MetroloJ: an ImageJ plugin to help monitor microscopes' health.

Cédric Matthews and Fabrice P. Cordelières

3rd ImageJ User and Developer Meeting
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CM : CNRS-IBDML-UMR 6216, Service Imagerie, Marseille, France
FPC : Institut Curie/CNRS UMR 3348, PICT-IBiSA@Orsay, Orsay, France

CM & FPC : Mission Ressources et Compétences Technologiques du CNRS, Meudon, France

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- All plugins provide a unified output in the form of spreadsheets and a pdf file (requires the iText library).

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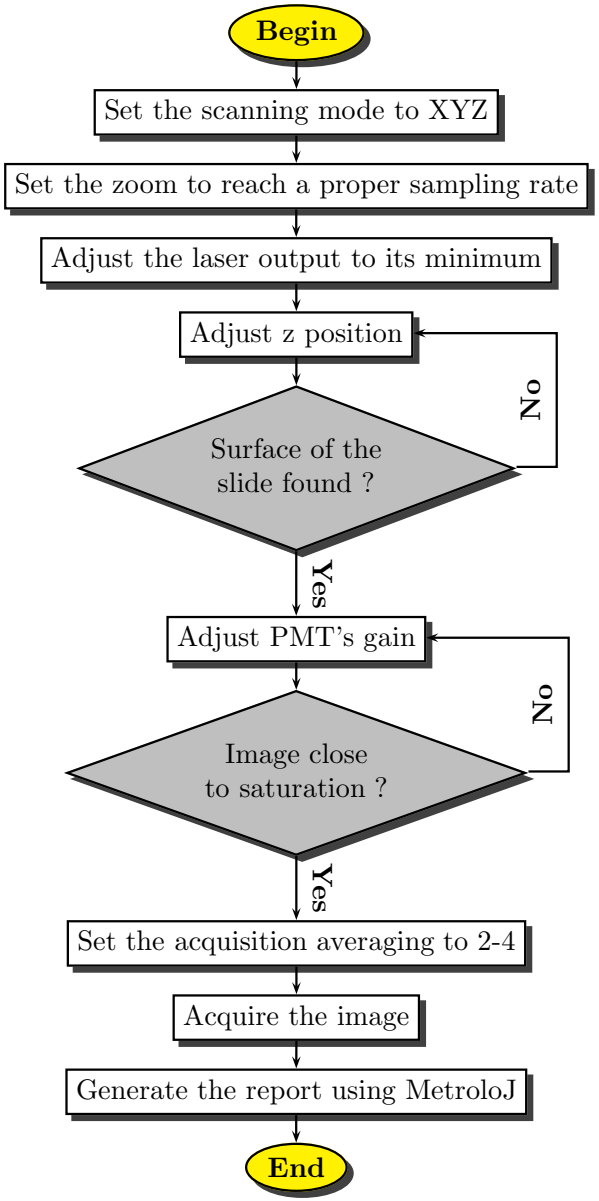

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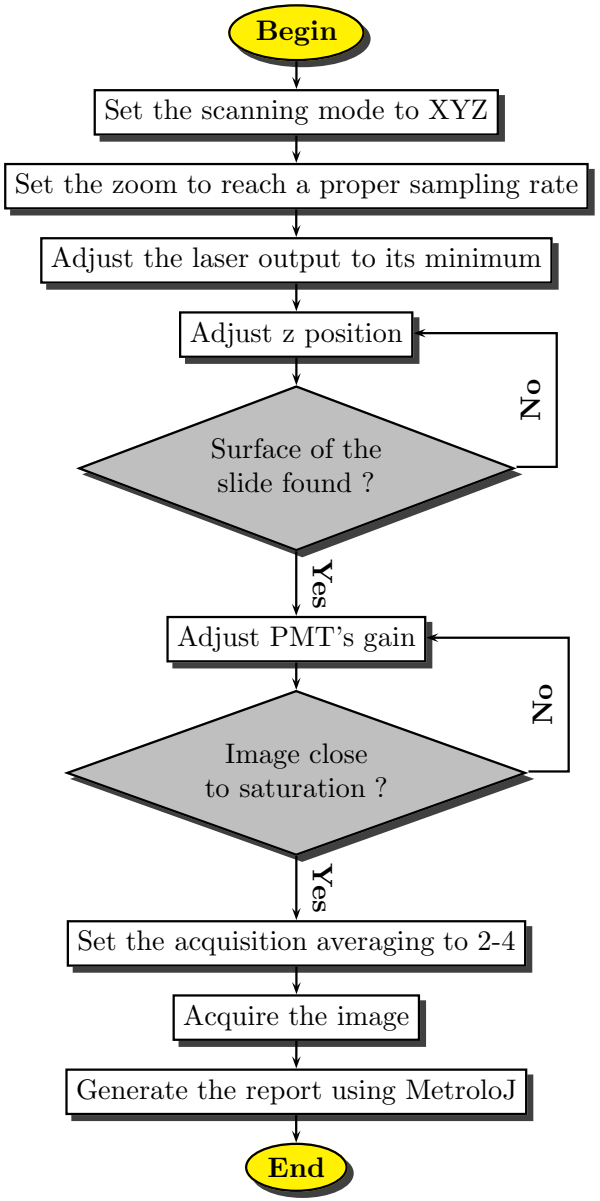

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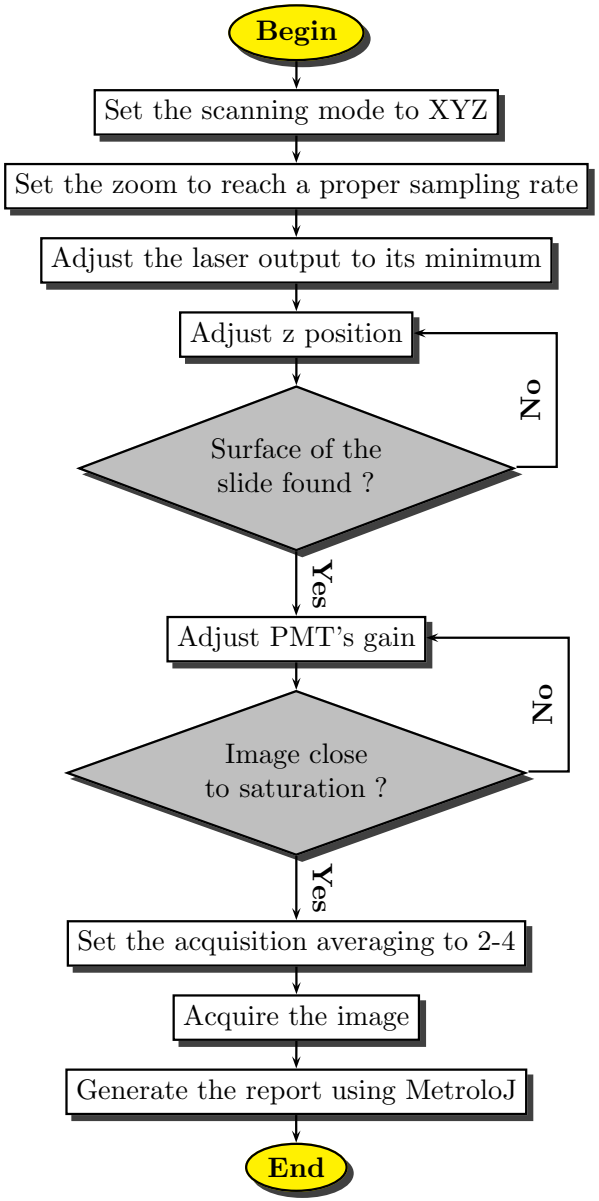

