## **AOData Walkthrough**

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The purpose of this tutorial is to demonstrate, step-by-step, how to get started with AOData. The walkthrough will take you through the process of setting up a custom AOData package. Before beginning, the walkthrough you should follow the instructions in the documentation for **Installation** and for adding AOData to your search path. You should also get the "aodata-tutorial-package" from Github and dowload the experiment files from the Dropbox link in the repository information.

This is a live script. You should step through it, running the code blocks one-by-one (Ctrl+Enter) and reading the associated text. I personally learn by doing and I want AOData to be accessible to people who aren't super comfortable programming, so I've included some little exercises. They would be especially helpful if you aren't super comfortable with MATLAB or object oriented programming in MATLAB. Or if, like me, you start to zone out when confronted with this much text/information to read through. There's a second file "AODataWalkthrough WithAnswers.mlx" if you get stuck on something.

Finally, the walkthrough is meant to be completed in order, and later sections will assume that you've read the previous sections.

#### **Documentation (where to find help)**

This walkthrough is meant to be a companion to the Documentation PDF as well as the in-code documentation (that is, documentation written as code comments in specific places). The PDF can be reached at the link above and, to access the in-code documentation, you can either use doc or help (or just open the code itself with edit).

```
% This will open up a detailed description of the methods and properties of
% the aod.core.Experiment class. Useful for an overview. You can always
% click on a method to get information on how to use it.
doc aod.core.Experiment
```

```
% For quick details on how to use a method/function, using "help" will
% print usage information to the command line.
help aod.core.Experiment.setHomeDirectory
```

To get a full sense of AOData, check out the accompanying documentation referenced at each step. At this step, check out the PDF Documentation's section on "Using the Documentation".

Opening each of the variables (particularly the core components in the AOData object model within MATLAB's variable viewer and exploring the contents will also help you make the most of the walkthrough. You can click on them in the "Workspace" tab, right click on the variable within the live script and press "Open", or use the openvar command from the command line (useful after you're done with the walkthrough and writing your own code). Within this live script, you can also your mouse over any variable to get a little detail on what it is, as long as you've run the code block that first creates the variable.

```
% Create a variable
a = struct('FieldOne', [1 2 3], 'FieldTwo', 'hello', ...
    'FieldThree', "hi", 'FieldFour', {'hello', 'hi'});
% Open in the variable viewer. The classes created by AOData will be
% similar to structs. You can click on the individual fields to get more
% information about the contents.
openvar('a');
```

The object's created by AOData classes will be similar to the struct above - each field will be a property of the class. You can click the individual fields to get more information about the contents.

If you aren't comfortable with MATLAB or object oriented programming, opening up the underlying code and checking out how the underlying code is implemented can be helpful too. The properties are listed at the top. The first method is always the constructor (i.e. it "constructs" the variable). Scroll through the rest to see the various functions you can use with the class, particularly the "Public" methods. Functions are organized into methods blocks. "Private" methods "methods (Access = private)" can only be used by code within the class, never from the command line or from subclasses. "Protected" methods "methods (Access = protected)" can be accessed by code within the class and code within subclasses. They can even be redefined or redefined by subclasses, but not from the command line. "Public" methods "methods (Access = public)" are like protected methods but you can also use them from scripts and the command line. These are the ones you'll be working with the most and that deserve the most attention.

```
% Open up the underlying code for aod.core.Experiment in the editor
edit aod.core.Experiment
```

Keep in mind that classes may be inheriting methods from parent classes (in the case of aod.core.Experiment that is aod.core.Entity). If you can't find the method you're looking for, check the superclass (it's the class name on the first line after "<").

#### **AOData Basics**

AOData is a framework for managing experimental data, metadata and code. In other words, AOData is meant to provide a strong, standardized foundation geared towards maximizing reproducibility, accessibility and collaboration. It's a platform for end-users to customize for their individual experiments and workflows. At this point, make sure you've read the documentation sections on "AOData Object Model" and how it maps to an HDF5 file. Opening up an example file in AODataViewer and clicking around will be helpful too.

You will need to ask a few questions about your data:

- 1) Conceptually, how does your experiment map onto the AOData object model?
  - What are the different types of Calibrations you perform (i.e. power measurements, AO calibrations, PMT optimizations)?
  - Is there any information you need to log about the status of your System (i.e. ad hoc additions like NDFs and filters not recorded in the system diagram, serial numbers of devices like PMTs, etc)?

