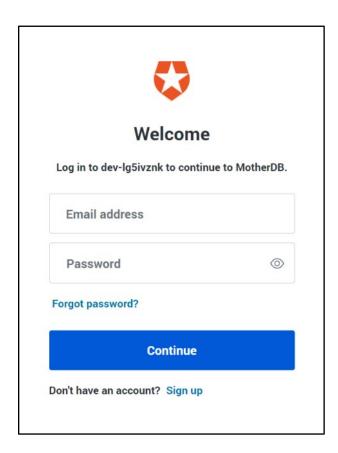
ezEML+MOTHER User Guide Version 1.1

Introduction

Sharing an ovary histology image with MOTHER requires the submission of its associated metadata. The Web-based tool, called ezEML+MOTHER, assists the user with the specification of the metadata. This tool leverages the ezEML tool, developed by Environmental Data Initiative (EDI), for the data provenance of the image (e.g., Contact, Project, Intellectual Rights, etc.) and adds specific metadata for MOTHER. Using ezEML+MOTHER, the image with its metadata can be submitted to the MOTHER project one image at a time. Alternatively, to share a collection of histology images, consider creating a project on the Open Science Framework (OSF) at osf.io and uploading the images and metadata to a project there, which creates an additional opportunity for discovery of your data. Let MOTHER know when you are ready to share your OSF project. MOTHER can then upload all images and metadata for your project, provided that you add motherdbosf@gmail.com to the project with read and write privileges.

Create an ezEML+MOTHER account

The use of the ezEML+MOTHER Web application requires a login, which can be automatically requested with email verification required. This login allows you to work on documents across multiple sessions, in addition to being able to upload an image with its associated metadata. If you have not created an account with ezEML+MOTHER, click "Log In" to be redirected to the login page, then click "Sign up" below the continue button. After entering an email and password, you will receive a confirmation email to complete the process.



Navigation in ezEML+MOTHER

You can think of using ezEML+MOTHER as filling out one big form that's organized as a number of separate content pages. The **ezEML+MOTHER** link at the top left of each page serves as a "Home" button. Your work is saved in the context of a Document, which can be worked on across sessions. There is a navigation toolbar at the top of the page to essentially navigate documents, and a content navigation on the left-hand side of the page to navigate the content pages within a document.

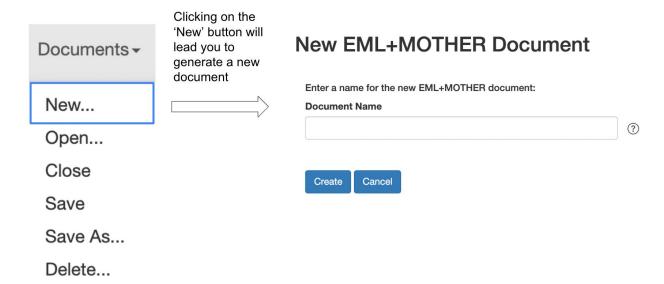
Top Navigation Toolbar

The top toolbar in ezEML+MOTHER provides access to creating or opening **Documents**, **Upload/Download** of xml metadata files, the **User Guide** for the tool, and an **About** informational page.



Documents

Select *New* to create a new document, which will ask for a name for the document. *Open* will open an existing document that you have already saved in ezEML+MOTHER. *Close* exits out of the current open document. *Save* will save the current open document as is, while *Save As* will allow you to change the current document name.



The name of the document will be set as the name of the project folder when downloaded. Any desired name may be utilized at your discretion.

Upload/Download

The metadata generated by the tool consists of an XML file that conforms to the requirements of the Ecological Metadata Language (EML) for data provenance and MOTHER's additional metadata requirements. Although your document will be stored across sessions, you may want to *Download* the XML metadata as a backup or as a template for slides from the same donor animal. For subsequent slides, you can create a *New* document and *Upload* the downloaded metadata for that donor. The upload will automatically complete the form with the saved information, requiring you to only enter the title and slide id for that image along with uploading the new histology image.

User Guide

The ezEML+MOTHER User Guide is essentially this document broken down into sections to access within the tool. There is also a quick overview for ezEML+MOTHER.

About

About provides a brief overview of the background of ezEML+MOTHER.

Navigation of Contents

The left navigation bar indicates the high-level content pages within the tool. Essentially, the items above the first line, are the topics leveraged from ezEML, whereas the Donor and Immunohistochemistry pages are specific to MOTHER.



