# Report from the LungMAP Image Annotation Work Group (IAWG)

## Contents



## Summary

Representatives from the LungMAP Data Coordinating Center (DCC) and Research Centers (RC) formed a working group, the Image Annotation Work Group (IAWG), to investigate the use of a 2-dimension image annotation tool developed in-house for use with images submitted to the LungMAP BREATH database. The tool consists primarily of a drawing mechanism that allows the association of the LungMAP ontology term(s) and notes with anatomical features that are rendered on a 2-dimensional image. The resulting annotations provide a mechanism that facilitates the visual recognition and identification of anatomical structures and cells, as well as future search capabilities in BREATH.

The group performed a series of tasks, including 1) use of PowerPoint slides to demonstrate desired annotation of representative images from a scientific perspective, without any limits from a tool, 2) testing and providing feedback on the tool under development by the DCC, 3) developing instructions for annotating images using the tool, 4) training in the use of the tool, 5) completing an annotation exercise(s) using the tool and terms from the LungMAP ontologies, and 6) meeting to review experiences and best practice.

Based upon the outcomes of these activities, the IAWG recommends that the image annotation tool be released with minimal change for use by LungMAP researchers. The IAWG also recommends that the RC groups depositing images to BREATH identify a small number of individuals who will annotate and/or approve image annotations for their respective images for the public website. The IAWG also recognizes that the annotation tool itself requires additional development to be effective for both data integration and the development of instructional/educational images. Observations and

recommendations are presented and best practices will be developed to serve as a foundation for use of the tool.

## Background & Purpose

The LungMAP project has the remit to develop a molecular atlas of the developing lung to serve as a reference resource for the research community. To achieve such a resource, there must be an integration of various experimental results with established knowledge to create a "picture" of lung development, particularly in the stages of alveologenesis.

An important step in achieving this capability was the development of a detailed anatomical ontology that can be coupled to the images and other data types. This was considered a high priority. During the past two years the LungMAP Ontology Subcommittee, currently led by Drs. Susan Wert (CCHMC), Gail Deustch (Seattle/HTC), and Helen Pan (RTI), has developed rich ontologies for mouse developmental and human post-natal time points. These robust ontologies are now available for annotating experimental data, including images.

The LungMAP project, currently in its 3rd year, has amassed over 5000 images deposited into the consortium BREATH database. The images are grouped at the sample and experiment level (a series of images) with minimal metadata descriptions to inform the viewer. BREATH users can link to images from the Data Inventory based on the image type (e.g., immunofluorescence, in situ hybridization) and can search on gene probes associated with the images. Upcoming enhancements will enable additional search features including anatomical terms.

In March 2016, the BREATH development team initiated an effort to create an image annotation tool that integrates with the LungMAP anatomical ontologies. The IAWG was formed to evaluate the image annotation tool, whether the LungMAP ontologies were effective in annotating images, and determine how best to annotate the images to maximize the time spent at the task.

## Process & Summary of Results

The IAWG members from the DCC and the IAWG lead developed a charter, obtained agreement from the LungMAP Steering Committee to proceed, and outlined a series of tasks to be performed as part of the working group. Volunteers for the IAWG were solicited. The group used a forum website (http://discourse.lungmap.net/c/annotation) to manage the tasks. During the tasks, a core group of individuals evaluated the image annotation tool under development and provided feedback to the development team. The full team met a total of 3 times during the course of the workgroup, from March through July 2016.

The following tasks and results of each task are summarized below.

**Exercise 1:** A series of mouse P07 histology images were selected based upon clarity of the image and the representative structures. Instructions were provided as well as

copies of the appropriate ontology. Individuals were free to annotate the images as they saw appropriate.
*Result:* Two workgroup members completed the task. The scientists found the task was cumbersome, but provided insights into the number of annotations on an image that were useful as well as colors and the type of drawing features they liked (e.g., arrows). The exercise results were reviewed with the workgroup.

**Exercise 2:** A series of human histological images were selected for the exercise. Instructions again were provided as well as copies of the appropriate ontology.
*Result:* No one completed this exercise due to time constraints on members.