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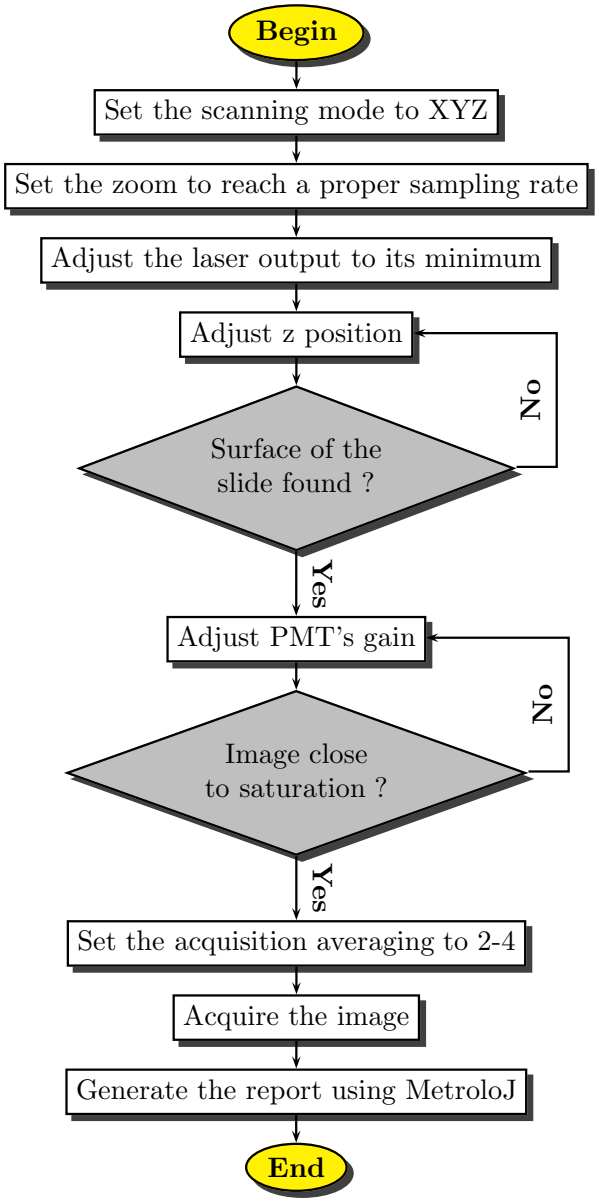

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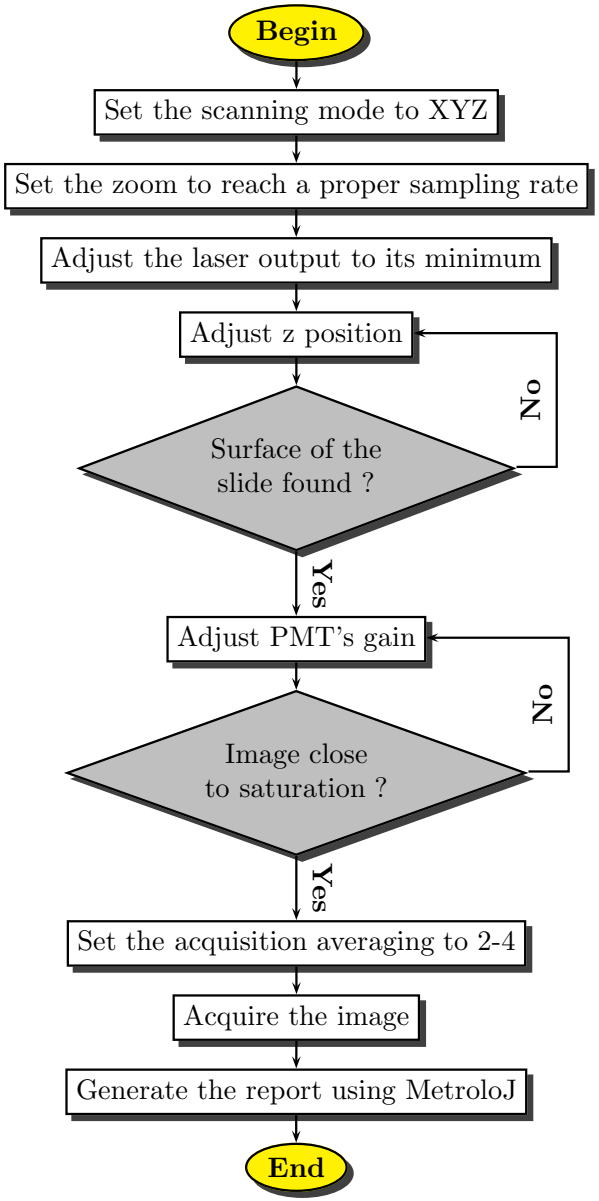

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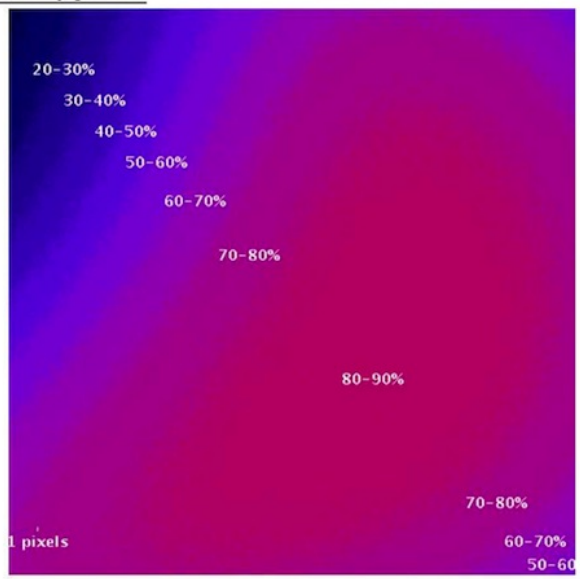
- **Preventive/Pro-active actions :** Check the optical path, re-align the light source, ..., call after-sale service.

Field illumination : what's on the report ?

12 août 2010 14:17
Field illumination report on Field illumination

Normalised intensity profile:



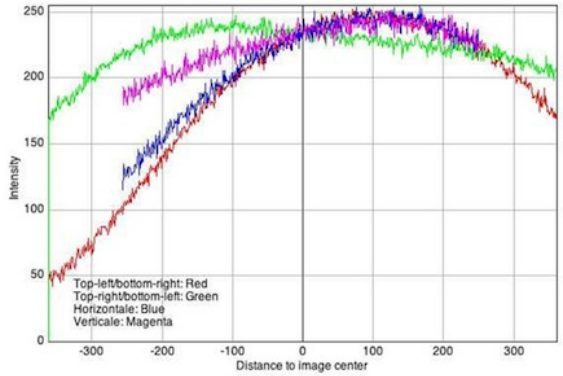
Microscope infos:

Microscope: Confocal
Wavelength: 510.0 nm
NA: 1.4
Sampling rate: 1.0x1.0x1.0 pixel
Pinhole: 1.0 Airy Units

Centers' locations:

	Image centre	Centre of intensity	Centre of the max intensity	Centre of the 100% zone
Coordinates	(256.0, 256.0)	(274.366, 270.451)	(263.0, 441.0)	(338.799, 309.725)
Distance to image centre		23.369μm	185.132μm	98.702μm

Intensity profiles:



Profiles' statistics:

Location	Intensity	Intensity relative to max
Maximum found at (263,441)	255	1.0
Top-left corner	39	0.153
Top-right corner	175	0.686
Bottom-left corner	175	0.686
Bottom-right corner	177	0.694
Upper bound, middle pixel	182	0.714
Lower bound, middle pixel	230	0.902
Left bound, middle pixel	131	0.514
Right bound, middle pixel	224	0.878

