

SOP: Construction of Organ Mapping Antibody Panels for Multiplexed Antibody-Based Imaging of Human Tissues

How to construct Organ Mapping Antibody Panels (OMAPs)

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Introduction

Optical imaging provides critical spatial data for the vast network of cell types and anatomical structures present in the human body. One such approach, highly multiplexed antibody-based imaging, targets protein biomarkers *in situ* using dozens of antibodies, allowing for the comprehensive profiling of cell phenotypes at single cell resolution within normal and diseased tissues. Beyond preserving cell-cell interactions and tissue architecture, this approach offers insight into the spatial patterns of complex tissues. This technique also permits assessment of cell states using probes for post-translationally modified molecules generated by active signaling events or by imaging of nuclear localization of transcription factors. When coupled with advanced analytical methods, high content imaging allows for the quantification of rare and difficult to extract cell types such as tissue-resident macrophages and fibroblasts. While these methods may vary in antibody format (fluorophore-, metal-, or oligo-conjugated), mode of imaging (all-in-one or cyclic), or mode of immunolabeling (all-in-one or cyclic), all employ antibodies for protein biomarker detection (see [Figure 1b](#)) in (Hickey et al. 2021). Given the wealth of information provided by multiplexed antibody-based imaging, these techniques are foundational to international

efforts aimed at building a comprehensive human reference atlas (HRA) (Snyder et al. 2019; Regev et al. 2017).

Spatial mapping approaches pose significant challenges as they are: 1) targeted: antibodies must be carefully validated before data acquisition, 2) fallible: non-reproducible immunolabeling and/or off target labeling is well-described (Uhlen et al. 2016; Bordeaux et al. 2010), 3) resource-intensive: a panel of 50 unique antibodies may require thousands of US dollars in reagent costs and months to build (Hickey et al. 2021), and 4) expertise-dependent: relying on Subject Matter Experts for their construction and optimization (Du et al. 2019; Radtke, Chu, et al. 2022; Radtke et al. 2020). To overcome these limitations, we are building Organ Mapping Antibody Panels (OMAPs) that identify the major anatomical structures and cell types present in various human tissues. Furthermore, we are integrating OMAPs with their respective protein biomarkers and cell types in the Anatomical Structures, Cell Types plus Biomarkers (ASCT+B) tables. To facilitate tissue mapping efforts within and beyond the HuBMAP community, OMAPs are designed for integration with the [ASCT+B Reporter](#) (Börner et al. 2021), a state-of-the-art visualization tool. We strongly encourage inclusion of blood endothelial markers to empower construction of a human reference atlas using the vasculature common coordinate framework (VCCF), originally proposed by (Galis 2020) and expanded upon here (Weber, Ju, and Börner 2020). The inclusion of antibodies directed against one or more lymphatic endothelial markers (e.g., LYVE1) is highly recommended to further our understanding of the human lymphatic system (Radtke, Lukacs, et al. 2022; National Heart, Lung, and Blood Institute (NHLBI) and National Institutes of Health (NIH) 2021).

Roles and Responsibilities

Different types of expertise are required to author and approve OMAPs and major roles are listed below. [Table 1](#) identifies the people occupying these roles.

OMAP Authors are Subject Matter Experts with backgrounds in multiplexed antibody-based imaging techniques.

OMAP Reviewers are Subject Matter Experts in pathology, cell biology, anatomy, histology, and/or multiplexed antibody-based imaging.

The **Lead OMAP Assembly Contact** is the main contact and is responsible for communicating deliverables among OMAP authors and reviewers.

The **Lead Antibody Validation Report (AVR) Contact** is the main contact responsible for communicating deliverables among AVR contributors.

The **Lead Pathologist** is the main contact responsible for reviewing OMAPs for their coverage of anatomical structures, cell types, and biomarkers based on domain expertise in tissue anatomy and pathology.

The **Lead ASCT+B Integration and Publication Contact** is a person who works with subject matter experts on ASCT+B table authoring, revision and validation with expertise in the ASCT+B Reporter, its functionality, and application to OMAP visualization and review. This

person coordinates communication between ASCT+B and Affinity Reagent Imaging and Validation Working Groups.

The **Lead OMAP Visualization Contact** is the main contact responsible for defining the requirements for visualization and annotation of datasets accompanying published OMAPs. This person additionally creates graphics and display items for publications and presentations related to OMAPs.

The **Principal Investigator (PI)** approves budgets associated with OMAP construction, sets high-level goals and associated deadlines, attends meetings, and reviews notes and recordings from relevant meetings between the Lead OMAP Contact, Subject Matter Experts, and other key personnel as needed.

The **Affinity Reagent Working Group (ARWG) Technical Project Manager** communicates the agenda for monthly meetings and facilitates the integration of antibody validation reports (AVRs) and OMAPs.

The **Human Reference Atlas (HRA) Technical Project Manager** works with ARWG personnel to define goals, monitor progress, facilitate communication across project leads, and provide project updates to PIs.

Table 1. Roles, names, and email addresses of key personnel.

Role	Name	Email
OMAP Author(s)	Depends on organ and assay	
OMAP Reviewer(s)	Depends on organ and assay	
Lead OMAP Assembly Contact	Andrea J. Radtke	andrea.radtke@nih.gov
Lead Antibody Validation Report (AVR) Contact	Elizabeth McDonough	elizabeth.mcdonough@ge.com
Lead Pathologist	Christopher Werlein	Werlein.Christopher@mh-hannover.de
Lead ASCT+B Integration and Publication Contact	Ellen M. Quardokus	ellenmq@indiana.edu
Lead OMAP Visualization Contact	Diane Saunders	diane.saunders@vumc.org

Principal Investigators	Katy Börner Neil Kelleher Ronald N. Germain	katy@indiana.edu n-kelleher@northwestern.edu rgermain@niaid.nih.gov
ARWG Technical Project Manager	Michael Caldwell	michael.caldwell@northwestern.edu
HRA Technical Project Manager	Abraham Verdoes	averdoes@iu.edu

What is an Organ Mapping Antibody Panel (OMAP)?

