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I. INTRODUCTION

This test concerns only the microscopes equipped with a motorized XY stage.

The “Stage motion control” analysis gathers the “Stage drift during timelapse” and “Stage repositioning repeatability” analyses.

“Stage drift during timelapse”

When performing timelapse imaging, it is important to ensure that the XY stage do not drift too much. Sources of stage drift can origin from the environmental conditions, such as temperature fluctuations and air flow.

The “stage drift during timelapse” analysis allows to measure the XY **stage drift** during a timelapse imaging, to ensure it is within the specifications.

“Stage repositioning repeatability”



During the acquisition of image tiles, the lateral stage may not reposition to some location in a repeatable way. Therefore, it is important to ensure that the XY stage repositioning repeatability is sufficiently accurate when tiling and stitching large regions in 2D or 3D.

The “stage repositioning repeatability” analysis allows to determine the XY motorized ***stage repositioning repeatability*** after many round trips, to ensure it is within the specifications.



II. IMAGE ACQUISITION PROCEDURE

The “*stage motion control*” analysis is associated with any “*repositioning cross*” pattern (Pattern H) or any “*single ring*” (see Figure 1).

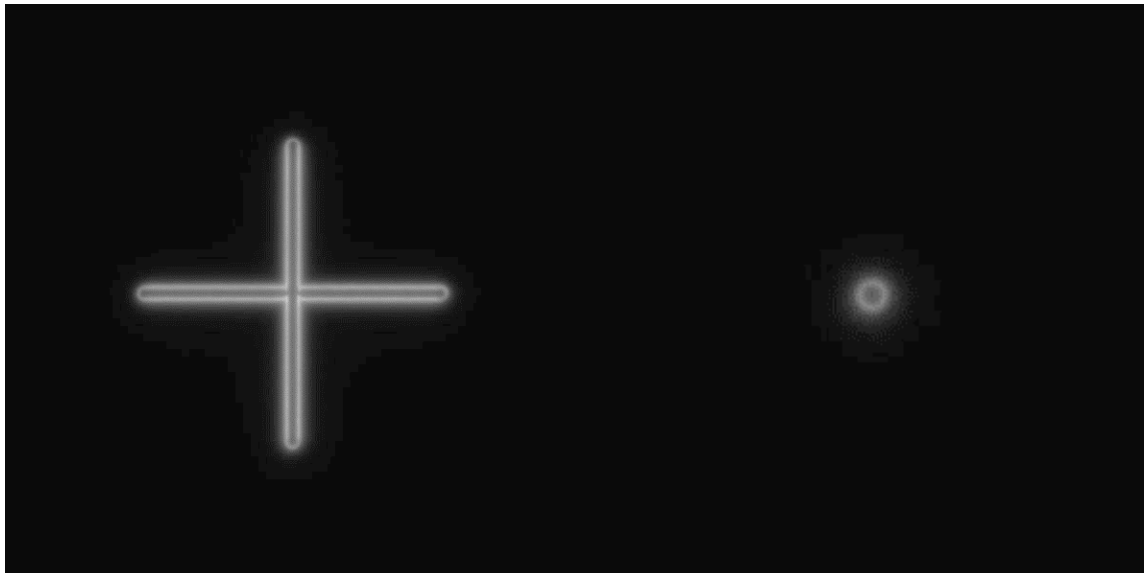


Figure 1: Images example of a “repositioning cross” pattern (left) and of a “single ring” pattern (right), fulfilling the acquisition recommendations.

1. ACQUISITION RECOMMENDATIONS

- **Recommended image type**

Z stack	No
Multi-channel	No
Tiles (or Scenes)	Yes (for “stage repositioning repeatability”)
T-stack	Yes (for “stage drift during timelapse”)

Do not zoom in, this could damage the pattern.

The area of the scanned zone should not be smaller than the area of the pattern.