Topic 2 : Variability in detection

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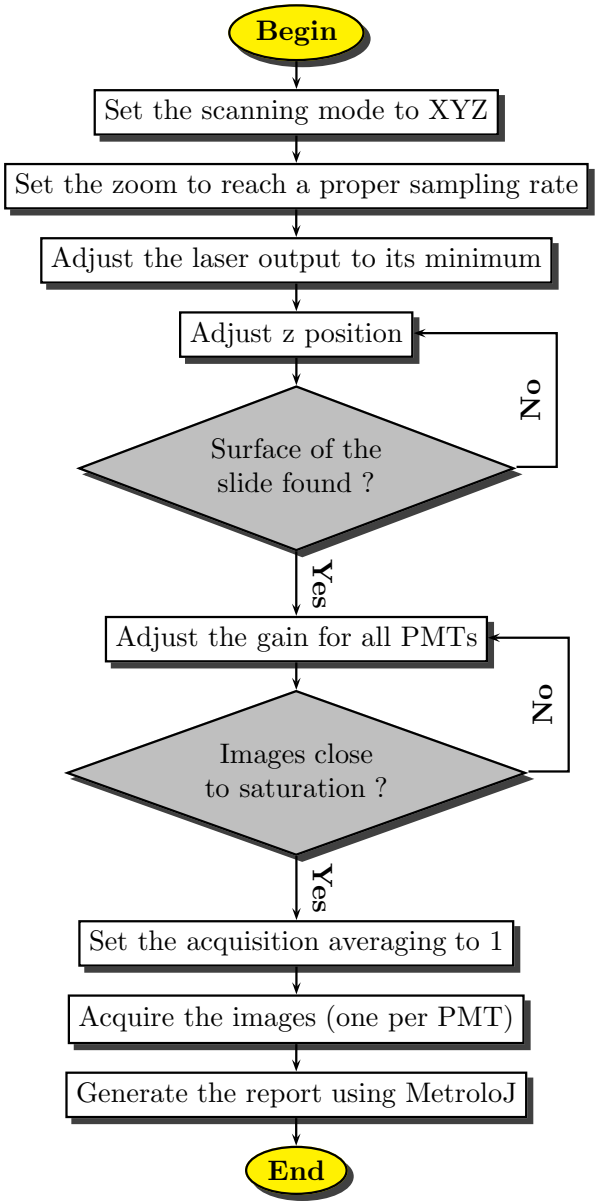

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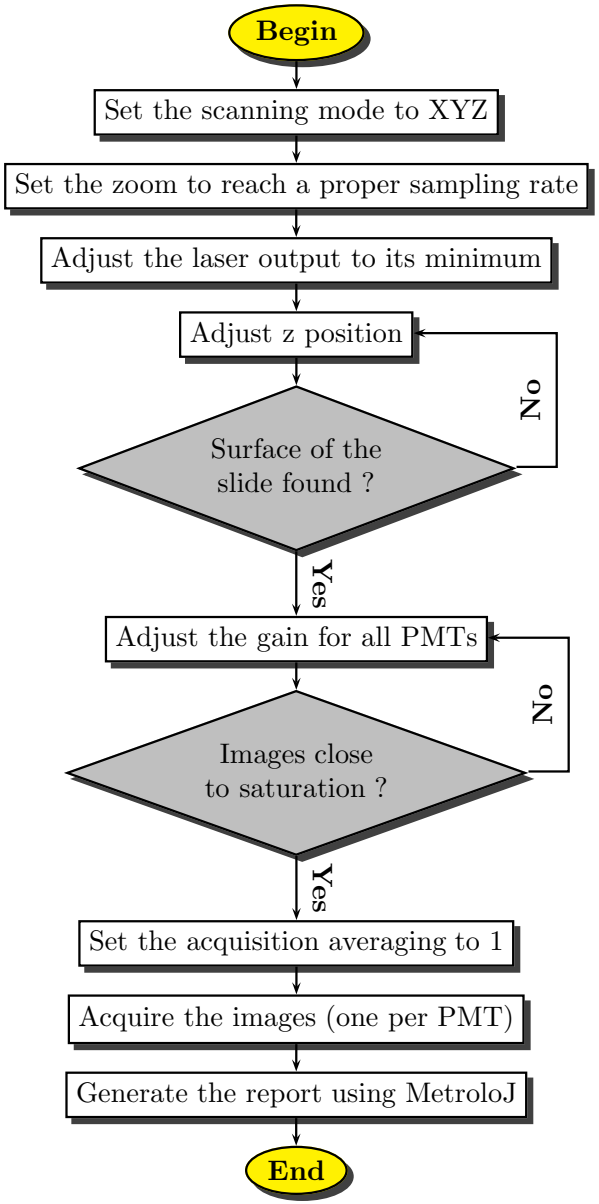

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- **Informations to be retrieved :** Within a region of interest, mean intensity (μ) and standard deviation .

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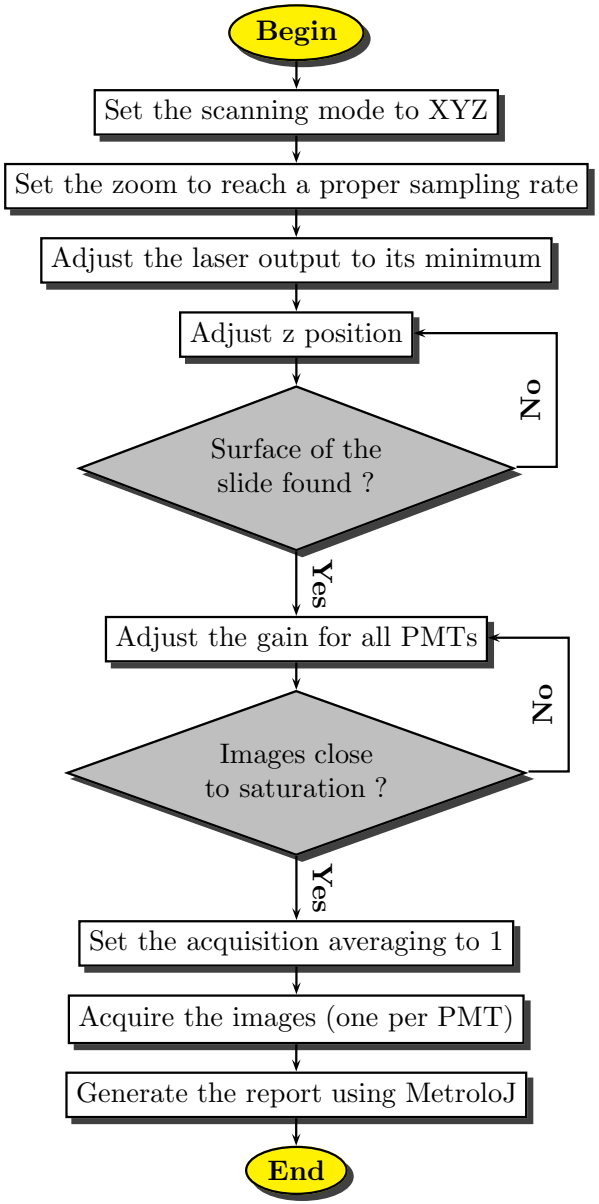

