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I. INTRODUCTION

The lateral resolution is the minimal distance between two objects close to each other that an imaging system can clearly measure.

There are different ways to measure the lateral resolution. The method used in this analysis relies on the contrast transfer function, *i.e.* the measured contrast versus the measured distance between gradually spaced lines.

The “lateral resolution” analysis provides uniquely the ***minimal resolvable distance*** between lines close to each other, for a given ***contrast*** value, with an associated ***signal-to-noise ratio*** (***SNR)*** value in the image.

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II. IMAGE ACQUISITION PROCEDURE

The ***"lateral resolution"*** analysis is associated with the ***"gradually spaces lines"*** patterns (Pattern family E - see Figure 1).

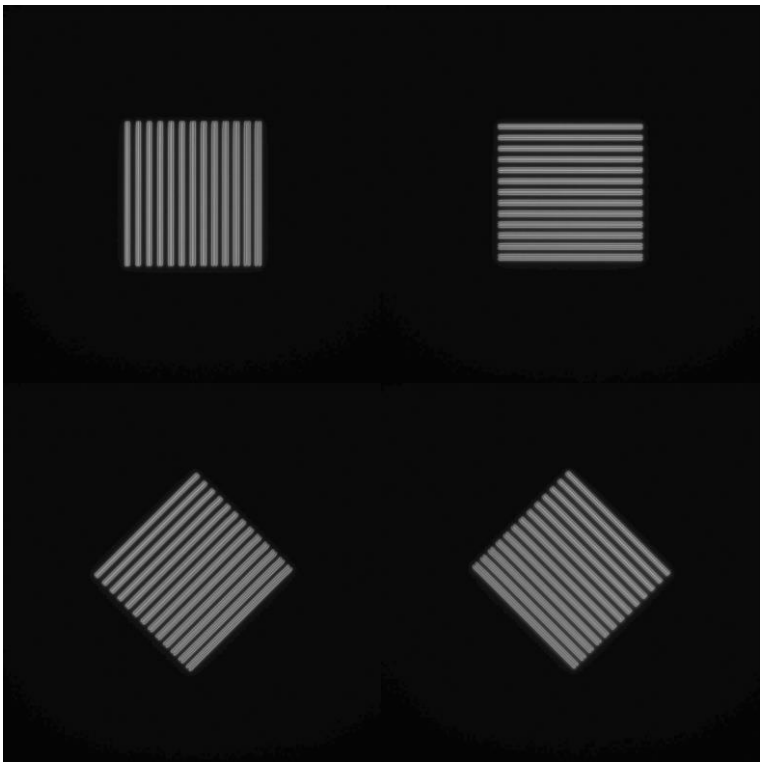

Figure 1: Example of images of the 4 "gradually spaced lines" patterns (vertical, horizontal, ascending and descending lines), fulfilling the acquisition recommendations.

1. ACQUISITION RECOMMENDATIONS

- **Recommended image type**

Z stack	Yes (if your microscope allows to do it)
Multi-channel	Recommended but not mandatory
Tiles	No

When a multi-channel Z-stack is acquired, the reader in Daybook separates each channel so that one Z-stack per channel can be analyzed.

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.

- **Lateral pixel size**
  The lateral pixel size of the Z-stack should be equal to the half of the theoretical lateral resolution limit (Nyquist criterion). However, if possible, we recommend that you adjust the image lateral pixel size to one-third of the theoretical lateral resolution limit.

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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) Align the center of the slide with 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 pattern looks the clearest (qualitative approach) and/or for which the intensity histogram is the broadest (quantitative approach).

3- Image the pattern and save the image

a) Image the pattern by following the acquisition recommendations.
b) Save the image into the proprietary format of the acquisition software or into a lossless compressed format. If saved into a compressed lossless format, the image file should have a dynamic range of 8 or 16 bits. Also, the metadata should be contained in the image file.

Important:
This pattern must be imaged entirely. Do not zoom into the pattern.
If the pattern is not imaged entirely, the analysis will not work.

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III. IMAGE ANALYSIS PROCEDURE

1. HOW TO LAUNCH AN ANALYSIS?

a) Select “Lateral resolution” in the “Select analysis” list.

b) Upload your image(s) using the “Upload file” button.
Select the image to be analyzed.

c) Set the required and optional settings (see section III.2 “Analysis Settings”).

d) Click on “Start the analysis”.

e) If needed, select a region of interest (ROI) and click on “Crop” to crop the image (*cf.* Figure 2).

f) Click on “Run”.
Results are displayed and can be saved as CSV, PDF, or transferred into Daybook Data Manager (if available in your package).

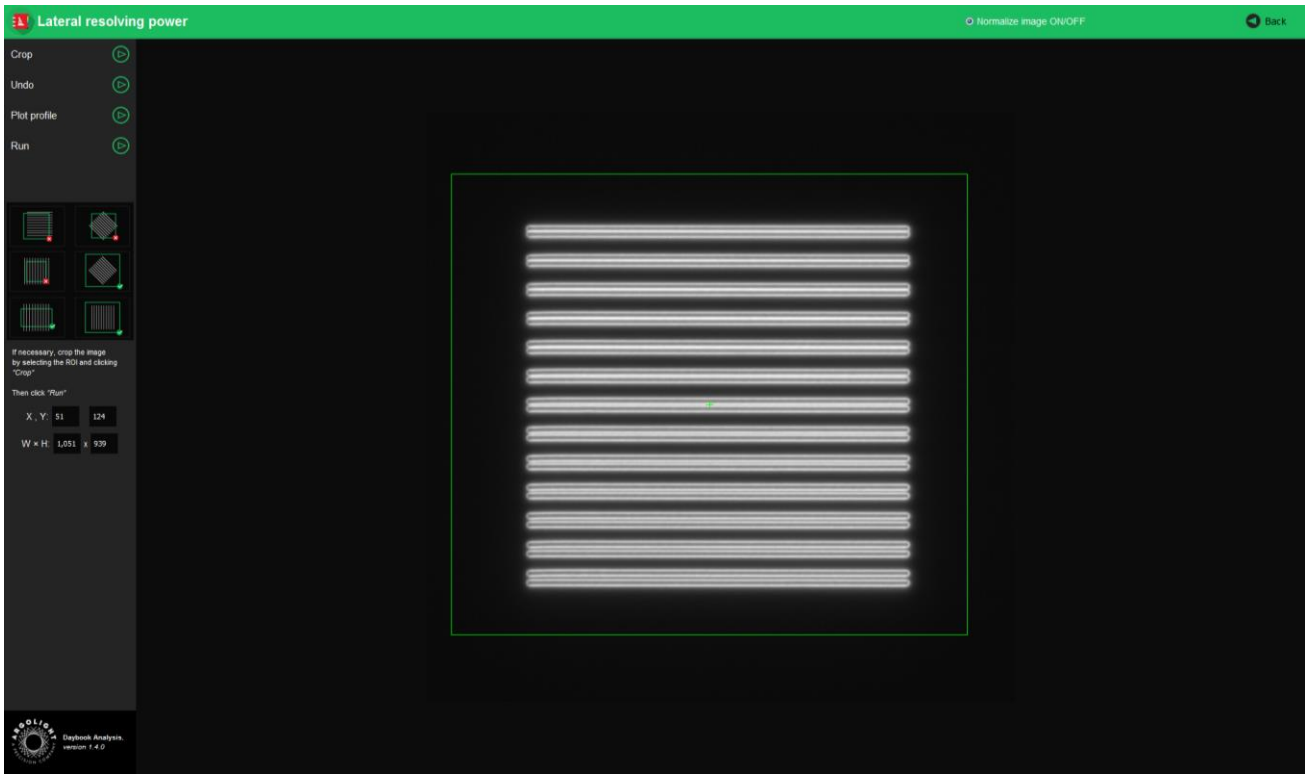

Figure 2: Crop window.

