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SegRef3D Lite: A browser-based workflow for artificial intelligence–assisted segmentation and quantitative three-dimensional morphological analysis

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Abstract

Segmentation is a major bottleneck in three-dimensional morphological analysis. Although artificial intelligence–assisted segmentation workflows can be used to address this issue, their implementations are constrained by dedicated software environments or local computing resources. To address these challenges, we developed SegRef3D Lite, a browser-based platform that integrates two complementary artificial intelligence–assisted segmentation approaches (namely, SegAnything and SegCT/MRI) within a common human-in-the-loop workflow. SegAnything provides general-purpose, prompt-based segmentation by using Segment Anything Model 2, and SegCT/MRI provides anatomy-specific segmentations of computed tomography and magnetic resonance imaging datasets by using TotalSegmentator. Both approaches generate segmentation masks that can be imported into the same browser-based workspace for visual review, manual correction, quantitative volume measurement, three-dimensional visualization, and surface-model export. In this workspace, routine image handling and refinement are performed locally in the browser, whereas artificial intelligence inference is executed through user-initiated Google Colaboratory workflows, thus eliminating the need for a compatible graphics processing unit on a local computer. We applied our platform to serial block-face photographs, volumetric computed tomography, serial light microscopy, serial electron microscopy, and human abdominal computed tomography. Across these diverse imaging modalities and spatial scales, our platform is able to process outputs from different segmentation approaches by using the same downstream workflow for review, refinement, quantitative analysis, and three-dimensional reconstruction. Therefore, SegRef3D Lite provides an accessible framework for integrating complementary artificial intelligence–assisted segmentation strategies with researcher-guided verification and quantitative morphological analysis.

Mini-abstract: SegRef3D Lite integrates general-purpose and anatomy-specific artificial intelligence–assisted segmentation with browser-based human refinement, volume measurement, and three-dimensional reconstruction across diverse morphological imaging modalities.

INTRODUCTION

Three-dimensional (3D) reconstruction is widely used in morphological research to visualize the spatial relationships between anatomical and histological structures that cannot be fully appreciated in individual two-dimensional sections. This approach has been applied to a broad range of imaging modalities, including computed tomography (CT) and magnetic resonance imaging (MRI) (Muro et al. 2025b), serial histological sections (Muro and Akita 2023), block-face imaging (Tajika et al. 2023), and light and electron microscopy (Koga et al. 2017; Tamada 2023). By integrating morphological information across serial images, 3D reconstruction enables investigators to analyze the shape, continuity, and spatial relationships of structures over a wide range of spatial scales.

Segmentation is a major obstacle in image-based 3D reconstruction. In conventional segmentation workflows, target structures are required to be delineated repeatedly across large numbers of sequential

images, thus making segmentation highly time-consuming and labor-intensive (Muro et al. 2025b). Although artificial intelligence (AI)–based segmentation can reduce this burden, morphological research presents several challenges that differ from standardized clinical image-analysis tasks. For example, the image modality, target structure, spatial scale, and image characteristics often vary between studies, and the precise shape and boundary of the segmented structure may constitute the object of morphological investigation. Therefore, researcher-based verification and correction of AI-generated segmentation results remain important even when automated segmentation demonstrates good performance (Muro et al. 2025a; Muro et al. 2025b).

To address these issues, we previously developed the Seg & Ref toolset, which combines AI-assisted segmentation by using Segment Anything Model 2 (SAM2) with interactive correction of segmentation masks and subsequent 3D reconstruction (Muro et al. 2025a). This toolset was designed as a human-in-the-loop methodology in which AI reduces repetitive segmentation work while researchers retain control over anatomical verification and refinement. Subsequently, we developed SegRef3D, which integrates AI-assisted segmentation, multiframe tracking, interactive refinement, volume measurement, and 3D reconstruction into a standalone desktop application (Muro et al. 2026b).

In parallel, we developed Instant3D, a lightweight graphical front end for TotalSegmentator-based CT and MRI segmentations and 3D reconstruction (Muro et al. 2026a). Instant3D simplifies the execution of anatomy-specific automatic segmentation and provides interoperability with SegRef3D, thus allowing automatically generated segmentation masks to be reviewed and refined after segmentation. Thus, two complementary workflows emerged: general-purpose, prompt-based segmentation centered on SAM2 and anatomy-specific automatic segmentation centered on TotalSegmentator.