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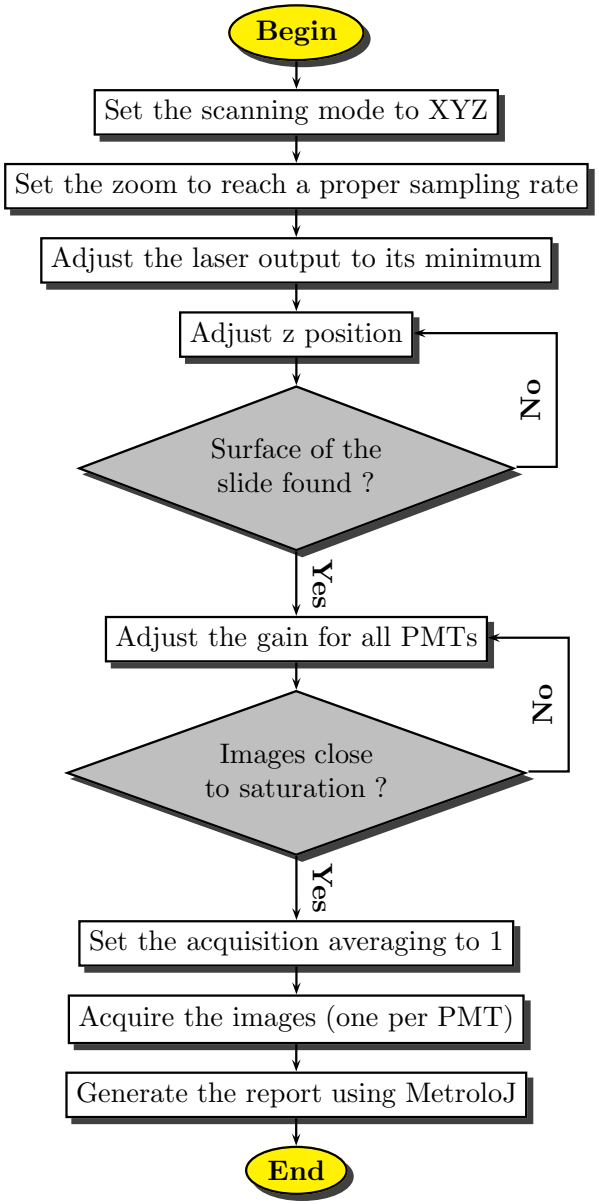

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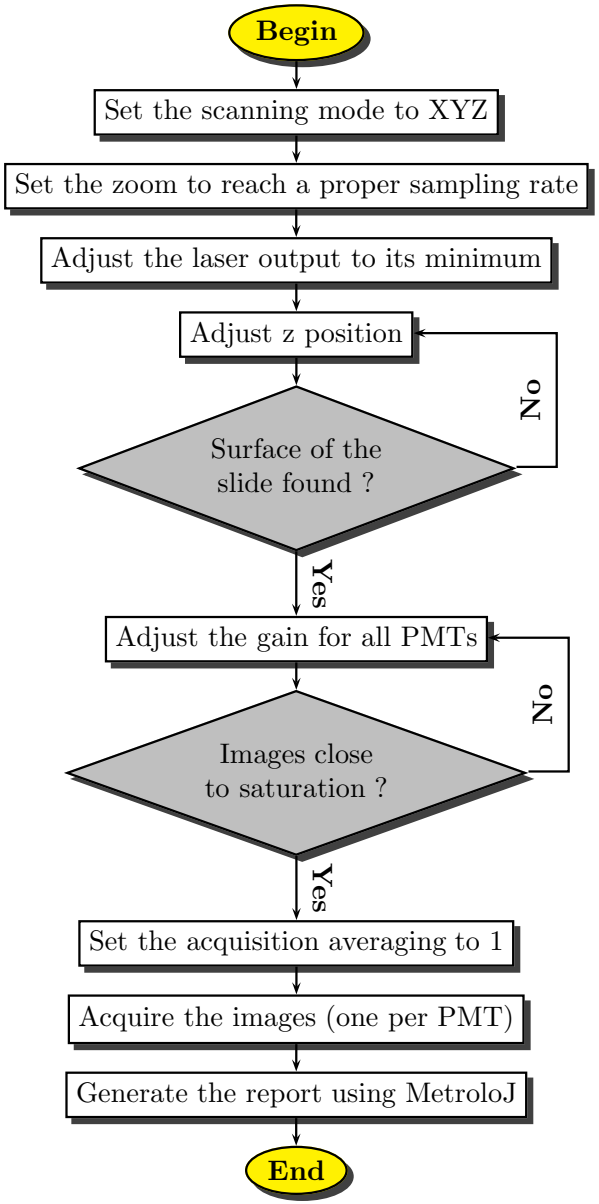

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  - CV=σ/μ.

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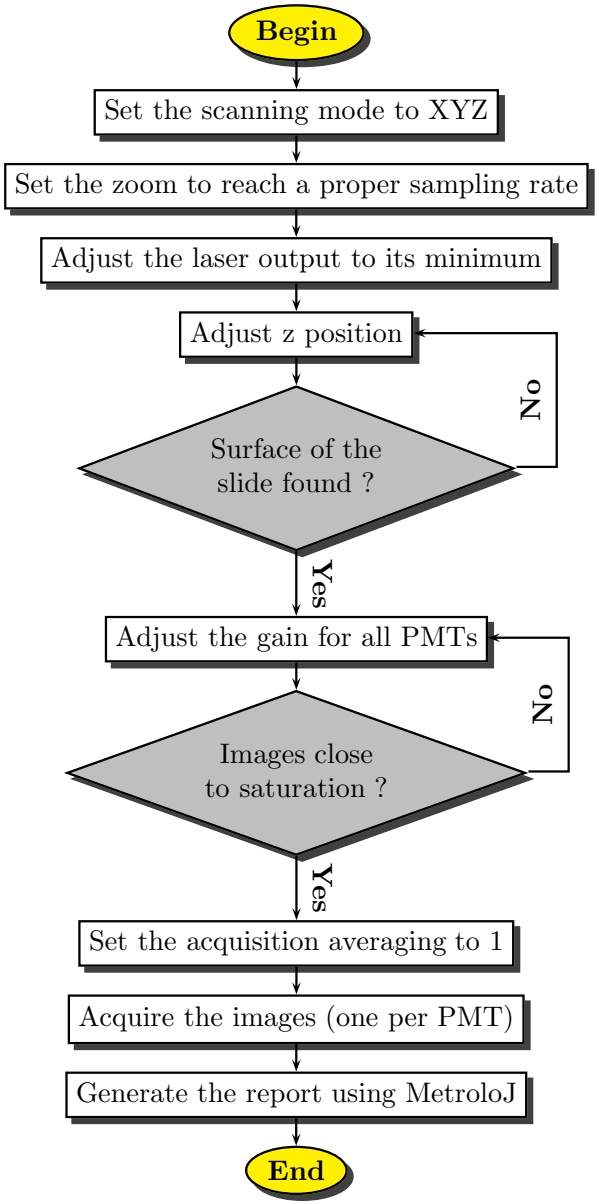

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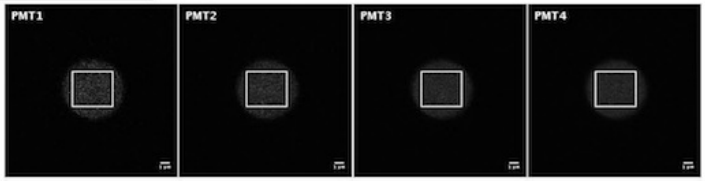
  - CV=σ/μ.
- **Preventive/Pro-active actions :** Check the optical path, ..., call after-sale service.

Variability in detection : what's on the report ?

12 août 2010 14:10
CV report on CV

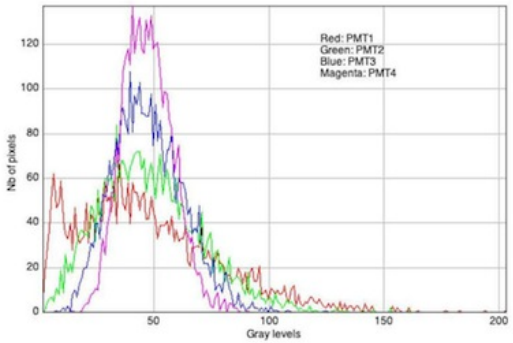
ROIs used for measures:



Microscope infos:

Microscope: Confocal
Wavelength: 510.0 nm
NA: 1.4
Sampling rate: 0.065x0.065x0.2 μm
Pinhole: 1.0 Airy Units

Histograms:



CVs table:

	Standard deviation	Average	Nb pixels	CV	CVs relative to min value
PMT1	30.401	47.784	3640	0.636	2.769
PMT2	22.194	48.653	3640	0.456	1.985
PMT3	15.538	47.88	3640	0.325	1.412
PMT4	10.941	47.617	3640	0.23	1.0

Resolution : PSF based measurements

- **Aim :** On the instrumental transfer function, mesure the X, Y and Z resolutions.

