the Strehl ≥ 0.8 criterion. Larger volumes are acquired via tiled imaging.
A detailed comparison with prior approaches is now provided in Supplementary Table 1, highlighting improved performance in resolution, field of view, and aberration correction under similar conditions.

We further note that FOV is inversely related to the detection NA. For example, comparing the 5x/0.14 NA (yielding ~1.2 µm lateral resolution) and 10x/0.28 NA (yielding ~0.75 µm lateral resolution) objectives, halving the nominal NA results in more than a twofold increase in diffraction-limited FOV (from ~0.74 mm to ~1.7 mm; ~2.3-fold improvement). Therefore, if needed, even larger diffraction-limited FOVs can be achieved by using slightly lower-NA air objectives while still maintaining excellent (<2 µm) lateral resolution. (For example, we simulated detection with 0.1 NA in air, yielding a ~2.16 mm diffraction-limited FOV, with lateral resolution still around 2 µm, i.e., at cellular resolution.)

For additional context for FOV usefulness, we note a recent elegant study from the Huisken lab (Aakhte et al., Nature Biotechnology, 2025), which reports a usable FOV of ~0.8 mm at ~0.85 µm resolution. In that work, detection was performed using a high-performance Special Optics/ASI multi-immersion objective (17x/0.4 NA, 12.1 mm WD), which is substantially more expensive (~$17k; Supplementary Table in Aakhte et al., compared to <$1k here) and provides a shorter working distance (12.1 mm vs. 34 mm).
In comparison, our SCOPE implementation enables the use of a Mitutoyo 10x/0.28 NA, 34 mm WD air objective, achieving <0.75 µm lateral resolution over a usable ~1.2 mm field, while providing >2.5-fold longer working distance and simpler integration without the need for sealed objective insertion.

Overall, we fully agree with the reviewer that further expansion of FOV while maintaining submicron resolution would be valuable for many experimental scenarios. The flexibility of the HySIL framework provides a clear path for future optimization toward this goal while preserving low cost, long working distance, system simplicity and scalability.

For light-sheet PSF characterization, we have updated Fig. 2d to include both lateral and axial FWHM analysis using 200 nm fluorescent beads across the field of view, providing a complete characterization of light-sheet detection performance consistent with the wide-field measurements. This is now stated explicitly in the Results section.

We have also added the depth color scale bars to Figs. 3 and 4.

Reviewer #3 (Remarks to the Author):

A. Summary of the key results
The manuscript addresses one of the challenges in modern light microscopy: the difficulty of imaging large, cleared samples with inexpensive air
objectives while still achieving aberration-free, subcellular or submicron resolution. The authors show that this limitation can be overcome through
a hybrid solid–liquid optical framework (HySIL), implemented as SCOPE and Super-SCOPE. These devices enable submicron-resolution,
aberration-free light-sheet microscopy using low-cost, long-working-distance air objectives, substantially enhancing effective numerical aperture
and correcting spherical aberrations across a wide range of refractive indices. Their approach supports centimeter-scale

imaging and is validated
through optical simulations, PSF measurements, and diverse biological demonstrations, including expansion microscopy, multiple tissue clearing
methods, organoid imaging, and 3D histopathology. Overall, the work offers a compelling and accessible solution for scalable, high resolution
volumetric imaging at the centimetre scale, and in my view the imaging community would benefit greatly from learning about it.

B. Originality and significance:
This work presents an original and impactful contribution to the field of volumetric microscopy. The HySIL framework introduces a hybrid solid–
liquid optical strategy that differs fundamentally from classical solid immersion lenses and from existing approaches to refractive-index-matched
imaging. By enabling submicron resolution, aberration free imaging with inexpensive long working distance air objectives, the work opens a new
design space for accessible high NA light sheet microscopy. The ability to maintain high image quality across a wide range of refractive indices
and sample preparation methods further underscores the practical significance of the approach. The combination of conceptual novelty, technical
elegance, and broad applicability positions this work to have a meaningful and lasting impact on the imaging community.