However, these workflows remained separate. The previously reported local implementation of SegRef3D requires a desktop application, and the local execution of SAM2-based segmentation and tracking additionally requires an NVIDIA graphics processing unit (GPU) with Compute Unified Device Architecture (CUDA) support (Muro et al. 2026b). To integrate these concepts into a single environment, we developed SegRef3D Lite, which combines general-purpose segmentation via SegAnything and anatomy-specific segmentation via SegCT/MRI. Our platform creates a common workflow for researcher-guided review and refinement, quantitative volume measurement, and 3D reconstruction. Compared with the desktop-based SegRef3D workflow, SegRef3D Lite eliminates the need for local software installation and a CUDA-capable local GPU while integrating SAM2- and TotalSegmentator-based segmentation within a common browser-based refinement and analysis environment.

In this study, we describe the design and workflow of SegRef3D Lite and demonstrate its application across diverse imaging modalities and spatial scales. We aim to establish an accessible browser-based workflow that integrates different AI-assisted segmentation approaches with human verification, quantitative morphological analysis, and 3D reconstruction.

MATERIALS AND METHODS

SegRef3D Lite system overview

SegRef3D Lite is a browser-based platform developed for the segmentation, interactive refinement, quantitative analysis, and 3D reconstruction of serial and volumetric images. It builds upon the previously developed Seg & Ref toolset, which established a human-in-the-loop workflow consisting of AI-assisted segmentation, researcher-based correction of segmentation masks, and subsequent 3D reconstruction (Muro et al. 2025a). These features were then integrated into a standalone desktop application in the form of SegRef3D (Muro et al. 2026b). Furthermore, we developed Instant3D, which allows automated segmentation masks to be reviewed and refined in SegRef3D (Muro et al. 2026a).

SegRef3D Lite runs in a modern web browser and does not require the installation of a local desktop application. The browser-based environment was tested on Microsoft Windows by using Google Chrome and on macOS by using Google Chrome and Safari. SegRef3D Lite performs routine image loading, mask editing, volume measurement, 3D visualization, and data export locally within the browser, and AI inference is performed separately in a user-initiated Google Colaboratory (Colab) environment. In contrast to SegRef3D, SegRef3D Lite allows for AI-assisted segmentation to be used without a CUDA-capable GPU on a local computer.

SegRef3D Lite incorporates two complementary AI-assisted segmentation workflows (Fig. 1): SegAnything provides general-purpose segmentation via SAM2 (Ravi et al. 2025), whereas SegCT/MRI provides anatomy-specific segmentation of CT and MRI datasets via TotalSegmentator. The original TotalSegmentator model was developed for the automatic segmentation of multiple anatomical structures in CT images (Wasserthal et al. 2023), and its subsequent MRI implementation provides sequence-independent automatic segmentation of anatomical structures in MRI (D'Antonoli et al. 2025). Both the CT and MRI implementations of TotalSegmentator are based on the nnU-Net framework (Isensee et al. 2021). SAM2 provides prompt-based segmentation and propagation across sequential images, whereas TotalSegmentator automatically identifies predefined anatomical structures.

After segmentation by either workflow, the generated masks are returned to the same SegRef3D Lite workspace, where they can be reviewed and manually corrected before quantitative analysis and 3D reconstruction. The source images are not uploaded to the SegRef3D server during routine local browser operations. For AI-assisted segmentation, image data are transferred only when the user explicitly uploads a request file generated by SegRef3D Lite to the corresponding Google Colab workflow.

General-purpose segmentation with SegAnything

SegAnything was designed for the general-purpose segmentation of user-defined structures without restricting the target to predefined anatomical classes. Users first load an image sequence or volumetric dataset into SegRef3D Lite and then select one or more objects for segmentation. For each object, a segmentation range and one or more box prompts can be defined for the representative images. The working image sequence and segmentation settings are then packaged into a ZIP file in the browser.

The request ZIP file is explicitly uploaded by the user to the SegAnything Google Colab workflow. SAM2 (Ravi et al. 2025) generates segmentation masks from user-defined box prompts and propagates the segmented objects through the specified image range. The resulting masks are then packaged into a ZIP file and imported back into SegRef3D Lite. The imported masks are not treated as final outputs; they remain editable and can be corrected using browser-based refinement functions.

To demonstrate the applicability across different image types and spatial scales, four datasets representing macroscopic imaging, CT, light microscopy, and electron microscopy were used (Fig. 2, Table 1).

