## **Using Bio-Formats in MATLAB**

This section assumes that you have installed the MATLAB toolbox as instructed in the <u>MATLAB user</u> <u>information page</u>. Note the minimum supported MATLAB version is R2007b (7.5).

As described in <u>Using Java Libraries</u>, every installation of MATLAB includes a <u>JVM</u> allowing use of the Java API and third-party Java libraries. All the helper functions included in the MATLAB toolbox make use of the Bio-Formats Java API. Please refer to the <u>Javadocs</u> for more information.

### Increasing JVM memory settings

The default JVM settings in MATLAB can result in java.lang.OutOfMemoryError: Java heap space exceptions when opening large image files using Bio-Formats. Information about the Java heap space usage in MATLAB can be retrieved using:

```
java.lang.Runtime.getRuntime.maxMemory
```

Default <u>IVM</u> settings can be increased by creating a <u>java.opts</u> file in the startup directory and overriding the default memory settings. We recommend using -Xmx512m in your java.opts file. Calling:

```
bfCheckJavaMemory()
```

will also throw a warning if the runtime memory is lower than the recommended value.

If errors of type <code>java.lang.OutOfMemoryError</code>: PermGen <code>space</code> are thrown while using Bio-Formats with the Java bundled with MATLAB (Java 6 or 7), you may try to increase the default values of <code>-</code> XX:MaxPermSize and <code>-XX:PermSize</code> via the <code>java.opts</code> file.

See also

http://www.mathworks.com/matlabcentral/answers/92813

How do I increase the heap space for the Java VM in MATLAB 6.0 (R12) and later versions?

[ome-users] Release of OMERO & Bio-Formats 5.1.1

# Opening an image file

The first thing to do is initialize a file with the <u>bfopen</u> function:

```
data = bfopen('/path/to/data/file');
```

This function returns an <u>n</u>-by-4 cell array, where <u>n</u> is the number of series in the dataset. If <u>s</u> is the series index between 1 and n:

- The  $data\{s, 1\}$  element is an m-by-2 cell array, where m is the number of planes in the m-th series. If t is the plane index between 1 and m:
  - The data(s, 1)(t, 1) element contains the pixel data for the t-th plane in the s-th series.
  - The data(s, 1)(t, 2) element contains the label for the t-th plane in the s-th series.
- The data(s, 2) element contains original metadata key/value pairs that apply to the s-th series.
- The data(s, 3) element contains color lookup tables for each plane in the s-th series.
- The data(s, 4) element contains a standardized OME metadata structure, which is the same regardless of the input file format, and contains common metadata values such as physical pixel

### Accessing planes

Here is an example of how to unwrap specific image planes for easy access:

```
seriesCount = size(data, 1);
series1 = data{1, 1};
series2 = data{2, 1};
series3 = data{3, 1};
metadataList = data{1, 2};
% etc
series1_planeCount = size(series1, 1);
series1_plane1 = series1{1, 1};
series1_label1 = series1{1, 2};
series1_plane2 = series1{2, 1};
series1_label2 = series1{2, 2};
series1_label3 = series1{3, 1};
series1_label3 = series1{3, 2};
```

### Displaying images

If you want to display one of the images, you can do so as follows:

```
series1_colorMaps = data{1, 3};
figure('Name', series1_label1);
if (isempty(series1_colorMaps{1}))
   colormap(gray);
else
   colormap(series1_colorMaps{1}(1,:));
end
imagesc(series1_plane1);
```

This will display the first image of the first series with its associated color map (if present). If you would prefer not to apply the color maps associated with each image, simply comment out the calls to colormap.