The concept of an antibody panel, a collection of antibodies used to define the cellular composition of tissues, is widely established in flow cytometry. Flow cytometry uses antibodies directed against protein biomarkers to phenotype cells prepared from dissociated tissues. A major obstacle shared by flow cytometry and multiplexed antibody-based imaging is the identification of appropriate reagents that yield robust performance while minimizing artifacts. Within the field of flow cytometry, commercial vendors and domain experts have responded to this challenge by generating online panel design tools and publishing peer reviewed Optimized Multicolor Immunofluorescence Panels (OMIPs) (Mahnke, Chattopadhyay, and Roederer 2010; Roederer and Tárnok 2010). We aim to expand these efforts by creating organ mapping antibody panels (OMAPs) for multiplexed tissue imaging. OMAPs will be rigorously tested to eliminate technical challenges such as steric hindrance, epitope loss, spectral overlap, and native tissue autofluorescence (Du et al. 2019; Radtke et al. 2020; Lin et al. 2018; Hickey et al. 2021). Furthermore, the deposited OMAPs will include details on 1) tissue preservation and imaging method, 2) antibody clones, isotypes, and conjugates, 3) rationale for selected markers in relation to organ-specific anatomy and/or cell types, 4) designation of 4-6 core markers that maximize cell type detection for lower parameter assays, and 5) relevant notes to aid other users in implementation, e.g., links to protocols and published work. Importantly, these OMAPs will be integrated with the Anatomical structures, Cell types plus Biomarkers (ASCT+B) Reporter <https://hubmapconsortium.github.io/ccf-asct-reporter/> to support tissue mapping efforts across consortia. Please see OMAPs frequently asked questions (FAQs) on the HRA portal for more details.

Prior Knowledge and Skills Required

Prior knowledge in the following areas is required for OMAP construction: 1) sample preparation and tissue handling, 2) antibody validation and titration, and 3) custom antibody conjugation. A keen understanding of factors influencing antibody performance such as antigen retrieval conditions, fluorophore conjugate, and epitope abundance and availability is helpful. In addition to skills associated with sample preparation, immunolabeling, and antibody validation, OMAP authors are expected to have microscopy expertise and/or prior success with imaging platforms used for the construction of their OMAP. Expertise related to minimizing common imaging artifacts such as spectral overlap between adjacent fluorophores, native tissue autofluorescence, and antibody cross-reactivity is additionally required to ensure collection of high quality data. If employing cyclic imaging methods,

authors must evaluate the potential impact of cycling order and dye inactivation on antibody performance before submitting their OMAP.

A *Nature Methods* Perspective authored by the HuBMAP consortium entitled, “Spatial mapping of protein composition and tissue organization: a primer for multiplexed antibody-based imaging”, provides guidelines and resources for the topics listed above (Hickey et al. 2021). This primer includes details and strategies for the following:

- 1) marker selection (see Table S2)
- 2) identifying suitable antibody clones (see Table S3 and Supplementary Dataset with clone list)
- 3) considerations for creating custom reagents (see Figure 4)
- 4) workflow and references for antibody validation
- 5) databases for assessing the tissue and subcellular location of a protein target (see Table S2)
- 6) strategies for minimizing native tissue autofluorescence (see Table S5).

This work also outlines the steps for panel construction and full panel validation (see Figure 3).

Additional guidance on constructing multiplexed imaging panels is found in a helpful protocol authored by developers of the cyclic imaging method t-CyCIF (Du et al. 2019).

A detailed protocol on how to gross examine and optimally prepare human tissues for highly multiplexed fluorescent imaging has also been reported (Radtke, Chu, et al. 2022).

If you are new to multiplexed tissue imaging and would like to know more, then please email the ARWG Technical Project Manager to receive an invite to our open working group.

Overview of Design Criteria

- **Core organ-relevant markers.** Authors should designate 4-6 markers that, when used together, allow profiling of major anatomical structures and cell types in a given tissue. This aids panel construction for lower-plex imaging approaches.
- **Blood vasculature marker(s).** The inclusion of antibodies directed against one or more blood endothelial markers (e.g., CD31) is strongly encouraged to support the construction of a human reference atlas using the Vasculature Common Coordinate Framework (VCCF) (Galis 2020; Weber, Ju, and Börner 2020; Boppana et al. 2022).
- **Lymphatic vasculature marker(s).** The inclusion of antibodies directed against one or more lymphatic endothelial markers (e.g., LYVE1) is highly recommended to further our understanding of the “yet to be charted” human lymphatic system (References and links: (Radtke, Lukacs, et al. 2022; National Heart, Lung, and Blood Institute (NHLBI) and National Institutes of Health (NIH) 2021; National Heart, Lung, and Blood Institute (NHLBI) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) 2022)).
- **Additional organ-relevant markers.** Beyond the 4-6 core markers, the OMAP will include antibodies that facilitate biological exploration of the specified organ. At a minimum, an OMAP should allow 10 or more unique biomarkers to be visualized in a single tissue section. An ideal panel will be as comprehensive as possible (20-60+ markers as technologies advance). We ask experts to select antibodies that capture

the greatest number of cell types and anatomical structures based on their domain knowledge, survey of the literature, consultation with pathologists, and review of the ASCT+B table for their organ.