C. Data & methodology: validity of approach, quality of data, quality of presentation
The methodological framework is sound and well aligned with the goals of the study. The combination of optical simulations, PSF measurements,
and biological imaging provides a coherent and logically structured validation pipeline. The optical modeling is appropriate for the questions being
addressed, and the experimental PSF data are of high quality and clearly demonstrate the expected performance improvements. The biological
datasets—including expanded tissues, cleared samples across multiple refractive indices, organoids, and human specimens—are well chosen and
effectively illustrate the versatility of the system.
The quality of data presentation is strong: figures are clear, well annotated, and appropriately scaled to highlight both optical performance and
biological detail. The manuscript communicates the methodology in a transparent and accessible manner, enabling readers to understand the
experimental workflow and the rationale behind key design choices. A few additional details such as alignment tolerances and robustness of the
hybrid solid–liquid interface would further enhance clarity, but overall the data and methodology are presented at a high standard.

D. The manuscript makes appropriate use of quantitative metrics for evaluating optical performance, particularly in the PSF characterization and
resolution analysis. The reported PSFs appear representative, though the treatment of measurement variability and uncertainty could be described
in more detail. For the biological imaging results, the figures effectively illustrate qualitative performance, but the manuscript would benefit from
clearer reporting of sample-to-sample variability or quantitative measures of consistency across preparations. While extensive statistical analysis is
not essential for this type of methodological paper, a brief discussion of uncertainties, both in optical measurements and in biological
demonstrations, would strengthen the rigor and transparency of the work.

E. The conclusions drawn in the manuscript are generally robust and well supported by the presented data. The combination of optical simulations,
PSF measurements, and diverse biological demonstrations provides a solid foundation for the authors' claims regarding the performance and
versatility of the HySIL framework. The system appears reliable across a wide range of refractive indices and sample preparation methods, and the
biological imaging results consistently reflect the expected improvements in resolution and aberration correction. While the overall validity of the
approach is strong, the robustness of the Super-SCOPE configuration would be further reinforced by including direct experimental PSF
measurements. Nonetheless, the conclusions are coherent, appropriately framed, and aligned with the evidence provided.

F. Suggested improvements: experiments, data for possible revision
1) Direct PSF measurements for Super-SCOPE would substantially reinforce the performance claims of this configuration.

The current manuscript
includes experimental PSFs only for SCOPE, leaving the Super-SCOPE characterization supported primarily by simulations. A measured PSF
would provide a more complete and balanced validation. Generally validation of Super-SCOPE is much less present in the paper comparing to
SCOPE and might be further improved, or alternatively - Super-SCOPE might be skipped in order to simplify the paper for readers.
2) Although the distinction between SCOPE and Super-SCOPE is described, a short summary table or schematic might help readers grasp the
differences more quickly - supposing that paper still wants to talk about both SCOPE and Super-SCOPE.
3)Additional benchmarking against a commercial high-NA immersion objective might help contextualize the practical performance gains of the
HySIL approach, especially for readers less familiar with the limitations of long working distance air objectives.
4) A brief comparison between HySIL and classical solid immersion lenses would help to introduce the novelty of the approach.
5) Additional information on alignment tolerances, chamber assembly, and the robustness of the hybrid solid–liquid interface would benefit users
aiming to adopt the method.
6) Benchmarking against commercial high-NA immersion objectives could further clarify the performance gains.
7) some image are missing scale bars and this should be improved

We thank the reviewer for the thorough, constructive, and highly positive evaluation of our work. We appreciate the recognition of the conceptual novelty, technical rigor, and practical significance of the HySIL framework, as well as the helpful suggestions to further strengthen clarity, validation, and usability. We have revised the manuscript accordingly, as detailed below.

F1. Experimental validation of Super-SCOPE

We agree that direct experimental validation of Super-SCOPE is important. In response, we have now included experimental PSF measurements of the Super-SCOPE configuration (Supplementary Fig. 3). These measurements confirm the expected resolution enhancement (~0.56 µm lateral) associated with the Weierstrass geometry. We have also clarified the current limitations of this configuration, including increased sensitivity to alignment and optical variations and reduced tolerance to chromatic variation. In the revised manuscript, we position Super-SCOPE as a proof-of-principle extension of the HySIL framework that is not yet a fully optimized configuration for routine imaging, and as best suited in its current form for high-resolution imaging under well-controlled, primarily single-color conditions.