Table 1
Demonstration datasets used in SegRef3D Lite

Dataset	Modality	Dataset size	Spatial information	Workflow	License
Apple	Serial block-face photographs	20 images	Approximate 4-mm interval	SegAnything	CC BY 4.0
RabbitCT	CT	256 voxels × 256 voxels × 256 voxels	1.0-mm isotropic	SegAnything	CC BY 4.0
HeLa	Serial block-face scanning electron microscopy	150 images, 512 pixels × 512 pixels	39.0625 nm × 39.0625 nm × 100 nm	SegAnything	CC0
Mouse brain	Light microscopy	132 images, 707 pixels × 553 pixels	User calibration required	SegAnything	CC BY-SA 4.0
PANCREAS_0080	Contrast-enhanced abdominal CT	181 images, 512 pixels × 512 pixels	From DICOM metadata	SegCT/MRI	CC BY 3.0

Abbreviations: CT, computed tomography; DICOM, Digital Imaging and Communications in Medicine; CC, Creative Commons; BY, Attribution; SA, ShareAlike

Anatomy-specific segmentation with SegCT/MRI

SegCT/MRI extends the previously reported Instant3D workflow by directly integrating the TotalSegmentator-based anatomy-specific segmentation into SegRef3D Lite (Muro et al. 2026a). In contrast to SegAnything, in which users specify arbitrary target structures by using prompts, SegCT/MRI allows users to select structures from a catalog supported by the incorporated TotalSegmentator

models. TotalSegmentator provides automatic anatomical segmentation by using nnU-Net-based models for CT and MRI (Isensee et al. 2021; Wasserthal et al. 2023; D'Antonoli et al. 2025).

After loading a compatible CT or MRI dataset, the user selects the structures to be segmented and generates a SegCT/MRI request ZIP file in SegRef3D Lite. The request file is explicitly uploaded by the user to the corresponding Google Colab workflow, where the TotalSegmentator is executed. The resulting segmentation data are returned as a ZIP file and imported into SegRef3D Lite, where individual anatomical structures are assigned to the editable SegRef3D objects. Subsequently, the imported masks can be reviewed, corrected, quantified, and reconstructed three-dimensionally within the same workspace.

A publicly available human abdominal CT dataset was used as a representative application of the SegCT/MRI workflow (Fig. 3, Table 1).

Review and refinement of segmentation masks

SegRef3D Lite was designed as a human-in-the-loop segmentation environment in which AI-generated segmentation masks serve as editable intermediate results rather than immutable final outputs. This design follows our previous observation that morphological research requires researcher-based verification and correction even when automated segmentation provides high apparent accuracy because the precise shape and boundary of the target structure are the objects of investigation (Muro et al. 2025b). In our previous evaluation of AI-powered serial segmentation, the segmentation performance varied according to the image modality and target structure, thus further supporting the need for researcher-guided verification and correction.

Segmentation masks generated by SegAnything or SegCT/MRI can be reviewed while overlaid on the original images. Users can manually add or erase labeled regions and transfer the labeled regions between objects. The mask-processing functions include filling holes, removing small isolated components, retaining the largest connected component, smoothing boundaries, dilation, and erosion. Mask editing is performed directly within the SegRef3D Lite workspace, thus allowing users to correct AI-generated results before performing quantitative measurements or 3D reconstruction. Figure 4 shows the representative examples of refinement using the Add and Transfer functions.

Quantification and 3D reconstruction

Spatial information is obtained from the source image metadata when available or can be entered by calibration when physical spacing information is not included in the source data. SegRef3D Lite calculates the voxel count and physical volume of the individual segmented objects from the reviewed segmentation masks.

Segmented structures can be reconstructed as 3D surface models and inspected directly using browser-based 3D previews. Individual structures can be displayed or hidden, and their opacity can be adjusted to visualize spatial relationships among multiple objects. Surface models can be exported in standard tessellation language (STL) format for subsequent visualization or analysis using external software. The segmentation data can also be exported as label-map files in Neuroimaging Informatics Technology Initiative (NIfTI) format or Tagged Image File Format (TIFF) image stacks. The complete workflow consists of AI-assisted segmentation, human review and correction, quantitative measurement, and 3D reconstruction (Fig. 1).

Demonstration datasets

Five openly available datasets incorporated into SegRef3D Lite were used for the workflow demonstration (Table 1). For general-purpose segmentation using SegAnything, four datasets were used: serial block-face photographs of an apple (Schut et al. 2023), volumetric rabbit CT (Rohkohl et al. 2009), serial block-face electron microscopy of HeLa cells (Peddie et al. 2020), and serial light microscopy of the mouse brain from the Brain Architecture Project (Bohland et al. 2009). For anatomy-specific segmentation using SegCT/MRI, we used the contrast-enhanced abdominal CT dataset PANCREAS_0080 from the Pancreas-CT Version 2 collection of The Cancer Imaging Archive (Roth et al. 2016). Table 1 summarizes the dataset characteristics and licensing information.

Software availability

SegRef3D Lite is openly available through the SegRef3D project website (https://satorumuro.github.io/SegRef3D/lite-web/) and the source repository (https://github.com/SatoruMuro/SegRef3D). The software is distributed under the Apache License 2.0. The SegRef3D Lite version used in this study corresponds to the Git commit e130f0213f94f9b76d841808d6c75989486aa8c4.