- **Link(s) containing imaging method details.** We do not require the reporting of imaging parameters or microscope configuration. Instead, we assume these details are included in your supporting protocols and accompanying peer-reviewed manuscript(s). Please ensure that these details are included and formatted according to guidelines in the field (Schapiro et al. 2022).
- **Supporting materials.** Beginning December 2023, each contributed OMAP will contain the following components in addition to the OMAP Table and OMAP Description Document: 1) completed AVR for each antibody included in the OMAP and 2) DOI for a representative dataset in a public repository such as the HuBMAP data portal or Zenodo.

Before You Begin: Gather Templates and Reference Materials

- To complete the OMAP Table, make a copy of the [table template](#).
- The first 10 rows of the OMAP table template are reserved for the following information:

Table 2. Author and reviewer details included in OMAP Table header.

Row	Description	Instructions and format	Examples
Row 1	Title	Authors should fill in the organ name and the multiplexed imaging technique. Authors should leave the OMAP-N ID blank (will be filled in upon DOI registration and publication to the HRA portal).	OMAP-1: Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lymph Node with IBEX
Row 2	<i>Intentionally left blank</i>		
Row 3	Author name(s) (up to 3)	Lead author first; co-authors following. First name, middle initial, last name.	Jane M. Doe; John Q. Public
Row 4	Author(s) ORCID(s)	Same order as author names in row 3. Use semicolons between IDs.	0000-0000-0000-0000; 0000-0000-0000-0000
Row 5	Reviewer name(s) (up to 6)	Include both internal and external reviewers. First name, middle initial, last name.	Jackie M. Doe; Jessie Q. Public
Row 6	Reviewer(s) ORCID(s)	Same order as reviewer names in row 5. Use semicolons between IDs.	0000-0000-0000-0000; 0000-0000-0000-0000
Row 7	General publication(s)	Please provide links to major papers validating the imaging method in DOI format. Separate citations using semicolons.	DOI:10.1016/j.exphem.2018.09.004
Row 8	Data DOI	Reserved for the DOI that will be registered for this OMAP.	<i>Leave this field blank</i>
Row 9	Date	Date that table is frozen for publication.	<i>Leave this field blank</i>
Row 10	Version number	Table version number added upon publication to the HRA Portal with DOI.	<i>Leave this field blank</i>

Row	Description	Instructions and format	Examples
Row 11	Column headers	Metadata fields harmonized between the ASCT+B Reporter visualization tool and AVRs. See Table 3 for more details.	Authors should NOT alter the names.

- OMAP authors should fill in the required information about their antibodies as outlined in [Table 3](#) Metadata schema for OMAP Table columns.
- To complete the OMAP Description Document, make a copy of the [description template](#) and fill in with a short description (<300 words) detailing the method, number of total markers, fiducial used for image alignment across cycles (if applicable), markers used for downstream data analysis, links to publications and protocols, and relevant information related to OMAP implementation.
- When your OMAP Table and OMAP Description Document are complete and ready for review, please notify the Lead OMAP Assembly Contact and Lead ASCT+B Integration and Publication Contact by email (see [Table 1](#) Roles, names, and email addresses of key personnel).
- All table authors and reviewers should provide their ORCID. To register, please visit <https://orcid.org>.
- For [Frequently Asked Questions \(FAQs\)](#) please visit the [OMAP page](#) on the HRA portal.

OMAP Construction Procedures

1. Marker Selection

There are several approaches for identifying protein biomarkers for spatial mapping efforts. The HuBMAP mapping component at Indiana University (MC-IU) has worked with domain experts to compile machine readable tables that capture essential anatomical structures and cell types plus biomarkers (ASCT+B) for a variety of organs which include semantic annotation (Börner et al. 2021).

The steps involved in the creation of an OMAP tailored to a tissue of interest are:

- Identify the major anatomical structures and cell types for your organ of interest using the [ASCT+B Reporter \(see documentation\)](#) and associated ASCT+B tables.
 - Select “All organs” or the specific organ of choice, making sure you choose the newest version for each organ for the most up-to-date information. To download a spreadsheet of the data displayed, use the [Report feature](#).
- If an ASCT+B table does not exist for your organ of interest, see the resources outlined below for more information:
 - Consider becoming an ASCT+B table author for your organ of interest by registering [here](#).
 - Review introductory slides for the original Common Coordinate Framework (CCF) NIH-HCA session [here](#).
 - Access video recordings of the ASCT+B working group [here](#).
 - Use Google Scholar and/or PubMed to identify the anatomical structures, cell types, and protein biomarkers others are targeting in your organ of interest.

- c. To identify essential biomarkers for major cell populations please see:
 - i. CellMarker database: (Zhang et al. 2019)
 - ii. [BioLegend essential mouse versus human markers](#)
 - iii. [Immune Cells](#)
 - iv. List of other cell types: [Antibody Review - Cell Markers Review](#)

2. Antibody Selection

- a. We recommend starting with highly cited antibody clones validated for your tissue preservation method (e.g., FFPE, fixed frozen) and antigen retrieval conditions, if applicable. Please see clone lists compiled below.
 - i. [List of community-validated antibody clones](#) targeting common human and mouse protein markers across different highly multiplexed imaging technologies.
 - ii. [Organ Mapping Antibody Panels on the HRA Portal](#)
- b. For markers not listed above, use the following resources for discovery efforts.
 - i. Antibody search engines: See Table S3 in (Hickey et al. 2021) for more details.
 - 1. [CiteAb - The Life Science Data Provider](#)
 - 2. [BenchSci: AI-Assisted Reagent Selection and Experiment Design](#)
 - 3. These websites allow you to compare antibodies from different companies. Narrow your results to include antibodies that are:
 - a. Validated for immunohistochemistry (IHC). Unconjugated antibodies validated for IHC of formalin-fixed paraffin-embedded (FFPE) samples often work as fluorophore-conjugated antibodies for FFPE or tissues fixed using 1-4% paraformaldehyde. Additional information on antigen retrieval conditions, recommended titrations, and appropriate positive and negative controls can often be found on vendor sites.
 - b. Prioritize monoclonal antibodies as they offer the least lot-to-lot variation as outlined in (Hickey et al. 2021).