2. ANALYSIS SETTINGS

1- <u>Required settings</u>

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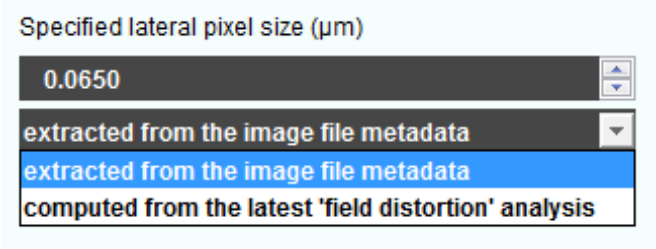
- **Specified lateral pixel size**
  There are two ways to get the lateral pixel size of the image to be analyzed:
  - Either from the image file metadata:
  Select *"extracted from the image file metadata"*.
  - Or from a previous *"field distortion"* analysis:
  Select *"computed from the latest 'field distortion' analysis"*.



- **Contrast criterion**
  The user can define here the contrast value that he would like to apply, from 0 to 100 %. There are many criteria (Rayleigh → 26.5 %, Schuster → 99.9 %, Sparrow → 0.1 %, etc.) that can be applied to determine the lateral resolution. Note that the human eye can distinguish intensity differences that have a minimal contrast value of 2 %.

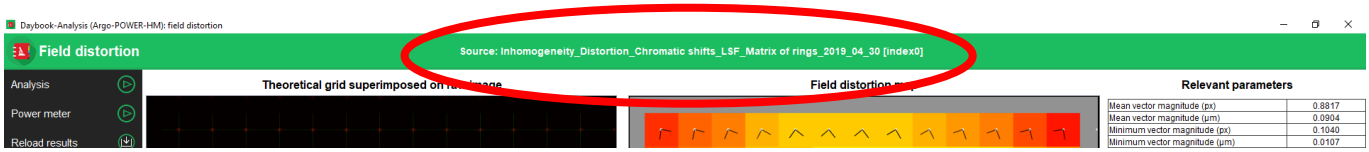
2- Optional settings

- **Background subtraction**
  Subtract the background in images where the signal-to-background ratio (SBR) is too low to be analyzed by Daybook Analysis.
  It requires acquiring an image of an area where there is no fluorescent pattern (*i.e.* a background image) with the same settings (channel, illumination power, exposure time, etc.) as the image of the pattern to be analyzed.
  For multi-channel tests, a background image for each channel is required.

- **Hot pixels removal**
  Remove the very intense (*i.e.* hot) pixels that may cause analysis issues.
  Use this option only if you have such hot pixels in the image.

- **Specified axial pixel size**
  On Z-stacks analysis, the axial pixel size is determined from the image file metadata.

- **Best focus selection**
  Il works only for mono- or multi-channel Z-stacks.
  It automatically selects from a Z-stack the image having the best contrast, corresponding to the best focus for the fluorescent pattern.
  The index of the selected image is displayed in the middle top of the results page (see figure below). Information about the selected image can also be found in the metrics and reports.



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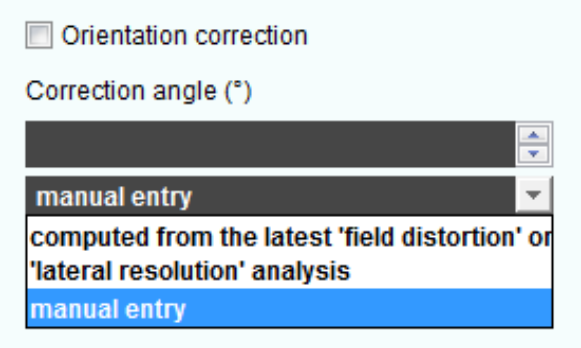
- **Orientation correction**
  There might sometimes be a tilt on the acquired images. Tick the “Orientation correction” button to enable the correction angle option.

- **Correction angle**
  The correction angle is computed from other analyses (*field distortion* or *lateral resolution*). It can also be set manually.



3- “Redo lateral resolution analysis” window

To optimize or to check the influence of the analysis settings (specified lateral pixel size, , fitting functions, contrast criterion, processed ROI width, interpolation factor, correction angle, fitting threshold level) on the results, you can click on the “Redo” button in the results page and re-run the analysis (*cf.* Figure 3).

In particular, the fitting functions, the interpolation factor and the processed ROI width can have a non-negligible influence on whether the analysis works or not. It is advised to increase the interpolation factor and/or the processed ROI width if the following situations occur:

- The detection of peaks and valleys does not work precisely.
- The image is under-sampled (lateral pixel size too large).
- The signal-to-noise ratio and/or the signal-to-background ratio are too low.

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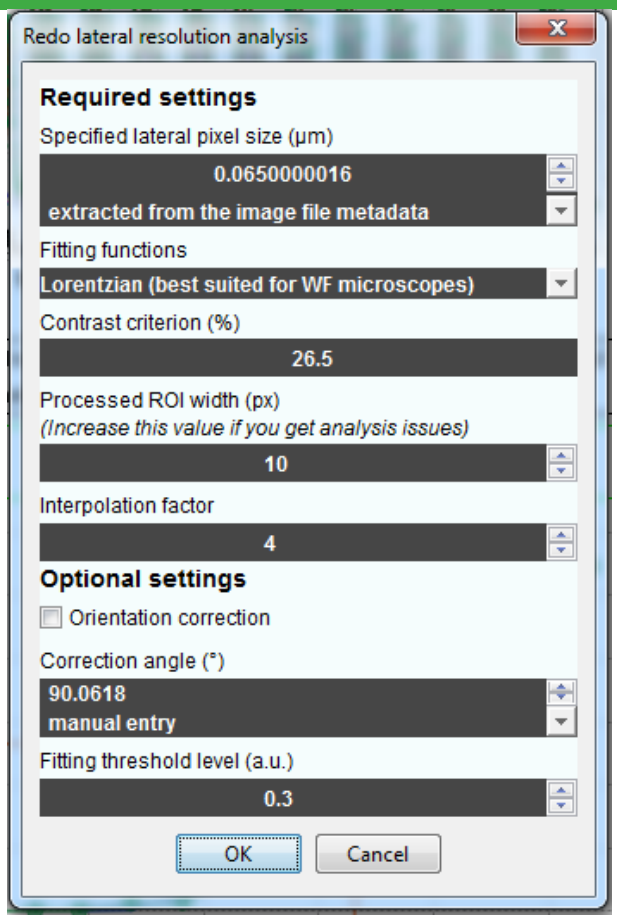

Figure 3: "Redo lateral resolution analysis" panel.

- **Fitting functions**
  The fitting functions are the mathematical functions used to fit the mean intensity profile perpendicular to the gradually spaced lines. Gaussian or Lorentzian fitting functions can be chosen. The Gaussian model is best suited for confocal (CF) and structured illumination microscopes (SIM). The Lorentzian model is best suited for wide-field (WF) microscopes.

- **Processed ROI width**
  The processed ROI width is the width of the Region Of Interest (ROI) analyzed by the algorithm.
  It is automatically calculated when launching the analysis, but it can also be set manually in the "Redo lateral resolution analysis" window.
  The automatic calculation sets the processed ROI width to about one-fourth of the length of the gradually spaced lines.

- **Interpolation factor**
  The interpolation factor, also known as resampling factor, allows the increase of the number of points of the intensity line profile, by applying a Lanczos interpolation.
  It is automatically calculated when launching the analysis, but it can also be set manually in the "Redo lateral resolution analysis" window.
  An interpolation factor of 1 does not change the data.

- **Fitting threshold level**

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The fitting threshold level is the intensity level above which the Gaussian of Lorentzian fitting is performed on the intensity line profile.
It is automatically computed when launching the analysis, but it can also be set manually in the “Redo lateral resolution analysis” window.