- How many different types of imaging do you do and are they performed in different videos (a.k.a. Epochs)?
- Are there important relationships between entities that cut across the AOData object model and are necessary for understanding the data? For example, are Epochs linked to specific Sources?

#### 2) Logistically, where and how is the information stored?

- Is it hand-written in an imaging log or saved as a file?
- What are the relevant files, where are they located in an experiment folder, what file formats, and how are they tied to the object they describe (e.g. saving the video number in the file name ties it to a specific Epoch)?
- Does any of this information rely on custom code (either to be generated or interpreted)? For example, did you write any code to generate a Stimulus or process a Calibration?

# 3) Programmatically, what code do you need to write to get that information into AOData's object model?

- · What custom classes do you need to create?
- · How will you read in your metadata files?
- Of the information related to an entity, what is best suited as metadata (an attribute in HDF5) and what is best-suited for an HDF5 dataset?

This walkthrough is focused on helping you with #3 while providing a familiarity with AOData's functionality that will help with #1. Ultimately, only you know the answers to #2. As you work through the walkthrough, keep an eye towards how the code and concepts introduced could applied/tailored to your own experiments.

#### Initialization

The first step is initializing AOData. If you haven't already, simply run the line below:

initializeAOData();

A GUI called **PackageManagerApp** should have opened up. There are 3 tabs which reflect the 3 settings AOData adds to your MATLAB user preferences:

- BasePackage: This is the location on your computer of the main AOData folder. If you move the folder for some reason, you'll want to re-run initializeAOData()
- 2. <u>SearchPaths</u>: This a list of the folders containing packages (i.e. the folder containing the first +folder. All subfolders that are packages will be added too). Some parts of AOData require knowledge of all the available classes and custom subclasses. For now, AOData's "src" folder should be already be added as this contains the "+aod" package. The subfolders that are packages do not need to be added here (e.g. "+core", "+persistent").

3. <u>GitRepos:</u> AOData tracks the commit IDs of any folders added here that are git repositories. When you create your own package, if you choose to track it with git (strongly recommended!), then you will add your git repository folder here.

For now, you can close out of it as initializeAOData() will have set the necessary initial values. You can return here at any time to add/remove custom packages and repositories by running:

AODataManagerApp();

## Creating a new package

Below is the information for creating a new custom package, which you can return to as you create custom packages (or check the PDF Documentation section on ""). If you want to follow along with the walkthrough, at least make a new folder on your computer to hold the files, even if it isn't tracked with git. At minimium, you should download the "tutorial package" from Github. Inside it, create a folder called "src" and within that a folder called "+tutorial" (explained below). Make sure to add it to your MATLAB path:

```
% Put the full file path for your package below:
addpath(genpath(''))
```

#### Create a git repository

Create a new git repository (see the <u>Git Tutorial</u> if you aren't familiar with this step). Name it something that identifies it as your personal AOData package (e.g. "yourname-aodata-package", "yourname-package", etc).

#### Create a namespace

Read the documentation section on "Namespaces" and refer to the linked MATLAB documentation for any additional questions that arise. As with all other concepts introduced here, looking at how AOData is organized can be helpful too.

An example of a class is aod.core.Device. The class is named Device and aod and core are the namespaces (Device is located in a folder called "+core" which is located in a folder called "+aod"). You will want to make your own namespace in your new repository folder. When in doubt, try the following template: "labname.yourname" (e.g. "williamslab.sara"). You want the namespace to be unique to you.

It can be useful to have packages contained within another folder so I'd recommend creating a folder named "src" within your main folder. If your chosen namespace is "labname.yourname", then inside "src", create a folder named "+labname". Inside that, create "+yourname".

#### Register with AOData

AOData needs to know your git repositories and packages exist. If you're using git, add the main folder to the "Git Repository" tab. Then make sure the folder containing the package folders is added to the "Search Path" tab (i.e. add "src" because it contains "+tutorial").

AODataManagerApp();

## **Example**

To familiarize you with process of mapping an experiment to AOData, we'll walk through an example experiment. First, we'll use just the core classes, then we'll develop some custom classes to make the process easier.

A small experiment folder is available at: <u>link</u>. Download this and save it somewhere to your computer. The location where you save it will be your experimentPath in the following code.

```
% Save the location of your experiment path
experimentPath = '';
```

The example is based on data from the Rochester one-photon AOSLO. While the processing is specific to these experiments, the resulting understanding of how an experiment maps to AOData should be generic. I've picked a complicated experiment on purpose, to maximally demonstrate as much of AOData's capabilities as possible.