3. Antibody Validation and Authoring Antibody Validation Reports (AVRs)

- a. Validate each antibody to be included in your OMAP using established guidelines which include:
 - i. Evaluating cellular location, spatial pattern, and co-localization with orthogonal markers in positive and negative control tissues.
 - ii. Resources for evaluating the cellular location and tissue distribution of protein biomarkers can be found below and Table S2 in (Hickey et al. 2021):
 - 1. [The Human Protein Atlas](#)
 - 2. [UniProt.org](#)
 - 3. [GeneCards - Human Genes | Gene Database | Gene Search](#)

- b. For cyclic immunofluorescence methods select fluorophore-conjugated antibodies or fluorescent reporters in non-overlapping channels, e.g., AF488, AF555, AF647.
- c. If custom reagents are required for your OMAP, then please compare the antibody labeling pattern of the unconjugated primary antibody to the custom reagent.
- d. If you are a HuBMAP member, you may complete an AVR for each antibody as [outlined on the AVR site](#); if you are a non-HuBMAP member, please contact the Lead Antibody Validation Report (AVR) Contact (table 1).
- e. Publish AVRs to the HuBMAP AVR portal by filling out the AVR CSV (one antibody per row and links to the AVR PDF file per antibody).
- f. Please document antibodies that were tested but not included in your OMAP in the AVR associated with the same protein marker, e.g., CD4 clones not recommended in the same AVR as the validated CD4 antibody.

4. Full Panel (OMAP) Validation

- a. Compare antibody performance in full panel (OMAP) to single-plex or standard immunofluorescence assay using serial tissue sections.
- b. For cyclic multiplexed imaging assays, evaluate the impact of cycle number on antibody performance by applying antibodies in different cycles (cycle 1 versus cycle 10). Place antibodies that are sensitive to dye inactivation solution or antibody stripping conditions in earlier cycles.
- c. Eliminate non-specific interactions by careful panel design including the placement of unconjugated primary antibodies in the first cycle and direct conjugates in later cycles after appropriate blocking of potential antibody cross-reactivity.
- d. Identify antibody formats that perform well in your assay and fit into available slots in your panel (Cycle 2 AF647 channel). Oftentimes, clone performance is dependent on the fluorophore conjugate. In these instances, one may need to evaluate multiple conjugates to identify the best option for a particular antibody and tissue depending on native tissue autofluorescence and target abundance.
- e. Please see ([Hickey et al. 2021](#); [Du et al. 2019](#); [Lin et al. 2018](#); [McDonough et al. 2020](#); [Eng et al. 2022](#); [Radtke et al. 2020](#); [Radtke, Chu, et al. 2022](#); [Black et al. 2021](#)).

5. Fields and Instructions for Completing OMAP Table

- a. Start by downloading a copy of the [OMAP Table template](#). See [Table 2](#) Author and reviewer details included in OMAP Table header for guidelines on completing the first 10 rows of information. Row 11 contains metadata columns harmonized for integration with the ASCT+B Reporter visualization tool and the AVR database. Starting on row 12, list individual antibodies in your OMAP. Think of each row as a database record, where each column represents a field in the record. See [Table 3](#) Metadata schema for OMAP Table for required format of each field.
- b. Please only include primary or secondary antibodies in the OMAP Table. Specify the nuclear label (e.g., Hoechst or DAPI) used in your [OMAP Description Document](#).

- c. For secondary antibodies please leave the following fields blank in the OMAP Table: uniprot_accession_number, HGNC_ID, and target_name. In the 'rationale' field, please state "Use for detection of [insert unconjugated primary antibody here]."
- d. Provide the names and ORCIDs of 2-3 subject matter experts who reviewed your OMAP. Reviewers should have expert knowledge of the anatomical structures, cell types, and biomarkers in the organ of interest. If you have difficulty identifying experts, please contact the Lead ASCT+B Integration contact for assistance.

6. Instructions for Completing Supporting Documents

- a. OMAP Description Document
 - i. See [Table 4](#) for required and optional information to include.
 - ii. Specify the method, number of markers used, and nuclear label.
 - iii. You are encouraged to link to publications and protocols, as well as any relevant information related to OMAP implementation.
 - iv. Please report any challenges encountered in OMAP construction, e.g., antibody sensitivity to cycle order, the need to amplify with a secondary antibody for low abundance epitopes, or inability to identify an appropriate antibody for a key cell type.
- b. OMAP AS, CT, and BProtein Table
 - i. In the [ASCT+B Reporter](#), choose the most recent version of the ASCT+B table for your organ. If your organ is not represented in the current ASCT+B tables, please contact the Lead ASCT+B Integration contact for assistance.
 - ii. Click on the 'Report' icon in the top right menu, then click the download icon.