As you move from page to page using the **Save and Continue** button, your changes are automatically retained. There is a **Cancel** button if you decide to exit the page without saving. You can select a **Contents** topic on the left to jump to that page. Just make sure that you have saved any changes on the current page. It's usually best to refrain from using your browser's forward and back buttons. Using them may cause you to lose unsaved edits.

Required Fields *

On each content page, the fields on the form that are required are marked by an asterisk (*).

Help Hints

There are also helpful hints on the form that can be selected for reminders, which are indicated by the ? symbol enclosed in a circle.

Title

EML requires that there is a unique title for each slide. Thus, you should use a descriptive title that also includes information about the lab, the species, and the unique donor and slide designation.

Examples:

- Zelinski Lab: Rhesus macaque 14736 Unspecified Ovary 020a
- Busycotypus canaliculatus reproductive data, Nantucket Sound, 2020: BC_2020-08-25_2_F 002

Title



Image

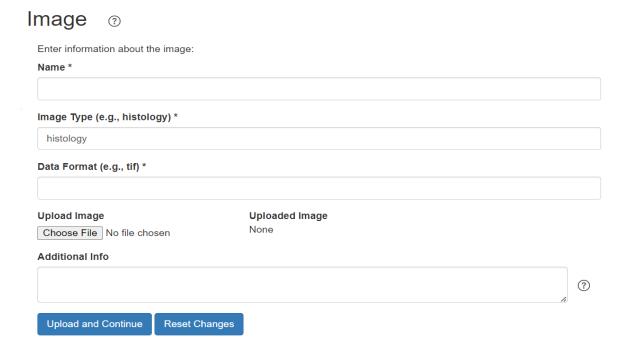
The histology image is uploaded on this page from your local computer. The **Name** and **Data Format** are automatically filled in based on the name of the file and its file extension that you upload, under **Upload Image**. The **Image Type** is by default *histology*, since MOTHER is only accepting histology images at this time. Note that **Additional Info** is an optional field where you may want to include information about the image that is not already specified as part of MOTHER on the Donor and Immunohistochemistry forms.

For example, are there any standard measurements of the donor taken that you have as part of your own metadata? The following example shows measurements typically taken for whelk using a JavaScript Object Notation (JSON)-like syntax, which uses **key: value** pairs separated by semicolons. The use of this format instead of free-form text opens up the future possibility of accessing this information computationally. Note that units have been included as part of the key.

Body Weight (g): 96.75; Gonad Weight (g): 2.13; Shell Length (cm): 13.5; Shell Width (cm): 8.9; Gonad Length (cm): 4

As another example, is this image part of a published study? If so, you can include a citation to the paper here using an appropriate citation style for your discipline.

Zelinski-Wooten, M. B., Hess, D. L., Wolf, D. P. Stouffer, R. L. (1994). Steroid reduction during ovarian stimulation impairs oocyte fertilization, but not folliculogenesis, in rhesus monkeys. Fertility and Sterility 61, 1147-1155.



People

There are many types of people that can be associated with an histology image: Creators, Contacts, Associated Parties, and Metadata Providers. At least one Creator and one Contact is required, while Associated Parties and Metadata Providers are optional. All People share a similar form as shown below, except that an Associated Party has an additional **Role** field. The EML specification requires the specification of at least one of the following fields: Last Name, Organization, or Position Name. Note that including additional fields to identify a person is useful, e.g. ORCID.

	ORCID ID (Recommended) e.g., 0000-0002-5143-7857 Middle Name/Initial (Optional)		
First Name (Optional)			
Org ID (Optional)	Org ID Type (Required for Org ID		
Online URL (O	Online URL (Optional)		
State (Optional)	Postal Code (Optional)		
	Org ID (Optional)		

Creators

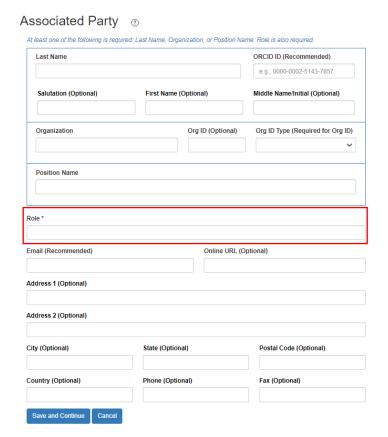
The creator is considered to be the owner of the image, i.e. the person(s) responsible for its creation. At least one creator is required.