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- **Sample to be used :** Well dispersed, uniformly fluorescent labelled, infra-resolution beads.

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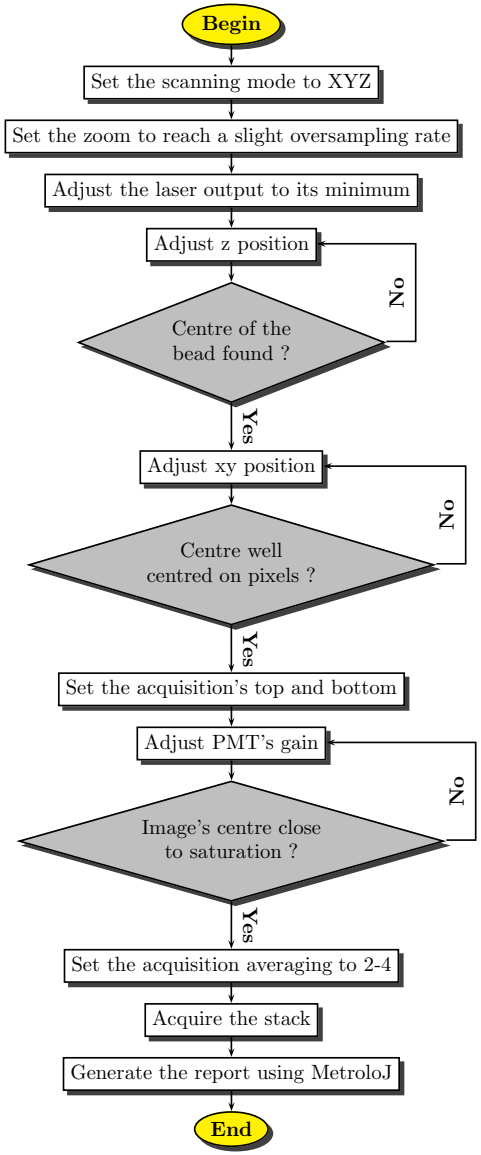

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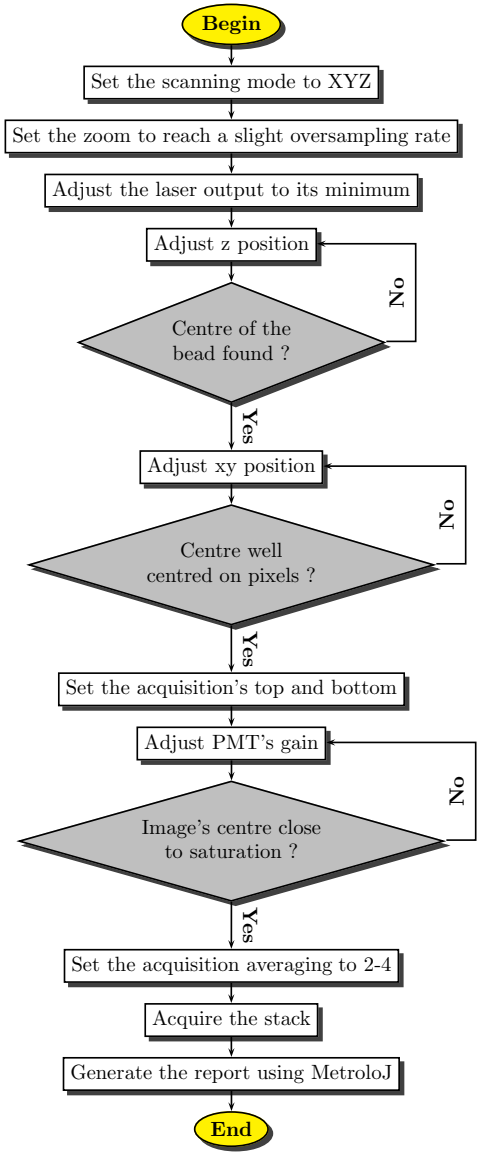

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- **Informations to be retrieved :** Based on intensity profiles passing through the bead's center, fitted on a Gaussian function, determine the FWHM (estimate of the resolution).

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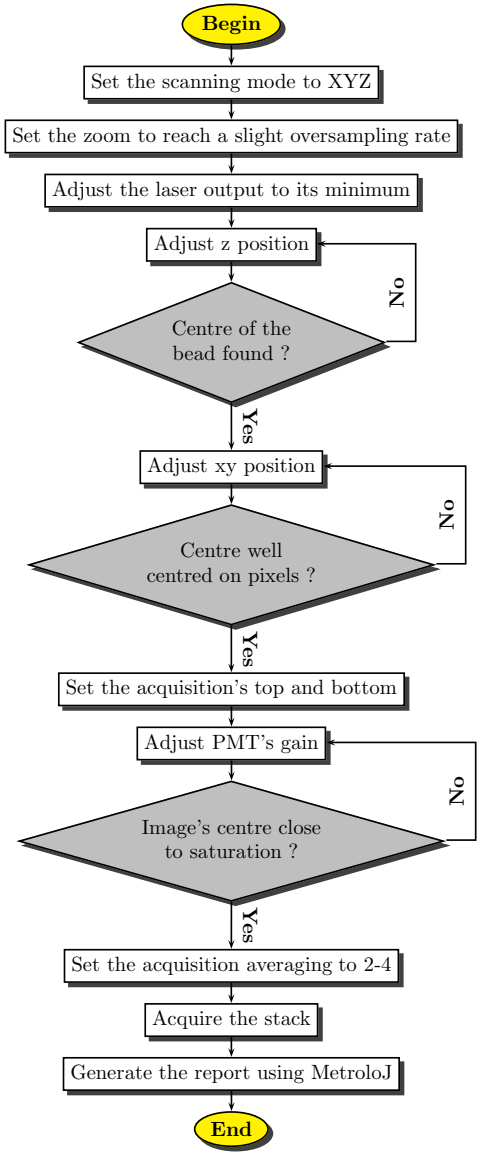

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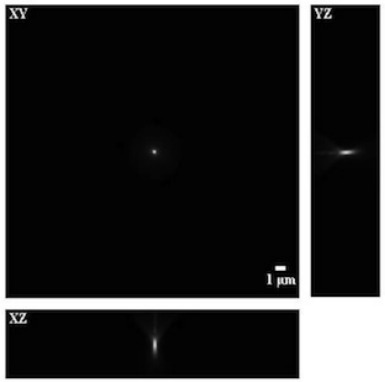
PSF based measurements : what's on the report ?