RESULTS

Application of general-purpose segmentation to diverse image modalities

For each of the four datasets used in the SegAnything workflow, the target structures were specified using box prompts and then segmented using SAM2 (Fig. 2, Table 1). The generated segmentation masks were imported into SegRef3D Lite and displayed as overlays on the original images. The imported masks remained editable within the browser-based workspace and could be refined prior to subsequent analysis.

Segmentation and 3D reconstruction were performed using the same SegRef3D Lite workflow across all four datasets, including serial macroscopic block-face photographs (Figs. 2a, 2b), volumetric CT

(Figs. 2c, 2d), serial electron microscopy (Figs. 2e, 2f), and serial light microscopy (Figs. 2g, 2h). Therefore, the workflow is applicable to macroscopy, radiology, light microscopy, and electron microscopy image data.

Anatomy-specific segmentation using SegCT/MRI

The SegCT/MRI workflow was applied to a human abdominal CT dataset to demonstrate the automatic segmentation of predefined anatomical structures. Multiple abdominal structures were segmented using TotalSegmentator and then imported into SegRef3D Lite as separate editable objects (Fig. 3a).

The imported segmentation masks were displayed directly on the corresponding CT images, thus allowing the anatomical boundaries of the individual structures to be reviewed within the same workspace. The segmented structures included the liver, spleen, stomach, gallbladder, kidneys, pancreas, aorta, inferior vena cava, duodenum, small bowel, and colon.

The segmentation results were subsequently reconstructed three-dimensionally by using SegRef3D Lite (Fig. 3b). Individual structures can be displayed simultaneously or selectively, thus enabling the visualization of their spatial relationships. The same imported masks can also be manually corrected and used for quantitative volume measurements or STL export without being transferred to a separate editing environment.

Researcher-guided refinement of segmentation masks

AI-generated segmentation masks can be directly reviewed and corrected within the SegRef3D Lite browser workspace (Fig. 4). Representative examples demonstrate the addition of a missing region by using the Add function and the reassignment of an incorrectly labeled region between objects by using the Transfer function. Therefore, AI-generated masks can be refined within the same workspace prior to quantitative analysis or 3D reconstruction.

DISCUSSION

SegRef3D Lite integrates the general-purpose SegAnything and anatomy-specific SegCT/MRI within a common browser-based workflow in which AI-generated masks can be reviewed, corrected, quantified, and reconstructed three-dimensionally.

A major feature of SegRef3D Lite is its applicability to heterogeneous image data. The SegAnything workflow was used with serial macroscopic block-face photographs, volumetric CT, serial light microscopy, and serial electron microscopy, with the datasets differing markedly in terms of spatial scale, image contrast, color characteristics, and acquisition method. Nevertheless, these datasets can be handled within the same SegRef3D Lite workflow from segmentation to refinement and 3D reconstruction. This versatility is particularly relevant to morphological research, in which investigators frequently work with unique datasets that differ between studies and cannot be addressed using segmentation models optimized for a single image modality or anatomical structure.

The integration of two AI-assisted segmentation strategies further extends the scope of the platform. SegAnything supports arbitrary prompt-defined targets using SAM2 (Ravi et al. 2025), whereas SegCT/MRI uses TotalSegmentator for predefined anatomical structures in CT and MRI (Wasserthal et al. 2023; D'Antonoli et al. 2025). These complementary approaches accommodate study-specific targets and structures that are supported by predefined anatomical models. More importantly, the outputs of both approaches are returned to the same SegRef3D Lite workspace and can be handled identically thereafter.

This common refinement step is central to the design of SegRef3D Lite. In morphological research, segmentation is not merely a technical preprocessing step: the exact shape, boundary, and continuity of the segmented structure may constitute a scientific observation. Therefore, automatic segmentation results should not necessarily be regarded as final even when the underlying AI model achieves high performance. In our previous evaluation of AI-powered serial segmentation, segmentation accuracy varied substantially according to image modality and target structure, and errors were particularly evident in small or branching structures (Muro et al. 2025b). Subsequently, we proposed the Seg & Ref toolset as a labor-complementing approach rather than as a labor-replacing one, i.e., the toolset allows AI to reduce repetitive segmentation work while researchers retain responsibility for verification and correction (Muro et al. 2025a). SegRef3D Lite preserves this human-in-the-loop principle while allowing the masks generated by different AI approaches to be corrected in the same browser-based environment.