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IV. RESULTS PAGE DESCRIPTION

1. INTERFACE

The picture below shows the results page for this analysis (*cf.* Figure 4).
Results are displayed in the form of images, maps, graphs and tables.

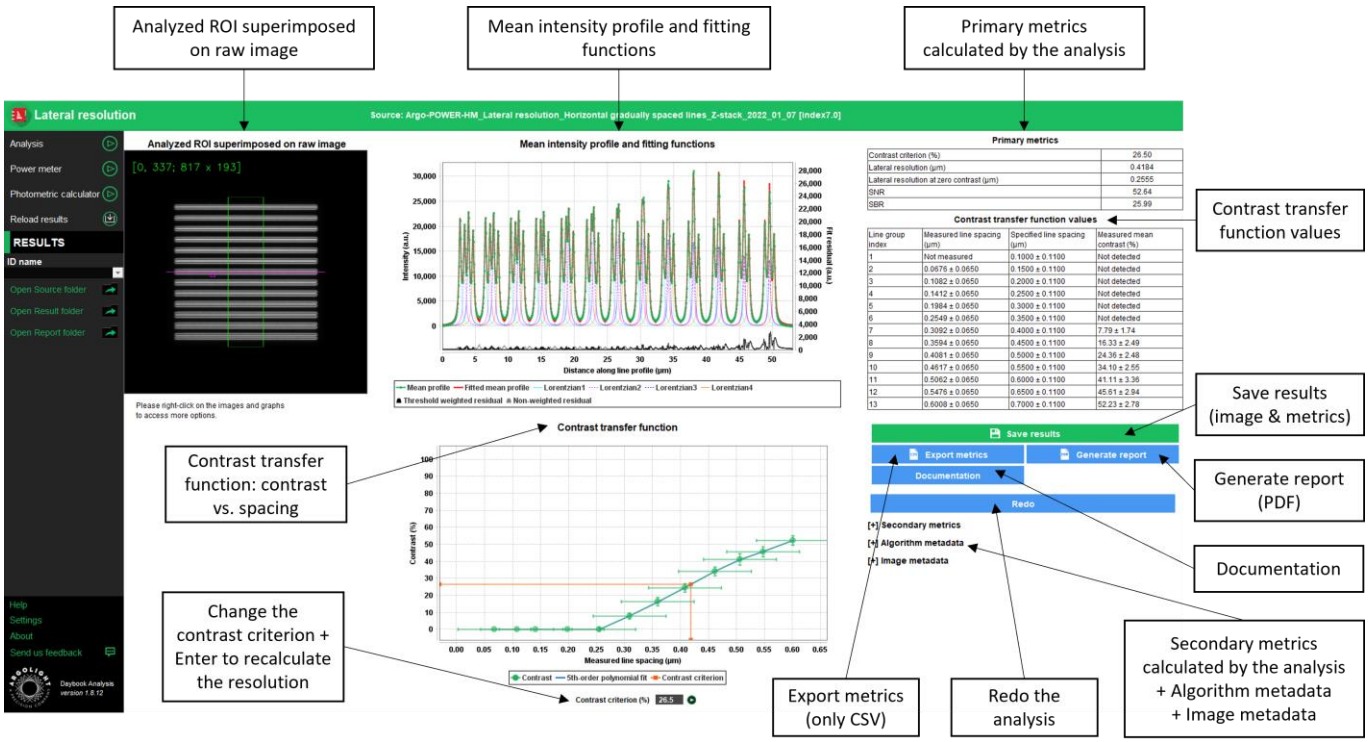

Figure 4: Results page.

2. OPTIONS

- **Saving options:**

When Daybook Data Manager is disabled, the results can be saved into a CSV file thanks to the "Save results" or "Export metrics" buttons.

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 4).

By default, the results will be saved in the "Daybook Analysis\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, the acquisition profile and the associated channel whose results you wish to save.

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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 upload date (date of the image upload), at the present date (date of the image analysis) or at a custom date (*cf.* Figure 5).

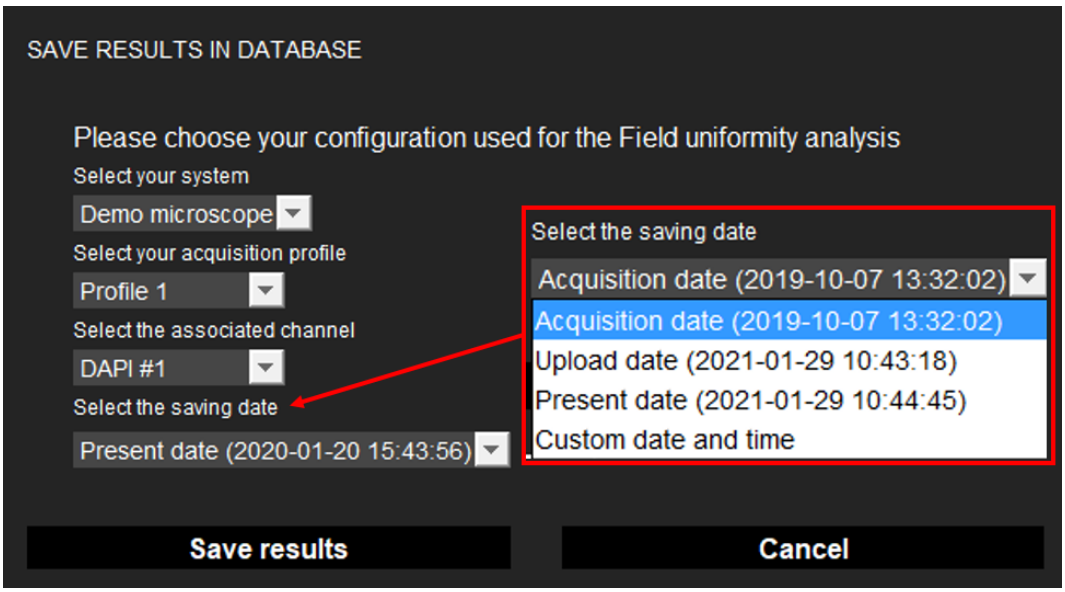

Figure 5: Interface window for saving the results in the database.

- **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.

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V. ANALYSIS ALGORITHM DESCRIPTION

1. DIAGRAM

The diagram below describes the algorithm that allows the extraction of the lateral resolution from the “gradually spaced lines” image (*cf.* Figure 6).