Contacts

Include here people that researchers looking to use your data can reach out to. Ideally, you'll want to include the person responsible for managing the data. At least one contact is required.

Associated Parties

Associated parties can include lab technicians who performed work on the slides, lab assistants, other students or professors involved with the project, etc.
Associated parties are not required. However, if specified, there is a required Role field, e.g. scanned slide.



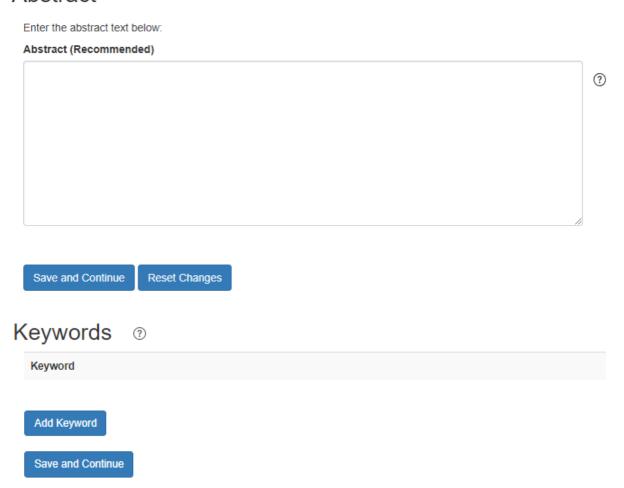
Metadata Providers

These are the people responsible for creating or providing the metadata itself. Metadata providers are not required.

Abstract and Keywords

Both an abstract and keywords are optional, though it is highly recommended to include both to make it easier for researchers to search for your image.

Abstract



Note that Keywords are entered one at a time by choosing the **Add Keyword** button. Choose keywords that will likely be used in a textual search for your image.



Intellectual Rights

We recommend that the data you provide comes with few or no use restrictions to maximize accessibility for future research. As such, it is encouraged that collaborators release their data under one of the two provided Creative Commons License options, CC0 or CC BY. CC0 is a declaration that you waive copyright claims to the data, that the data falls under the category of the public domain, and the data DOES NOT require users to use proper citations.

CC BY, on the other hand, requires users to provide appropriate credit for your work and indicate if they made any changes to your work. Both licenses allow for commercial use by users. If you decide to release your data under another license, you can input the description in the "Other" text field. For more about all the different types of Creative Commons licenses, visit https://creativecommons.org/about/cclicenses/.

MOTHER requires the specification of intellectual rights.

Intellectual Rights ①

Choose from one of the intellectual rights options shown below:

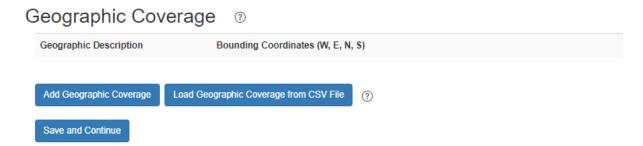
- This data package is released to the "public domain" under Creative Commons CC0 1.0 "No Rights Reserved" (see: https://creativecommons.org/publicdomain/zero/1.0/). It is considered professional etiquette to provide attribution of the original work if this data package is shared in whole or by individual components. A generic citation is provided for this data package on the website https://portal.edirepository.org (herein "website") in the summary metadata page. Communication (and collaboration) with the creators of this data package is recommended to prevent duplicate research or publication. This data package (and its components) is made available "as is" and with no warranty of accuracy or fitness for use. The creators of this data package and the website shall not be liable for any damages resulting from misinterpretation or misuse of the data package or its components. Periodic updates of this data package may be available from the website. Thank you.
- This information is released under the Creative Commons license Attribution CC BY (https://creativecommons.org/licenses/by/4.0/). The consumer of these data ("Data User" herein) is required to cite it appropriately in any publication that results from its use. The Data User should realize that these data may be actively used by others for ongoing research and that coordination may be necessary to prevent duplicate publication. The Data User is urged to contact the authors of these data if any questions about methodology or results occur. Where appropriate, the Data User is encouraged to consider collaboration or co-authorship with the authors. The Data User should realize that misinterpretation of data may occur if used out of context of the original study. While substantial efforts are made to ensure the accuracy of data and associated documentation, complete accuracy of data sets cannot be guaranteed. All data are made available "as is." The Data User should be aware, however, that data are updated periodically and it is the responsibility of the Data User to check for new versions of the data. The data authors and the repository where these data were obtained shall not be liable for damages resulting from any use or misinterpretation of the data. Thank you.