Another important advancement is accessibility. The previously reported SegRef3D integrates SAM2-based segmentation, interactive refinement, and 3D reconstruction into a standalone desktop application (Muro et al. 2026b). However, the local execution of SAM2-based segmentation and tracking requires a CUDA-capable NVIDIA GPU (Muro et al. 2026b). By contrast, SegRef3D Lite separates routine image handling and refinement from GPU-dependent AI inference. Image loading, mask editing, volume measurement, 3D visualization, and export are all performed in the browser, and AI inference can be executed separately in a user-initiated Google Colab environment. Consequently, the local computer does not require a CUDA-capable GPU. The browser-based interface was tested on Microsoft Windows using Google Chrome and on macOS using Google Chrome and Safari.

This study was designed to demonstrate the workflow and applicability of SegRef3D Lite rather than to benchmark the accuracy of the underlying AI models independently. The performance of SAM-based segmentation in anatomical and histological image datasets has been evaluated previously (Muro et al. 2025b), whereas SAM2 and TotalSegmentator have been independently described and validated in their original reports (Wasserthal et al. 2023; Ravi et al. 2025; D'Antonoli et al. 2025). Accordingly, the segmentation examples presented here should be interpreted as demonstrations of workflow integration rather than as accuracy validation. In addition, SegAnything and SegCT/MRI require internet access and the explicit uploading of request files to the Google Colab for AI inference. Institutional policies may restrict the transfer of research or medical image data to external cloud computing environments. Finally, the segmentation performance remains dependent on the characteristics and limitations of the

underlying AI models, and researcher review remains necessary when the resulting masks are used for quantitative morphological analysis.

SegRef3D Lite extends SegRef3D from a local desktop application to a browser-based morphological analysis environment that integrates general-purpose and anatomy-specific AI-assisted segmentation. By combining diverse AI segmentation strategies with researcher-guided refinement, volume measurement, and 3D reconstruction in a common workspace, SegRef3D Lite provides a flexible approach for quantitative morphological analysis across a wide range of imaging modalities and spatial scales.

Declarations

Author contributions: Satoru Muro: Conceptualization, Methodology, Software, Investigation, Visualization, Writing – original draft. Takuya Ibara: Methodology, Validation, Writing – review and editing. Akimoto Nimura: Supervision, Writing – review and editing. Keiichi Akita: Supervision, Writing – review and editing. All authors read and approved the final manuscript.

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During the preparation of this manuscript, the authors used ChatGPT (OpenAI) for assistance in language refinement, manuscript structuring, and drafting. The contents, scientific statements, and references that were created with assistance from AI were critically reviewed and verified. All authors take full responsibility for the final version of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

This study used only publicly available, de-identified datasets. No new human participants, human specimens, or animals were recruited or collected for this study.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Data availability

All datasets used in this study are publicly available from the repositories cited in Table 1 and the references. No new primary datasets were generated in this study.

Code availability

SegRef3D Lite is openly available at https://satorumuro.github.io/SegRef3D/lite-web/, and its source code is available at https://github.com/SatoruMuro/SegRef3D under Apache License 2.0. The SegRef3D Lite version used in this study corresponds to the Git commit e130f0213f94f9b76d841808d6c75989486aa8c4.