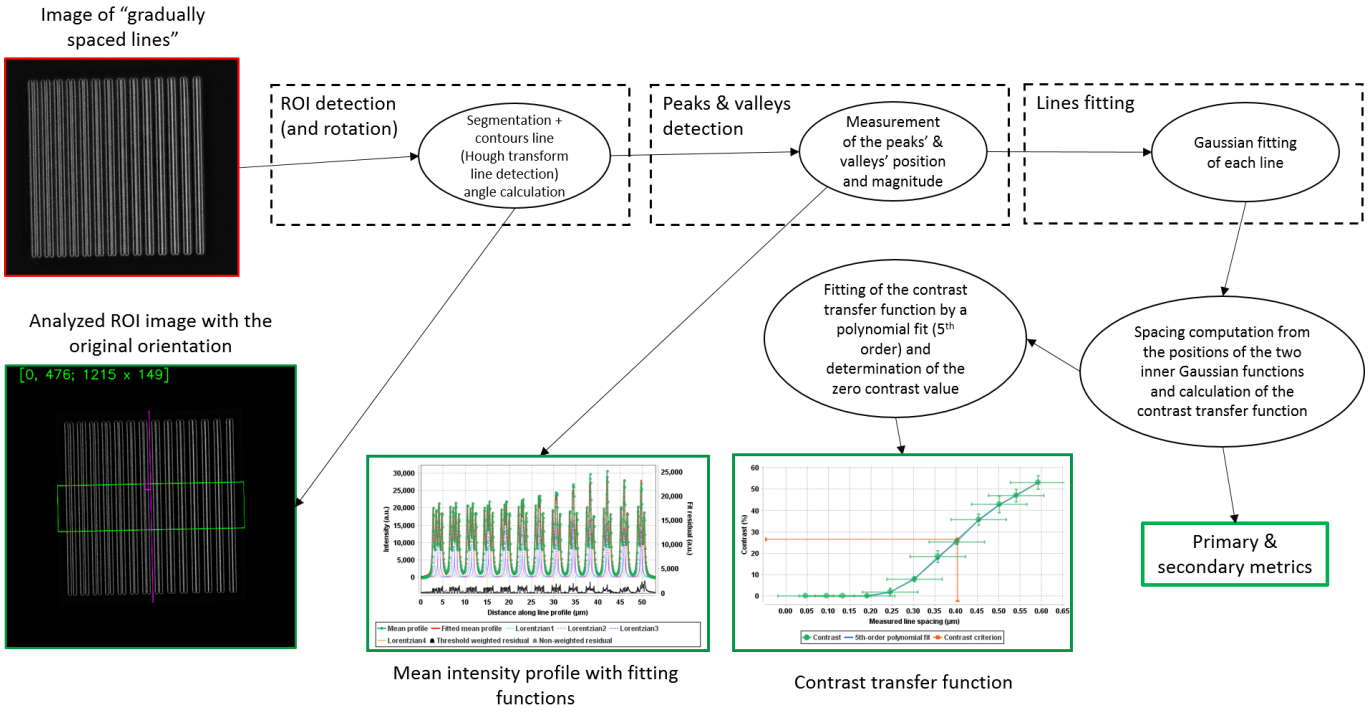

Figure 6: Schematic description of the different steps of the analysis algorithm.

2. DESCRIPTION

In short, the algorithm works as follows:

- It detects and segments the “gradually spaced lines” in the image.
- It determines the orientation of the “gradually spaced lines”: vertical, horizontal, ascending or descending.
- It applies an automatic orientation correction, if necessary.
- It plots an intensity line profile perpendicular to the lines.
- It applies a slight smoothing to the intensity line profile.
- It detects the peaks (in red) and the valleys (in blue) for each group of lines.
- It computes the mean contrast for each group of lines. A low pass filtering is applied when the number of peaks per group of lines is higher than 4.
  The line groups for which their associated measured contrast is lower than 1 % are labeled as “Not detected”, while the line groups for which the number of detected peaks is lower than 3 or higher than 4 are labeled as “Not measured”, in the “Measured mean contrast” column of the table “Contrast transfer function” values.
  Note that the offset (background) is not subtracted prior the mean contrast calculation. If you want the offset (background) to be subtracted, you need to acquire a background image and use the optional setting “Background subtraction” before starting the analysis.

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- It fits each line group with a sum of Gaussian or Lorentzian functions, from which it measures the spacing between the two inner lines of each group. An automatic Lanczos interpolation is applied, so that there are about 50 points per Gaussian or Lorentzian fitting function.
- It displays the mean contrast versus the measured line spacing into the “Contrast transfer function” graph.
- It also computes the SNR, by dividing the average value of the intensity of each pixel along one line of each group with the standard deviation of the intensity values.

A more detailed description of the three main parts of the algorithm (contrast, line spacing and SNR measurements) is presented below.

1- Contrast measurement

In the mean intensity profile perpendicular to the gradually spaced lines, the peaks and the valleys between the inner lines are detected and labeled in red and blue on the graph, respectively (*cf.* Figure 7).

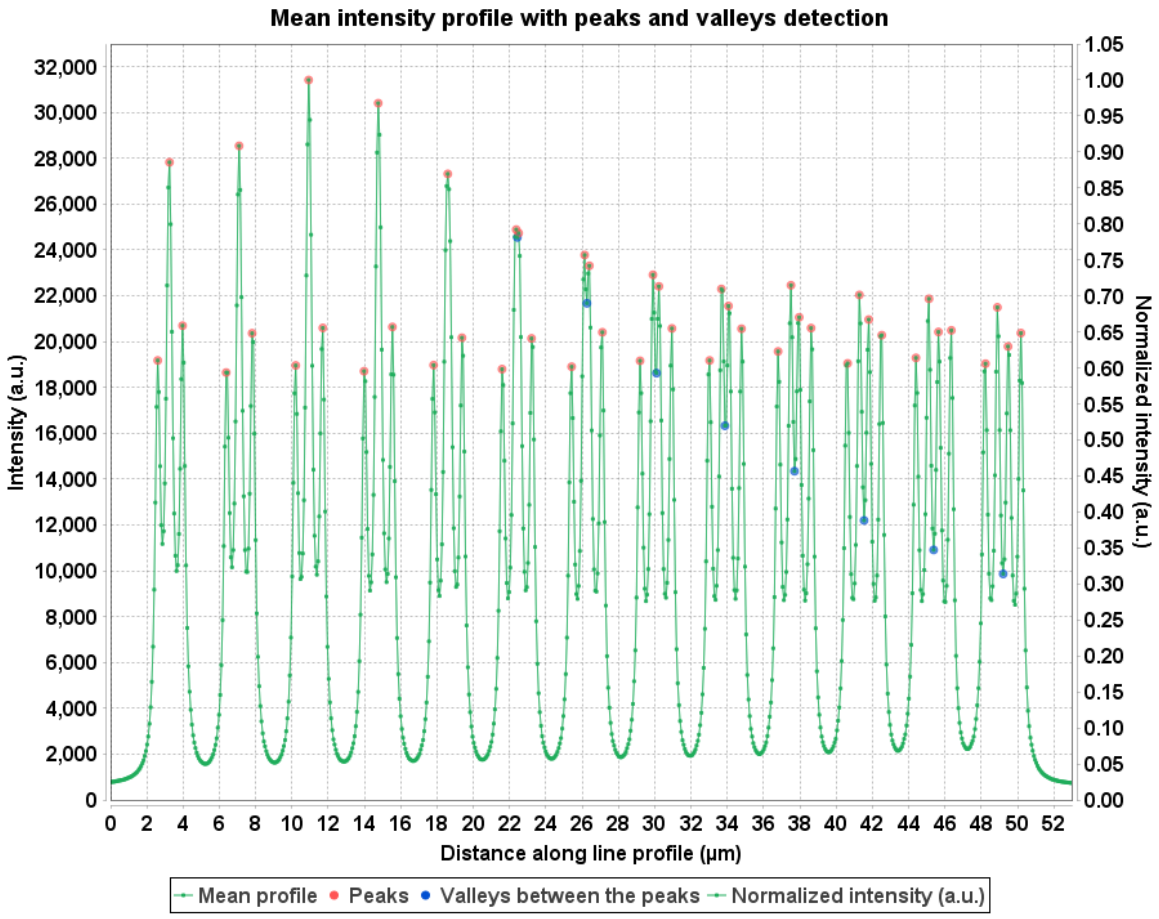

Figure 7: Mean intensity profile with peaks and valleys detection.

For each line group in which four lines are resolved, the mean contrast is calculated, based on the peaks and valleys intensities, according to the following equation:

$$Mean\ contrast = 100 \times \frac{\overline{I_{peak}} - I_{valley}}{\overline{I_{peak}}}$$

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Where: $\overline{I_{peak}}$ is the average intensity of the four peaks' magnitude and I_{valley} is the local minimum intensity between the two inner lines.

2- Line spacing measurement

To determine the spacing between the inner lines of each group, the mean intensity profile is fitted with Gaussian or Lorentzian functions. The fitting consists of two steps, described hereafter.

STEP 1: INITIALIZATION FITTING OF THE LINE GROUP WITH THE LARGEST INNER LINE SPACING

The line group (*i.e.* the double pair of lines) with the largest inner line spacing is fitted with one of the following models: a sum of four Gaussian functions or a sum of four Lorentzian functions.