 - iii. In the generated Excel file, delete all columns except the two entitled 'Unique Anatomical Structures' and 'Unique Cell Types' (these are usually the first two columns). Bold all AS and CT that are identifiable by the markers in your OMAP.
 - iv. For each cell type that you bold, list in the next column all protein biomarkers that can be used to identify that cell type.
 1. If applicable, bold the marker that is considered a single "core" marker for this cell type. List the bolded "core" marker first.
 2. Please capitalize each biomarker name and separate multiple markers with commas.
- c. Representative dataset
 - i. Upload a dataset (image and metadata) using your OMAP to a public repository of your choice (e.g., Zenodo, HuBMAP Data portal). For assistance, please contact the Lead OMAP Visualization Contact ([Table 1](#)).
 - ii. Requirements for the representative dataset and associated metadata are based on the [Minimum Information about Highly Multiplexed Tissue Imaging \(MITI\)](#) (Schapiro et al. 2022) guidelines. They include:

1. Image(s) that can be separated and/or viewed by individual biomarkers
 - a. Example formats: OME-TIFF, QTIFF
 - b. Please rename each channel in your image with the corresponding target name (target_name) in your OMAP Table.
2. Include a description of the sample (e.g., tissue microarray, cross-section, etc.), relevant imaging parameters, and experimental details (or link to associated publication and/or protocol).
 - a. Example: Dataset is a 24 parameter IBEX experiment performed on a human jejunum section labeled with the nuclear marker Hoechst and antibodies directed against the indicated markers. Images were acquired using an inverted Thunder 3D Cell Culture microscope with a high precision (Quantum) stage, a high quantum efficiency sCMOS camera (DFC9000 GTC), and a 40x (1.3) NA oil objective. The light source was an LED8 with 8 individual LED lines for excitation with millisecond triggering. All images were captured at a 16-bit depth with the following pixel dimensions: x (0.160 μm), y (0.160 μm), and z (1 μm). Images were tiled and merged using the LAS X Navigator software (LAS X 3.7.1.21655).
- iii. Recommended (optional) data:
 1. Cell segmentation or other masks derived from segmentation algorithms
 - a. Example: Dataset [HBM443.FDHZ.888](#); see file reg001_mask.ome.tif
 2. Spatial feature table (e.g., rows represent single cells and columns represent image parameters such as intensity values)
 - a. Example: <https://doi.org/10.5061/dryad.dfn2z352c>
- iv. Include a link to your dataset (in DOI format, <https://doi.org/...> or <https://dx.doi.org/...>) in your [OMAP Description Document](#).

7. OMAP Review Process

Prior to publication, all OMAPs undergo a two step review process consisting of internal and external evaluation. The roles and responsibilities of each review step are outlined below:

1. Internal Review
 - a. Responsible parties: OMAP authors and members of contributing laboratory
 - b. Tasks:
 - i. Please identify 2-3 experts in pathology, cell biology, anatomy, histology, and/or multiplexed tissue imaging to review your OMAP before submission. If you cannot find suitable reviewers, then please contact the Lead ASCT+B Integration expert for assistance. All reviewers should register for an [ORCID](#).
 - ii. Quantify the number of AS, CT, and BProtein profiled by your OMAP using the ASCT+B table for your organ as a reference (see Section 6b). Please contact the Lead OMAP Assembly contact, Lead ASCT+B

Integration and Publication contact, and Lead OMAP Visualization contact via email ([Table 1](#)) for support. Record these details in your OMAP Description Document. These details will be published on the portal along with your OMAP.

2. External Review
 - a. Responsible parties: pathologists with understanding of the organ profiled by your OMAP
 - b. Tasks:
 - i. Pathologists (Identified by HuBMAP consortium)
 1. Review OMAP for accuracy regarding coverage of anatomical structures and cell types.
 2. Review rationale for marker inclusion provided in the OMAP Table and suggest edits as needed.
 3. Comment on use of antibodies and/or markers used to phenotype cell types.
 4. Highlight cell types and anatomical structures not covered in OMAP that should be discussed in the OMAP Description Document.

8. OMAP Submission and Publication Process

Please contact the Lead OMAP Assembly Contact, Lead ASCT+B Integration and Publication Contact, and Lead OMAP Visualization Contact via email ([Table 1](#) Roles, names, and email addresses of key personnel) when these required documents are ready for review:

Required Documents

(see detailed instructions in indicated sections)

- a. OMAP Table (Section 5 Fields and Instructions for Completing OMAP Table)
- b. OMAP Description Document (Section 6a)
- c. OMAP AS, CT, and BProtein Table (Section 6b)
- d. Completed AVRs for each antibody included in the OMAP (Section 3; required by December 2023)
- e. DOI for representative dataset in a public repository (Section 6c; required by December 2023)

These steps will be followed to ensure that the submitted OMAP conforms to the standards described in this SOP:

1. The OMAP Table will be evaluated for proper formatting and completion of all fields.
2. The OMAP Description Document will be evaluated for content and adherence to the instructions provided.
3. The completion of AVRs for each antibody included in the OMAP will be verified (required by December 2023).
4. The OMAP AS, CT, and BProtein Table will be evaluated for completion.

5. The inclusion of a representative dataset in a public repository will be confirmed (required by December 2023).
6. The OMAP will be given a unique identifier based on the date of approval, e.g., OMAP-1, OMAP-2.
7. The OMAP will be given a unique DOI and published on the HuBMAP Human Reference Atlas (HRA) portal [OMAPs page](#).

Table 3. Metadata schema for OMAP Table columns.