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Figures

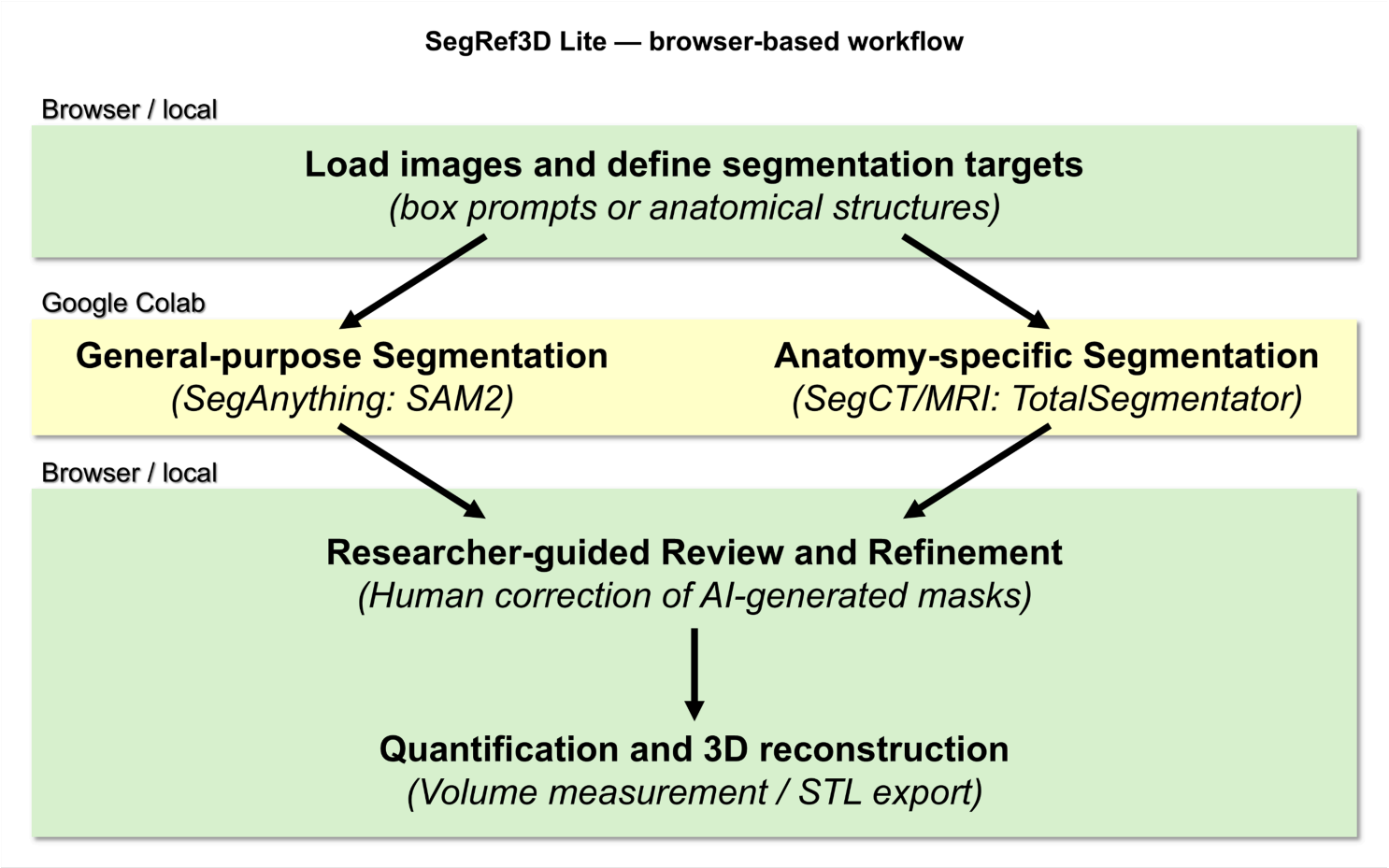


Figure 1

Conceptual workflow of SegRef3D Lite

Image loading, definition of segmentation targets, researcher-guided review and refinement, quantitative analysis, and 3D reconstruction are performed locally in the browser. AI inference is performed separately in Google Colab by using either SegAnything via SAM2 or SegCT/MRI via TotalSegmentator. Outputs from both segmentation workflows are returned to the common browser-based workspace for review and refinement. AI: artificial intelligence; Colab: Colaboratory; SAM2: Segment Anything Model 2