12 août 2010 14:21
PSF profiler report on PSF

Profile view:



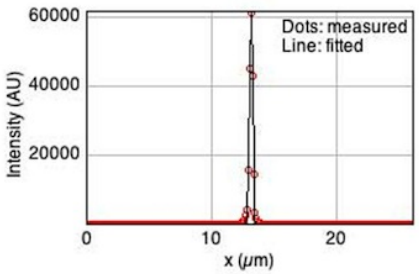
Microscope infos:

Microscope: WideField
Wavelength: 510.0 nm
NA: 1.4
Sampling rate: 0.102x0.102x0.2 µm

Resolution table:

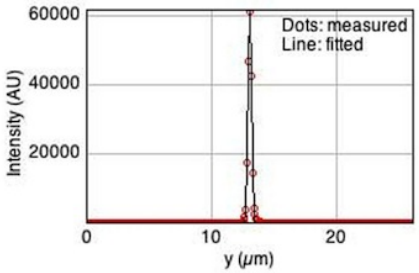
	FWHM	Theoretical resolution
x	0.293 µm	0.222 µm
y	0.299 µm	0.222 µm
z	1.047 µm	0.52 µm

X profile & fitting parameters:



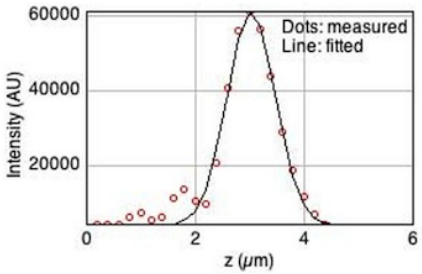
Fitted on y = a + (b-a)*exp(-(x-c)^2/(2*d^2));
Number of iterations: 491 (8000)
Number of restarts: 2 (2)
Sum of residuals squared: 19148092.8669
Standard deviation: 274.0266
R^2: 0.9976
Parameters:
a = 101.5712
b = 61829.8944
c = 13.2059
d = 0.1246

Y profile & fitting parameters:



Fitted on y = a + (b-a)*exp(-(x-c)^2/(2*d^2));
Number of iterations: 542 (8000)
Number of restarts: 2 (2)
Sum of residuals squared: 18141301.4980
Standard deviation: 266.7252
R^2: 0.9978
Parameters:
a = 97.6990
b = 61962.6559
c = 13.1004
d = 0.1270

Z profile & fitting parameters:



Fitted on y = a + (b-a)*exp(-(x-c)^2/(2*d^2));
Number of iterations: 243 (8000)
Number of restarts: 2 (2)
Sum of residuals squared: 275294350.0796
Standard deviation: 3029.2703
R^2: 0.9724
Parameters:
a = 4092.1677
b = 61552.2355
c = 3.0364
d = 0.4445

Resolution : Mirror slide based measurement

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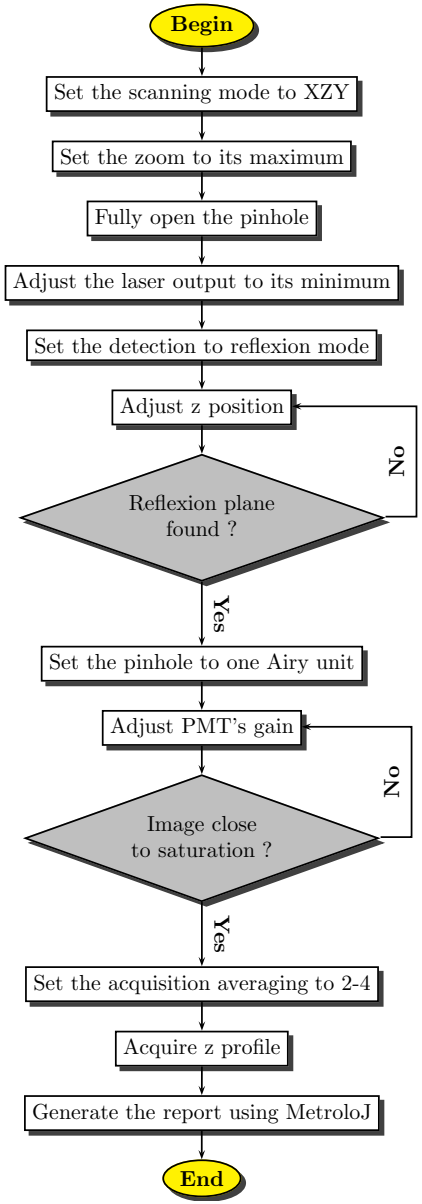
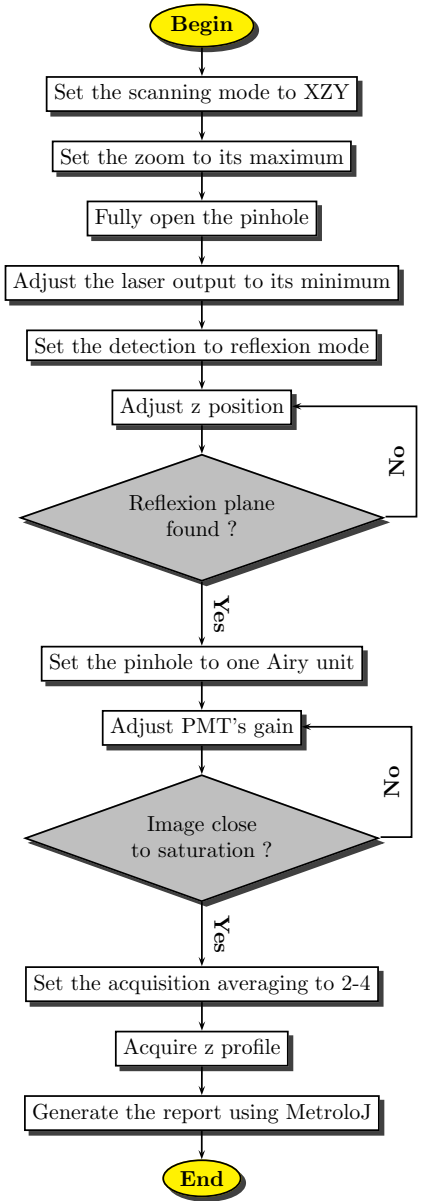
- **Aim :** On a XZ reflexion pattern obtained imaging a mirror, mesure the Z resolution.
- **Sample to be used :** A plane mirror slide.

Resolution : Mirror slide based measurement



- **Aim :** On a XZ reflexion pattern obtained imaging a mirror, mesure the Z resolution.
- **Sample to be used :** A plane mirror slide.
- **Acquisition of a standardized image**.

Resolution : Mirror slide based measurement



- **Aim :** On a XZ reflexion pattern obtained imaging a mirror, mesure the Z resolution.
- **Sample to be used :** A plane mirror slide.
- **Acquisition of a standardized image**.
- **Informations to be retrieved :** Based on an averaged intensity profiles across the reflexion profile, fitted on a Gaussian function, determine the FWHM (estimate of the resolution).

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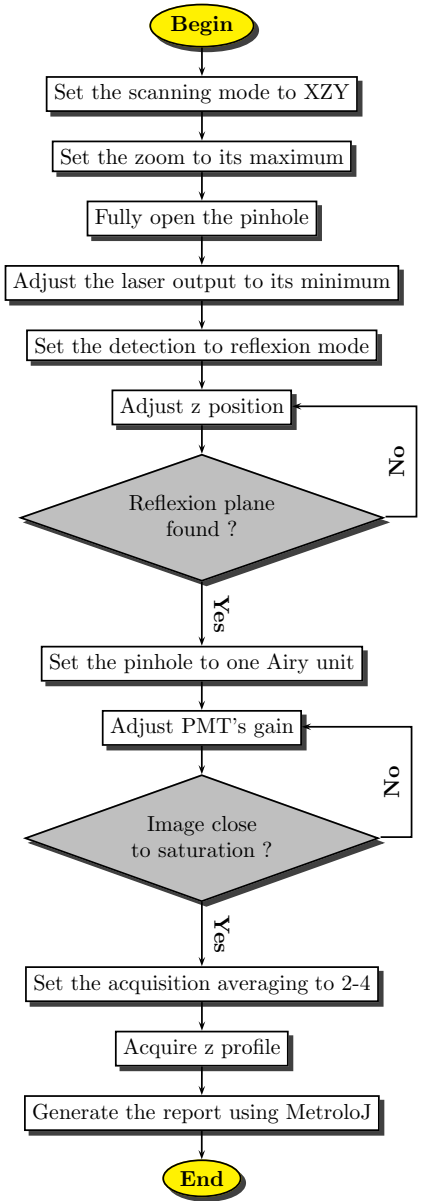

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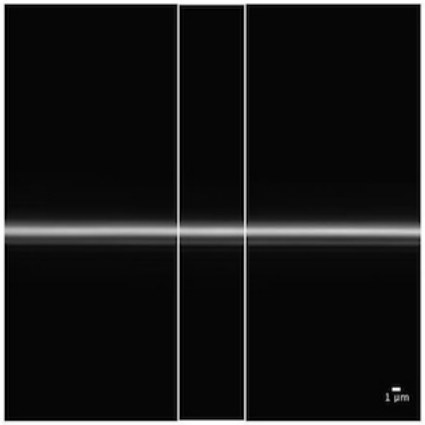
- **Preventive/Pro-active actions :** Check the optical path, check for index mismatches (ex : RI of the immersion oil, mounting medium...), ..., call after-sale service.

