Quick Guide to Cryo-Correlative Microscopy

Vladan Lucic

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Max Planck Institute of Biochemistry
Email: vladan@biochem.mpg.de

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1 Summary

The goal of the correlation approach is to locate an object of interest in different imaging modes. (typically light microscopy) in another imaging mode. This document describes concepts and procedure for cryo-correlative microscopy procedures as implemented in Pyto. The imaging modes for which correlation procedures are currently implemented are light / fluorescent miscroscopy (2D and 3D), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and focused ion beam (FIB). These procedures are meant for TEM, SEM and FIB imaging of cryo-preserved samples (imaged in the fully hydrated, vitrified state).

2 Introduction

2.1 General

The correlation procedures described here are coordinate system-based. Namely, given the location (coordinates) of one or more objects of interest (targets) in one imaging mode (initial system), the correlation procedure yields its / their location(s) in another imaging mode (final system). In order to achieve this, it is necessary to establish the correlation between the two imaging systems, that is to find a coordinate transformation between the two systems. This transformation is then used to obtain the coordinates of targets in the final system from their coordinates in the initial system.That is, the correlation procedure consists of two general steps:

1. Establish a correlation between the initial and the final system.
2. Correlate positions of objects of interest (targets) from one system to the other.

Typically the initial system is a light microscopy image (single 2D image or a 3D confocal stack) and the final system is one of the EM modes (transmission EM image, EM stage coordinates, or electron / ion beam scanning image), but other choices are also possible.

2.2 Direct vs. indirect correlation

2.2.1 Direct correlation

A direct correlation requires only the initial and the final system, that is it does not involve intermediate systems. It is easier to use than the indirect correlation, but it has limited applicability. Conceptually, a direct correlation procedure is composed of the following steps:

1. Get the marker positions (coordinates) in the initial and the final systems

2. Establish the correlation, that is find the coordinate transformation between the systems
3. Get the positions (coordinates) of the objects of interest (targets) in the initial system
4. Calculate the positions (coordinates) of the objects of interest in the final system by correlating the target points to the final system using the transformation between the systems

Steps 1 and 2 need to be done by the user (see section 3.1), while steps 3 and 4 are performed by the scripts.

2.2.2 Indirect correlation

If the initial and the final systems are very different, so that it is not possible to find markers that can be detected in both systems, direct correlation can not be used. In these cases one or more intermediate systems need to be used, so that a chain of direct transformations that connect the initial and the final transformation via the intermediate systems is created. This procedure consists of the following steps:

- Get corresponding markers in the initial and the first intermediate system and find the transformation between them
- Get corresponding markers in the first and the second intermediate systems and find the transformation between them
- ...
- Get corresponding markers in the last intermediate and the final systems and find the transformation between them
- Get the transformation between the initial and the final systems by the composition of the transformations obtained above
- Get the positions (coordinates) of the objects of interest (targets) in the initial system and correlate them to the final system using the transformation found in the previous step

Finding the individual and the final transformation, as well as correlating target points are done by the scripts, while the user needs to get all marker coordinates.

3 Usage

3.1 Markers and targets

In order to establish a direct correlation between two systems, the user needs to find suitable markers that can be detected in both systems and to extract their coordinates. Importantly, marker coordinates in one system have to correspond to the marker coordinates in the other system, that is the same markers have to be chosen in both systems. How the markers are obtained and saved generally depends on the image viewer used. In some cases, such as for TEM stage position, it depends on the software that exposes the coordinates. Target coordinates are obtained in the same way.

The simplest way is to have marker coordinates stored in a file, where each marker takes one row and some of the columns contain x, y, ... coordinates. It is recommended, but not necessary, to use ImageJ / Fuji to record marker and target coordinates and save them in a file. The advantage of following the ImageJ / Fiji procedure given below is that the correlation scripts are already set to read the coordinate file generated in that way. Alternatively, other software can be used, but the user then has to set the file format parameters, such as the column number for x, y, ... coordinates, comment sign, or the field separator.

Generally, marker coordinates for all systems, as well as target coordinates can be stored in an arbitrary order in one (recommended) or multiple files.