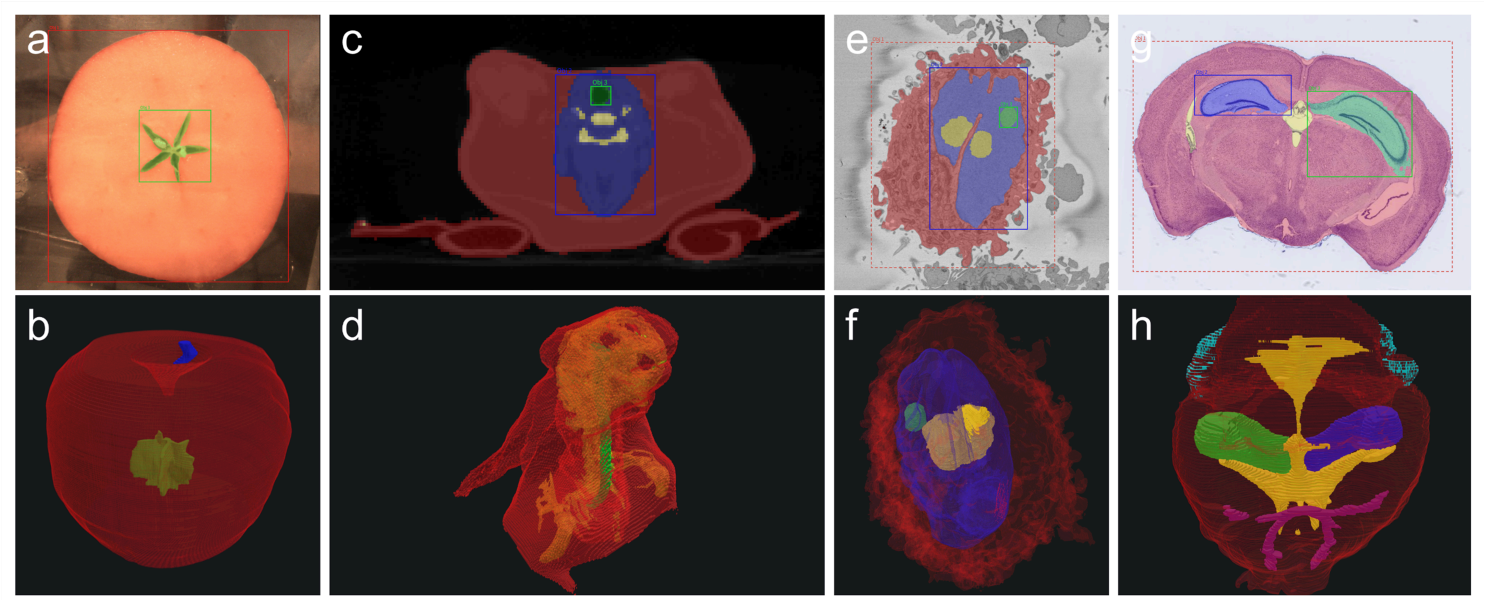


Figure 2

Application of SegRef3D Lite to diverse image modalities

Representative segmentation results and 3D reconstructions are shown for serial block-face photographs of an apple (a, b), rabbit CT (c, d), serial electron microscopy of HeLa cells (e, f), and serial light microscopy of the mouse brain (g, h). The upper panels (a, c, e, g) show representative images with segmentation masks, and the lower panels (b, d, f, h) show 3D reconstructions generated from the resulting masks.

The mouse brain images used in panels (g, h) were obtained from the Brain Architecture Project (https://brainarchitecture.org/; Bohland et al. 2009), licensed under CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0/). The source images were modified through AI-assisted segmentation and 3D reconstruction. The adapted material in panels (g, h) is distributed under CC BY-SA 4.0.

3D: three-dimensional; CT: computed tomography.