	The data authors and the repository where these data were obtained shall not be liable for damages resulting from any use or misinterpretation of the data. Thank you.
0	Other (Enter text below)
	save and Continue Reset Changes
'	ave and Continue Reset Changes

Geographic/Temporal Coverage

Geographic and Temporal Coverage is optional. However, for some images this may be an important component of your metadata. For example, if the donors were retrieved at a particular geographic location on a specific date, this can be captured as part of the metadata.

Geographic



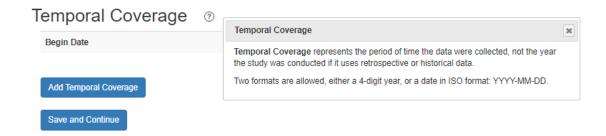
If you know the general geographic location where the donor was found, you can provide the bounding coordinates as latitude and longitude values. All coordinates are typed as decimals. Use latitude as the North and South bounding coordinate, and longitude as the West and East bounding coordinate. East should be greater than or equal to West and North should be greater than or equal to South. Since all four elements are required, a bounding area that is a single point should use the same latitude values for North Bounding Coordinate and South Bounding Coordinate, and the same longitude for West Bounding Coordinate and East Bounding Coordinate.

Geographic Coverage ②

Enter the geographic description and I	bounding coordinates below:		
Geographic Description *			
			?
		h	
West Bounding Coordinate *	East	Bounding Coordinate *	
			?
North Bounding Coordinate *	Sour	th Bounding Coordinate *	
Bounding Altitudes (Optional) B	ounding Altitudes is optional, b	out if any fields are filled in, they all must be.	
Minimum Altitude	Maximum Altitude	Altitude Units	_
		~	?
Save and Continue Cancel			

Temporal

Likewise, if you have the time period in which your donors were collected, you can provide it here. For a single collection date, enter the start date and leave the end date blank.



Temporal Coverage



Taxonomic Coverage

Taxonomic Coverage is required for MOTHER, which records the Genus and Species. For a taxonomic search on species, enter the binomial name. Upon entering the binomial name into the "Taxon Scientific Name" field, you will need to specify its taxonomic rank. You can also automatically create the hierarchy by specifying which Taxonomic Authority to reference and then clicking "Fill Hierarchy".

Taxonomic Coverage Enter a description of the range of taxa addressed in the data set or collection. For example, "All vascular plants were identified to family or species, mosses and lichens were identified as moss or lichen.": General Taxonomic Coverage (Optional) Enter the most specific relevant taxon, below, then click "Fill Hierarchy." Taxon Rank will be filled in automatically, unless the taxon is unknown to the Taxonomic Authority. Filling the hierarchy takes a few seconds. Taxon Scientific Name Taxon Rank Taxonomic Authority Fill Hierarchy macaca mulatta NCBI - National Center for Biotechnology Informa Species Taxon Rank Taxon Name Common Name Links Species Macaca mulatta Rhesus monkey External Link Genus Macaca macaques External Link Subfamily External Link Cercopithecinae Family Cercopithecidae Old World monkeys External Link Superfamily Cercopithecoidea External Link Infraorder Simiiformes External Link Suborder Haplorrhini External Link Order Primates External Link Superorder Euarchontoglires External Link Class Mammalia mammals External Link Superclass Sarcopterygii External Link Subphylum Craniata External Link Phylum Chordata External Link chordates External Link Kingdom Metazoa metazoans Save and Continue Cancel

To proceed when the taxon scientific name cannot be found in the system, simply enter the taxon scientific name and taxonomic rank, and click on the 'Save and Continue' button. Please contact MOTHER if you have manually entered in the taxonomic coverage.

Methods

Method steps are descriptions of specific steps of the method employed when collecting data. They are intended to be descriptive, i.e., human-readable rather than machine-readable. The method steps document a series of procedures followed. These may include text descriptions of the procedures for an experiment, relevant literature, software, instrumentation, source data and any quality control measures taken. Methods are not required. However, if a Method Step is added, then a description is required.