- **Alignment prior image acquisition**
Align precisely the detector orientation and/or the scanning with respect to the XY translation stage. The analysis, however, can correct a low XY orientation misalignment (a few degrees).
- **Signal-to-background ratio (SBR)**
Acquire images with enough contrast between the pattern and the background, e.g. a signal-to-background ratio higher than 2:1.



- **Signal-to-noise ratio (SNR)**
Acquire images with enough contrast between the pattern and the noise, *e.g.* a signal-to-noise ratio higher than 10:1.
- **Image intensity**
Acquire images within the linear response range of the detector, that is above the detection limit and below the saturation limit. If available in the acquisition software, use the color-coded pixels to adjust properly the image intensity. Note that Daybook Analysis cannot analyze images containing negative values.
- **Image dynamic range**
When possible, acquire images with a detector that captures raw data with a bit depth of 8 or 16 bits, the allowed image dynamic range for computers (1-byte and 2-byte chunks, respectively). If the detector captures raw data with a bit depth different from 8 or 16 bits, convert the images into 8- or 16-bit-dynamic range without losing any information. Note that if the image file weight is too big for the computational capacity of your computer, the analysis may not succeed.
- **Stacking**
Single channel stack images should be acquired using a high numerical aperture objective. The channel should be chosen to provide the best signal-to-background and signal-to-noise ratios. The stack can be:
 - A time series (or time-lapse or T-stacking) of the pattern imaged at a fixed position to measure the XY stage drift over time.
 - Tiles (or scenes) of the pattern imaged after each round trip of the XY stage, for N round trips. The round trips can be in two directions simultaneously (X and Y at the same time), or in one direction (X or Y).

2. HOW TO IMAGE THE PATTERN?

1- Find the patterns

- a) Start with a low mag objective (such as 10× or 20×). Set the DAPI (405 nm) or GFP (488 nm) channel.
- b) Make coincide the center of the slide with respect to the objective.
- c) Adjust focus through the eyepieces.
- d) Switch to the objective you would like to use. Move the slide to the pattern.

2- Adjust your setup

- a) Match the center of the pattern with the center of the field of view.
- b) Adjust the focus.



The best focus usually corresponds to the Z-plane for which the central cross looks the clearest (qualitative approach) and/or for which the intensity histogram is the broadest (quantitative approach).

3- Image your pattern

- a) Image the pattern by following the acquisition recommendations.
- b) Save images into a raw, non-compressed format (for example, the acquisition software proprietary format) or into a lossless compression format (*e.g.* TIFF). The image file must have a dynamic range of 8 or 16 bits.



III. IMAGE ANALYSIS PROCEDURE

1. HOW TO LAUNCH AN ANALYSIS?

- a) Select “Stage motion control” in the “Select analysis” list.
- b) Upload your image(s) using the “Upload file” button.
Select one image of the T-stack of the suitable pattern.
- c) Select the analysis type, *i.e.* “Stage drift during timelapse” or “Stage repositioning repeatability”.
- d) Set the required and optional settings (see chapter 2 “Analysis Settings”).
- e) Click on “Start the analysis”.



- f) If needed, select a region of interest (ROI) and click on “Crop” to crop the image (*cf.* Figure 2).
 - g) Click on “Run”.
- Results are displayed and can be saved as CSV, PDF, or transferred into Daybook Data Manager (if available in your package).