***Model 1*: Sum of 4 Gaussian functions with 4 different magnitudes**

$$f(x) = K_1\, exp\left[-2\, ln(2)\frac{(x-x_1)^2}{FWHM_{1-3}^2}\right] + K_2\, exp\left[-2\, ln(2)\frac{(x-x_1-d_{1-2})^2}{FWHM_{2-4}^2}\right]$$
$$+K_3\, exp\left[-2\, ln(2)\frac{(x-x_4+d_{3-4})^2}{FWHM_{1-3}^2}\right] + K_4\, exp\left[-2\, ln(2)\frac{(x-x_4)^2}{FWHM_{2-4}^2}\right]$$

***Model 2*: Sum of 4 Lorentzian functions with 4 different magnitudes**

$$f(x) = K_1\frac{1}{1+\left[\frac{(x-x_1)}{0.5FWHM_{1-3}}\right]^2} + K_2\frac{1}{1+\left[\frac{(x-x_1-d_{1-2})}{0.5FWHM_{2-4}}\right]^2}$$
$$+K_3\frac{1}{1+\left[\frac{(x-x_4+d_{3-4})}{0.5FWHM_{1-3}}\right]^2} + K_4\frac{1}{1+\left[\frac{(x-x_4)}{0.5FWHM_{2-4}}\right]^2}$$

The following parameters are set as free:
x_1: position of the 1st line.
x_4: position of the 4th line.
d_{1-2}: spacing between the first two lines (1st and 2nd line).
d_{3-4}: spacing between the last two lines (3rd and 4th line).
K_1, K_2, K_3, K_4: respective magnitudes of the four lines.
$FWHM_{1-3}$: full width at half-maximum of the 1st and 3rd lines (these widths are supposed to be the same).
$FWHM_{2-4}$: full width at half-maximum of the 2nd and 4th lines (these widths are supposed to be the same).

The intensity profiles of the lines are locally normalized with an offset correction determined at the edges of all the analyzed double pairs of lines (*i.e.* the line groups).

The interline mean spacing between the 1st and 2nd lines and the 3rd and the 4th lines, denoted *d*, is calculated from $\boldsymbol{d = \frac{d_{1-2}+d_{3-4}}{2}}$.

This initialization fitting (*cf.* Figure 8) allows to determine the parameters $\boldsymbol{d = \frac{d_{1-2}+d_{3-4}}{2}}$, $\boldsymbol{FWHM_{1-3}}$ and $\boldsymbol{FWHM_{2-4}}$ that will be applied afterwards in the fitting of the second step.

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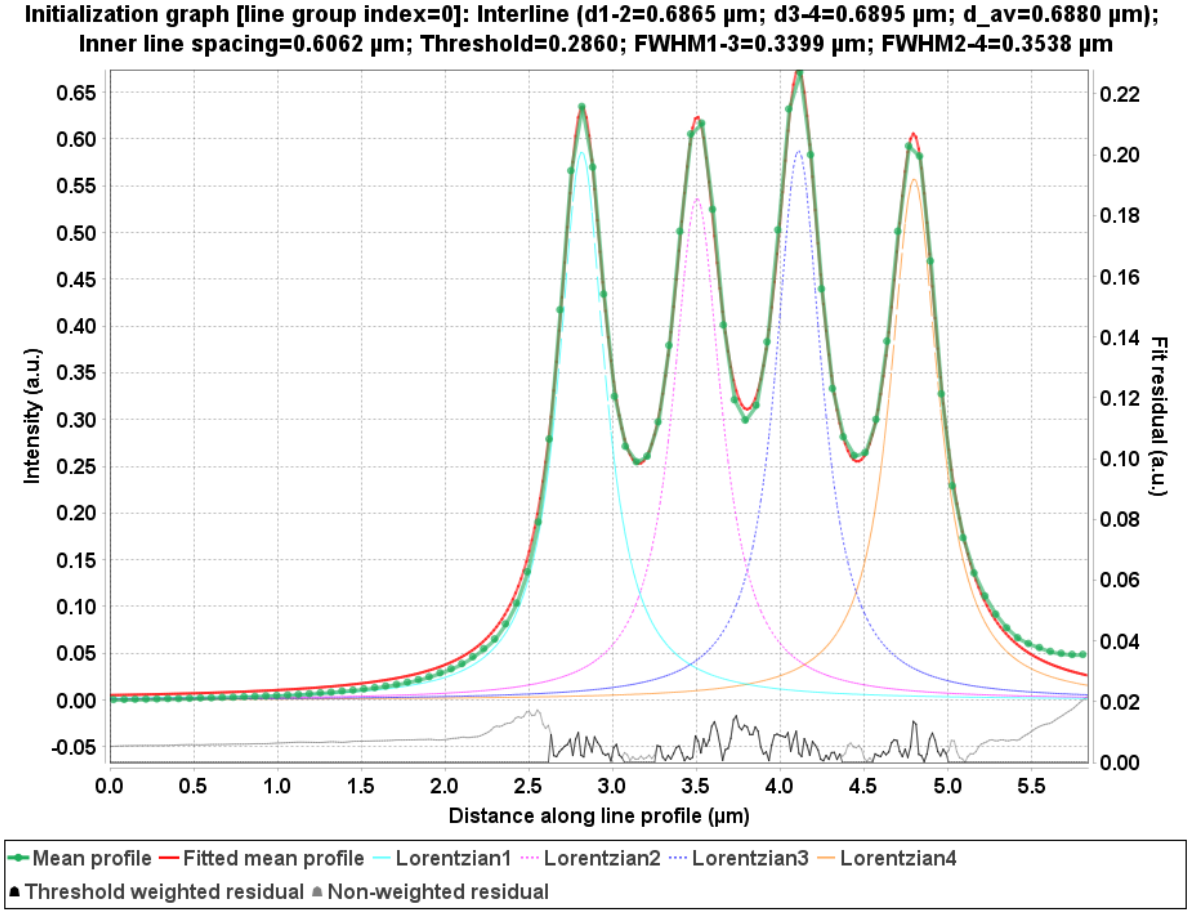

Figure 8: Fitting with four Gaussian or Lorentzian functions of the intensity profile of the line group (*i.e.* the double pair of lines) with the largest inner line spacing. From this initialization fitting, the parameters d, $FWHM_{1\text{-}3}$ and $FWHM_{2\text{-}4}$ are calculated and set for the fitting in the second step.

STEP 2: FITTING OF THE COMPLETE MEAN INTENSITY PROFILE

All the line groups (*i.e.* the double pairs of lines) are fitted with one of the two following models, depending on the cases whether four or three lines per group are resolved (*cf.* Figure 9).

If four lines per group are resolved:

Model 1-a: Sum of 4 Gaussian functions with 4 different magnitudes

$$f(x) = K_1\, exp\left[-2\, ln(2)\frac{(x-x_1)^2}{FWHM_{1-3}^2}\right] + K_2\, exp\left[-2\, ln(2)\frac{(x-x_1-d)^2}{FWHM_{2-4}^2}\right]$$
$$+K_3\, exp\left[-2\, ln(2)\frac{(x-x_1-d-\Delta)^2}{FWHM_{1-3}^2}\right] + K_4\, exp\left[-2\, ln(2)\frac{(x-x_1-2d-\Delta)^2}{FWHM_{2-4}^2}\right]$$

Model 1-b: Sum of 4 Lorentzian functions with 4 different magnitudes

$$f(x) = K_1\frac{1}{1+\left[\frac{(x-x_1)}{0.5FWHM_{1-3}}\right]^2} + K_2\frac{1}{1+\left[\frac{(x-x_1-d)}{0.5FWHM_{2-4}}\right]^2}$$
$$+K_3\frac{1}{1+\left[\frac{(x-x_1-d-\Delta)}{0.5FWHM_{1-3}}\right]^2} + K_4\frac{1}{1+\left[\frac{(x-x_1-2d-\Delta)}{0.5FWHM_{2-4}}\right]^2}$$

The following parameters are set as free:

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x_1: position of the 1st line.
K_1, K_2, K_3, K_4: respective magnitudes of the four lines.
Δ: spacing between the 2nd and 3rd lines (the two inner lines which are resolved).