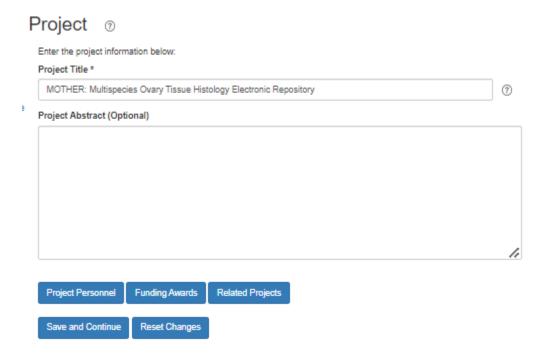
Select **Add Method Step** to add for a step that requires at least a method step description.

Method Step Enter the method step description and (optionally) instrumentation and data sources below: Description * Instrumentation (Optional) Data Sources (Optional) Save and Continue Cancel

Project

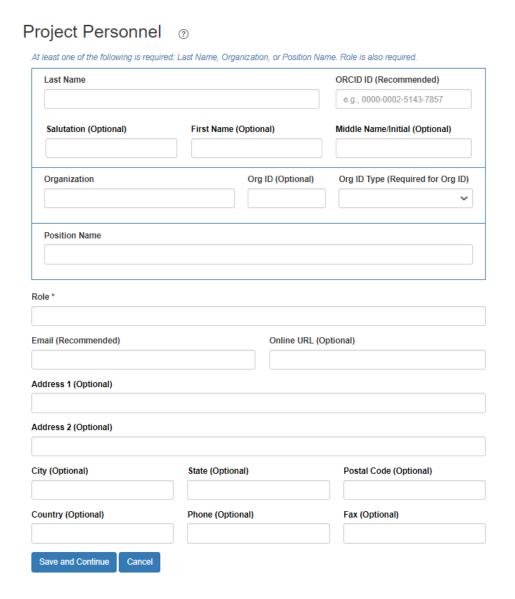
If the contributed histology image is related to a project, then include information about the project, including its title, abstract, project personnel, funding awards, and related projects. While the 'Project' element is optional, if included, it must contain a 'Project Title' and 'Project Personnel' information. If you have decided to skip the project element, you may proceed to the next element, 'Donor', by clicking on it.

Note that after entering **Project Personnel**, **Funding Awards**, or **Related Projects**, you need to select **Back to Project** to access the other components of the project.



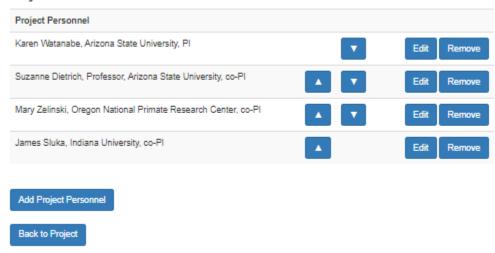
Project Personnel

Project Personnel is similar to the People content pages (Creators, Contacts, Associated Parties, Metadata Providers) and should include people/organizations that were involved in the research project. You must also include their Role should you choose to include Project Personnel, such as PI, Co-PI, Senior Personnel, etc.



Save and Continue shows the Project Personnel page with the added personnel. The order of the personnel can be updated using the up/down arrow icons. You can also **Edit** or **Remove** personnel.

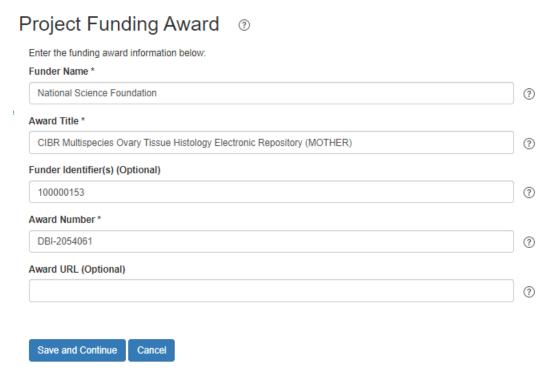
Project Personnel ®



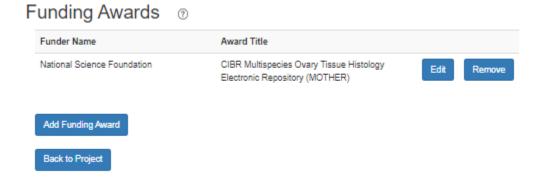
Select Back to Project to add Funding Awards or Related Projects.