Field name in OMAP table	Description	Required format	Examples
uniprot_accession_number	Identifies the target protein (see https://www.uniprot.org). For human protein panels, be certain to include human protein designation. If UniProt ID cannot be found for the antibody, please leave the field blank.	Alphanumeric (see https://www.uniprot.org/help/accession_numbers); for multiclonal (pan-) antibodies, list IDs for all targeted proteins as a comma delimited list.	A2BC19, P12345, Q9BZS1
HGNC_ID	Gene identification number from Human Gene Ontology Nomenclature Committee (HGNC) https://www.genenames.org/ encoding the target protein.	HGNC:####	HGNC:4947, HGNC:3612
target_name	Provides a common name for the target protein being detected by assay. Please spell out Greek letters (alpha, beta, gamma, delta, etc).	Commonly used name or protein abbreviation	CD20, ICAM, Somatostatin, Alpha smooth muscle actin
host	Identifies the species in which the antibody was raised.	Capitalize first letter	Rabbit, Donkey, Mouse
isotype	Describes the antibody isotype.	Please write out any symbols.	IgG, IgG1, IgG1 kappa
clonality	Provides the clone ID (if monoclonal) or identifies the antibody as polyclonal.	List clone as provided by the manufacturer. If polyclonal, enter 'Polyclonal.'	L26, EPR5386, Polyclonal
vendor	Provides information on the source of the antibody.	Vendor name	Cell Signaling Technology, Abcam, BioLegend
catalog_number	Provides catalog number from vendor for the source of the antibody.	Vendor catalog number	Ab9566, sc-20060, C6198
lot_number	Allows for monitoring of lot-to-lot variation.	Number listed on antibody vial. If lot numbers are not available, please leave this field blank.	B256112, 16A5T1, KPY01191
recombinant	Classifies the antibody as recombinant or not. Recombinant antibodies (rAbs) are monoclonal antibodies which are generated in vitro using synthetic genes.	Indicate Y for yes, N for no.	Y, N

Field name in OMAP table	Description	Required format	Examples
concentration_value	Provides a recommended usage in standardized units (µg/mL). If providing dilution instead, leave this field blank.	Numeric only (units standardized)	5, 2.6, 0.554
dilution	Provides a recommended dilution factor. If providing a concentration instead, leave this field blank.	1:#### (please use colon and not a slash or backslash)	1:100, 1:50, 1:2000
conjugate	Specifies addition to the antibody (e.g., fluorophore, heavy metal, oligonucleotide) enabling detection, if applicable.	For fluorophores, please use industry standard abbreviations (e.g., AF for Alexa Fluorophore™).	AF647, PE, Oligonucleotide, 146Nd, Atto550
rrid	Allows for universal identification of an antibody (search by catalog number at https://scicrunch.org/resources). If there is no entry, please register your antibody.	AB_#####	AB_793620, AB_10124480
method	Specific multiplexed antibody-based imaging technique.	Provide standard abbreviation of method	CODEX, IBEX, Cell DIVE, SIMS
tissue_preservation	Preservation technique used. If fixative other than formalin, indicate the percentage of fixative indicated (e.g., 1% or 4%).	Use a common abbreviation format (e.g., FFPE for formalin fixed paraffin embedded).	FFPE
cycle_number	Identifies the cycle number in which an antibody was either applied to the tissue or, in the case of CODEX, visualized with a fluorescent reporter. For non-cyclic methods use 1 for all cycles.	Numeric	6, 1, 14
fluorescent_reporter	For indirect visualization (e.g., oligo-conjugated antibodies), define the fluorescent reporter utilized in the corresponding cycle. For metal or fluorophore-conjugated antibodies, please leave blank.	Fluorophore only. Please use industry standard abbreviations (e.g., AF for Alexa Fluorophore™).	AF488, Cy3, Atto550
protocol_doi	Details the protocol used to validate the antibody, including positive and negative controls and example images. To enhance reproducibility, we recommend that all protocols be made publicly available via protocols.io or other open protocol repositories. Alternatively, a manuscript may be included if sufficient details are included in the methods and supplementary information.	https://doi.org/... or https://dx.doi.org/...	https://dx.doi.org/10.17504/protocols.io.bqjimuke
author_orcid	Identifies the individual who validated the antibody used in the assay.	####-####-####-#### (the last digit may be X)	See https://info.orcid.org/researchers/

Field name in OMAP table	Description	Required format	Examples
core_panel	Indicates whether the author considers the antibody to be part of the minimum (4-6) antibody panel for the tissue.	Indicate Y for yes (included in Core Panel), N for no.	Y, N
rationale	Why was the antibody included in the panel? What anatomical structures, cell types, or cell states of the tissue does it demonstrate? Cell state markers are highly context-specific and may be included, but are not the primary focus of the OMAP effort. Please indicate whether a marker should be prioritized based on its ability to define key cell lineages and/or multiple cell types and structures.	Preferably lists anatomical structures and cell types identified by the antibody in the panel.	For CD20 in the human lymph node OMAP, "Defines B cell follicles and is an essential marker of B cells. This marker represents a prioritized target in the lymph node OMAP.

Table 4. Information to include in OMAP Description Document (300 words or less).

Order in paragraph (Inclusion: Required or Optional)	Description	Example
1 (Required)	Include OMAP name, multiplexed imaging method, and tissue preservation method with protocols and publications DOIs (up to 3 for this field). If applicable, reference landmark study employing this OMAP.	OMAP-5 was designed for Secondary Ionization Mass Spectrometry (SIMS) imaging of fresh frozen human liver samples (https://doi.org/10.1101/2022.09.26.508878).
2 (Required)	Specify the number of antibodies included in your OMAP and the nuclear marker used for image alignment, if applicable.	The panel contains 54 antibodies and the nuclear marker Hoechst for image alignment and nuclear segmentation.
3 (Required)	Highlight overlap with ASCT+B tables and include link to organ-specific ASCT+B table (see Section 6b for detailed instructions).	This OMAP provides a spatial context for all anatomical structures and most cell types present in the ASCT+B lung table v1.1.
4 (Optional)	Discuss other OMAPs or multiplexed imaging studies relevant to your organ. Please include DOIs to existing OMAPs or closely related studies.	This OMAP builds from OMAP-1 (link to DOI) and additionally includes: [list markers]. The core and essential protein biomarkers detailed here overlap (>40%) with a panel developed for multiplexed imaging of kidney samples using imaging mass cytometry (IMC) (10.1172/jci.insight.129477).
5 (Required)	Discuss key cells that are missing from OMAP due to the lack of suitable reagents, if known.	Acinar and epsilon cells are not targeted in the existing panel due to the complexity of finding suitable antibodies.