F2. Clarification of SCOPE vs Super-SCOPE

We agree that clearer differentiation is beneficial. We have added a concise description in the main text outlining the trade-offs between SCOPE and Super-SCOPE, emphasizing that Super-SCOPE enables higher resolution under controlled conditions, whereas SCOPE provides a more robust and broadly applicable configuration. These distinctions are now reflected in the PSF characterization (Fig. 2, Supplementary Fig. 3) and in the comparison table (Supplementary Table 1).

F3 & F6. Benchmarking against immersion objectives

We agree that direct benchmarking is valuable. We have therefore included a side-by-side comparison on the same sample and imaging region (Supplementary Fig. 4), comparing SCOPE (10x/0.28 NA air objective) with a high-NA immersion objective (10x/0.6 NA). This comparison demonstrates comparable image quality in biological tissue, including similar signal levels, contrast, and resolution of fine structures, while SCOPE provides substantially longer working distance (~34 mm vs. ~8 mm), lower cost, and simpler system integration.

F4. Comparison with classical solid immersion lenses

We have expanded the Introduction and Discussion to more clearly position HySIL relative to classical SIL approaches. In particular, we clarify that classical SILs typically require direct optical contact and are limited to surface-proximal imaging, whereas HySIL extends this concept to a hybrid solid–liquid architecture enabling volumetric imaging of large specimens.

F5. Practical implementation details

We have added additional details in the Methods and Supplementary Information addressing alignment tolerance, interface maintenance, and temperature stability (Supplementary Fig. 2e). These additions clarify that the system is robust to small alignment deviations, does not require active temperature control under typical conditions, and can be maintained using standard optical cleaning procedures. In addition, we plan to set up a dedicated GitHub repository with mode details, including protocols, design files, troubleshooting guide, and updates to support community use and adoption.

F7. Scale bars
We thank the reviewer for noting this point. We have reviewed all figures and added missing scale bars where necessary.
We thank the reviewer again for their time and constructive feedback. The revisions, including experimental validation of Super-SCOPE, additional benchmarking, expanded comparisons, and improved methodological clarity, have strengthened the rigor, clarity, and practical relevance of the manuscript.

G. References: appropriate credit to previous work?
The manuscript cites relevant and up-to-date literature across light sheet microscopy, tissue clearing, expansion microscopy, and solid immersion lens design. Foundational work is appropriately acknowledged, and the authors position their contribution within the existing landscape in a balanced way.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
The abstract and summary are clear, well structured, and accurately reflect the scope and contributions of the work. They effectively communicate the motivation, the core innovation of the HySIL framework, and the main experimental findings without overstating the claims. The introduction provides appropriate context, clearly articulating the limitations of existing approaches and the need for a method that enables high resolution imaging across diverse refractive indices and large sample volumes. It situates the work within the broader landscape of light sheet microscopy and solid immersion lens design in a way that is accessible to both optical engineers and biological users.

Conclusion
We thank all reviewers for their thoughtful and constructive feedback and for their valuable time. The additional experiments, analyses, and clarifications have strengthened the manuscript and further clarified the conceptual advances of the HySIL framework and SCOPE imaging platform. We hope that the revised manuscript satisfactorily addresses all reviewer concerns.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I have carefully reviewed the authors' rebuttal and the revised manuscript. The authors have made substantial efforts to address the major concerns raised in my initial review. The most critical issue—the insufficient comparison to meniscus lens approaches—has been significantly improved through new experimental data, optical simulations, expanded discussion, and clear conceptual differentiation. The manuscript is now substantially stronger and, with minor additional revisions, suitable for publication in Nature Biotechnology.