Funding Awards

The funding awards for the project. Each funding award is described by the name of the funding agency, the title of the award, and its award number. Optional fields are provided as well for the Open Funder Registry identifiers for the agency and program that made the award and the URL to the award page describing the award.



Once entered, the Funding Awards page shows the funder name and award title. Select **Back to Project** to add Related Projects, if any.

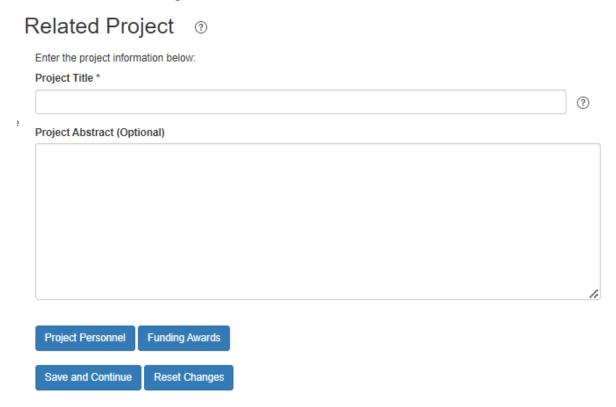


Related Project

Include citations to projects that are associated with or similar to yours. EML treats one project as the main or primary project, which can then have one or more related projects as "children". Splitting projects up in this way lets you associate different funding awards with different Principal Investigators.



Select **Add Related Project** to add details on a Related Project, which can have Project Personnel and Funding Awards.



Donor

The Donor content is unique to ezEML+MOTHER. Recall that all fields marked with an asterisk on the form are required.

Donor ID*

The Donor ID must be unique across ALL donors in the MOTHER database. Thus, this is NOT a simple number but an encoding of various information about the donor. The recommendation is to use the following information separated by an underscore:

- Initials for the lab or creator associated with the donor.
- The first letter of the genus and species of the donor.
- A unique identification for the donor used by the scientist. This can be the animal id for a lab animal. If the donor was collected from the field, it may be the combination of the date collected and its sequence number in the collection.

Some examples:

- A Rhesus Macaque (macaque mulatta) donor assigned an animal id of 12345 by the lab/creator MZ can be assigned a donor id of MZ_MM_12345.
- A whelk donor that was obtained on 14 September 2020 with a sequence number
 of 97 collected on that date can be assigned a donor id of JS_BC_2020-0914_097 where JS are the lab/creator's initals, BC stands for the first letter of the
 genus (busycotypus) and species (canaliculatus), followed by the collection date,
 and sequence number.

Sex*

Currently, *female* is the only value since MOTHER is storing only ovarian histology images.

Years and Days

You can specify the age of the donor, if known, as either just years, just days, or a combination of years and days.

Life Stage*

Values for life stage include: *fetal, neonatal, prepubertal, pubertal, adult, aging, and unspecified.* If you choose *unspecified*, please contact the MOTHER team with details for the life stage values for the donor species so that this information can be incorporated in future versions.

Specimen Sequence Number*

The specimen sequence number is typically 1. However, a donor may have multiple ovaries. If you are contributing slides from two ovaries of the same donor, then the specimen sequence number differentiates between the ovaries.

Specimen Tissue*

Currently, *ovary* is the only tissue for which MOTHER is storing histology images.

Ovary Position*

Choose Left, Right, or Unspecified from the drop-down.

Specimen Location*

The current values for specimen location are whole ovary, ovarian cortex, ovarian medula, follicle, corpus luteum, unspecified.

If the specimen location is chosen as corpus luteum, then the **Corpus Luteum Type** drop-down will be enabled to choose one of the following values: *early*, *mid*, *late*, *albicans*

If these values do not capture the location within the ovary of your donor, please contact the MOTHER team.

Day of Cycle

If known, enter the reproductive cycle day that the specimen was collected.

Cycle Type and Stage of Cycle

MOTHER currently supports the stages of the *menstrual* cycle and the *estrous* cycle in the drop down.