Data on the Rochester 1P-Primate system is acquired from two channels: a reflectance channel focused on the cone mosaic and a fluorescence channel focused on the ganglion cell layer (the RGCs express GCaMP6 (480/520) and a subset are retrogradely-labeled with Rhodamine (555/585). In addition, there's a wavefront sensing channel and a channel for providing visual stimuli to the cones.

The raw, acquired data consists of two simultaneously-acquired videos per epoch: one in the reflectance channel (e.g. Ref/838\_20221122\_ref\_0001.avi) and one from the fluorescence channel (e.g. Vis/838\_20221122\_vis\_0001.avi). The files are named "AnimalID\_Date\_Channel\_EpochID.avi".

I'll explain more about the dataset as it becomes relevant in the walkthrough.

First, we're going to set up some of the Experiment using just the base classes to get a feel for how those work. Next, we're going to start over and develop custom classes, which will streamline the process and customize it to the underlying dataset.

## **Experiment (aod.core.Experiment)**

First let's create an experiment object. Check out the Documentation within the aod.core.Experiment file as well as the PDF. As you'll see, there are 3 required inputs (experiment date, the file path to the experiment folder and ) and 2 optional parameters (Administrator and Laboratory).

```
EXPERIMENT = aod.core.Experiment('838_ODR_20221122', experimentPath, '20221122',...
'Administrator', 'Sara Patterson', 'Laboratory', '1P-Primate');
```

Take a look at what's inside EXPERIMENT...

```
openvar('EXPERIMENT');
```

You will see that the 3 required inputs are properties. Those will map to HDF5 datasets. The parameters, which map to HDF5 attributes, are within the parameters property:

```
disp(EXPERIMENT.parameters)
```

### Methods shared by all entities

All of the core classes inherit from aod.core.Entity which means they get all the properties and methods defined by aod.core.Entity and can add their own as well. The PDF documentation describes these in depth. The nice thing about inheritance is that it means many aspects of the core classes are identical - if you understand how to use aod.core.Experiment, then you understand a lot about how to use, for example, aod.core.Device. Let's go through some of these shared methods:

**Description.** All entities have an property called "description". Setting it is optional. Here let's use it to describe the experiment's purpose.

```
EXPERIMENT.setDescription('To demonstrate the use of AOData');
```

**Notes.** This is a good place to add those miscellaneous notes on the front page of the imaging log. You can add as many notes as you would like.

```
EXPERIMENT.addNote('First experiment after latest AO calibration');
EXPERIMENT.addNote('PMT Z position from model eye was off');
disp(EXPERIMENT.notes)
```

You can, for example, remove the first with removeNote(1). You can clear them all with clearNotes(). If you need practice with MATLAB, try adding removing a note or clearing all notes below, then add the notes back (you will want them for later steps).

**Additional Parameters**. You can also add additional parameters, beyond just 'Administrator' and 'Laboratory'. Parameters are specified as "key/value pairs". That is, each entry has a key (e.g. "Administrator") and a value ("Sara Patterson"). For more information on the underlying data structure, check out the PDF's section on "aod.util.Parameters", then follow the linked resource for learning about MATLAB's documentation for the MATLAB containers. Map class.

```
EXPERIMENT.addParam('ParamName', 1);
```

You can remove a parameter with removeParam('ParamName'), get the value of a parameter with get('ParamName') or ask whether a parameter exists with hasParam('ParamName'). If you want to change a parameter, just rerun setParam() with your different value.

Try changing, removing and re-adding "ParamName" below. Use disp(EXPERIMENT.parameters) to check the results. Use the help or doc functions introduced above if you get stuck.

You can also add file names to an entity. For example, "Experiment" is a good place to add the file name of the Imaging Log.

```
EXPERIMENT.setFile('ImagingLog', 'ImagingLog.pdf');
```

Why not add the full path? This is because AOData offers the option for *relative file names*, that is, file names that are relative to experimentPath, the value of which was stored in the Experiment's homeDirectory property. When you use the getExptFile() method, the homeDirectory property will be appended.

```
disp(EXPERIMENT.getExptFile('ImagingLog'))
```

In fact, if you included the whole file path, it would be stripped from the file name before storing it in files. See for yourself:

```
EXPERIMENT.setFile('ImagingLog', fullfile(experimentPath, 'ImagingLog.pdf'));
disp(EXPERIMENT.getExptFile('ImagingLog'))
```

Why do this? AOData is built for collaboration and the absolute file paths will vary depending on the computer used. This is also helpful if the absolute file path differs between, say your work computer and your laptop. You can always change the homeDirectory as demonstrated below in the "Experiment Methods" section.