**Evaluation of the Tool prior to release for the IAWG:** The DCC members and the IAWG Lead spent time reviewing the tool prior to the execution of exercise 3 (below). A spreadsheet (https://docs.google.com/spreadsheets/d/1elRKx4yOHD9GT3RNYdEQHjUUXle4jFQQNlQgD95-toM/edit?pref=2&pli=1#gid=0) was used to capture and prioritize enhancements and changes to the tool. The DCC development team completed the major tasks before Exercise 3 commenced.
*Result:* Changes to the tool included addition of the arrow, ability to rotate the arrows on the image, changes to the colors available for annotation, moving the detailed information pop-up box on the image to prevent obscuring the location of the drawing on the image, and addition of an "instance" feature to minimize redundancy in labeling more than one example of an anatomic structure or cell. Search (based upon the anatomical terms on the image) and the request for new terms was not fully functional.

**Exercise 3:** A series of mouse confocal immunofluorescence images were identified in BREATH testing area, and the IAWG members were asked to use the annotation tool to annotate the images. The group met for a training session on the tool, and the session was recorded on WebEx for those who could not attend or wanted to review. A script was prepared to give stepwise instructions on how to annotate an image. The IAWG members completed a survey developed by the DCC WG reps to collect their impressions on the tool. The group met to review the survey results and develop the recommendations.
Result: Six IAWG members participated. Even with training the tool was not intuitive for proper and complete use. The participants did find the tool was effective. In general, the favorite mechanism to find terms was the search feature (i.e., of the ontology terms and synonyms). All drawing types were useful, although the dot and arrow were the most used. The color pallet was effective, although there was difficulty using Apple products (mouse sensitivity). The group suggested that the ability to use high-end devices, such as high-precision tablets, would be useful. Lastly, the group found the ontology terms were adequate to annotate the images, although it was recognized that the group was familiar with the ontologies and may be biased. Additional group recommendations are summarized below.

## Recommendations

The table below summarizes recommendations from the IAWG. This list is not exhaustive but were commonly expressed observations. Additional recommendations may follow after broader use.

| c | Recommendation | *Rationale* |
|---|---|---|
| **Tool/Web** | Tag/flag annotated images for searching or aggregation. Provide visual at the experiment level indicating that an image is annotated. | *Most critical for public use. Currently you cannot directly search for an annotated image or see which images are annotated until you open the viewer.* |
| | Offer a 'Publish Annotations' feature at the image level. Enables all terms and drawings to be made available to the public in one step. | *Review at the image level allows for quicker release to the public, while review at the term level offers the opportunity for full review before release to the public. Approval of each single term could delay release depending upon efficiency of the review process.* |
| | Allow annotator to make their annotated image public. | *Annotator is best judge, incorrect annotations can be fixed later during a to be established quality control process* |
| | Add Notes at the image level | *Allows the annotator to explain why they selected certain items for annotation and explain the importance of the image. Hiding such notes in a single annotation as currently done is often missed.* |
| | Add a 'vote' feature for viewers to agree/disagree with annotation at image level with room for comment | *Elicits feedback from community and offers a form of quality control.* |
| | System should track changes to annotations once posted publicly | *Will allow history and fluidity of the process to be studied* |
| | Offer a link for the annotator to send a message to DCC regarding tool suggestions | *Allows systematic way of collecting suggestions for quarterly review of possible enhancements* |
| | Offer ability to annotate an image with additional information (e.g., hypertext links within the notes area of terms or the image) | *Allows the annotator to supplement the annotation with additional resources.* |
| | Establish process for submitting suggestions for enhancements/changes to tool | *Need standardized method that is clear to users, message button linked to the tool?* |
| | Develop database reports that provide information on image annotations, e.g. # images annotated, non-public annotations, annotations with comments, etc. | *Will provide metrics on progress and identify annotations that may require additional review or follow-up or support QC efforts.* |
| **Annotation guides** | The IAWG does not recommend limits to the number of annotations for repeating structures. However consider annotating across the image rather than crowding. Quality, not quantity is suggested. A minimal number is recommended, leaving the annotator to spend as much time as they are willing on annotation. | *Facilitates finding an image based upon the annotation, allows the viewer to see the notation without obscuring image, reduces the burden on annotator time of the annotator. For antibody-stained images, focus on structures according to the probe, structures where the probes overlap rather than all structures.* |
| | Establish optimal number of images to annotate within an experiment. For | *Fewer "complete" images annotated will be more powerful than attempting to note* |