The revised manuscript includes significant additions, such as several new supplementary figures. They substantially expanded the discussion throughout the Introduction, Results, and Discussion sections. The authors now clearly articulate the fundamental architectural difference between HySIL and meniscus-based approaches. This distinction is well-illustrated in Supplementary Fig. 2a-b. The authors effectively demonstrate that HySIL is a flexible framework, not a single implementation, by showing equivalent performance with plano-convex, biconvex, and meniscus lens geometries (Supplementary Fig. 2c-d). The performance comparisons involve different objectives (e.g., 10×/0.28 NA vs. 20×/0.40 NA), which complicates direct interpretation. However, the authors' argument that HySIL achieves superior resolution with lower NA objectives is compelling if substantiated. A brief note in Supplementary Table 1 explaining why these comparisons remain meaningful despite different NAs would enhance clarity.

The authors have added experimental PSF measurements for Super-SCOPE (Supplementary Fig. 3), confirming ~0.56 µm lateral resolution vs. ~0.75 µm for standard SCOPE with the same objective. Importantly, the authors honestly acknowledge current limitations and position Super-SCOPE as a 'proof-of-principle extension' that demonstrates the flexibility of the HySIL framework rather than a mature, fully optimized technology.

The authors have taken a more cautious approach and have removed the ~190 nm pre-expansion resolution claim for mouse and have added Supplementary Fig. 5 with quantitative expansion factor measurements for the salamander brain, showing ~2.5× expansion (not 4×) with good uniformity. The salamander measurement showing ~2.5× instead of the assumed 4× raises questions about whether the mouse sample also achieved less expansion than expected. The current

approach is more honest but leaves this question partially unresolved.

The remaining issues are minor:
1. Clarifying the rationale for performance comparisons across different NA objectives
2. Either measuring mouse ExM expansion factor or explicitly acknowledging this limitation

The manuscript now clearly establishes HySIL as a flexible, generalizable framework for aberration-corrected, high-resolution volumetric imaging using inexpensive air objectives. The work represents a genuine methodological contribution with strong potential for broad adoption and impact in the imaging community. I recommend acceptance pending these minor clarifications.

Reviewer #2

(Remarks to the Author)
The revised version is very much improved and all my concerns have been adequately addressed. This is a very interesting approach of high relevance to the field.

I only have a few last comments/suggestions:

Figure 1:
- Fig. 1a: units of W.D. missing. Were the values in the plot taken from the specs of the zoo of objectives? Please make that explicit that in the legend.
- Fig. 1b: the RI in the sample cuvette ('sample RI') should be indicated. In general, Figure 1 and the corresponding main text lack a proper introduction and details of the modeled sample. In the methods it says "The sample was modeled as a planar slab of defined thickness and RI" but this needs to be explained or illustrated in the main text or the legend (also for Fig. 1e). Similarly, information about the sample cuvette(s) in the experiments is sparse and hidden in the manuscript, which hinders comprehension by the reader. Please expand on this aspect in the main text or figure legends.

Figure 3:
- Fig. 3a claims to show resolved dendritic spines but there are hardly any discernible. The dendritic branches rather look a bit blebby. Perhaps use arrows to indicate what you consider spines or find a better example since this one is not compelling. Also, where is the inset in Fig. 3b from? It does not appear to correspond to the images left of it. It would be much better to show such inset together with its larger-view image. Overall, these 'spine results' so far appear anecdotal to me.

Author Rebuttal letter:

Response to Reviewers
We are grateful to the reviewers for recognizing the importance of our work and for providing very helpful minor suggestions to improve the accessibility and readability of the manuscript. Following their instructions exactly, we made these edits, as described below.

Reviewer #1 (Remarks to the Author):
I have carefully reviewed the authors' rebuttal and the revised manuscript. The authors have made substantial efforts to address the major concerns
raised in my initial review. The most critical issue—the insufficient comparison to meniscus lens approaches—has been significantly improved
through new experimental data, optical simulations, expanded discussion, and clear conceptual differentiation. The manuscript is now substantially
stronger and, with minor additional revisions, suitable for publication in Nature Biotechnology.