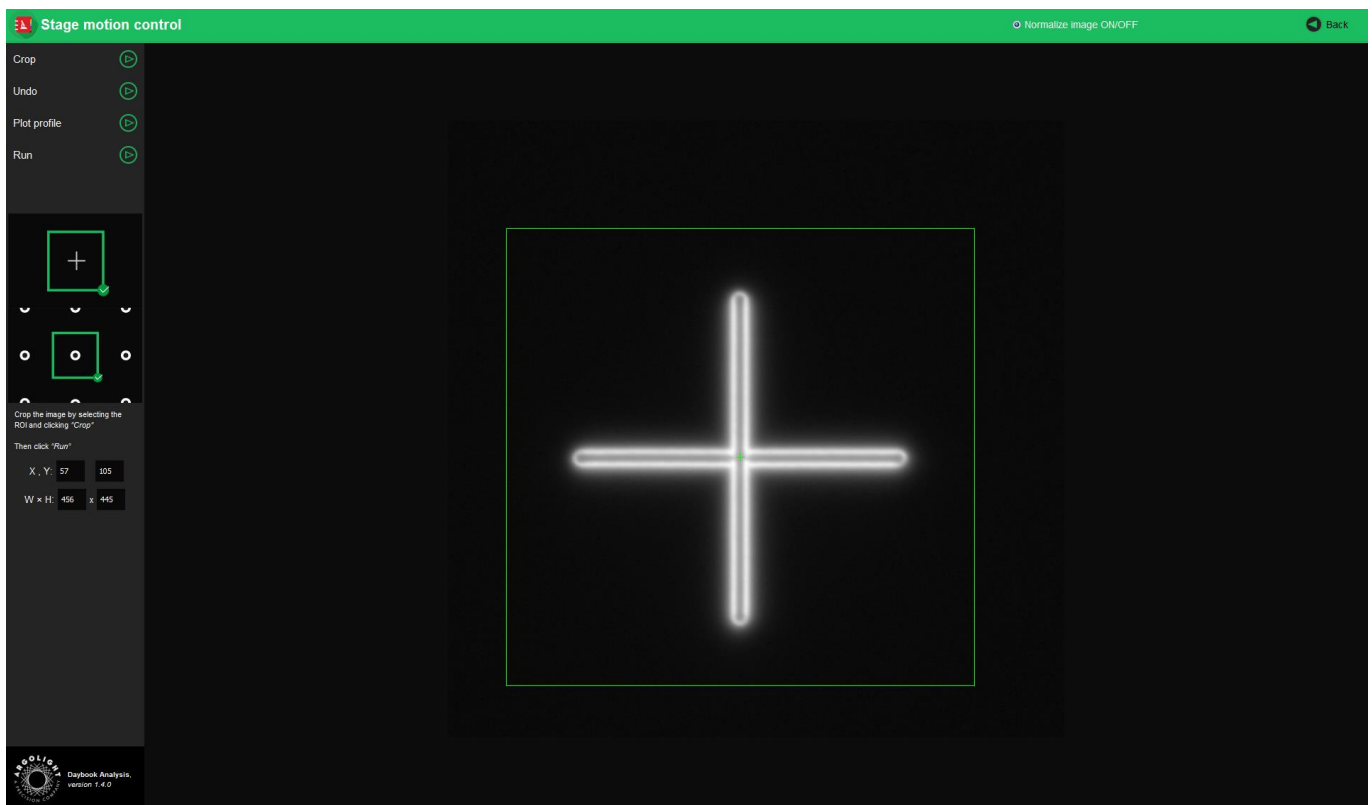


Figure 2: Crop window.



2. ANALYSIS SETTINGS

1- Required settings

- **Specified lateral pixel size**

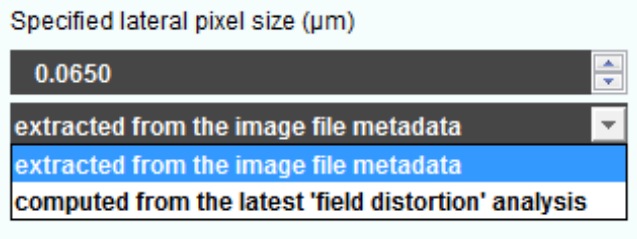
There are two ways to get the lateral pixel size of the image to be analyzed:

- Either from the proprietary file:

Select *“extracted from the image file metadata”*.

- Or from a previous *“field distortion”* analysis:

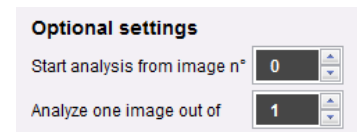
Select *“computed from the latest ‘field distortion’ analysis”*.