What if you want to log a file that was on the AO system's computer rather than within the experiment folder? The input to setFile() will only be altered *if the beginning matches the* homeDirectory *property.* If you want to get a file value without appending the homeDirectory, simply use getFile() instead of getExptFile().

```
EXPERIMENT.setFile('OtherImagingLog', 'X:\Users\somename\Documents\ImagingLog.pdf');
disp(EXPERIMENT.getFile('OtherImagingLog'));
```

Try comparing the outputs of getFile() and getExptFile() for 'ImagingLog':

You can play around some with the files above, adding/removing/clearing. For the purposes of demonstration later, make sure you that, if you clear all the files, you add them back before the next step.

## Mapping the Experiment to an HDF5 file

Usually you will want to add more to the experiment before writing it to an HDF5 file. But for the sake of demonstration, let's write it to the HDF5 file to see what it will look like.

```
aod.h5.writeExperimentToFile('Tutorial_JustTheExperiment.h5', EXPERIMENT, true);
```

You should now have a file called "Tutorial\_JustTheExperiment.h5" in your cd. If you want it to be saved elsewhere, include the full file path before the file name. Check the documentation for aod.h5.writeExperimentToFile for the other input information.

Now let's open the HDF5 file in **AODataViewer**. You can specify the file name or leave it blank. If you don't, you'll get a file directory option where you can select the HDF5 file you want to open.

```
AODataViewer('Tutorial_JustTheExperiment.h5');
```

By the end of the tutorial, you'll understand all the different aspects visible within AODataViewer. For now just click on the main Experiment folder, and check out the table on the bottom right. These are the HDF5 attributes. Grayed out ones are system attributes (i.e. ones AOData uses behind the scenes). Ones that aren't grayed-out are the entity's parameters and you should be able to see all the parameters within your experiment's parameters property.

By expanding the Experiment node (click on arrow next to it), you can see the contents. You can also see the properties we defined: "homeDirectory", "Name" and "experimentDate" as well as the ones we modified after creating EXPERIMENT ("notes" and "files"). If you click on one, you can see the contents in the top right panel. You'll notice files is handled a bit differently: the contents are visible in the attributes panel instead of the data panel - see the "HDF5" section of the PDF Documentation for more details on why.

As you work through this tutorial, you can write to HDF5 at any point and then open it in a new AODataViewer window to get a sense of how the HDF5 file is built. I'd recommend doing this any time you aren't sure what the last step meant for the file.

### **Methods specific to Experiment**

Subclasses of aod.core.Entity get all the properties and methods defined there by default. In addition, subclasses can add on new properties and methods. In other words, aod.core.Experiment is "customizing" aod.core.Entity. You will do the same with the core classes (like aod.core.Experiment) when you will do when develop your own packages. aod.core.Experiment has a few of these methods (in fact, more than any other entity as it is the root for the entire experiment dataset). Here are some relevant ones:

**homeDirectory.** The path to the experiment folder is stored in Experiment's homeDirectory property. As you add new entities to the experiment hierarchy.

## **The Persistent Hierarchy**

As mentioned above, Experiment is the "root" for the entire AOData file. By root, I mean that every other entity you create will be added to Experiment or to an entity that is added to Experiment. If you check out the contents of Experiment again in the Variable UI (or looking at the properties in the code), you will see 5 properties called "Analyses", "Calibrations", "Segmentations", "Sources" and "Systems". If you look back at the AOData object model in the PDF documentation, you will see these fall directly under Experiment in the hierarchy.

## Source (aod.core.Source)

Sources are the most complex parts of the AOData object model, because you can have nested Sources - that is, a Source that contains other Sources. As described below, this is needed to describe imaging locations at the appropriate level of detail.

Each experiment involves just 1 primate. Within that primate, there are two eyes, both of which may be imaged during an experiment and which have eye-specific metadata like axial length. Finally, within each eye, there are multiple locations imaged. These may not have metadata but still need to be recorded so the data can be sorted by location later.

This situation demonstrates why Source offers a nested hierarchy of Sources. The animal should be a Source within the Experiment. The eye or eyes imaged should be a Source within the animal's Source. Finally the locations imaged should be a Source within the eye. The aod.core.sources package contains classes for this exact situation: Subject, Eye, and Location. All are customized subclasses of aod.core.Source.