The revised manuscript includes significant additions, such as several new supplementary figures. They substantially expanded the discussion
throughout the Introduction, Results, and Discussion sections. The authors now clearly articulate the fundamental architectural difference between
HySIL and meniscus-based approaches. This distinction is well-illustrated in Supplementary Fig. 2a-b. The authors effectively demonstrate that
HySIL is a flexible framework, not a single implementation, by showing equivalent performance with plano-convex, biconvex, and meniscus lens
geometries (Supplementary Fig. 2c-d). The performance comparisons involve different objectives (e.g., 10×/0.28 NA vs. 20×/0.40 NA), which
complicates direct interpretation. However, the authors' argument that HySIL achieves superior resolution with lower NA objectives is compelling
if substantiated. A brief note in Supplementary Table 1 explaining why these comparisons remain meaningful despite different NAs would enhance
clarity.

The authors have added experimental PSF measurements for Super-SCOPE (Supplementary Fig. 3), confirming ~0.56 µm lateral resolution vs.
~0.75 µm for standard SCOPE with the same objective. Importantly, the authors honestly acknowledge current limitations a nd position Super-
SCOPE as a 'proof-of-principle extension' that demonstrates the flexibility of the HySIL framework rather than a mature, fully optimized technology.

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Supplementary Fig. 5 with quantitative expansion factor measurements for the salamander brain, showing ~2.5× expansion (not 4×) with good
uniformity. The salamander measurement showing ~2.5× instead of the assumed 4× raises questions about whether the mouse sample also achieved
less expansion than expected. The current approach is more honest but leaves this question partially unresolved.

The remaining issues are minor:
1. Clarifying the rationale for performance comparisons across different NA objectives
2. Either measuring mouse ExM expansion factor or explicitly acknowledging this limitation

The manuscript now clearly establishes HySIL as a flexible, generalizable framework for aberration-corrected, high-resolution volumetric imaging
using inexpensive air objectives. The work represents a genuine methodological contribution with strong potential for broad adoption and impact
in the imaging community. I recommend acceptance pending these minor clarifications.

Following the reviewer's instructions exactly, we revised the legend of Supplementary Table 1 to improve clarity and provide a comparison framework across these published studies. We believe this is more useful to readers than providing a separate Supplementary Note, because it directly guides them to two relevant comparison scenarios with previously published data: performance using objectives with similar NA, and the NA required to achieve similar resolution. Apart from this guidance for comparison, nothing else in the table has changed relative to the previous version of the manuscript.
Specifically, we highlight that the 5x/0.15 NA objective used with SIMlens (yielding ~2.6 µm in x and ~1.7 µm in y) is comparable in NA to the 5x/0.14 NA objective used in this study (yielding ~1.2 µm lateral resolution), directly demonstrating improved resolution with similar-NA objectives.
We also note that the 20x/0.40 NA objective used with SIMlens (yielding ~1.09 µm in x and 0.68 µm in y) is comparable to the 10x/0.28 NA objective used in this study (yielding <0.75 µm lateral resolution) in terms of achieved resolution, but with substantially lower NA in HySIL, as well as much longer working distance and larger field of view.
Modified Supplementary Table 1 legend:
Supplementary Table 1 | Comparison of HySIL/SCOPE with meniscus lens-based approaches.
Comparison of optical performance and system characteristics between the HySIL framework (SCOPE and Super-SCOPE) and
meniscus lens-based methods, including SIMlens-based open-top light-sheet microscopy and recent off-the-shelf meniscus implementations. Because the detection-objective NA are not exactly matched across the source publications, comparisons should
be interpreted in two ways: performance for similar-NA objectives, and the NA required to achieve similar resolution. For similar-
NA comparison, the 5x/0.15 NA objective used with SIMlens (yielding ~2.6 µm in x and ~1.7 µm in y) is comparable to the 5x/0.14
NA objective used in HySIL/SCOPE (yielding ~1.2 µm lateral resolution), indicating improved resolution at similar NA. For similar-resolution comparison, the 20x/0.40 NA objective used with SIMlens (yielding ~1.09 µm in x and 0.68 µm in y) is comparable to the 10x/0.28 NA objective used in this study (yielding <0.75 µm lateral resolution), indicating that HySIL achieves
similar resolution with lower NA, longer working distance, and larger field of view.