2- Optional settings

- **Partial analysis**

If necessary, the analysis can be set to start from a given image and to analyze a fraction of the images out of the entire T-stack.



IV. RESULTS PAGE DESCRIPTION

The picture below shows the results page for this analysis (cf. Figure 3). Results are displayed in the form of maps, graphs, and tables.

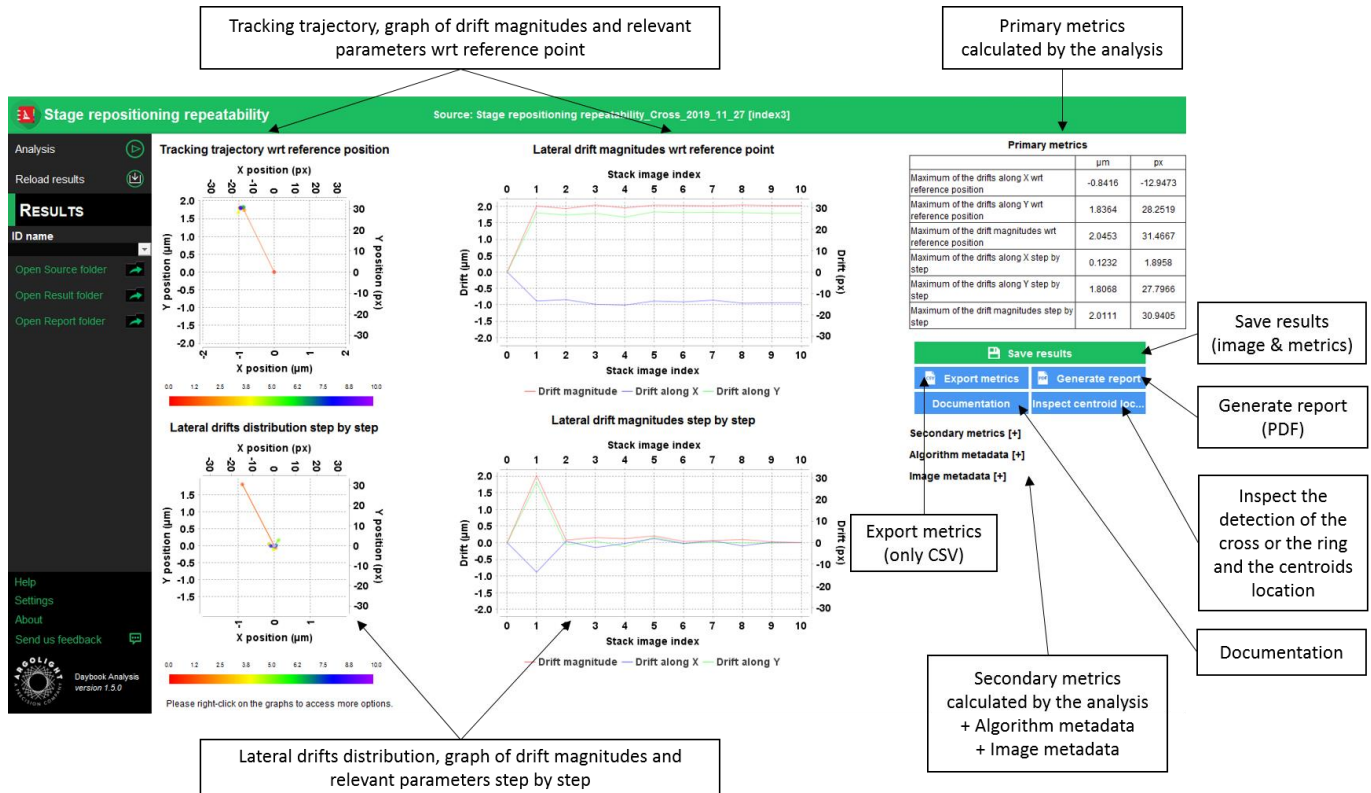


Figure 3: Results page.