The following parameters have been set beforehand, in the first step:
d: interline mean spacing between the 1st and 2nd lines and the 3rd and the 4th lines.
$FWHM_{1-3}$: full width at half-maximum of the 1st and 3rd lines (these widths are supposed to be the same).
$FWHM_{2-4}$: full width at half-maximum of the 2nd and 4th lines (these widths are supposed to be the same).

If three lines per group are resolved:
Model 1-b: Sum of 4 Gaussian functions with 3 different magnitudes:

$$f(x) = K_1\, exp\left[-2\, ln(2)\frac{(x-x_1)^2}{FWHM_{1-3}^2}\right] + K_{2-3}\, exp\left[-2\, ln(2)\frac{(x-x_1-d)^2}{FWHM_{2-4}^2}\right]$$
$$+K_{2-3}\, exp\left[-2\, ln(2)\frac{(x-x_1-d-\Delta)^2}{FWHM_{1-3}^2}\right] + K_4\, exp\left[-2\, ln(2)\frac{(x-x_1-2d-\Delta)^2}{FWHM_{2-4}^2}\right]$$

Model 2-b: Sum of 4 Lorentzian functions with 3 different magnitudes:

$$f(x) = K_1\frac{1}{1+\left[\frac{(x-x_1)}{0.5FWHM_{1-3}}\right]^2} + K_{2-3}\frac{1}{1+\left[\frac{(x-x_1-d)}{0.5FWHM_{2-4}}\right]^2}$$
$$+K_{2-3}\frac{1}{1+\left[\frac{(x-x_1-d-\Delta)}{0.5FWHM_{1-3}}\right]^2} + K_4\frac{1}{1+\left[\frac{(x-x_1-2d-\Delta)}{0.5FWHM_{2-4}}\right]^2}$$

The following parameters are set as free:
x_1: position of the 1st line.
K_1, K_4: respective magnitudes of the 1st and 4th lines.
K_{2-3}: magnitude of the 2nd and 3rd lines (these magnitudes are supposed to be the same).
Δ: spacing between the 2nd and 3rd lines (the two inner lines which are ***not*** resolved).

The following parameters are set beforehand:
d: interline mean spacing between the 1st and 2nd lines and the 3rd and the 4th lines.
$FWHM_{1-3}$: full width at half-maximum of the 1st and 3rd lines (these widths are supposed to be the same).
$FWHM_{2-4}$: full width at half-maximum of the 2nd and 4th lines (these widths are supposed to be the same).

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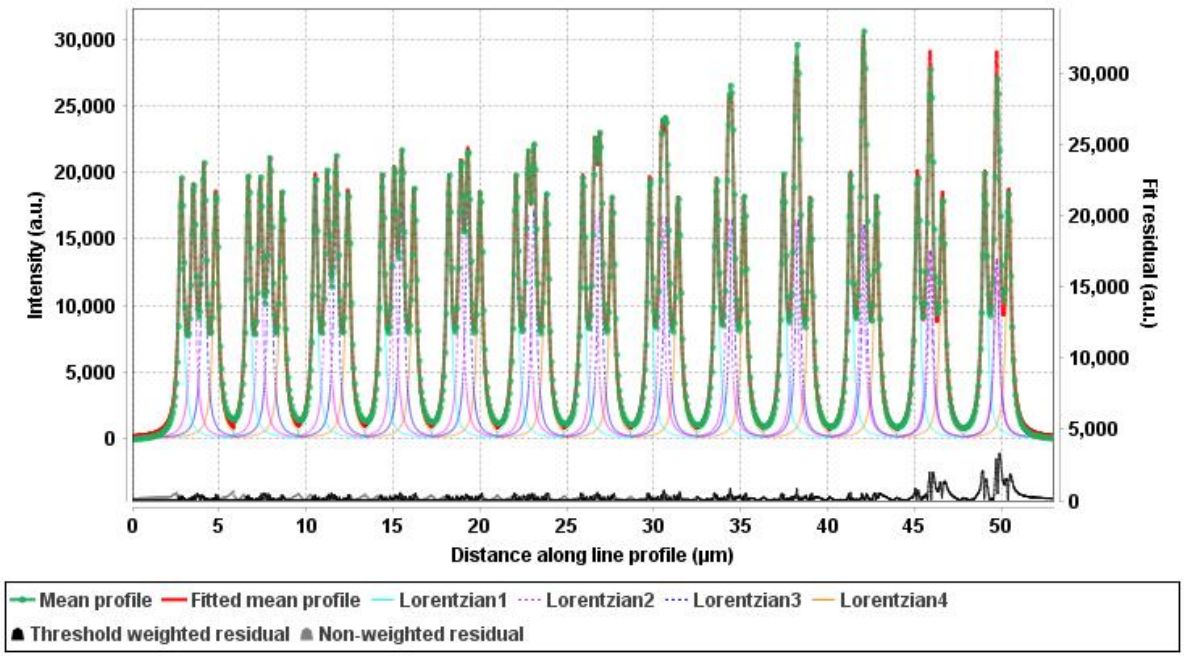

Figure 9: Fitting with four Gaussian or Lorentzian functions of the mean intensity profile of all the line groups (*i.e.* all the double pairs of lines).

To choose which fitting model to apply, the number, the position, the magnitude and the width of the lines are first determined.
Once the fitting model is applied, the number and the position of the lines is then cross-checked.

A fitting threshold parameter, set automatically, allows fitting only the data points that are above the dip between the 2nd and 3rd lines (the two inner lines), determined during the first step.
It is however possible to set manually this threshold parameter, in terms of fraction (from 0 to 0.9) of the maximum value of the detected peaks.

At the end, the spacing between the inner lines (*i.e.* the 2nd and 3rd lines) of each line group is determined and associated to the corresponding contrast values previously measured. The "contrast versus line spacing" data are the plotted in the "Contrast transfer function" graph and fitted with a 5th-order polynomial fit (*cf.* Figure 10).

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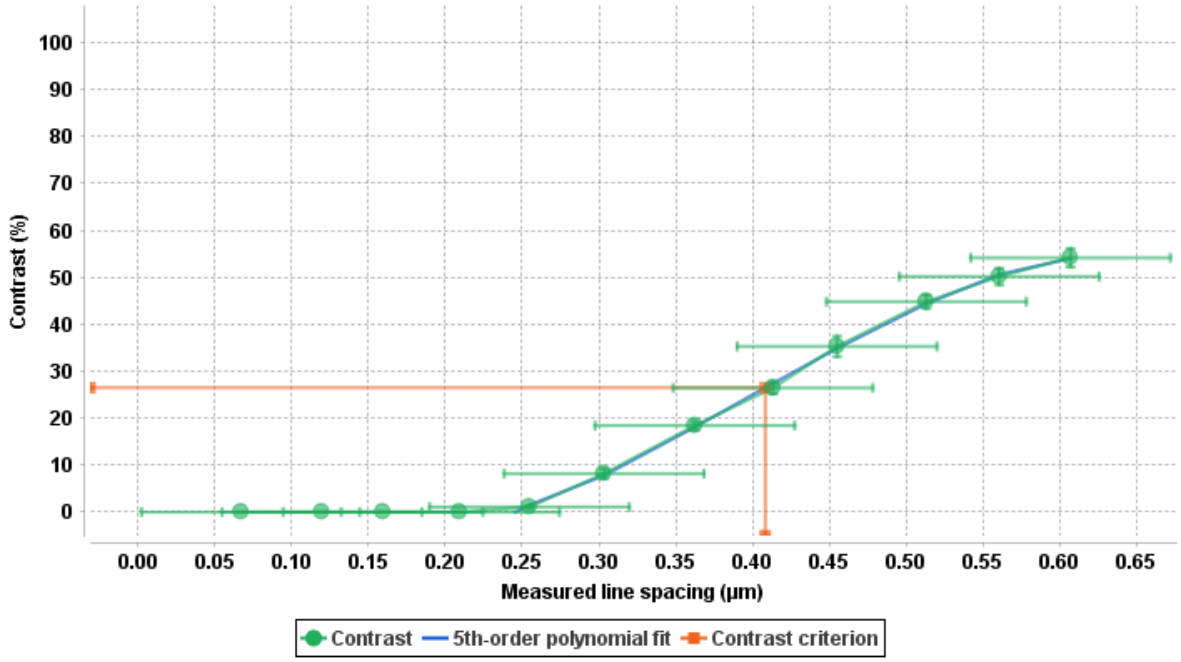