3.1.1 Coordinates by ImageJ / Fiji

1. (optional) Set to pixels: ImageJ / Image / Properties: Units = pix; x width = y width = 1
2. Open measurement options by Analyze / Set measurements
3. Set measurements: mean grey and stack position are sufficient; display label, add to overlay, decimal places=1 are useful
4. To store selected points to ROI (useful): Edit / Options / Point tool (or double click on point tool): Auto measure, add to ROI, label
5. Activate point tool
6. For each point (markers in both systems and objects of interest): Click on a point (if auto measure was not set need Ctrl-M to put it in the results; shift-click might also work)
7. When ROI manager opens or Analyze / Tools / ROI manager check ROI manager / More / Options / Associate ... with slices in order that points are shown only on the corresponding slices
8. Save results: Results / File / Save as. The name of this file
9. Save ROIs (in zip format) : ROI manager / More / Save

Steps 4, 7 and 9 are useful because they allow saving ROIs and retrieving them at alater point, but are not strictly necessary. Picks saved at ROIs can be displayed on the image at a later point but it's hard to read the pick coordinates. On the contrary, the coordinates are easily accessible in the results file, but it is difficult to display the picks on the same or on another image.

3.2 Scripts and examples for specific procedures

Scripts (in pyto/scripts/) and usage examples (in pyto/examples/correlation/) are provided for several cases. All scripts and examples contain the following sections:

- Instructions: please be sure to read them
- Parameters: Should be edited to enter file names, coordinates and correlation parameters
- Functions (optional) and the main function: Functions used in the scripts that invoke classes and methods of Pyto (should not be modified)

In addition, the input coordinate files and the result files are provided for the examples.

The scripts and examples can be run directly from the command line, or from a Python shell.

3.3 Advanced usage

The above scripts (including functions) can be modified for cases when (other) intermediary systems are used, or the coordinates are specified in files of different formats. Reusing the code from the scripts may suffice for many different applications.

If needed, other methods and attributes of the relevant classes (pyto.geometry.Affine and those that inherit from it) can be used. Please see the relevant doc files.

3.4 Specific procedures

All scripts mentioned here are in pyto/scripts/ and usage examples in pyto/examples/correlation/.

3.4.1 Direct correlation, general

1. Script correlation_simple.py and example correlation_simple.py: Basic correlation between two arbitrary systems where all coordinates are entered directly in the script file. The two systems can have an arbitrary dimensionality (both systems need to be the same dimensionality).

2. Script correlation_simple_fileio.py and example correlation_simple_fileio.py: Basic correlation between two arbitrary systems where all coordinates are read from a file. The two systems can have an arbitrary dimensionality (both systems need to be the same dimensionality).

3.4.2 Indirect correlation LM - TEM

Script correlation_two_step_lm_em.py allows correlating targets such as fluorescent spots detected on 2D light microscopy images (initial system) to the coordinates of TEM stage (2D). A low magnification TEM image (overview) is used as an intermediate system. Different variants of this correlation are shown in the following examples:

1. two_step_move-search.py: move search variant

2. two_step_move-search_gl.py: move search variant with separate general linear (gl) and translation

3. two_step_move-overview.py: move overview variant with a single overview image

4. two_step_mosaic_move-overview.py: move overview variant with a mosaic overview image

Please see the script or the examples for detailed description of these variants.

3.4.3 Indirect correlation LM (2D) - SEM - FIB

Coordinates of targets on a 2D LM images (such as fluorescence images) can be correlated to a FIB image, where one or two SEM images are used as intermediate systems. This method was described in Fukuda et al 2014 (see below) and is intended for a rough correlation. It can be executed by extending the direct correlation script or by calling Pyto methods directly.

3.4.4 Direct correlation: LM (3D) to FIB (2D)

Script correlation_3d_2d.py allows correlating targets such as fluorescent confocal spots in 3D (initial system) to their positions on 2D images such as those produced by FIB, SEM or TEM imaging.This method is intended for a precise correlation.

4 Citing

Please consider citing us if you do correlative work using Pyto.

For 3D to 2D correlation, please cite: Arnold, J., J. Mahamid, V. Lucic, A. d. Marco, J.-J. Fernandez, Laugks, H.-A. Mayer, Tobias, W. Baumeister, and J. Plitzko, 2016. Site-specific cryo-focused ion beam sample preparation guided by 3-dimensional correlative microscopy. Biophysical Journal 110:860-869

For all other correlative work: Fukuda, Y., N. Schrod, M. Schaffer, L. R. Feng, W. Baumeister, and V. Lucic, 2014. Coordinate transformation based cryo-correlative methods for electron tomography and focused ion beam milling. Ultramicroscopy 143:15– 23.

Thank you.