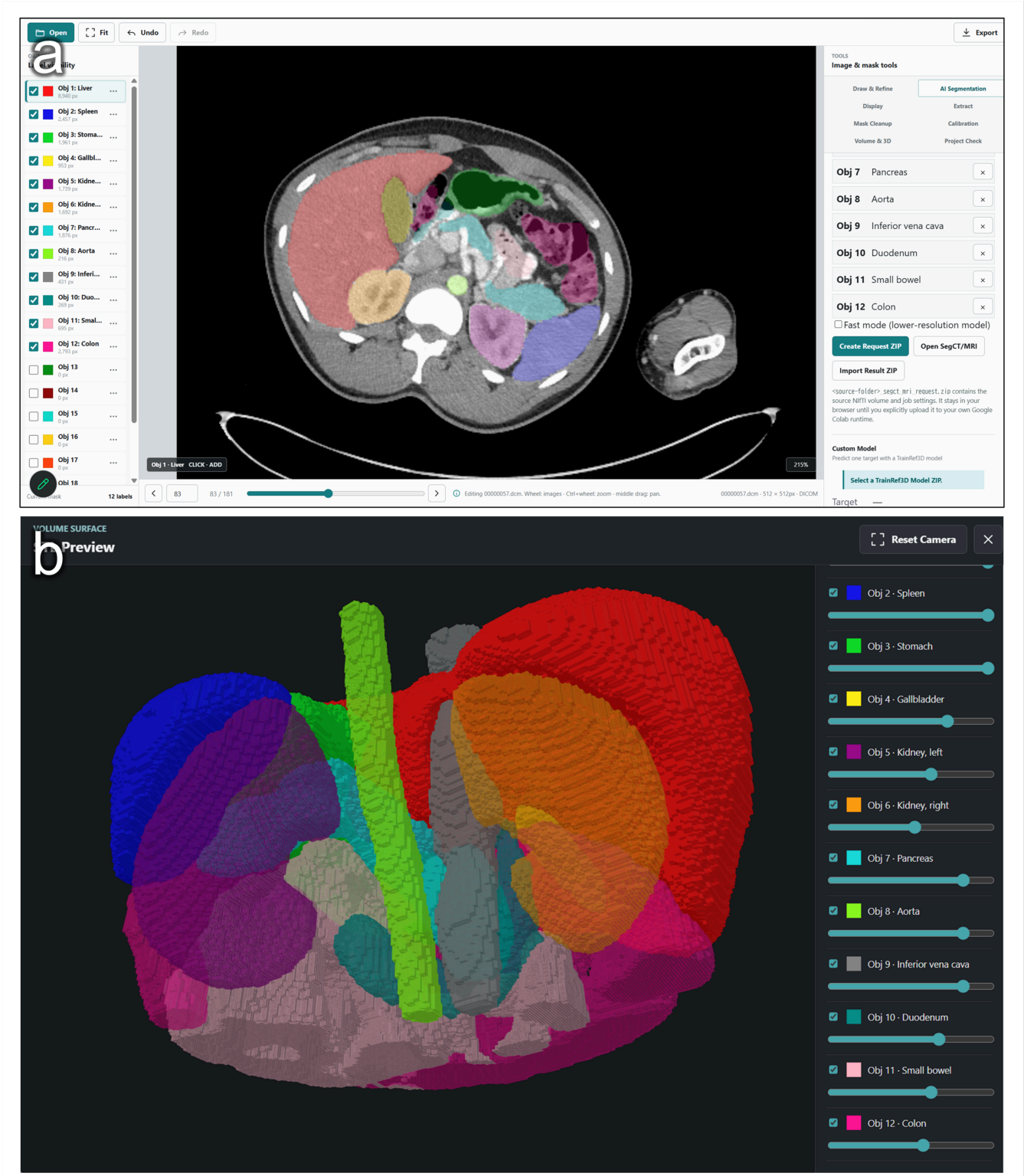


Figure 3

Example of anatomy-specific automatic segmentation using SegCT/MRI in SegRef3D Lite

(a) Representative axial abdominal CT image with multiple anatomical structures segmented using SegCT/MRI and imported into SegRef3D Lite. (b) Three-dimensional reconstruction of the segmented structures in SegRef3D Lite. CT: computed tomography

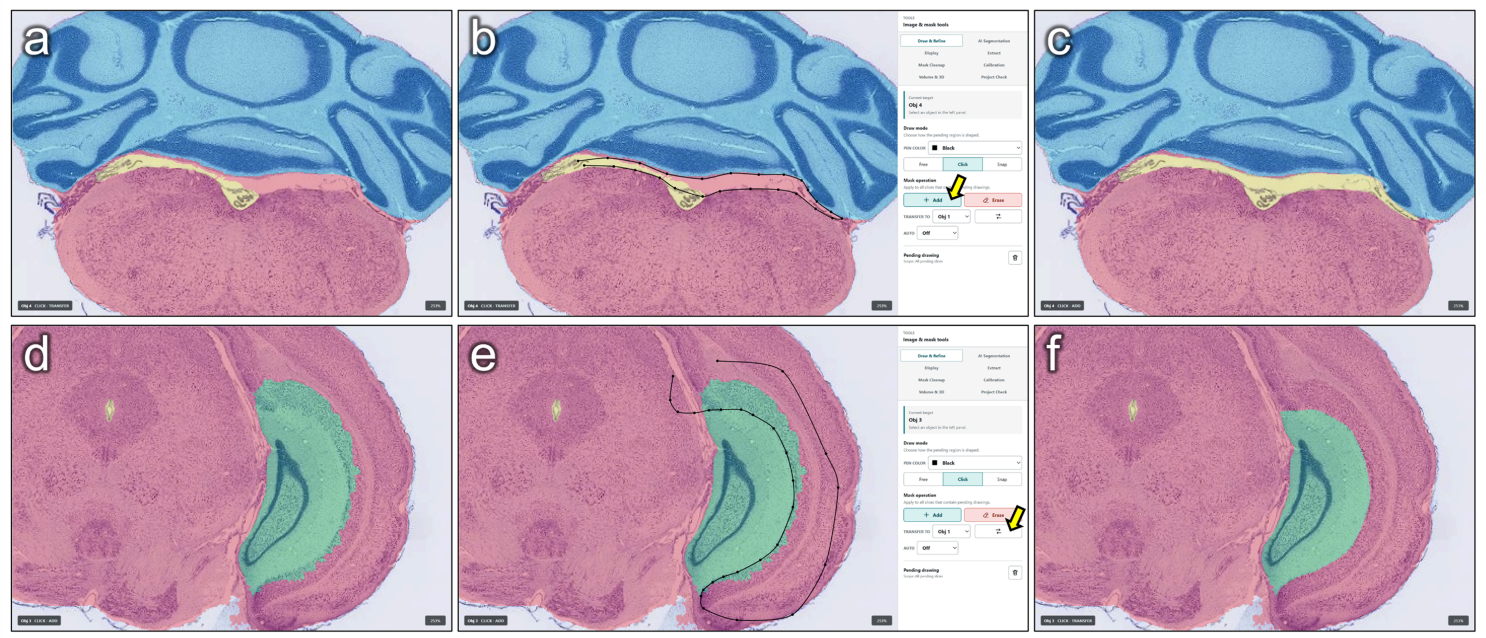


Figure 4

Representative examples of researcher-guided refinement of AI-generated segmentation masks in SegRef3D Lite

(a–c) Refinement using the Add function; a missing region in the yellow object was manually delineated and added. (d–f) Refinement using the Transfer function; an excessive region in the green object was manually delineated and reassigned to the red object.