- menstrual stage values: follicular, pre-ovulatory, ovulation, luteal, unspecified.
 - If follicular, then the Follicular Values drop-down is enabled with the following values: early, mid, late.
 - o If luteal, then the **Luteal Values** drop-down is enabled with the following values: *early*, *mid*, *late*, *albicans*.
- estrous stage values: proestrus, estrus, metestres, diestrus, anestrus, unspecified

There is also an *other* cycle type if your donor does not use menstrual or estrous cycles. Choose *other* and enter the stage of cycle manually. Please contact the MOTHER team so that the cycle type and stages for the donor species can be incorporated in future versions.

Slide ID*

The slide ID must be unique within the donor and specimen sequence number. If you are contributing multiple images from the same physical slide, then the slide id value must be identified by the physical slide number and the section designation of the image. For example, if slide 1 has sections A and B and you want to contribute both images, then the slide id for the image for section A is 1A whereas the slide id for the image for section B is 1B.

Section Sequence Number

If you are submitting a collection of images that allow for a 3d reconstruction of the ovary, then please enter its section sequence number for the reconstruction.

Section Thickness*

Enter the thickness of the section on the slide.

Section Thickness Units*

Choose Microns or NM from the drop-down.

Fixation*

Choose a fixation drop-down value: *Neutral Buffered Formalin 10, Paraformaldehyde, Davidsons, Neutral Buffered Formalin5 acetic Acid, Bouins, Other*If *Other* is selected, enter the name of the fixation used in the **Other Fixation** field.

Stain*

Choose a stain drop-down value: Light Microscopy Stain, Fluorescent Microscopy Stain, Electron Microscopy Stain. The appropriate drop-down will be enabled based on the type of microscopy chosen:

- Stain Light Type values: Eosin only, Hematoxylin only, Hematoxylin and Eosin, Masons Trichrome, Mallorys Trichrome, Periodic Acid Schiff, Sudan, Acid Fuschin, Alcian Blue, Azan Trichrome, Casans Trichrome, Cresyl VioletNissl, Giemsa, Methylene Blue, Neutral Red, Nile Blue, Nile Red, Orcein, Reticulin, Toluidine Blue, Van Gieson, Other
 - If Sudan, choose the Sudan Stain Value: III, IV, Black B, Oil Red O, Osmium tetroxide
 - o If Other, enter the name of the Other Light Stain
- Stain Fluorescent Type values: Acridine Orange, Calcein, DAPI, Hoechst, Propidium Iodide, Rhodamine, TUNEL, Other
 - o If Other, enter the name of the Other Fluorescent Stain

- Stain Electron Type values: Colloidal Gold, Osmium tetroxide, Phosphotundstic Acid, Silver Nitrate, Other
 - o If Other, enter the name of the Other Electron Stain

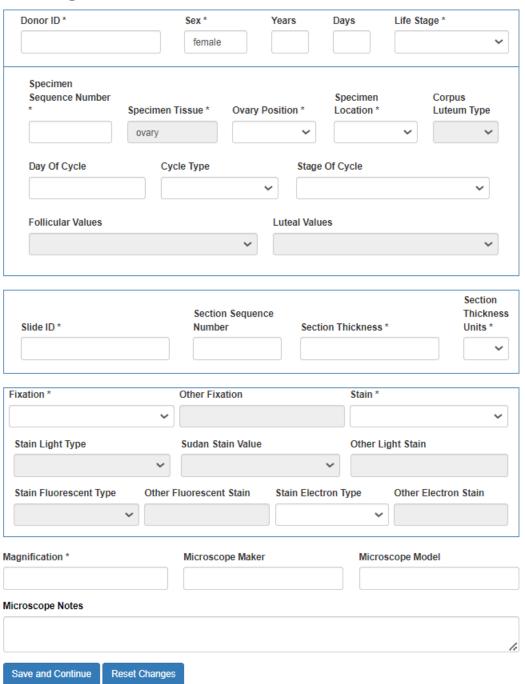
Magnification*

Enter the magnification value used to digitize the image.

Microscope Maker, Microscope Model, Microscope Notes

Enter the information about the microscope, if known.