Order in paragraph (Inclusion: Required or Optional)	Description	Example
6 (Optional)	Highlight specific application of OMAP, if any, besides mapping healthy tissues.	The inclusion of protein biomarkers DDB2 and p53 allow profiling of skin damage and aging effects.
7 (Optional)	Discuss the analytical pipeline and markers used for downstream image analysis, if applicable, with DOIs for relevant publications (up to 3 for this field).	Object-based cellular segmentation was performed using a convolutional neural network (CNN)-based approach with Mask R-CNN architecture and ResNet-50 as a backbone. As an input three channels were used: Hoechst for nuclei, CD45 as a base membrane, and composite of several other membrane markers (CD138, CD163, CD94, CD69, CD8, CD4) (https://doi.org/10.1101/2022.06.03.494716).
8 (Required)	Include links to relevant protocols, accompanying datasets, and other resources, e.g., validated antibody repository for your method (up to 3 for this field).	Additional details on sample preparation, antigen retrieval, and the Cell Dive method can be found here (Protocols.io DOI). Representative datasets using this OMAP can be found here (Public repository DOI, e.g., 10.5281/zenodo.5244551). See (web link or DOI) for antibodies validated for this method.
9 (Required for FFPE samples)	Include the method (buffer, temperature, time, device) used for antigen retrieval and link to a detailed protocol or publication (see 8).	Following antigen retrieval (citrate buffer pH=6, heating at high pressure 114-121°C for 20 minutes), tissue autofluorescence was reduced using a photobleaching protocol (https://doi.org/10.1038/s41596-019-0206-y , modified by Derek Oldrige).
10 (Optional)	Discuss any custom conjugations or special detection methods, if applicable.	Protein targets were detected using either primary antibodies with fluorophore-labeled secondaries or fluorescent dye-conjugated antibodies. Several targets required custom conjugation to unique fluorophores by commercial suppliers or antibody conjugation kits (e.g., AF532, Thermo A20182).
11 (Required)	If applicable, note if an antibody should be placed in an earlier cycle to avoid loss of immunogenicity with cycle number.	Antibodies directed against CD106 (RRID: AB_314561) are known to perform better in cycle 1 than in later cycles.
12 (Required) By December 2023	Include a link to a PDF with Antibody Validation Reports (AVRs) for each antibody included in your OMAP.	AVRs for each antibody in OMAP can be found here (PDF).

Acknowledgments

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Glossary

Anatomical Structures (AS): Parts of the body in defined locations and regions, including the surface, internal organs, and tissues. These structures may be described by gross or microscopic morphology and include functional tissue units and highly organized cellular ecosystems (such as alveoli in the lungs).

Antibody Validation Report (AVR): Document providing details on the characterization of individual antibodies for multiplexed antibody-based imaging assays. These details include target information (e.g., target name, UniProt accession number, and antibody information (e.g., RRID, host organism, vendor, catalog number, lot number). AVRs also provide details on

controls used for antibody characterization (positive and negative tissues, cell lines, isotype controls, etc), exemplar imaging data, and information on other antibodies tested.

Biomarkers (B): Molecular, histological, morphological, radiological, physiological or anatomical features that help to characterize the biological state of the body. Here we focus on the molecular markers that can be measured to characterize a cell type.

Cell DIVE: The Cell DIVE multiplexed imaging solution is an integrated system for imaging 60+ biomarkers from one tissue sample at the single-cell level. The tissue-preserving staining and dye inactivation workflow and advanced image processing enables a more comprehensive understanding of the tissue microenvironment than has been available with traditional chromogenic- or fluorescence-based imaging. ([Gerdes et al. 2013](#)).

Cell Types (CT): Biological units of metazoans with a defined function, typically having a nucleus and cytoplasm surrounded by a membrane. Each cell type may have broad common functions across organs and specialized functions or morphological or molecular features within each organ or region (for example, epithelial cells in the skin, lungs and kidneys may have shared and specialized functions according to tissue localization).

CO-Detection by indEXing (CODEX): Multiparameter imaging method that relies on oligonucleotide-conjugated antibodies and the cyclic addition and removal of complementary fluorescently labeled oligonucleotide probes. The commercial fluidics and high-plex image acquisition platform (Akoya Biosciences, Marlborough, MA) for this method enables whole slide imaging of up to 100 biomarkers on tissue samples in situ (Black et al. 2021; Goltsev et al. 2018; Kennedy-Darling et al. 2021).

Conjugate: Fluorescent dye, oligonucleotide, metal or enzyme covalently attached to a primary or secondary antibody that is used for downstream detection of the antibody bound to its target in/on a cell.

Fixed Frozen: Method of tissue preservation where samples are placed in fixative (e.g., formalin), cryoprotected with sucrose, frozen in molds with optimal cutting temperature (OCT) media, and stored at -80C.

Formalin Fixed Paraffin Embedded (FFPE): Most common form of tissue preservation where tissues are saturated with formalin and then embedded in a block of paraffin wax. Samples are stored at room temperature.

Fresh Frozen (FF): Method of tissue preservation where samples are frozen at ultralow temperature without fixative (fresh), immobilized in cryosafe media (e.g., OCT, carboxymethyl cellulose), and stored at -80C.