Click on “Inspect centroid locations” to see the detection of the crosses/rings, as well as the location of the centroids in the T-stack.

When a valid Daybook Data Manager license is registered, the results are transferred into Daybook Data Manager thanks to the “Save in Data Manager” button.

Reports (in a PDF format) containing the results (maps, graphs, metrics) can be generated and saved by clicking on the “Generate report” button (cf. Figure 3).

By default, the results will be saved in the “/Daybook results” folder, located within the Daybook directory. To modify the default folder, go to the “Settings” menu at the bottom left corner.

When a valid Daybook Data Manager license key is registered, the “Save results” button becomes “Save into Data Manager”. Results are therefore transferred into Daybook Data Manager when clicking the “Save in Data Manager” button. To do that, in the saving window interface, select the system, acquisition profile and associated channel for which you would like to save the results.



By default, the results are saved at the acquisition date of the image. If the acquisition date is not in the metadata of the image, it is possible to save the results at the present date (date of the analysis) or at a custom date (cf. Figure 4).

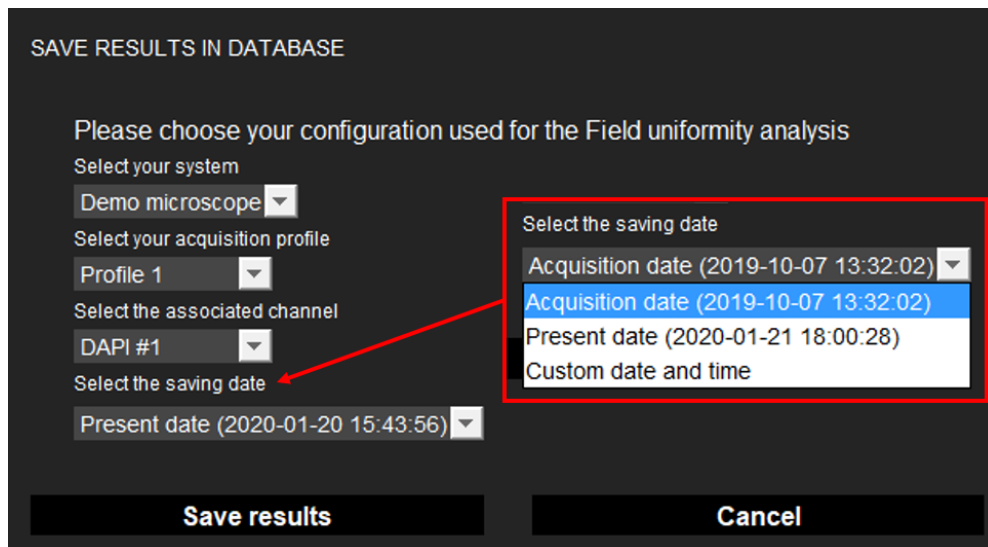


Figure 4: Interface window for saving the results in the database.

- **Image options:**
 - Zoom in and out. The images can be zoomed in and out by using the mouse roller.
- **Graph options:**
 - Zoom in and out: Hold the left or right button of the mouse and move it towards the bottom right to create a selection rectangle. To go back to the initial size, hold the left or right button of the mouse and move it towards any direction.
 - Optional features. Right click on the graph to have access to:
 - “Properties”: Edit the chart properties.
 - “Save as”: Save an image into a PNG or JPEG file, or the graph values into a TXT file.
 - “AutoRange”: Adjust automatically the ranges of the axes.



V. ANALYSIS ALGORITHM DESCRIPTION

The diagram below describes the algorithm that allows the extraction of the stage drift during a timelapse or the stage repositioning repeatability from a T-stack of a “single cross” or a “single ring” (cf. Figure 5).