Mirror slide based measurement : what's on the report ?

12 août 2010 14:27
Axial resolution report on Axial resolution

Profile view:



Microscope infos:

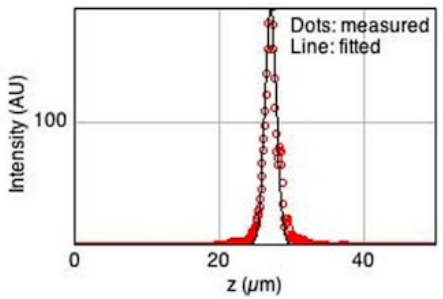
Microscope: Confocal
Wavelength: 510.0 nm
NA: 1.4
Sampling rate: 0.098x0.098x1.0 µm
Pinhole: 1.0 Airy Units

Resolution table:

ROI: from (214, 0) to (296, 512)

	FWHM	Theoretical resolution
z	1.87 µm	0.364 µm

Z profile & fitting parameters:



Fitted on y = a + (b-a)*exp(-(x-c)^2/(2*d^2))
Number of iterations: 364 (8000)
Number of restarts: 2 (2)
Sum of residuals squared: 15748.1863
Standard deviation: 5.5514
R^2: 0.9669
Parameters:
a = 4.2723
b = 188.4883
c = 27.2543
d = 0.7939

Topic 4 : Co-alignement

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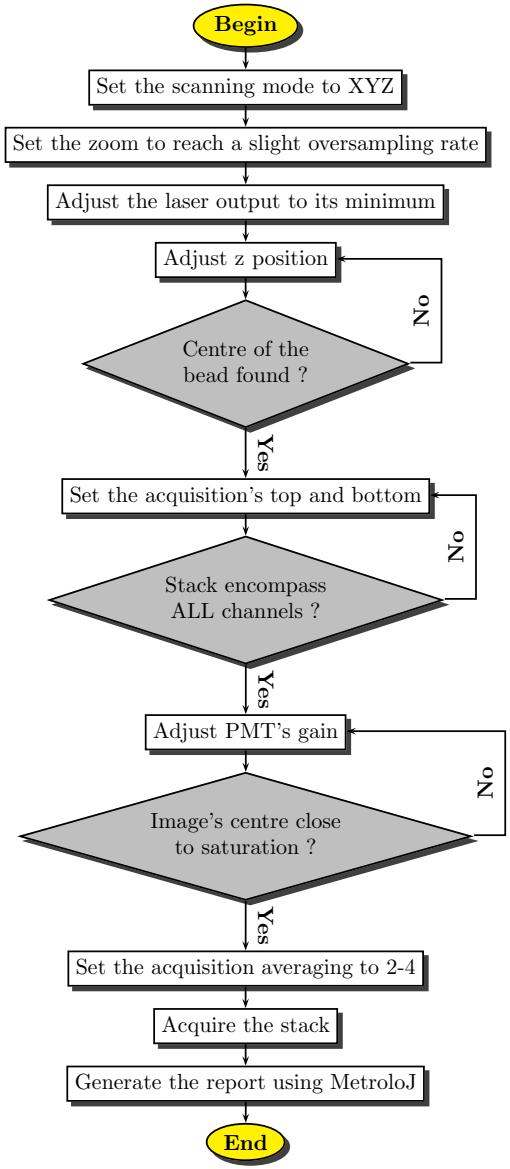

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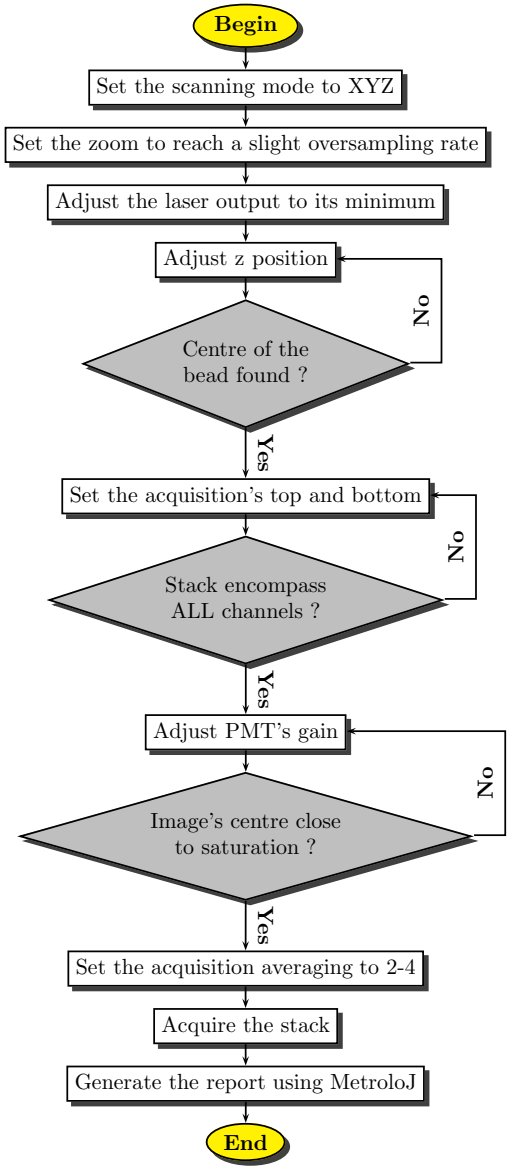

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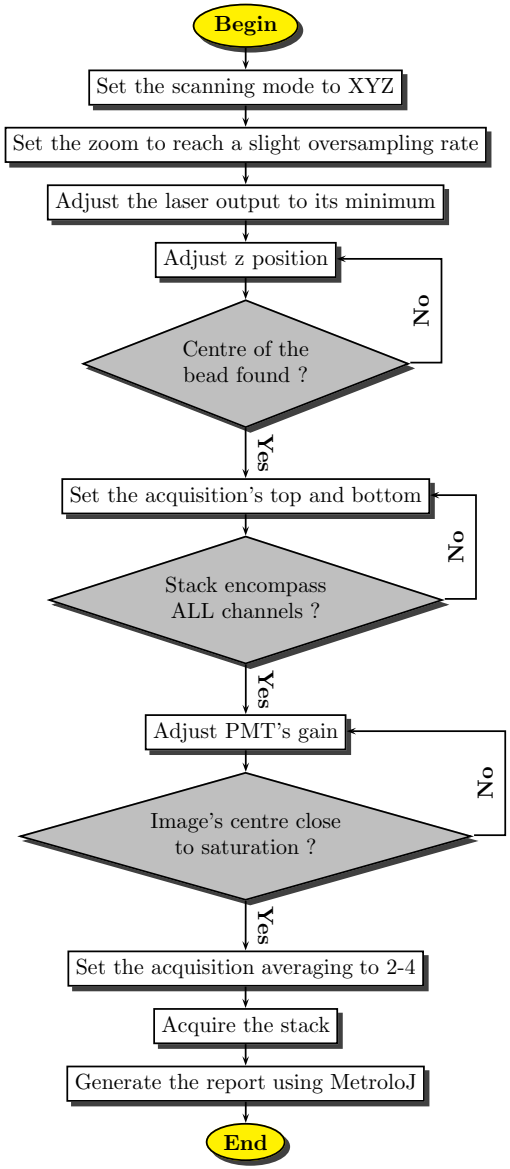