Following the reviewer's instruction exactly - "Either measuring mouse ExM expansion factor or explicitly acknowledging this limitation" - we measured the approximate expansion factor of the mouse brain sections by comparing the outlines of pre-expansion and post-expansion sections, yielding an expansion factor of ~2.45x, consistent with the salamander brain expansion factor obtained using the same protocol. We therefore added this information for clarity.
In Results:
"Under these conditions, expanded (~2.45-fold, see methods) Thy1-GFP mouse brain sections .."
In Methods:

"..imaging device. The approximate overall expansion factor (~2.45x) was estimated by comparing the outlines of multiple pre-
and post-expansion mouse brain sections."

Reviewer #2 (Remarks to the Author):
The revised version is very much improved and all my concerns have been adequately addressed. This is a very interesting approach of high
relevance to the field.
I only have a few last comments/suggestions:
Figure 1:
- Fig. 1a: units of W.D. missing. Were the values in the plot taken from the specs of the zoo of objectives? Please make that e xplicit that in the
legend.
- Fig. 1b: the RI in the sample cuvette ('sample RI') should be indicated. In general, Figure 1 and the corresponding main text lack a proper
introduction and details of the modeled sample. In the methods it says "The sample was modeled as a planar slab of defined thickness and RI" but
this needs to be explained or illustrated in the main text or the legend (also for Fig. 1e). Similarly, information about the sample cuvette(s) in the
experiments is sparse and hidden in the manuscript, which hinders comprehension by the reader. Please expand on this aspect in the main text or
figure legends.

Following the reviewer's instructions exactly, we added units for W.D. (mm) and clarified in the Figure 1 legend that the objective specifications used in the illustrative schematic of representative objectives were compiled from common commercial sources, including Edmund Optics, Thorlabs, and major optical manufacturers such as Nikon and Olympus.
Updated Figure 1 legend: ".. resolution and scalability. The objective specifications were manually compiled from commercial
catalogs, including those of Edmund Optics, Thorlabs, and major manufacturers such as Nikon and Olympus. "

Following the instructions exactly, we also added the sample cuvette RI information in Fig. 1d. We believe the reviewer intended Fig. 1d, as there is no sample cuvette in Fig. 1b, only in Fig. 1d.
We added the following clarifying statements in the Results, Methods, and Fig. 1 legend.
Results: "We next assessed tolerance to variations in sample RI (1.38-1.54) and thickness (2-6 mm). In the simulations, a generic
sample with thicknesses of 2, 4, and 6 mm and refractive indices ranging from 1.38 to 1.54 was modeled as a planar slab adjacent
to the sample plane (Fig. 1g) to evaluate the tolerance of SCOPE to variations in sample optical properties. As shown in Fig. 1g
and Supplementary Fig. 1c, …"
Methods: "Specifically, samples with thicknesses of 2, 4, and 6 mm and refractive indices ranging from 1.38 to 1.54 were modeled
as planar slabs adjacent to the sample plane. "
Fig. 1 legend: "Tolerance analysis of air-objective detection in immersion, SCOPE, and Super-SCOPE configurations. For the
simulation, samples with thicknesses of 2, 4, and 6 mm and refractive indices ranging from 1.38 to 1.54 were represented as planar
slabs adjacent to the sample plane. "

To avoid confusion, we now state in multiple places that all sample imaging was performed in quartz glass cuvettes (RI ~1.46):
Results:
"Samples were mounted in quartz cuvettes (RI ~1.46; 10 × 10 mm, 10 × 20 mm, or 20 × 20 mm base dimensions, as appropriate)
to maintain refractive-index continuity with the SCOPE system. "
"Brains immunostained for tyrosine hydroxylase (TH) were mounted in quartz cuvettes (RI ~1.46) and imaged using.."
"All imaging experiments were performed with samples mounted in quartz cuvettes (RI ~1.46). As shown in Supplementary Fig.
1d, SCOPE enabled .."
Methods:
"For all imaging experiments, samples were mounted in quartz cuvettes (RI ~1.46; 10 x 20 mm or 10 x 10 mm base dimensions) to

ensure RI continuity across optical interfaces."