Imaging Mass Cytometry (IMC): Multiplexed imaging method that employs metal-conjugated antibodies applied in a single master mix to tissues. Target-specific antibodies are detected using laser ablation coupled to mass cytometry ([Giesen et al. 2014](#)).

Immunofluorescence (IF): Laboratory technique used to visualize proteins in cells or tissues using specific primary antibodies conjugated to fluorescent dyes.

Immunohistochemistry (IHC): Laboratory technique used to visualize proteins in cells or tissues using specific primary antibodies conjugated to chromogens.

Immunostaining with Signal Amplification by Exchange Reaction (Immuno-SABER): A highly multiplexed and signal amplification method free of in situ enzymatic reactions that utilizes oligonucleotide-conjugated antibodies and complementary fluorescent imagers ([Saka et al. 2019](#)).

Iterative Bleaching Extends Multiplexity (IBEX): An open source iterative immunolabeling and chemical bleaching method for high-content imaging of diverse tissues ([Radtke et al. 2020](#); [Radtke et al. 2022](#)).

Iterative Indirect Immunofluorescence Imaging (4i): A cyclic high-content imaging method for visualizing cellular and subcellular structures in situ ([Gut, Herrmann, and Pelkmans 2018](#)).

MACSima Imaging Cyclic Staining (MICS): Immunofluorescent imaging method for hundreds of protein targets across a single specimen at subcellular resolution based on cycles of staining, imaging, and erasure. This method uses photobleaching of fluorescent labels of recombinant antibodies (REAffinity Antibodies) or release of antibodies (REAlase Antibodies) or their labels (REAdye_lease Antibodies). The commercial platform (MACSima Imaging System) is a fully automated instrument based on fluorescence microscopy, recombinant ready-to-use antibody-fluorochrome conjugates, sample carriers and a MACS iQ View Analysis Software for data analysis ([Kinkhabwala et al. 2022](#); [Schäfer et al. 2021](#)).

Multiplexed Ion Beam Imaging (MIBI): Multiplexed imaging method that employs metal-conjugated antibodies applied in a single master mix to tissues and an ion beam for tag ionization ([Angelo et al. 2014](#); [Lomeli et al. 2021](#)).

Open Researcher and Contributor ID (ORCID): <https://orcid.org/>; unique identifier number researchers own and control that connects the iD with professional information – affiliations, grants, publications, etc. and can be used during the publication process.

Organ Mapping Antibody Panel (OMAP): A comprehensive panel of curated antibodies that identifies the major anatomical structures and cell types present in a specific organ. The selected antibodies are optimized for a tissue preservation method and multiplexed imaging modality.

OMAP Table: File required for all contributed OMAPs. The file is created in a shareable Google sheet that summarizes all antibodies and reagents used in the OMAP and provides details on the successful use and rationale for selected antibodies. The published DOI'd versions exist as both .csv and .xlsx.

OMAP Description Document: File required for all contributed OMAPs. The text file provides a short description (< 300 words) of the OMAP with details on the tissue preservation method, multiplexed imaging method, fiducial (e.g., DAPI), antigen retrieval conditions, and total number of antibodies used. This document also lists cell types and

structures not covered by the OMAP, names of contributors (authors and reviewers), and funding sources.

Primary Antibody: An immunoglobulin made in response to a specific antigen (often a protein) that causes an immune response in an animal. These antibodies can be used for research purposes to detect the corresponding antigen in or on cells in a tissue. An antibody binds to an antigen or protein target directly. Primary antibodies are most often conjugated covalently to a fluorescent dye, oligonucleotide, metal or enzyme for downstream detection methods.

Prioritized OMAP Target: A protein target that defines major anatomical structures and/or cell types within an OMAP. OMAP authors and reviewers can designate up to 25 biomarkers in their OMAP as Prioritized OMAP Targets in the “rationale” section of their OMAP Table. All core markers should be designated as Prioritized OMAP Targets.

Research Resource Identifiers (RRIDs): Unique identifiers for referencing a biomedical reagent or resource. See <https://www.rrids.org/>.

Secondary Antibody: An antibody that is used to indirectly visualize a primary antibody. Secondary antibodies are made by immunizing a host animal with antibodies of a different species (e.g., goat anti-rabbit secondary antibodies derived from goat immunized with rabbit IgG) and are used to detect species- and isotype-specific primary antibodies (e.g., goat anti-rabbit IgG paired with primary antibody made in rabbit). Secondary antibodies are most often conjugated covalently to a fluorescent dye, oligonucleotide, metal or enzyme for downstream detection methods and are sometimes used to amplify the signal of the antigen-bound primary antibody.

Secondary Ion Mass Spectrometry (SIMS): A technique capable of imaging tissues, single cells, and microbes revealing chemical species with sub-micrometer spatial resolution ([Gamble and Anderton 2016](#)). SIMS is used as a multiplexed imaging method by detecting metal-conjugated antibodies where an ion beam generates ejected ions detected with a mass spectrometer.

Subject Matter Expert (SME): A person with extensive knowledge about a given topic. In the procedures addressed here, the SME may have substantial experience with one, or all, aspects related to the cellular composition of human tissues, tissue preservation methods, multiplexed imaging techniques, integration with ASCT+B Reporter, and affinity reagents such as longitudinally stable antibodies.

Tissue-based Cyclic Immunofluorescence (t-CyCIF): A cyclic high-content imaging method employing commercial antibodies and diverse microscope configurations, based on the approach of chemically inactivating fluorophores after iterative rounds of immunofluorescence ([Lin et al. 2018](#); [Lin, Fallahi-Sichani, and Sorger 2015](#)). For more information, see <https://www.cycif.org/>.