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| | example, annotate one high and one low magnification, or two images that are in different regions or showing different structures. | *redundant information on many images. Annotating subset of images in an experiment is reasonable, and is dependent on how divergent.* |
| | Establish optimal magnification level for specific type of annotation and associated terms. | *Certain annotation requires higher magnification to be optimally noted, e.g., cell types need higher magnifications.* |
| | Ensure that probe presence and intensity is consistent with cells/structures annotated | *Can act as a QC on displayed information for image.* |
| | Add Notes where appropriate to aid a person viewing the image. | *Can add additional information, similar to a figure legend in a paper.* |
| | For H&E and in situ, consider focusing on the marker genes and/or larger structures for annotation. | *Identification of specific cells may be more difficult on H&E and in situ hybridization images. Additional discussions on this will be beneficial.* |
| **Process/workflow guides** | Annotate your own image; get agreement from RC/PI to annotate their images | *Allows collaborative annotation with expectation that you work with others on joint interests* |
| | Annotate images going forward; annotate backlogged images or all images at RC discretion. | *Since annotation is an added task for centers, performing this activity is at RC discretion based on time and budget* |
| | Recognizing that there may not be time & resource to annotate images, the IAWG does not recommend any enforcement of annotation for images. | *Images without direct annotation remain valuable. Information provided in the experiment, sample, and image detail boxes can be used for initial interpretation. Grouping of images between lower and higher magnification may help to make the associations easier for a person viewing the experiment image series.* |
| | Allow for annotation at data submission stage, not wait until public posting of image. | *Will insure annotation gets done, otherwise there could be a backlog. Note: this will require additional development in BREATH data submission.* |
| | Display contact info for annotator so that comments can be provided | *Issue can be resolved b/t annotator and commenter and annotation can be changed if agreed* |
| **QC** | Establish a small number of 'administrators' who can make changes to annotations | *Requires high expertise in lung anatomy and intense interest in conveying to public. Dialogue should be established between original annotator and administrator before changes made.* |
| | RCs nominate annotators for images and determine mechanism for QC of their institution's images, and when to make annotations public. | *By having a select group, the annotator is best judge and control is at the institution level.* |
| | Establish a process to enable any individual to comment on an annotation, can be thumbs up (agree) or not as a counter. | *This will require additional system functionality, but is a way to identify if the larger community agrees to an annotation or not. May have to be monitored to prevent misuse.* |

## Next Steps: Documentation

Before the tool is offered for use, clear instructions need to be written describing the steps involved in its use. In addition, we will draft documentation on the expected workflow and other procedural issues needed for proper implementation of the annotation tool.

Instructions for tool use

As noted in the Recommendations table, we will draft a simple bullet list of steps to follow in order to create annotations on an image (along with illustrative examples). A few examples of items to address are the interaction of the selected symbol and the ontology, how to close out of the view without losing where you are, how to delete notations made in error, how to use the "instance" feature to eliminate redundancy. For the benefit of the annotator, we advise creating a short web tutorial to demonstrate tool use.

Best practices by image type

At this time, we are not certain whether the specific approach to annotation should vary by image type, but we do want to recognize that the basic differences in the types of information contained in immunofluorescence and other types of stained images may warrant different approaches to what is presented through annotation. Likewise, eventually we will need to consider the innate differences in annotating 2D versus 3D images. A major question will be whether the difference is simply a matter of expanded terminology or if a fundamentally different perspective is needed.

Workflow

The large number of images submitted to the DCC, suggest that allowing annotation at submission may be most efficient and effective. We envision inclusion of annotation terms as metadata when images are submitted to the DCC. Such information could act as a guide for actual annotation if the delay between submission and posting impedes immediate use of the tool and might also serve as "tags" that will allow searching and linkage to other data. Terms selected as metadata for annotation would be identified using the same ontology as used with the tool. Thus, the question of versioning is relevant to this step as well as the actual image annotation step.

Review pre and post public display

Discussions with the WG came to the conclusion that those who annotate should take responsibility for ensuring the annotation is done as accurately as possible. Approval prior to public posting should not be a requirement. If in doubt, annotators are able to consult within their center or across the consortium to ensure their confidence in the information provided.

After public posting, a small number of "super annotators" should have the ability to make changes if they detect errors. We will also look to the lung community to provide feedback on annotations. The best method for collecting this will be explored.