Figure 3:
- Fig. 3a claims to show resolved dendritic spines but there are hardly any discernible. The dendritic branches rather look a b it blebby. Perhaps use
arrows to indicate what you consider spines or find a better example since this one is not compelling. Also, where is the inset in Fig. 3b from? It
does not appear to correspond to the images left of it. It would be much better to show such inset together with its larger-view image. Overall, these
'spine results' so far appear anecdotal to me.

Following the reviewer's instructions exactly, we made the following edits.

Because Fig. 3a shows a less magnified view, we removed "including spines" from the Fig. 3a legend.
Original: "The bounding box is 3.41 mm × 2.14 mm × 0.6 mm. Insets (bottom): magnified views reveal fine axonal and dendritic
processes, including spines. "
Edited: "The bounding box is 3.41 mm × 2.14 mm × 0.6 mm. Insets (bottom): magnified views reveal fine axonal and dendritic
processes."

We also clarified explicitly that Supplementary Video 1 is derived from the same dataset and provides a more detailed volumetric view for readers to assess these fine structures. In addition, we added a white dashed box in panel a to indicate the location of the data subset shown in panel b, and a magenta box in panel b to indicate the location of the dendritic shaft shown in the inset.
Consistent with standard practice in the field, we also qualified the detected dendritic spines as "punctate protrusions consistent with putative dendritic spines" in the Fig. 3 legend. We note that full structural details are difficult to appreciate in static 2D snapshots, which is why we included the high-resolution volumetric rendering in Supplementary Video 1, where these features can be assessed more clearly by readers.
Updated Figure 3 legend:
"Figure 3 | High-resolution volumetric imaging of expanded mouse brain. (a) Light-sheet imaging of an expanded Thy1-GFP mouse brain section using the SCOPE imaging device. Left: 3D depth color-coded rendering of a selected region of interest within
the hippocampal area, corresponding to the whole-section dataset shown on the right. The bounding box is 3.41 mm × 2.14 mm ×
0.6 mm. Insets (bottom): magnified views reveal fine axonal and dendritic processes. See also Supplementary Video 1 for a detailed
visualization of this dataset. The dashed white box indicates the data subset shown in b. (b) Automated tracing of individual neurons, dendritic arbors, and putative dendritic spines. Left: raw fluorescence image; middle: overlay of data and reconstructed
traces; right: isolated traced neuron showing punctate protrusions consistent with putative dendritic spines. Insets: magnified views of a dendritic shaft (magenta box) highlighting putative spines (cyan). Scale bars, 1 mm (a, upper-right panel), 100 µm (b,
left), and 5 µm (b, insets)."

Reviewer 1 comments on Reviewer 3's concerns:

I have also reviewed the authors' responses to Reviewer 3's concerns and find them comprehensive and satisfactory. Reviewer 3 requested mainly
six improvements: (1) experimental PSF measurements for Super-SCOPE, (2) clearer differentiation between SCOPE and Super-SCOPE
configurations, (3) benchmarking against commercial high-NA immersion objectives, (4) comparison with classical solid immersion lenses, (5)
practical implementation details including alignment tolerances and interface robustness, and (6) addition of missing scale bars.
The authors have systematically addressed all six points with substantive additions. Most notably, they added new Supplementary Figure 3 with
experimental Super-SCOPE PSF validation, Supplementary Figure 4 providing direct side-by-side comparison with a high-NA immersion objective
(exactly as requested), expanded discussion of classical SIL approaches, comprehensive practical implementation details including temperature
stability simulations (Supplementary Figure 2e), and added all missing scale bars. The responses demonstrate genuine

engagement with Reviewer
3's constructive feedback, and I believe Reviewer 3 would be satisfied with these revisions and likely recommend acceptance.