Donor ②



Immunohistochemistry

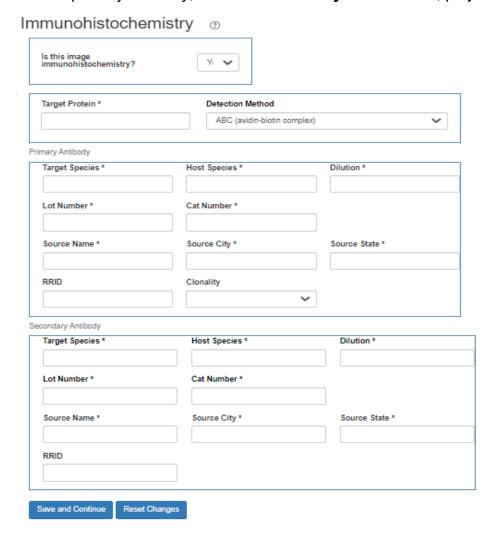
This section is only required to fill out if immunohistochemistry was used on the slide. If you did not, you will just need to answer "No" to the top question.

Target Protein:

Enter the name and description, e.g. CD

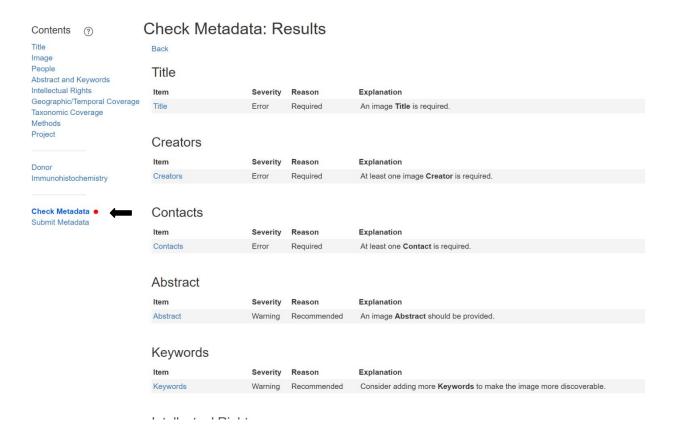
Detection Method Values: ABC (avidin-biotin complex), Alkaline Phosphates, Diaminobonzidine, FITC, Horseradish Peroxiduse, LSAB (labeled streptavidin-biotin), RPE

For each antibody, enter the following: **Target Species, Host Species, Dilution, Lot Number, Cat Number, Source Name, Source City, Source State**, and **RRID**For the primary antibody, choose its **Clonality**: *monoclonal, polyclonal*



Check Metadata

Once you've gone through all of the sections listed above, you may be ready to submit your metadata. You'll notice a colored circle next to **Check Metadata** on the right. Red means that you have an error, most often being a missing section that is required to be filled out. Yellow-Orange means you only have warnings, usually a recommendation to add or improve upon your metadata. Green means there are neither any errors or warnings and that your metadata is ready to be submitted. You may submit your metadata with warnings. However, you should review each warning before submission.



Submit Metadata

After clicking on the **Submit Metadata** text, a window will appear displaying the name of the Image File and its XML File. The Submit Metadata button creates a zip of these two files, named with the Document Name – not the Image name, and submits the zip to MOTHER.



The successful submission will give you the opportunity to download the ZIP file submitted to MOTHER. This step is highly recommended for you to maintain a record of the submitted zip.



When you have submitted all of the images that you want to contribute, please contact the MOTHER team to start the curation process.

Summary of Required/Optional Metadata for ezEML+MOTHER

Topic/Contents	EML		MOTHER	
* indicates required	Required	Optional	Required	Optional
Title*	✓			
Image*			Name, Image Type, Data Format, Image	Additional Info
People* [At least last name, organization, or position name]	Contact, Creator	Associated Parties, Metadata Provider		
Abstract and Keywords		Abstract, Keywords		
Intellectual Rights*			✓	
Geographic/ Temporal Coverage		Geographic, Temporal		
Taxonomic Coverage*			✓	
Methods				
Project	Project Title	Project Abstract Personnel		
		Funding Awards		
		Related Projects		
Donor*		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	Donor ID	Years
			Sex	Days
			Life Stage	Day of Cycle
			Specimen Seq Number	Stage of Cycle
			Specimen Tissue	Section Seq Number
			Ovary Position	Microscope Maker
			Specimen Location	Microscope Model
			Slide ID	Microscope Notes
			Section Thickness	
			Section Units	
			Fixation	
			Stain	
			Magnification	
Immunohistochemistry			Target Protein	Detection Method
[primary & secondary antibody]			Target Species	RRID
			Host Species	Clonality [primary]
			Dilution	
			Lot Number	
			Cat Number	
			Source Name, City, State	