Requests for new ontology terms

Although rare, there may be instances when the desired term is not found. This will be dependent on the version of the ontology available to the annotators. It will be important to make the most complete version linked to the tool. We will define the process to follow when a term is truly missing and must be requested.

Requests for upgrades to tool

Changes to a released tool are not simple to incorporate. For this reason, we will collect suggestions and review carefully for upgrades no more frequently than once per quarter. This cycle does NOT apply to problems/bugs that are impeding its use. This refers only to suggestions for making the tool better for the user and/or better as a way of conveying important image information to the public user.

## Future Considerations

A number of steps are needed to make full use of image data and to achieve the goals of LungMAP. These efforts cross the boundary of image annotation but spring from discussions related to annotation as well and raised by the Imaging SubCommittee. The ideas below can serve as a springboard to move along several important considerations as we enter the mid-point of the project period. Two related, yet distinct objectives must be weighed: (1) educational/illustrative purposes and (2) data mining/integrative discovery and visualization.

What is the role of image data in LungMAP?

Since the goal of LungMAP is construction of an atlas, visual information is a critical piece and perhaps the most effective way of presenting the process of lung development to a broad base of users. However, much of the information that informs discovery of development processes is non-image data, i.e., data that describes gene expression through methylation, presence of proteins, lipids and metabolites. When these processes converge to describe morphogenesis and cell specification, differentiation, and function, we move into the area where image becomes the best language. Our challenge is to integrate these tissue and cellular events with the molecular events found at the tissue and cell level.

What is the role of image annotation?

Image annotation reflects a choice on the part of the annotator in order to convey specific selected information. For this reason we have coined the phrase *purposeful annotation.* This choice is shaped by the priorities of the annotator and will vary by person. Purposeful annotation is notation of image details to convey specific information about the image to the viewer and also to convey these details **in the**

**context of the image**. Ideally, the annotator will consider the reasons the image was created, the use of the particular material chosen and identify aspects of the image that best express the story behind this work. The story can be very complex. For instance, the overlap of immunofluorescent signals for HOPX and proSPC expression in the developing acinar tubules at E16.5 in the mouse reveals that these expression patterns are the inverse of one another. HOPX is concentrated in the proximal tubule and is undetected in the distal acinar tubules, whereas proSP-C is concentrated in the distal tubule and is undetected in the proximal acinar tubule. In between these two regions, there is a "transition zone" where both genes are expressed in the epithelium at decreasing levels. This is correlated with the single cell RNA seq data for this time point, which show 3 different cell expression patterns: 1) HOPX only, 2) SFTPC only and 3) coexpression of both genes in the same cell.

Approaches to image annotation

For Lungmap, there are 3 distinct and important perspectives that can shape image annotation, each having unique concerns, approaches and requiring different skills. The LungMAP SC should agree on how best to use its resources across the different approaches.

1. Select terms to link images to non-image data (data-driven annotation)
2. Select terms through an automated process (machine annotation)
3. Select terms to describe image for educational purposes (educational annotation)

Data-driven annotation is critical for BREATH. Links to other relevant data allow a user to make comparisons to better understand processes at work in lung development and to make connections between data for further analysis. This data linking can occur based on metadata, but may be more interesting if the link is displayed in a structural context within the image. However, integration of molecular data with image data is a big challenge requiring high levels of quality control. Navigating and connecting 3-dimensional images will also require additional thought and effort.

Machine annotation is being attempted through two separate LungMAP related efforts at CCHMC and Duke. We are hopeful that segmentation through algorithms based on metadata and aspects of the image will allow more images to be annotated in a standard way that is understood and agreed upon by the consortium. This is an ambitious goal that will no doubt continue for the duration of the project. However, its use will impact the task of image annotation by human researchers. These two efforts should receive due scrutiny for possible integration.

Educational annotation has its role in providing novice users with the state of current knowledge as compiled by the experts. Groups such as Allen Brain Atlas and GUDMAP make use of illustrative reference maps and models as a tool to guide the user community.

Formation of a new work group

As discussed above, annotation can be approached from a variety of perspectives. In order to continue exploring the best way to use image annotation to achieve the goals of

LungMAP, we propose to establish a new group and seek input from the consortium for priorities.