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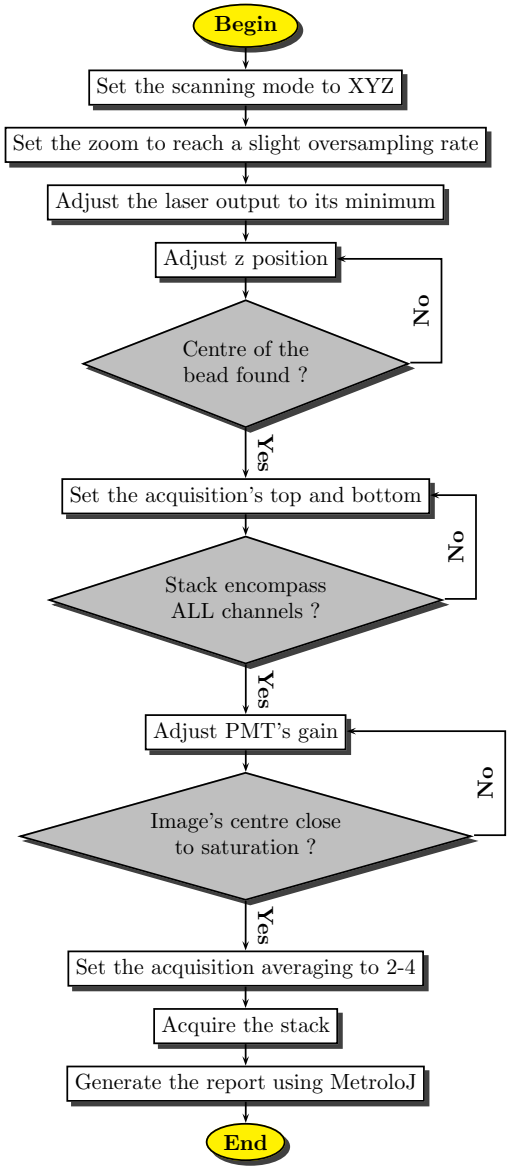

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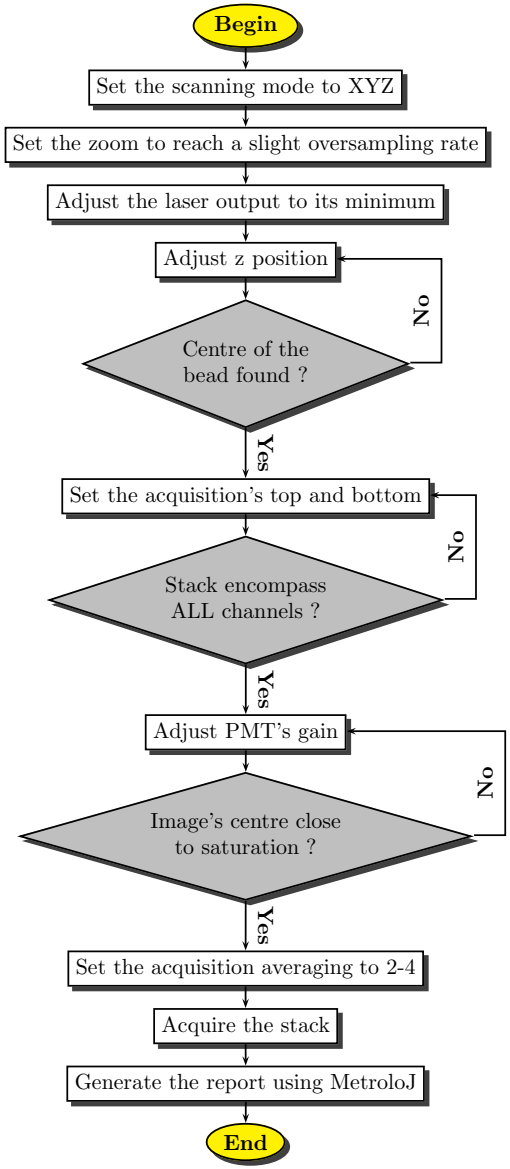

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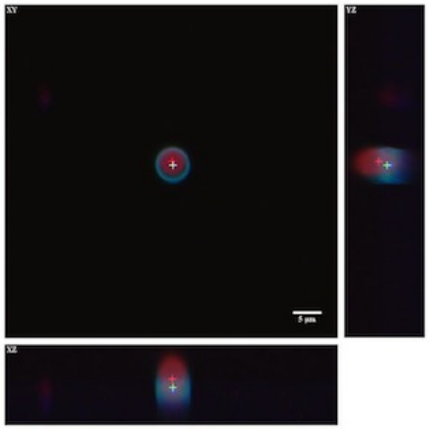
- **Preventive/Pro-active actions :** Check the optical path, re-align the light source, check for index mismatches (ex : RI of the immersion oil, mounting medium...), ..., call after-sale service.

Co-alignement : what's on the report ?

12 août 2010 14:30
Co-Alignement report
Co-alignement

Profile view:



Microscope infos:

Microscope: Confocal
Wavelengths: 600.0, 500.0, 400.0 nm
NA: 1.4
Sampling rate: 0.112x0.112x1.0 μm
Pinhole: 1.0 Airy Units

Pixel shift table:

Shift (pix.)	Red (Ref.)	Green (Ref.)	Blue (Ref.)	Titles
Red	0 0 0	-0.5 -5.5 -1.5	-2.0 -5.5 -2.0	Co-alignement (red).tif
Green	0.5 5.5 1.5	0 0 0	-1.5 0.0 -0.5	Co-alignement (green).tif
Blue	2.0 5.5 2.0	1.5 0.0 0.5	0 0 0	Co-alignement (blue).tif

Distances table (uncalibrated):

Dist. (pix.)	Red	Green	Blue	Resolutions (pix.)	Centres' coord.	Titles
Red	-	5.723	6.185	1.524 1.524 0.429	258.0 241.0 5.5	Co-alignement (red).tif
Green	5.723	-	1.581	1.27 1.27 0.357	258.5 246.5 7.0	Co-alignement (green).tif
Blue	6.185	1.581	-	1.016 1.016 0.286	260.0 246.5 7.5	Co-alignement (blue).tif

Distances table (calibrated):

Dist. (Ref. dist.) μm	Red	Green	Blue	Resolutions (μm)	Centres' coord.	Titles
Red	-	1.624 (0.334)	2.106 (0.274)	0.171 0.171 0.429	258.0 241.0 5.5	Co-alignement (red).tif
Green	1.624 (0.334)	-	0.528 (0.273)	0.143 0.143 0.357	258.5 246.5 7.0	Co-alignement (green).tif
Blue	2.106 (0.274)	0.528 (0.273)	-	0.114 0.114 0.286	260.0 246.5 7.5	Co-alignement (blue).tif

Why and when should the MetroloJ package be used ?

The MetroloJ package is a collection of plugins aimed at extracting quantitative data out of images taken on standardized samples, using standardized procedures.

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Ongoing/future works

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- **Data archiving, systems' benchmarking :**
  - Option : send measures to a database
  - From the database, get an estimate of the "normal" situation to which each single measure might be compared to.

Acknowledgments

Thanks to all the members of the 'groupe de travail Métrologie du RT-MFM" and/or of the MetroloJ package's beta-testers... (and all apologies for the potentially forgotten ones).

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