If you have the image processing toolbox, you could instead use:

```
imshow(series1_plane1, []);
```

You can also create an animated movie (assumes 8-bit unsigned data):

```
v = linspace(0, 1, 256)';
cmap = [v v v];
for p = 1 : size(series1, 1)
   M(p) = im2frame(uint8(series1{p, 1}), cmap);
end
if feature('ShowFigureWindows')
   movie(M);
end
```

### Retrieving metadata

There are two kinds of metadata:

- Original metadata is a set of key/value pairs specific to the input format of the data. It is stored in the data{s, 2} element of the data structure returned by bfopen.
- **OME metadata** is a standardized metadata structure, which is the same regardless of input file format. It is stored in the <a href="mailto:data{s, 4}">data{s, 4}</a> element of the data structure returned by <a href="mailto:bfopen">bfopen</a>, and contains common metadata values such as physical pixel sizes, instrument settings, and much more. See the <a href="mailto:OME Model and Formats">OME Model and Formats</a> documentation for full details.

#### Original metadata

To retrieve the metadata value for specific keys:

```
% Query some metadata fields (keys are format-dependent)
metadata = data{1, 2};
subject = metadata.get('Subject');
title = metadata.get('Title');
```

To print out all of the metadata key/value pairs for the first series:

```
metadataKeys = metadata.keySet().iterator();
for i=1:metadata.size()
  key = metadataKeys.nextElement();
  value = metadata.get(key);
  fprintf('%s = %s\n', key, value)
end
```

#### **OME** metadata

Conversion of metadata to the OME standard is one of Bio-Formats' primary features. The OME metadata is always stored the same way, regardless of input file format.

To access physical voxel and stack sizes of the data:

```
omeMeta = data{1, 4};
stackSizeX = omeMeta.getPixelsSizeX(0).getValue(); % image width, pixels
stackSizeY = omeMeta.getPixelsSizeY(0).getValue(); % image height, pixels
stackSizeZ = omeMeta.getPixelsSizeZ(0).getValue(); % number of Z slices
voxelSizeXdefaultValue = omeMeta.getPixelsPhysicalSizeX(0).value();
                                                                                % returns valu
voxelSizeXdefaultUnit = omeMeta.getPixelsPhysicalSizeX(0).unit().getSymbol(); % returns the
voxelSizeX = omeMeta.getPixelsPhysicalSizeX(0).value(ome.units.UNITS.MICROM); % in \( \mu m \)
voxelSizeXdouble = voxelSizeX.doubleValue();
                                                                                % The numeric
voxelSizeY = omeMeta.getPixelsPhysicalSizeY(0).value(ome.units.UNITS.MICROM); % in \( \mu m \)
voxelSizeYdouble = voxelSizeY.doubleValue();
                                                                                % The numeric
voxelSizeZ = omeMeta.getPixelsPhysicalSizeZ(0).value(ome.units.UNITS.MICROM); % in μm
voxelSizeZdouble = voxelSizeZ.doubleValue();
                                                                                % The numeric
```

For more information about the methods to retrieve the metadata, see the <u>MetadataRetrieve</u> Javadoc page.

To convert the OME metadata into a string, use the dumpXML() method:

```
omeXML = char(omeMeta.dumpXML());
```

# Reading from an image file

The main inconvenience of the <u>bfopen.m</u> function is that it loads all the content of an image regardless of its size.

To access the file reader without loading all the data, use the low-level <u>bfGetReader.m</u> function:

```
reader = bfGetReader('path/to/data/file');
```

You can then access the OME metadata using the getMetadataStore() method:

```
omeMeta = reader.getMetadataStore();
```

Individual planes can be queried using the <u>bfGetPlane.m</u> function:

```
series1_plane1 = bfGetPlane(reader, 1);
```

To switch between series in a multi-image file, use the <u>setSeries(int)</u> method. To retrieve a plane given a set of (z, c, t) coordinates, these coordinates must be linearized first using <u>getIndex(int, int, int)</u>

```
% Read plane from series iSeries at Z, C, T coordinates (iZ, iC, iT)
% All indices are expected to be 1-based
reader.setSeries(iSeries - 1);
iPlane = reader.getIndex(iZ - 1, iC -1, iT - 1) + 1;
I = bfGetPlane(reader, iPlane);
```

## Saving files

The basic code for saving a 5D array into an OME-TIFF file is located in the <u>bfsave.m</u> function.