Here's how you can use those to create a Source hierarchy for the situation above. The first input is assigned to the Name property. See the underlying the code for Subject, Eye and Location or their help files to learn more about the extra inputs for each.

```
subject = aod.core.sources.Subject('MW00851',...
    'Sex', 'female', 'Age', 6, 'Species', 'macaca fasciularis');
OD = aod.core.sources.Eye('OD',...
    'AxialLength', 16.56);
rightOD = aod.core.sources.Location('Right');
leftOD = aod.core.sources.Location('Left');
% The right side has GCaMP6 and the left side has both GCaMP6 and rhodamine
% expression. Let's add some custom parameters to for that.
rightOD.setParam('Flurophores', "GCaMP6");
leftOD.setParam('Flurophores', "GCaMP6", "Rhodamine"]);
```

Now you have created the object, but you'll need to link them to each other, and the experiment. This is accomplished with the add() function. First, add your subject to EXPERIMENT. It will go in the Sources property. In addition, EXPERIMENT will be set to the Parent property of subject. As expected from the AOData Object Model, all entities will have a Parent, except for Experiment as it's the root/top-level entity.

```
EXPERIMENT.add(subject);
% Now your subject is within the Sources property
disp(EXPERIMENT.Sources)
disp(subject.Parent)
```

Now, we'll add the child Sources to subject. They will now be visible in the Sources property of subject.

```
subject.add(OD);
disp(subject)
% You can access OD through Experiment:
disp(EXPERIMENT.Sources(1).Sources);
```

To understand why we can add these to subject and see the changes reflected in EXPERIMENT. Sources, see the section of the PDF Documentation on "Handle vs. Value Classes".

```
OD.add([rightOD, leftOD]);
% There should now be two sources within OD
```

```
Experiment.Sources(1).Sources(1)
```

When you ask for the Sources within 0D, you get an array of two Sources - rightOD will be first because it was the first added to the Experiment, left0D will be the second.

```
disp(Experiment.Sources(1).Sources(1))
disp(Experiment.Sources(1).Sources(2))
```

#### Searching an Experiment

The syntax for getting right0D and left0D was pretty cumbersome and requires that you remember which Source within 0D was 1 and which was 2 (or at least using disp to check before using one in subsequent code). This is where the search() function comes in. Search allows you to request entities of a specific type that meet some criteria - this could be name, class, subclass or even a specific parameter. I'll demonstrate some here and you can check out the PDF Documentation section "Searching within the Core Interface".

The first input to search() is the entity type you want.

I've used MATLAB's new key/value pair syntax for subsequent inputs as I think it makes the code more readable.

```
out = EXPERIMENT.search('Source', Name="Right");
```

## aod.core.Segmentation

The Annotation class is designed for you to

## aod.core.Epoch

The example dataset contains 3 types of imaging that I typically perform within the same experiment. Some metadata is unique to each imaging type and other metadata is shared by all imaging types. There is also both shared and unique post-processing.

- <u>Anatomy:</u> data is acquired from both channels but there is no visual stimulus presented to the cones. The goal is only to visualize the fluorescently-labeled cells.
- <u>Spectral physiology:</u> data is acquired from both channels and a spatially-uniform visual stimulus is presented to the cones through a 3 LED Maxwellian View
- <u>Spatial physiology:</u> data is acquired from both channels. GCaMP6 responses are being measured to a spatial stimulus presented through the scanning system with a 561 nm laser. The stimulus is stabilized on the cone mosaic so remains locked on the same cones in the presence of eye movements.

First, let's just add the epoch IDs (these could be the IDs associated with each video.

```
epochIDs = [1, 2, 4:6];
for i = 1:numel(epochIDs)
    experiment.add(aod.core.Epoch(epochIDs(i)));
end
disp(experiment)
```

You should see the 5 epochs in the "Epochs" property and the IDs of each Epoch in the "epochIDs" property

```
disp(experiment.Epochs)
disp(experiment.epochIDs)
```

## aod.core.Response

A response is extracted from a specific spatiotemporal region of an Epoch's acquired data. Some examples include an image representing the average for each pixel or a timecourse average over a specific region of pixels (typically specified as an Annotation). The Response must specify the data from which the response is extracted - two approaches are enabled in in aod.core.Response, but subclasses could define alternatives, if needed.

- 1. By specifying the name of a file within the parent Epoch's files property. If the file requires specialized import, a aod.util.FileReader subclass can be provided as well. If not, one of the built-in readers will be assigned based on the file's extension (see findFileReader for supported extensions).
- 2. By specifying a Dataset associated with the parent Epoch.