Figure 10: Contrast transfer function, *i.e.* the mean contrast versus the measured line spacing.

3- SNR measurement

The SNR is defined as the ratio between the mean signal $\bar{S}$ and the mean noise $\bar{N}$. It is given by the following equation:

$$SNR = \frac{\bar{S}}{\bar{N}} = \frac{Mean(\overline{S_n})}{Mean(\overline{N_n})}$$

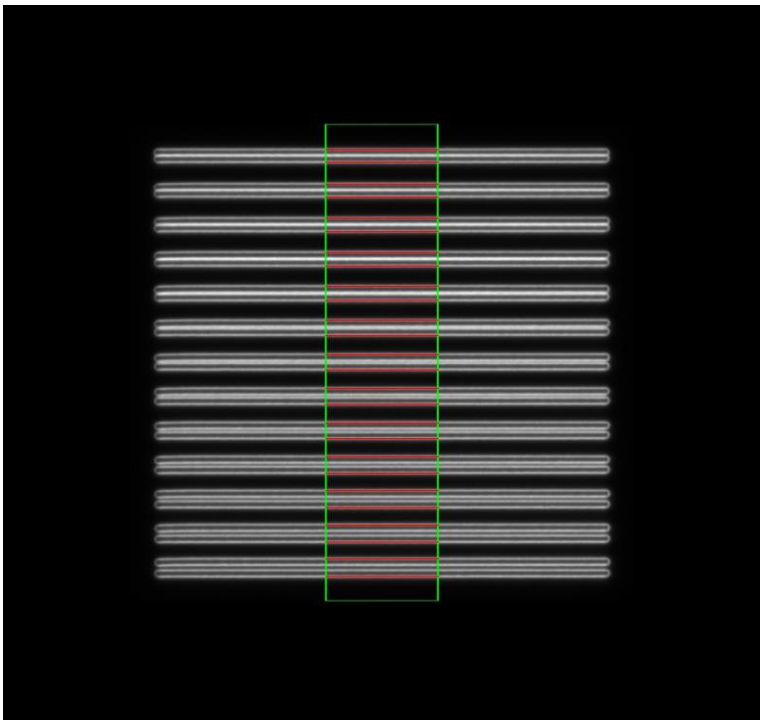

Figure 11: Line profiles (in red), superimposed on the raw image within the analyzed ROI, along which the average signal $\overline{S_n}$ and the average noise $\overline{N_n}$ are measured.

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For the two outer lines of each group (*cf.* Figure 11), the average signal of each line profile *n* with the raw intensity *I* at pixel *y* is expressed as:

$$\overline{S_n} = \frac{1}{ROI\ width} \sum_{y=1}^{ROI\ width} I_n(y)$$

For the two outer lines of each group (*cf.* Figure 11), the average noise of each line profile *n* with the raw intensity *I* at pixel *y* is expressed as:

$$\overline{N_n} = \sqrt{\frac{1}{ROI\ width} \sum_{y=1}^{ROI\ width} [I_n(y) - \overline{S_n}]^2}$$

The mean signal is the average of the *n* average signals calculated here for each line profile, and is expressed as:

$$\bar{S} = Mean(\overline{S_n}) = \frac{1}{2 \times number\ of\ line\ groups} \sum_{n=1}^{2 \times number\ of\ line\ groups} S_n$$

The mean noise is the average of the *n* average noises calculated here for each line profile, and is expressed as:

$$\bar{N} = Mean(\overline{N_n}) = \frac{1}{2 \times number\ of\ line\ groups} \sum_{n=1}^{2 \times number\ of\ line\ groups} N_n$$

The measurement uncertainty of the SNR is given by the following equation:

$$\Delta(SNR) = SNR \times \sqrt{\left[\frac{SD(\bar{S})}{\bar{S}}\right]^2 + \left[\frac{SD(\bar{N})}{\bar{N}}\right]^2}$$

Where: $SD(\overline{S_n}) = \frac{1}{2 \times number\ of\ line\ groups} \sum_{n=1}^{2 \times number\ of\ line\ groups} [\overline{S_n} - \bar{S}]^2$ is the standard deviation (SD) of the average signals.

And: $SD(\overline{N_n}) = \frac{1}{2 \times number\ of\ line\ groups} \sum_{n=1}^{2 \times number\ of\ line\ groups} [\overline{N_n} - \bar{N}]^2$ is the standard deviation (SD) of the average noises.

4- SBR measurement

The SBR is defined as the ratio between the mean signal $\bar{S}$ and the mean background $\bar{B}$. It is given by the following equation:

$$SBR = \frac{\bar{S}}{\bar{B}}$$

The mean signal is the average of the peaks intensity values of the outer lines of each group and the mean background is the average of the intensity values at each extremity of the mean intensity profile (*cf.* Figure 12).

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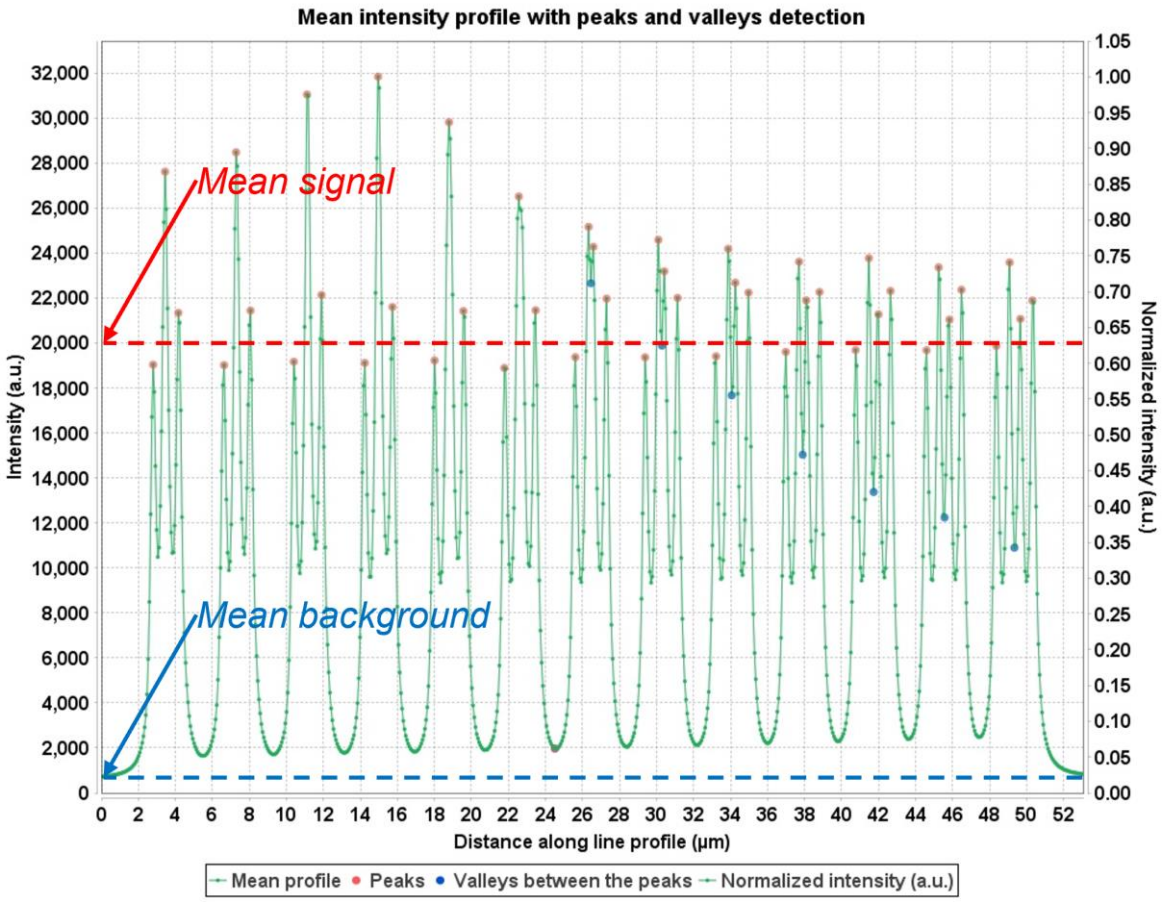

