

DEF_AST_ESTIMATOR reference manual

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1. Quick start

This software is an implementation of algorithms described in M.Vulović, E. Franken, R.B.G. Ravelli, L.J. van Vliet, and B. Rieger, *Precise and Unbiased Estimation of Astigmatism and Defocus in Transmission Electron Microscopy* (Ultramicroscopy, 2012, accepted)

The algorithm to estimate defocus and astigmatism with their uncertainties have been implemented in DipImage, a publicly available software toolbox (www.diplib.org) for Matlab (The MathWorks, Inc.). Matlab needs to include also Statistics toolbox and Optimization toolbox. Thon ring averaging is optional and we provide precompiled mex files for windows 64 bit and linux 64 bit. For other architectures: If a C compiler is installed, the code is automatically compiled.

The following input parameters are required for the function **estimate_df_ast**:

- 1) Image of an amorphous sample. The Fourier transform of the image (PSD) should show at least one Thon ring.
- 2) The list of physical and processing parameters given in a structure params

Have a look at the file `example1.m` which shows how to set obligatory physical parameters and call the function. This example demonstrates the defocus and astigmatism estimation without Cs correction, for underfocus ($df > 0$, $A1 > 0$), high magnification and relatively small astigmatism. `example2.m`, `example3.m` and `example4.m` show three more examples for the different acquisition conditions and how to change default processing parameters. `example2.m` shows the estimation for overfocus and small astigmatism; `example3.m` demonstrates the estimation with Cs correction; while `example4.m` shows the estimation from just a couple of rings. Additionally, `example_check_rings.m` could be used as a help to find best processing parameters to make Thon rings more prominent (semi-automated approach).

2. How to use the program

DEF_AST_ESTIMATOR is intended to estimate defocus and astigmatism in TEM from the images of an amorphous sample together with corresponding uncertainties.

The program is run by executing the script (e.g. `example1-4`) that calls the function `estimate_df_ast.m` :

```
[df, std_df, ellipticity, angle, ast, std_ast] = estimate_df_ast(a,params);
```

INPUT:

a - the input image
params - structure with input parameters

OUTPUT:

df - estimated defocus
std_df - uncertainty of defocus estimate
ellipticity - estimated ellipticity of the Thon rings
angle - orientation of astigmatism
ast - estimated astigmatism
std_ast - uncertainty of astigmatism estimation

The input image and appropriate parameters must be specified in that script. The parameters are organized in structure file params and split in two groups.

In the first group are the obligatory input parameters such as:

1. pixel size in the specimen plane (`params.pixsize`)
2. voltage of the electron source (`params.voltage`)
3. spherical aberration coefficient (`params.Cs`)

The second group includes the processing parameters (optional). Their default values are specified in `estimate_df_ast.m`. However, if you want to change them to suit better your acquisition (see section 3 of this manual and `example_check_rings.m` for some recommendations), you can uncomment the lines in the script (e.g. lines 54-85 in `examples2-4.m`) and change any value. If you want to use only default values keep those lines commented. For more details about the parameters look at the Table1 and [Recommendations for parameters adjustment](#) section.

Table1. Description of the input parameters. Obligatory parameters are given in red.

parameters (structure)	Description
<code>params.pixsize</code>	Pixel size in the specimen plane in [m]. It can be specified directly, or indirectly by specifying magnification, physical pixel size of the camera, binning of the acquired image and post magnification factor (from the film plane to the camera plane).
<code>params.voltage</code>	Voltage of the electron source in [V]
<code>params.Cs</code>	Spherical aberration coefficient of the objective lens in [m]
<code>params.prepro</code>	Preprocessing of the PSD (suppressing the background and inverting the Thon rings contrast) Options: 'adaptive', 'bilateral', 'adaptivespec', 'bilateralspec'.
<code>params.b_c2</code>	Additional binning in the spatial domain
<code>params.b_f</code>	Additional binning in the Fourier domain

<code>params.sigmas</code>	Sigmas of filter derivatives (used if <code>params.prepro</code> is 'adaptivespec' or 'bilateralspec')
<code>params.spa_sig</code>	Spatial sigma for <code>params.prepro</code> = 'bilaterspec'
<code>params.tonal_sig</code>	Tonal sigma for <code>params.prepro</code> = 'bilaterspec'
<code>params.signdf</code>	Use the knowledge that expected defocus is underfocus (<code>params.signdf</code> = 1), overfocus (<code>params.signdf</code> = -1), or unknown (<code>params.signdf</code> = 0). Default = 0.
<code>params.use_df_mic</code>	Use defocus value from the microscope as a help (if you think it is reasonable) (=1 for yes, =0 for no). In case of 1, please provide that value as <code>params.defocus</code> . Default=0.
<code>params.defocus</code>	Defocus value displayed by the microscope. Keep in mind that defocus in the microscope is usually given with negative sign for underfocus, and positive for overfocus. Provide it as such.
<code>params.parav_sem</code>	Periodogram averaging (=1 for yes, =0 for no). Default =0.
<code>params.iterNo</code>	Number of patches used for periodogram averaging
<code>params.patchSz</code>	Patch size used for periodogram averaging
<code>params.DISPLAY</code>	Display graphs and the results (=1 for yes, =0 for no)
<code>params.equi</code>	Outlier rejection based on CTF zeros equidistance in q^2 space (=1 for yes, =0 for no)
<code>params.cs_correct</code>	Use the correction (2-step approach) if the Cs influence on aberration function is more than 10% of the defocus influence (=1 for yes, =0 for no)
<code>params.ast_strength</code>	Expected strength of the astigmatism. Options: 'large' (Ellipticity R=1.2), 'small' (R=1.02), 'verysmall' (R=1.002), 'none' (R=1), 'specify' (specify <code>params.expectedratio</code>).
<code>params.expectedratio</code>	Expected ellipticity. After the initial estimate, the estimation can be 'refined' once again by the estimated value of ellipticity. In this case <code>params.ast_strength</code> must be 'specify'.
<code>params.TRAv</code>	Calculates and displays the circular, elliptical and Thon ring averaging. (=1 for yes, =0 for no)
<code>params.TRAs</code>	Thon ring assessment. (=1 for yes, =0 for no). Possible only if <code>params.TRAv</code> =1