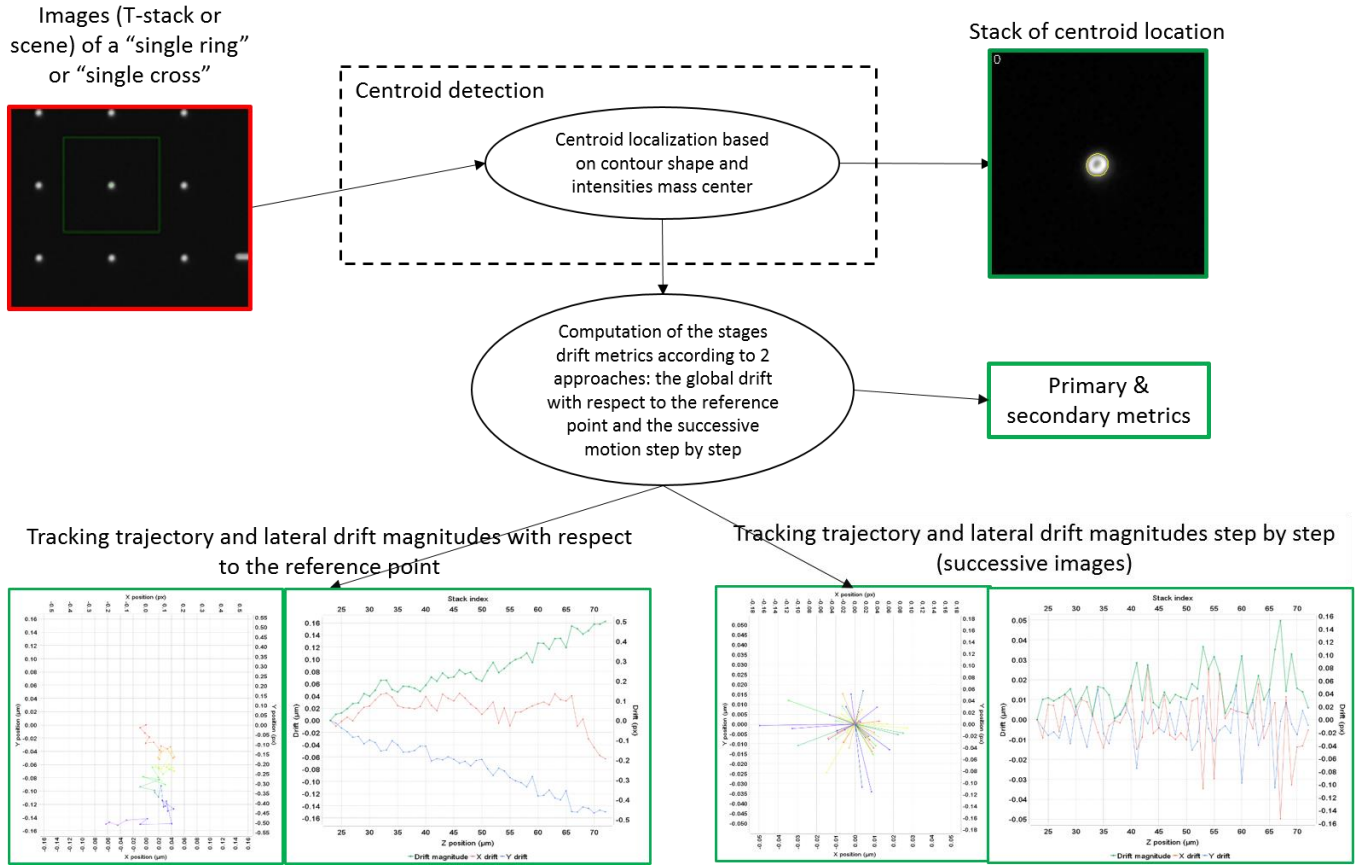


Figure 5: Schematic description of the different steps of the analysis algorithm.

In short, the algorithm works as follows:

- It detects and segments the “single cross” or “single ring” for each image.
- It determines the XY coordinates of the centroid of the “single cross” or “single ring” in each image.
- It measures the drift between these coordinates all along the T-stack.
- It displays these drifts into graphs. The graphs show lateral drift magnitudes: one with respect to a reference point, and the other with respect to the previous value (step by drift).