We have heard from several centers that our data tells a story and we hope to be able to create a structure that lends itself to telling these stories. This would require going beyond straightforward notation of image features to present information about the image, which describes aspects of development in a more narrative form, incorporating and highlighting data that relate to the morphogenetic changes in the images displayed. We think this type of presentation would enrich LungMAP as an educational resource by making the complexity of development more accessible to non-scientists and students.

This approach could be part of a broader outreach effort that focuses on education, including more traditional illustrations of anatomy and narrative tutorials that walk the viewer through various aspects of lung development and explain the molecular data that trigger and regulate development.

With all of the talent across centers and the number of researchers who hold teaching positions, we feel fortunate that LungMAP has the capability to tackle this educational challenge. We look forward to ongoing discussion and decisions that will move LungMAP forward.

## IAWG Members

**IAWG RC Lead**: Susan Wert
**LungMAP DCC Representatives:** Carol Hill, Mary-Anne Ardini
**IAWG RC Contributors:** Namasivayam Ambalavanan, Kathryn Wikenheiser-Brokamp, Tom Mariani, Ravi Misra, Charles Frevert, James Carson, Joseph Kitzmiller, Wei Shi.

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# LungMAP Image Annotation Addendum Report

## Contents



## Summary

Since the report of activities of the Image Annotation Work Group distributed in September 2016, the DCC has continued working to implement tool modifications and produce the documentation needed for effective annotation. The tool has undergone refinements recommended by the group and was released in late December on the test server for use by a small number of annotators. New documentation is described in the next section. The IAWG completed its mission as defined by charter and disbanded in September 2016. All further work on the annotation tool and its website presentation and use will be the responsibility of the DCC.

## Accomplishments: Documentation

Documentation created since the IAWG report is included as an Appendix and includes:

- Quick Reference Guide. A short summary that presents key steps for use of the tool, along with demonstrational exhibits. A few examples of items addressed are the interaction of the selected symbol and the ontology, how to close out of the view without losing where you are, how to delete notations made in error, how to use the “instance” feature to eliminate redundancy.
- Guidelines. A brief summary of the aims of image annotation in general and some best practices to serve as a foundation for use of the tool.
- FAQs. Q and A basics to facilitate use of the tool.

Documentation will be posted on the private area of the website. Documents are available only to the consortium since annotation, at this time, is restricted to RCs.

## Accomplishments: Advancement of Concept

A number of future considerations were raised in the IAWG Report of September 2016. Since that time, we have explored ways of addressing these issues in a way that results in concrete progress. An outgrowth of the IAWG based on recommendations of the group and agreed upon by the SC was formation of another work group devoted to initial development of a narrative based tool for LungMAP researchers to create "stories" about the data. Consortium members responded positively during the in person SC meeting in September, several volunteering to be members of the new work group, which was formed immediately and held its first meeting in early October. A summary of those activities is contained in a separate report to be released in mid-January 2017.

In a parallel approach to image annotation, also noted in the IAWG report, the machine annotation effort led by Cliburn Chan of the DCC, continued to evolve, culminating in a presentation-ready product in December 2016. Work continues on refinement of this approach as efforts will take place to integrate the tool into the LungMAP image workflow so that output can be quality checked by the community of LungMAP users. Effectiveness of this approach will have a significant impact on the feasibility of annotation of all images and may shape presentation of information about images to website users with varying levels of scientific training.

## Outstanding Issues

Versioning: An image may speak differently to viewers. To allow more than one annotation of a single image or to allow updating of an annotation if new knowledge becomes available, we suggest allowing multiple versions of an annotation that can be accessed by viewers for comparison.

Annotation terms as metadata when submitting image experiments: Such information could act as a guide for actual annotation if the delay between submission and posting impedes immediate use of the tool and might also serve as "tags" that will allow searching and linkage to other data. Terms selected as metadata for annotation would be identified using the same ontology as used with the tool.

Tutorial: Create a short web tutorial to demonstrate how the tool is used to create annotations. Such a tutorial can be shared for public viewing as informational video.

## IAWG Membership

**DCC Members**: Carol Hill, Mary-Anne Ardini
**RC Members:** Susan Wert, Namasivayam Ambalavanan, Kathryn Wikenheiser-Brokamp, Tom Mariani, Ravi Misra, Charles Frevert, James Carson, Joseph Kitzmiller, Wei Shi.