3. Recommendations for adjustment of some processing parameters

Default processing parameters can be adjusted (by uncommenting a parameter in the script) to suit better your acquisition. Here are recommendations to adjust some of your parameters. Please look also at `example_check_rings.m`.

Table2. Processing parameters

Parameters	Description and recommendation
<code>params.prepro</code>	Preprocessing of the PSD. Options: 'adaptive', 'bilateral', 'adaptivespec', 'bilateralspec'. Default is 'adaptive'. In a case that SNR is poor and only a couple of rings are to be detected, 'bilateral' could be a better option as it segments better lower frequency rings. The sigmas of 'adaptive' and 'bilateral' filters can be varied using 'adaptivespec', 'bilateralspec'. Have a look at <code>example_check_rings.m</code> how these are used. There are also options 'adaptive1', 'adaptive2-6', as well as 'bilateral1-11'. They use different blurring parameters of the filter. 'laplace' is a simple second- order Gaussian derivative filter.
<code>params.b_c2</code>	Spatial binning. Options: 1,2,4,8. It is used to enhance the SNR and reduce the processing time. By default it is set to 2 for images =>4k and to 1 otherwise. However, If the PSD has rings that are narrow and close to each other, but they do not extend to a high fraction of Nyquist frequency (e.g. relatively high magnification and high defocus), spatial binning is beneficial.
<code>params.b_f</code>	Binning in Fourier domain. Options: 1,2,4,8. It is used to enhance the SNR and reduce the processing time. By default it is set to 2 for images >=1k, and to 1 otherwise. The lower values (e.g.1 or 2) can be used if magnification and/or defocus is large. The higher values (e.g. 4,8) are recommended for relatively low magnification (e.g.<100k x) or not excessive defocus (e.g. <10 μm) when the distance between neighboring CTF zeros is not small.
<code>params.sigmas</code>	Sigmas of filter derivatives (used if <code>params.prepro</code> is 'adaptivespec' or 'bilateralspec') . For 'adaptivespec' it is an array (e.g. [3 1] blurs in the direction of a Thon ring 3 times more than perpendicular to it). See help <code>gaussf_adap.m</code> and <code>prefilt.m</code> for more details. For 'bilateralspec' <code>params.sigmas</code> is isotropic and therefore scalar.
<code>params.spa_sig</code>	Spatial sigma for <code>params.prepro</code> = 'bilaterspec'. See help of <code>bilateral.f.m</code> for more details
<code>params.tonal_sig</code>	Tonal sigma for <code>params.prepro</code> = 'bilaterspec'. See help of <code>bilateral.f.m</code> for more details
<code>params.signdf</code>	Use the knowledge that expected defocus is underfocus (<code>params.signdf =1</code>), overfocus (<code>params.signdf =-1</code>). Default is unknown (<code>params.signdf =0</code>).

<code>params.use_df_mic</code>	Use defocus value from the microscope as a help (if you think it is reasonable) (=1 for yes, =0 for no). Default is 0. In case of 1, please provide that value as params.defocus
<code>params.defocus</code>	Defocus value displayed by the microscope. Keep in mind that defocus in the microscope is usually given with negative sign for underfocus, and positive for overfocus. Provide it as such.
<code>params.parav_sem</code>	Periodogram averaging (=1 for yes, =0 for no). Default is 0. It is used to enhance the SNR and reduce the processing time alternatively and/or complementary to the binning in Fourier domain. Larger <code>params.b_f</code> corresponds to periodogram averaging (with smaller patches). If the image is not homogeneous, it is recommended to avoid periodogram averaging or to use larger number of patches.
<code>params.ast_strength</code>	Expected strength of the astigmatism. Options: 'large' (Ellipticity R=1.2), 'small' (R=1.02), 'verysmall' (R=1.002), 'none' (R=1), 'specify' (specify params.expectedratio). Default: 'large'. If the estimated astigmatism is small, rerun the estimation with 'small' to get potentially more accurate estimation. If you want to specify the ellipticity that was found from the estimate and use it for the second (maybe refined) estimation fill in the next field <code>params.expectedratio</code>
<code>params.expectedratio</code>	Expected ellipticity. After the initial estimate, the estimation can be 'refined' once again by the estimated value of ellipticity. In this case params.ast_strength must be 'specify'. This new value is used to make templates.

4. Conditions of use

The Quantitative Imaging Group of the TU Delft has developed and is the owner of the image processing routines distributed in this package, hereafter called SOFTWARE. The SOFTWARE is free for non-commercial use by students and staff in universities or non-profit research institutes. Redistribution of SOFTWARE or parts thereof in any form is not permitted. This SOFTWARE is distributed in the hope that it will be useful, but without any warranty; without even the implied warranty of merchantability or fitness for a particular purpose.