VI. OUTPUT METRIC DESCRIPTION

The images, graphs and metrics provided by this analysis are gathered in two groups: “wrt reference position” and “step by step”:

- “wrt reference position” means that any drift is measured with respect to a reference position. This reference position is the initial position, *i.e.* the coordinates of the centroid of the cross/ring in the **first analyzed image**.
- “step by step” means that any drift is measured with respect to the drift value preceding it.

- *Drift magnitude* is expressed both in pixel and in μm , and is given by the following equation:

$$\text{Drift magnitude} = \sqrt{(\text{Drift along } X)^2 + (\text{Drift along } Y)^2}$$

1. PRIMARY METRICS

- *Maximum of the drifts along X or Y* is the maximum value of the drifts along X or Y. It is expressed both in pixel and in μm .
- *Maximum of the drift magnitudes* is the maximum value of the drift magnitudes. It is expressed both in pixel and in μm .

2. SECONDARY METRICS

- *Minimum of the drifts along X or Y* is the minimum value of the drifts along X or Y. It is expressed both in pixel and in μm .
- *Mean of the drifts along X or Y* is the mean (average) value of the drifts along X or Y. It is expressed both in pixel and in μm .
- *Standard deviation of the drifts along X or Y* is the standard deviation of the drifts along X or Y. It is expressed both in pixel and in μm .
- *Minimum of the drift magnitudes* is the minimum value of the drift magnitudes. It is expressed both in pixel and in μm .
- *Mean of the drift magnitudes* is the mean (average) value of the drift magnitudes. It is expressed both in pixel and in μm .
- *Standard deviation of the drift magnitudes* is the standard deviation of the drift magnitudes. It is expressed both in pixel and in μm .



3. ALGORITHM METADATA

- *Analysis date* is the date at which the analysis has been performed.
- *Software version* is the version of the software.
- *Product type* is the type of Argolight product selected in the panel settings.
- *Estimated limit of the algorithm* is the evaluated practical limit of the algorithm on the measurement of the shifts. It is expressed both in pixel and μm .
- *Index of the first analyzed image in the stack* is the index value entered in “Start analysis from image n°” from the analysis settings.
- *Index of the discarded images in the stack* is the index value entered in “Analyze one image out of” from the analysis settings.
- *X coordinate of the ROI* is the coordinate along X (starting from the top left corner) of the cropped area in the image. A null value corresponds to an uncropped image. It is expressed in pixel.
- *Y coordinate of the ROI* is the coordinate along Y (starting from the top left corner) of the cropped area in the image. A null value corresponds to an uncropped image. It is expressed in pixel.
- *ROI width* is the width of the cropped area in the image. A value equal to the image width corresponds to an uncropped image. It is expressed in pixel.
- *ROI height* is the height of the cropped area in the image. A value equal to the image height corresponds to an uncropped image. It is expressed in pixel.

4. IMAGE METADATA

- *Acquisition date* is the date at which the acquisition of the image has been performed. If this information is not contained in the metadata of the image, then the note “unknown” is displayed.
- *Specified lateral pixel size* is the size of one pixel, provided by the metadata associated to the raw image. It is expressed in μm .
- *Specified axial pixel size* is the interval between each slice of the stack, provided by the metadata associated to the raw image. It is expressed in μm .



- *Image dynamic range* is the dynamic range of the image, provided by the metadata associated to the raw image. It is expressed in bits (8 or 16 bits).
- *Detector bit depth* is the data capturing range of the detector, provided by the metadata associated to the raw image. It is expressed in bits. For example, a 16-bit detector can capture $2^{16} = 65536$ intensity levels.
- *Image width* is the width of the image, provided by the metadata associated to the raw image. It is expressed in pixel.
- *Image height* is the height of the image, provided by the metadata associated to the raw image. It is expressed in pixel.



Encountered an issue or a question when using Daybook Analysis?

Please send a screenshot and your issue description at:

customer@argolight.com