Figure 12: Mean signal (in red) and mean background (in blue) measured from the mean intensity profile, extracted from the analyzed ROI in the raw image.

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VI. OUTPUT METRIC DESCRIPTION

1. PRIMARY METRICS

- *Contrast criterion* is the value of the contrast set by the user to determine the corresponding lateral resolution. It is expressed in %.
  For instance, the well-known Schuster, Rayleigh and Sparrow criteria correspond to contrast thresholds of 99.9 %, 26.5 %, and 0.1 %, respectively.
  The PSF FWHM criterion (which is the contrast criterion equivalent to the FWHM measurement of a PSF for ideal imaging conditions, *i.e.* no aberration, no noise nor background) corresponds to contrast thresholds of 3.8 % for a wide-field microscope, and of 7.3 % for a confocal microscope.

- *Lateral resolution* is the value of the lateral resolution determined for the contrast criterion set by the user. It corresponds to the minimum separation distance that is distinguishable. It is obtained from the intercept between the horizontal line at the set contrast and the 5^{th}-order polynomial fitting function of the contrast transfer function. It is expressed in µm.

- *Lateral resolution at zero contrast* is the value of the lateral resolution for a 0 % contrast criterion. It is obtained from the intercept between the abscissa axis and the 5^{th}-order polynomial fitting function of the contrast transfer function. In other words, it is the minimum resolvable distance by the imaging system. It is expressed in µm.

- *SNR* is the signal-to-noise ratio measured in the analyzed image. It is unitless.

- *SBR* is the signal-to-background ratio measured in the analyzed image. It is unitless.

2. CONTRAST TRANSFER FUNCTION VALUES

The values of both the measured line spacings and the measured mean contrasts, that are plotted in the “Contrast transfer function” graph, are displayed in the “Contrast transfer function values” table.

3. SECONDARY METRICS

- *Maximum detectable line spacing* is the maximum spacing value between two inner lines of a group of lines that can be detectable, *i.e.* that can be measured. It is expressed in µm.

- *Minimum detectable line spacing* is the minimum spacing value between two inner lines of a group of lines that can be detectable, *i.e.* that can be measured. It is expressed in µm.

- *Intensity maximum* is the maximum intensity value in the mean intensity profile. It is expressed in arbitrary unit.

- *Intensity minimum* is the minimum intensity value in the mean intensity profile. It is expressed in arbitrary unit.

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4. 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.
- *Angle value used for the orientation correction* is the angle value applied to the analyzed image to correct a small rotation/tilt of the pattern, usually due to camera or laser scanning misalignment in microscopes. This angle value can either be automatically calculated by some of the algorithms and/or previously set in the analysis settings. It is expressed in degree.
- *Measurement uncertainty of the lateral resolution* is the uncertainty of the algorithm on the measurement of the lateral resolution from the analyzed image. It is equal to one specified lateral pixel size. It is expressed in µm.
- Measurement uncertainty of the SNR is the uncertainty on the measurement of the signal-to-noise ratio from the analyzed image. It is unitless.
- *Background subtraction* indicates if the "Background subtraction" option has been activated or not.
- *Hot pixels removal* indicates if the "Hot pixels removal" option has been activated or not.
- *Best focus selection* indicates if the "Best focus selection" option has been activated or not.
- *Index of the selected image in the stack* indicates the index of the image in the stack that has been selected when activating the "Best focus selection" option.
- *Fitting functions* are the Gaussian or Lorentzian mathematical functions used to fit the mean intensity profile perpendicular to the gradually spaced lines.
- *Processed ROI width* is the width set for the region of interest within the "gradually spaced lines" pattern. It is expressed in pixel, and automatically calculated to about one-fourth of the length of the gradually spaced lines.
- *Interpolation factor* is the factor used to interpolate the mean intensity profile. It is unitless, and automatically calculated according to the following formula:

$$Interpolation\ factor = \text{Round up}\left\{\frac{1}{Processed\ ROI\ length\ (39\ or\ 52\ \mu m)} \times 4 \times Number\ of\ line\ groups\ (10, 13\ or\ 14) \times 50\ (number\ of\ points\ per\ Gaussian) \times Specified\ lateral\ pixel\ size\ (\mu m)\right\}$$

It can also be set manually in the "Redo lateral resolution analysis" window.

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- *Fitting threshold level* is the intensity level above which the Gaussian or Lorentzian fitting is performed on the mean intensity line profile. It is automatically computed.
  It can also be set manually when there is a problem with the analysis in the “Redo” window.

- *Detected number of line groups* is the detected number of line groups. For the analysis to provide correct results, it must be the same as the *expected number of line groups*.

- *Expected number of line groups* is the expected number of line groups, for a given slide type: 13 for Argo-HM, 14 for Argo-SIM and 10 for Argo-Z.

- *Specified minimum line spacing* is the minimum line spacing specified by Argolight: 0.10 ± 0.11 for Argo-HM, 0.00 ± 0.11 for Argo-SIM and 0.10 ± 0.11 for Argo-Z. It is expressed in µm.

- *Specified maximum line spacing* is the maximum line spacing specified by Argolight: 0.70 ± 0.11 for Argo-HM, 0.39 ± 0.11 for Argo-SIM and 0.55 ± 0.11 for Argo-Z. It is expressed in µm.

- *Specified line spacing increment* is the line spacing increment specified by Argolight: 0.05 for Argo-HM, 0.03 for Argo-SIM and 0.05 for Argo-Z. It is expressed in µm.

- *RMSE for the fitting function of the CTF* is the root mean square error between the 5th-order polynomial fit and the experimental data in the contrast transfer function. It is a measurement of the goodness of the fit. It is expressed in % and is given by the following equation:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(C_{measured_i} - C_{fit_i}\right)^2}$$

  Where *n* is the number of experimental points, $C_{measured_i}$ the measured contrast value of the *i*th experimental point and C_{fit_i} the contrast value of the *i*th point from the 5th-order polynomial fit.

- *Status of the contrast detection* provides information on the contrast transfer function. This metadata can take the following values: 0, -1 or -2. If it is equal to 0, the lateral resolution can be measured; if it is equal to -1, the lateral resolution cannot be measured and takes therefore the maximum possible value; if it is equal to -2, the lateral resolution cannot be measured and takes NaN as a value.

- *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.

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- *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.

5. 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.

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