For instance, the following code will save a single image of 64 pixels by 64 pixels with 8 unsigned bits per pixels:

```
plane = zeros(64, 64, 'uint8');
bfsave(plane, 'single-plane.ome.tiff');
```

And the following code snippet will produce an image of 64 pixels by 64 pixels with 2 channels and 2 timepoints:

```
plane = zeros(64, 64, 1, 2, 2, 'uint8');
bfsave(plane, 'multiple-planes.ome.tiff');
```

By default, <u>bfsave</u> will create a minimal OME-XML metadata object containing basic information such as the pixel dimensions, the dimension order and the pixel type. To customize the OME metadata, it is possible to create a metadata object from the input array using <u>createMinimalOMEXMLMetadata.m</u>, add custom metadata and pass this object directly to bfsave:

```
plane = zeros(64, 64, 1, 2, 2, 'uint8');
metadata = createMinimalOMEXMLMetadata(plane);
pixelSize = ome.units.quantity.Length(java.lang.Double(.05), ome.units.UNITS.MICROM);
metadata.setPixelsPhysicalSizeX(pixelSize, 0);
metadata.setPixelsPhysicalSizeY(pixelSize, 0);
pixelSizeZ = ome.units.quantity.Length(java.lang.Double(.2), ome.units.UNITS.MICROM);
metadata.setPixelsPhysicalSizeZ(pixelSizeZ, 0);
bfsave(plane, 'metadata.ome.tiff', 'metadata', metadata);
```

For more information about the methods to store the metadata, see the MetadataStore Javadoc page.

# Improving reading performance

Initializing a Bio-Formats reader can consume substantial time and memory. Most of the initialization time is spend in the <u>setId(java.lang.String)</u> call. Various factors can impact the performance of this step including the file size, the amount of metadata in the image and also the file format itself.

One solution to improve reading performance is to use Bio-Formats memoization functionalities with the <u>loci.formats.Memoizer</u> reader wrapper. By essence, the speedup gained from memoization will only happen after the first initialization of the reader for a particular file.

The simplest way to make use the Memoizer functionalities in MATLAB is illustrated by the following example:

```
% Construct an empty Bio-Formats reader
r = bfGetReader();
```

```
% Decorate the reader with the Memoizer wrapper
r = loci.formats.Memoizer(r);
% Initialize the reader with an input file
% If the call is longer than a minimal time, the initialized reader will
% be cached in a file under the same directory as the initial file
% name .large_file.bfmemo
r.setId(pathToFile);
% Perform work using the reader
% Close the reader
r.close()
% If the reader has been cached in the call above, re-initializing the
% reader will use the memo file and complete much faster especially for
% Large data
r.setId(pathToFile);
% Perform additional work
% Close the reader
r.close()
```

If the time required to call <u>setId(java.lang.String)</u> method is larger than <u>DEFAULT\_MINIMUM\_ELAPSED</u> or the minimum value passed in the constructor, the initialized reader will be cached in a memo file under the same folder as the input file. Any subsequent call to <u>setId()</u> with a reader decorated by the <u>Memoizer</u> on the same input file will load the reader from the memo file instead of performing a full reader initialization.

More constructors are described in the <u>Memoizer javadocs</u> allowing to control the minimal initialization time required before caching the reader and/or to define a root directory under which the reader should be cached.

As Bio-Formats is not thread-safe, reader memoization offers a new solution to increase reading performance when doing parallel work. For instance, the following example shows how to combine memoization and MATLAB parfor to do work on a single file in a parallel loop:

```
% Construct a Bio-Formats reader decorated with the Memoizer wrapper
r = loci.formats.Memoizer(bfGetReader(), 0);
% Initialize the reader with an input file to cache the reader
r.setId(pathToFile);
% Close reader
r.close()
nWorkers = 4;
% Enter parallel loop
parfor i = 1: nWorkers
    % Initialize a new reader per worker as Bio-Formats is not thread safe
    r2 = javaObject('loci.formats.Memoizer', bfGetReader(), 0);
    % Initialization should use the memo file cached before entering the
    % parallel loop
    r2.setId(pathToFile);
    % Perform work
    % Close the reader
    r2.close()
end
```